THE APPLICATION OF PHYTOPLANKTON IN ECOLOGICAL ASSESSMENT OF THE BALKHASH LAKE (KAZAKHSTAN)

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Abstract. The Balkhash Lake is a unique natural water body with a gradient of the total content of dissolved salts along its longitudinal axis and the increased level of toxic pollutions. To assess the spatial distribution of environmental factors along the Balkhash Lake surface, methods of bioindication and statistical mapping were used. An assessment of the overall level of toxic pollution was carried out using the Water Ecosystem State Index (WESI). Phytoplankton has been used to assess the ecological state of the Balkhash Lake in the summer of 2004. A total of 92 species were found in the algal communities, with prevalence of Chlorophyta in the western, fresher water part, and Bacillariophyta in the eastern, brackish part. Mapping showed a positive relationship between the species richness in algal Divisions and macrophytes. Changes in the proportion of indicator species within the composition of phytoplankton reflected the spatial gradient of depth, TDS, pH-values, organic pollution. According to bioindication data, the waters of the Balkhash Lake are moderately polluted, water quality class III. The WESI index calculation revealed the effect of the total toxic pollution on the phytoplankton of the Western Balkhash. The spatial distribution of the WESI index showed that in the zone the influence of river flow has stimulating effect of biogenic elements on phytoplankton but was partially leveled by the increased level of toxic pollution.
Keywords: *bioindication, heavy metals, organic pollution, total dissolved solids, WESI index, phytoplankton, Balkhash Lake, Kazakhstan*

Introduction

The Balkhash Lake is located in arid zone of the southeastern Kazakhstan, on sedimentary carbonate soils with granites, porphyries, tuff and slates in its northern and western parts (Sidorenko, 1972; Leonov, 2013). The water area is the third largest following the Caspian Sea and Aral Sea. One distinct feature about the lake is its spatial heterogeneity of the content of Total Dissolved Solids (TDS) in water (Tarasov, 1961). The arid climate also conditions interannual changes of TDS associated with fluctuations of level of the Balkhash Lake.

Due to the rich reserves of polymetallic ores (Mazurov, 2005), there are mining and processing industry facilities in the region (Kudekov, 2002). The territory south of Balkhash Lake is used for agriculture and pasture cattle. The combination of natural

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and anthropogenic factors determines the increased level of pollution and substantial heterogeneity of ecological conditions on various parts of the water area of the Balkhash Lake.

Recently, methods of the biological analysis have been used more and more for assessment of quality of water. Phytoplankton serves as a good instrument for that purpose (Barinova et al., 2006; Barinova and Chekryzheva, 2014; Klymiuk and Barinova, 2016; Krupa et al., 2016; Krupa and Barinova, 2016; Barinova and Krupa, 2017), and its application has not exhausted its potential (Barinova et al., 2015a). Water quality assessment is carried out both on the basis of structural indicators of biological communities (abundance, biomass, ratio of divisions in quantitative indicators, average cell volume, diversity indices), and with indicator species distribution.

The basis for using indicator algae for bioindication is their relation to environmental factors - the amount of organic matter, oxygen conditions, salinity of water, etc. The system for assessing the level of organic pollution of water bodies on the basis of the ratio of indicator species of various systematic groups was developed by V. Sládeček (1973). S. S. Barinova (Barinova et al., 2006; Barinova, 2011; Barinova and Fahima, 2017; Barinova, 2017a) compiled the electronic database on algal indicators with species-specific ecological preferences in the world. This database on the ratio of algal species, in addition to the level of organic contamination, allows assessing a wider range of environmental factors such as temperature, oxygen conditions, salinity, prevailing depths, and water pH.

When assessing the ecological status of any waterbody, it is most difficult to detect the effect of toxic pollution, in particular the effect of heavy metals, on biological communities. This is due to the fact that the toxicity of heavy metals depends on the multidirectional action of a complex of environmental factors. So, high temperature intensifies, and increased salinity and pH reduce the toxic properties of heavy metals for living organisms (Li et al., 2013). These same environmental factors affect the absorption of heavy metals by submerged plants (Fritioff et al., 2005) and thereby to self-purification processes in aquatic ecosystems.

One of the new methods, allowing in an integrated form to assess the effect of the general level of toxic pollution on phytoplankton communities, is the Water Ecosystem State Index (WESI) (Barinova et al., 2006; Barinova, 2011, 2017a, b). The indicator significance of this index was demonstrated by us earlier when assessing the ecological state of water bodies in Kazakhstan and other regions (Barinova et al., 2011, 2015a, b; Barinova and Chekryzheva, 2014; Barinova and Krupa, 2017).

The research on phytoplankton of Balkhash Lake has begun in 1929. In the years 1929-1930 the microalgae were represented by 308 species and forms (Abrosov, 1973). The main contribution to the diversity of algal flora was made by benthic species of algae, which did not play a significant role in plankton. Data on quantitative parameters of algal flora during this period are absent. The Kapshagay reservoir began to fill from the Ili River in 1970 and the Ili River is the main filling source of the Balkhash Lake. Reduction of the volume of river flow led to a decrease in the water level and intensification of toxic pollution of the lake. The biomass of phytoplankton in the summer of 1976-1978 after the completion of the Kapshagay reservoir filling, reached 0.51-1.64 g m^{-3} (Vorobyeva et al., 1982; Fokina, 1982). During the period from 1998 to 2012, the variety of planktonic algae ranged from 82 to 146 species (Ponomareva et al., 2005; Krupa et al., 2014a). The mean annual biomass averaged 0.94 g m^{-3}, fluctuating from 0.17 to 1.86 g m^{-3} across different years. The diatom algae dominated the biomass

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most often, followed by blue-greens. Studies of long-term dynamics (1972-2012) revealed the influence of the invader, the bivalve *Monodacna colorata* on phytoplankton biomass (Krupa et al., 2013), and the relationship between the long-term variability of phytoplankton biomass and regional and global climatic factors (Krupa et al., 2014b). The spatial distribution of total species richness (Barinova et al., 2017) and structural variables of phytoplankton (Krupa et al., 2017a, 2018) was studied along the Balkhash Lake in a gradient of abiotic factors based on the materials of 2004.

There is no information in the recent publications on the indicator species of planktonic algae and their using for assessment of organic and toxic pollution level of the Balkhash Lake.

The aim of the present work is to assess the organic contamination, hydrochemical and oxygen conditions, and water pH by the mapping of spatial distribution of indicator species of planktonic algae in the Balkhash Lake as well as assess the overall level of toxic pollution of the reservoir on the basis of spatial distribution of the Water Ecosystem State Index (WESI). For Lake Balkhash this will be done for the first time.

Materials and methods

Sampling and chemical analysis

The research of phytoplankton, the content of biogenic elements and heavy metals in the water was carried out by means of a grid of 58 stations (*Figs. 1* and *2*) in June and July of 2004. The measures of the temperature and pH values of the surface water layers were taken in the field environment. Water transparency was measured with Secchi disk. Coordinate referencing of the stations was done by Garmin eTrex GPS-navigator. The samples for heavy metals were fixed in the site by adding nitric acid; samples for biogenes were fixed with chloroform. All collected samples were transported to the lab in an icebox. One-liter samples of phytoplankton were sampled from surface layer of the lake water.

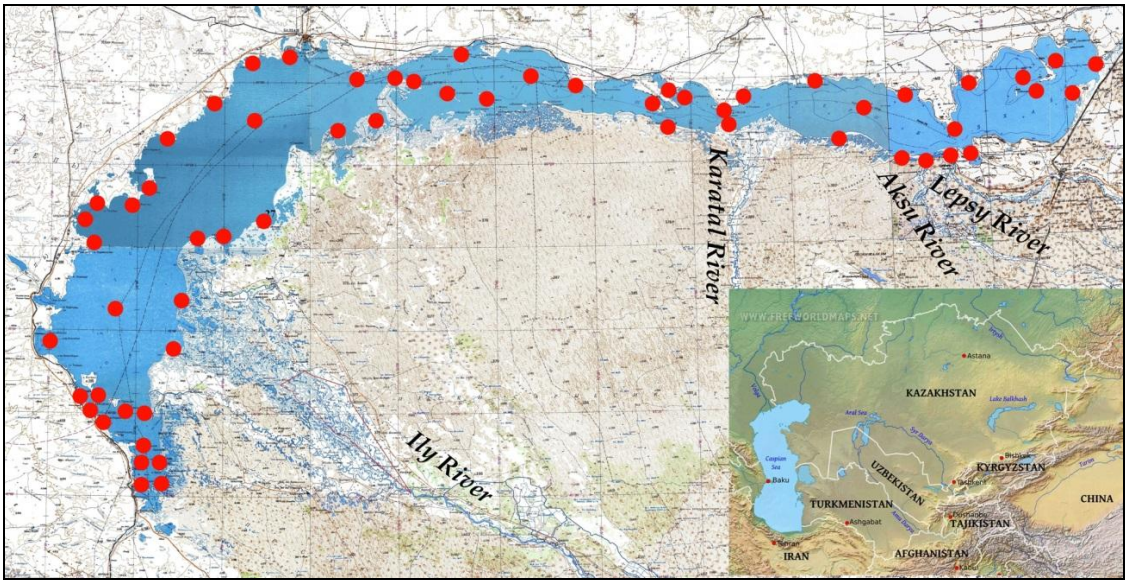


Figure 1. *Map of sampling stations on the Balkhash Lake, 2004*

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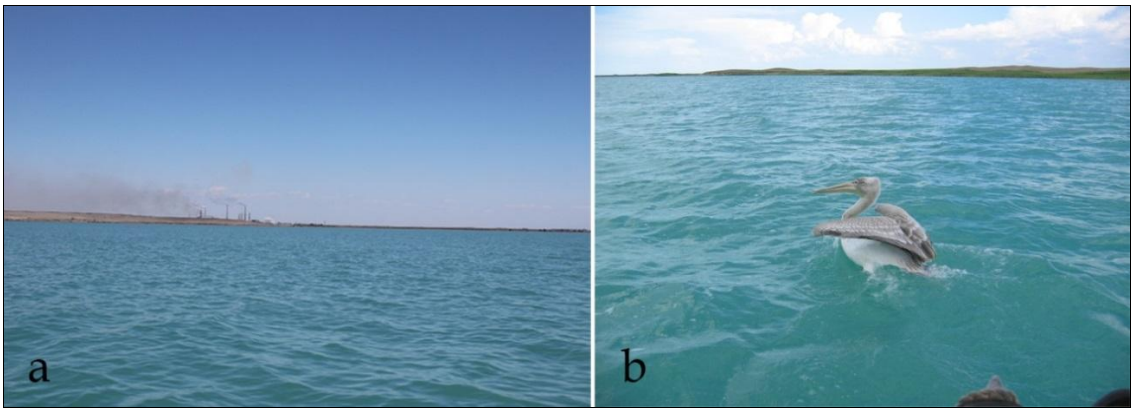


Figure 2. *Western (a) and eastern parts (b) of the Balkhash Lake, 2004*

Conventional methods of chemical analysis of water were used (Semenova, 1977; Fomin, 1995). Water samples were analyzed in three – four replications. The error of estimate for major ions in the water was 0.5–5.0%, depending on the analysis. Concentrations of heavy metals were determined by the atomic absorption method using an AAS-1N spectrophotometer (Germany). The device allows for the detection of the various chemical elements in complex matrices, including those in the sea and grey water and in the biological objects in micro-trace quantities Test-sensitivity of AAS-1N spectrophotometer – 0.001–0.0025% Mass.

The settling method was used (Kiselev, 1956) to process phytoplankton samples. Species identification of planktonic algae was performed using determinant handbooks for relevant divisions (Gollerbach et al., 1953; Zabelina et al., 1951; Popova, 1955; Moshkova et al., 1986; Palamar-Mordvintseva, 1982; Matvienko, 1954).

Bioindication

The ecological characteristics of algal species were obtained from the database compiled for freshwater algae of the world from multiple analyses of algal biodiversity by Barinova et al. (2006), according to substrate preference, temperature, oxygenation, pH, salinity, organic enrichments, as well as N-uptake metabolism and trophic states according Van Dam et al. (1994). The ecological groups were separately assessed according to their significance for bioindications. Species that respond predictably to environmental conditions were used as bioindicators for particular variables of aquatic ecosystems, the dynamics of which are related to environmental changes.

The correspondence between the classificational indicators of ecosystem according to the biogenic elements and those of saprobity indices was conducted through calculating the index of the ecosystem state (WESI, Water Ecosystem State Index) as the quotient from the classification rank on biota divided by the classification rank on the nitrates. The WESI index ranges from 0 to 9. If it is less than one, then the ecosystem is exposed to the toxic effects; if equal to or greater than 1 – the self-purification is not suppressed (Barinova et al., 2006, Barinova, 2011).

Statistical methods

The statistical methods used were those recommended by Heywood (2004) for the development of taxonomic studies. The innovative approach with spatial mapping in

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wafer plots construction was applied using the Statistica 12.0 program for analysis of biological and environmental variables distribution over the lake surface. For this type of calculation we used GIS coordinates of each sampling station. Data of biological variables for the Balkhash lake sampling stations were inserted to the Statistica 12.0 program and maps were created for each variable. Statistically generated maps are better if we have numerous sampling points. The maps have some prognostic properties and let to assume the level of each mapped variable between sampling points.

Description of the study site

The Uzun-Aral Channel divides the Balkhash Lake into two parts: western, which is shallow and wide (Western Balkhash), and eastern, which is narrow and deep (Eastern Balkhash). The lake is elongated from southwest to northeast (*Fig. 1*). Its length makes up to 614 km, and the area is approximately 16400 km^2 if the water level is 342 m above sea level. Width of the lake reaches 9-19 km in the eastern part and up to 74 km in western part (Kudekov, 2002). The lake is fed by rivers Ili, Karatal, Lepsy and Aksu. There is constant flow of water from the west to the east (flow of run-off) (Tarasov, 1961). The movement of water masses begins from the mouth of Ili River and flows into the Western Balkhash. Encroaching on the opposite shore, the current is directed to the northeast and reaches the Uzun-Aral Channel. Further flow is caused by a difference of levels between the western and eastern parts as the Karatal, Lepsy and Aksu rivers do not fully compensate the evaporation from a surface of the Eastern Balkhash.

Results

Hydrophysical characteristics

The Western and Eastern Balkhash significantly differ in environmental variables (*Table 1*). The shallow western part of the lake is characterized by lesser transparency and pH-values of water, poorer distribution of stiff macrophytes (reed, cane, cattail), but it is more overgrown with soft macrophytes (pondweed plants, *Myriophyllum*). Macrophytes developed mainly in the southern and southeast parts of the lake where rivers discharge their waters such as the Ili River in the Western Balkhash, and the Karatal, Aksu and Lepsy rivers in the Eastern Balkhash.

Table 1. *Hydrophysical and morphometric characteristics of Balkhash Lake, summer 2004*

Variable	Whole Balkhash	Western Balkhash	Eastern Balkhash
Temperature, °C	24.14±0.14	23.49±0.16	24.77±0.14
Depth, m	5.58±0.49	4.34±0.28	6.86±0.81
Transparency, m	1.13±0.15	0.53±0.02	1.74±0.25
Macrophytes Hard, %	20.05±3.03	18.03±3.00	22.14±5.36
Macrophytes Soft, %	25.18±2.68	28.28±3.29	22.00±4.24
pH	8.63±0.04	8.52±0.02	8.74±0.09

Hydrochemical and toxicological characteristics

Total Dissolved Solids (TDS) content in the East Balkhash is statistically significantly higher, than in the western part (*Table 2*). In Western Balkhash, the

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concentration of nitrates, nitrites, total phosphorus, iron, zinc and copper is higher than in the eastern part. Phosphates, easily oxidized organic matter, cadmium, lead, nickel and cobalt were present in the waters of the East Balkhash in large concentration. The content of ions of ammonium and silicon did not differ in the two parts of the lake.

Table 2. *Chemical water variables, toxicants, index S and index WESI for the Balkhash Lake, summer 2004*

Variable*	Whole Balkhash	Western Balkhash	Eastern Balkhash
TDS	2286.0±236.0	1080.3±83.4	3436.8±286.7
Cl	473.3±58.9	170.5±18.6	762.4±71.3
NH_4	0.102±0.017	0.103±0.013	0.102±0.033
NO_3	0.945±0.244	1.379±0.410	0.492±0.226
NO_2	0.042±0.011	0.060±0.013	0.024±0.016
$P\text{-}PO_4$	0.017±0.004	0.011±0.003	0.023±0.007
P_{tot}	0.064±0.001	0.106±0.012	0.021±0.007
Oxydability	7.39±0.46	5.07±0.40	9.13±0.45
Zn	0.028±0.009	0.039±0.018	0.017±0.002
Cu	0.018±0.003	0.022±0.005	0.013±0.002
Cd	0.0036±0.0002	0.0028±0.0002	0.0044±0.0002
Pb	0.034±0.003	0.021±0.002	0.047±0.004
Ni	0.039±0.001	0.037±0.002	0.042±0.002
Co	0.013±0.001	0.010±0.0005	0.017±0.001
Index S	1.85±0.04	1.94±0.06	1.78±0.04
Index WESI	1.35±0.14	0.86±0.20	1.86±0.20

*All variables units as mg dm^{-3}, Oxydability as mg O_2 dm^{-3}, indices S and WESI are unitless

Phytoplankton

Altogether 92 species of phytoplankton were revealed, among them green algae were prevail with 29 species, then follow diatoms (27), cyanobacteria (21), charophytes and euglenophytes (4 each), dinophytes (3), chrysophyte algae (1) (*Table 3*).

Table 3. *Species diversity with ecological preferences in phytoplankton of the Balkhash Lake, summer 2004*

Taxa	T	Hab	Reo	pH	pH-range	Hal	D	Tro	Aut-Het	Sap	Index s
Bacillariophyta											
Achnanthes sp.	-	-	-	-	-	-	-	-	-	-	-
Amphora sp.	-	-	-	-	-	-	-	-	-	-	-
Asterionella formosa Hassall	-	P	st-str	alf	7.4	i	sx	o-m	ate	o	1.35
Aulacoseira granulata (Ehrenberg) Simonsen	temp	P-B	st-str	ind	5.8-9.4	i	es	me	ate	b	2.0
Chaethoceros sp.	-	-	-	-	-	-	-	-	-	-	-
Cocconeis sp.	-	-	-	-	-	-	-	-	-	-	-
Coscinodiscus sp.	-	-	-	-	-	-	-	-	-	-	-

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Cyclotella meneghiniana Kützing	-	P-B	-	-	-	hl	-	-	-	-	-
Cymbella cymbiformis C. Agardh	temp	B	str	ind	6.2-9	i	sx	o-m	ats	b	2.0
Cymbella sp.	-	-	-	-	-	-	-	-	-	-	-
Diatoma hyemalis (Roth) Heiberg	temp	B	st-str	alf	6.2-9.0	i	sx	me	ate	o-b	1.5
Entomoneis paludosa (W. Smith) Reimer	-	P-B	-	alf	-	hl	-	m	-	b-a	2.5
Eunothia sp.	-	-	-	-	-	-	-	-	-	-	-
Fragilaria acus (Kützing) Lange-Bertalot	-	P	st-str	alb	-	i	es	-	-	o-a	1.8
Fragilaria crotonensis Kitton	-	P	st-str	alf	7.5	I	es	m	ate	o-b	1.5
Gyrosigma acuminatum (Kützing) Rabenhorst	cool	B	st-str	alf	-	i	es	me	ate	o-a	1.95
Melosira sp.	-	-	-	-	-	-	-	-	-	-	-
Navicula sp.	-	-	-	-	-	-	-	-	-	-	-
Nitzschia acicularis (Kützing) W. Smith	temp	P-B		alf	7.85-8.15	i	es	e	hce	a-o	2.7
Nitzschia sigmoidea (Nitzsch) W. Smith		P-B	st-str	alf	6.2-8.8	i		e	ate	b-a	2.5
Nitzschia sp.	-	-	-	-	-	-	-	-	-	-	-
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	-	B	st-str	alf	6.7	i	es	me	ate	o-a	1.9
Rhopalodia gibba (Ehrenberg) Otto Müller	temp	B	-	alf	6.2-9.0	i	es	o-m	-	o-b	1.4
Surirella sp.	-	-	-	-	-	-	-	-	-	-	-
Tabellaria sp.	-	-	-	-	-	-	-	-	-	-	-
Tryblionella angustata W. Smith		P-B	st	alf	7.7	i	sx	m	ats	o-b	1.5
Tryblionella hungarica (Grunow) Frenguelli	-	P-B	-	alf	-	mh	sp	e	ate	a-o	2.9
Charophyta											
Cosmarium sp.	-	-	-	-	-	-	-	-	-	-	-
Desmidium sp.	-	-	-	-	-	-	-	-	-	-	-
Staurastrum sp.	-	-	-	-	-	-	-	-	-	-	-
Staurastrum gracile Ralfs ex Ralfs	-	P-B	st	acf	-	i	-	m	-	o	1.3
Chlorophyta											
Ankistrodesmus sp.	-	-	-	-	-	-	-	-	-	-	-
Binuclearia lauterbornii (Schmidle) Proschkina-Lavrenko	-	-	-	-	-	-	-	-	-	o-a	1.8
Botryococcus braunii Kützing	-	P-B	st	ind	-	i	-	-	-	o-b	1.5
Characiopsis sp.	-	-	-	-	-	-	-	-	-	-	-
Characium sp.	-	-	-	-	-	-	-	-	-	-	-

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Chlamydomonas sp.	-	-	-	-	-	-	-	-	-	-	-
Chlorella sp.	-	-	-	-	-	-	-	-	-	-	-
Chlorococcum sp.	-	-	-	-	-	-	-	-	-	-	-
Cladophora sp.	-	-	-	-	-	-	-	-	-	-	-
Coelastrum microporum Nägeli	-	P-B	st-str	ind	-	i	-	-	-	b	2.3
Dictyosphaerium simplex Korshikov	-	P	-	-	-	-	-	-	-	b-a	2.5
Franceia sp.	-	-	-	-	-	-	-	-	-	-	-
Lagerheimia sp.	-	-	-	-	-	-	-	-	-	-	-
Monoraphidium minutum (Nägeli) Komárková-Legnerová	-	P-B	st-str	-	-	i	-	-	-	b-a	2.5
Mucidosphaerium pulchellum (H.C. Wood) C. Bock, Proschold & Krienitz	-	P-B	st-str	ind	-	i	-	-	-	b	2.3
Oocystis solitaria Wittrock	-	P	st	ind	-	i	-	-	-	b-o	1.7
Oocystis sp.	-	-	-	-	-	-	-	-	-	-	-
Oocystis borgei J.W. Snow	-	P-B	st-str	ind	-	i	-	-	-	o-a	1.9
Palmella sp.	-	-	-	-	-	-	-	-	-	-	-
Pseudoschroederia robusta (Korshikov) E. Hegewald & E. Schnepf	-	P-B	st-str	-	-	i	-	-	-	o-a	1.9
Scenedesmus arcuatus (Lemmermann) Lemmermann	-	P-B	st-str	-	-	i	-	-	-	o-a	1.9
Scenedesmus balatonicus Hortobagyi	-	-	-	-	-	-	-	-	-	b	2.0
Scenedesmus quadricauda (Turpin) Brébisson	-	P	-	ind	-	i	-	-	-	b	2.1
Selenastrum sp.	-										
Tetradesmus obliquus (Turpin) M.J. Wynne	-	P-B	st-str	ind	-	i	-	-	-	b	2.05
Tetraëdron caudatum (Corda) Hansgirg	-	P-B	st-str	ind	-	i	-	-	-	b	2.0
Tetraëdron triangulare Korshikov	-	P-B	st-str	-	-	i	-	-	-	b	2.0
Tetraëdron minutissimum Korshikov	-	-	-	-	-	-	-	-	-	-	-
Tetrapedia gothica Reinsch	-	-	-	-	-	-	-	-	-	-	-
Tetrapedia sp.	-	-	-	-	-	-	-	-	-	-	-
Chrysophyta											
Dinobryon sp.	-	-	-	-	-	-	-	-	-	-	-
Cyanobacteria											
Anabaena sp.	-	-	-	-	-	-	-	-	-	-	-

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Aphanizomenon flosaquae Ralfs ex Bornet & Flahault	-	P	-	-	-	hl	-	m	-	o-a	1.95
Aphanothece sp.	-	-	-	-	-	-	-	-	-	-	-
Chroococcus turgidus (Kützing) Nägeli	-	P-B,S	aer	alf	-	hl	-	-	-	x-b	0.8
Coelosphaerium kuetzingianum Nägeli	-	P	-	-	-	i	-	m	-	b-o	1.6
Coelosphaerium pusillum Van Goor	-										
Gloeocapsa sp.	-	-	-	-	-	-	-	-	-	-	-
Gloeocapsopsis crepidinum (Thuret) Geitler ex Komárek	-	B	-	-	-	hl	-	-	-	-	-
Gloeocapsopsis magma (Brébisson) Komárek & Anagnostidis ex Komárek	-	S	-	ind	-	i	-	-	-	-	-
Gomphosphaeria aponina Kützing	-	P-B	st-str	alf	-	hl	-	o	-	o	1.2
Limnococcus limneticus (Lemmermann) Komárková, Jezberová, O. Komárek & Zapomelová	-	P	-	-	-	i	-	o-m	-	b-o	1.65
Merismopedia glauca (Ehrenberg) Kützing	-	P-B	-	ind	-	i	-	o-m	-	b-o	1.75
Merismopedia smithii De Toni	-	P-B	-	-	-	i	-	o	-	o-b	1.5
Merismopedia tenuissima Lemmermann	-	P-B	-	-	-	hl	-	e	-	b-a	2.4
Merismopedia minima G. Beck	-	B,S	aer	-	-	-	-	o	-		
Microcystis aeruginosa (Kützing) Kützing	-	P	-	-	-	hl	-	e	-	b	2.1
Microcystis pulverea (H.C. Wood) Forti	-	P-B,S	-	-	-	i	-	-	-	o-b	1.5
Microcystis sp.	-	-	-	-	-	-	-	-	-	-	-
Phormodium sp.	-	-	-	-	-	-	-	-	-	-	-
Pseudanabaena endophytica (Elenkin & Hollerbach) Anagnostidis	-	Ep	-	-	-	i	-	-	-	-	-
Snowella lacustris (Chodat) Komárek & Hindák	-	P	-	-	-	i	-	me	-	b-o	1.6
Spirulina sp.	-	-	-	-	-	-	-	-	-	-	-
Dinophyta											
Gymnodinium sp.	-	-	-	-	-	-	-	-	-	-	-
Peridinium sp.	-	-	-	-	-	-	-	-	-	-	-
Prorocentrum cordatum (Ostenfeld) J.D. Dodge	-	P	-	-	-	hl	-	-	-	-	-

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Euglenophyta											
Euglena sp.	-	-	-	-	-	-	-	-	-	-	-
Phacus sp.	-	-	-	-	-	-	-	-	-	-	-
Strombomonas sp.	-	-	-	-	-	-	-	-	-	-	-
Trachelomonas sp.	-	-	-	-	-	-	-	-	-	-	-

Note: Temperature preferences (T): temp – temperate temperature, eterm – eurythermic, Substrate preferences (Hab): P – planktonic, P-B – plankto-benthic, B – benthic. Oxygenation and water moving (Reo): st – standing water, str – streaming water, st-str – low streaming water, aer – aerophiles. Acidity (pH) degree according to Hustedt (1957): alf – alkaliphiles, ind – indifferents; acf – acidophiles; alb – alkalibiontes. Halobity degree according to Hustedt (1938-1939) (Hal): i – oligohalobes-indifferent, hl – halophiles, mh – mesohalobes. Organic pollution indicators according to Watanabe et al. (1986) (D): sx – saproxenes, es – eurysaprobes, sp – saprophyles. Trophic state (Tro) (Van Dam et al., 1994): o – oligotraphentic; o-m – oligo-mesotraphentic; m – mesotraphentic; me – meso-eutraphentic; e – eutraphentic; o-e – oligo-eutraphentic. Nitrogen uptake metabolism (Aut-Het) (Van Dam et al., 1994): ats – nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate – nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne – facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen; hce – nitrogen-heterotrophic taxa, needing elevated concentrations of organically bound nitrogen. Species-specific Index of Saprobity (s). Self-purification zone preferences (Sap): x – xenosaprob; o – oligosaprob; o-b – oligo-beta-msosaprob; o-a – oligo-alpha-mesosaprob; b – beta-mesosaprob; b-o – beta-oligo-mesosaprob; b-a – beta-alpha-mesosaprob; a-o – alpha-oligo-mesosaprob; a – alpha-mesosaprob

In the Western Balkhash, where Chlorophyta prevailed (*Fig. 3*), the number of species of planktonic algae (74) was higher than in the Eastern Balkhash (69). Bacillariophyta primarily formed the species richness in communities in brackish eastern part. Despite the specified distinctions, trends of distribution of the abundance of species per taxonomic Division almost coincided in eastern and western parts of the water area, which testified to the similarity of species structure in the lake as a whole. In the Western Balkhash, dinophyte *Peridinium* sp., diatom *Cyclotella meneghiniana*, euglenophyte *Trachelomonas* sp. were found rather often. In the Eastern Balkhash, besides the two latter species, diatom *Navicula* sp., green *Francei*a sp., cyanobacteria *Snowella lacustris*, *Gomphosphaeria aponina,* and *Gloeocapsa* sp. were widespread.

In relation to the habitat preferences, planktonic-benthic and planktonic species were prevalent within phytoplankton. Revealed species-indicators prefer alkaline waters (alkaliphiles) or are indifferent to pH, and serve as indicators of moderately oxygenated waters, temperate temperatures, and are indifferent to salinity (*Fig. 3*). In comparison with the western part, the Eastern Balkhash had more planktonic algae species that are associated with temperate temperature waters, not cold, more indicators of oxygenated waters, and halophiles with presence of mesohalobes. Despite these differences, *Figure 3* illustrates that the lines of polynomial trends nearly coincided for all the indicator distributions in the Eastern and Western Balkhash, except pH-indicators. Here, there is an essential difference: the water of the eastern part of the lake, according to bioindication, is more alkaline than in the western part, and tops of trend lines are shifted into opposite directions of pH-spectrum.

Distribution of algae species-indicators of organic pollution of water show that the water quality of the lake generally corresponded to Class III (*Fig. 4*) according to Sládeček (1973), or Watanabe et al. (1986). In comparison with the west, the eastern part of the lake was characterized by slightly higher level of trophy, and that has been

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reflected by the presence of saprophiles (according to Watanabe et al., 1986) within phytoplankton species. Among algae-indicators of the nutrition type, there were mostly species that use photosynthesis and are stable against moderate concentration of organic nitrogen (ate). The number of species of this ecological group of algae was higher in the Eastern Balkhash. It should be noted that in the context of difference in species-indicators structure (*Fig. 3*), the distributions trends of the nutrition type indicators in the Eastern and Western Balkhash almost coincided (*Fig. 4*).

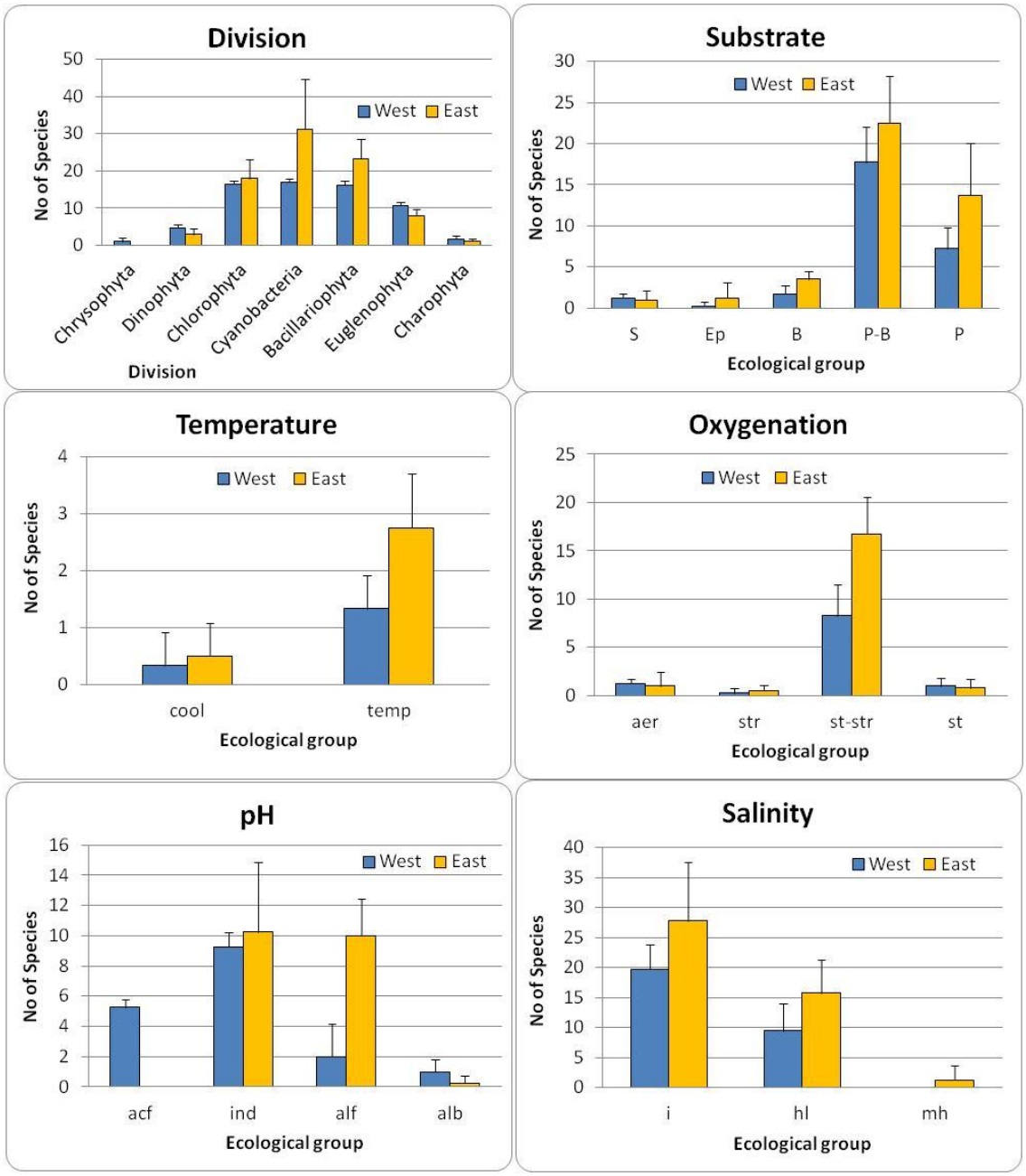


Figure 3. *Distribution of the number of species-indicators over taxonomic divisions and ecological groups in the Western and Eastern Balkhash according to phytoplankton species' preferences to habitat type, water temperature, pH, water mobility and saturation by oxygen, water pH, and salinity in summer 2004*

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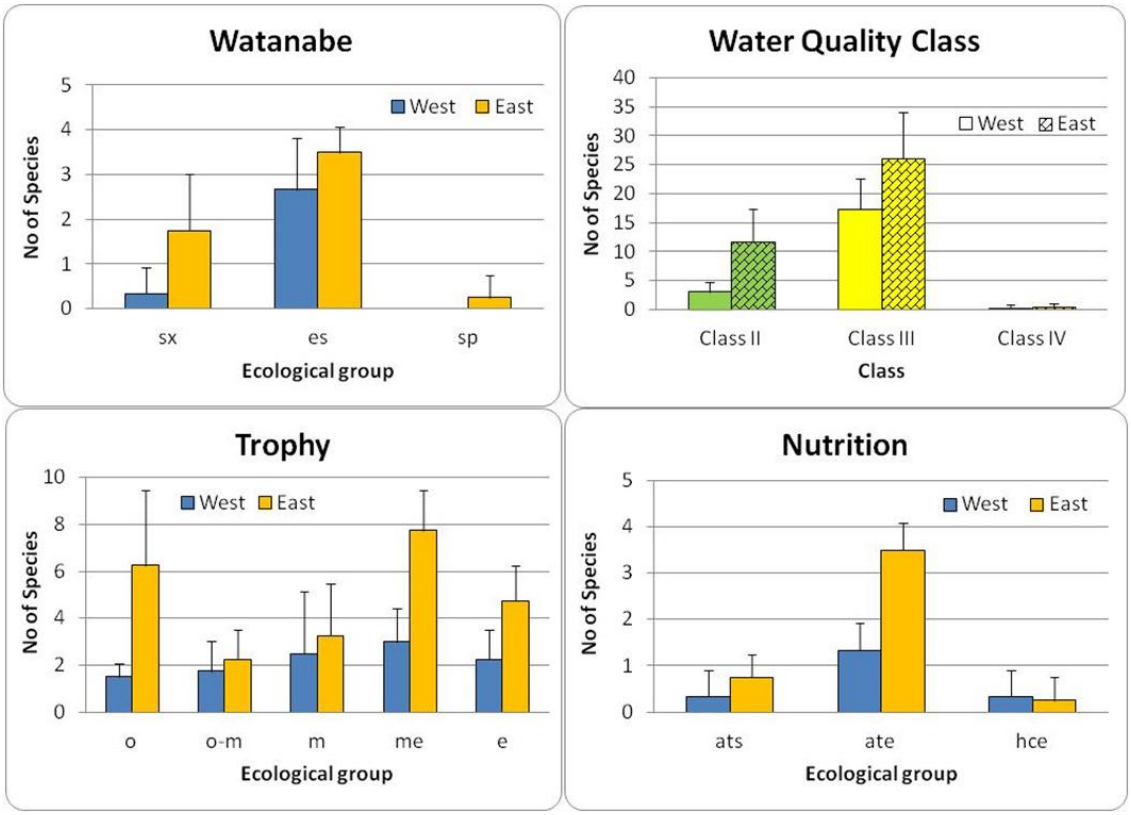


Figure 4. *Assessment of the ecological state of the Balkhash Lake according to phytoplankton species-indicators in relation to organic pollution according to Watanabe (1986) and Sládeček (1973), trophic status and nutrition type, summer 2004. Coloring of the histogram in the figure of water quality classes corresponds to the codes of the EU scale: Class II – green; Class III – yellow; Class IV – orange. Abbreviations for indicator groups of algae as in Table 3*

Wide groups of algae ranging from oligotrophs to eutrophs (*Fig. 4*) represented indicators of trophy. In Eastern Balkhash, mesotrophs had larger number of species, and the presence of eutrophs shifted the trend line of distribution of the indicators for this part of the water area towards a eutrophy.

Spatial distribution

The set of ecological maps have been constructed to assess the spatial distribution of indicator species across the water area of the Balkhash Lake. The majority of planktonic algae species has been spread unevenly, with outbursts of species richness on limited areas. Bacillariophyta was prevail in species number in areas of the Ili River discharge (the Western Balkhash), as well as the Karatal, Lepsy and Aksu rivers (the Eastern Balkhash) (*Fig. 5a*).

Chrysophyta was found in the Western Balkhash only, and together with Charophyta, it was most represented in area of the Ili River discharge (*Fig. 5b)*. The Cyanobacteria was discovered with high species richness in the eastern part of the water area (*Fig. 5c*). The spatial distribution of Dinophyta (*Fig. 5d*), Euglenophyta (*Fig. 5e*) and Chlorophyta species richness (*Fig. 5f*) did not follow certain patterns, but there were more of them in the western part.

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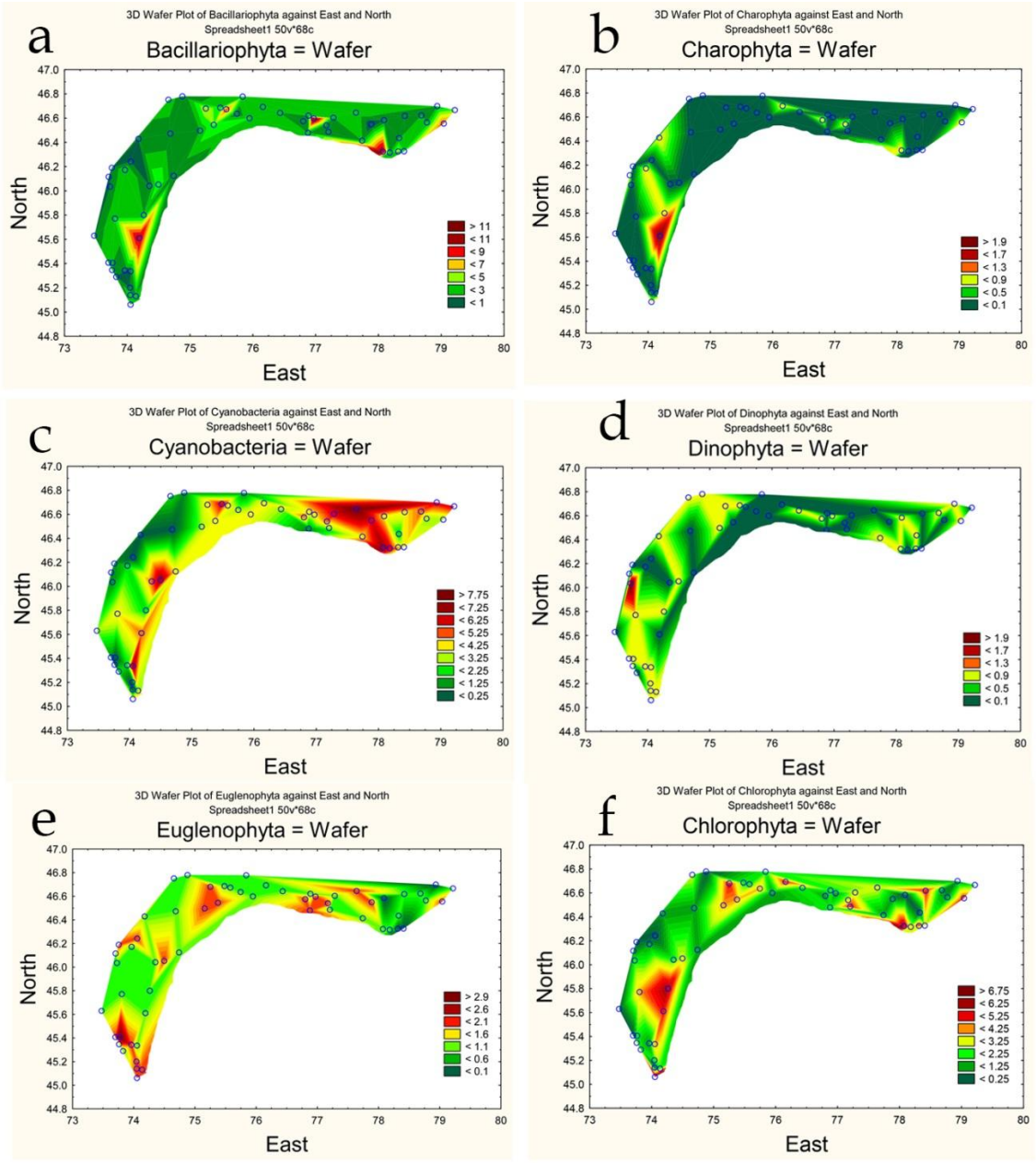


Figure 5. *The distribution of total phytoplankton species richness in the algal Divisions over the water surface of the Balkhash Lake, summer 2004*

We analyzed distribution of species indicators of substrate preferences. Planktonic algae prevailed in communities of the eastern deep-water part of the lake (*Fig. 6a*). Planktonic-benthic algae were widespread across the entire water area, with the highest species richness being recorded near the southeastern coast of the Western Balkhash and with the homogenous distribution of the number of species in the eastern part of the water area (*Fig. 6b*). Benthic species of algae preferred the areas of river discharge (*Fig. 6c*). Sparse species that have vital connection with a substrate such as epiphytes and soil related species were found across the whole lake water area with higher concentration near the coastal zones (*Fig. 6d*).

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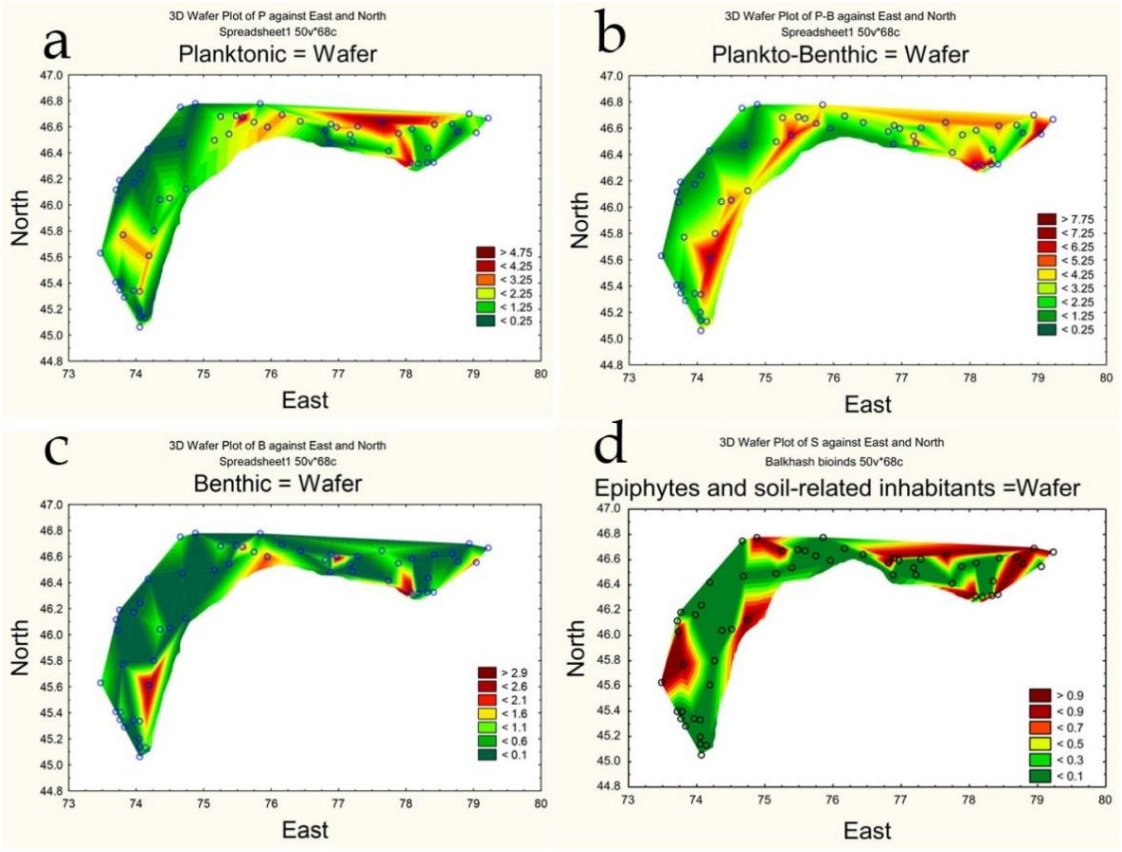


Figure 6. *The distribution of algal species-indicators of habitat over the water surface of the Balkhash Lake, summer 2004*

Species-indicators of moderate temperature conditions prefer water areas near the river discharge as well as shallow waters (*Fig. 7a*). Only few indicators of cold waters were found on some areas of water surface (*Fig. 7b*).

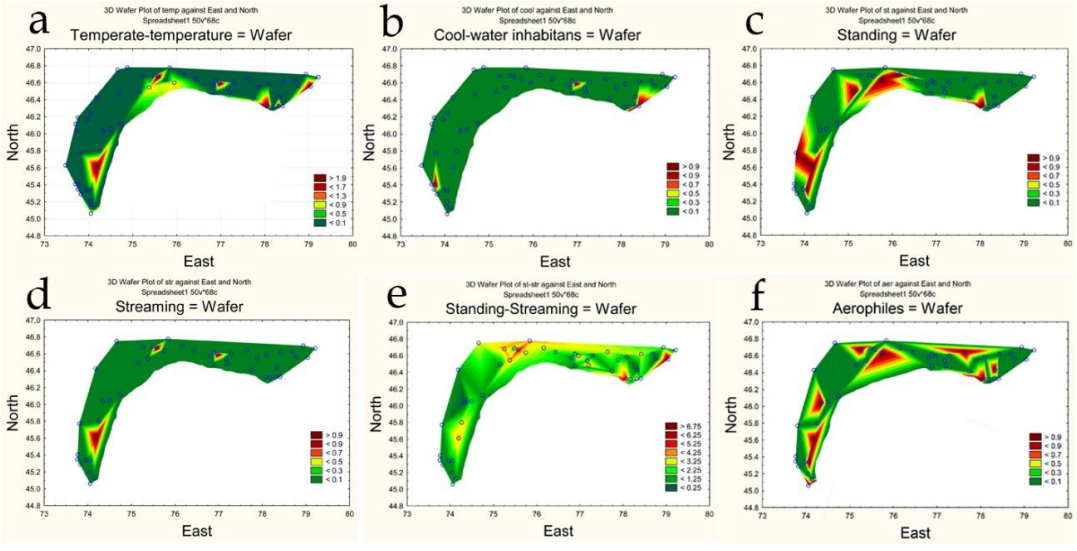


Figure 7. *The distribution of algal species-indicators of the temperature and water oxygenation over the water surface of the Balkhash Lake, summer 2004*

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Algae – inhabitants of the poorly oxygenated standing waters were represented by the greatest number of species in the western relatively shallow part of the lake (*Fig. 7c*), whereas the distribution of algae that prefer moving and oxygenated waters did not adhere to clear patterns (*Fig. 7d, e, f*).

Distribution maps of water pH indicators show that acidophiles (*Fig. 8a*) and alkalibionts (*Fig. 8c*) preferred the Western Balkhash, whereas alkaliphiles – the Eastern Balkhash (*Fig. 8d*). Species-indifferents were found across the whole water area, and they were drawn towards the southeastern coast of the Western Balkhash, towards northern and partially the southern and eastern coast of the Eastern Balkhash (*Fig. 8b*). There is notable absence of extreme groups of pH-indicators (acidophiles and alkalibionts) in the eastern part of the lake. That is, the western part of the water area is more varied regarding of water pH. Apparently, it is connected with the inflow of fresh water from the Ili River, and also with the general heterogeneity of conditions in the Western Balkhash.

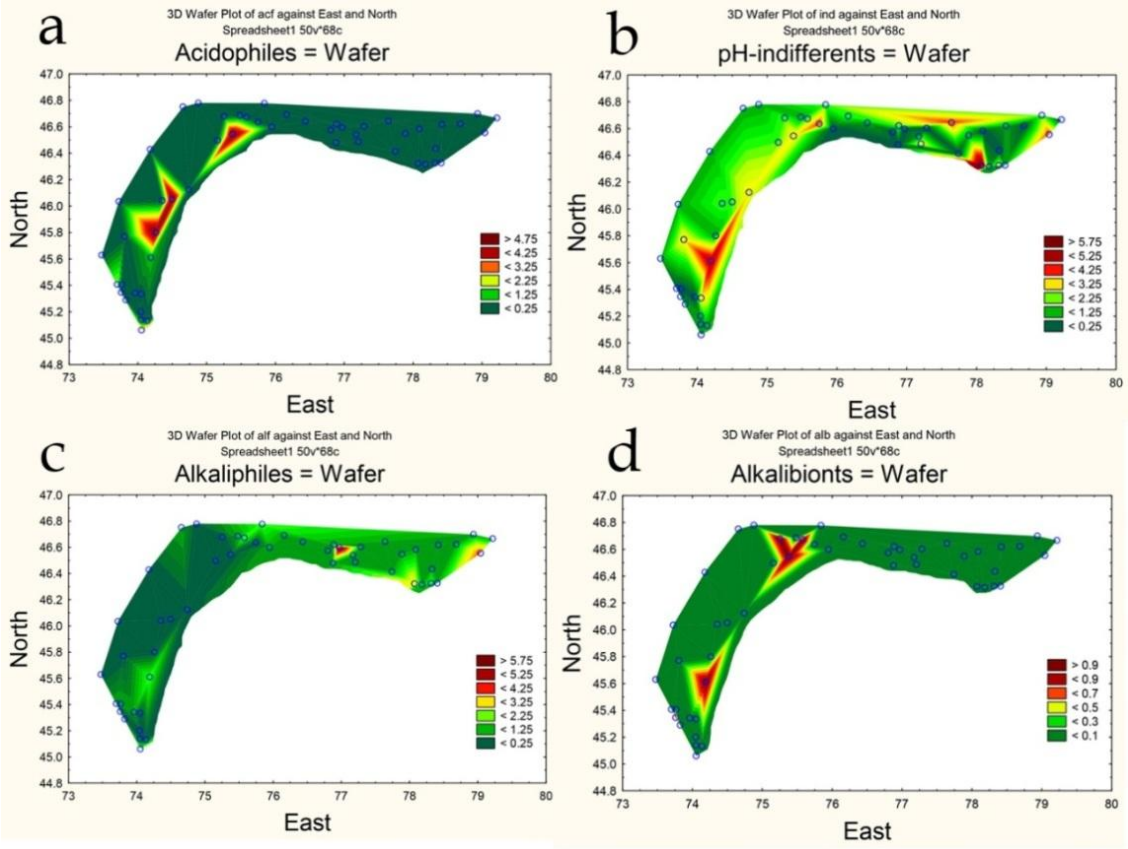


Figure 8. *The distribution of algal species-indicators of water pH over the water surface of the Balkhash Lake, summer 2004*

Maps of salinity indicators distribution demonstrated that mesohalobes were revealed only in the east brackish part of the water area (*Fig. 9a*). Species salinity-indifferents were widespread across the whole water area (*Fig. 9c*), and halophiles preferred the Eastern Balkhash and adjacent part of the water area of the Western Balkhash (*Fig. 9b*).

Algae – indicators of trophic state ranged from oligotraphentic to eutraphentic. Oligotraphentic species were most various in the Eastern Balkhash (*Fig. 10a*), oligo-

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mesotraphentic – in the Western Balkhash (*Fig. 10b*). Mesotraphentic, meso-eutraphentic and eutraphentic algae were recorded across the whole water area (*Fig. 10c, d, e*).

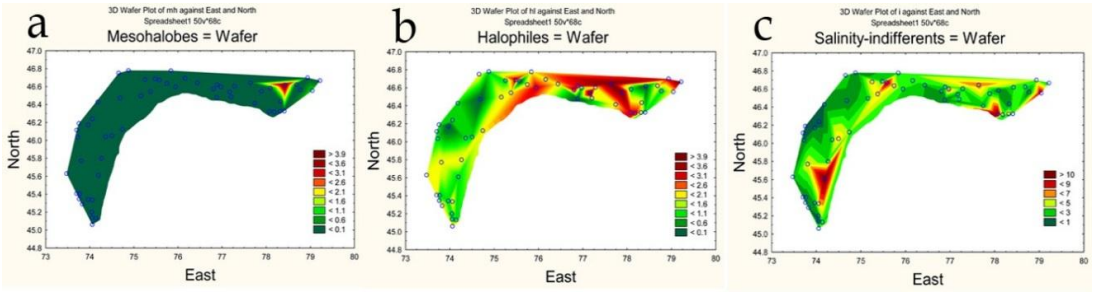


Figure 9. *The distribution of algae species-indicators of salinity over the water surface of the Balkhash Lake, summer 2004*

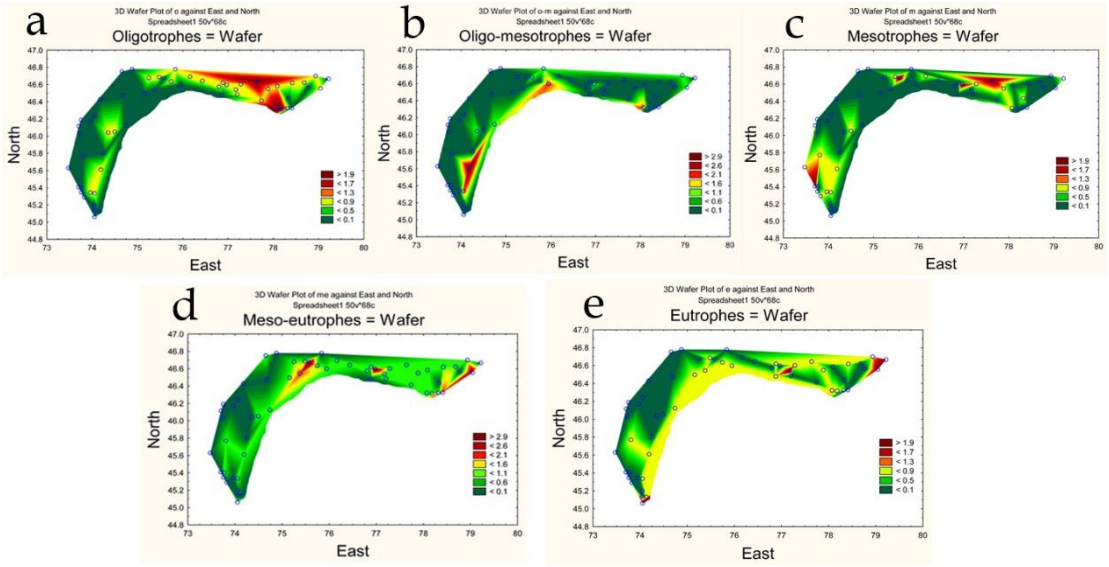


Figure 10. *The distribution of algae species – trophic indicators over the water surface of the Balkhash Lake, summer 2004*

The maps of distribution of species-indicators of nitrogen uptake metabolism show that nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen (*Fig. 11a*) and nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen (*Fig. 11b*) preferred areas of river drain discharge and shallow areas of the Eastern Balkhash. Trace amounts of facultative heterotrophs were found across the whole water area of the lake (*Fig. 11c*). Indicators of organic pollution (according to Watanabe, 1986) demonstrated similar distribution patterns for inhabitants of pure waters – saproxenes (*Fig. 11d*) and those of waters moderately saturated with organic matter – eurysaprobes (*Fig. 11e*).

The majority of algal indicators of organic pollution according to Sládeček (1973) pointed to the moderate level that corresponds to water quality class III (*Fig. 12*). Average values of saprobity index S (*Table 2*) confirmed this conclusion, even though they were slightly higher in the western part of the lake.

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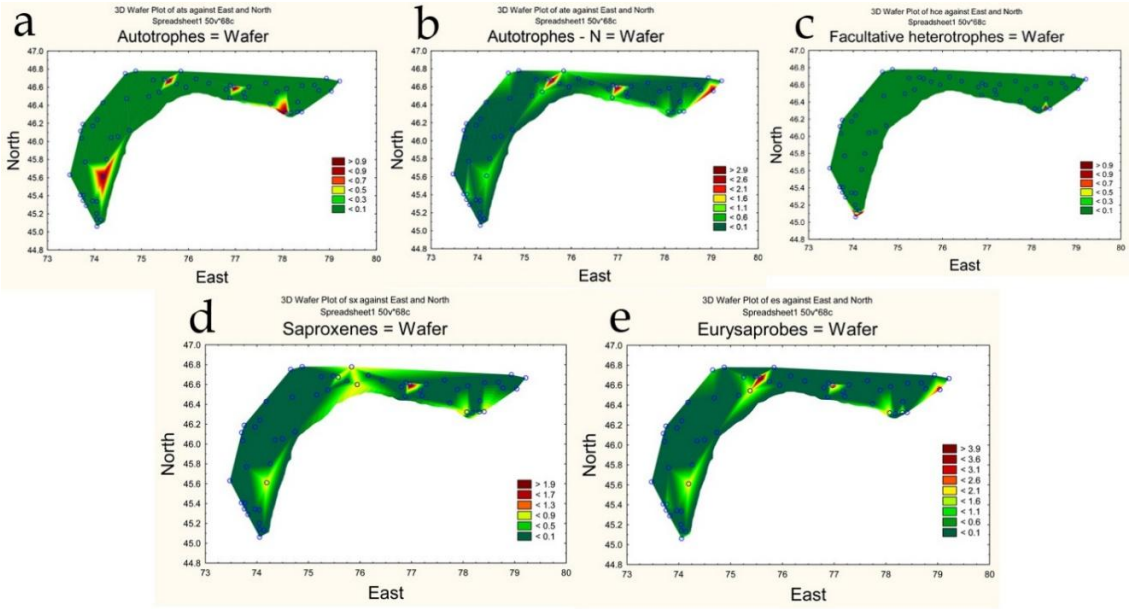


Figure 11. *The distribution of algal species – nutrition type indicators over the water surface of the Balkhash Lake, summer 2004*

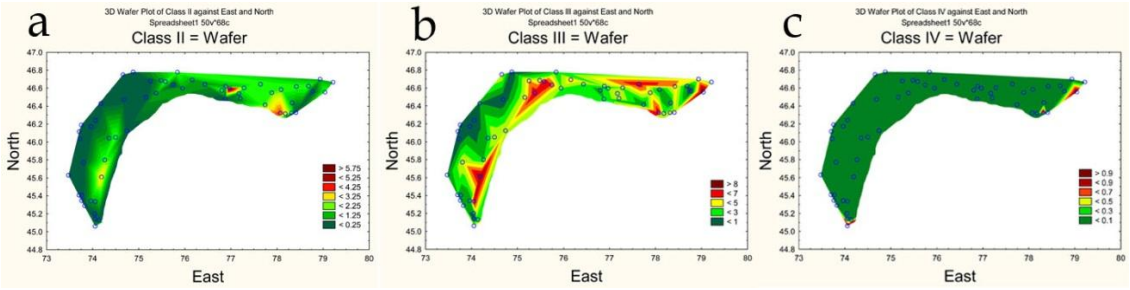


Figure 12. *The distribution of algal species-indicators of the water quality class over the water surface of the Balkhash Lake, summer 2004*

No significant correlation was revealed in the distribution of indicator species of phytoplankton and the content of heavy metals in water of the Balkhash Lake. The influence of the total concentration of toxicants on phytoplankton was evident using WESI index. The considerable part of the water area of the Western Balkhash was characterized by the WESI index being less than one, and that demonstrated an oppression of photosynthetic activity of algae (*Fig. 13*). According to index values, the toxic pollution did not affect the phytoplankton communities in the Eastern Balkhash.

Discussion

The Balkhash Lake is a unique natural water body with gradient of salt saturation of water along a longitudinal axis of the lake. Besides hydrochemical conditions, the western and eastern parts of Balkhash differ in other environmental variables. As can be seen on summer 2004 investigation results, water of the Western Balkhash contained increased concentrations of nitrates, nitrites, total phosphorus, iron, zinc and copper,

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and in the Eastern Balkhash, phosphates, easily oxidized organic matter, cadmium, lead, nickel and cobalt were prevalent.

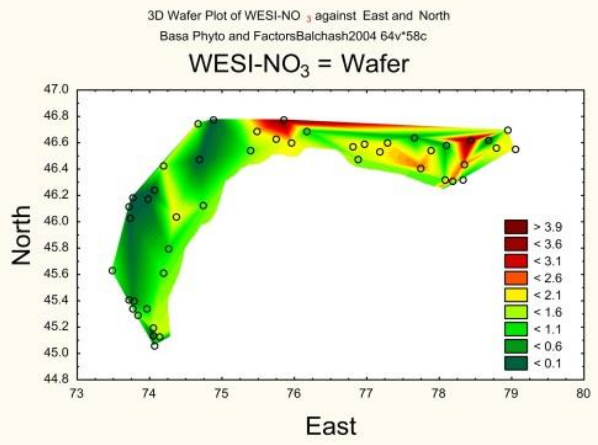


Figure 13. *The distribution of the WESI index values over the water surface of the Balkhash Lake, summer 2004*

Phytoplankton in the Balkhash Lake was represented by 92 species of algae in summer 2004. Bacillariophyta prevailed in species richness in brackish eastern part, whereas Chlorophyta in the Western Balkhash. Differences between total number of algal species in western freshwater part (74) and eastern brackish part of Balkhash (69) were insignificant. In the same time, a negative correlation was found between the number of species in phytoplankton community and water salinity in water body of another regions such as Eastern Mediterranean (Barinova et al., 2009), Northern (Klymiuk et al., 2014; Barinova et al., 2015b) and Eastern Black Sea region (Bilous et al., 2016), and Northern Kazakhstan (Barinova et al., 2011). Mapping illustrated that local outbreaks of the species richness Bacillariophyta, partly Charophyta, Cyanobacteria, Chlorophyta are associated with the shallow, macrophytic areas of the water surface that are under the influence of river discharge. We have previously demonstrated a positive relationship between the spatial distribution of the total number of species of plankton algae along the Balkhash Lake and macrophytes coverage (Barinova et al., 2017). Detailing the results obtained earlier in the present study showed that the species of the Bacillariophyta, Charophyta, Cyanobacteria, and Chlorophyta divisions contribute roughly to the increase in species richness in the areas where macrophytes are developed.

The analysis of published data showed that the relationship between the species richness of hydrocenosis and macrophytes could be both positive and negative (Peretyatko et al., 2009; Muylaert et al., 2010; Pieczyńska et al., 1999; Kuczyńska-Kippen and Joniak, 2015). Previously noted positive relationship between macrophytes and the number of species of phytoplankton in the water bodies in the arid zone of Kazakhstan (Barinova and Krupa, 2017) may have been because the presence of macrophytes enhances the role of small species in zooplankton, mainly rotifers (Krupa, 2012), that has no significant effect on algae.

The data visualization showed that the planktonic species are more widely represented in the Eastern Balkhash, which is consistent with the increase in depth in this part of the water area. The active reaction of water clearly divided the

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phytoplankton communities into two parts: of the Western Balkhash with predominance of pH-indifferent species and of the Eastern Balkhash with predominance of alkaliphiles, which is coherent with our pH measurements. The predominance of mesohalobes and halophiles in the phytoplankton of the Eastern Balkhash reflected a higher concentration of dissolved salts in this part of the lake water. The response of phytoplankton to the temperature and water oxygen saturation was approximately the same for the Eastern and Western Balkhash, while the zones of influence of river discharge and shallow waters substantially affected the distribution of indicator species.

Bioindication data has identified the Balkhash Lake as moderately polluted, with water quality class III. The distribution of algal species – indicators of the trophic state (the presence of nutrients) and the ratio of species to the total level of organic pollution of water (according to Sládeček) made for different estimates of the eastern and western parts of the lake. In comparison with the west, the eastern part of Balkhash has been characterized by a higher level of trophy, as evidenced by the appearance of saprophiles in the phytoplankton composition, the presence of more eutrophic species and species resistant to moderate concentrations of organic nitrogen.

This evaluation was supported by the spatial dynamics of phosphates (*Table 2*) which are present in low concentrations in the reservoirs of Kazakhstan (Nikolaenko, 1989) and therefore have a stronger limiting effect on phytoplankton compared to nitrogen compounds. Maps of the spatial distribution of algal species – trophic indicators showed a higher content of nutrients in the mouths of rivers and in shallow waters. The water quality assessment by Sládeček (1973) also characterized a level of organic pollution of the Balkhash Lake as moderate and mesosaprobic, however, with slightly higher values of the saprobity index in the western part. This assessment showed that pollutants primarily come with the waters of the Ili River which flows into the Western Balkhash. This confirms the conclusion we made earlier when analyzing the spatial distribution of biogenic elements and total phosphorus in the waters of the Balkhash Lake (Krupa et al., 2017b). The supply of nutrient elements with river waters stimulated an increase in the quantitative variables of phytoplankton communities in this zone, which we showed earlier (Krupa et al., 2018).

The effect of total toxic pollution on phytoplankton of the Balkhash Lake has not been tracked when analyzing the distribution of indicator species, but it was evident when using the WESI index. The small values of the index – 0.46 on average, have been recorded in the zone of the Ili River discharge (*Fig. 1*) and on the opposite coast of the lake – 0.97 on average, where the discharge current flows directly from the estuarine zone. The main contribution to the inhibition of algal communities in this part of the western Balkhash water area was by zinc and copper that come with river waters at concentrations of 0.080-0.264 mg dm^{-3} and 0.02-0.06 mg dm^{-3}. Thus, the pollutants entering the lake along with the river runoff have a mutually opposite effect on the communities of Lake Balkhash. Biogenic elements have a stimulating effect on phytoplankton, whereas an increased level of toxic pollution partially suppresses its development. This was only possible to reveal using the WESI index.

The average content of zinc and copper was also higher in the western part of the water area compared to the eastern. In the Eastern Balkhash, there was cadmium, lead, nickel and cobalt; however, the total amount of toxic substances did not adversely affect the phytoplankton of this part of the lake, as evidenced by the WESI index values. That means high self-purification capacity of the Balkhash Lake ecosystem. This may be due to the increased content of dissolved salts in the Eastern Balkhash, which together with

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high pH-values (*Table 1*) weaken the toxic properties of heavy metals by making the latter insoluble (Nikanorov and Zhulidov, 1991). The stress that we have identified for algal communities in the Western Balkhash is not only related to anthropogenic influence, but is also caused by natural increased potentially toxic elements background due to the lake being located in the metallogenic province (Mazurov, 2005). Earlier, we have detected an increased content of some heavy metals and their negative impact on algal communities of small lakes in Kazakhstan, both in protected areas (Krupa et al., 2016; Krupa and Barinova, 2016) and in conditions of moderate anthropogenic pollution (Barinova et al., 2011). That said the alkaline reaction of the lake water partially neutralizes the toxic effect of heavy metals on phytoplankton as evidenced in the eastern part of the Balkhash Lake.

Conclusion

In summer 2004, phytoplankton of the Balkhash Lake was represented by 92 species. In the western, freshwater part of the lake, green algae prevailed, in the eastern, brackish part it was diatoms. The larger total number of algal species in the Western Balkhash (74), compared to the Eastern one (69). Mapping showed a positive relationship between the species richness of algal Divisions and macrophytes. Changes of the proportion of indicator species within the phytoplankton composition reflected the spatial gradient of environmental variables – an increase in depth, TDS and pH-values in the direction from west to east. According to bioindication analysis, the water of the Balkhash Lake is moderately polluted, water quality class III with high self-purification of its ecosystem. The WESI index calculation revealed the effect of the total toxic pollution on the phytoplankton of the Western Balkhash. The spatial distribution of the WESI index showed that the pollutants entering the lake with river flow had a mutually opposite effect on the phytoplankton communities: the stimulating effect of biogenic elements from one hand, and the partial suppression by an increased level of toxic pollution from the other.

In general, the method of statistical mapping of indicators in combination with bioindication based on the algae can be recommended for use of future monitoring of changes in water quality and identify sources of pollution of the unique natural water body – the Balkhash Lake.

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ASSESSING SOIL QUALITY THROUGH SOIL CHEMICAL PROPERTIES AND ENZYME ACTIVITIES IN SEMIARID AREA, IRAN

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Abstract. Semiarid ecosystems are more sensitive to environmental changes than other terrestrial ecosystems, which makes their monitoring very important. Determining soil quality is critical to precise monitoring of semiarid area, and its evaluation involves the assessment of soil properties. In this study, the soil chemical properties and enzyme activities were measured and compared in order to determine soil quality. Four plots were chosen in the cold and warm sites in the Khabr national park and Ruchun wildlife refuge with grazed and not grazed areas. Samples of surface soil (0–10 cm) were collected in spring and autumn. The results showed that chemical properties of the soils were significantly affected by site (soil organic carbon, total nitrogen, available phosphorus and soil moisture) and season (pH, total nitrogen, available phosphorus and soil moisture) but not by grazing. All assayed enzyme activities were significantly influenced by site and season. Alkaline phosphatase activity was affected by grazing, too. Soil chemical properties (soil organic carbon, total nitrogen, and available phosphorus content and soil moisture) and enzyme activities (acid phosphatase, alkaline phosphatase, invertase, β-glucosidase, urease and arylsulfatase) showed higher value at cold sites. The positive correlation between all assayed enzymes and soil organic carbon (r= 0.189-0.639) indicated the important role of soil organic carbon availability in soil enzyme activities. Seasonal variation was observed in soil chemical properties except for electrical conductivity. In addition, temporal variation was observed in enzyme activities with more activity in spring samples except for arylsulfatase activity. According to more value in nutrient content and enzyme activities, it can be concluded that soil at cold sites have higher quality than warm sites. In conclusion, the warm sites with poor soil quality need more concern to be protected.
Keywords: *grazing, monitoring, seasonal, semiarid, soil quality*

Introduction

Semiarid ecosystems approximately cover 40% of the Earth's land and are increasing in proportion due to global warming and consequent desertification. The soils, in these ecosystems, characteristically contain less organic matter and plant biomass (Reyes-Reyes, 2007). The fragility of soil in semiarid environments, caused by low organic matter content, lack of vegetation exposes soil to wind and soil erosion, and salty crusts impede root growth, makes soil quality monitoring and management imperative.

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Various physical, chemical, biological and biochemical indicators have been used to estimate soil quality. It is important to take into account sensitivity to soil management in a wide range, sampling error, cost of measurement and required time. The measurement of soil enzyme activities provides an early indication of changes in soil quality, as they are involved in the mineralization of nutrients such as N, P and C (Trasar-Cepeda et al., 2008). Soil enzymes participate in almost every transformation process of decomposition and play a central role in maintaining soil fertility by releasing mineral nutrients from complex organic resources (Baldrian and Stursová, 2010). Since soil enzyme activities are linked with several ecosystem processes and exhibit rapid response to both natural and anthropogenic disturbances, they have been adopted as a suitable indicators for soil quality (Das and Varma, 2010).

Iran, with an area of 164 million hectares, is located in the mid-latitude belt of arid and semiarid regions of the Earth. The arid and semiarid regions cover more than 60% of the country, which is vulnerable to land degradation and, in consequence, desertification due to the increasing population pressure on the land due to grazing and the consumption of water resources (Amiraslani and Dragovich, 2011). The Khabr National Park and the Ruchun Wildlife Refuge is located in the southeast of Iran with various ecological systems and different habitats for plants and animals (Najmizadeh and Yavari, 2006). While the quality of a soil is related to its physical, chemical and biological properties, only several studies has been conducted on physical processes, chemical properties and the biology of soil flora and fauna in the semiarid areas of Iran (Bagheri et al., 2009; Rajabi et al., 2011; Shirvani, 2012; Sharafatmandrad et al., 2014; Mirzaei et al., 2017). There is very little information on soil biological indicators in the Iranian semiarid areas (Raiesi and Riahi, 2014; Kabiri et al., 2016). Indeed, there has not been any study on soil enzyme activities in the Khabr National Park and the Ruchun Wildlife Refuge. Therefore, this study addresses the information gap that exists on the effects of site, season and grazing on soil enzyme activities as important soil quality indicators in this park, which is needed to aide in the overall quality assessment of the ecosystems contained within this park. It was hypothesized that soil chemical properties and enzyme activities are different at cold and warm sites and is also affected by season and grazing. For this purpose, the variation in soil chemical properties and enzyme activities was studied of selected cold and warm sites, which were either grazed or not grazed in spring and autumn seasons.

Materials and methods

Field site, experiment design and soil sampling

The study area is located in the southeast of Iran within the semiarid steppe region of Khabr National Park and Ruchun Wildlife Refuge, Kerman province, Iran. This park extends from 28°28' to 28°58' N and from 56°02' to 56°38' E. The mean annual temperature and precipitation varies between 17.5-21.0°C and 200-350 mm, respectively. Mean monthly temperature and precipitation has been given in *Figure 1*. Two plots at the cold sites and two plots at the warm sites were selected as sampling plots for this study. The altitude of the warm site is 1,707 m above sea level (a.s.l) and the vegetation is dominated by *Artemisia siberi*. The cold sites have an altitude of 2,365 m (a.s.l), and the vegetation is dominated by *Stipa hassknechti* and *A. siberi*. At both the cold (C) and warm (W) sites, grazed (G) and not grazed (NG) areas were selected (*Fig. 2*). Thus, in the analysis, four treatments were considered as follows: (1) cold-grazed

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(CG), (2) cold-not grazed (CNG), (3) warm-grazed (WG) and (4) warm-not grazed (WNG).

Eight soil samples were collected from the top 10 cm of soil from a plot area 100× 100 m in mentioned treatments in spring (S, June) and autumn (A, November). In the laboratory, collected samples were thoroughly mixed and homogenized by sieving through a 2 mm sieve. Room-temperature, dried soil was used in chemical analysis. Enzyme activities assays were done on soil samples stored at -20 °C.

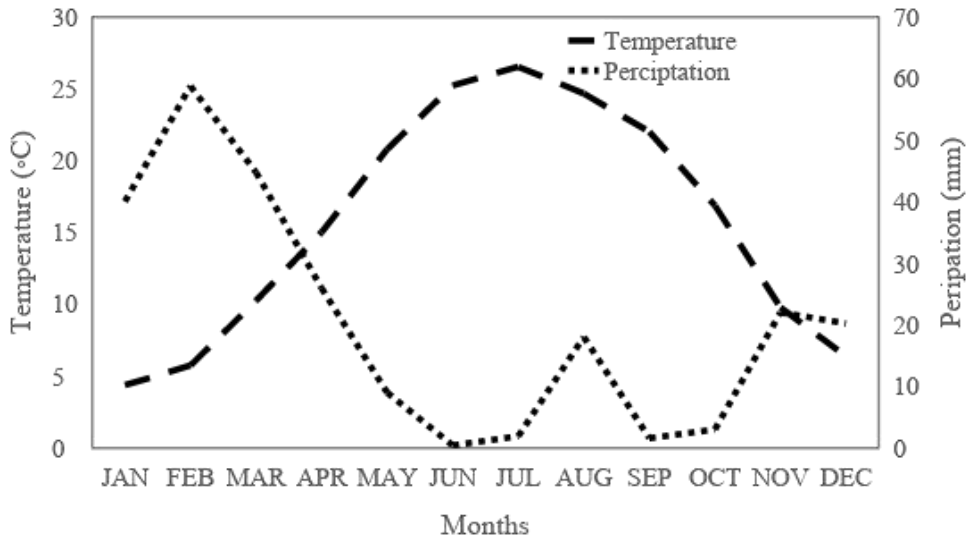


Figure 1. *Mean monthly temperature and precipitation during 2007-2017 at the Khabr National Park and Ruchun Wildlife Refuge*

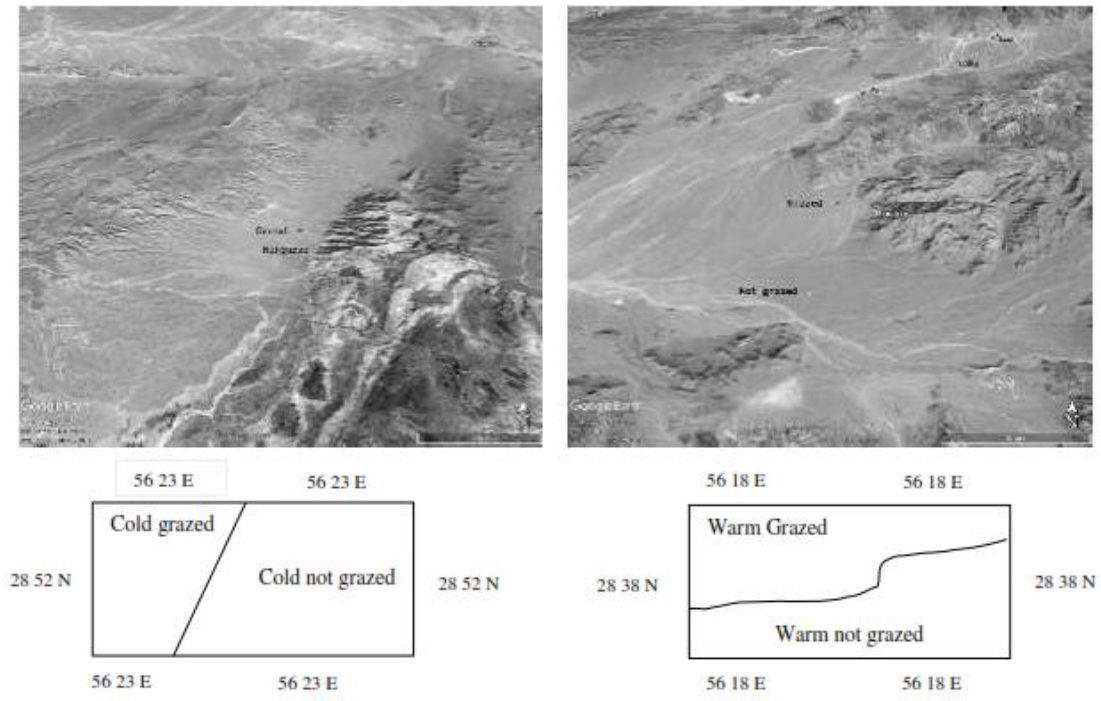


Figure 2. *Precise location of grazed and not grazed areas at the cold (left) and the warm (right) sites (Google Earth)*

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Soil chemical properties

Soils chemical properties values were measured based on standard methods. The soil pH and electrical conductivity were measured in soil suspensions (Mclean, 1982). Soil water content was measured by drying soil samples at 105 °C (Schlichting and Blume, 1966). The soil texture was determined by the hydrometric method (Toogood, 1958). A wet digestion method (Walkley and Black, 1934) was used to measure soil organic carbon. The soil available phosphorus (AP) was determined using the molybdenum blue method (Olsen and Summers, 1982) following extraction by sodium bicarbonate. A micro Kjeldahl method (Bremner and Mulvaney, 1982) was used to measure total nitrogen (TN) in soil samples.

Soil enzyme activities

The activity of seven hydrolytic enzymes was determined using their specific substrates. Acid phosphatase, alkaline phosphatase, arylsulfatase and β-glucosidase activity were measured in soil samples based on assays described by Schinner et al. (1996). Invertase and urease activity were determined as described by Schinner and von Mersi (1990) and Kandeler and Gerber (1988), respectively. The enzyme activities were assayed in duplicates at their optimal pH. The controls were soil samples to which substrate were added after the incubation step. An outline of studied enzymes, their substrate and assay pH has been given in *Table 1*.

Table 1. *Soil enzymes assayed in this study with their substrates and assay conditions*

Enzyme	Substrate	Assay condition (pH)
Alkaline Phosphatase	p-nitrophenyl phosphate hexahydrate	11
Acid Phosphatase	p-nitrophenyl phosphate hexahydrate	6.5
β-glucosidase	p-Nitrophenyl-β-D-glucopyranoside	6
Invertase	Sucrose	5.5
Urease	Urea	7
Aryl Sulfatases	p-Nitrophenyl sulfate	5.8

Statistical analysis

The data of variables were checked for their normality with Shapiro-Wilk test. If necessary, the variables were transformed by Jonson transformation using Minitab software to improve normality. Three -way analysis of variance (ANOVA) was used to assessment significant effects of site, season and grazing on soil chemical and enzyme activities. Non-normal data were analyzed using Mann-Whitney U test to evaluate the main effects of studied factors. Mean comparisons were evaluated by the Least Significant Difference (LSD) test at the 5% level of significance. In order to explore links between soil chemicals and enzyme activities, Pearson's correlations were calculated. Reporting of data as histograms was conducted using Microsoft Excel 2010.

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Results

Soil chemical properties

The soil types are Haplic Calcisoils according to according to the soil classification of the World Reference Base (WRB) for Soil Resources. All soils were characterized as sandy loam texture with an average of 10% clay, 30% silt and 60% sand. None of soil chemical properties was affected by grazing (*Table 2*). Only the effect of season was significant ($p< 0.01$) on soil pH (*Table 3*). The pH value was higher in the soil samples, collected in autumn (November) than soil samples collected in spring (June) samples (*Table 4*). The effect of site and season was significant ($p< 0.01$) on total nitrogen (TN) and available P (AP) but not grazing (*Table 2*). Whereas the spring samples had higher available P than autumn samples, the value of TN was more in autumn samples than spring samples (*Table 4*). In addition, site and season interaction was significant ($p< 0.01$) on AP (*Table 2*) with the highest available AP in samples taken from cold sites in spring (*Fig. 3*).

Table 2. *Mean squares of studied factors and their interactions for normalized parameters*

Treatments	Independent variables			
	EC	**TN**	**AP**	**SM^T**
Site	0.001^{ns}	37.20^{**}	324.00^{**}	13.88^{**}
Season	0.08^{ns}	6.45^{**}	196.00^{**}	26.62^{**}
Season × site	0.049^{ns}	0.20^{ns}	676.00^{**}	0.03^{ns}
Replication × (season× site)	1.57	0.49	12.28	0.14
Grazing	0.03^{ns}	0.04^{ns}	36.00^{ns}	0.03^{ns}
Season × grazing	0.41^{ns}	0.09^{ns}	36.00^{ns}	0.23^{ns}
Site × grazing	0.02^{ns}	0.01^{ns}	36.00^{ns}	0.12^{ns}
Season × site× grazing	0.07^{ns}	0.03^{ns}	36.00^{ns}	0.04^{ns}
Error	0.001	0.27	12.35	0.15
Total	53.73	66.07	2030.00	49.36

Significant effect at $p< 0.05$ and $p < 0.1$ levels are indicated by * and **, respectively. ^{ns} indicates no significant effect. EC: electrical conductivity, TN= total nitrogen, AP: Available phosphorus and SM: Soil moisture. Transformed data were used in EC and TN analysis.

Table 3. *Level of significance non-normalized parameters by using non-parametric Mann-Whitney U test*

Comparison source	Independent variables		
	pH	**SOC**	**ACP**
Site (cold and warm)	37.00^{ns}	2.00^{**}	319.00^{**}
Season (spring and autumn)	1.00^{**}	418.00^{ns}	0.00^{**}
Grazing (Grazed with not grazed)	443.00^{ns}	474.50^{ns}	494.00^{ns}

Significant effect at $p< 0.05$ and $p< 0.1$ levels are indicated by * and **, respectively. ^{ns} indicates no significant effect SOC: soil organic carbon, ACP= acid phosphatase.

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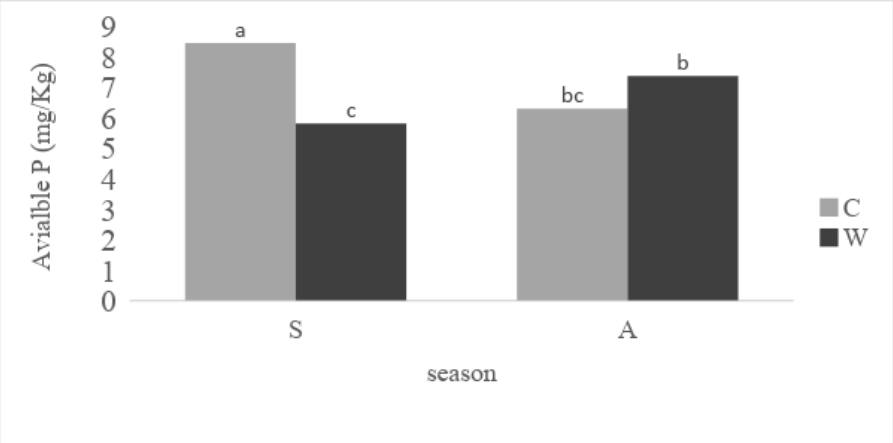


Figure 3. *Phosphorus availability in soil developed under interaction of site (C= cold and W= warm) and season (S= spring and A= autumn) conditions. Different letters indicate significant difference at the p< 0.05 level*

As given in *Table 3,* only site had significant (p< 0.01) effect on soil organic carbon (SOC) with more value in cold sites (*Table 4*). Measured soil water content showed significantly higher values in cold sites and autumn samples in comparison to warm sites and spring samples, respectively (*Tables 2* and *4*).

Table 4. *Mean comparison (n= 8) between sites and season in soil chemical properties*

Soil chemical	Site		Season	
	Cold	Warm	Spring	Autumn
pH	7.75[a]	7.85[a]	7.9[b]	8.3[a]
Total nitrogen (%)	0.08[a]	0.05[b]	0.06[b]	0.07[a]
Available phosphorus	35.00[a]	30.5[b]	34.50[a]	31.00[b]
Soil organic carbon (%)	0.65[a]	0.29[b]	0.39[a]	0.45[a]
Soil moisture (%)	4.44[a]	1.32[b]	0.93[b]	4.83[a]

Different letters show significant difference at p< 0.05 level.

Soil enzyme activities

As shown in *Table 5,* the effect of site (p< 0.01), season (p< 0.01) and grazing (p< 0.05) was significant on alkaline phosphatase activity (*Table 5*). Activity of soil alkaline phosphatase decreased by 28, 22 and 15% in warm sites, autumn samples and not grazed areas in comparison to cold sites, spring samples and grazed areas, respectively (*Table 6*). The effect of site and season was significant on acid phosphatase but not grazing (*Table 3*). Acid phosphatase activity decreased by 32 and 66% in warm site and autumn samples in comparison with cold sites and spring samples (*Table 6*), respectively.

The results indicated significant effect (p< 0.01) of site and season on β-glucosidase and invertase activity (*Table 5*). The β-glucosidase activity decreased by 33 and 52% in warm site and autumn samples (*Table 6*). Grazing did not affect significantly β-glucosidase activity. However, the interaction of site and grazing was significant (p< 0.05) on its activity (*Table 5* and *Fig. 4*).

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Table 5. *A summary of the levels of treatment significance on soil enzyme activities*

Treatments	ALP	β-Glu	INV	UR	AS
Site	95256.33**	63936.3**	13.88**	35.57ns	12121.1**
season	40290.02**	186981.6**	26.62**	5353.56*	7333.90**
Season × site	4.29ns	6306.15ns	0.03ns	111.22ns	107.53ns
Replication × (season× site)	875.566	2127.32	0.14	547.58	128.46
Season × grazing	64.98ns	219.99ns	0.24ns	178.05ns	191.01ns
Grazing	6331.98*	3715.97ns	0.03ns	850.08ns	21.59ns
Site × grazing	503.16ns	20155.13*	0.12ns	67.22ns	387.17ns
Season × site × grazing	474.31ns	6395.40ns	0.04ns	861.35ns	15.38ns
Error	943.27	316.98	0.15	1000.82	162.56
Total	193852.42	43578.24	49.35	50811.55	28586.39

Significant effects at the $p < 0.05$ and $p < 0.1$ levels are indicated by * and **, respectively. ns indicates no significant effect. AlP: alkaline phosphatase, β-Glu: β-glucosidase, INV: invertase, UR: urease and AS: arysulfatase. Transformed data were used in INV analysis.

Table 6. *Mean comparison (n = 8) between sites, season and grazing on soil enzyme activities*

Enzyme	Site		Season		Grazing	
	Cold	Warm	Spring	Autumn	Grazed	Not grazed
Alkaline phosphatase	240.68a	163.71b	227.37a	177.19b	162.95a	147.71b
Acid phosphatase	262.04a	187.89b	374.00 a	75.93b	245.12a	238.75a
β-Glucosidase	186.91a	123.73b	209.38a	101.28b	189.45a	175.12a
Invertase	2248.39a	528.04b	339.53a	243.69b	1491.45a	1284.7a
Urease	52.01a	53.99a	61.99a	43.70b	56.49a	49.2a
Arylsulfatase	120.7a	99.54b	102.60b	124.01a	111.04a	115.4a

Different letters show significant difference at $p < 0.05$ level.

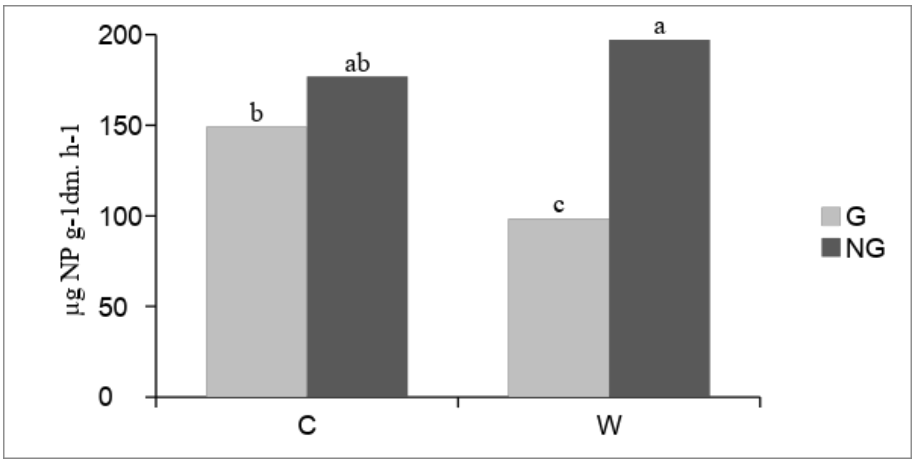


Figure 4. *The significant effect of site (C= cold, W= warm) and grazing (G= grazed, NG= not grazed) interaction on β-glucosidase activity. Different letters show significant difference at P< 0.05 level*

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There was similar trend in invertase activity, as its activity decreased by 76 and 28% in warm site and autumn samples (*Tables 5* and *6*).

Measurement of urease activity showed that only the season caused significant difference on its activity (*Table 5*). The urease activity decreased by 29% in autumn samples in compared with spring samples (*Table 6*).

The effect of site and season was significant ($p < 0.01$) on arylsulfatase activity (*Table 5*). As shown in *Table 6,* there was a decrease in value of arylsulfatase activity in warm site (17.5%) and spring samples (17%).

The correlation between soil properties and enzyme activities

According to Pearson correlation coefficient values, soil electrical conductivity did not show any correlation with other soil chemical properties (*Table 7*). Soil pH correlated negatively with soil available phosphorus and soil moisture (*Table 7*). Whereas the correlation between soil organic carbon and total nitrogen was positive ($r = 0.774$, $p < 0.001$), they correlated negatively with soil moisture (*Table 7*).

The Pearson correlation indicated no significant correlation between soil EC and enzyme activities (*Table 7*). Whereas there was no correlation between soil pH and alkaline phosphatase activity, other assayed enzyme activities correlated negatively with soil pH except for arylsulfatase (*Table 7*). The correlation between soil available phosphorous and enzyme activities was positive except for urease and arylsulfatase activity. Total nitrogen showed positive correlation with alkaline phosphatase and arylsulfatase activity (*Table 7*) with higher correlation with arylsulfatase activity ($r = 0.609$, $p < 0.001$). The activity of all assayed enzymes correlated positively with SOC contents (*Table 7*). The highest correlation was observed with arylsulfatase activity ($r = 0.630$, $p < 0.001$). Based on Pearson values, soil moisture positively correlated with acid phosphatase, β-glucosidase, invertase and urease activity (*Table 7*) except for arylsulfatase activity ($r = -0.689$. $p < 0.001$).

Discussion

In this study, 11 soil variables including soil chemical properties and enzyme activities were measured to determine soil quality in a semiarid area.

Soil pH and EC are known as the principal indicator of the chemical characteristic of a particular soil, playing significant role in soil biogeochemical processes, solubility of soil nutrients, plant growth, microbial growth and enzyme activities (Aciego Pietri and Brookes, 2008; Sinsabaugh et al., 2008). Our results indicated that some of soil chemical properties did not easily change, which supported by other studies (Zarekia et al., 2012; Mureithi et al., 2014; Vargas-Gastelum et al., 2015). The less value of pH in spring samples can be explained by production of CO_2 by plant roots and bacteria that temporarily lower the pH value in natural ecosystem (Subba Rao, 2009).

Available phosphorus (AP), total nitrogen (TN) and soil organic carbon (SOC) are used as important indicators of soil fertility and long-term ecosystem sustainability (da Silva et al., 2008; Wienhold et al., 2009). There are some evidences that plant cover slows losing nutrients from soil (Porder and Chadwick, 2009; Deekor et al., 2012), supporting more TN, AP and SOC content in cold sites with dense plant cover in comparison with warm sites. The less concentrations of SOC at warm sites is attributed to faster turnover of organic C in warm sites and negative correlation between temperature and soil organic carbon (Friedlingstein et al., 2006). Whereas there was no

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seasonal variation in SOC content, TN and AP changed seasonally. The temporal alteration of available P was contradictory to total nitrogen seasonality. Plants uptake more P with increasing temperature (Yan et al., 2012), depleting the available phosphorus in soil from spring to autumn which is in accordance with our results. The more TN in autumn can be related to more activity of nitrogen fixing bacteria, which possibly increase the TN content (Zeng et al., 2009). In general, seasonal differences in AP and other nutrients are due to change in soil pH, moisture and temperature (Turner et al., 2013).

Table 7. *Correlation values for soil chemical properties and enzyme activities*

Variables	EC	pH	TN	AP	SOC	SM	ACP	ALP	βG	IN	UR	AS
EC	1	0.015	-0.096	-0.022	-0.035	0.142	-0.008	-0.053	-0.009	0.065	-0.123	-0.172
pH	0.015	1	0.089	-0.340**	-0.110	-0.567***	-0.835***	0.595	-0.709***	-0.702***	-0.312*	0.290*
AP	-0.022	-0.340**	0.078	1	0.206	0.097	0.344**	0.457**	0.414**	0.479***	0.157	0.155
TN	-0.096	0.089	1	0.078	0.774***	-0.564***	0.059	0.365**	0.053	0.192	-0.178	0.609***
SOC	-0.035	-0.110	0.774***	0.206	1	-0.508***	0.189*	0.524***	0.274*	0.384**	0.023*	0.630***
SM	0.142	-0.567***	-0.564***	0.097	-0.508***	1	0.568***	0.040	0.321**	0.305*	0.307*	-0.689***
ACP	-0.008	-0.835***	0.059	0.344**	0.189*	0.568***	1	0.681***	0.683***	0.784***	0.308*	-0.232
ALP	-0.053	-0.595***	0.365**	0.457**	0.524***	0.040	0.681***	1	0.578***	0.706***	0.172	0.316*
BG	-0.009	-0.709***	0.053	0.414**	0.274*	0.321**	0.683***	0.578***	1	0.709***	0.222	-0.068
IN	0.065	-0.702***	0.192	0.479***	0.384**	0.305*	0.784***	0.706***	0.709***	1	0.151	0.020
UR	-0.123	-0.312*	-0.178	0.157	0.023	0.307*	0.308*	0.172	0.222	0.151	1	-0.148
AS	-0.172	0.290*	0.609***	0.155	0.630***	-0.689***	-0.232	0.316*	-0.068	0.020	-0.148	1

*, ** and *** indicate significant correlation at $p< 0.05$, $p< 0.01$ and $p< 0.001$, respectively. EC: electrical conductivity, AP: available phosphorus, TN: total nitrogen, SOC: soil organic carbon, SM: soil moisture, ACP: acid phosphatase, AlP: alkaline phosphatase, β-Glu: β-glucosidase, INV: invertase, UR: urease and AS: arysulfatase.

The soil enzyme activities is the metrics predominantly used to provide information on soil quality. We studied the activity of six extracellular enzymes, involved in P, C, N and S cycling. Soil phosphatases play a major role in the mineralization processes of organic phosphorus substrates. The activity of alkaline phosphatase was more than acid phosphatase, possibly related to soil pH (> 7), which makes condition more favourable for alkaline phosphatase activity than acid phosphatase activity. Whereas phosphatases show pH-dependent activity profile, with specific optimum pH for their maximum activity and stability, only acid phosphatases showed strong negative correlation ($r= -0.835$, $p< 0.001$) with soil pH. The soil pH values in studied areas were in the range of near optimum pH for the alkaline phosphatase activity, explaining why this enzyme did not correlate with soil pH (Olander and Vitousek, 2000; Renella et al., 2007).

The positive correlation between alkaline and acid phosphatase activity with SOC and available P is attributed to more activity of these enzymes in cold sites. Generally, a positive correlation is expected between enzyme activities and soil organic carbon, increasing soil enzyme activities as organic carbon increase in soil. The major reason for increased enzyme activities with increasing soil organic carbon could be attributed to the greater availability of soluble organic C, nutrients which stimulate microbial growth and activity as main source of soil enzymes (Debnath et al., 2015). Although

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our results showed a positive correlation between available P content and both phosphatase activities, this relationship is usually complicated and not constant, since a positive, a negative or no relationship between them has been reported (Kang et al., 2009; Lemanowicz et al., 2011; Piotrowska-Długosz and Wilczewski, 2014). A significant and positive relationship between phosphatase activity and available phosphorus is observed usually in natural soils and those with a low content of nutrients, where phosphorus deficiency occurs (Šarapatka, 2003). Furthemore, the uptake of available phosphorus by plants may complicate this correlation. The observed positive correlation between total nitrogen and alkaline phosphatase is supported by Piotrowska-Dlugosz and Wilczewski (2014) and Shi et al. (2008) findings. The significant and positive correlation between the acid phosphatase activity and the soil moisture is in agreement with Zheng et al. (2015) and but in contrast with Brockett et al. (2012) findings. The soil moisture content, by altering conditions for soil microbiota, causes changes in soil microbial growth and enzyme activities (Kim et al., 2008; Borowik and Wyszkowska, 2016).

Invertase and β-glucosidase play critical role in C cycle and release low molecular weight sugars that are important as energy sources for microorganisms. The positive correlation between β-glucosidase and invertase activity with soil pH was in consistent with other studies (Piotrowska and Koper, 2010; Shao et al., 2015; Zhang et al., 2015). Although a negative or no correlation between soil pH and invertase and β-glucosidase activity has been reported (Shi et al., 2008; Tan et al., 2014). The positive correlation between β-glucosidase and invertase activity with soil phosphorus is in agreement with Zheng et al. (2015) and Cheng et al. (2013). β-glucosidase and invertase activity were positively correlated with the soil organic carbon content. Similarity, Shi et al. (2008), and Böhme and Böhme (2006) observed significant and positive correlation between β-glucosidase and invertase activities with organic carbon content in soil samples, indicating the important role of organic matter in maintaining their activities. In addition, the observed positive correlation between soil organic matter and available phosphorus with invertase and β-glucosidase can explain more activity of these enzymes in cold sites, which is in agreement with various studies (Böhme and Böhme, 2006; Shi et al., 2008; Piotrowska and Koper, 2010). Our results did not confirm any significant correlation between soil total nitrogen and β-glucosidase and invertase, which is in agreement with Shi et al. (2008) and Zheng et al. (2015) but in contrast with Cheng et al. (2013) and Zhang et al. (2015) findings.

Urease is associated with the transformation, biological turnover and bioavailability of nitrogen in soil (Piotrowska-Długosz and Wilczewski, 2014). According to our results, there was a correlation between soil urease activity with soil pH ($r= -0.312$, $p< 0.05$), soil organic carbon ($r= 0.023$, $p< 0.05$), total nitrogen ($r= 0.178$, $p<0.05$) and soil moisture ($r = 0.307$, $p < 0.05$). Similar results have been reported by Tan et al. (2014), Zhang et al. (2013), and Piotrowska-Dlugosz and Wilczewski (2014). Soil urease activity did not show any correlation with soil EC and available phosphorus, which is in agreement with Melero et al. (2006) and Tan et al. (2014) findings.

Arylsulfatase activity, involved in S cycle, had a significant correlation with soil pH ($r= 0.290$, $p< 0.05$), soil organic carbon ($r= 0.630$, $p< 0.001$), total nitrogen ($r= 0.609$, $p< 0.001$) and soil moisture ($r= -0.689$, $p < 0.001$) which are supported by Mankolo et al. (2012) and Green et al. (2007). The strong positive correlation between SOC and arylsulfatase highlights the important role of soil organic carbon in arylsulfatase activity (Mankolo et al., 2012).

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There is no constant pattern in seasonal variation in soil enzyme activities, depending on assayed enzymes, soil properties and ecosystem types. The enzyme seasonal variation is governed by parameters that regulate enzyme activities such as soil temperature, moisture and substrate availability (Wittmann et al., 2004; Niemi et al., 2005; Baldrian et al., 2008). Although seasonal variations in enzyme activities was not supported by Boerner et al. (2005) and Wallenstein et al. (2008), there was seasonal variation in all studied enzymes. The observed seasonal variation in acid and alkaline phosphatase, β-glucosidase, invertase and urease can be explained by more plant root exudates and seasonal variation in the microbial biomass which is in agreement with Devi and Yadava (2006), Yang et al. (2010) and Wallenstein and Weintraub (2008) findings. The all enzymes had more activity in spring except for arylsulfatase activity. Our finding on more intense activity of arylsulfatase in autumn is opposite with Margesin et al. (2014) report, but can be supported by Whalen and Warman (1996) findings. The more arylsulfatase activity in autumn samples can be related to presence of non-competitive inhibitors, possibly removed by plants during growth season and increasing arylsulfatase activity in autumn samples (Whalen and Warman, 1996).

Although the soil enzyme activities values were more in grazed area but the difference was not significant between grazed and not grazed areas except for alkaline phosphatase activity, showing its more sensitivity to environmental changes. It has been approved that the effect of grazing on soil properties depends on grazing intensity and period (Steffens et al., 2008; Fang et al., 2013).

Conclusion

It has been accepted that soil chemical properties and enzyme activities are important indicators in soil quality assessment. This study was conducted to illustrate the effect of site, season and grazing on soil chemical properties and enzyme activities. The results revealed the significant influence of site and season on some of soil chemicals and extracellular enzymes activities in semiarid areas but not grazing. The higher activity of all assayed enzyme activity in soils at cold sites can be attributed to the more soil organic carbon as there was a positive correlation between SOC and all assayed enzymes except for urease. There were also significant seasonal dynamics among different enzyme activities. Among the enzymes studied alkaline phosphatase was affected by grazing, which shows its higher sensitivity to soil management. In conclusion, it seems the soil quality is poor in warm sites according to both soil chemical and enzyme activities. Then soil in warm sites are more vulnerable to degradation and need more concern to be protected.

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PHYSICAL AND CHEMICAL VARIABLES, SPECIES COMPOSITION AND COVERAGE OF MACROPHYTES IN PONDS (CASE STUDY IN EASTERN POLAND)

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Abstract. Studies were conducted on the eleven largest and oldest ponds in the mesoregion Western Polesie in Eastern Poland. The aim of the study was to analyze the influence of environmental variables on species richness and vegetation cover of ponds. The analyzed variables included hydraulic parameters of ponds, water quality and buffer zone management. Detrended correspondence analysis (DCA), principal component analysis (PCA) and redundancy analysis (RDA) were applied to reveal principal variation patterns within the data. There were a different number of species comprising the phytolittoral zone in the investigated ponds. This study also showed that ponds are valuable for plant biodiversity. In total 75 species of plants occurred in the studied complex. The total number of species ranged from 10 to 49 in pond. The water of the ponds is characterized by very low value for most tested water quality indicators. The ponds are characterized by statistically significant differences of the buffer zone management. They have similar environmental conditions (temporariness of flooding period, low depth, water quality) and they potentially can share similar species. Our results also showed that the relationships between macrophyte species and cover depends on buffer zone management and some physicochemical variables of water.
Keywords: *water body, plant community, water quality, buffer zone management, ordination methods*

Introduction

Owing to their important contribution to aquatic biodiversity, ponds should be considered as an important target system in strategic plans that aim at conserving or developing aquatic biodiversity at the landscape scale. Such plans can only be effective if based on a solid knowledge of the factors that affect pond community structure and diversity. In issue, several studies document clear associations between the aquatic community characteristics and a variety of ecologically relevant, such as hydroperiod (Della Bella et al., 2008), surface area (Céréghino et al., 2008a), salinity and pond connectivity (Oertli et al., 2007; Boix et al., 2008; Gascón et al., 2008). In the first European Pond Workshop devoted to the Conservation and Monitoring of Pond Biodiversity launched a European network of people and institutions to collaboratively work on solving fundamental scientific issues and developing practical applications to protect ponds (Heino et al., 2015).

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Ponds, particularly temporary ones, are aquatic habitats with multiple constraints due to their great abiotic spatial variability. However, this abiotic variability can provide certain, well adapted species, advanced opportunities to succeed (Lemmens et al., 2013). Drought is the principal constraint limiting macrophytes development and establishment in ponds. This constraint is even greater because of its unpredictability and alternation of dry and wet phase varies (Della Bella et al., 2008). The development of life cycles with different length, the dominance of one form of reproduction, the major or minor investment in seed production and the germination patterns, might concur in structuring the macrophyte assemblages in wetlands with different hydroperiod length (Ierotheos et al., 2010).

Research, driven both by the need to improve pond conservation strategies and by increasing interest in fundamental aspects of pond ecology (Heino et al., 2015), has started to shed new light on pond ecosystem structure and function. As a result, there is growing evidence that ponds are functionally different from larger lakes (Bornette et al., 2011) and that despite their small size they are collectively biologically diverse (Ierotheos et al., 2010). Ponds also show greater biotic and environmental temporal amplitudes than rivers and lakes (Céréghino et al., 2008b; Søndergaard et al., 2010).

In the mesoregion Western Polesie several pond complexes exist among which are the Sosnowica and Libiszów pond complexes. These pond complexes have been altered for harbouring and breeding carp. From an economic point of view, eutrophication is beneficial as it stimulates the growth of organisms that serve as the base of the food chain for fish. Hypertrophy is an adverse phenomenon resulting from over-fertilizing of ponds. In addition the data generated, so that this information may be used as the decision maker for conservation and effective utilization of water bodies particularly the reservoirs (Grzywna, 2013).

Studies were conducted on the eleven largest and oldest ponds in this region. They have been for one fish farming since World War I (Grzywna and Nieścioruk, 2016). The aim of the study was to evaluate the richness and distribution of aquatic macrophytes in fishing farm and to examine the relationships between habitat characteristics, environmental conditions, and aquatic macrophyte compositions. The ponds were all located within the border parts of protected natural areas of the Natura 2000 zone – the Forest of Parczew PLB060006. Data concerning physical and chemical properties of water, as well morphology, landscape and macrophytes were collected.

The aim of the study was to analyze the influence of environmental variables on species richness and vegetation cover of ponds. The analyzed variables included hydraulic parameters of ponds, water quality and buffer zone management. Hydraulic parameters of ponds were characterized by 2 values. Water quality was determined based on 10 physical and chemical properties of water. Five ways of using the buffer zone were identified.

Materials and methods

The paper presents the results of water quality tests, buffer zone management and richness of macrophyte species. Studies were carried out in July 2016 in two pond complexes in Western Polesie (Eastern Poland). The first complex, Sosnowica, consists of 6 fish ponds located in the Piwonia river basin (51°30'N, 23°05'E). The second complex, Libiszów, consists of 5 fish ponds located in the Konotopa river basin (51°40'N, 22°50'E). Both ponds in spring time are filled with water from the Wieprz

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and Krzna Canal (*Fig. 1*). They were all shallow with a different water area – WA, and depth – D (*Table 1*). The three largest ponds in each of the investigated complexes were flowing ponds connected by a system of ditches with the river Piwonia and river Konotopa. The rest of the studied ponds were standing ponds. All the studied ponds were temporary, having shorter wet-phase duration < 200 days. The ordination analysis covered 75 species, 11 study sites and 17 environmental variables.

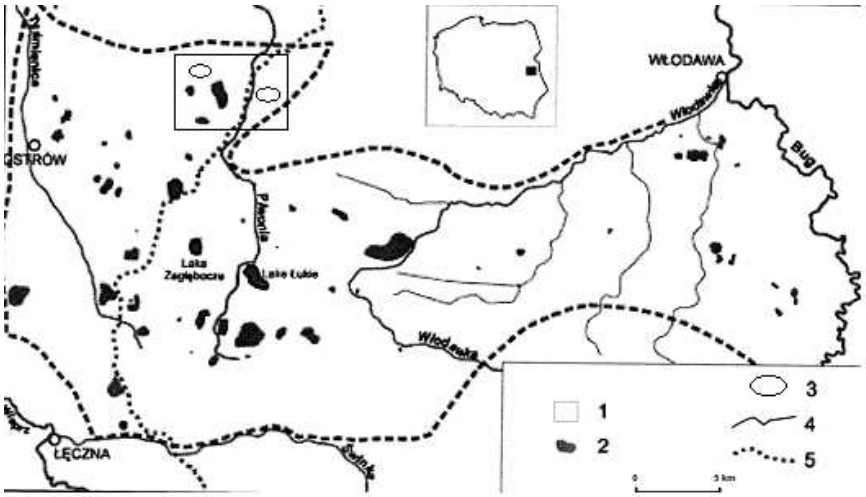


Figure 1. *Location of the study area in the Western Polesie. 1 – study area, 2 – lakes, 3 – ponds, 4 – river, 5 – Wieprz-Krzna Canal*

Minimum and maximum water depth was measured in situ using a meter stick. Pond area, water surface and buffer zone management (BZM) were estimated using ortho-image interpretation. Detailed analysis of land use types were carried out in ponds buffer zones, assuming the same radius length for ponds, 100 m, measured from the centroid of ponds and set automatically by the ArcGIS 10.0 software. Five ways of using the buffer zone were identified – Infrastructure (I), Water surface (W), Grasslands (G), Forests (F), Bushes (B).

Table 1. *Hydraulic parameters of ponds*

No. ponds	Name of pond	Total area (ha)	Water area (ha)	Depth min-max (m)	Volume of the water (m^3)	Power supply
1	Hetman	90	54.5	0.8-1.2	530	Flowing pond
2	Anielski	83	44.7	0.8-1.2	440	Flowing pond
3	Kłoda	64	31.6	0.6-1.0	250	Flowing pond
4	Jedlina	41.5	30.4	1.0-1.5	360	Standing pond
5	Renta	15.5	13.3	0.7-1.2	120	Standing pond
6	Rumieniec	34	25.7	0.7-1.2	230	Flowing pond
7	Płonne	37	28.6	0.5-0.9	220	Flowing pond
8	Wiklik	40	30.8	0.8-1.2	300	Flowing pond
9	Morski	35	29.5	0.6-1.0	240	Standing pond
10	Kościuszko	27	23.8	0.6-1.0	190	Standing pond
11	Racławice	24	19.5	0.6-1.0	150	Standing pond

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Conductivity (Con), dissolved oxygen (DO) and pH were measured in situ with a multi-parameter probe. Three subsamples were taken and preserved at 4 ^{o}C to be analyzed in the laboratory later. Two of the subsamples were glass filtered to determine chlorides (Cl), sulphates (SO_4), nitrate nitrogen ($N\text{-}NO_3$) and ammonia nitrogen ($N\text{-}NH_4$). The third subsample, which was unfiltered, was used to determine total phosphorus (TP) and total nitrogen (TN). Water transparency was measured by visual evaluation Secchi disc (SD) (Grzywna et al., 2015).

Total surface covered with all studied groups of plants was calculated on the basis of macrophyte analysis in horizontal transects, extending from the edge of the embankment of the pond to the maximum colonization depth in pond. Aquatic transects radiated inward towards the centre of the pond for the length of macrophyte occurrence and were uniformly distributed along the entire shoreline every 100 m. The spatial structure of macrophytes was analysed in detail from 9 (the smallest pond) to 25 (the biggest pond) transects within the each pond. In each transect we collected emergent macrophytes using frames with sampling area of 0.5 m^2. Each measurement was repeated five times. Submerged and floating leaves macrophytes were sampled using a floristic Bernatowicz type of sampler (area 0.16 m^2) (Sender, 2016). On this basis, we determined species composition and the range of occurrence of particular groups of macrophytes in ponds. We recorded all species among 4 groups of macrophytes (emergent – EM, submerged – SM, floating – FM and flood meadow taxa – MT).

To calculate the present proportion of the different plant groups in ponds ArcGIS 10.0 software was used. Total coverage and range of particular groups of plants occurrence was done by a retrospective photointerpretative analysis (Chmielewski et al., 1996) using orthophotomap (2013), bathymetry maps of ponds, as well as field investigations. Maximum depth and GPS location were determined using the sonar Elite no. 5 of LOWRENCE. Aquatic plants were grouped in five class of coverage (1 = 1–5%, 2 = 5–25%, 3 = 26–50%, 4 = 51–75% and 5 = 76–100%), following the phytosociologic approach (Braun-Blanquet, 1976). Phytosociological units were distinguished using a systematic and nomenclature system by Matuszkiewicz (2008).

The data set was first subjected to detrended correspondence analysis (DCA) which showed a first-axis gradient length of 0.68 in standard deviation units. Therefore we decided to apply linear methods of ordination as principal component analysis (PCA) and redundancy analysis (RDA). PCA with standardized environmental variables was used to visualize patterns of environmental variation across ponds. Prior to the analysis, redundant environmental variables (with linear correlation coefficient $r > 0.7$) were omitted to avoid collinear rite (Blanchet et al., 2008). Further analysis took the following environmental variables into account: W, G, F (buffer zone management); TN, TP and Con (water quality). Significant PCA axes were selected with both Kaiser-Gutman criterion and the broken-stick model (Borcard et al., 2011). In the next stage, redundancy analyses with forward selection were performed. We carried out an RDA with all environmental explanatory variables, as well as for two subsets of variables (BZM and plant cover). We decided to apply Hellinger transformation to the species data (Legendre and Gallagher, 2001). Environmental data was cube root transformed and standarized to fulfill the assumption of normality. The RDA results were used to produce an ordination triplot. Statistical analyses were performed using vegan package in R (R Core Team, 2015; Oksanen et al., 2016).

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Results

The water of the ponds is characterized by very low value for most studied water quality indicators. It applies mainly concentrations of such indicators as: Cl, SO_4, N-NO_3, N-NH_4, conductivity (con.), water reaction (pH), as well as TN. The concentration of DO and TP were different and rather high in studied ponds. The DO concentration reached average value 2.3 $mgO_2 \cdot dm^{-3}$ (range from 1.9 to 2.8 $mgO_2 \cdot dm^{-3}$), while, TP concentration ranged from 0.62 to 4.35 $mgP \cdot dm^{-3}$ (*Table 2*).

Statistically insignificant differences in physical and chemical parameters of water are characteristic of the analyzed ponds.

The ponds are characterized by statistically significant differences of the buffer zone management. The zone is predominantly overgrown with forests and bushes (accounting for > 50% of the total area). In the case of ponds situated in the central part of the complexes the water of the neighbouring ponds constitutes more than 40% of the ecotone area (*Table 3*).

Table 2. *Physical-chemical variables of water in ponds*

Feature	Unit\Pond	1	2	3	4	5	6	7	8	9	10	11
SD	m	0.7	0.7	0.7	0.4	0.3	0.5	0.6	0.5	0.5	0.4	0.5
pH		7.1	6.9	6.9	7	7.1	7.4	7.3	7.7	8.2	8	7.6
DO	$mgO_2 \cdot dm^{-3}$	2.5	2.3	2.0	1.9	2.6	2.1	2.8	2.5	2.7	2.8	2.4
Con	$\mu S \cdot cm^{-1}$	389	209	237	220	302	360	370	380	300	340	321
N-NH_4	$mg \cdot dm^{-3}$	0.45	0.09	0.11	0.08	0.06	0.37	0.29	0.22	0.17	0.25	0.3
N-NO_3	$mg \cdot dm^{-3}$	0.46	0.65	0.48	0.6	0.61	0.5	0.53	0.55	0.59	0.51	0.6
TN	$mg \cdot dm^{-3}$	1.4	0.5	0.9	0.6	0.9	0.97	0.82	0.93	0.61	0.55	0.8
TP	$mg \cdot dm^{-3}$	0.62	1.88	2.77	4.35	0.84	1.98	1.67	1.82	1.24	1.11	1.5
SO_4	$mg \cdot dm^{-3}$	25	30	32	21	69	27	24	20	31	33	40
Cl	$mg \cdot dm^{-3}$	18	16	14	12	4	13	11	10	8	7	15

Table 3. *Buffer zone management*

Land use [%]	1	2	3	4	5	6	7	8	9	10	11
Infrastructure (I)	8	2	3	1	0	3	12	10	1	3	1
Water (W)	40	31	7	19	14	0	0	0	45	40	26
Grasslands (G)	3	0	5	10	1	12	20	18	10	5	7
Bushes (B)	29	59	60	37	19	25	18	9	4	7	18
Forest (F)	20	8	25	33	66	60	50	63	30	45	48

Distribution of plants in the ponds was heterogeneous (mosaic). Phytolittoral zones often developed from one bank to another. Four different growth groups of macrophytes were found: submerged, emergent, floating and flood-meadow taxa. In total, 75 species of plants occurred in the studied complex. The number of species ranged from 10 in Morski pond to 49 in Anielski pond. There was a different number of species comprising the phytolittoral zone in the investigated ponds. The most abundant aquatic plant species found in the ponds were flood-meadow species (*Table 4*). They were represented by 49 (up to 67%) of aquatic species in some ponds. The species richness of

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the pond was up 6 to 34 species. The most common species were: *Bidens trpartita, Lycopus europaeus, Scirpus sylvaticus.* They overgrow the embankment area, beyond the permanent water surface. Emergent macrophytes (EM) were represented by 10 species, occurring in all ponds. The dominant species among emergent macrophytes were *Phragmites australis* and *Typha angustifolia*. Submerged macrophytes (SM) comprised the poorest group, representing only 7 species. Species of this group occur in only 6 ponds. The most common species of SM was *Ceratophyllum demersum*. Floating macrophytes (FM) were represented by 9 species occurring in 9 ponds. The species richness of the pond was up to 5 species. The most common FM species were: *Lemna minor* i *Polygonum amphbium*. Among the species present in the investigated ponds were protected ones including: *Nuphar lutea*, and *Nymphea alba*. The total pond area covered with vegetation ranged from 5.0 to 39.6%.

Table 4. *Characteristic of the macrophytes occurrence*

Pond	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Macrophyte cover [%]											
Floating	2.7	8.9	3.7	0	1.0	1.9	0.4	0	0.4	0	0.5
Submerged	2.2	3.2	3.9	0	0	2.8	4.9	4.4	0	0	0
Emergent	5.4	13.2	12.1	5.0	2.2	4.3	5.2	7.4	3.2	2.1	1.1
Flood-meadows	9.2	4.7	19.9	6.9	2.6	4	3.2	2.3	2.3	3.1	3.4
Total cover	19.5	30	39.6	11.9	5.8	13	13.4	14.1	5.9	5.2	5.0
Species number											
Floating	2	6	7	0	3	3	1	0	2	0	2
Submerged	0	2	3	0	0	3	3	3	0	0	0
Emergent	7	7	6	3	3	5	2	3	2	4	4
Flood-meadows	25	34	22	10	12	20	17	15	9	6	12
Total number	35	49	38	13	18	31	23	22	13	10	18

PCA axes 1 and 2 explained 72.06 % of a total variance (*Fig. 2*). Axis 1 explained 48.55 % of the total variance with forest and grassland coverage and conductivity showing high positive loading ($\lambda = 1.04, \lambda = 0.81, \lambda = 0.77$, respectively) and total phosphorus and total nitrogen showing a negative loadings ($\lambda = 0.75, \lambda = 0.71$, respectively). Axis 2 explained 23.52 % of the total variance and showed high negative loading for water coverage ($\lambda = -0.87$) and a positive loading of total nitrogen ($\lambda = 0.73$). Forest coverage and conductivity are highly positively correlated. Water and grassland coverage are negatively correlated. Total nitrogen and water coverage have nearly orthogonal arrows, indicating a correlation close to 0.

Redundancy analysis results are visualized in an ordination diagram (*Fig. 3*). All environmental variables explained 76.28% of the total variation (Legendre and Birks, 2012). The first two RDA axes explained 35.58% of the total variation in the species data (RDA1 = 18.8%, RDA2 = 16.78%). We performed a statistical significance test using the *anova* function in the vegan package for all variables and received a significant result (p = 0.039). The results of the forward selection and permutation tests indicated that only total phosphorus are significantly related to the species data (p = 0.02; R^2 = 0.67). When the subset of variables related to plant cover was considered, a total inertia of 26.85% was explained (p = 0.03; R^2 = 0.29). After the

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forward selection procedure the variable total phosphorus was selected as significant. However, when the subset BZM of environmental variables were taken into account, the explained total inertia was slight (34.87%) and not significant ($p = 0.105$; $R^2 = 0.35$). Among the strongest species-environment associations, we found that the species *Iris pseudoacorus* was positively associated with total phosphorus. *Typha angustifolia* and *Eupatorium cannabinum* were negatively associated to total phosphorus. Variable total phosphorus seemed to be associated to the negative part of Axis 1.

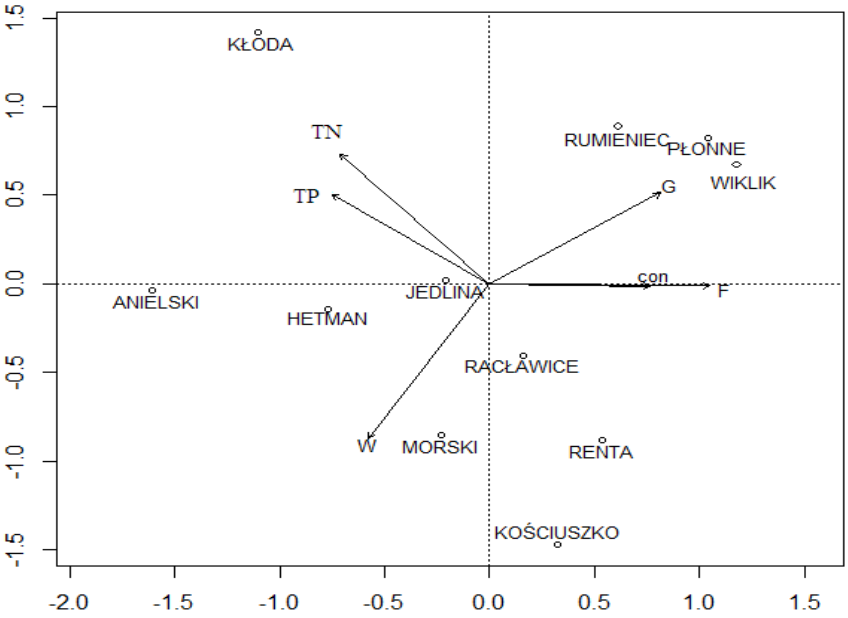


Figure 2. *Principal components analysis (PCA) of the environmental variables included in the study and ordination of the sites*

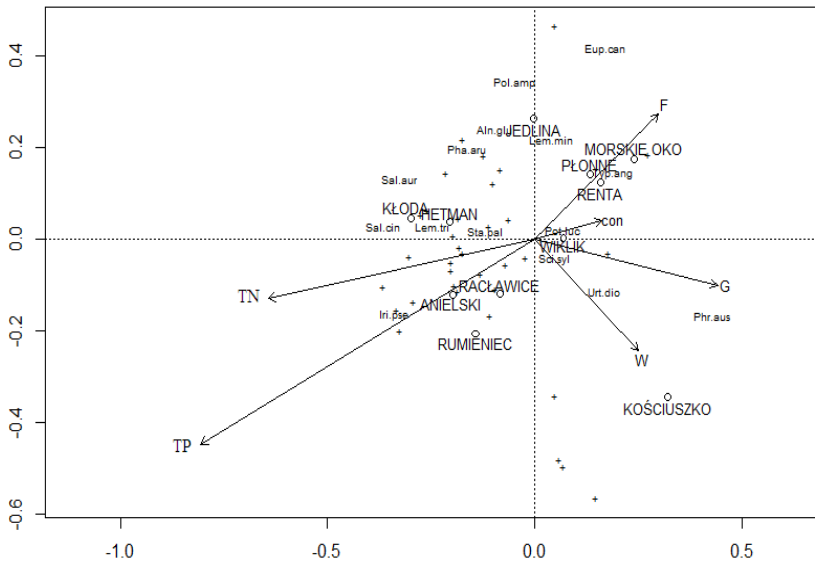


Figure 3. *RDA ordination diagram of the environmental variables was included in the study and the sites. All species were included in the analysis, but for clarity the most poorly represented species were displayed on scatter plots as "+"*

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Discussion

Biodiversity in ponds is frequently studied because ponds are rich in microhabitats (Céréghino et al., 2008a) and provide appropriate conditions for aquatic vegetation development and establishment. Specifically, occasional breaks in the surrounding canopy on pond banks create changes in sediment properties that foster the development of plants (Gallego et al., 2014; Gallego et al., 2015).

Ordination analysis included 11 study fish ponds and 17 environmental variables. This study also showed that ponds are valuable for plant biodiversity. In total 75 species of plants occurred in the studied complex. The total number of species ranged from 10 in Morski pond to 49 in Anielski pond. In the studied ponds, a high number of aquatic plants were found according to the most recent works in Europe. In the studied ponds in the Mediterranean there were 51 species (on average 24 species per pond). In a study of lowland ponds in Great Britain an average number of 6 species in standing ponds and 11 species in flowing ponds was recorded and in ponds located in central Italy the average was 9 species (Della Bella et al., 2008; Gallego et al., 2014).

Maintaining the greatest diversity of macrophytes, as well as stable quality water, provides high economic effects in the studied ponds (Lemmens et al., 2013). Landscape characteristics mainly played an important role in species composition-environmental conditions relationships, ale chemical characteristics were significant (Hrivnák et al., 2013). The chemical parameters of the water to a large degree determine the occurrence of macrophytes; however, this is not the only type of pressure. Also, the development of the catchment area and of the littoral zone of the aquatic ecosystems has a significant effect on water and rush plant communities. Studies on the effect of the method of development of the littoral zone of lakes in the state of Wisconsin show that housing development definitely has a negative effect on submerged and floating-leaf plants. Such an effect was not observed for emergent vegetation. On the other hand, a lower share of forests in the catchment basin reduced the development of all macrophyte groups (Ahrenstarff et al., 2009; Akasaka et al., 2010).

The results of the forward selection tests indicated that only floating leaves are significantly related to the cover. They cover the water's surface and in this way provide fish the benefits of shade, protection and breeding areas. Ponds as a rule are shallow and during the summer their temperature rises very fast. This could cause a rapid increase in algal (Lin-lin et al., 2012). The proper buffer zone management (forest mixed meadow) could limit the penetration of sunlight (Sender, 2016). In these places, where the buffer zone is without shading vegetation, they cool the water for the fish and give fish a place to hide. But they could decrease the occurrence of submerged macrophytes. Submerged macrophytes, in the investigated ponds, were not numerous. So, in our opinion a compensation phenomenon appeared in these ponds, like in the shallow lakes from the Polesie region (Sender et al., 2015). Floating leaves may grow well in water with low transparency, well sunlit (Feldmann and Nöges, 2007), so it seems that ponds create the best habitat for them. The litoral and especially the zone of floating leaved plants decrease with increasing lake area due to wind stress related to the height and strength of waves (Feldmann, 2012; Kolada, 2014).

Ponds are ecosystems created by man and like each type of such ecosystem they require man's constant interference (regulation of water level, feeding fish). They are usually formed in unattractive agricultural places, while their presence significantly increases the natural values of the area. Antrophogenic waters provide suitable environmental conditions for the potential establishment and growth of macrophytes,

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floating-leaved and flood-meadow taxa. The analysed ponds could be an excellent example of sustainable development.

Conclusions

In conclusion, this study highlighted that temporary ponds in Western Polesie have different macrophyte species composition, with aquatic species exclusively found in each pond. Temporary ponds, while capable of hosting some hydrophytes, are tightly linked to humid meadows. They have similar environmental conditions (temporariness of flooding period, low depth, water quality) and they potentially can share similar species. Our results also showed that the relationships between macrophyte species and cover depends on buffer zone management and some physico-chemical variables of water. The PCA analysis showed a positive effect of the presence of grassland and forests in the buffer zone on the diversification of plant vegetation. We observe a negative effect on the diversity of vegetation in the case of high concentration of nitrogen and total phosphorus and increasing surface of the ponds. The RDA analysis showed a statistically significant negative effect of the total phosphorus concentration on macrophyte differentiation.

For conservation purposes, the studied temporary ponds hosted some species of conservation interest at regional scale: *Nuphar lutea*, *Nymphea alba* and *Epipactis palustris* (Lower Risk Category) are exclusively found more than in only one of pond. Therefore, the ponds should be preserved or created because both could give an irreplaceable contribution to the conservation of aquatic plant diversity of small water bodies.

Studies on physical and chemical parameters and occurrence of aquatic vegetation should be carried out in subsequent years at different times of the year. These factors are subject to seasonal and long-term changes. In the future, these studies will allow to determine the directions of changes and their causes.

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ASSESSMENT OF THE CONTENT OF SELECTED TRACE ELEMENTS IN COCKSFOOT AND MAIZE MANURED WITH SEWAGE SLUDGE MIXED WITH HARD COAL ASH

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Abstract. The objective of the study was to determine changes in the content of selected trace elements in the biomass of test plants as affected by manuring with fresh and composted sewage sludge, hard coal ash, sewage sludge mixed with coal ash and liming at the background of mineral fertilisation. The experimental design was a completely randomised arrangement with three replicates. The following factors were examined: fertilisation with organic and mineral materials (fresh sewage sludge; composted sewage sludge; hard coal ash; calcium carbonate) and mineral fertilisation (no fertilisation; NPK fertilisation). An application of sewage sludge, hard coal ash, and sludge-ash mixtures significantly enhanced lithium content in the biomass of the 1st cut of cocksfoot, and molybdenum content in the 2nd cut grass. Hard coal ash mixed with sewage sludge contributed to an increase in lithium contents in the cocksfoot biomass of the 1st cut, titanium content in the 2nd cut of grass. Soil liming significantly increased lithium content in the plant biomass of 1st and 2nd cuts, as well as molybdenum contents in maize biomass. NPK nutrition significantly increased lithium, and titanium concentrations in the biomass of test plants.
Keywords: *organic fertilisation, mineral fertilization, liming, heavy metal*

Introduction

In recent years, a decline in the amount of organic manures applied to crops has been observed, which can frequently result in a negative soil balance of micro-elements. The phenomenon may disturb the appropriate content of micro-elements in crop plants which, in turn, is a very important qualitative characteristic taken into account while evaluating consumption- (Wojciechowska-Solis and Soroka, 2017) and feedstuff-related characteristics of the crops. Molybdenum and titanium are the elements which are indispensable for plant growth and development because they take part in metabolic processes. Additionally, molybdenum is a component of enzymes participating in transformations of nitrogen, phosphorus and sulphur compounds (Williams and Frausto, 2002; Bambara and Ndakidemi, 2010). The elements are also necessary for an appropriate development of animals and man (Kabata-Pendias and Mukherjee, 2007; Han et al., 2016).

Natural products for crop nourishment (Ciepiela and Godlewska, 2014; Ciepiela et al., 2016), industrial and municipal waste may be a valuable source of micro-elements and organic matter (Kalembasa and Godlewska, 2009a; Gondek, 2010; Kardos et al., 2011; Niewiadomska et al., 2015). Sewage sludge is of particular interest as it can be used for manuring purposes. However, sewage sludge may contain not only valuable nutrients but also harmful and toxic compounds (Usman et al., 2012) which, in turn, can be harmful for the environment, particularly if soil is acidic (Delibacak et al., 2009). The influence of these undesirable compounds may be reduced by an addition of substances such as hard coal ash or calcium carbonate, which contributes to an increase

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in sewage sludge pH thus reducing the solubility and phytoavailability of the majority of metals (Maksimovic et al., 2008; Kalembasa et al., 2008). It would make it possible to achieve two important economic goals, that is remediation of ash landfills and sewage sludge management.

The objective of the study was to determine changes in the content of selected trace elements in the biomass of test plants as affected by manuring with fresh and composted sewage sludge, hard coal ash, sewage sludge mixed with coal ash and liming at the background of mineral fertilisation.

Materials and methods

A pot experiment was established in a glasshouse located at the experimental unit of Siedlce University of Natural Sciences and Humanities in Poland (52.169° N, 22.280° E). The experimental design was a completely randomised arrangement with three repetitions. The following factors were examined:

I – nutrition with organic and mineral materials (sewage sludge – raw and composted during three months) obtained from the sewage treatment plant in Siedlce – municipal and industrial sewage; hard coal ash from Energy Works in Siedlce; calcium carbonate). Sewage sludge was applied once, adding 5% relative to soil weight. Sewage sludge and coal ash were mixed at the ratio of 2:1 (DM).

II – mineral nutrition (with or without NPK fertilization) Mineral fertilisers: urea, triple superphosphate and potassium sulphate, were applied pre-plant.

The soil used in the experiment was very loamy sand obtained from the 0-20 cm layer of grey brown podzolic soil. Before the experiment was set up, soil contents of nitrogen, carbon, available phosphorus and potassium were determined (respectively: 1.10, 8.20, 0.052 and 0.071 g kg^{-1}). Pots were filled with the soil at the amounts of 10 kg and humidity was maintained at the level of 60% of the full water capacity during the vegetation period.

Cocksfoot (*Dactylis glomerata*) was the test plant in the first study year; it was sown at the amount of 1.0 g pot^{-1}, and three cuts per growing season were harvested. In the second study year, maize (*Zea mays*) was grown at the density of 3 plants per pot, and harvested at the flowering stage to determine the relationship between mineralization rate of the organic materials tested in the study and plant uptake of nutrients. In the dried and powdered plant biomass, the total quantities of Mo, Ti, and Li were determined by means of the ICP–AES technique (Boss and Fredeen, 2004) after 'dry' mineralisation. Plant material (1 g) was digested in a muffle furnace at 450 °C with gradually increased temperature; then digested samples were dissolved in water solution of hydrochloric acid ($HCl:H_2O$, 1:1) and evaporated until dry to decompose carbonates and separate silicates. The ash obtained was transferred to measure flasks containing 10% HCl solution through a hard filter paper. Total molybdenum, titanium and lithium contents in sewage sludge and hard coal ash were also determined by the ICP-AES method following 'dry' mineralisation.

The program STATISTICA (data analysis software system), version 12 (www.statsoft.com) was used to statistically analyse the results. Significance of differences between means for the experimental factors was checked using Tukey's test at the significance level of $\alpha \leq 0.05$.

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Results and discussion

Hard coal ash and sewage sludge are composite materials whose chemical content and properties depend on many factors (Kalembasa et al., 2008). Sewage sludge applied in the experiment (*Table 1*) contained several times smaller amount of Li and Ti as hard coal ash. Molybdenum content in the sewage sludge and hard coal ash was similar and amounted to 5.67 and 3.76 mg kg^{-1}, respectively.

Table 1. *The content of Mo, Ti and Li in D.M. in sewage sludges and ash hard coal*

Element	Sewage sludge (mg kg^{-1})	Ash hard coal (mg kg^{-1})
Mo	3.76	5.67
Li	43.6	53.0
Ti	92.6	617

The cocksfoot grown in the experiment contained 2.74 mg molybdenum kg^{-1} DM, on average, the amount varying in particular cuts and depending on the experimental factors (*Table 2*). In the first study year, molybdenum content increased in subsequent grass cuts in plots with no NPK nutrition, but the opposite tendency was observed in NPK-fertilised units. It may indicate that the process of organic compound mineralisation accelerated due to additional mineral nutrition. An application of fresh sewage sludge significantly increased molybdenum quantity in the biomass of the 2nd cocksfoot cuts, whereas composted sludge and hard coal ash contributed to a decline in molybdenum content in the 1st-cut plants compared with the control. Also McBride (2005) reported increased Mo contents after sludge application.

By contrast, according to Wichard (Wichard et al., 2009) molybdenum in soils can bind to organic matter, which can cause limiting its availability to plants.

Table 2. *The content of Mo (in mg kg^{-1} DM) in cocksfoot*

Organic and mineral materials	NPK fertilisation								Means			Means
	0				NPK							
	Cuts			Means	Cuts			Means				
	I	II	III		I	II	III		I	II	III	
1	1.97cd	2.33	4.06a	2.79	3.51abc	2.67	1.14d	2.44	2.74c	2.50cd	2.60bc	2.61ab
2	2.70bc	3.84	3.42abc	3.32	3.43bc	3.36	1.52cd	2.77	3.07bc	3.60ab	2.47bc	3.05ab
3	0.85e	2.06	2.24bc	1.72	2.26d	1.82	2.12bcd	2.07	1.56d	1.94d	2.18c	1.89 b
4	1.66de	2.29	2.55bc	2.17	2.54cd	1.76	1.41cd	1.9	2.10d	2.03cd	1.98c	2.04 b
5	1.20de	1.83	2.07c	1.7	3.62ab	1.93	1.50cd	2.35	2.41c	1.88d	1.79c	2.03 b
6	2.32bc	3.56	3.56ab	3.15	3.88ab	2.05	3.07ab	3	3.10b	2.81bc	3.32ab	3.07ab
7	1.91cd	3	4.07a	2.99	3.59ab	2.75	2.86abc	3.07	2.75c	2.88abc	3.47ab	3.03ab
8	3.42ab	3.39	3.63ab	3.48	3.57ab	2.64	1.43cd	2.55	3.50ab	3.02abc	2.53bc	3.01ab
9	3.82a	4.73	3.75a	4.1	4.45a	2.83	3.84a	3.71	4.14 a	3.78a	3.80a	3.90ab
Means	2.21*	3.00*	3.26*	2.82	3.42*	2.42*	2.10*	2.65	2.82	2.71	2.68	2.74

1 - control object, 2 - sewage sludge, 3 - fermented sewage sludge, 4 - ash hard coal, 5 - sewage sludge + ash, 6 - ferment. sewage sludge + ash, 7 -liming, 8 - liming + sewage sludge + ash, 9 - liming + ferment. sewage sludge + ash

Values in columns for individual factors indicated with different small letters, differ significantly.

Asterisks within the same line indicate significant differences ($\alpha \leq 0.05$).

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Ash mixed with composted sludge significantly increased molybdenum content in cocksfoot but its mixture with fresh sewage sludge contributed to a decline in molybdenum concentration in test plants. Liming of the soil to which sludge-ash mixtures were incorporated, significantly enhanced the molybdenum uptake by cocksfoot. Mineral nutrition of the 1st cut plants significantly increased molybdenum content in cocksfoot whereas in the 2nd and 3rd cuts a decline was observed.

Average molybdenum concentration in the biomass of maize grown in the second study year was 0.786 mg kg^{-1} DM (*Table 3*), on average, and significantly depended on the waste materials applied. An application of sewage sludge, ash, and a sludge-ash mixture – both fresh and composted – significantly increased molybdenum content in maize compared with control plants. Soil liming increased the amount of the element in maize biomass as compared to control plants. Also, liming significantly enhanced molybdenum uptake by maize in plots where fresh and composted sludge-ash mixtures had been applied. Kalembasa and Godlewska (2009b) found that soil liming increased Mo content in plants, which was also confirmed in the present study.

Table 3. *The content of Mo (in mg kg^{-1} DM) in maize*

NPK fertilization / Organic and mineral materials	0	NPK	Means
1	0.294	0.138	0.216b
2	0.636	1.01	0.823ab
3	0.517	0.896	0.707ab
4	0.71	0.34	0.525b
5	0.794	0.852	0.823ab
6	0.54	1.1	0.820ab
7	0.994	0.696	0.845ab
8	1.25	0.827	1.04a
9	1.41	1.14	1.28a
Means	0.794	0.778	0.786

For explanations see *Table 2.*

Titanium content in cocksfoot biomass (*Table 4*) was 2.88 mg kg^{-1} DM, on average, and increased in subsequent cuts. The waste materials applied significantly reduced the titanium concentration in the 1st cut grass as compared to control plants. An addition of ash to fresh and composted sewage sludge resulted in a significant decline in titanium content in the biomass of the 1st cut cocksfoot. However, ash added to sludge increased titanium quantity in the 2nd cut of test grass. NPK nutrition had a significant effect only for the 1st cut grass whose titanium content was significantly higher.

The average titanium content in maize (*Table 5*) was 2.40 mg kg^{-1} DM. Liming significantly increased titanium content in plants fertilised with fresh sewage sludge mixed with hard coal ash. Titanium concentration in maize grown in plots treated with composted sludge, ash, and fresh sludge mixed with ash was lower as compared to control plants. Also Kalembasa and Wysokiński (2009) reported higher titanium amounts in control maize compared with sewage sludge-treated plants. NPK nutrition significantly increased titanium concentration in the test plants.

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Table 4. *The content of Ti (in mg kg^{-1} DM) in cocksfoot*

Organic and mineral materials	NPK fertilisation								Means			Means
	0				NPK							
	Cuts			Means	Cuts			Means				
	I	II	III		I	II	III		I	II	III	
1	1.58b	2.04	4.13a	2.58	3.72a	3.34	3.11a	3.39	2.65a	2.69abc	3.62	2.99
2	1.56b	2.09	4.03a	2.56	2.79b	2.85	3.23a	2.96	2.18b	2.47bc	3.63	2.76
3	1.70ab	2.32	3.18b	2.4	1.92de	4.1	4.24a	3.42	1.81bc	3.21abc	3.71	2.91
4	1.58b	3.65	3.45ab	2.89	2.60bc	3.91	3.25a	3.25	2.09bc	3.78ab	3.35	3.07
5	0.96c	3.96	4.72a	3.21	1.40ef	4.07	3.31a	2.93	1.18d	4.02a	4.02	3.07
6	1.18bc	3.87	3.88ab	2.98	0.87f	4.1	3.52a	2.83	1.03d	3.99a	3.7	2.9
7	1.22bc	2.54	3.05b	2.27	2.01cd	1.91	3.53a	2.48	1.62c	2.23c	3.29	2.78
8	2.30a	3.94	3.79ab	3.34	1.12f	3.59	3.95a	2.89	1.71c	3.77ab	3.87	3.12
9	0.92c	3.92	2.43b	2.42	1.33f	3.81	3.89a	3.01	1.13d	3.87a	3.16	2.72
Means	1.44*	3.15	3.63	2.72	1.97*	3.52	3.56	3.02	1.71	3.34	3.6	2.88

For explanations see *Table 2.*

Table 5. *The content of Ti (in mg kg^{-1} DM) in maize*

NPK fertilization / Organic and mineral materials	0	NPK	Means
1	2.12ab	2.51bc	2.32ab
2	2.22ab	2.90abc	2.56ab
3	1.76b	2.39bc	2.08b
4	2.02ab	2.06c	2.04b
5	1.61b	2.50bc	2.06b
6	1.86ab	3.45a	2.66ab
7	2.38ab	2.67abc	2.53ab
8	2.44ab	3.15ab	2.80a
9	2.70a	2.35bc	2.53ab
Means	2.12*	2.66*	2.4

For explanations see *Table 2.*

The average lithium content in cocksfoot biomass (*Table 6*) amounted to 23.4 mg kg^{-1} DM, and it was affected by the experimental factors. Waste materials contributed to significant differences between the cuts, but lithium content did not exceed 50 mg kg^{-1} DM, which is assumed as the limit for root crops. The content was lower in all the cuts of control cocksfoot biomass compared with the remaining units. Hard coal ash mixed with fresh and composted sewage sludge contributed to increased lithium contents in the cocksfoot biomass of each cut the differences being significant for the 1st cut only. Soil liming in plots where sludge-ash mixtures were used, enhanced the quantity of the element in all the cuts of the test plants. Moreover, there were recorded significantly higher lithium concentrations in 1st and 2nd cut plants harvested from limed plots, and a significantly higher mean concentration calculated across the three cuts for limed units

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compared with the control grass, which was not consistent with other authors' reports. According Aral and Vecchio-Sadus (2008) plant uptake of lithium is greater in acidic soils than in alkali soils. By contrast, Wysokiński and Kalembasa (2009) found a higher lithium content in sunflower grown in plots manured with fresh and composted sewage sludge mixed with brown coal and CaO, compared with plants manured with sewage sludge only. Mineral nutrition increased the lithium content in the 1st and 2nd cuts but it insignificantly affected the lithium uptake by cocksfoot in the 3rd cut.

Lithium level in maize biomass (*Table 7*) was 14.5 mg kg^{-1} DM, on average. Although no significant differences were found, lithium content tended to increase in maize plants due to an application of waste materials and their mixtures compared with the control. Hard coal ash mixed with fresh and composted sewage sludge had no effect on the uptake of lithium by plants. Mineral nutrition was the only factor which contributed to a significant increase in the maize content of lithium.

Table 6. *The content of Li (in mg kg^{-1} DM) in cocksfoot*

Organic and mineral materials	NPK fertilisation								Means			Means
	0				NPK							
	Cuts			Means	Cuts			Means				
	I	II	III		I	II	III		I	II	III	
1	18	12.1	11.4e	13.8	17.7	23.4	28.1b	23.1	17.9c	17.8c	19.8abc	18.5b
2	26.7	19.4	26.6a	24.2	28.6	19.4	21.7d	23.2	27.7ab	19.4abc	24.2abc	23.7ab
3	27.2	24.2	25.4a	25.6	26.7	25.6	28.1bc	26.8	27.0ab	24.9abc	26.8ab	26.2a
4	23.9	25.1	20.7c	23.2	24.6	22.7	20.1e	22.5	24.3ab	23.9abc	20.4abc	22.9ab
5	27.3	14.3	24.1b	21.9	31.2	26.6	30.9a	29.6	29.3a	20.5abc	27.5a	25.7ab
6	25.7	21.7	24.2b	23.9	33.3	33.2	22.7cd	30	29.5a	27.5ab	23.5abc	26.8a
7	27	25.2	20.6c	24.3	28.5	30.5	23.5c	27.5	27.8ab	27.9a	22.1abc	25.9a
8	21.2	16.9	20.6c	19.6	24.3	24.7	17.3f	22.1	22.8bc	20.8abc	19.0bc	20.8ab
9	21.1	16.8	17.8d	18.6	28	21.4	16.3f	21.9	24.6ab	19.1bc	17.1c	20.2ab
Means	24.2*	19.5*	21.3	21.7*	27.0*	25.3*	23.2	25.2*	25.6	22.4	22.3	23.4

For explanations see *Table 2.*

Table 7. *The content of Li (in mg kg^{-1} DM) in maize*

NPK fertilization / Organic and mineral materials	0	NPK	Means
1	16.6a	9.83c	13.2
2	14.7a	13.5abc	14.1
3	16.3a	10.6bc	13.5
4	13.9a	17.4ab	15.7
5	18.1a	11.5bc	14.8
6	16.4a	14.7abc	15.6
7	17.9a	9.12c	13.5
8	11.7a	15.7abc	13.7
9	13.5a	18.8a	16.2
Means	15.5*	13.5*	14.5

For explanations see *Table 2.*

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Conclusions

An application of sewage sludge, hard coal ash, and sludge-ash mixtures significantly enhanced lithium content in the biomass of the 1st cut of cocksfoot, molybdenum content in the 2nd cut grass. Hard coal ash mixed with sewage sludge contributed to an increase in lithium contents in the cocksfoot biomass of the 1st cut, titanium content in the 2nd cut of grass. Soil liming significantly increased lithium content in the plant biomass of 1st and 2nd cuts, as well as molybdenum contents in maize biomass. NPK nutrition significantly increased lithium, titanium and barium concentrations in the biomass of test plants.

In summary, it should be noted that the degree of organic matter decomposition and origin has a considerable effect on the characteristics discussed in the work. Further studies are needed to develop more comprehensive results and evaluate the impact of different rates of organic and mineral materials.

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SEASONAL AND ANNUAL CROP EVAPOTRANSPIRATION IN EUROPE OVER 2011–2070

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Abstract. The knowledge of evapotranspiration variation is necessary not only for climatology reasons but also for agriculture and hydrology purposes to understand the amount of water resources and its distribution in territory. Here, we present an exhaustive study of spatial distribution of crop evapotranspiration (ET_c) in Europe, both for seasonal and annual time-shifts related to the present (2011–2040) and the future (2041–2070). The method is based on the crop coefficients (K_c) and the climate modes. The seasonal ET_c varies in Europe from 0 mm to 772 mm in the present period and from 0 mm to 848 mm in the future period. The annual ET_c ranges from 0 mm to 1385 mm year^{-1} for the present while the future calculations indicate values which range from 0 mm to 1496 mm year^{-1}. Elevated values of annual ET_c were depicted in the Iberian Peninsula, Balkan Peninsula, Italian Peninsula, and Pannonian basin. These findings are the base for the water resources quantity in Europe for the 21st century, with major help in the hydrological and climatological studies, but also for the agriculture and planning strategies, policymakers' decisions, and considerable scientific contribution.
Keywords: *climate change, spatial climate data, evapotranspiration mapping, land cover, crop coefficients, GIS*

Abbreviations: GIS: geographical information systems, K_c: crop coefficients, ET_c: crop evapotranspiration, ET_0: potential evapotranspiration, $K_{c\ ini}$: initial season coefficient, $K_{c\ mid}$: mid-season coefficient, $K_{c\ end}$: end season coefficient, $K_{c\ cold}$: cold season coefficient

Introduction

The changes in the planet's geospheres are continuously also in relations with the climate. A lot of natural systems and artificial conceptions depend on an optimum standard to maintain its functionality. The Earth's climate is warming (Haeberli et al., 1999; Oerlemans, 2005), a fact for which the equilibrium of different entities is triggered in many places, regions, and continents. Increase of the mean air temperature in the last 140 years was observed on the climate trend (IPCC, 2001). Moreover, for the 21st century, an increase in the mean air temperature up to 3 °C and a decrease of precipitation in different places across the globe were predicted (IPCC, 1996, 2001; Stavig et al., 2005; The Canadian Climate Models, 2014). The obvious findings about global warming have been carried out by studying the glaciers, which are the most sensitive indicators of climate change (Haeberli et al., 1999; Kargel et al., 2005; Dong et al., 2013; Nistor and Petcu, 2015). An increase in the mean sea level was calculated, taking into account the Arctic regions and negative trends of the ice field areas, and ice caps were depicted. However, the impact of climate change on the Earth is not related only to the glaciers melting; it also implies numerous negative outcomes on agriculture, land use, and hydrology. Parmesan and Yohe (2003), Campos et al. (2013) and Prăvălie et al. (2014) indicated the climate change negative impact on ecosystems and on water

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resources. The components of ecosystems and the ecolines could be easily affected by climate change. Due to this reason, many ecosystems and several natural reservations are exposed to major changes in their composition and biodiversity (Nistor, 2013; Nistor and Petcu, 2014).

Regarding the water resources, the negative impact of climate change on surface water has been claimed by scientists to be indicated by the decrease in river discharge and the reduction in runoff (Piao et al., 2010). These facts have a direct impact on water availability (Čenčur Curk et al., 2014). On the other hand, the northern sides of Europe and the Alpine region are faced with the increase of the stream flow discharge in the spring period due to the melting of glaciers and snow, which leads to floods and erosion in many places due to extreme events (IPCC, 2007). Numerous surveys on groundwater and climate change were completed in the last two decades as a consequence of global warming by numerous scientists (Loàiciga et al., 2000; Bachu and Adams, 2003; Brouyère et al., 2004; Nistor et al., 2014; Prăvălie et al., 2014). The majority of them have found that the negative effects of climate change on groundwater recharge were mainly related to the reduction in spring flow discharge and seawater intrusion into coastal areas due to sea level rise. The negative impact of climate change on groundwater quality is related to the increase in groundwater temperature (Taylor and Stefan, 2009; Kløve et al., 2014) and "dissolved oxygen concentrations" (Figura et al., 2011; Kløve et al., 2012, 2014; Haldorsen et al., 2012). Jiménez Cisneros et al. (2014) summarize a series of quantity and quality problems related to climate change in the "IPCC 2014 report". The most important issue of the surface waters and groundwater quantity is directly related to the evapotranspiration amount. Mojid et al. (2015) report the contribution of the impacts of climate change on crop water demand.

In the Southeast Europe region, climate change also negatively affects the evapotranspiration (Nistor et al., 2017a). Several hydrological and climatological studies used the potential evapotranspiration (ET_0) and actual evapotranspiration for the water balance calculations and water surplus determination (Li et al., 2007; Rosenberry et al., 2007; Gowda et al., 2008). These calculations are more accurate and realistic if the climate data are well combined with the vegetative pattern. This task contributes to find the appropriate values of the ET_c. Allen et al. (1998) and Allen (2000) proposed a method for ET_c calculation using K_c values, extracted from the evapotranspiration ratio of the ET_c and ET_0. They calculated the K_c values for different large varieties of crops, including also the tropics and temperate zones. In the same period, Grimmond and Oke (1999) have conducted a significant research on the K_c values related to the urban areas of different cities from the United States. Later, considering Allen et al.'s (1998) method and Grimmond and Oke's (1999) additional work, Nistor and Porumb-Ghiurco (2015) have calculated and completed the spatial distribution of the ET_c in the Emilia-Romagna region from Italy. They used the climatological data grids to calculate the ET_0 and the land cover layer to create the evapotranspiration rate in four seasons. Due to its high applicability, their method has been widely used in ET_c mapping for many regions of Europe. Thus, Nistor et al. (2017b) found the ET_c in the Pannonian basin using the climate models of 30 years for the present (1991–2020) and future (2021–2050) and the K_c values. They observed an increase of ET_c for the future period due to increase in the mean air temperature. In the mountain area of the Carpathian region, the ET_c have been calculated by Nistor et al. (2016) and the research findings indicate the increase of ET_c values for recent decades. These results were connected to the negative climate warming effects on the evapotranspiration and possible decrease in water availability

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for the next half century. Using the groundwater modelling under climate change, the water table levels in Geer Basin from Belgium will be expected to decrease in future (Brouyère et al., 2004). A comprehensive study about the evapotranspiration parameters, based on accurate methods in Western Macedonia from Greece, was done by Ambas and Baltas (2012). The REMO and ALADIN regional climate model simulations have been used by Ladányi et al. (2015) in the Kiskunság National Park from Hungary to investigate the future drought hazard. Anda et al. (2015) and Blanka et al. (2017) have completed the investigations related to evapotranspiration and water management in Central Europe. Kurnik et al. (2014) applied a model of Soil Water Balance European Water Accounting to assess the evapotranspiration of main agricultural regions from Europe. Recently, using the regional fuzzy chain model, Güçlü et al. (2017) calculated the evapotranspiration in the Kingdom of Saudi Arabia. Based on Geographical Information System (GIS) and remote sensing technology, Zhan et al. (2015) have calculated the reference evapotranspiration at a regional scale in China. Ramírez-Cuesta et al. (2017) did the appropriate work in the South of Spain. In the Hungarian-Serbian cross-border area, Fiala et al. (2014) analyzed the severity of drought and its influence on agricultural production.

Considering the threats posed by climate change to agriculture and hydrological resources in one of the most important and diversified continents of the world, our work aimed to calculate ET_c in two time periods (2011–2040 and 2041–2070) based on the high-resolution climate models and land cover data.

Materials and methods

Study area

The European continent extends from 34°35’ to 80°42’ latitude N and from 0°0’ to 8°59’ longitude W and from 0°0’ to 66°42’ longitude E (*Fig. 1a*). Here, we were able to complete the ET_c only for the main realm of Europe and few islands. The Eastern extremity of Europe and some islands such as Iceland have not been included in this investigation due to a lack of climate data.

Europe is a very varied continent from the relief, climate, and land cover points of view. The northern, western and southern coastal line is unregulated, with many peninsulas and islands. The altitudes of the continent range from slightly below 0 m in the Netherlands and some lowlands such as the Danube Delta to 4810 m in Mont Blanc Peak from the Alps Range.

The most extended landforms in Europe are the plains, followed by hilly areas and mountains. Thus, in the eastern part of Europe is the East Europe Plain while in the central and northern sides is located the North European Plain. Other plains are present in different regions of the continent, e.g. the Po Plain extends in the North of Italy, Île-de-France in the North of France, the Romanian Plain in the South of Romania, and the Pannonian basin in the South-central part of Europe.

More important for the land cover biodiversity and for the air mass movement are the mountain ranges too. In the South there are the Alps Mountains which extend on approximately 1000 km in length; in the central and eastern sides there is the Carpathian Mountains; the elevated southern sides of Europe are represented by the Dinaric Mountain and in the North are located the Scandinavian Mountains. In the West of Europe, between France and Spain, there are the Pyrenees Mountains, in the South of Europe are the Apennines Mountains, while in the North of the British Islands there are

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the Scottish Mountains. In Spain, the high plateau can be found as "Meseta Central", but also other hilly areas extend in Europe, generally interposed between the mountains and plains (e.g. Prealps between the Alps Range and Po Plain, Sub-Carpathians between the Carpathian Range and the Romanian Plain).

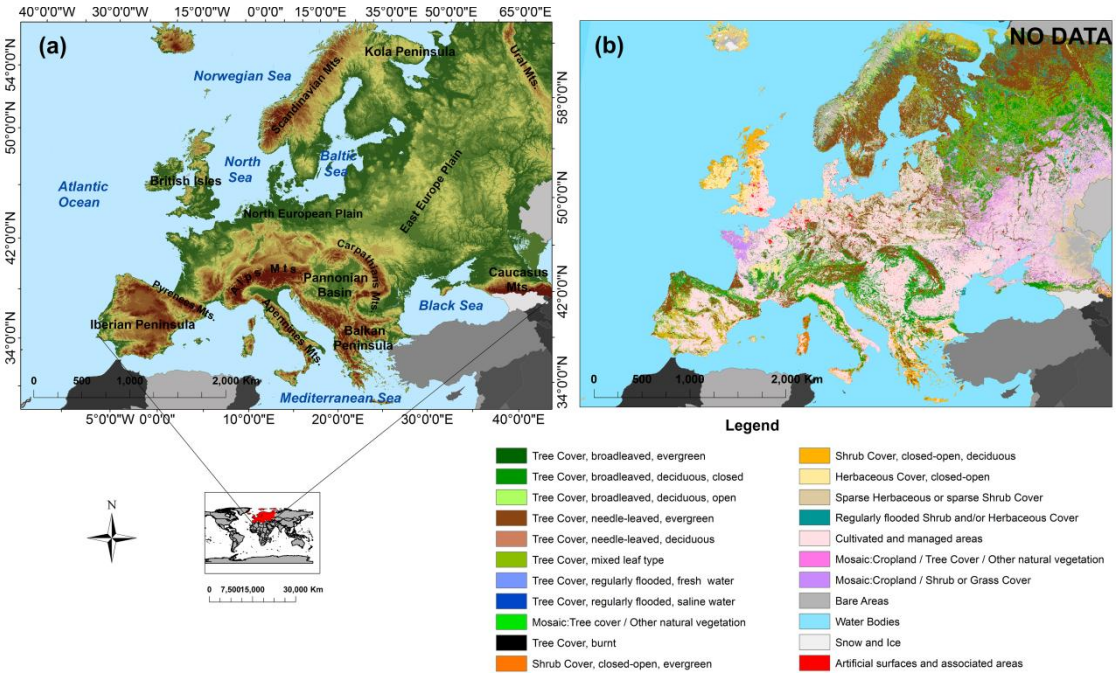


Figure 1. *(a) The physical map of European continent and its location on global map. (b) Land cover of Europe*

The high extension in latitude and also the presence of the Atlantic and Arctic Oceans in Europe's surroundings are going to influence the different types of climate. In the North of Europe, the climate is Baltic; in the West of Europe there is the ocean influence. In the South of the continent, the Saharan and Mediterranean influences are felt. The continental influences that occur in the East of Europe and the transitional climate between oceanic and continental is more present in the Romanian landscape. The local climates may be depicted in the mountain and near the coastal areas (e.g. in the Alps, in the Carpathians, near the Black Sea, around the Mediterranean Sea). Cfa climate was verified in the central, North-central, Southern and some territories of the southeastern parts of Europe (Kottek et al., 2006). This type of climate is characterized by the warm temperate climate with hot summer and a fully humid period during one year. The Dfa and Dfb climates, which represent the cool climate with hot and warm summers, could be found in the eastern and southeastern sides of Europe. In the Scandinavian Peninsula and northeastern sides, the Dfc cool climate with the cool summers has been identified (Kottek et al., 2006). The South of Europe, mainly the southern sides of the Italian and Balkan Peninsulas, has the Cfc climate. The Csb climate, characterized by dry and warm summers extends to the North of the Iberian Peninsula while the Csa climate, characterized by hot summers and limited precipitation, extends to the South of the Iberian Peninsula. Due to the very low

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temperatures during the winter season, in the Scandinavian Peninsula and in the elevated areas of the Alps Range, the polar tundra climate is easy to find.

In the direct relationship with the climate and relief conditions, the vegetation pattern shows an intense biodiversity in Europe. First, the favourable plain lands and coastal areas are highly recognized for their agriculture lands and urban developed areas. Generally, in the mountains, the coniferous and evergreen forests are growing. In the hilly areas and low mountains, the land cover is mainly composed of mixed and deciduous forests and includes such species as oak (Quercus), beech (Fagus), hornbeam (Carpinus), and elms (Ulmus) (European Environment Agency, 2007). Pasture, transnational woodland, and shrubs predominantly cover the alpine areas. The agricultural crops, hay, and herbaceous vegetation are typically for the lowlands and plain areas. The northern sides of Europe are covered by tundra vegetation. In the North of the Mediterranean Sea, the Mediterranean vegetation of stone pine (Pinus pinea) and maquis vegetation are presented. In the beach areas and drylands such as the South of Europe, the sclerophyllous vegetation is characteristic. The harbour areas, deltas, lagoons, marshes, beaches and dunes are also located in the coastal areas. *Figure 1b* shows the main land cover of Europe.

Climate data models

Climate models of the monthly air temperature and annual precipitation at high spatial resolutions have been used for the understanding of Europe's climatic parameters and for the seasonal and annual ET_c calculations. The models related to the present (2011–2040) and future periods (2041–2070) are the two main datasets for which the ET_c was computed. Climate projections were obtained from a publicly available database of high resolution climate grids for Europe (*http://tinyurl.com/ClimateEU*, Hamann et al., 2013). The database builds on the Parameter Regression of Independent Slopes Model (PRISM) to generate monthly precipitation grids for the 1961–1990 climate normal period, generated by Daly (2006). The ANUSplin interpolation method was used to generate monthly temperature grids for the 1961–1990 normal period. Future projections for temperature and precipitation are based on 15 AOGCMs of the CMIP5 multi-model dataset overlayed on the 1961–1990 baseline grids using the delta method. Further details on the climate database are described by Hamann et al. (2013). Both datasets are bias corrected and calibrated by using an anomaly method. The eastern extremity of Europe does not have available climate data, thus our study does not include this part of the continent.

Land cover data

A significant dataset in the ET_c calculation is the land cover layer, which expresses in the best way the vegetation spatial distribution in a certain field. To identify the main crops of Europe and the existing categories of the land cover type, here we used the CORINE Land Cover 2000 database. Even if it dates from 2000s years, CORINE database detailed up to the 3rd level is a raster data 250 m in spatial resolution elaborated and revised by the European Environment Agency (2016). The main advantages of the CORINE are the details and spatial resolution and the fact that this database covers the whole of Europe better than other versions of the CORINE 2006 and 2012 in that half the territory of Europe is not represented. Moreover, the raster grid of land cover is easy to integrate into the GIS environment. Both land cover and

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climatological data were set at 1 X 1 km spatial resolution. This resolution was adopted also by Nistor et al. (2016) in their mapping ET_c study.

Potential evapotranspiration (ET_0)

To calculate the seasonal ET_c, the monthly ET_0 was performed both for present and future periods. Due to the worldwide use and efficient performance, the Thornthwaite (1948) method was applied for this study. The monthly raster grid data of mean air temperature for present and future periods have been used to carry out the monthly ET_0. Seasonal and annual ET_0 were computed from the monthly raster data of ET_0.

The Thornthwaite (1948) formula (*Eq. 1*) is widely considered in the climate, hydrology and agricultural studies (Čenčur Curk et al., 2014; Nistor et al., 2015) but also provides good results at a regional scale (Nistor et al., 2016).

$$ET_0 = 16\left(\frac{10T_i}{I}\right)^{\alpha} \quad \text{(Eq.1)}$$

where:
ET_0 monthly potential evapotranspiration [mm]
T_i average monthly temperature [°C], $ET_0 = 0$ if $\bar{Tm} < 0$
I heat index (*Eq. 2*)
α complex function of heat index (*Eq. 3*)

$$I = \sum_{i=1}^{12} \left(\frac{T_i}{5}\right)^{1.514} \quad \text{(Eq.2)}$$

where:
T_i monthly air temperature

$$\alpha = 6.75 \times 10^{-7} I^3 - 7.71 \times 10^{-5} I^2 + 1.7912 \times 10^{-2} I + 0.49239 \quad \text{(Eq.3)}$$

where:
I annual heat index

Crop coefficients (K_c)

The key to the land cover evapotranspiration research and ET_c calculation at spatial scale in this approach are the values of K_c. For each type of land cover was assigned a specific K_c, accordingly with the specific literature (Allen et al., 1998) and previous scientific works (Nistor and Porumb-Ghiurco, 2015; Nistor, 2016a; Nistor et al., 2016, 2017a, b).

Understanding it as being the ratio between ET_c and ET_0, the K_c could have a slight variation for the same crops, depending on the annual period, local micro-climate conditions, dry or wet state of the Earth surface, latitude, and crop growth (Allen et al., 1998; Grimmond and Oke, 1999; Nistor et al., 2017b). For this reason in the present paper we adopt the standard methodology of the single crop (Allen et al., 1998) and also the values proposed by Nistor (2016a). Allen et al. (1998) published testing values of the K_c for different zones of the world in the Food and Agriculture Organization Paper 56 report. Regarding the evapotranspiration in the bare soil and urban areas, Grimmond

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and Oke (1999) carried out the K_c values by measurements in different locations across the United States. Appropriate values of K_c have been chosen for Europe, considering the latitude as the main factor of relevance.

An important aspect of the spatial representation of the K_c is coming from the calendar crops growth. For the temperate zone, the plant growth stage has been set in four seasons during one year: (i) initial season from March to May, (ii) the mid-season from June to August, (iii) the end season during September and October, and (iv) the cold season during January, February, November, and December. Nistor (2016b) also inserts one season between initial season and mid-season, the so-called developed season, but in his work the daily ET_c for a not-so-extensive area was done. Due to the overlapping of the crop calendar (Nistor et al., 2016), at the regional and continental scales the seasonal ET_c may be divided into four seasons. Thus, in function of the crops and land cover components, the specific values for the initial season coefficient ($K_{c\ ini}$), mid-season coefficient ($K_{c\ mid}$), end season coefficient ($K_{c\ end}$), and cold season coefficient ($K_{c\ cold}$) were set for the whole land cover pattern of Europe. *Figure 2* indicates the spatial distribution of K_c on the European continent. *Table 1* reports the values of K_c in the four chosen stages.

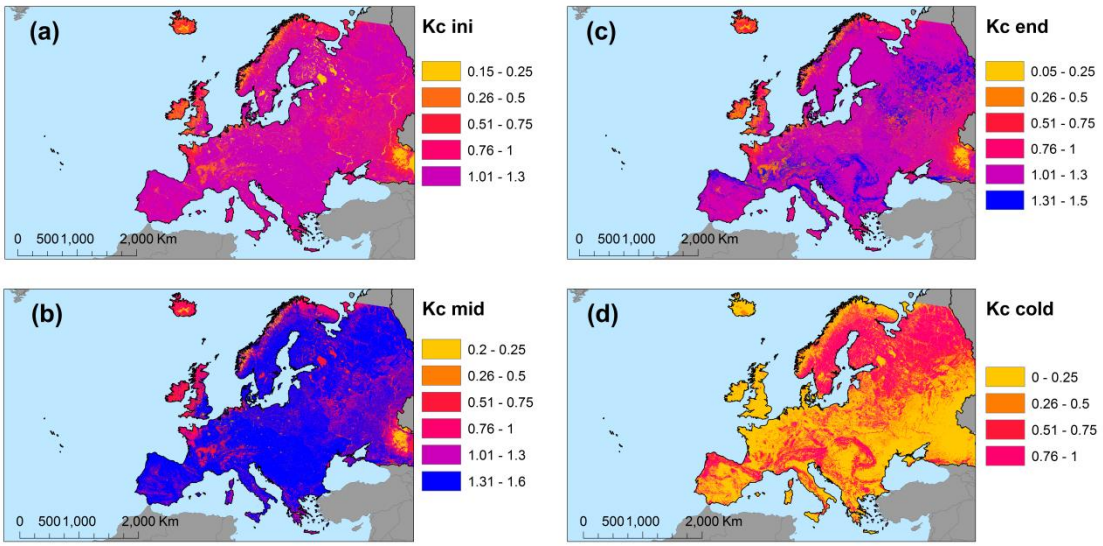


Figure 2. *Spatial distribution of K_c in Europe related to the land cover. (a) $K_{c\ ini}$ for the initial stage. (b) $K_{c\ mid}$ for the mid-season stage. (c) $K_{c\ end}$ for the end stage. (d) $K_{c\ cold}$ for the cold stage*

Crop evapotranspiration (ET_c)

The original procedure to calculate the ET_c is to multiply the K_c with ET_0 (*Eq. 4*). The mathematical calculations were completed using the 'Raster Calculator' function, from the ArcGIS 10.3 environment. The powerful spatial data analysis, climatological phenomena mapping and environmental surveys are the main advantages of the Spatial Analyst Tools from the ESRI software (Baltas, 2007; Dezsi et al., 2015; Nistor and Petcu, 2015).

Both seasonal and annual ET_c for Europe are related to the 2011–2040 and 2041–2070 periods, with the purpose of observing the effects of climate change on the amount of evapotranspiration during the 21st century. The four seasons of ET_c have been calculated, multiplying the K_c of the respective season with the seasonal ET_0 from the

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same period stage. Thus, the initial ET_c ($ET_{c\ ini}$) (*Eq. 4*), the mid-season ET_c ($ET_{c\ mid}$) (*Eq. 5*), the end season ET_c ($ET_{c\ end}$) (*Eq. 6*), and the cold ET_c ($ET_{c\ cold}$) (*Eq. 7*) were computed for Europe. Successively, the sum of the seasonal ET_c was done to calculate the annual ET_c for the present and the future (*Eq. 8*).

$$ET_{c\,ini} = ET_{0\,ini} \times K_{c\,ini} \quad \text{(Eq.4)}$$

$$ET_{c\,mid} = ET_{0\,mid} \times K_{c\,mid} \quad \text{(Eq.5)}$$

$$ET_{c\,end} = ET_{0\,end} \times K_{c\,end} \quad \text{(Eq.6)}$$

$$ET_{c\,cold} = ET_{0\,cold} \times K_{c\,cold} \quad \text{(Eq.7)}$$

$$\text{Annual } ET_c = ET_{c\,ini} + ET_{c\,mid} + ET_{c\,late} + ET_{c\,cold} \quad \text{(Eq.8)}$$

Table 1. *Corine Land Cover classes and representative seasonal Kc coefficients*

Corine Land Cover 2000	**Kc ini season**	**Kc mid season**	**Kc end season**	**Kc cold season**
CLC description	**Kclc**	**Kclc**	**Kclc**	**Kclc**
Tree cover, broadleaved, deciduous, closed	1.3	1.6	1.5	0.6
Tree cover, broadleaved, deciduous, open (open 15–40% tree cover)	1.2	1.5	1.4	0.5
Tree cover, needle-leaved, evergreen	1.1	1.4	1.25	1
Tree cover, needle-leaved, deciduous	1.25	1.45	1.35	0.7
Tree cover, mixed leaf type	1.2	1.5	1.3	0.8
Tree cover, regularly flooded, fresh water (& brackish)	1.2	1.4	1.3	0.7
Tree cover, regularly flooded, saline water (daily variation of water level)	1.05	1.3	1.2	0.6
Mosaic: Tree cover/other natural vegetation	1	1.15	1.05	0.5
Tree cover, burnt	0.4	0.6	0.3	-
Shrub cover, closed-open, evergreen (sparse tree layer)	0.9	1.15	1	-
Shrub cover, closed-open, deciduous (sparse tree layer)	0.8	1	0.95	-
Herbaceous cover, closed-open	0.4	0.6	0.5	-
Sparse herbaceous or sparse shrub cover	0.6	0.8	0.7	-
Regularly flooded shrub and/or herbaceous cover	0.7	0.9	0.8	-
Cultivated and managed areas	1.1	1.35	1.25	-
Mosaic: cropland/tree cover/other natural vegetation	0.9	1.1	1.05	0.3
Mosaic: Cropland/shrub or grass cover	0.55	0.8	0.7	-
Bare areas	0.15	0.2	0.05	-
Water bodies (natural & artificial)	0.25	0.65	1.25	-
Snow and ice (natural & artificial)	0.48	0.52	0.52	0.48
Artificial surfaces and associated areas	0.2	0.4	0.3	-

Kclc - crop coefficient for land cover including: Kc - crop coefficient for plants, Ks - evaporation coefficient for bare soils, Ku - crop coefficient for urban areas, Kw - evaporation coefficient for open water. Source: From Allen et al. (1998); Nistor and Porumb-Ghiurco (2015); Nistor and Mîndrescu (2017); Nistor et al. (2017b)

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Results

Climate models indicate a rise in mean air temperature trend for the current century from 21.4 °C (*Fig. 3a*) to 22.8 °C (*Fig. 3b*) in Europe. For both the present and future periods, the maximum values of the mean annual temperature could be found mainly in the South of Europe, on the Mediterranean coast, in the West sides of the continent, especially in the Iberian Peninsula, and in some places from the southeastern sides of Europe, near the Black Sea and Marmara Sea. The Scandinavian Peninsula, North of Great Britain Island, and the mountain ranges (the Alps, Carpathians, Dinaric, Pyrenees, and North of Caucasus) are the territories with low values of temperature in Europe (below 6 °C). Annual precipitation pattern varies from 160 mm to 3539 mm and from 140 mm to 3487 mm in the present respective future periods (*Fig. 4a, b*). The main changes of the spatial distribution of annual precipitation were depicted in the central and eastern sides of the continent. The high values (over 2500 mm) of the annual precipitation are frequently in the West of the Scandinavian Peninsula, Alps Range, Dinaric Range and North of the British Island. The lands with low precipitation are located in the South of the Iberian Peninsula, East and South-East of Europe near the Black Sea, and in the South of the Balkan Peninsula. As a consequence of the temperate regime, the annual ET_0 reaches values over 1200 mm year^{-1} in the present and over 1400 mm year^{-1} in the future period. The highest values of annual ET_0 were depicted in the southern and western sides of Europe while the lowest values of ET_0 were depicted in the northern and northeastern sides of the continent, but also in the mountain areas. *Figure 5* shows the annual spatial distribution of ET_0 in Europe for the current and future set time periods.

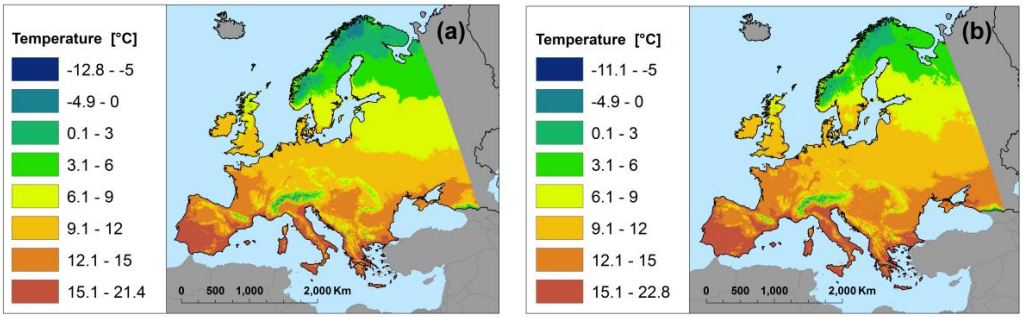


Figure 3. *(a) The average of mean annual air temperature between 2011 and 2040. (b) The average of mean annual air temperature between 2041 and 2070*

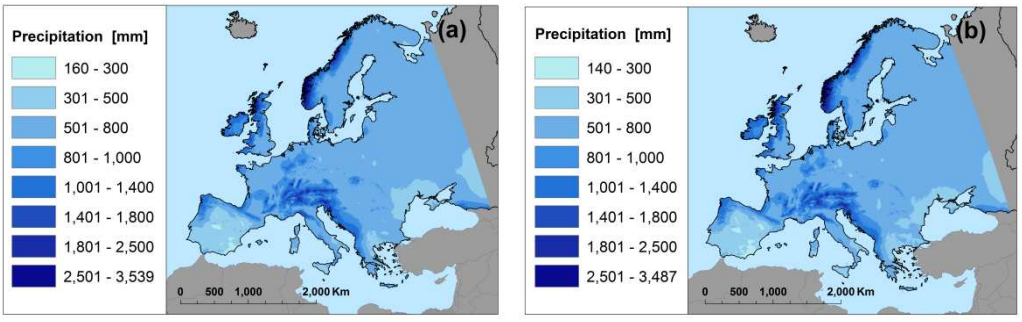


Figure 4. *(a) The average of mean annual precipitation between 2011 and 2040. (b) The average of mean annual precipitation between 2041 and 2070*

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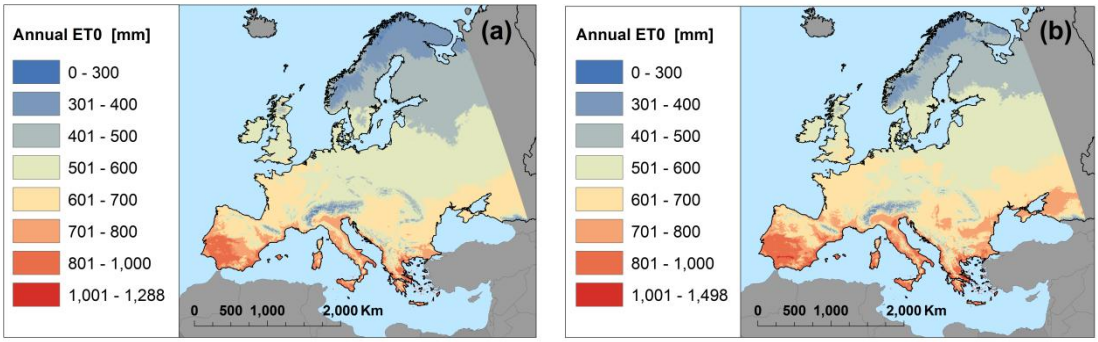


Figure 5. *(a) The average annual ET_0 between 2011 and 2040. (b) The average annual ET_0 between 2041 and 2070*

Based on climate models and land cover data, the seasonal ET_c mapping has been completed and analysed for the periods 2011–2040 and 2041–2070. In both times shifts, the seasonal ET_c calculations illustrate the maximum values during the mid-season, as a consequence of high temperatures and ET_0 values in the summer months but also because of the $K_{c\ mid}$ values, which exceed 1.3 in major parts of the continent.

In the present period (2011–2040) the $ET_{c\ ini}$ values range from 0 to 240 mm, whereas in the future period (2041–2070) the $ET_{c\ ini}$ ranges from 0 to 248 mm (*Fig. 6a, b*). The few changes between the present and the future may be explained by the contribution of climate change to the increase of ET_0 in the spring months (March–May). The high values of $ET_{c\ ini}$ were found in the West of the Iberian Peninsula and in the south-central part of the continent. The lowest values of the $ET_{c\ ini}$ are overlapping to the North and northwestern sides of the Scandinavian Peninsula, Alps Range, Pyrenees and Caucasus Mountains. Low $ET_{c\ ini}$ values were depicted also in the West of the British Islands and in the northwestern sides of Europe.

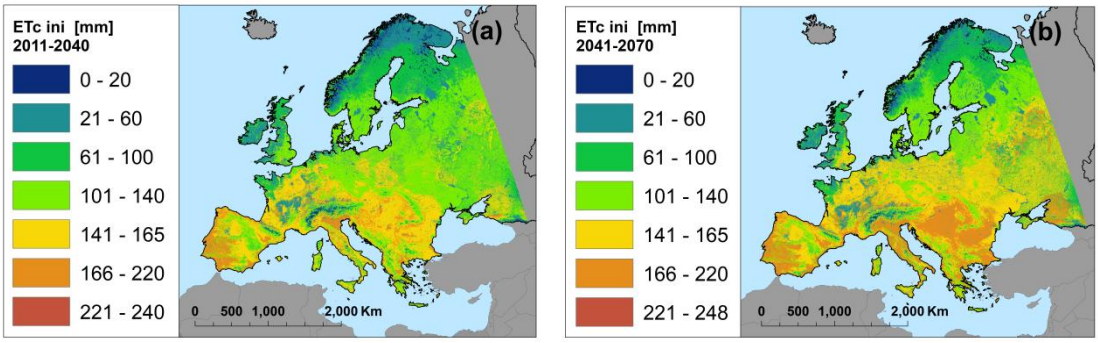


Figure 6. *Spatial distribution of $ET_{c\ ini}$ in Europe. (a) $ET_{c\ ini}$ for the initial stage (2011–2040). (b) $ET_{c\ ini}$ for the initial stage (2041–2070)*

The $ET_{c\ mid}$ ranges in the present period from 0 to 772 mm, whereas in the future period the $ET_{c\ mid}$ values range from 0 to 848 mm. The highest values of the $ET_{c\ mid}$ (over 600 mm) were depicted in the South of the Iberian Peninsula and in the South of the Balkan Peninsula. The more significant aspect of the $ET_{c\ mid}$ spatial distribution in Europe is that in the future period the areas of high values extend in a large territory, especially in the South and central sides of the Iberian Peninsula and also on the northern coast of the

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Aegean Sea. The sides with lower $ET_{c\ mid}$ are found in the elevated mountains of Europe such as the Alps and the Caucasus ranges, in the North and West of the Scandinavian Peninsula, in the central and northern parts of France, and in some sides of northeastern Europe. *Figures 7a* and *7b* illustrate the spatial distribution of $ET_{c\ mid}$ in Europe.

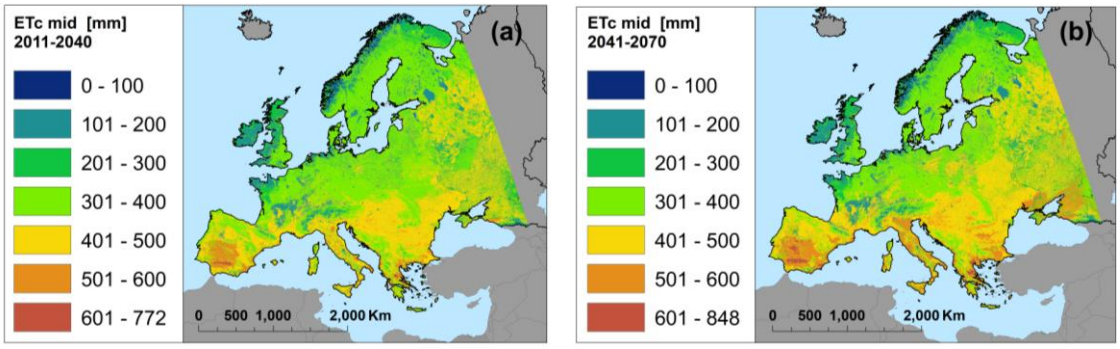


Figure 7. *Spatial distribution of $ET_{c\ mid}$ in Europe. (a) $ET_{c\ mid}$ for the mid-season stage (2011–2040). (b) $ET_{c\ mid}$ for the mid-season stage (2041–2070)*

For the present period the $ET_{c\ end}$ varies from 0 to 334 mm and for the future period the $ET_{c\ end}$ varies from 0 to 359 mm. The high values of $ET_{c\ end}$ are located in the West and South of Europe, mainly in the Iberian and Italian Peninsulas, but several places with high $ET_{c\ end}$ values were depicted in the Balkan Peninsula and Mediterranean Islands such as Sardinia, Sicily, and the Aegean Islands. The lower values of $ET_{c\ end}$ are sparsely located in the centre of the continent, the Northern and Eastern parts of Europe, and on the western sides of the British Islands. No significant differences in the spatial distribution pattern between the present and the future are observed. Spatial distribution of $ET_{c\ end}$ in Europe is shown in *Figure 8a, b*.

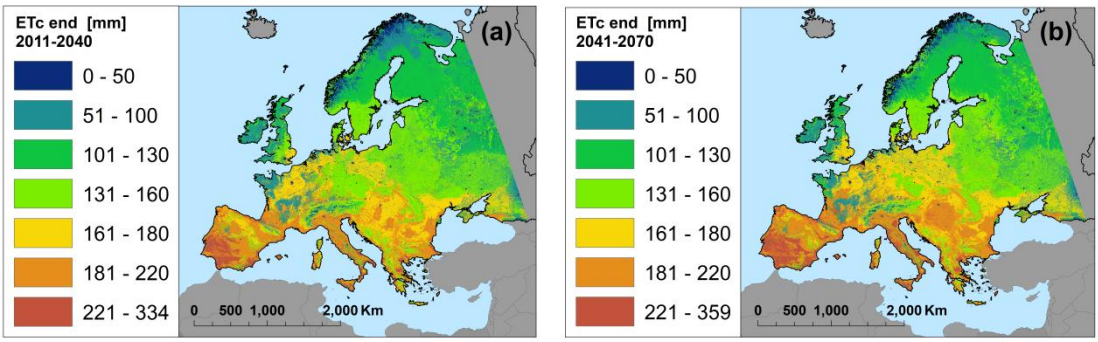


Figure 8. *Spatial distribution of $ET_{c\ end}$ in Europe. (a) $ET_{c\ end}$ for the end stage (2011–2040). (b) $ET_{c\ end}$ for the end stage (2041–2070)*

As we expected, the low values of seasonal ET_c were depicted during the cold season. In the present period the $ET_{c\ cold}$ ranges from 0 to 178 mm, whereas in the future period the $ET_{c\ cold}$ ranges from 0 to 181 mm. The high values of $ET_{c\ cold}$ were found in the center, South, South-East and West of Europe. In contrast to other seasons, the spatial distribution of ET_c overlaps mainly to the elevated areas covered by forest and vegetation that have a significant $K_{c\ cold}$. Interestingly, the maximum values of the ET_c

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$_{cold}$ could be easily identified in the North, North-West, and North-East of the Iberian Peninsula. Significant values of $ET_{c\ cold}$ are also in the South of the Scandinavian Peninsula, North of the Caucasus Mountains, Carpathian Mountains, and sparsely in the Balkan Peninsula (*Fig 9a, b*).

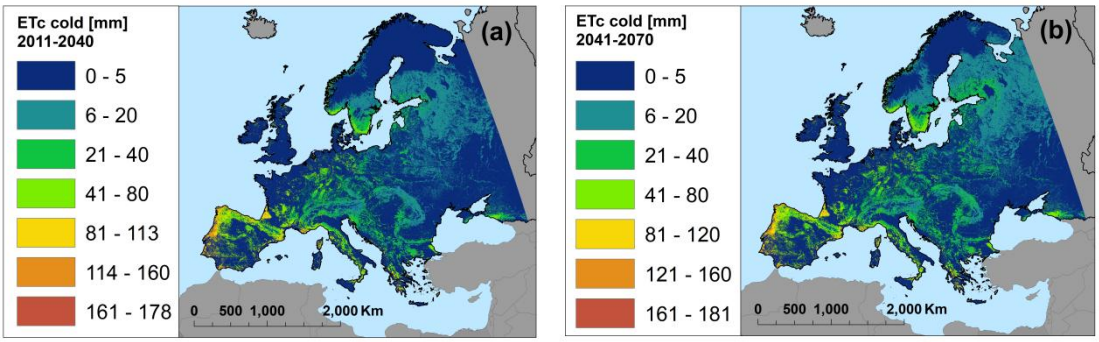


Figure 9. *Spatial distribution of $ET_{c\ cold}$ in Europe. (a) $ET_{c\ cold}$ for the cold stage (2011–2040). (b) $ET_{c\ cold}$ for the cold stage (2041–2070)*

The annual ET_c ranges in the present period from 0 to 1385 mm year^{-1} and in the future period from 0 to 1496 mm year^{-1}. The high values (over 1000 mm) of the annual ET_c may be found in the South, West, and South-East of Europe, mainly on the Mediterranean coast. In the central and eastern sides of the continent, the medium values (400 mm year^{-1} to 800 mm year^{-1}) of the annual ET_c extend, while the low values (below 400 mm year^{-1}) of the annual ET_c could be found in the North of Europe, the Alps and the Caucasus Ranges, the central land of France, and the West of the British Islands. The elevated values of the annual ET_c in the future period occupy more territory than in the present, especially in the Iberian Peninsula, Balkan Peninsula, Pannonian basin, and on northern sides of the Black Sea. *Figures 10a* and *10b* show the spatial distribution of the annual ET_c in Europe.

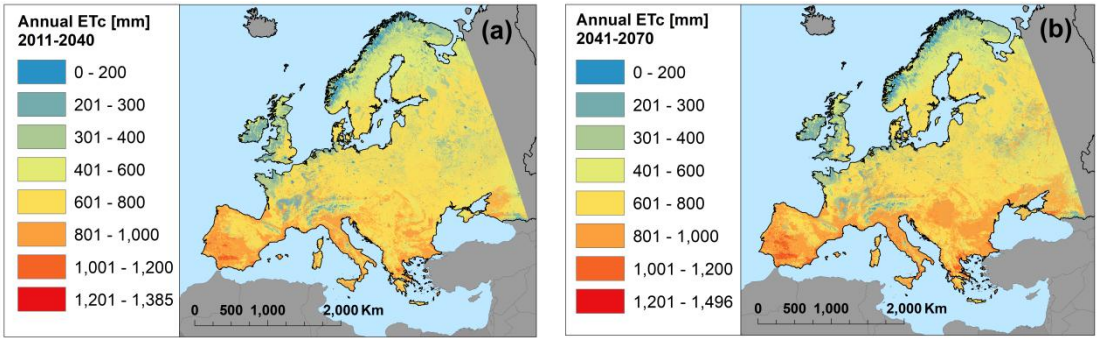


Figure 10. *Spatial distribution of annual ET_c in Europe. (a) Annual ET_c for the period 2011 and 2040. (b) Annual ET_c for the period 2041 and 2070*

Discussion

The main goal of the work was to determinate and complete the spatial distribution of the seasonal and annual ET_c in Europe during the 21st century. Numerous calculations and few presences in the specialty literature of this type of research are

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available. The applied method, based on the land cover and climate models, allow us to bring forward one of the most important climatic parameters at European scale, the seasonal and annual ET_c. Considering the recent climate change (Haeberli, 1999; IPCC, 2001, 2007; Oerlemans, 2005; Nistor, 2014, 2017; Nistor and Petcu, 2015), the first observations about the general climate of Europe have come to affirm the continental warming about 1.4 °C for the future period (2041–2070). Additionally, the precipitation amount is expected to be slightly lower with a minimum of 50 mm $year^{-1}$ and an increase in the annual ET_0 by 200 mm $year^{-1}$ is shown for the 2041–2070 period. These findings are acceptable while we use the climate models based on the RCP4.5 and not RCP8.5, which may provide the worst scenarios.

The climate variables used in the present study are in line with the findings of existing regional models. Čenčur Curk et al. (2014) and Cheval et al. (2017) carried out the mean air temperature, mean annual precipitation, and annual ET_0 for South East Europe. In this part of Europe, Cheval et al. (2017) carried out a maximum difference of 1.4 °C in the mean air temperature between 1991–2020 and 2021–2050 periods, while Čenčur Curk et al. (2014), in their studies about climate change and mitigation of the water resources in South East Europe, illustrate the reduction of the annual precipitation pattern by 60 mm and increases of the ET_0 between the future and present periods. Thus, in the present period Čenčur Curk et al. (2014) indicated values of annual ET_0 up to 957 mm $year^{-1}$, while in the future they have found 1040 mm $year^{-1}$ of annual ET_0. In the present work, the values of annual ET_0 are more appropriate than the annual values of ET_0 carried out by Čenčur Curk et al. (2014).

Best for our contribution to the European researches, up to the present, no studies regarding ET_c were done on a Europe scale due to the complex procedure of monthly ET_0 calculations and K_c integration. Few regional works of ET_c bring forward the effects of climate change on evapotranspiration. Annual values of ET_c obtained in the present paper are comparable to the values obtained by Nistor et al. (2017b) in South East Europe. They determined the annual ET_c in two time shifts (1991–2020 and 2021–2050) in which the maximum values of the annual ET_c registered 1297 mm, respective 1410 mm. More than this, even if Nistor et al. (2017b) have used regionally coupled models (RCMs) for the climate data, the spatial distribution of high values of ET_c were found in the South of the Balkan Peninsula, like in the present paper. Appropriate results of the annual ET_c at European scale are in line with annual ET_c carried out at the regional scale of the Pannonian basin and Carpathian region by Nistor et al. (2017a), respective Nistor et al. (2016). In the first case, the maximum annual values of 1083 mm for the present period (1991–2020) and 1148 mm for the future period (2021–2050) have been calculated for the ET_c in the Pannonian basin, while in the Carpathian region the annual ET_c reached 1019 mm in the period of 1991–2010. At the European scale, in these territories the annual ET_c values indicate 600–1000 mm for the present and 800–1200 mm for the future. Based on the historical data of the 1991–2005 period, Nistor and Porumb-Ghiurco (2015) have found maximum values of annual ET_c around 1118 mm $year^{-1}$ in the Emilia-Romagna region from Italy, while in our study, the annual ET_c for the same region indicates 1000–1200 mm $year^{-1}$.

Between present and future periods, the seasonal ET_c indicates higher difference in the mid-season and end season. Thus, an increase from 772 mm to 842 mm it was calculated during the mid-season for 2011–2040 respective 2041–2070. Regarding the end season, the values of $ET_{c\ end}$ are expected to increase between present and future from 334 mm to 359 mm. These results may have drastic influences on the hydrology of Europe but also

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on the biodiversity of the territory mainly in the summer and early autumn. The negative impact of the current and future climate in Europe on the evapotranspiration is quite alarming, especially for the western and southern sides of Europe. The above-mentioned comparison with the previous surveys in various regions of Europe is a good outcome in agreement with the European map of ET_c. However, our work has some limitations, which are closer to the direct evapotranspiration measurements that can improve the results, but only in the local sites. To generalize the field measurements of each crop type to the whole of Europe is not a suitable method for the fact that we have chosen to integrate the standard values proposed by Allen et al. (1998), Nistor and Porumb-Ghiurco (2015), Nistor (2016), Nistor et al. (2017a). Moreover, these shortcomings do not affect the research because the study was done on a continental scale and the land cover and climate data are represented using high resolutions.

The results of the advanced calculations of the seasonal ET_c related to the four stages show not only an increase in the ET_c amount for all stages but also the widening of the territories with high values. In the initial season, the higher values of $ET_{c\ ini}$ are often found in warm areas with the land covered by broad-leaved forests, cultivated lands, and managed agricultural areas. The herbaceous areas and croplands backed with the high mean air temperature indicate the highest values for the $ET_{c\ mid}$ season such as in the South of the Iberian Peninsula and east-central sides of Greece. During the $ET_{c\ end}$ season, tree cover, needle-leaved forest, deciduous forests and agricultural areas from West, South and South-East sides of Europe represent the main vegetation with high evapotranspiration capacity. The $ET_{c\ cold}$ pattern looks very interesting, both for the present and future periods. The elevated values of the $ET_{c\ cold}$ are located especially in the areas covered by the mixed leaf associated trees, needle-leaved and deciduous forests, and evergreen forests which have the $K_{c\ cold}$ higher than 0.6 and the hilly and mountainous areas. In this season, the agriculture and urban lands indicate very low values (below 20 mm) of the $ET_{c\ cold}$ due to the low temperatures, but in major cases the $ET_{c\ cold}$ is 0 mm due to no value for the $K_{c\ cold}$. In the cold months, due to the negative values of air temperature, the monthly index I of the Thornthwaite formula cannot return the positive values for the respective territories. Corrections of the index I raster data including the "0" values in that parcels was done with the aim to complete the ensemble of 12 months which served as the annual heat index I. These operations were needed because the seasonal and annual ET_0 indicated "no data" in certain areas with low-temperature values, but in reality the ET_0 is null there. Integrating the monthly heat index I and alpha parameter into the ET_0 equation, the seasonal and annual ET_0 and ET_c were found. In broad terms, both annual ET_0 and ET_c have a correlated spatial distribution with the latitude and altitudes; moreover, good correlation exists between West and East, respectively the oceanity and continentality influences. Thus, on the southern and western sides of Europe, at low latitudes and closer to the Atlantic Ocean, the ET_0 and ET_c have high values, whereas on the northern and eastern sides, at high latitudes and close to the Asia continent, the ET_0 and ET_c have lower values. In the central parts of Europe, likely as a strike from West to East, the ET_0 and ET_c register medium values, slightly decreasing over the East. Comparing both maps, the details of the annual ET_c indicates a more realistic situation of the European evapotranspiration because it incorporates the vegetation factor. As we expected for the British Islands, the annual ET_c reaches values between 300 and 500 mm, with decreasing spatial distribution from West to East due to the ocean air mass direction, but also the higher mean air temperature in the eastern areas.

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A significant observation should be made on the annual ET_c in the urban areas and land with water bodies. Due to the low K_c and no values of this coefficient during the cold season for the urban and water body lands, the annual map of ET_c shows evapotranspiration amount below 200 mm year^{-1}.

Conclusions

Overall, the seasonal and annual ET_c in Europe were mapped for the 2011–2070 period, using long-term climate models and detailed CORINE Land Cover data. Variations of seasonal ET_c values between the present and future are found for all four set stages, with significant increase in the mid-season and end season. The most striking seasonal findings refer to the $ET_{c\ mid}$ when the maximum values increase from 772 mm to 848 mm and the distribution of high values extends considerably in the area. Significant changes of the annual ET_c could also be shown between the present and future periods. The annual ET_c values increase from 1385 mm to 1496 mm due to the impact of climate change in the second half of the 21st century.

Using the K_c and climate models, the present study shows a reliable approach to assessing the ET_c at the spatial level even if the crop calendar may have some overlapping times due to the cultivations and complexity of vegetation development in the European continent. Nistor and Porumb-Ghiurco (2015) and Nistor et al. (2017a) illustrated in their studies how the methodology at a regional scale works.

Based on the findings, the impact of climate change indicates a negative aspect in terms of drought and water availability in several areas of Europe. The southern, western, southeastern, and central sides of Europe are faced with the high annual ET_c (over 1000 mm year^{-1}), a fact which may produce problems on the surface and groundwater resources, also a slight desertification may also occur in those areas. Even if few, the important areas with low annual ET_c were found in the Alps Range, Pyrenees Range, Caucasus Range, Scandinavian Peninsula, western and northern sides of the British Islands, centre of France and sparse parts of East Europe. These areas are not drastically affected by global warming and can be a considerable source of water supply. In this sense, our work highlights the spatial variation of evapotranspiration under climate change. The findings could be important keys for the scientists in climatology, hydrology, and agriculture. Moreover, the maps made in this survey represent a tool for urban and environmental planning, water resources and land irrigation strategies, reports and further scenarios of derivate indices regarding the land use policy and climate change mitigation. We strongly recommend to complete more investigations of water availability in the areas where the annual ET_c exceed 1000 mm year^{-1}, especially in the southern, western, southeastern, and central sides of Europe. The investigation of these areas should include groundwater monitoring and vulnerability assessment to climate change. Surface waters quality evaluation through in-situ methods (water sampling from rivers, irrigation channels) in the South of Europe will allow providing sustainable water resources and durable ecosystems. The aquifers located near the coastal areas are supposed to be in danger due to sea level rise and seawater intrusions. For a proper management and sustainable groundwater resources in the coastal areas, we suggest to each region to complete temporal water sample analysis (once two years) regarding the salinity variation and to organize workshops to provide solutions for fresh water sustainability in the regions where the salinity in groundwater increases.

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LAND COVER CHANGE AND ITS INFLUENCE ON THE ASSESSMENT OF THE ECOLOGICAL STABILITY

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Abstract. In this contribution, a simple way of quantification of ecological changes in a territory is proposed. Six degrees of ecological stability (from 0 for an anthropic element to 5 for natural and nature-close environments) and a coefficient of ecological stability (CES) related to land cover have been used to measure local ecological stability. The cadastral territory Báb (near Nitra, Slovakia) was segmented into 1 ha sized squares (1,870) to estimate ecological situation by fuzzy clustering. For each of the three time horizons, well documented time snapshots (distant past, present and possible future) of the model territory, three clusters with negative, neutral and positive ecological characteristics (CES based) were identified. Positive as well as negative changes have been detected and localized in space. Negative (from the past to the present state) and positive (landscape consolidation project based state) landscape development has been observed in the study area. Introduced procedures can help to assess effects of proposed ecological measures in land consolidation projects.
Keywords: *coefficient of ecological stability, land time horizons, multicriterial analysis, clustering, Slovakia*

Introduction

Monitoring of particular land elements, their spatial representation, spatial configuration, and dynamics (Lipský and Kvapil, 2000; Angelovičová et al., 2015) is used when observing landscape changes in time. Investigation of land development from the distant past to the present times is important for ecological analyses leading to proposals of sustainable land utilization. Many factors as climate change (Lovejoy and Hannah, 2005), urbanization (Izakovičová et al., 2017), deforestation (Padonou et al., 2017), water quality deterioration (Dumbrovský et al., 2016; Petrovič et al., 2017), natural disasters (Yu et al., 2010), soil degradation (Van Oost et al., 2000), loss of biodiversity (Verburg et al., 2009) do play a role.

Spatial heterogeneity expressed by the landscape structure is linked to functional properties of land and is determined by natural, semi-natural, and anthropogenic factors, see e.g. Hoang and Denmukhametov (2015). Landscape structure can be described by land use classes in a certain time (e.g. Munteanu et al., 2014). Detection of changes is possible by comparing several time horizons. Every landscape is characterized by two processes: the process of the destruction of the original structure and the consequent development of a new one (e.g. Špulerová et al., 2016).

Slovakia's landscape significantly changed particularly since the 50's of the 20th century when the large-scale farming was introduced (Tarasovičová et al., 2013; Žabenský et al., 2016). For about 50 years, any obstacle (balks, unpaved roads, drainage) was removed that hampered the large-scale land management. A landscape with an average land unit of approximately 250 ha causes increased water and wind erosion (Húska et al., 2017), deterioration of the water source quality (Pekárová and

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Pekár, 1996), low ecological stability and consequent decline in biodiversity (e.g. Bezák et al., 2016), whirlwinds, floods (Urban et al., 2013), and droughts (Tárník, 2017).

Since 1991, land consolidations (LCs) address the landscape issues in Slovakia, Muchová et al. (2017). LCs are considered in many countries (Hartvigsen, 2014, 2016) as unique tools, able to harmonize ownership (Noszczyk and Hernik, 2016; Jin et al., 2016), functionality and ecological measures (Moravcová et al., 2017). Land ownership defragmentation (Kwinta and Gniadek, 2017; Janus and Markuszewska, 2017; Muchová and Jusková, 2017; Muchová, 2017) is a prerequisite for wider regional development of rural areas, which includes improvements to public facilities in compliance with the protection of natural resources (Pasakarnis and Maliene, 2010; Sallaku et al., 2010).

In this contribution, the level of anthropogenic impacts on the landscape is quantified by detection, measuring, spatial localization, evaluation, and visualization of land use / land cover changes. Presented procedures are intended for objective assessment of A) the quality of ecological measures in a territory (proposals and implementations, current and historical situation) and B) for directing decision-making activities in compliance with the environmental protection.

Material and methods

Quantification of anthropogenic impacts on a landscape was processed as follows:

1. Selection of study area: A territory with finished land consolidation project was chosen to allow for assessment of possible future changes in the landscape.
2. Land cover classification: Refined land use classes from Slovak cadaster of real estates have been used as a basis for estimation of ecological stability of particular landscape elements.
3. Use of time horizons: Three well documented time snapshots (distant past, present and possible future) of the study area provided means for determining landscape changes.
4. Determination of local ecological stability: Six degrees of ecological stability and a coefficient of ecological stability have been introduced to measure and visualize the state of the landscape in a given time.
5. Segmentation and clustering: A grid with 1 ha sized squares was used to segment the study area. Similarity of the ecological stability in particular squares was estimated using a fuzzy analysis.

Study area

Cadastral territory Báb, 2,009.09 ha, (*Fig. 1*) is located in Nitra region, Nitra district. Agricultural land covers 83.4% of Báb total area (arable land 76.4%), forests 6.9%, water bodies 2.2%, build-up areas 5.1% and other areas 2.5%.

From the geographical point of view, the territory belongs to a hilly area with the relief formed by medium to mildly steep slopes with significant manifestations of water erosion and floods (*Fig. 2A*). The territory belongs to a dry and warm climatic area with frequent droughts. It is a typical land of intensive agricultural use with top-quality soils. The Báb creek flows through the territory with a small irrigation reservoir (*Fig. 2B*) of 18 ha and a capacity of 600,000 m^3. Flora and fauna are dominated by xerophytic and thermophilic species, especially of the Pannonian origin.

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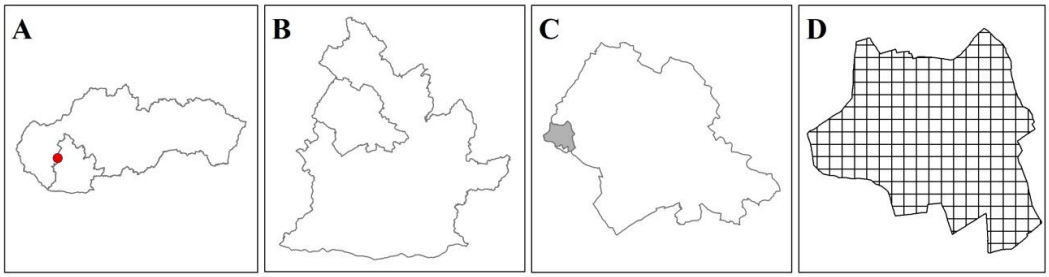


Figure 1. *Báb location in A) Slovakia and region, B) region and district, C) district and cadastral area; D) border of the cadastral area and the grid with 1 ha sized squares*



Figure 2. *A) Báb landscape, B) irrigation reservoir*

Land cover classification

The Slovak cadaster of real estates recognizes 10 land use (LU) classes (arable land, hop garden, vineyard, garden, orchard, permanent grassland, forest, water area, build-up area, and other area). All official maps use this nomenclature. LU classes are not suitable for quantification of ecological stability on a small scale, therefore refined LU elements have been used (Muchová et al., 2009).

Time horizons

Three time horizons, well-documented snapshots of the land use in Báb cadaster in distant past, present and possible future describe the landscape evolution. The first time horizon (TH1, distant past) represents historic land use (*Fig. 3*) in the year 1839 (second military mapping, also called Francis mapping, in the Austro-Hungarian Empire).



Figure 3. *Distant past land use, TH1: A) military mapping II (geoportal.gov.sk), B) vectored map of land use classes, C) land use classes in ha*

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The second time horizon (TH2, present) represents current land use (*Fig. 4*). Detailed mapping of the entire cadastral area was carried out in 2006 by Geotop Nitra and Geodézia Bratislava (with accuracy of 0.14 m). The update from 2006 to 2016 was performed by additional measurements by the authors of this article. Only minor changes (on 1% of the territory) have been recorded.



Figure 4. *Present land use, TH2: A) orthophotomap (Eurosense), B) special purpose topographic mapping with land use classes, C) land use classes in ha*

The third time horizon (TH3, possible future) represents proposed land use (*Fig. 5*). New functional arrangement of the territory after the land consolidation (LC) project with new roads, erosion control, water management, and ecological measures is binding and expected to be implemented in near future.

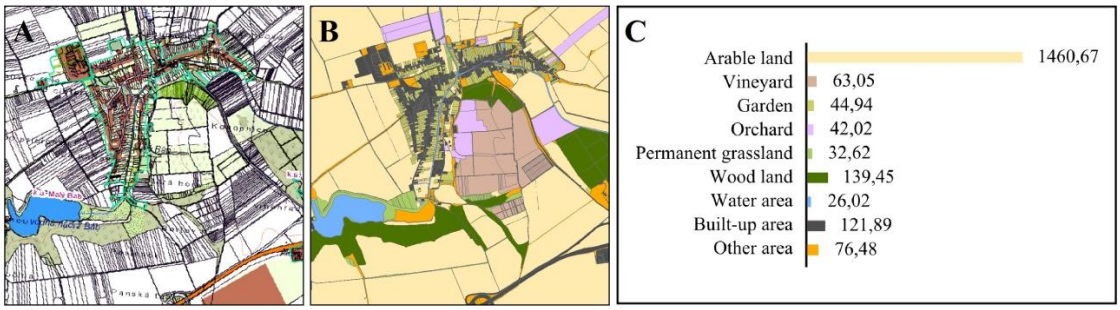


Figure 5. *Possible future land use, TH3: A) cadastral map (https://zbgis.skgeodesy.sk), B) revised cadastral map with land use classes, C) land use classes in ha*

Determination of local ecological stability

The ecosystems are best to be studied through elements of land use, as stated in several sources (e.g. Asner et al., 2004). In general, individual elements (refined land use classes) have a different character and different environmental quality (environmental stability). Each element is assigned a degree of ecological stability from 0 to 5 according to the origin of vegetation (Löw, 1995); 0 for an anthropic element, without vegetation and artificially designed (e.g. built-up areas, paved roads), 1 (e.g. intensively utilized, large-scale blocks of arable land), 2 (e.g. intensively used orchards, vineyards, reclaimed meadows), 3 (e.g. lawn orchards), 4 (e.g. forests, meadows with the predominance of naturally occurring species, natural water areas with semi-natural vegetation) and 5 for natural and nature-close environments (e.g. natural forests, natural

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herbaceous communities, wetlands, peat bogs, watercourses with natural bottom, banks and with characteristic water and coastal flora). Degrees of local ecological stability for past, present and future are depicted in *Figure 6*.

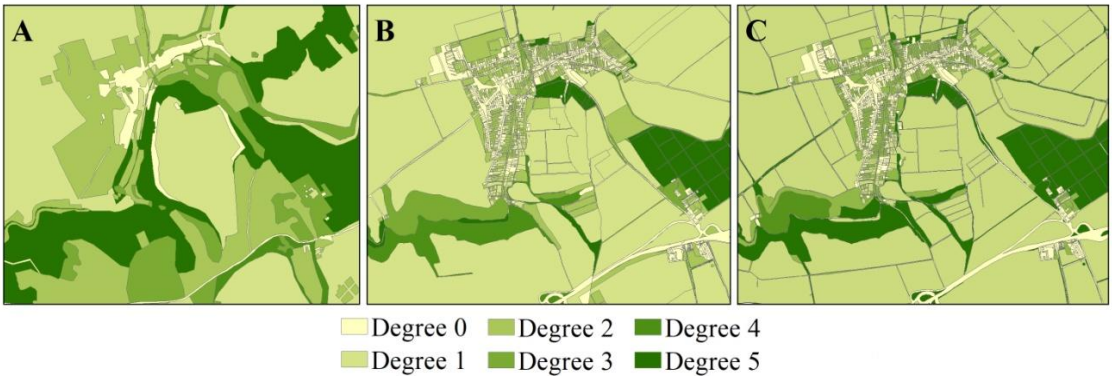


Figure 6. *Degree of ecological stability for A) distant past land use, TH1, B) present land use, TH2, C) possible future land use, TH3*

A coefficient of ecological stability (CES) can be defined (Muchová et al., 2009; Muchová et al., 2016; Kupková, 2001; Streďanský and Šimonides, 1995) as (*Eq. 1*):

$$CES = \frac{P_5 + P_4 + P_3}{P_2 + P_1 + P_0} \quad \text{(Eq.1)}$$

P_i variables (i from 0 to 5) denote the areas of refined land use classes of a given degree of ecological stability. *CES* is < 0.4 for a landscape / area with very low ecological stability, 0.41 – 0.80 low ecological stability, 0.81 – 1.20 stable, > 1.21 high ecological stability.

Segmentation and clustering

A grid with 1 ha sized squares was used to segment (see e.g. Muchová et al., 2015; Stejskalová et al., 2013) the Báb cadaster (*Fig 1D*). Local ecological stability (degrees of ecological stability of land use classes / elements and coefficient of ecological stability) was determined for each segment as described above.

Fuzzy clustering (R Core Team, 2017; Maechler et al., 2017) was applied to determine similarity of individual squares.

Ecological risk was estimated for each cluster using the CES value. Spatial analyses and map sets were processed by the GIS tool ArcMap 10.2 (ESRI, 2017).

Results and discussion

1,870 defined squares were assigned into three clusters (i.e. three for each time horizon) based on the similarity in local ecological stability (*Fig. 7*).

The clusters can be described as follows:

- Cluster characterized as negative contains 945 squares mostly in the degree 1 of ecological stability for the historical land use (TH1), i.e. 50% of the territory. 1,161 squares belong to it in present land use (TH2), also primarily in

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the degree 1 of ecological stability, covering 62% of the territory. 778 squares are members of it in the possible future land use (TH3), mostly degree 1 of stability (41% of the total area).

- Cluster with neutral characteristic contains 592 mostly ecological stability degree 2 squares (31%) for the historical land use (TH1). 361 predominantly stability degree 2 and 3 squares (19%) are assigned to it in present land use (TH2). 599 (32%) mostly ecological stability degree 2 squares are members of it in the possible future land use (TH3).
- Cluster with positive characteristic contains 333 mostly ecological stability degree 5 segments (17%) for the historical land use (TH1), 348 squares (18%) of predominantly stability degree 3 and 5 in present land use (TH2) and 493 mostly ecological stability degree 5 squares (26%) in the possible future land use (TH3).

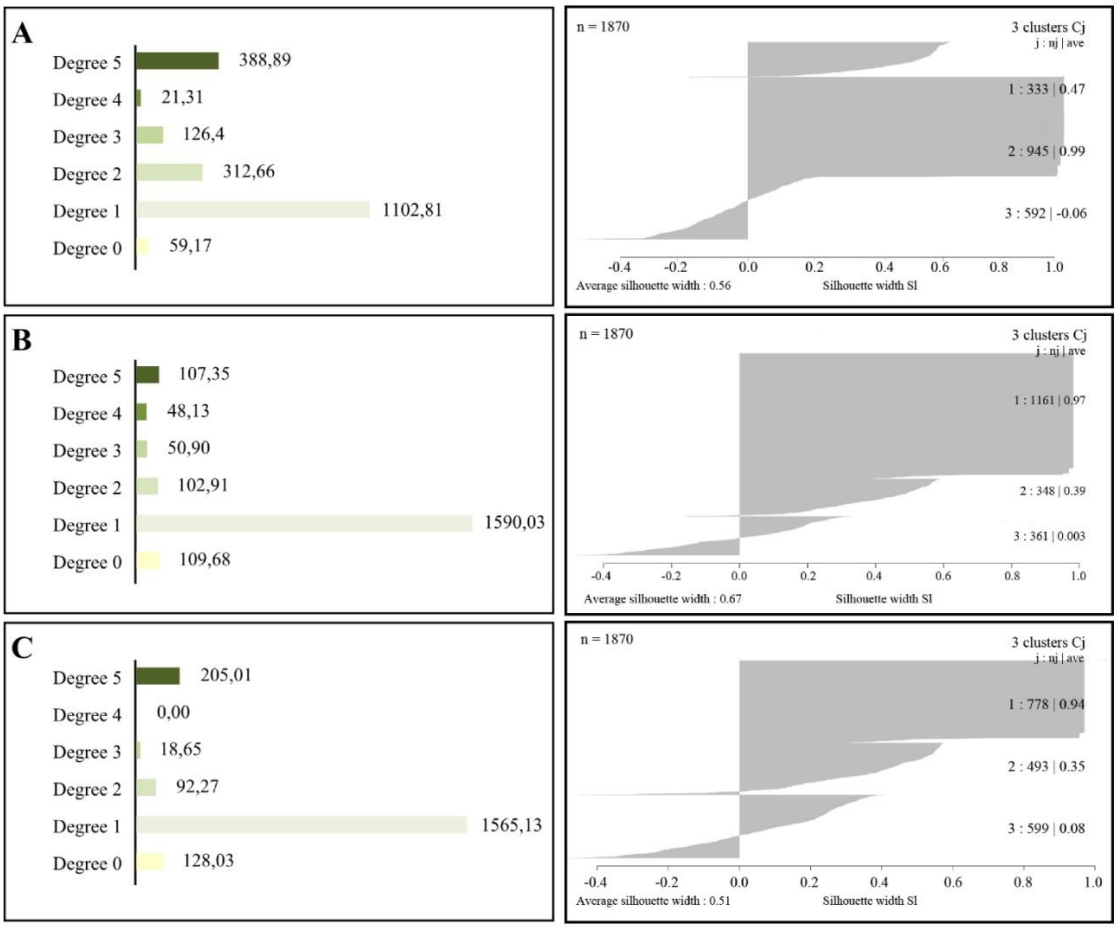


Figure 7. *Local ecological stability for time horizons (in ha) and results of the cluster analysis A) TH1 (past), B) TH2 (present), C) TH3 (future)*

The results of clustering have been visualized by marking individual segments according to cluster membership (*Fig. 8*).

Based on the spatial distribution of ecologically similar areas, the time evolution of the landscape can be observed (*Fig. 9*). 805 squares of land changed between past (TH1) and present (TH2) and 686 between TH2 and possible future (TH3).

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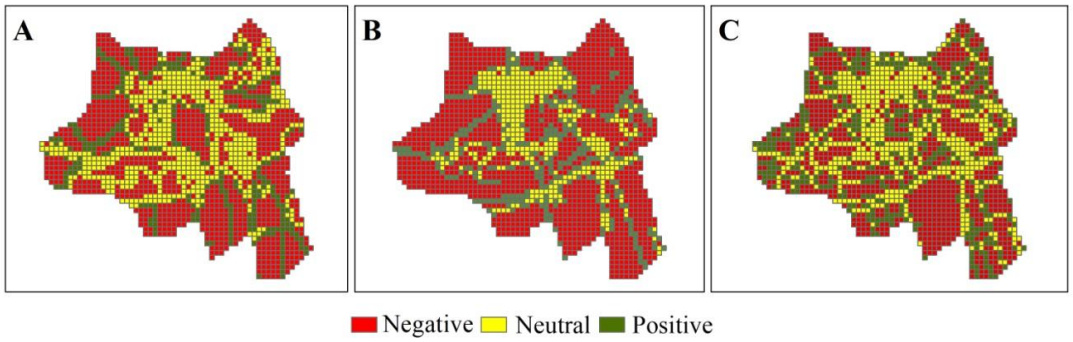


Figure 8. *Cluster membership of individual segments A) historical land use (TH1), B) present land use (TH2), C) possible future land use (TH3)*

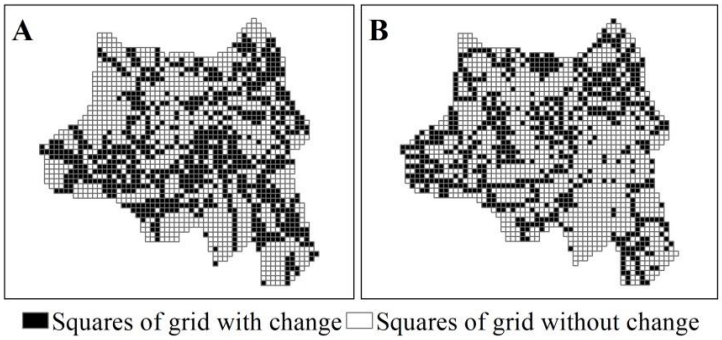


Figure 9. *Local land cover changes: A) between past (TH1) and present (TH2), B) between TH2 and possible future (TH3)*

A desirable (positive) local change can be identified when a segment moves from a lower to a higher category of cluster in time (*Fig. 10A, B*), negative change (*Fig. 10C, D*) means moving in the opposite direction in time.

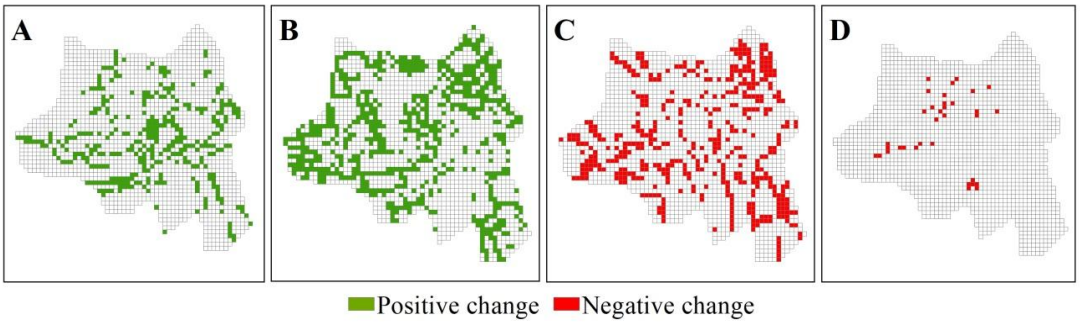


Figure 10. *Positive changes A) from TH1 to TH2, B) from TH2 to TH3. Negative changes C) from TH1 to TH2, D) from TH2 to TH3*

Ecologically negative changes clearly dominate in the landscape evolution from the past (TH1) to the present (TH2) state (463 squares, *Fig. 10C*). Positive changes are rarer (341 segments, *Fig. 10A*) and are connected to construction of road bodies with natural elements increasing the ecological stability such as tree lines, windbreaks or other

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habitat corridors (*Fig. 11*). The negative transformations are related to: ploughing the balks, groves, and small-scale elements of non-forest woody vegetation; construction of new buildings; turning marshlands and water areas into large-scale arable land or removing forest covers (*Fig. 12*).

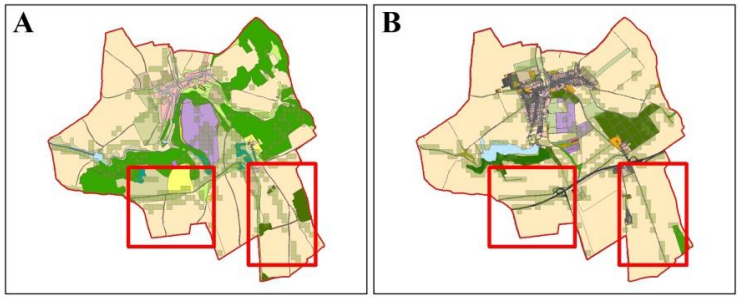


Figure 11. *Unpaved roads with accompanying vegetation as an example of a positive change between the past (A, TH1) and the present (B, TH2) landscape*

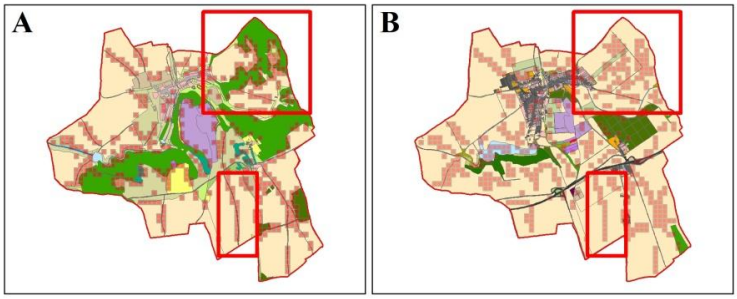


Figure 12. *Examples of negative transformations (e.g. removing forest covers, ploughing balks) between the past (A, TH1) and the present (B, TH2) landscape*

Land consolidation project (possible future land use, TH3) in the Báb area did not neglect ecological aspects as witnessed by the number of segments with positive changes between the present (TH2) state and the proposed (TH3) one (652, *Fig. 10B*). Through the LC project in the model territory, positive ecological changes were proposed on 35% of the total land area. Positive transformations (*Fig. 13*) include e.g. reinforced unpaved roads enriched by accompanying green such as windbreaks, habitat corridors, disruption of slopes by ditches; grassing the valley lines.

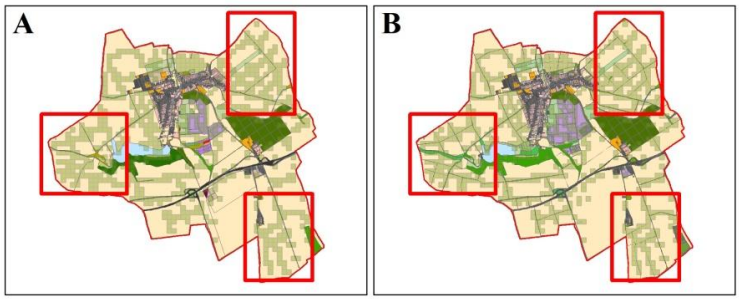


Figure 13. *Localization of some positive changes (protective vegetation mostly) between the present (A, TH2) state and the proposed (B, TH3) one after the LC project*

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Negative changes (between the present state and the proposed one) have been detected on 34 squares only (*Figs. 10D* and *14*).

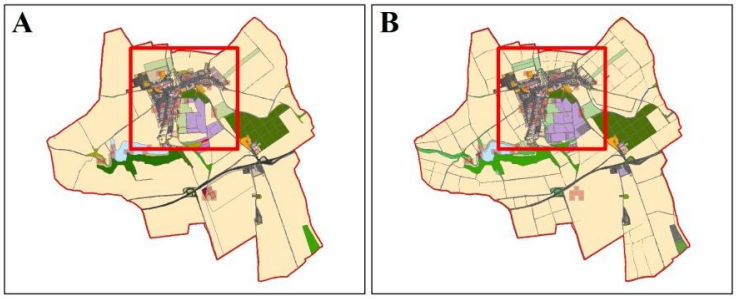


Figure 14. *Localization of main negative changes (construction related) between the present (A, TH2) state and the proposed (B, TH3) one after the LC project*

The changes observed when moving from the historical land structure (TH1) to the present day one (TH2), see *Table 1*, can be characterized by a significant increase in arable land (+45.24%) and built-up areas (+324.95%) accompanied by a decrease in permanent grassland (-97.74%) and forests (-67.18%).

Table 1. *Changes (in ha) between TH1 (past land use) and TH2 (present land use),* ***AL*** *- arable land,* ***V*** *- vineyard,* ***G*** *- garden,* ***O*** *- orchard,* ***PG*** *- permanent grassland,* ***F*** *- forest,* ***WA*** *- water area,* ***BA*** *- build-up area,* ***OA*** *- other area*

TH2/TH1	AL	V	G	O	PG	F	WA	BA	OA	SUM TH2
AL	946.05	32.52	3.05	0.00	241.32	255.53	3.13	5.18	48.09	**1534.87**
V	0.26	31.42	0.30	0.00	1.76	4.38	0.00	0.14	0.56	**38.82**
G	10.82	0.00	8.22	0.00	11.59	6.24	0.45	8.70	2.40	**48.42**
O	17.93	11.69	0.12	0.00	4.49	8.58	0.00	0.00	2.89	**45.70**
PG	1.72	1.44	0.29	0.00	1.47	0.54	0.05	0.25	1.60	**7.36**
F	12.68	0.41	0.03	0.00	7.43	113.18	0.01	0.10	1.47	**135.31**
WA	14.54	0.00	0.94	0.00	13.55	11.49	3.08	0.60	0.13	**44.33**
BA	36.45	0.33	8.66	0.00	31.01	5.91	0.75	15.71	4.19	**103.01**
OA	16.32	4.65	1.79	0.00	12.06	6.46	1.84	1.02	6.63	**50.77**
SUM TH1	**1056.70**	**82.46**	**23.4**	**0.00**	**324.68**	**412.31**	**9.31**	**31.70**	**67.96**	

Land use changes from TH1 to TH2 definitely represent a negative development trend (particularly in relation to the ecological stability of the landscape). Many authors also do consider land interventions of the 50's of the 20th century negative due to the application of radical land reforms (Schmitt, 1993; Mathijs, 1997). According to Kabát and Hagedorn (1997), the primary goal of collectivization was to provide large-scale agricultural production and production increase at the expense of the land. Homogenization of the land and removal of its microstructure are typical features of the socialistic agriculture, see e.g. Bezák and Mitchley (2014). The original mosaic of

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arable land, meadows and pastures, marshlands, balks and groves, and unpaved roads turned into homogeneous areas of arable lands or meadows. Availability of the land both for biota and for humans decreased due to it (Young et al., 2007). According to McDonald (2013), a problem of the land future is also the farmer – their relationship with the land was destroyed during the socialist period by the forced collectivization with the consequent much more inconsiderate behavior of land users towards the environment.

Changes between present land structure TH2 and the possible future one (as proposed in the LC project) TH3 (*Table 2*) include a slight decrease of the arable land areas (-5.00%). Splitting of large-scale land units by green areas can help to limit consequences of natural disasters. Permanent growth of built-up areas (+18.00%) is caused mainly by establishing industrial zones and housing construction, which is typical for those cadastral territories located in close vicinity of district towns (Báb lies near Nitra on a highway). There is an expected increase of green spaces (+444.42%), mostly in the form of protective vegetation – functional vegetation, original and unoriginal non-woody vegetation.

Table 2. *Changes (in ha) between TH2 (present land use) and TH3 (projected future land use),* ***AL*** *- arable land,* ***V*** *- vineyard,* ***G*** *- garden,* ***O*** *- orchard,* ***PG*** *- permanent grassland,* ***F*** *- forest,* ***WA*** *- water area,* ***BA*** *- build-up area,* ***OA*** *- other area*

TH3/TH2	AL	V	G	O	PG	F	WA	BA	OA	SUM TH3
AL	1453.95	0.42	2.19	0.00	0.01	0.10	1.61	1.60	0.79	**1460.67**
V	23.06	36.49	0.00	2.82	0.00	0.00	0.00	0.00	0.68	**63.05**
G	0.04	0.00	44.54	0.03	0.00	0.00	0.02	0.00	0.31	**44.94**
O	0.01	0.00	0.00	41.88	0.00	0.00	0.00	0.00	0.13	**42.02**
PG	15.04	0.01	0.13	0.21	3.39	0.04	9.85	1.77	2.18	**32.62**
F	0.00	0.00	0.00	0.00	0.00	134.24	0.00	0.00	5.21	**139.45**
WA	0.19	0.00	0.00	0.01	0.00	0.00	25.59	0.00	0.23	**26.02**
BA	15.05	1.28	0.65	0.33	0.62	0.07	0.57	97.43	5.89	**121.89**
OA	26.75	0.08	0.95	0.44	3.32	0.87	6.75	2.51	34.81	**76.48**
SUM TH2	**1534.09**	**38.28**	**48.46**	**45.72**	**7.34**	**135.32**	**44.39**	**103.31**	**50.23**	

Since 1991, 427 LC projects and 23 implementations have been started in Slovakia. Correctly conceived LC projects are tackling the landscape issues as a whole. The realization of expected benefits requires interdisciplinary coordination (e.g. Pasakarnis and Maliene, 2010) and compliance with a methodology since the projects are related to areas as regional development, environment, agriculture, infrastructure… The ratio of positive to negative changes in properly designed land consolidation projects, as is the case for the Báb area, objectively shows that a project can bring positive (ecological) landscape interventions and reduce impacts of anthropogenic land interventions. Extensive land consolidations in the Czech Republic (Jusková and Muchová, 2014) with numerous realizations of proposed ecological measures (Moravcová et al., 2017) also provide experiences that point to a positive impact (Kaulich, 2013) of comprehensive transformations of rural landscape. The success in resolving landscape

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issues (including the ecological ones) is so predominant (Sklenička et al., 2009) that comprehensive landscape consolidation projects have priority and are expanding there. Correct quantification (detection, measurement, evaluation, localization, visualization) of impact of proposed measures on ecological stability is evidently possible (as documented above) and can help to ensure the materialization of expected benefits of regulated landscape interventions.

Conclusion

In order to be able to assess the ecological stability of a territory in the course of (anthropogenic) land interventions a correct quantification of the changes is needed. The aim of this paper was to propose a way to do it. Measurement of the ecological stability was based on six degrees of ecological stability and a coefficient of ecological stability (CES) related to land cover / origin of vegetation (from 0 for an anthropic element to 5 for natural and nature-close environments). The study area (cadastral territory Báb near Nitra, Slovakia) was segmented into 1 ha sized squares (1,870) for the evaluation purposes. Similarity of ecological situation was estimated using fuzzy clustering. Three clusters with negative, neutral and positive ecological characteristics (CES based) were identified for each of the three time horizons, well-documented time snapshots (distant past, present and possible future) of the model territory. Positive (segment moves from a lower to a higher category of cluster in time) and negative changes (segment moves from a higher to a lower category of cluster in time) have been detected and localized in space. The cadastral territory of Báb served as an example of both a negative landscape development (from past to the present) as well as positive interventions (proposed future state from the finished landscape consolidation project). The second case documented that properly designed land consolidation projects can bring about significant improvement of ecological stability (on 35% of the total area). Procedures introduced in this contribution can help to quantify effects of proposed ecological measures in land consolidation projects and thus aid in correct decision making.

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EVALUATION OF TRACE ELEMENT SUPPLEMENTATION FOR METHANE PRODUCTION AND ECOTOXICOLOGICAL ASPECTS OF SUGAR BEET PRESSED PULP

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Abstract. The aim of this study was to evaluate the effects of trace element supplementation on semi-continuous anaerobic digestion processes of sugar beet pressed pulp. We also investigated the phytotoxicity of anaerobic sludge via an ecotoxicological test for white mustard (*Sinapis alba*) and spring barley (*Hordeum vulgare* L.). To determine the accumulation of microelements, the spring barley test was followed by microelement analysis of sludge, soil and barley biomass samples. The results show that average values of specific methane yields were 11.0 and 11.7% higher in the supplemented and supplemented plus iron trials compared to the non-supplemented trial, respectively.
In the root elongation test of white mustard, the highest stimulation rate (-59.41%) was observed for the 10-fold dilution in the treatment with trace element and iron supplementation. We observed major stimulation of spring barley biomass production in all trials, with the strongest effect (stimulation of -62.80%) in the trace element supplementation plus iron trial. In sludge samples, cobalt levels increased by 14 and 16 times, nickel by 10 and 14 times, boron by 1.8 and 1.9 times, iron by 1.6 and 2.1 times, respectively, in the supplemented and supplemented + Fe trials. The bioaccumulation factors of different elements varied widely (0.07-110.8).
Keywords: *anaerobic sludge, microelement, phytotoxicity, Sinapis alba, Hordeum vulgare*

Introduction

The use of sugar beet pressed pulp (SBPP) in anaerobic digestion is one way to abrogate sugar production waste (Tukacs-Hájos et al., 2014). The obtained renewable energy can be applied for heat or electricity production, but there is a secondary derivative, such as fermented sludge, which has a great potential in the application on agricultural and forestry fields for fertilisation (Govasmark et al., 2011; Demirel et al., 2013; Dong et al., 2013). By this complex use we can nearly reach a waste-free technology.

The digester effluents could provide the essential nutrients for plant growth when used as organic fertilisers (Achiba et al., 2010). Since sludges are complex mixtures of

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different substances, speciation of toxic materials would be expected to increase or reduce toxicity (Gómez et al., 2001; García-Delgado et al., 2007; Tu et al., 2012; Li et al., 2010). Effluents used for irrigation water may cause a risk of decreasing crop production due to high salinity (Ullman and Mukhtar, 2007).

Optimal anaerobic digestion can be reached by addition of different trace elements based on to microbial requirements (Zhang and Jang, 2012; Qiang et al., 2013). The most commonly studied elements are iron, cobalt and nickel (Choong et al., 2016). Apart from the positive impact on methane production, the accumulated heavy metals in the sludge could have adverse effects on the soil ecosystem (Walter et al., 2006; Salazar et al., 2012). This negative effect can be prevalent for soil flora and fauna (Carbonell et al., 2009). The most critical point of view is the availability of microelements by plants from the soil, which could be affected by anaerobic digestion and lower mobility of metals from digested slurry (Marcato et al., 2009; Pagnanelli et al., 2009). Zhu et al. (2014) state that Cu, Zn, Mn, Ni and Cd may be grouped as toxic and active components in sludge. The different trace elements can appear in food chain systems through cultivated plants from contaminated soil (Wahsha et al., 2014; Le et al., 2015).

Information on the phytoavailability changes with trace element supplementation of anaerobic sludge are scarce. There are two main methods to assess bioavailability: chemical measurements in the soil matrix and biological measurements using organisms exposed to soil (Walsh et al., 1991; Harmsen, 2007). To investigate the injurious effects, different acute and chronic ecotoxicological tests are applied in practise (Rosa et al., 2007; Roig et al., 2012; Young et al., 2012). *Lactuca sativa* seed germination and root elongation inhibition tests are used for assessing the toxicity reduction from anaerobic digestion based on measuring main chemical operational parameters (Young et al., 2012). Hybská et al. (2017) examined the ecotoxicological properties of granulated slag from the pilot production of gray iron with red mud addition and concrete composites with the application of this slag by testing highly-cultivated plant *Sinapis alba*. Their results confirmed that the application of the hazardous waste in the production of gray iron produced waste slag with no toxic properties.

The main aims of this study were to investigate the improved methane yield by supplementation of trace elements and to evaluate the ecotoxicological effects on fermented sludge in the application of nutrient replacement for soils.

The biological approach consisted of microelement uptake assessment in growth chamber experiments with spring barley.

Materials and methods

Anaerobic digestion

Biogas reactors

The anaerobic digesters were 1-L working volume bottles (2.5 L capacity threaded brown bottles, Merck, Germany). The digesters were incubated in a water bath (Memmert WNB 14 Basic, Memmert GmbH. & Co.) at a constant temperature of 38°C. The anaerobic digester inoculum was active, adapted to sugar beet pressed pulp substrate (HRT 20 days) obtained from a biogas plant (Sugar factory, Kaposvár, Hungary). The reactors were manually mixed three times per day and the produced biogas was collected by Tedlar® gas sampling bags; volume was measured with a

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Hamilton gas-tight syringe (Sigma Aldrich Co). Biogas and methane yields were recalculated to the standard conditions for pressure and temperature.

Experimental design

Sugar beet pressed pulp as substrate was applied in our semi-continuous lab-scale experiments. The organic loading rate was raised to 5.2 $gVSL^{-1}d^{-1}$ by day 19; subsequently, because of the high tVFA concentration in all trials (up to 10 000 mg L^{-1}), feeding was stopped for two days. After this, a moderate loading rate was performed from day 22 to day 44, with a maximum of 3.8 $gVSL^{-1}d^{-1}$; subsequently, the organic loading rate (OLR) remained stable until day 81.

Three parallel reactors were run in all cases of non-supplemented (without trace element supplementation), supplemented (2 $\mu L\ L^{-1}d^{-1}$ of trace element solution) and supplemented + Fe (2 $\mu L\ L^{-1}d^{-1}$ of trace element solution + 82 $\mu LL^{-1}d^{-1}$ of Fe(III)-chloride 40% aqueous solution – technical grade). The application of high doses of iron is explained by its detoxification effect on sulphide inhibition, as described by (Gonzalez-Silva et al., 2009).

The trace element supplement solution contained 1 625 mg of zinc, 13 640 mg of manganese, 93 mg of boron, 20 000 mg of nickel, 600 mg of copper, 50 000 mg of cobalt, 228 mg of molybdenum and 113 mg of selenium in a special organic complex form per kg of solution (42.2% TS). Dosing of microelements was based on our former experiment (Tukacs-Hájos et al., 2014).

Analytical methods

Sludge samples (10 mL) were taken for chemical analysis before feeding. Samples were centrifuged for 10 min at 3 420 RCF (EBA 21, A. Hettich Co, Germany). From the resulting supernatant, 5 mL were used for determination of titrated VFA (tVFA) levels (every 5th day), using a potentiometric pH meter (EuTech PC 510, Thermo Fisher Scientific), and calculated in acetic acid equivalents (Rétfalvi et al., 2011).

The components of biogas were analysed using an Ecoprobe 5-IR (RS Dynamics Ltd, Czech Republic).

Determination of COD was carried out according to the Hungarian standard protocol (MSZ ISO 6060). Determination of ammonium and total phosphorus was carried out according to the Hungarian standard protocols MSZ ISO 7150-1 and MSZ 488/18-77, respectively (Rétfalvi et al., 2015).

Ecotoxicological tests

Preparation of aqueous extracts

The quantities of original sludges were weighted, corresponding to 100 ± 0.1 g of dry matter, and 1 L of demineralised water was added. Subsequently, the samples were shaken for 6 h. The extracts were then left for 18 h to sediment and after sedimentation, solid phases were separated by filtration through a paper filter with a pore size of 3 ± 2 µm.

Root elongation test with white mustard

The acute toxicity test was applied according to the Slovakian standard of STN 83 8303:1999. The tested organism was white mustard (*Sinapis alba*), a member of the *Brassicaceae* family. The sorted white mustard seeds were ochre-yellow, with a size of

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1.5 to 2.5 mm, and germination power was higher than 99%. Thirty seeds were placed uniformly into Petri dishes (diameter of 120 to 140 mm) and 10 mL of the prepared aqueous extracts were added (*Table 1*).

Three parallel tests were applied in all dilution rates (dilution of 10, 50, 100 and 200 x) of non-supplemented, supplemented and supplemented + Fe sludges, respectively.

As diluent, water stock solutions of salts were prepared and also used for the control. The pH of the diluent water varied around 7.6-8.0.

Table 1. *Test conditions of white mustard root growth inhibition*

Testing organism	*Sinapis alba*, ochre yellow, size 1.5–2 mm, germination > 99%, 30 seeds in Petri dish, 10 mL sample
Temperature	20 ± 1°C, incubator TS 606 CZ/2-Var (WTW, Germany)
Control	Reconstituted water
Exposition period	72 hours
Monitored response	Root growth inhibition compared to control, IC

The basis of inhibition/stimulation effect on root growth was the average length of root determined in the individual concentrations of sludge in comparison with the average root length in the control.

The inhibition–stimulation effect (IC%) on root growth was calculated using the following equation:

$$IC\% = \frac{L_k - L_v}{L_k}.100,$$

where
L_v is the average length of root in the tested concentration of aqueous sludges in cm,
L_k is the average length of root in the control in cm.

Spring barley

The effect of microelement accumulation on terrestrial flora was tested using the Slovakian standard STN EN 14735:2006-03 (83 8300). The static acute toxicity test was designed for investigation of spring barley (*Hordeum vulgare* L.) biomass in a growth chamber experiment; the germination power of seeds was higher than 97% (*Table 2*).

Table 2. *Conditions of spring barley static acute toxicity test*

Testing organism	*Hordeum vulgare* L., germination > 97%, six seeds in pots, artificial soil mixture
Temperature	22 ± 1°C
Humidity	28-30%, HH2 Moisture Meter (Delta-T Devices of Cambridge, England)
Control	Distilled water
Exposition period	21 days
Monitored response	Grown biomass, trace element accumulation

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The experiment was carried out in pots filled with 500 g of artificial soil mixture of 10% peat, 20% kaolin clay and 70% industrial quartz sand. The soil was irrigated with aqueous extracts and control samples were watered with distilled water; subsequently, six spring barley seeds were planted into each pot.

The controlled conditions were: constant temperature of 22 °C and constant humidity of 28-30%, which was ensured by daily measurement with a HH2 Moisture Meter (Delta-T Devices of Cambridge, England) hygrometer.

After an incubation time of 21 days, the grown biomass was weighted for the inhibition-stimulation test and dried for trace element analysis.

Trace element analysis

Air-dried soil and sludge samples were ground, sieved through a 2-mm sieve, dried at 105 °C and ground in a mortar. The plant samples were also dried at 105 °C and ground in a mortar. About 1 g of each sample was measured in a Teflon bomb and we added 5 mL cc HNO_3 and 2 mL H_2O_2 (30% m/m). During the digestion period of 3 h, the bombs were kept in an oven at 110 °C. After filtration, the supernatants were acidified with cc HNO3 and filtrated again. Subsequently, 50 ml of supernatant were evaporated, followed by adding 1 ml cc HNO_3 and washing into a 25-ml volumetric flask. Elemental analysis was carried out by ICP-OES (iCAP 6300 Duo ICP-OES, Thermo Fisher Scientific Inc, Waltham, MA USA).

Statistical analysis

The software package STATISTICA 10 (Version 10, StatSoft, Tulsa, OK, USA) was used for performing (analysis of variance) and single factor dispersion analysis to evaluate the results of the ecotoxicological tests. Graphical presentation of ANOVA and Duncan's test results was performed using 95% confidence intervals for average immobilisation and inhibition values for individual samples (Hybská et al., 2017).

Results and discussion

Methane production

The effect of microelement supplementation was examined via methane production (*Fig. 1*) The average values of specific methane yield (SMY) (403.8 ± 4.51^{a} mL CH_4 gVS^{-1} in case of non-supplemented, 448.3 ± 9.91^{b} mL CH_4 gVS^{-1} in case of supplemented and 451.1 ± 9.16^{b} mL CH_4 gVS^{-1} in case of supplemented + Fe trial, different letters indicate significant difference at $P \leq 0.05$) represent the 11.0% and 11.7% benefit effect of supplementations. This observation is in agreement with the findings of Wall et al. (2014). They reported an increase in SMY in the mono-digestion of grass-silage by 12% to 404 L CH_4 kg^{-1} VS by the effects of Co, Fe and Ni addition. The specific methane yields were balanced during the uploading stage, with average values being 285.2, 317.6 and 328.9 mL CH_4 g VS^{-1} for the non-supplemented, supplemented and supplemented + Fe trial, respectively (*Fig. 1*). Values of the average SMY values of supplemented and supplemented + Fe compared to non-supplemented increased by 12.6 and 23.0% during the drastic overloading period (day 12-19). The most significant effect of microelement supplementation on SMY was recognised during the reloading stage. In the first seven days of this period, SMY values increased by 22.2 and 32.3% and then decreased by 6.5 and 29.3% in the supplemented

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fermenters. During the stable run at moderate OLR levels, the effects of microelement supplementation on SMY rates were lower (5.8 and 1.8%, respectively).

The methane content of biogas was not significantly influenced by microelement supplementation. Average methane values and ranges of daily biogas production were 53.95 (51.68-56.74%), 54.19 (51.66-58.32%) and 53.49% (50.36-58.69%) for the non-supplemented, supplemented and supplemented + Fe trial, respectively.

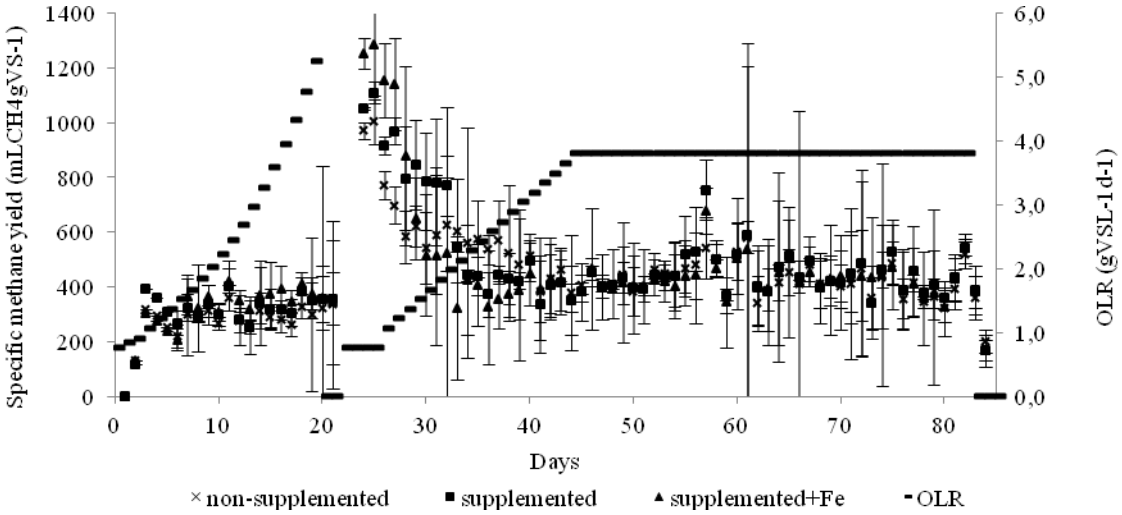


Figure 1. *Alteration of specific methane yields of non-supplemented, supplemented and supplemented + Fe trials during the experiment*

The initial tVFA level of the sludge was 2 440 mg L^{-1}. By increasing the OLR rate, the tVFA levels raised, but remained under 4 500 mg L^{-1} during the first 1 days of the experiment in all cases. On day 20 at 5.2 g VSL^{-1} d^{-1}, OLR average and standard deviation of tVFA levels increased to 12 496 ± 1 452 mg L^{-1}, 11 421 ± 917 mg L^{-1}, 9 943 ± 1 211 mg L^{-1} in the non-supplemented, supplemented and supplemented + Fe trial, respectively. Based on the effect of interrupted OLR, acid levels decreased by 12.9, 21.2 and 17.6%, respectively, thus moderate uploading (constant 0.76 $gVSL^{-1}$ d^{-1} for four days, then increasing up to 3.80 g $VSL^{-1}d^{-1}$ in the 19-day period) was started at day 22. Before reaching a stable run period, tVFA levels decreased rapidly by 58.1, 69.4 and 70.3%, respectively, by day 30 of the experiment. Until the end of the experiment, tVFA concentrations remained at low levels of 2 500-3 000 mg L^{-1} in all cases. Similar effects of microelement supplementation were described in the study of Qiang et al. (2013), who found that at 6.3 kg m^{-3} d^{-1} OLR level of high-solid food waste, the addition of 10 mg L^{-1} Fe^{2+}, 1 mg L^{-1} Co^{2+} and 1 mg L^{-1} Ni^{2+}, approximately every 45 days, resulted in efficient methane fermentation. A comparison of our microelement supplementation level with the above-mentioned date is not relevant due to the different bioavailability of the chelate-form and inorganic ions.

The most relevant chemical parameters (COD, soluble NH_4^+ and total P) of the sludges were determined every two weeks during the experiments; no significant difference was found between the groups. Average values of sludge COD, NH_4^+ and total P used for ecotoxicological experiments were 4 920 ± 173 mg L^{-1}, 1 457 ± 106 mg L^{-1} and 40 ± 4 mg L^{-1}; 5 104 ± 147 mg L^{-1}, 1 422 ± 93 mg L^{-1} and 36 ± 4 mg L^{-1}; 5 157

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± 214 mg L^{-1}, 1 349 ± 71 mg L^{-1} and 35 ± 2 mg L^{-1} in the non-supplemented, supplemented and supplemented + Fe trials, respectively.

Zhang and Jahng (2012) reported the essential role of iron in the long-term anaerobic digestion of food waste. They found that the operational parameters (CH_4 yield, pH value, tVFA concentration) of anaerobic digestion of food waste remained stable in a year-long experiment in the case of microelement (Co, Mo, Ni, Fe) supplementation. Contrary to our results, they did not find increasing SMY levels with microelement supplementation.

Ecotoxicological tests

Root elongation test with white mustard

A decreased inhibitory effect could be determined in the non-supplemented treatment with increasing dilution rates (*Fig. 2*). The highest stimulating rate (-59.41%) was found for a 10-fold dilution in the supplemented + Fe trial. Iron completion had no significant effect on IC% values.

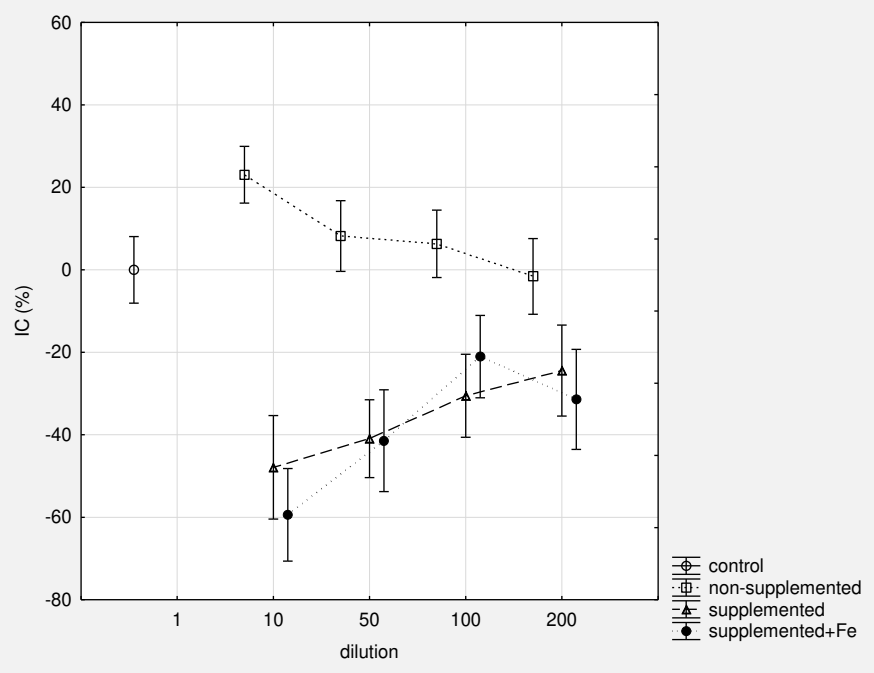


Figure 2. *Effects of dilution rates of anaerobic sludge on root growth of white mustard (0-100 IC%: inhibition, 0-100 IC%: stimulation)*

The preliminary test was negative as root growth inhibition was less than 30% and stimulation was lower than 75% compared to the control; therefore, further tests were not necessary (STN 83 8303:1999).

To correct the two-sample t-test, which, if used in more couples, would produce incorrect results, Duncan´s probability of equality test was used. Based on the results in *Table 3,* the difference between control and non-supplemented (50 x dilutions) was 28.7%, while the difference between control and non-supplemented (100 x dilution) was 38.2% and that between control and non-supplemented (100 x dilution) was 82.6%. All other dilution combinations and samples were significantly different from the control.

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Table 3. *Confidence levels for Duncan´s test - P-value for Duncan´s test and for the root growth inhibition test in Sinapis alba*

Sample		Control	Non-suppl.	Non-suppl.	Non-suppl.	Non-suppl.	Suppl.	Suppl.	Suppl.	Suppl.	Suppl.+Fe	Suppl.+Fe	Suppl.+Fe	Suppl.+Fe
	Dilution	1	10	50	100	200	10	50	100	200	10	50	100	200
		0	23.07	8.196	6.296	-1.583	-30.53	-24.41	-40.94	-47.87	-59.41	-41.44	-21.03	-31.41
Control	1		0.002	0.287	0.382	0.826	0.000	0.001	0.000	0.000	0.000	0.000	0.005	0.000
Non-suppl.	10	0.002		0.039	0.026	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Non-suppl.	50	0.287	0.039		0.792	0.221	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Non-suppl.	100	0.382	0.026	0.792		0.306	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Non-suppl.	200	0.826	0.001	0.221	0.306		0.000	0.002	0.000	0.000	0.000	0.000	0.007	0.000
Suppl.	10	0.000	0.000	0.000	0.000	0.000		0.396	0.174	0.030	0.000	0.171	0.216	0.903
Suppl.	50	0.001	0.000	0.000	0.000	0.002	0.396		0.034	0.003	0.000	0.033	0.639	0.364
Suppl.	100	0.000	0.000	0.000	0.000	0.000	0.174	0.034		0.368	0.017	0.946	0.012	0.186
Suppl.	200	0.000	0.000	0.000	0.000	0.000	0.030	0.003	0.368		0.109	0.372	0.001	0.035
Suppl. + Fe	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.109		0.017	0.000	0.000
Suppl. + Fe	50	0.000	0.000	0.000	0.000	0.000	0.171	0.033	0.946	0.372	0.017		0.011	0.191
Suppl. + Fe	100	0.005	0.000	0.000	0.000	0.007	0.216	0.639	0.012	0.001	0.000	0.011		0.193
Suppl. + Fe	200	0.000	0.000	0.000	0.000	0.000	0.903	0.364	0.186	0.035	0.000	0.191	0.193	

Spring barley

The effect of trace element supplementation of anaerobic sludge was studied using the static acute toxicity test for spring barley. There was no inhibition on grown biomass, and a major stimulation on biomass production was observed in all trials. The stimulation ratio increased with supplementation; the strongest effect (stimulation of -62.80%) was determined in the case of supplementation with iron, as shown in *Table 4*. Iron is a part of the active groups of enzymes and involved in many physiological processes. The most important process is the formation of chlorophyll, which is a photosynthetically active dye. In our study, supplementation affected net assimilation rate and thus the amount of biomass produced. Regarding to the results of biomass production based on Duncan´s probability test, equality of samples different significantly from the control and between the pairs of samples, respectively.

Table 4. *Stimulation effects of anaerobic sludge on biomass production of spring barley (0-100 IC%: inhibition, 0-100 IC%: stimulation)*

Sample	Stimulation (%)			
	Average	Standard deviation	-95.00%	95.00%
Non-supplemented	-10.17	0.12	9.65	10.68
Supplemented	-26.37	0.27	25.22	27.51
Supplemented + Fe	-62.80	0.76	59.54	66.06

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Our results partly contradict by the findings of Roig et al. (2012), who did not observe a correlation between heavy metal contamination and ecotoxicology of different sewage sludges due to the low metal content. They suggested that organic pollutants, such as phenols, have a higher significance than inorganic compounds.

Trace element analysis

The effect of microelement-enriched anaerobic sludge on element accumulation during the spring barley test was followed by microelement analysis of sludge, soil and barley biomass samples (*Table 5*). Application of microelement supplementation during anaerobic digestion enhanced element levels in the sludge (*Table 5*). Cobalt levels increased by 14 and 16 times, nickel by 10 and 14 times, boron by 1.8 and 1.9 times, iron by 1.6 and 2.1 times higher in the supplemented and supplemented + Fe trial, respectively. Despite zinc supplementation with iron addition, the concentration of zinc decreased by 33%. Interpretation of this phenomenon has not revealed yet. The metal contents of the sludges were significantly lower than those in the study by Roig et al. (2012), considering 10-100 times higher metal concentrations in sewage sludge.

Table 5. *Trace element concentrations of sludge, artificial soil and spring barley samples (different letters indicate significant difference at $P \leq 0.05$)*

Trace element concentrations of sludge samples															
Mg kg TS^{-1}		Al	B	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Ni	Pb	Sr	Zn
Non-supp	**Average**	**0.26**	**0.61^{a}**	**0.14^{a}**	**n.d.**	**0.03^{a}**	**n.d.**	**0.06^{a}**	**1.95^{a}**	**0.01^{a}**	**0.37^{a}**	**0.03^{a}**	**0.01^{a}**	**0.64^{a}**	**0.45^{a}**
	SD	0.030	0.090	0.010	-	0.002	-	0.02	0.18	0.000	0.07	0.01	0.002	0.07	0.13
Supp	**Average**	**n.d.**	**1.13^{b}**	**0.10^{a}**	**n.d.**	**0.50^{b}**	**0.01**	**0.07^{a}**	**3.16^{ab}**	**0.04^{a}**	**0.30^{a}**	**0.42^{b}**	**0.01^{a}**	**0.60^{a}**	**0.41^{a}**
	SD	-	0.040	0.010	-	0.110	0.002	0.01	0.46	0.01	0.02	0.08	0.002	0.02	0.06
Supp + Fe	**Average**	**n.d.**	**1.15^{b}**	**0.08^{a}**	**n.d.**	**0.42^{b}**	**0.01**	**0.05^{a}**	**4.09^{b}**	**0.04^{a}**	**0.41^{a}**	**0.32^{b}**	**0.01^{a}**	**0.57^{a}**	**0.30^{a}**
	SD	-	0.040	0.020	-	0.080	0.003	0.01	0.38	0.005	0.04	0.04	0.001	0.20	0.08
Trace element concentrations of artificial soil samples															
Mg kg TS^{-1}		Al	B	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Ni	Pb	Sr	Zn
Control	**Average**	**2688^{a}**	**0.94^{a}**	**10.8^{a}**	**0.03^{a}**	**1.21^{a}**	**4.97^{a}**	**3.80^{a}**	**3304^{a}**	**4.88^{a}**	**86.5^{a}**	**3.69^{a}**	**50.0^{a}**	**30.8^{a}**	**29.8^{a}**
	SD	189.4	0.099	0.505	0.007	0.145	0.425	0.245	448.1	0.020	8.655	0.193	7.566	4.701	5.940
Non-supp	**Average**	**3031^{a}**	**1.49^{a}**	**24.0^{b}**	**0.02^{a}**	**1.96^{a}**	**5.19^{a}**	**3.31^{a}**	**3253^{a}**	**4.86^{a}**	**84.3^{a}**	**4.00^{a}**	**24.6^{a}**	**31.5^{a}**	**28.3^{a}**
	SD	397.2	0.223	1.310	0.009	0.296	0.667	0.463	51.13	0.086	5.579	0.320	9.851	6.496	5.561
Supp	**Average**	**2533^{a}**	**3.87^{b}**	**25.4^{b}**	**0.01^{a}**	**19.2^{b}**	**4.17^{a}**	**4.81^{b}**	**2971^{a}**	**4.86^{a}**	**95.1^{a}**	**10.5^{b}**	**33.0^{a}**	**31.4^{a}**	**30.3^{a}**
	SD	109.2	0.338	1.615	0.000	0.784	0.554	0.098	150.3	0.011	2.980	1.386	15.31	3.032	2.044
Supp + Fe	**Average**	**3103^{a}**	**3.17^{b}**	**35.9^{c}**	**0.03^{a}**	**13.9^{c}**	**4.52^{a}**	**4.69^{b}**	**3484^{a}**	**4.71^{a}**	**98.9^{a}**	**9.11^{b}**	**39.9^{a}**	**28.3^{a}**	**30.7^{a}**
	SD	775.0	0.519	4.367	0.009	1.925	0.404	0.043	110.5	0.099	5.050	0.50	6.507	1.064	5.464
Trace element concentrations of spring barley samples															
Mg kg TS^{-1}		Al	B	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Ni	Pb	Sr	Zn
Control	**Average**	**408^{a}**	**47.7^{a}**	**25.4^{a}**	**0.00^{a}**	**0.16^{a}**	**1.19^{a}**	**19.1^{a}**	**350^{a}**	**2.21^{a}**	**105^{a}**	**0.69^{a}**	**5.76^{a}**	**24.2^{a}**	**104^{a}**
	SD	180.0	1.463	13.53	0.000	0.067	0.251	8.230	37.19	0.196	6.427	0.127	1.608	3.613	48.92
Non-supp	**Average**	**213^{a}**	**53.2^{a}**	**17.0^{a}**	**0.27^{a}**	**0.67^{a}**	**0.91^{a}**	**10.3^{a}**	**249^{a}**	**2.07^{a}**	**66.4^{b}**	**1.20^{ab}**	**2.48^{a}**	**17.2^{b}**	**59.0^{a}**
	SD	35.37	3.374	1.908	0.151	0.020	0.028	4.669	16.17	0.228	8.187	0.511	0.851	1.036	12.89
Supp	**Average**	**543^{a}**	**48.2^{a}**	**21.9^{a}**	**0.13^{a}**	**3.36^{b}**	**1.29^{a}**	**22.3^{a}**	**238^{a}**	**2.64^{a}**	**76.1^{ab}**	**1.66^{b}**	**4.53^{a}**	**24.2^{ab}**	**84.8^{a}**
	SD	187.5	29.12	6.338	0.000	0.024	0.566	2.464	76.03	0.581	3.493	0.490	3.906	4.722	15.19
Supp + Fe	**Average**	**423^{a}**	**38.8^{a}**	**16.7^{a}**	**2.95^{b}**	**6.35^{c}**	**3.77^{b}**	**17.7^{a}**	**325^{a}**	**5.46^{a}**	**75.1^{ab}**	**3.99^{c}**	**5.83^{a}**	**22.2^{ab}**	**77.2^{a}**
	SD	144.7	0.635	0.014	0.563	1.726	1.626	0.103	33.28	1.342	7.504	0.749	0.289	0.135	7.025

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Similar observations can be found while studying the effect of irrigation with anaerobic sludges on the microelement composition of soil samples (*Table 5*). Due to the initial high iron content of the soil, sludge amendment did not elevate iron levels. Barium levels were increased through sludges originating from SBPP digestion.

The results obtained in alteration of metals in soil by the effect of sludge amendment are similar to previous study (Carbonell et al., 2009). The amendment of sludges originating from municipal wastewater treatment resulted in significantly higher concentrations of Zn and Cu in soils due to the high Zn and Cu content of the sludge. No significant difference was observed for Cd, Cr and Ni concentrations.

Bioaccumulation factors (BF) of different elements varied widely (0.07-110.8). Extremely high values were found for cadmium (more than 100 times higher) in the supplemented + Fe treatment, which were 10 times higher than those in the supplemented or non-supplemented samples. This can be explained by the synergic effect of iron on cadmium accumulation. Similar impacts were found for cobalt and chromium deposition. Microelement supplementation of sludge had a negative effect on boron BF; the accumulation of microelements in spring barley samples is shown in *Table 5*. Cobalt concentrations increased by 4.2, 21.0 times and 40.0 times in the non-supplemented, supplemented and supplemented + Fe trial, respectively. A similar tendency (1.7, 2.4 and 5.8 times higher) was found for nickel. The significant accumulation of cadmium, chrome and lithium in barley samples, observed in the supplementation + Fe trial, cannot be explained by the relevant values of the sludges.

Conclusion

This study has highlighted the importance of trace element supplementation for anaerobic digestion of SBPP. In addition, we investigated the ecotoxicological effects of sludges on root elongation (white mustard) and accumulation on terrestrial flora (spring barley).

Based on the average values of SMY, supplementation (supplemented and supplemented + iron) increased the values by 11.0 and 11.7% compared to the non-supplemented control trial. The methane content of the produced biogas was not significantly influenced by microelement supplementation. This means that anaerobic digestion with trace element supplementation is more stable and flexible, while during an imbalance caused by overload stress, tVFA values remained 9-11% lower in the supplemented trials compared to the control, indicating that the systems could regenerate faster.

Ecotoxicological assessment of anaerobic sludges is extremely important to determine application possibilities that could adversely affect forestry and agriculture.

The applied tests were appropriate indicators to determine the differences of inhibition-stimulation effects. In the white mustard root elongation test, the highest stimulation rate (-59.41%) was found for the 10-fold dilution in the supplemented + iron trial. The results of static acute toxicity test of spring barley showed that the stimulation ratio increased with supplementation; the strongest effect (stimulation of -62.80%) was determined supplementation with iron. This stimulation effect of sludge originating from SBPP metal-supplemented anaerobic digestion highlights the fact that plant-derived sludges have a lower ecotoxicology compared to sludges with high metal contents (Roig et al., 2012).

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Bioaccumulation factors (BF) of microelements in spring barley/soil samples varied widely between 0.07 and 110.8. According to the results of the sludge trace element analysis, irrigation of barley with sludge impacts soil metal concentrations. Therefore, additional physico-chemical and microbiological data should also be considered in order to better understand the long-term implications of plant-derived anaerobic sludge addition to forest and agricultural soils.

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EFFECTS OF MIXTURE LEVEL OF SORGHUM HAY WITH FORAGE LEGUMES ON PERFORMANCE AND BLOOD METABOLITES OF YEARLING INDIGENOUS PEDI BUCKS

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Abstract. Four experiments were conducted at the University of Limpopo Experimental farm, Limpopo province, South Africa to determine the effect of legume inclusion on the performance of Pedi bucks on a basal diet of forage sorghum hay. *Vigna ungiculata* (cowpea), *Lablab purpureus* (lablab) and *Mucuna pruriens* (mucuna) hays were used in this trial at different inclusion levels. Fifteen yearling Pedi bucks with average weights of 15 ± 4 kg (Experiment 1), 18 ± 2 kg (Experiment 2) and 17 ± 3 kg (Experiment 3) were used in the first three experiments in a completely randomised design. Nine goats with an average weight of 20 ± 4 kg were used in Experiment 4. Mucuna hay had the highest ($P < 0.05$) hydrolysable tannin contents. Lablab hay had higher ($P < 0.05$) total polyphenolics than mucuna, cowpea and sorghum hays. Dry matter intake and digestibility increased with increasing levels of the respective forage inclusion levels. Cowpea and mucuna hay improved ($P < 0.05$) final live weights and feed conversion ratio, while goats on lablab hay lost weight. Diet intake, digestibility and final live weights of the goats were optimized at different forage inclusion levels. This may indicate that legume inclusion levels for optimal productivity will depend on the particular parameter of interest.
Keywords: *legume inclusion, optimal intake, polyphenolic, digestibility, goats*

Introduction

Goats are economically, nutritionally and culturally important livestock species among the agrarian rural communities of South Africa. Additionally, the indigenous Pedi goats in Limpopo Province of South Africa constitute a valuable source of genetic material because of their relative adaptation to harsh climatic conditions and their ability to better utilise the limited and poor quality feed resources (Brown and Ngambi, 2017). However, their productivity is limited by acute shortages of good quality feed, especially during the dry and winter seasons (Brown et al., 2017). Available fodder during this period is fibrous and low in nitrogen resulting in low voluntary intake and digestibility (Tolera et al., 2000). Poor nutrition results in loss of body weight and reproductive performance (Matlebyane et al., 2010). Supplementation with forage legumes may be appropriate alternative for increasing intake and digestibility of these poor quality roughages (Mupangwa, 2002).

Forage legumes such as *Vigna ungiculata* (cowpeas), *Lablab purpureus* (lablab) and *Mucuna pruriens* (mucuna) are commonly grown by farmers in Limpopo province of South Africa. These legumes have high nitrogen contents and hence there is potential to increase the productivity of indigenous goats feeding on low quality roughages. Legumes can supply the limiting nitrogen in the form of amino acids and peptides. Abule et al. (1995) reported increased total dry matter (DM) intake in calves fed straw supplemented with either cowpea or lablab hay. Umunna

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et al. (1995) reported increased average daily gain in sheep fed oat hay supplemented with lablab leaf meal. Similar results were reported in sheep fed scrubland herbage supplemented with lablab leaf meal (Ndlovu and Sibanda, 1996). Mupangwa et al. (2002) concluded that inclusion of *Mucuna pruriens*, (mucuna) in low quality grass hay diets increased protein availability in sheep by increasing the supply of both rumen degradable and undegradable proteins. However, some of these legumes may contain polyphenolic compounds which may reduce diet intake and digestibility (Reed, 1995). The effects of such compounds in tropical legumes on diet intake and digestibility have not been extensively studied in indigenous goat breeds. Thus, extensive evaluation of these forage legumes in terms of their polyphenolic effects on diet intake, digestibility and productivity is appropriate. Therefore, the objective of this study was to determine the effect of mixture level of forage sorghum with cowpea, lablab or mucuna legumes on diet intake, digestibility growth and blood metabolites of indigenous Pedi goats of South Africa.

Materials and methods

Study site

This study was conducted at the University of Limpopo Experimental Farm, South Africa (latitude 27°55 S and longitude 24°77 E). The ambient temperature at the study site ranges between 20 and 36°C in summer (November - January) and between 5 and 25 °C in winter (May – July). Mean annual rainfall ranges between 446.8 and 468.44 mm. The vegetation is a mixture of shrubs (mainly *Vachellia* species), trees and grass.

Forages

Forage sorghum, cowpea (*Vigna ungiculata*), lablab (*Lablab purpureus*) and mucuna (*Mucuna pruriens*) hays were grown in large plots under irrigation in January, 2012. At harvesting, the legumes were at the flowering stage while forage sorghum was at early grain filling stage. The forages were harvested using a mower, and dried under the shade to prevent nutrient loss. After drying, the forages were chopped (12.7 mm length) to reduce selection by animals when feeding (Mentu et al., 2001). The dried and chopped forages were then thoroughly mixed according to the different ratios and packed into sacks as hay for winter-feeding.

Experimental procedures, treatments and designs

This study was divided into a series of experiments due to lack of equipment to conduct the whole study in one session. The experimental design was a completely randomized design, with individual animals placed in metabolic cages as experimental units. A total of four experiments were conducted. Experiments 1 to 3 involved cowpea, lablab and mucuna hays, respectively, while Experiment 4 determined the inclusion level for optimal intake from each of the first three experiments, as indicated below. The dietary treatments for optimal intake were determined from the quadratic equations described under statistical analysis. The experimental treatments were as listed below:

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Experiment 1	**Treatments**
Code	**Description of the treatment**
$FS_{90}C_{10}$	A mixture of 90% forage sorghum and 10% cowpea
$FS_{80}C_{20}$	A mixture of 80% forage sorghum and 20% cowpea
$FS_{70}C_{30}$	A mixture of 70% forage sorghum and 30% cowpea
$FS_{60}C_{40}$	A mixture of 60% forage sorghum and 40% cowpea
$FS_{50}C_{50}$	A mixture of 50% forage sorghum and 50% cowpea
Experiment 2	**Treatments**
Code	**Description of the treatment**
$FS_{90}L_{10}$	A mixture of 90% forage sorghum and 10% lablab
$FS_{80}L_{20}$	A mixture of 80% forage sorghum and 20% lablab
$FS_{70}L_{30}$	A mixture of 70% forage sorghum and 30% lablab
$FS_{60}L_{40}$	A mixture of 60% forage sorghum and 40% lablab
$FS_{50}L_{50}$	A mixture of 50% forage sorghum and 50% lablab
Experiment 3	**Treatments**
Code	**Description of the treatment**
$FS_{90}M_{10}$	A mixture of 90% forage sorghum and 10% mucuna
$FS_{80}M_{20}$	A mixture of 80% forage sorghum and 20% mucuna
$FS_{70}M_{30}$	A mixture of 70% forage sorghum and 30% mucuna
$FS_{60}M_{40}$	A mixture of 60% forage sorghum and 40% mucuna
$FS_{50}M_{50}$	A mixture of 50% forage sorghum and 50% mucuna
Experiment 4	**Treatments**
Code	**Description of the treatment**
$S_{61}C_{39}$	A mixture of 61% forage sorghum and 39% cowpea
$S_{68}L_{32}$	A mixture of 68% forage sorghum and 32% lablab
$S_{77}M_{23}$	A mixture of 77% forage sorghum and 23% mucuna

Animals

All procedures involving animals were approved by the Animal Research Ethics Committee of the University of Limpopo, South Africa. Fifteen growing yearling Pedi bucks (a local breed in Limpopo province of South Africa) with mean live weights of 15 ± 4 kg (Experiment 1), 18 ± 2 kg (Experiment 2), and 17 ± 3 kg (Experiment 3) were used. Nine goats with a mean live weight of 20 ± 4 kg were used in Experiment 4. In each experiment, different goats were used but of similar ages. Thus, a total of 54 goats were used in the study. Each treatment had three replicates with one goat per replicate as described by Brown and Ng'ambi (2017). The animals were housed in individual metabolic cages that allowed the separation of faeces and urine (5.5' x 2.0') and were given the experimental diets during the study period. The goats were drenched with an anthelmintic (Valbazen® broad-spectrum dewormer, Pfizer Inc, NY, USA) before the start of the experiment.

An adjustment period of 2 weeks allowed goats to become acclimatized to the metabolic cage before the start of the study. Each goat was initially fed 700 g of feed per day in individual troughs. This amount was adjusted accordingly so that a 10% feed refusal was maintained to avoid selection. Goats were fed once a day at 08.00 hours and feeds offered and refused were determined. Each experiment lasted for 21 days, the last 7 days being collection period. Animals had access to feed and water *ad libitum*. The goats were weighed weekly. Average daily gain was determined. The weighing of the goats was carried out before morning feeding to avoid feed effect (Sarwatt et al., 2003). Feeds were collected from each diet and stored for laboratory analysis. During collection, daily faecal outputs for each animal

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were collected, mixed and recorded. Ten percent of the faeces were sub-sampled and kept for analysis. All the sub-sampled faeces were pooled on animal basis, dried and kept until required for chemical analysis. From the food eaten and faecal matter secreted, apparent digestibility of the nutrients was calculated (McDonald et al., 2010). Blood samples were taken from each goat via the jugular vein just before the morning feeding on the first and last day of the experiment (Olafadehan, 2011). The blood samples were collected from each animal, before feeding at the beginning and end of the study, into anticoagulant free bottles, allowed to coagulate at room temperature and centrifuged at 1500ˈx *g* for 10 min. The supernatant sera were then harvested and stored at -20°C for subsequent analysis.

Laboratory analyses

Samples of the forages (cowpea, lablab, mucuna and sorghum) were ground to pass a 1 mm screen for nutrient analysis. Samples for tannin analysis were further ground to pass through a 0.2 mm screen. Dry matter of feeds, refusals and faeces were determined by the method described by AOAC (2005). Ash was determined by the method described by AOAC (2005). Acid detergent fibre and neutral detergent fibre were determined by the method described by Van Soest et al. (1991). Nitrogen content was determined for feeds and faeces using the Kjedahl procedure (AOAC, 2005). Sodium oxalate fluoride was used for glucose preservation, while the blood glucose was determined by enzymatic colorimetric test (Quimica Clinical Applicada, SA). Serum urea was obtained by the method of Valley et al. (1980). Extraction of polyphenolics from plant material was done using the method described by Makkar et al. (1995). Condensed tannins, hydrolysable tannins and total polyphenols were determined using the methods described by Porter et al. (1986).

Statistical analysis

All data on diet intake, digestibility, weight change, feed conversion ratio, nitrogen balance, and blood metabolites were analysed using the General Linear Model procedures of SAS (SAS, 2010). Covariance analysis was done using initial bodyweight of the goats as covariate. Treatment means were compared using Tukey's HSD and significant differences were declared at $P < 0.05$. The responses in optimal intake, digestibility, weight change, feed conversion ratio, nitrogen balance, and blood metabolites were modelled using the following quadratic equation:

$$Y = a + b_1x + b_2x^2$$

where Y = Intake, digestibility, weight change, feed conversion ratio, nitrogen balance or blood metabolites; a = intercept; b = coefficients of the quadratic equation; x = level of inclusion and $-b_1/2b_2$ = level of inclusion value for optimal production response (Brown and Ng'ambi, 2017).

Results

Nutrient composition of feeds

Results of the chemical composition of the experimental feeds are presented in *Table 1*. Cowpea, lablab and mucuna hay had higher ($P < 0.05$) protein contents than sorghum hay. There were traces of tannins and polyphenolic compounds in all the forages. However,

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mucuna hay had higher ($P < 0.05$) condensed and hydrolysable tannin contents when compared to sorghum, lablab and cowpea. Lablab hay had higher ($P < 0.05$) total polyphenolics (1.24 mg/g) when compared to mucuna (0.35 mg/g), cowpea (0.05 mg/g) and sorghum (0.17 mg/g) hays. Cowpea hay had lower ($P < 0.05$) total polyphenolics, condensed and hydrolysable tannin contents.

Table 1. *The chemical composition of the experimental feeds*[#]

Nutrient (% DM)	Forage				SEM	Probability
	Cowpea	Lablab	Mucuna	Sorghum		
Dry matter	90	93	94	94	2.10	NS
Organic matter	83[c]	87[b]	90[a]	90[a]	1.50	*
Crude protein	22[a]	19[b]	18[c]	13[d]	0.00	*
Neutral detergent fibre	38[bc]	34[c]	40[b]	64[a]	1.82	*
Acid detergent fibre	32[bc]	31[c]	36[b]	52[a]	2.35	*
Condensed tannins[##]	0.03[d]	0.05[c]	0.40[a]	0.06[b]	0.00	*
Hydrolysable tannins (mg/g)	81.3[d]	113.3[b]	284.3[a]	111.1[c]	1.30	*
Total polyphenols (mg/g)	0.05[d]	1.24[a]	0.35[b]	0.17[c]	0.00	*

SEM: Standard error of the mean
[#]Four samples per replicate were used
[##]Condensed tannins as percentage DM leucocyanidin equivalent
[a,b,c]Means in the same row with different superscripts are significantly different
NS: Not significant
*$P < 0.05$

Cowpea inclusion

The results of the effect of cowpea hay inclusion level on diet intake, digestibility, live weight change, nitrogen balance and blood serum chemistry of indigenous Pedi bucks fed a basal diet of forage sorghum hay are presented in *Table 2*. Daily dry matter intakes of cowpea were similar ($P > 0.05$) across dietary treatments, ranging from 370 to 511 g per goat per day. Similarly, goats consumed similar ($P > 0.05$) amounts of organic matter (OM) and neutral detergent fibre (NDF) contents. However, Pedi bucks on diets 20, 30, 40 or 50% cowpea hay inclusion levels had higher ($P < 0.05$) crude protein (CP) and acid detergent fibre (ADF) intakes than those on diets with 10% cowpea hay. Pedi goats on a diet with 50% cowpea hay inclusion level had higher ($P < 0.05$) DM, OM and CP intakes per metabolic weight as compared to those on other dietary treatments. Goats on diets having a 30% cowpea hay inclusion level had lower ($P < 0.05$) NDF and ADF intakes per metabolic weight than those on other dietary treatments. Dry matter, OM, NDF and ADF digestibility values were similar ($P > 0.05$) across the dietary treatments. However, goats on diets having 20, 40 or 50% cowpea hay inclusion levels had higher ($P < 0.05$) dietary CP digestibility values than those on diets having 10 or 30% cowpea hay inclusion levels. Pedi goats fed diets having 20, 30, 40 or 50% cowpea hay inclusion levels had higher ($P < 0.05$) nitrogen intakes than those on a diet with 10% cowpea hay inclusion level. Similarly, goats on diets having 20, 40 or 50% cowpea hay inclusion levels retained higher ($P < 0.05$) amounts of nitrogen than those on 10 or 30% cowpea diets. Cowpea inclusion level did not ($P > 0.05$) have effects on blood glucose concentration of the bucks. However, goats fed diets having 20, 40 or 50% cowpea hay inclusion levels had higher ($P < 0.05$) blood

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urea than those on diets with 10 or 30% cowpea hay inclusion levels. Cowpea hay inclusion level did not ($P > 0.05$) have effect on weight gain of goats. However, dietary treatment had effect ($P < 0.05$) on the final live weight of goats. Goats fed diets having a 10% cowpea hay inclusion level had the lowest final weight as compared to those on 30% inclusion level. Feed conversion ratio was improved ($P < 0.05$) in goats fed diets having a 50% cowpea hay inclusion level. Cowpea hay inclusion levels of 39, 39, 54 and 33% optimized DM, OM, CP and ADF intakes while nitrogen retention and blood glucose were optimized at 56 and 33% levels, respectively (*Table 3*).

Table 2. *Effect of cowpea hay inclusion levels on diet intake, digestibility, live weight change, nitrogen balance and blood metabolic profiles of Pedi bucks fed a basal diet of forage sorghum hay*

Variable	Treatment					SEM	P
	$FS_{90}C_{10}$	$FS_{80}C_{20}$	$FS_{70}C_{30}$	$FS_{60}C_{40}$	$FS_{50}C_{50}$		
Intake (g/goat/day)							
Dry matter	370	511	469	476	500	50.59	NS
Organic matter	320	426	394	395	417	40.49	NS
Crude protein	53^{b}	76^{a}	74^{a}	79^{a}	88^{a}	7.53	*
Neutral detergent fibre	370	345	285	291	292	36.09	NS
Acid detergent fibre	194^{b}	283^{a}	245^{a}	247^{a}	242^{a}	24.34	*
Intake ($g/kgW^{-0.75}$)							
Dry matter	80^{d}	99^{b}	80^{d}	93^{c}	101^{a}	0.28	*
Organic matter	70^{d}	83^{b}	68^{e}	75^{c}	85^{a}	1.01	*
Crude protein	12^{d}	14^{bc}	13^{cd}	17^{ab}	18^{a}	1.02	*
Neutral detergent fibre	81^{a}	68^{b}	48^{e}	56^{d}	59^{c}	0.68	*
Acid detergent fibre	43^{d}	55^{a}	42^{e}	48^{c}	49^{b}	0.30	*
Digestibility (decimal)							
Dry matter	0.57	0.67	0.65	0.69	0.65	0.04	NS
Organic matter	0.59	0.66	0.59	0.65	0.68	0.07	NS
Crude protein	0.70^{b}	0.76^{a}	0.69^{b}	0.80^{a}	0.75^{a}	0.03	*
Neutral detergent fibre	0.65	0.69	0.59	0.66	0.62	0.07	NS
Acid detergent fibre	0.43	0.52	0.40	0.50	0.53	0.01	NS
N-intake (g/goat/day)	8.4^{b}	12.1^{a}	11.8^{a}	12.7^{a}	14.0^{a}	1.20	*
N-retention (g/goat/day)	5.9^{c}	9.2^{ab}	8.2^{b}	10.1^{a}	10.5^{a}	1.01	*
Blood metabolites (mmol/L)							
Blood glucose	2.5	3.3	3.1	3.4	3.4	0.37	NS
Blood serum urea	7.3^{c}	7.8^{ab}	6.6^{c}	7.3^{ab}	8.3^{a}	0.5	*
Live weight							
Initial (kg/goat)	14.9	17.7	19.7	16.7	15.0	0.92	NS
Final (kg/goat)	15.6^{b}	18.2^{ab}	20.4^{a}	17.4^{ab}	16.03^{ab}	1.42	*
Gain (g/goat/day)	140	100	140	140	212	308.1	NS
Feed conversion ratio	2.57^{c}	5.11^{a}	3.35^{b}	3.40^{b}	2.36^{d}	0.002	*

SEM: Standard error of the means
[a,b,c]Means in the same row with different superscripts are significantly different
P: Probability
NS: Not significant
*$P < 0.05$

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Table 3. *Cowpea hay inclusion levels for optimal diet intake, nitrogen retention and blood glucose in Pedi bucks on a basal diet of forage sorghum hay*

Factor	Formula	Ratio level	Y- Level	r^2	P
Intake (g/goat/day)					
DM	$Y=320.747+9.279x+ -0.120x^2$	38.66	500	0.307	0.111
OM	$Y=274.000+7.416x + -0.096x^2$	38.63	417	0.568	0.432
CP	$Y=41.600+1.630x+ -0.015x^2$	54.33	56	0.847	0.153
ADF	$Y=150.200+6.943x+ -0.106x^2$	32.75	264	0.479	0.521
N-retention (mmol/L)					
	$Y=4.300x+0.225x+ -0.002x^2$	56.25	11	0.599	0.200
Blood glucose (mmol/L)					
	$Y=2.020+0.066x+ -0.001x^2$	33.00	3	0.782	0.218

r^2: Coefficient of determination
P: Probability

Lablab inclusion

The results of the effect of lablab hay inclusion level on dietary intake, digestibility, live weight change, nitrogen balance and blood serum chemistry of indigenous Pedi bucks fed a basal diet of forage sorghum hay are presented in *Table 4*.

Table 4. *Effect of lablab hay inclusion levels on diet intake, digestibility, live weight change, nitrogen balance and blood metabolic profiles of Pedi bucks fed a basal diet of forage sorghum hay*

Variable	Treatment					SEM	P
	$FS_{90}L_{10}$	$FS_{80}L_{20}$	$FS_{70}L_{30}$	$FS_{60}L_{40}$	$FS_{50}L_{50}$		
Intake (g/goat/day)							
DM	517	552.	583	565	539	55.63	NS
OM	324	465	494	489	434	75.71	NS
CP	70	78	86	87	86	8.08	NS
NDF	424	402	400	378	337	30.56	NS
ADF	191	227	217	214	211	17.50	NS
Intake ($g/kgW^{-0.75}$)							
DM	92^{c}	100^{bc}	111^{ab}	112^{a}	104^{ab}	5.38	*
OM	57^{d}	84^{c}	93^{b}	97^{a}	85^{c}	0.93	*
CP	14^{c}	14^{b}	17^{a}	17^{a}	17^{a}	0.75	*
NDF	76	72	67	75	65	7.57	NS
ADF	33^{b}	40^{a}	41^{a}	43^{a}	41^{a}	1.33	*
Digestibility (decimal)							
DM	0.66	0.67	0.68	0.69	0.70	0.02	NS
OM	0.58	0.63	0.74	0.73	0.69	0.10	NS
CP	0.72^{b}	0.73^{b}	0.77^{a}	0.79^{a}	0.78^{a}	0.02	*
NDF	0.74	0.71	0.78	0.73	0.71	0.05	NS
ADF	0.22	0.27	0.73	0.59	0.54	0.23	NS
N-intake (g/goat/day)	11.3	12.5	13.8	13.8	13.9	1.29	NS
N-retention (g/goat/day)	8.1^{c}	9.1^{bc}	10.6^{ab}	11.1^{a}	10.7^{a}	0.83	*

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Blood metabolites (mmol/L)							
Blood glucose	2.7	2.9	3.3	2.3	3.2	0.53	NS
Blood urea	5.9	7.9	7.0	7.4	7.9	1.25	NS
Live weight							
Initial (kg/goat)	19.7	20.4	17.9	18.3	18.6	1.92	NS
Final (kg/goat)	19.5	19.5	17.8	18	18	1.71	NS
Loss (g/goat/day)	-40	-180	-20	-60	-120	318.1	NS

SEM: Standard error of the means
[a,b,c]Means in the same row with different superscripts are significantly different.
P: Probability
NS: Not significant
*P < 0.05

Daily DM, OM, CP, NDF and ADF intakes were similar (P > 0.05) across dietary treatments. Pedi goats fed a diet having a 40% lablab hay inclusion level had higher (P < 0.05) dry matter intakes per metabolic weight than those on 10 or 20% inclusion levels. Goats fed diets having 30, 40 or 50% lablab hay inclusion levels had higher (P < 0.05) CP intakes per metabolic weight than those on other dietary treatments. Pedi goats had similar (P > 0.05) DM, OM, NDF and ADF digestibility values across the dietary treatments. Goats on diets having 30, 40 or 50% lablab hay inclusion levels had higher (P < 0.05) crude protein digestibility values than those on diets having 10 or 20% lablab hay inclusion levels. Daily nitrogen intakes were similar (P > 0.05) across dietary treatments. However, goats fed diets having 40 or 50% lablab hay inclusion levels had higher (P < 0.05) nitrogen retention values than those on 10 or 20% lablab hay inclusion levels. Lablab hay inclusion level did not have effect (P > 0.05) on blood glucose and serum urea of goats. Similarly, lablab hay inclusion level did not have effect (P > 0.05) on the live weights of Pedi goats. However, weight losses were observed across all dietary treatments. Dry matter, OM, CP and ADF intakes were optimized at 32.29, 34.04, 41.14 and 32.48% lablab hay inclusion levels, respectively, while nitrogen retention was optimized at 39.17% lablab hay inclusion level (*Table 5*).

Table 5. *Lablab hay inclusion levels for optimal diet intake and nitrogen retention in Pedi bucks on a basal diet of forage sorghum hay*

Factor	Formula	Ratio level	Y- Level	r^2	P
Intake (g/goat/day)					
DM	$Y=448.913+7.879x+ -0.122x^2$	32.29	576	0.532	0.010
OM	$Y=155.000+20.697x+ -0.304x^2$	34.04	507	0.974	0.026
CP	$Y= 56.600+1.481x+ -0.018x^2$	41.14	87	0.989	0.011
ADF	$Y=168.400+3.313x+ -53.80x^2$	32.48	222	0.622	0.378
Intake ($g/kgW^{-0.75}$)					
DM	$Y=72.000+2.160x+ -0.030x^2$	36	111	0.937	0.63
OM	$Y=21.000+4.247x+ -0.059x^2$	36	97	0.991	0.009
ADF	$Y=25.400+0.919x+ -0.012x^2$	38.29	43	0.958	0.042
N-retention (mmol/L)					
	$Y=5.860+0.235x+ -0.003x^2$	39.17	11	0.964	0.036

r^2: Coefficient of determination
P: Probability

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Mucuna inclusion

The results of the effect of mucuna hay inclusion level on dietary intake, digestibility, live weight change, nitrogen balance and blood serum chemistry of indigenous Pedi bucks fed a basal diet of forage sorghum hay are presented in *Table 6*.

Table 6. *Effect of mucuna hay inclusion levels on diet intake, digestibility, live weight change, nitrogen balance and blood metabolic profiles of Pedi bucks fed forage sorghum hay*

Variable	Treatment					SEM	P
	$FS_{90}M_{10}$	$FS_{80}M_{20}$	$FS_{70}M_{30}$	$FS_{60}M_{40}$	$FS_{50}M_{50}$		
Intake (g/goat/day)							
DM	468	460	447	492	550	41.11	NS
OM	405	400	393	430	482	0.106	NS
CP	63^{b}	64^{b}	65^{b}	74^{b}	85^{a}	5.921	*
NDF	341	343	343	334	364	32.66	NS
ADF	180	180	176	189	184	14.86	NS
Intake (g/kgW$^{-0.75}$)							
DM	79^{e}	92^{c}	85^{d}	102^{a}	95^{b}	1.03	*
OM	68^{e}	80^{c}	75^{d}	89^{a}	84^{b}	0.23	*
CP	11^{c}	13^{b}	13^{b}	16^{a}	15^{ab}	0.98	*
NDF	58^{e}	69^{b}	66^{c}	70^{a}	64^{d}	0.09	*
ADF	30^{d}	36^{b}	33^{c}	40^{a}	33^{c}	1.02	NS
Digestibility (decimal)							
DM	0.59	0.60	0.59	0.62	0.62	0.02	NS
OM	0.64^{a}	0.60^{bc}	0.57^{c}	0.65^{ab}	0.69^{a}	0.03	*
CP	0.68	0.68	0.67	0.68	0.71	0.02	NS
NDF	0.67	0.63	0.48	0.65	0.67	0.11	NS
ADF	0.17	0.12	0.32	0.24	0.33	0.16	NS
N-intake (g/goat/day)	10.1^{b}	10.3^{b}	10.4^{b}	11.8^{b}	13.6^{a}	0.95	*
N-retention (g/goat/day)	6.0^{bc}	7.0^{b}	7.0^{b}	8.0^{b}	9.7^{a}	0.70	*
Blood metabolites (mmol/L)							
Blood glucose	2.8^{c}	3.7^{c}	3.3^{c}	4.0^{ab}	4.5^{a}	1.03	*
Blood urea	7.9^{bc}	9.7^{a}	8.6^{b}	9.7^{a}	9.8^{a}	1.12	*
Live weight							
Initial (kg/goat)	20.5	16.8	17.8	16.1	19.0	1.09	NS
Final (kg/goat)	21.1^{a}	17.5^{b}	18.6^{ab}	17.1^{b}	19.9^{a}	1.37	*
Gain (g/goat/day)	120	140	183	200	180	118.6	NS
Feed conversion ratio	3.90^{a}	3.29^{ab}	2.44^{c}	2.46^{c}	3.06^{bc}	0.39	*

SEM: Standard error of the means
^{a,b,c}: Means within a row with different superscripts differ significantly
P: Probability
NS: Not significant
*P < 0.05

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Daily DM, OM, NDF and ADF intakes were similar ($P > 0.05$) across the dietary treatments. However, goats on a diet having 50% mucuna hay inclusion level had higher ($P < 0.05$) crude protein intakes than those on diets having 10, 20, 30 or 40% dietary treatments. Goats on a diet having a 40% mucuna hay inclusion level had higher ($P < 0.05$) DM, OM, CP, NDF and ADF intakes per metabolic weight than those on other dietary treatments. Similarly, goats on a diet with 50% mucuna hay inclusion level had higher ($P < 0.05$) DM and OM intakes per metabolic weight than those on 10, 20 or 30% inclusion levels. Goats had similar ($P > 0.05$) DM, CP, NDF and ADF digestibility values across dietary treatments. However, goats on diets with 10 or 50% mucuna hay inclusion levels had higher ($P < 0.05$) OM digestibility than those on other dietary treatments. Goats on diets having a 50% mucuna hay inclusion level had higher ($P < 0.05$) nitrogen intake and nitrogen retention than those on diets with 10, 20, 30 or 40% inclusion levels. Goats on diets with 40 or 50% mucuna hay inclusion levels had higher ($P < 0.05$) blood glucose than those on 10, 20 and 30% inclusion levels. Similarly, goats on diets having 20, 40 or 50% mucuna hay inclusion levels had higher ($P < 0.05$) serum urea concentrations than those on 10 or 30% inclusion levels. Goats on diets having 10 or 50% mucuna hay inclusion levels had higher ($P < 0.05$) final body weights than those on other dietary treatments. Goats on diets having 30 or 40% mucuna hay inclusion levels had better ($P < 0.05$) feed conversion ratio than those on 10, 20 or 50% mucuna hay inclusion levels. Mucuna hay inclusion levels of 50.27, 47.86, 59.75, 33.87 and 35.73% optimized DM, OM, CP, NDF and ADF intakes per metabolic weight of the goats, respectively, while live weight change and feed conversion ratio of the goats were optimized at 41.7 and 41.5% inclusion levels, respectively (*Table 7*).

Table 7. *Mucuna hay inclusion levels for optimal diet intake, live weight change and feed conversion ratio in Pedi goats on a basal diet of forage sorghum hay*

Factor	Formula	Ratio level	Y -Level	r^2	P
Intake ($g/kgW^{-0.75}$)					
DM	$Y=70.000+1.106x+ -0.011x^2$	50.27	99	0.614	0.386
OM	$Y=59.400+1.053x+ -0.011x^2$	47.86	85	0.701	0.299
CP	$Y=8.800+0.239x+ -0.002x^2$	59.75	16	0.838	0.162
NDF	$Y=48.000+1.287x+ -0.019x^2$	33.87	70	0.756	0.244
ADF	$Y=23.400+0.786x+ -0.011x^2$	35.73	37	0.495	0.505
LWC (g/d)	$Y=57.600+6.343x+-0.076x^2$	41.7	190	0.91	0.086
FCR	$Y=5.423+-6.89x+3.44x^2$	41.5	2	0.938	0.062

r^2: Coefficient of determination
P: Probability
LWC: Live weight change

Optimal intakes

The results of the effect of cowpea, lablab, and mucuna hay inclusion level at optimal intake are presented in *Table 8*. Daily DM, OM, CP and ADF intakes of the diets were similar ($P > 0.05$) across dietary treatments. However, goats on diets having cowpea or lablab had higher ($P < 0.05$) NDF intakes than those on a diet having mucuna hay. Crude protein and ADF intakes per metabolic weight were not affected ($P > 0.05$)

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by dietary treatment. Pedi goats on diets having cowpea had higher ($P < 0.05$) DM, OM and NDF intakes per metabolic weight than those on diets having lablab and mucuna hays. Dietary treatments had no effect ($P > 0.05$) on diet DM, OM, CP, NDF and ADF digestibility. Similarly, dietary treatment had no effect ($P > 0.05$) on diet nitrogen intake, nitrogen retention and weight gain of the goats. However, goats fed a diet containing lablab had higher ($P < 0.05$) final body weights than those on diets containing cowpea hay. Goats on a diet having mucuna hay had better ($P < 0.05$) feed conversion ratio than those on diets containing lablab or cowpea hay.

Table 8. *Effect of cowpea, lablab and mucuna hay inclusion levels on diet intake, digestibility, live weight change and nitrogen balance in Pedi bucks fed forage sorghum hay as a basal diet*

Variable	Treatments			SEM	P
	Cowpea ($S_{61}C_{39}$)	Lablab ($S_{68}L_{32}$)	Mucuna ($S_{77}M_{23}$)		
Intake (g/goat/day)					
DM	521	527	499	58.02	NS
OM	424	434	422	41.53	NS
CP	86	79	71	6.61	NS
NDF	162^{a}	149^{ab}	134^{c}	7.0	*
ADF	134	142	129.41	11.90	NS
Intake (g/kgW$^{-0.75}$)					
DM	91^{a}	73^{c}	81^{b}	0.88	*
OM	74^{a}	60^{c}	68^{b}	0.27	*
CP	15	12	12	1.30	NS
NDF	29^{a}	21^{b}	22^{b}	1.04	*
ADF	24	19	21	1.33	NS
Digestibility (decimal)					
DM	0.51	0.54	0.52	0.05	NS
OM	0.47	0.52	0.50	0.05	NS
CP	0.67	0.73	0.68	0.03	NS
NDF	0.39	0.12	0.19	0.37	NS
ADF	0.28	0.45	0.50	0.33	NS
N-intake (g/goat/day)	13.3	12.6	13.8	1.06	NS
N-retention (g/goat/day)	7.8	8.9	9.4	0.82	NS
Live weight					
Initial (kg/goat)	19.2	24.4	20.1	1.67	NS
Final (kg/goat)	20^{b}	25.4^{a}	21.2^{ab}	1.63	*
Gain (g/goat/day)	160	200	220	251.0	NS
Feed conversion ratio	3.26^{a}	2.64^{b}	2.27^{c}	0.005	*

SEM: Standard error of the means
^{a,b,c}: Means within a row with different superscripts differ significantly
P: Probability
NS: Not significant
*P < 0.05

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Discussion

The legume species have higher protein contents (18% and above) than sorghum hay (13%). The protein values of cowpea, lablab and mucuna hays were similar to the protein values reported by Nielsen et al. (1997) and Adjorlolo et al. (2001), respectively. Thus, these legumes can be used as protein supplements where low quality roughage is used as a basal diet for goats. Forage sorghum hay contained higher amounts of acid detergent fibre than the legumes. Higher acid detergent fibre contents may indicate lower digestibility values (Buxton et al., 1996). Lablab hay had the highest concentrations of total polyphenols. Mokoboki (2007) reported similar high values of total polyphenols and extracted condensed tannins in lablab hay. Mucuna hay had the highest concentrations of both condensed tannins and hydrolysable tannins. Chikanga-Malunga et al. (2009) reported similarly high concentrations of total tannins in whole mucuna plants at 123 days after planting.

Cowpea hay inclusion increased crude protein intake and digestibility by goats. This result is similar to the findings of Ravhuhali (2010) who reported higher intake responses in goats fed a basal diet of buffalo grass supplemented with cowpea hay. Anelea et al. (2010) also reported increased dry matter and crude protein intakes by goats fed cowpea haulms. The authors attributed this to the high protein content in cowpea hay. In the present study, the level of inclusion for optimal intake was 38.66% of the diet. The optimal intake at this level was calculated to be 500 g per goat per day. This high diet intake may be attributed to the high protein content in cowpea hay. It may, also, be attributed to increases in DM and OM digestibility with increasing levels of cowpea hay inclusion as observed in the present study. In the present study, cowpea inclusion improved nitrogen retention and feed conversion ratio. Nitrogen retention was optimized at 56.25% level of cowpea inclusion, while feed conversion ratio improved linearly with increasing level of cowpea. Live weight of goats also increased linearly with increasing level of cowpea hay inclusion. This linear increase in live weight may be explained in terms of increases in diet intake and digestibility of the legume. This observation has been reported elsewhere in the literature (Savadogo et al., 2000; Ravhuhali, 2010). Cowpea hay inclusion significantly increased blood serum urea. This is similar to the results of Turner et al. (2005) who found increase of urea in the blood serum of goats given *Lespedeza cuneata* or alfalfa (*Medicago sativa*) hays.

Lablab hay inclusion increased diet DM intake and digestibility by goats. The level of inclusion for optimal intake was 32.29% of the diet. The optimal intake at this level was calculated to be 576 g per goat per day. According to Goodchild and McMeniman (1994), animals on supplements with higher protein contents exhibit better intake, digestibility and live weight gain responses. However, lablab inclusion had a negative effect on live weight gain. Weight lost was observed at all inclusion levels. Nsahlai and Umunna (1996) reported no effect on live weight gain of sheep fed a basal diet of oat hay supplemented with lablab. This adverse effect may be explained in terms of the high concentrations of polyphenols in lablab hay. High concentrations of polyphenols in a diet tend to reduce nutrient availability to the animals, thus adversely affecting its live weight gains (Tanner et al., 1990). It was possible that the protein in lablab hay was not available to the animals due to the high tannin content which tend to bind with proteins and hence reducing their digestion (D'Mello, 1992). Similarly, other dietary nutrients such as carbohydrates and vitamins may have been adversely affected by the total polyphenols in the digestive tract, thus negatively affecting nutrient availability and growth at tissue level (Makkar, 2003). Other studies have

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shown that lablab supplement to low quality hay increased average daily gain of ruminants (Ndlovu and Sibanda, 1996; Festus et al., 2008). In the present study, lablab hay inclusion improved nitrogen retention. A low inclusion level of 39.17% optimized nitrogen retention. This observation may be ascribed to high concentrations of total polyphenols in lablab hay. Tannin-protein complexes in an animal's gut are believed to be responsible for low protein digestibility and increased faecal nitrogen concentrations (McNabb et al., 1993).

Mucuna hay inclusion improved diet CP intake and OM digestibility. The results are in agreement with that of Mupangwa et al. (2002). Other studies have shown that mucuna hay is a good crude protein supplement for ruminants fed low quality forages such as maize stover and tropical grass hays (Mupangwa et al., 2002; Nyambati and Sollenberger, 2003). Additionally, mucuna hay inclusion improved feed conversion ratio of the goats. Level of inclusion for optimal live weight change and feed conversion was 42%. Such a level is recommended when performing dose-response trials. Mucuna hay inclusion improved live weight of the animals despite the presence of high levels of tannins in the diet. The improvement in live weight may be explained in terms of increases in diet intake and digestibility by goats. This result is similar to the findings of Brown et al. (2017) who reported higher live weight gains in indigenous Pedi bucks fed tanninferous *Acacia karroo* leaf meal. Solaiman et al. (2010) also reported improved weight gains in goats fed forages high in condensed tannins. Usually, tannins tend to reduce dietary intake and digestibility (Reed, 1995). However, there is evidence that low to moderate tannin content in diet may increase the efficiency of protein utilisation by ruminants (McNabb et al., 1993). Tannins bind with proteins at the rumen pH of 5.5 to 7.0 and protect protein from microbial degradation. These complexes are unstable at low pH in the abomasum, thus the proteins become available for digestion in the small intestines upon appropriate change in pH (McArthur et al., 1992). Mucuna hay significantly increased blood serum urea level. This observation may be ascribed to an increase in diet digestibility. Increased serum urea would, thus, suggest increased absorption of amino acids (Silanikove et al., 1996).

When levels of forage inclusion for optimal intake were used (Experiment 4)**,** goats on cowpea or lablab hays had similar NDF intakes which were higher than those on mucuna hay. Dry matter, OM and NDF intakes per metabolic weight of cowpea hay were higher than those of lablab and mucuna hays. Goats fed lablab hay had the highest final live weight (25.4 kg). Goats fed mucuna hay had better feed conversion ratio than those on cowpea and lablab hay inclusions. However, these values could not be compared due to lack of sufficient information in literature.

Conclusion

All the legumes used in this study had higher protein contents than sorghum hay and thus, have the potential to be utilised as protein supplements, where low quality hay is used as a basal diet for goats. Feed intake, digestibility and final live weights of the goats were optimized at different levels for all the legumes. This may indicate that levels for optimal productivity will depend on the particular parameter of interest. Lablab hay had negative effects on live weight gain of goats. The high concentration of polyphenol in the diet may have exerted adverse effects on the performance of animals. Further studies are required to ascertain this observation.

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INTRASPECIFIC VARIATIONS IN THE IVORY FEATHERLEG *PLATYCNEMIS DEALBATA* (INSECTA: ODONATA) FROM TURKEY

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Abstract. Intraspecific variations can be observed in many types of animal species in Turkey due to the geographical and ecological characteristics of the region. The aim of this research is to study the *Platycnemis dealbata* populations in Turkey and to survey how the Anatolian Diagonal affects them. The *Platycnemis dealbata* specimens were collected from Kahramanmaraş and Iğdır Provinces of Turkey in 2010, 2012, 2015, and 2017. Variations were found between these two populations. The variations seen in the *Platycnemis dealbata* populations were examined and demonstrated with illustrations.
Keywords: *Odonata, damselfly, Turkey, taxonomy, biodiversity*

Introduction

The genus *Platycnemis* is represented by three species (*P. pennipes*, *P. kervillei,* and *P. dealbata*) in Turkey (Kalkman et al., 2003; Kalkman, 2006). *P. dealbata* males have enlarged white legs and an ivory body. *P. dealbata* males and females differ from other species (*P. pennipes* and *P. kervillei*) in this region by the unmarked tibia of the mid and hind legs (Kalkman, 2006; Boudot et al., 2009). *P. dealbata* can be found from Northwest India through Kashmir and Afghanistan and west to the Caucasus, southeastern Turkey, Iran, Iraq, Lebanon, Israel, Syria and Jordan (Askew, 1988; Steinmann, 1997; Boudot et al., 2009). It is most abundant in Syria, Lebanon, and Israel (Dumont, 1977). The species occurs in southeastern Turkey and in the coastal area in the southeast (but does not occur beyond the Taurus range in the northwest of Turkey) (Kalkman, 2006; Boudot et al., 2009).

Anatolia is located in the Alpine-Himalayan mountain belt between Europe, Asia, Africa, and Arabia. Its landform is primarily due to its geological history of collision of the Arabian and African plates with the European plate, resulting in the closure of the Tethys Sea (Rögl, 1998; Mutun, 2010; Kapli et al., 2013). Because of that, Anatolia experienced the upheaval and folding of the Caucasus and the Taurus Mountains, and the uplift of the Central Anatolian Highlands (Mutun, 2010). Anatolia is considered to be either a bridge or a barrier for species dispersal between Asia, Europe, and the Ethiopian region because of its position and geological history (Kapli et al., 2013).

There is a highland dividing Anatolia, known as the Anatolian Diagonal, and it has been suggested that the Anatolian Diagonal is an important geographic barrier contributing to the current composition of various species across Anatolia and dividing species distribution in the east and west (Davis, 1971; Çıplak et al., 1993; Mutun, 2010). This barrier has isolated many species in East and West Anatolia (Çıplak et al., 1993). Anatolia has a mountainous land and various climate types, so various subpopulations were generated there for all insect groups (Çıplak et al., 1993).

According to Martens (1996), *P. dealbata* and *P. kervillei* can be observed to the east of the Anatolian Diagonal, while *P. pennipes* shows distribution to the west (*Fig. 1*). *P.*

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dealbata and *P. kervillei* show distribution together in Turkey, Syria, Iraq and Iran (the east of Anatolian Diagonal) (Boudot et al., 2009; Kiany et al., 2016; Schneider and Ikemeyer, 2016, 2017).

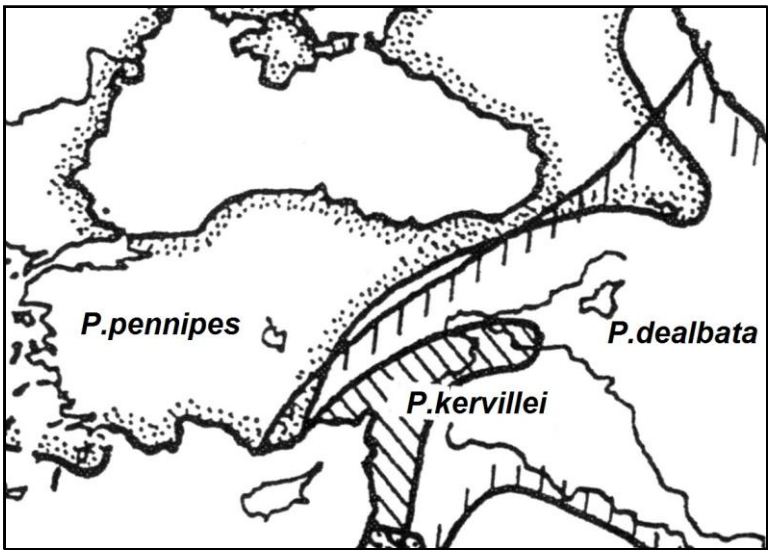


Figure 1. *Distribution of genus Platycnemis in Turkey and neighbouring countries (Martens, 1996)*

This study is mainly focusing on the populations of the ivory featherleg *P. dealbata* in Turkey and the observed effect of the Anatolian Diagonal on these populations. For this reason, samples were collected from Kahramanmaraş and Iğdır Provinces. As a result of the study, it was determined that there are variations between these two populations and these variations are revealed. It was mentioned that this could be caused by the Anatolian Diagonal.

Material and methods

The *P. dealbata* specimens were collected and identified from Kahramanmaraş and Iğdır Provinces (Turkey) in 2010, 2012, 2015, and 2017 (*Fig. 2*). The examined specimens were collected with aerial nets. The habitat consists of running water and is associated with weeds. Relevant literature (Martens, 1996; Dijkstra and Lewington, 2006; Kalkman, 2006) was used for taxonomic examination and identification of the specimens.

The material examined: 52 male and 26 female specimens, collected from Kahramanmaraş (20 male and 12 female specimens) and Iğdır (32 male and 14 female specimens) Provinces, Turkey in 2010, 2012, 2015, and 2017; leg. and det. Miroğlu, A. All materials are deposited in the Museum of Fatsa Faculty of Marine Sciences, Ordu University, Ordu, Turkey.

Results

The variations of the *P. dealbata* male include spots on the head (especially on the frons), on the abdominal segments (S7-9), and on the pronotum. Its variations have been presented by illustrations (*Figs. 3, 4* and *5*). In some specimens, these areas are all

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white, while others have black spots. The spreading of the black spots varies from narrow to wide.

When the head is viewed from the dorsal aspect, a weak and continuous or discontinuous black stripe can be seen on the frons (especially between the antennae). In some specimens (especially from Iğdır Province), the black stripe is stretching between the antennae. At the same time, the spreading of the black transverse band on the occiput and on the vertex varies from narrow to wide (*Fig. 3a, b*).



Figure 2. *Topographic position of P. dealbata collection localities, with dashed line showing the "Anatolian Diagonal"*

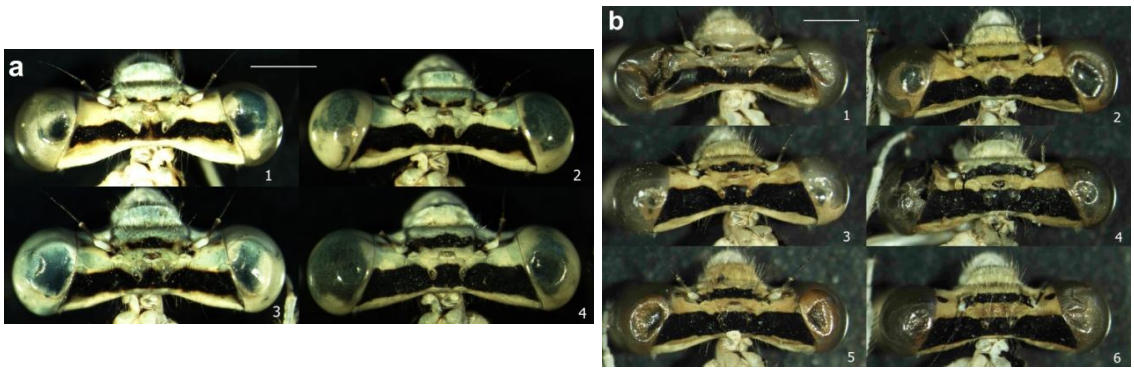


Figure 3. *Head dorsal view (a) Kahramanmaraş Province (b) Iğdır Province; scale bars 1.0 mm*

Also, black spots are exhibited on the pronotum similar to those on the frons and on the occiput-vertex. However, especially in the Kahramanmaraş Province specimens, those spots are seen to be different (*Fig. 4a, b*).

In *P. dealbata*, the spots and the color on the abdomen are very important. While the specimens from Iğdır Province have pale blue abdomen, their abdomen is ivory colored in Kahramanmaraş Province. In both localities (Iğdır and Kahramanmaraş Provinces), the specimens have black spots on the S7-9, but, those spots are observed much less on the Kahramanmaraş Province specimens (*Fig. 5a, b*).

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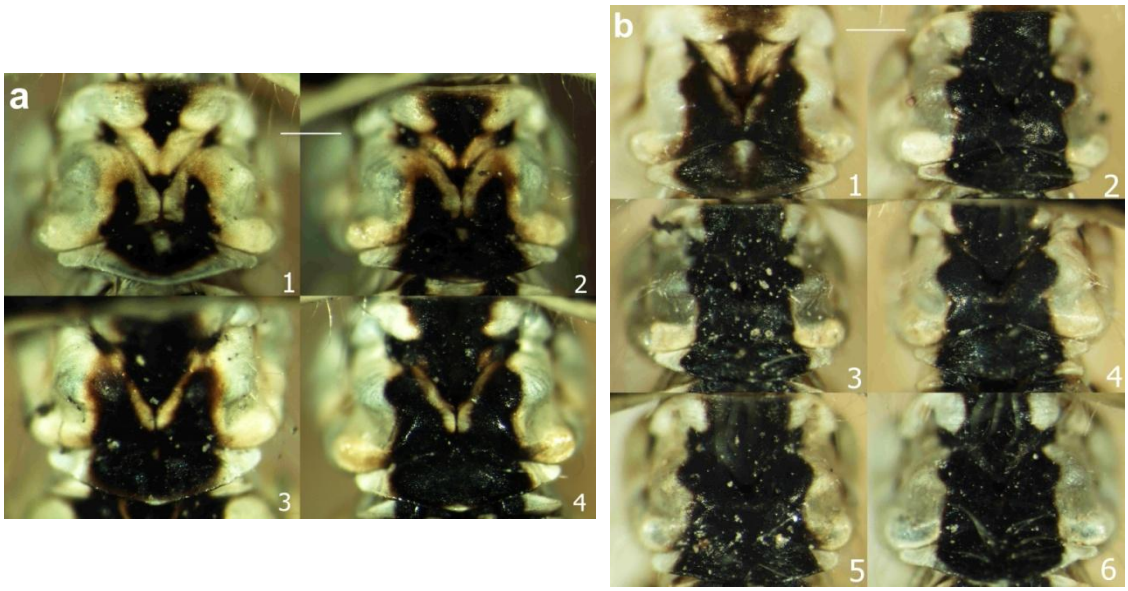


Figure 4. *Pronotum dorsal view (a) Kahramanmaraş Province (b) Iğdır Province; scale bars 0.5 mm*



Figure 5. *Abdomen (S7-10) lateral view (a) Kahramanmaraş province (b) Iğdır Province; scale bars 2.5 mm*

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Discussion

In the genus *Platycnemis*, although many characteristics for identification are taken into account, the most important characteristics are the spots and the color on the abdomen. According to Dijkstra and Lewington (2006), males of these species are identified by their ivory bodies and enlarged white legs. In this case, the abdomen is almost all white, with the S7-9 being seldom marked with black, never with blue and pruinose. Although the S7-9 is marked with black in the examined specimens, it is seldom so. This situation has been determined in many samples. Furthermore, a pale blue abdomen has been observed in the Iğdır Province specimens.

Although differences in color and spots have been observed within the species, there are also differences between the two populations (Kahramanmaraş and Iğdır). Specimens in the Iğdır population have more intense spots than specimens in the Kahramanmaraş population. Besides, Iğdır specimens have pale blue coloration, particularly on the abdomen.

As described previously, the Anatolian Diagonal may lead to diversity in species, and it may also contribute to intraspecific variations. At this point, Kahramanmaraş is located in an important region. While Kahramanmaraş is located on the Anatolian Diagonal, Iğdır is found on the eastern part of the Diagonal. According to Çıplak (2008), this region is known as the Maraş triangle, and it is rich in species. At this time, the region has a high intraspecific diversity (Mutun, 2010). It may be considered that the differences (both ecological and geographical) between the two populations (Kahramanmaraş and Iğdır) are probably due to the Anatolian Diagonal.

Conclusion

Samples should be collected from more regions where *P. dealbata* is distributed to find out whether there are variations in these populations. Phylogenetic studies are also needed to reveal whether there is a genetic diversity between the Kahramanmaraş and Iğdır populations.

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PROMOTER EFFECT OF MICROBES IN SLOPE ECO-ENGINEERING: EFFECTS ON PLANT GROWTH, SOIL QUALITY AND EROSION RATE AT DIFFERENT VEGETATION DENSITIES

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Abstract. Slope revegetation is claimed to be accelerated with the right choice of plants and effective planting techniques for the root establishment. In addition, microorganisms also promote plant growth via nutrient intensification and soil-root enhancement, hence may alleviate soil surface erosion. Thus, this study is aimed to identify the effects of microbial application on plant growth, soil properties and soil erosion rate at different vegetation densities. Six experimental plots were set up at the Guthrie Corridor Expressway, Selangor, Malaysia, with different amount of microbial application and vegetation density. Dense vegetation cover with microbial application (DM) plot exhibited the highest soil microbes abundance and fungal/bacteria (F/B) ratio after 24 months of observation. Meanwhile, photosynthetic rate and root length density of *Lantana camara* in the DM plot revealed the highest rate, followed by *Melastoma malabathricum* and *Bauhinia purpurea*. Moreover, the soil of DM plot had also increased in CEC, total N, and respiration rate, reflecting the soil quality. Consequently, erosion rate of DM plot exhibited the 52.6% of total decrement from the initial experiment. Thus, microbes are proven to be relatively good promoters for the improvement of plant growth performance, the soil quality and alleviate the soil erosion of the slope.
Keywords: *microbes, vegetation cover, plant growth, soil properties, erosion rate, slope stability*

Introduction

Soil is claimed to be degraded via two domains on slopes; weathering processes and intervention of human activities (Guerra et al., 2017). The torrential rains in tropical regions may elevate the frequency and magnitude of surface erosion processes and subsequently, slope failures (Crozier, 2010). Soil erosion alters the slope environment in various ways, from degrading the soil structure and nutrient content, to contributing to serious landslide tragedies. Although the technical or engineering approaches can be used to provide a significant contribution to mitigate slope instability, replacement of sustainable control of soil erosion such as plant communities is highly desirable. The establishment of vegetative elements has become an alternative approach for slope stabilizing against erosion as well as minimizing the occurrence of landslides (Normaniza and Barakbah, 2011; Liu et al., 2016). Moreover, soil structure itself is not static, but is affected by the activity of plant materials, microbes and plant-microbial interaction (Hawkes et al., 2005).

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The key ecosystem processes of soil habitats are driven by microbial communities, where local assemblages of microorganisms interact with each other and their environment (Konopka, 2009). Generally, similar land-use types usually have similar soil properties and microbial communities (Tian et al., 2017). Hence, we predict that the heterogeneity of cut slope soils may contributes to varying the microbial community and their composition will be highly localized. Soil microorganisms are sensitive to the changes of some environmental factors and play critical part in regulating many ecological processes such as litter decomposition, soil nutrient turnover as well as the plant growth performance (Liu et al., 2010; Wardle et al., 2004). Apart from that, soil microbes have also been reported to have dependency upon soil water availability (Chen et al., 2005; Liu et al., 2009). The soil water availability indirectly influences soil microorganisms via increasing decomposition of litter and soil organic matter (SOM) and subsequently alters the carbon substrates (Risch et al., 2007; Garcia-Pausas et al., 2008). On the other hand, previous studies have found that the bacterial diversity and abundance are influenced by ecological and geological factors such as vegetation type, soil temperature and pH level that establish the complex interaction among them (Bryant et al., 2008; Young and Crawfor, 2004). Given the revegetation process is an ecologically challenging task of restoring the ecosystem on the "problematic area" (cut slope), together with additional use of commercial biofertilizers, we predict that it will positively affect soil microbial activities and influence on revegetation success as well as the slope stability.

Topography is one of the most important factors controlling revegetation success on slope. Nevertheless, how the vegetation adjusts to terrain effects and the implications of the vegetation-hydrology processes and stability are the most substantial. Vegetation is known to have a major role in slope stability through hydrological and mechanical processes. Mechanical and hydrological processes are strongly interacted to each other and these two processes need to be considered together when assessing the stabilization of slopes by vegetation (Ghestem et al., 2011). Plants establishment on slopes typically intercepts rainfall through dense vegetation cover (Liu et al., 2016; Park and Cameron, 2008), their roots improve the soil structure by modifying aggregate stability (Fattet et al., 2011); and these functions will improve the stability of the slope and enhancing the biodiversity of landscapes (Zou et al., 2012). Additionally, since the topsoil and vegetation were destroyed during the slope cuttings, the slope soil properties are also changed (Chen et al., 2016). Rutigliano et al. (2004) has reported that the vegetation covers significantly affect the soil properties through the input of organic matter to the soil via the carbon and energy supplied from root exudates and plant remains. Whilst other studies have also discovered that as the age of vegetation increased, the soil physical and chemical properties are also enhanced (Zhu et al., 2010; Wang et al., 2015). Therefore, the ability to adapt on the harsh conditions and balance the soil water consumption need to be considered when implementing the vegetation screening for the slope.

From a practical perspective, it is imperative to select the optimal vegetation type that not only effective to control soil erosion, but can also maintain sustainable vegetation restoration (McVicar et al., 2010; Duan et al., 2016). Despite of considering the in situ environmental conditions (e.g. soil and temperature etc.), the easily measurable plant traits with a sound geotechnical basis are also need to be considered (Mickovski et al., 2006). Typically, trees have been used for erosion control, however, they are not recommended for planting on steep slopes because of the risk of uprooting that can cause mass movement (Pawlik, 2013). While the grasses that usually applied

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via hydroseeding are also not guaranteed as the extreme microclimates (e.g. heat stress and shade) can inhibit the establishment of sustainable grass covers and subsequently impair their capabilities for erosion mitigation (Bunnell et al., 2005). In this study, we had chosen shrubs as alternative pioneers that are capable of forming relatively dense evergreen cover on the cut slopes in tropical climate of Malaysia. *Lantana camara* is a perennial shrub, which is useful in covering the ground with a fine leaf mulch and was found to improve the fertility of soil (Ghisalberti, 2000) as well as exhibited a significantly higher photosynthetic capacity at higher temperatures (Zhang et al., 2014). Whilst *Melastoma malabathricum* was chosen due to its outstanding capacity to survive in extreme soil conditions and known as an aluminum accumulating plant (Saifuddin et al., 2016; Watanabe et al., 2008). This species exhibited the best physiological performance on slope, which is attributed to high growth rate and the higher planting density contributed in alleviating the erosion rate of slope soils (Aimee and Normaniza, 2015). Another shrub, *Bauhinia purpurea* was also been selected as it can grow on a dry on hill slopes and has deep root system and high root: shoot ratio, which is suitable for slope stabilization (Orwa et al., 2009). Therefore, these species exhibited the best potential as the slope species. All of the species studied have the root type M and VH, contributing to surface erosion control and soil reinforcement for slope stabilisation (Saifuddin and Normaniza, 2016)

To date, there is a lack of integral concept related to the interactions between microbes and the soil hydrological properties, soil nutrients content, and plants physiological performance. Thus, this study attempts to relate the microbes (bio-fertilizer) application on slope towards the growth performance (above-ground and below-ground properties) of the plants chosen and with respect to different plant density. It is anticipated that these findings would enhance the ability of the life growing plant on slopes, modify and improve the soil properties on the slope and reduce soil erosion, resulting in a more stable slope.

Materials and methods

Study area

The study area (*Fig. 1*) is located at the Guthrie Corridor Expressway, Selangor, Malaysia, at the geographical coordinates of N 03°13’24.1” and E 101°30’50.2” and annual average precipitation is 2663.34 mm. It has minor rill and minor to moderate gully erosions on the cut slope area with a slope gradient ranging between 26° to 35° (temperature 25–32 °C, maximum Photosynthetically Active Radiation (PAR) 2100 mE m^{-2} s^{-1}, and relative humidity of 60–75%). Most of the soil texture was dominated by sandy clay loam (moderately to slightly acidic; pH 3.78–5.37). Moreover, this area consisted of Kenny Hill formation, an agglomeration of mixed silts, sands, clays, and even some phyllites which vary from stiff to soft in consistency.

Experimental setup

In order to avoid the influences of slope aspect, the experimental plots were established on the similar slope properties and aspects (*Table 1*). Six experimental plots were assigned to three different vegetation coverages; bare (control), less dense (50% of plant coverage), and dense (80% of plant coverage) with the size of 8 m × 8 m of each in a complete randomized design (CRD). The buffer zone between the plots was 3 m.

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The second main factor was the bio-fertilizer application with microbe-M and non-microbe-NM were applied on each plant coverage; bare (BM and BNM), less dense (LDM and LDNM), and dense (DM and DNM).

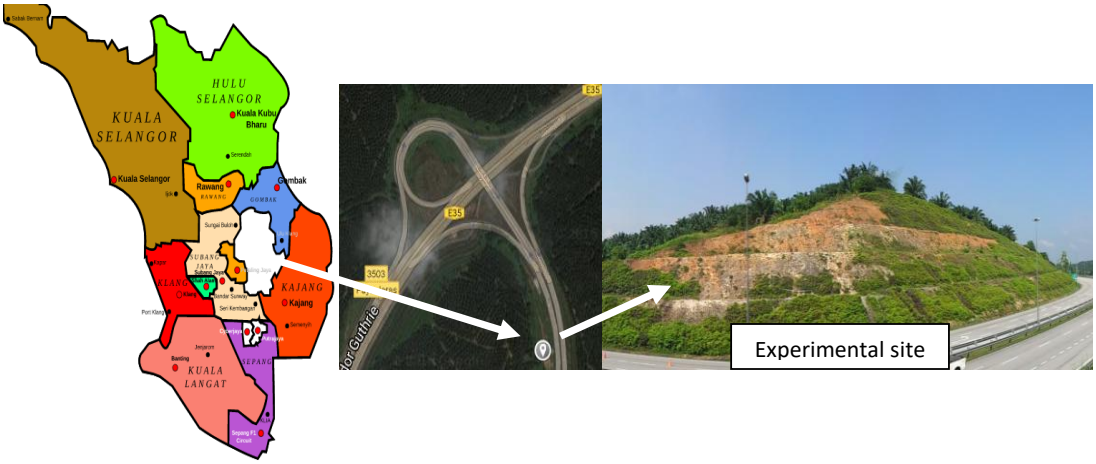


Figure 1. *Location of the study area at the Guthrie Corridor Expressway, Selangor, Malaysia*

Table 1. *Basic information of the selected plots*

Plot	Slope (%)	Elevation (m)	Vegetative coverage (%)	Number of plants
Bare	53	63.5	0	0
Less dense	55	64.1	50	32
Dense	57	64.5	80	52

Transplanting technique and microbes (bio-fertilizer) amendment

Three potential slope plants (as described in the introduction); *Lantana camara, Melastoma malabathricum,* and *Bauhinia purpurea* were transplanted onto slopes using a Microclimate Plant Propagation Technique with modified soil depth (Normaniza and Barakbah, 2011). Each seedling was transplanted into holes by using soil coring machine (Eijelkamp Agrisearch Equipment, Model Cobra, The Netherlands) at 0.6 m of soil depth. Plant supplements: NPK fertilizer, sphagnum moss and rock-phosphate (15 g each/hole) were applied only in the beginning of treatment in order to initiate the establishment of the roots and other physiological processes to take place in a "micro-environment", which could be more conducive for plant establishment and adaptation.

After a week of transplanting, 50 g bio-fertilizer (containing a consortium of bacteria and fungi) was applied at 10 cm of soil depth near the transplanted plants, representing the microbe treated plots. Applications were carried out in a single dose and the transplanted plants grew without any management practices.

Measurements

Isolation and enumeration of soil microbes

The soil samples (n = 3) for each plot were collected four times throughout the two-year observation. A Soil sample was taken at 10 cm radius from the stem of the studied plant (n = 3) using sterile spatula and containers. Bacteria and fungi were isolated on

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soil extract agar and potato dextrose agar media, respectively. Soil sample (1 g) was ten-fold serially diluted with quarter strength Ringer's solution and 100 µl of the soil suspension were spread on to agar plates in triplicates. All the agar plates were incubated for 3 to 7 days at 28 °C. Growth of bacteria and fungi were enumerated as colony forming unit per gram of soil (cfu/g).

Plant growth

Physiological parameters such as photosynthetic rate of the species studied were measured within six months interval and the root length density was measured at the end of the experiment. Photosynthetic rate was measured using a portable photosynthesis system (Li6400XT, LICOR, U.S.A.) in an open system mode, between 1030 and 1230 h with a Photosynthetically Active Radiation (PAR) ranged between 400 and 2100 µE m^{-2} s^{-1}.

After twenty-four months, the soils were sampled in triplicates at 0.45 m of soil depth and 0.1 m from the plant stem using a soil coring machine (Eijelkamp Agrisearch Equipment, Model Cobra, The Netherlands) to determine the root length density.

Soil physico-chemical properties, respiration rate and erosion rate

The soil samples of each plot were taken at every six months interval. The samples were air-dried and milled to pass through a 2 mm sieve and stored in plastic bags prior to laboratory analysis.

Cation Exchange Capacity (CEC) was measured by summing the calcium, magnesium and potassium measured in the soil testing procedure with an estimate of exchangeable hydrogen obtained from the buffer pH (Warncke et al., 1980; Raman and Sathiyanarayanan, 2009). Furthermore, nitrogen content in soil was quantified as the important soil nutrients that affects the plant growth (Sardans et al., 2008) and the microbes are the one that providing a basic mechanism for producing plant-available N (Huang et al., 2015). Total N was determined using the modified Kjeldahl method (Bremner and Mulvaney, 1982). Soil respiration was measured at the study site between 1030 and 1230 h using the closed respiration chamber system (EGM-4CO_2, PP Systems, U.S.A.). Each plot was enclosed with 25 cm height of wooden wall and the eroded soils was channelled to the sediment tank. The eroded soil deposited at the bottom of the tank was calculated in g/m^3.

Statistical analysis

All the data obtained were analysed by a two-way analysis of variance (ANOVA) test ($P < 0.05$) using SPSS 20.0 software. Where significant, the first post hoc, least significant difference (LSD) test was calculated to compare the mean values. All results presented in the text are expressed as mean values ($n = 3$). Meanwhile the relationships between variables were determined by using regression analyses.

Results

Microbial abundance in soil

The fungal and bacterial abundances were ranged from 4.3×10^2 to 1.8×10^4 and 3.3×10^2 to 3.7×10^6 cfu/g, respectively. The F/B ratio varied between 0.02 and 1.52, being

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the highest in DM at the end of the experiment (*Fig. 2*). Fungal and bacterial abundances and the F/B ratio were shown to be affected by microbial application and vegetation density. The F/B ratio decreased in all treatments after two years of experiment except for DM and BM treatments.

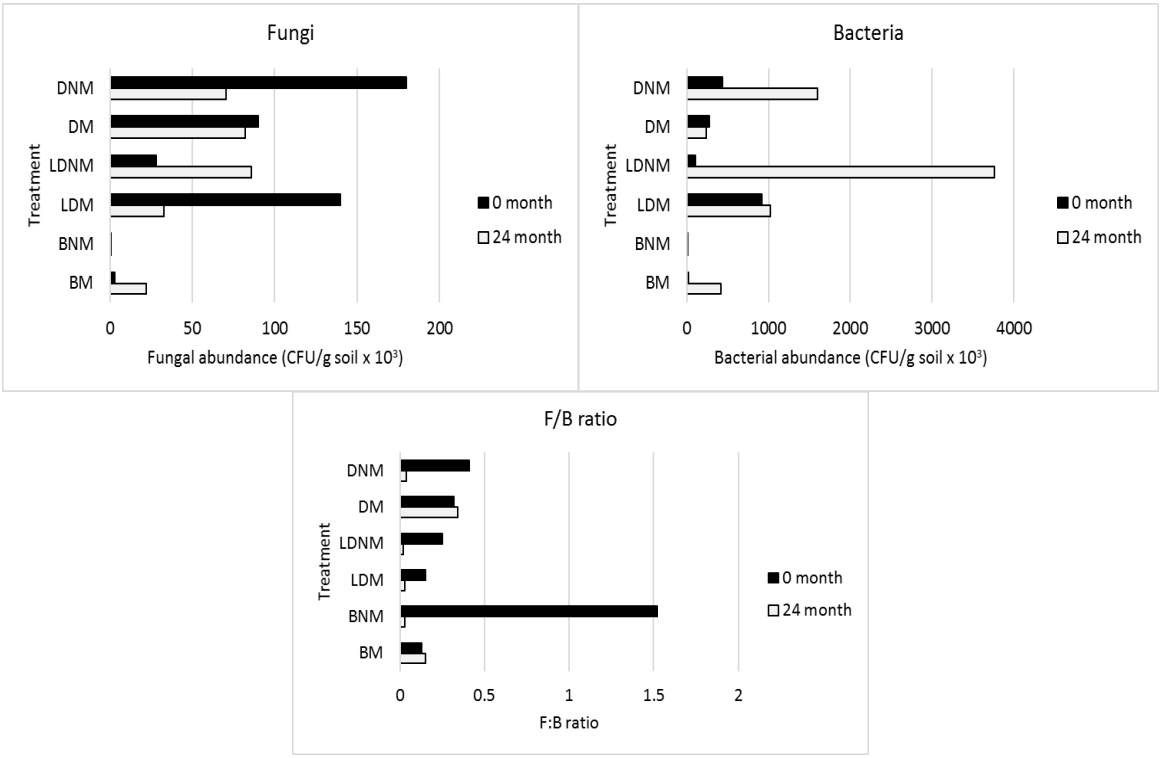


Figure 2. *Fungal and bacterial abundances and F/B ratio for microbes and vegetation density treatments on slope*

Plant growth performance

Figure 3 shows the photosynthetic rate and root length density performances of the species studied after a two-year bio-fertilizer application on the vegetation density treatment plots. *L. camara* in DM plot exhibited the highest photosynthetic rate followed by *M. malabathricum* and *B. purpurea*. In comparison between plots, photosynthetic rate of *L. camara* in DM plot was higher by 22%, 26% and 29% than that of in LDM, DNM and LDNM plots, respectively. Similarly, roots of *L. camara* grew rapidly in DM plot, attaining root length density of 284 cm m^{-3}, in which 66% of a total increment from the early of experiment. Moreover, all the species studied showed the higher total values of root length density in DM plot, followed by the plants in DNM, LDM, and LDNM plots.

Soil properties

Table 2 shows soil physico-chemical properties and respiration rate at the planting site after 24 months of bio-fertilizer application, grown with dense and less dense of vegetation densities. The microbial application had shown the prominent effects in reducing the porosity especially in the plots with vegetation density treatments (DM and LDM plots). Furthermore, vegetation coverage treatment with the bio-fertilizer

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amendment positively influenced the cation exchange capacity, indicating a greater capacity to hold cations. Whilst the soil N contents exhibited slightly increment at the end of the experiment except for control plot (BNM). A further analysis of soil performance in comparing between the initial and a 2-year treatment data had gave a better perspective on the changes in respiration rate. A significantly higher respiration rate was observed in microbe-treated soil, with dense plants density (DM plot).

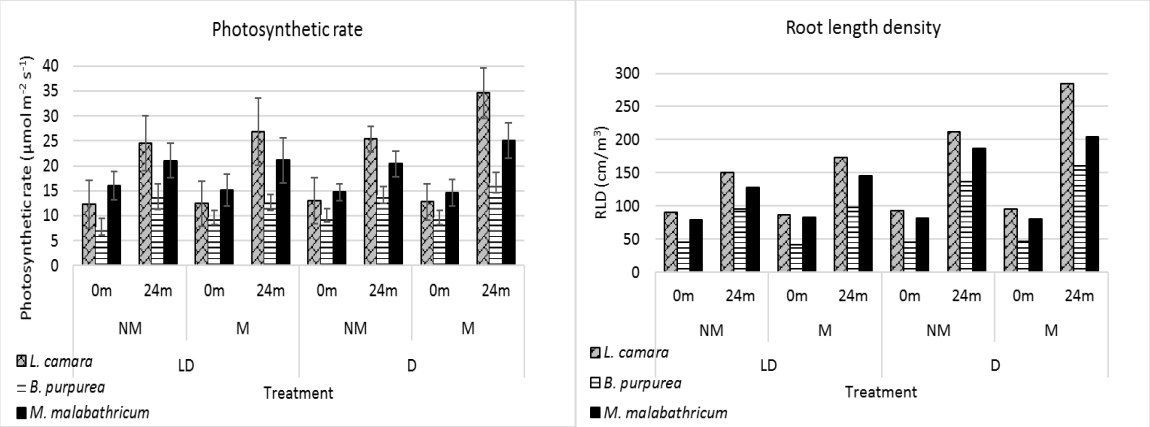


Figure 3. *Photosynthetic rate (left) and root length density (right)of the species studied at 0 and 24 month*

Table 2. *Descriptive data (mean ± standard deviation) for soil water content, physico-chemical properties and respiration rate of the experimental plots*

	CEC ($cmol^{(+)}/kg$)		**Total N (%)**		**Respiration rate ($mg\ CO_2\ m^2\ h^1$)**	
	0 m	**24 m**	**0 m**	**24 m**	**0 m**	**24 m**
DNM	7.68±0.3	10.61±0.4	0.11±0.03	0.15±0.04	421.5±198.6	837.8±480.3
DM	8.81±0.4	15.83±0.6	0.1±0.01	0.19±0.03	418.3±201.1	1103.5±577.3
LDNM	7.13±0.4	9.55±0.5	0.09±0.03	0.11±0.05	367.8±115.6	577.7±268.3
LDM	7.58±0.2	10.33±0.4	0.14±0.02	0.17±0.02	401.2±187.6	595.6±331.1
BNM	8.16±0.6	8.04±0.5	0.12±0.09	0.11±0.03	368.9±200.5	377.4±189.6
BM	6.97±0.4	7.52±0.4	0.09±0.02	0.11±0.04	388.1±211.7	402.5±225.7

Erosion rate

In general, the erosion rates were reduced through time in all treatments except for fluctuated trend observed in bare plot (*Fig. 4*). It was clear that erosion rate was strongly affected by vegetation type and total plant coverage. DM plot showed the lowest erosion rate, exhibiting the 52.6% of a total decrement from the initial experiment. Meanwhile, BM and BNM plots were presented inconsistent in erosion rate as no vegetation in these plots, hence, expose to the direct impact of rainfall to the soil surface.

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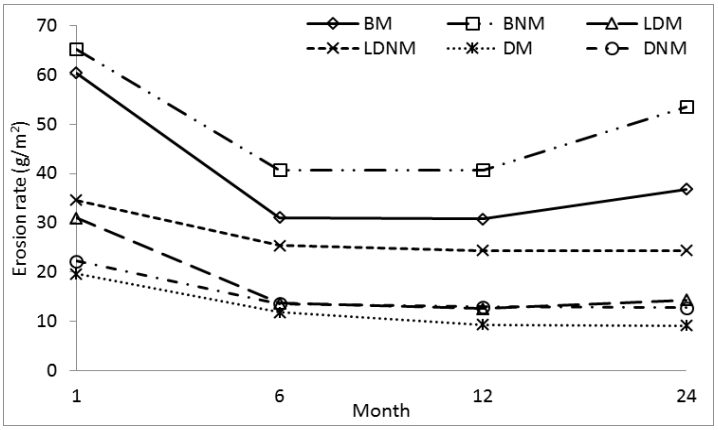


Figure 4. *The erosion rates of the experimental plots*

Relationship analysis

Some correlations were also observed in this study. The results showed that there is a relationship between microbes abundance and erosion rate (R = 0.7; *Fig. 5*). Likewise, microbes abundance seemed to have a weak correlation with plant physiology (photosynthetic rate) (R = 0.3; *Fig. 5*).

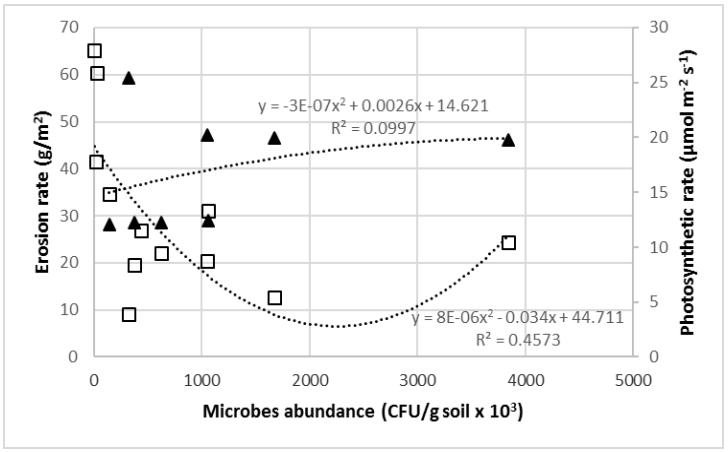


Figure 5. *Relationship between microbes, erosion rate and photosynthetic rate of the experimental plots*

Discussion

Our study showed an application of bio-fertilizer (microbes) in higher vegetation density on the slope with the species chosen as the pioneers, marked a positive response in microbe abundance after 24 months of observation (*Fig. 2*). Generally, in most cases, bacteria dominate under conventional tillage due to a direct contact between bacteria and substrate hence, encouraging the bacterial growth, whereas the fungi dominate under no tillage (Beare et al., 1997). In contrast, based on the abundance of the microbes observed throughout the experiment (*Fig. 2*), the bacterial abundance that we found were somewhat far higher than the fungal abundance since our study area is considered under no tillage. Finding by Malik et al. (2016) revealed that a lower fungal

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biomass has been linked to lower capacity of such soils to sequester C, material basis of soil fertility. Thus, our finding suggested that the vegetated-plot and the microbe amendment would establish a good condition for plant growth, which resulted in a good plant-soil interaction. Furthermore, a higher vegetation density contributed to the increase of decomposition processes in litter, in which the main decomposition pathways in soil are either bacterial-based or fungal-based (de Vries et al., 2006). Therefore, the biomass of fungi compared to bacteria can be considered as an indicator for the activity of two pathways of the soil food web, represented by fungal: bacterial (F/B) ratio (Malik et al., 2016).

The F/B ratio has been extensively used in soil ecology particularly in the context of land management (Strickland and Rousk, 2010). While it was not entirely clear the cause in the difference of F/B ratio on the studied plots, it appears to be due to subtle differences in the total of aboveground plants coverage. It indicates that microbial application and higher vegetation density in DM enhance the F/B ratio of the microbial community through ecological complementarity effects such as higher supply of resources for microorganisms (Lange et al., 2014). It was also observed that F/B ratio was extremely decreased in the control plot (BNM), implying that the bare surface of soil and direct raindrops impact suppressed the microbial community. Hence, we verify that microbial application and dense vegetation density can probably serve a good condition for microbial communities in soil hence, improve the fertility of soil and provide surface erosion protection.

Apart from that, the microbial application and dense vegetation cover had also shown a significant enhancement in photosynthetic rate and the root length density in plants chosen (*Fig. 3*). We do not anticipate the results as the high vegetation densities were expected to thrive in the highly competitive plant community. However, our finding verifies the prominent contribution of the species chosen as the outstanding pioneers on the slopes. On the other hand, microbial communities influence the plants by modifying soil structure (e.g. aggregate stability) via litterfall supply (Wallenstein et al., 2007), and by releasing photosynthetic products into the soil, which feeds heterotrophic soil microorganisms (Staddon et al., 2003). In return, soil microbial communities help in obtaining the nutrients for the plant use (Dobermann and Fairhust, 2000) as well as enhancing the soil fertility on slope.

Meanwhile, root reinforcement has been considered as the main mechanical contribution of vegetation to the stability (Yildiz et al., 2015). Throughout the 24 months of experiment, the total root length density (RLD) of DM plot was higher compared to the root densities in other plots (*Fig. 3*). The results were mainly attributed to high in microbial community and dense vegetation cover, which most likely resulted in increased of water absorption by root and soil water loss through transpiration. Consequently, these plant growth patterns; photosynthetic rate and root length density, had positive effects indirectly and directly, respectively, in increasing the safety aspect of the slope and enhancing the soil stability of the slope.

Based on the two-year observation of physiological performances of the species chosen, *L. camara* has shown the greater plant growth performance as compared to other species (*Fig. 3*). This finding was in line with Sharma et al. (2005), in which has described that this invasive species showed an outstanding performance in growth rate on the harsh environment. Thus, this result implies that the sun tolerant feature of *L. camara* as well as having higher number of branching and leaves, resulting in the increased of stomata and subsequently lead to the significant light utilization efficiency

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of the plants in photosynthesis. The second prominent species in this study was shown by *M. malabathricum.* Study by Aimee and Normaniza (2015) revealed that this species plays an important role as erosion control plant especially on highly eroded areas where it is low in nutrients content. This species also has dense root system that implies the ability of the species to absorb water from the soil. In addition, as the current global warming trend has remarkable changes in temperature and precipitation as well as rising the concentrations of CO_2 that may inhibit the plant growth (Parmesan, 2006), thus, we suggest that these two species can serve as good pioneers at the initial phase of succession on the slope.

The microbial application and vegetation density treatments were relatively effective in enhancing soil properties in the studied slope (*Table 2*). Cation exchange capacity (CEC) is an important measure of the soil's ability to retain and to supply nutrients (Raman and Sathiyanarayanan, 2009). The CEC values of the soils in the study area were affected by microbial amended and vegetation density. Adding microbes to the soil with dense vegetation cover caused a significant increase in the CEC of the soil, suggesting an improvement in soil fertility and nutrient retention (Jien and Wang, 2013). Additionally, further analysis of soil nutrient level comparing the initial and the final properties would give a better perspective on changes in fertility level. Soil with microbial amended and dense vegetation cover showed important improvement in total N, indicating that the presence of heavy litter and humus contents in the surface layers of the studied plot. Hence, the high amount of organic matter may also enhance the richness of N in the vegetated area as compared to bare surface.

Soil respiration from the soil surface is one of the most important components of the C cycle in terrestrial ecosystems (Adachi et al., 2006). Soil respiration rates observed in 24-months were greater than those observed at the initial study. Several studies had found that the soil respiration is composed of respiration from both roots and microbes, and there was a relationship between soil respiration and the underground environment (e.g. root and soil microbial biomass) (Søe and Buchmann, 2005; Neergaard et al., 2002). Thus, the high soil respiration rate observed in the microbes applied and dense vegetation cover treatment plots may have been due to high microbial activities.

Concomitant to the positive microbial-vegetation density feedback, soil erosion rates were steadily decreased in all treatments except in the bare plots (*Fig. 4*). The soil erosion rate decreased significantly in dense vegetation cover, indicating that the microbial incorporation reduced the potential of soil to be eroded. Therefore, based on our results, we deduced that the major reason for reduction of soil loss after the addition of microbes was the extensive integration between microbial community with the plants properties (root system), contributing to the aggregate stability and macroaggregate formation (Cantón et al., 2009), maintaining soil porosity and subsequently decrease the soil erosion.

A strong relationship between microbes abundance and erosion rate (R = 0.7; *Fig. 5*) implies that microbes-plant interaction improve the soil porosity and dense root length density (*Fig. 3*) could help to reinforce the soil by increasing its shear strength and alleviate the erosion. On the other hand, a weak correlation of microbes abundance with plant physiology (photosynthetic rate) (R = 0.3; *Fig. 5*) indicates that the dense vegetation cover is attributed to the higher in decomposition process, hence, enhance the nutrients available for the plant growth.

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Conclusion

A two-year observation revealed a positive influence of the microbial application and dense vegetation cover on the slope stability. The performance of the treated plots was accelerated when the promising pioneers, *Lantana camara, Melastoma malabathricum,* and *Bauhinia purpurea* were grown together in the plots. In particular, the increased microbes abundance lead to increase in CEC, increase in total N and respiration rate, indicating the outstanding microbes-plant interaction. The increase of photosynthetic rate and root length density of the species studied was considerably indirect influenced by the microbial activities in soil. In aftermath, positive effect on slope stability was proven in the negative relationship between microbe abundance and erosion rate. It is concluded that microbial amendment in soil with dense vegetation cover could positively influence the plant growth performance and soil quality, which subsequently may alleviate the soil erosion problem of the slope.

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CLIMATE CHANGE CAUSING FOOD INSECURITY IN EAST AFRICA: TRADITIONAL AND NON-TRADITIONAL STRATEGIES TO SOLVE THE PROBLEM

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Abstract. In East Africa, climate change is understood as a period of drought or floods. In real sense, it is the universal changes in the behavior of weather patterns based on statistical predictions for a long period of time. The region has experienced severe climatic changes of droughts, floods that negatively affect agricultural production causing food insecurity. This has caused both internal and regional migrations across East African borders especially by pastoral groups. These impelled migrations often cause insecurity. Literature searches have been used to gather information about what has been done and what should be done to safeguard communities from food insecurity caused by climate shocks. Results show that over 31% of the people are affected by undernourishment, an indicator of food insecurity and this has been to a great extent brought about by destruction of ecosystems which has enhanced climate change. Proposed mitigation and adaptation strategies include traditional and non-traditional measures. Policy issues have also been emphasized. In conclusion, destruction of fragile ecosystems contributes to climate change. Failure to adopt new technologies and neglect of some traditional technologies are greatly responsible for food insecurity in East Africa.
Keywords: *adaptation, anthropogenic, ecosystem, migrations, mitigation*

Introduction

In many parts of East Africa, most of the people perceive climate change as real due to variability in terms of temperature rise, rainfall reductions, erratic rains and prolonged droughts (Gichangi et al., 2015; Bryan et al., 2013; Souverijns et al., 2016; Leal et al., 2017). This is also a result of catastrophic rains characterized by storms, floods and landslides in some areas. Although perceived in such terms, it is understood inadequately. It is the universal changes in the behavior of weather patterns based on statistical predictions for a long period of time. It is any long term change in the statistics of weather over periods of time that range from decades to millions of years (IPCC, 2001). These changes are attributed directly or indirectly to the anthropogenic activities which affect sustainability of the biosphere in natural state, thus causing disruptive effects. In East Africa and Sub-Saharan Africa as a whole, there is an

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increase in awareness about climate change even when the initiative to take adaptation and mitigation measures are still low. Surprisingly, as most of the people in the developing world increasingly understand this phenomenon, there is a decline about climate concerns alongside an increase in skepticism regarding its seriousness and anthropogenic causes in the developed countries (Spence, 2011).

The National Aeronautics and Space Administration (NASA) observed that the Earth's 2016 surface temperatures were the warmest since modern record keeping began in 1880, and it may continue over years if no measures are put in place to counteract the effects (Potter, 2016). Due to these climatic variations, people have faced food insecurity as result of crop failure and livestock death. Warming and prolonged droughts in North Eastern Uganda, Kenya, Ethiopia, and Tanzania have caused movements of people across borders searching for water and pastures. This is because of insufficient rainfall which drives them to migrate to new areas (Salerno, 2016). This has created conflicts and insecurity, cattle raids, land degradation, killing of park animals, bush burning in traditionally cattle raiding areas and food insecurity (Ojore, 2017; Okoroafor, 2017; Johnston, 2017; IAWG, 2017) and has put pressure on the natural resource base.

The main objective of this paper therefore, is to suggest possible traditional and non-traditional adaptation and mitigation measures to address food insecurity as a result of climate changes.

Climate change in East Africa and its effects

East Africa has been hit by climate change leaving millions at the verge of starvation as thousands of livestock die and crops fail due to drought. There is continuous decline in precipitation amounts (Souverijns et al., 2016) as temperatures continuously rise. In many areas of the region, rain-fed agriculture has practically failed. It is estimated that about 11 million people are dangerously food insecure and in need of humanitarian assistance (OXFAM, 2017). Although most areas have been affected by drought, some areas have been affected by floods i.e. Eastern Uganda where over 400 people died due to floods (The International Federation's Disaster Relief Emergency Fund, 2010). Climate change will influence human mobility, resulting in humanitarian crises (Baldwin, 2017). This will create climate migrants or climate refugees and may result into disease outbreak and competition for resources in the recipient areas. Mobility of people with large herds of animals will not only cause conflict but also poor management of animal manure. This will increase greenhouse gases (GHGs). In Ethiopia which has the largest livestock population in Africa (Yohannes, 2016; Amdu, 2010), the large numbers of livestock contribute greatly to emission of GHG. According to a report published by Ethiopian government, 50% of GHG emissions in the country are attributed to agriculture. Livestock was estimated to have contributed 65 Mt CO2 equivalent in 2010 (FDRE, 2011).

Agricultural production in the region contributes greatly to the Gross Domestic Product (GDP) and is dominated by smallholder farmers. The adverse climatic effects will affect food production and will substantially reduce the economic base of all the countries in the region. This will affect job creation for the population, majority being the youth, affecting their livelihood. The population growth in the region is among the highest in the world (African Union, 2017) and employment is a big problem and any effect on agricultural production will exacerbate the situation. Furthermore, malnutrition and high mortality in children is likely to increase due to failed agricultural system

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which does not produce sustainably for such a large population. The prices for staple food crops like cereals will rise at the detriment of poor consumers (Karugia et al., 2011).

Climate change will increase human suffering by affecting infrastructure especially in city suburbs as well as agricultural production. This will negatively impact the health of many people (Labbé et al., 2015) and food security respectively.

Materials and methodology

Profile of the study area

East Africa is a region that begins in Tanzania in the south and extends north through the great grasslands of Kenya and Uganda and Ethiopia, including the Great Rift Valley (*Fig. 1*).

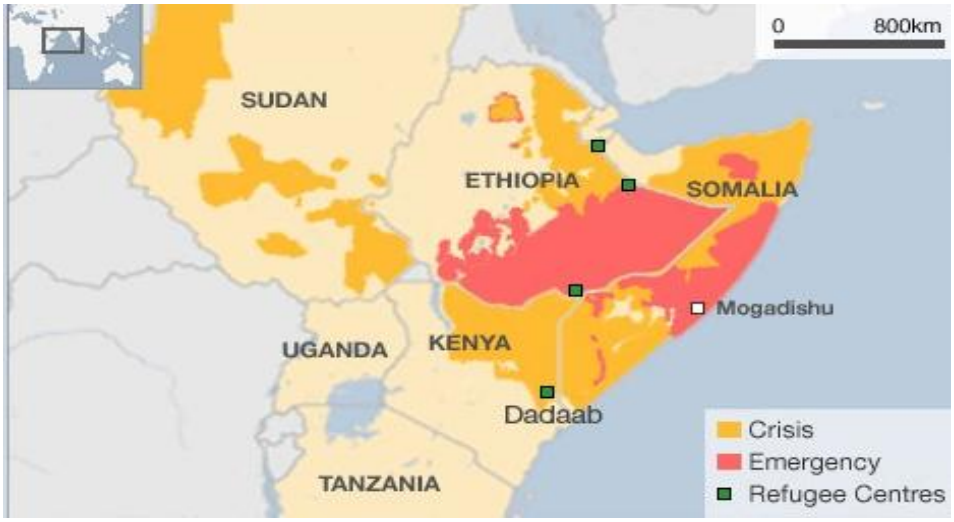


Figure 1. *Areas affected by food insecurity and climate change in East Africa. (Source: UNHCR/USAID, 2011)*

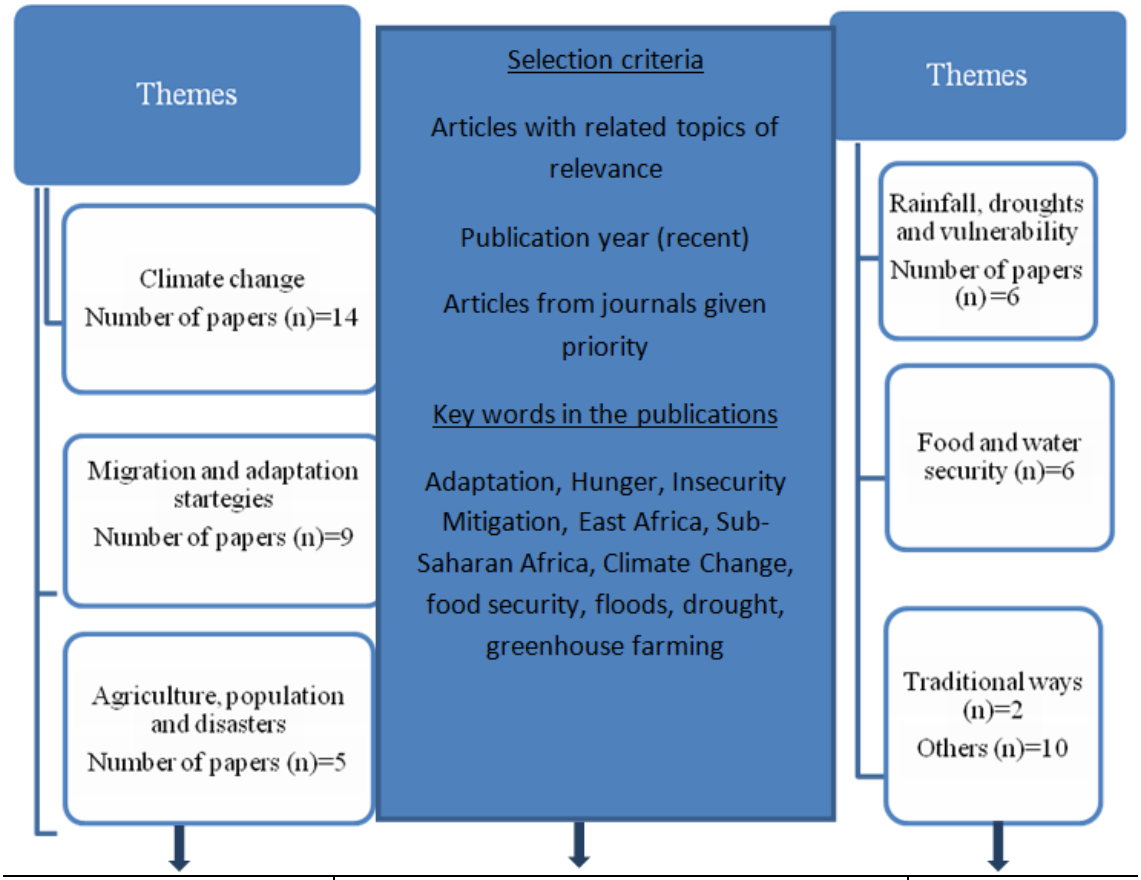
The region also comprises the countries of South Sudan, Somalia, Djibouti, Eritrea, Rwanda and Burundi.

The world's second-largest lake by surface area is Lake Victoria, which borders Uganda, Tanzania, and Kenya.

In this article, we obtained data from different sources, mostly indexed journals such as Climate change, Environmental Management and other popular sources. UN Organizations such as FAO were also searched to get reports. In our search, we focused on papers that were published in English and are recent 2010-2017. However, some papers were published in 1996, 1999, 2001 and 2005. These were included to highlight on what was previously done and recent work. The terms commonly used in the search strategy were climate change, adaptation, mitigation and food security in East Africa. To manage and screen data well, the articles were grouped into themes and sub-themes. In total, 128 papers were retrieved for possible inclusion from where 52 papers were selected as seen in *Table 1*.

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Table 1. *Summary of the key main articles selected. (Source: Authors)*



Sub-themes	Key findings	Authors
Silos/post harvest handling	Currently, most food produce is sold from the garden directly. There is nothing to keep in silos and no silos anymore	Afedraru, 2013
Climate change responses	Climate change has affected and changed the Masai traditional culture	Leal et al., 2017
Population	The population growth rate in East Africa is one of the fastest on the continent	African Union, 2017
Farmers perception	Farmers in Ethiopia recognize temperatures, frequency of drought and flood. Rainfall pattern has become unpredictable, a sign of climate change	Amdu, 2010
Climate change and migrations	Migrations due to climate change have resulted into climatic refugees	Baldwin, 2017
International trends in public perception about climate change	The seriousness of climate change is exaggerated, with an almost doubling of this perception over the 5-year period	Capstick et al., 2015
Adaptation to climate change by smallholder farming communities	The key adaptation measures include: practices that improve soil fertility and moisture, increase soil carbon storage, reduce soil degradation and increase crop diversification	Chandra et al., 2016
Hydrological cycle	Human activities affect hydrological cycle which influence global climate changes and agricultural production	Evans, 1996
Food insecurity and vulnerability	Food and water security of countries still need to improve despite slight progresses	FAO, 2001

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Climate change, water and food security	Policy response to climate variability and change should be flexible and sensible	FAO, 2011
Climate-smart agriculture	Most of the impacts of climate change in agriculture and rural livelihoods are expected to result from changes in the water cycle hence rainfall variability	FAO, 2013
Communities and climate change adaptation	Policies should undergo periodic evaluation to incorporate traditional knowledge and science among different communities to adapt to climate change	Ingty, 2017
Agriculture and water management in Uganda, Burkina Faso and Morocco	Water harvesting holds a great potential in increasing yields and improving food security in rain-fed farming areas	FAO, 2016
Edible insects	Insect rearing for food remains a sector in its infancy	FAO Forestry Paper, 2013
The state of food insecurity in the world	Prevalence of food insecurity and malnutrition is significantly higher in protracted crisis contexts resulting from conflict and natural disasters	FAO, IFAD and WFP, 2015
Ethiopia's climate-resilient green economy:	Livestock emissions are estimated to be 65 Mt CO2e in 2010 – more than 40% of total emissions today. Crop cultivation contributes to concentration of GHGs by requiring the use of fertilizers	Federal Democratic Republic of Ethiopia (FDRE) (2011)
Hidden hunger	A new focus must be undertaken to address longer-term determinants of nutrition insecurity and hidden hunger	Francesco et al., 2011
Temperature and climate	Due to increasing climate change, there is a likelihood of air temperatures exceeding the optimum range for many species of crops. Cool season crops will have a constrained growing season due to high temperatures	Hatfield and Prueger, 2015
Drought crisis	More than 15 million people in Ethiopia, Kenya and Somalia are facing food and water shortages	IAWG, 2017
Adaptation and vulnerability	Developing countries, particularly the least developed countries, have lesser capacity to adapt than do developed countries	IPCC, 2001
Encroachment of wetlands	Multi-faceted approach, coordination of the various stakeholders and engagement of wetland-dependent communities as part of the solution, might require zoning out the wetland and restricting certain activities to specific zones	Isunju, 2016
Tackling climate change	Without global efforts to reduce emissions and to help the world's poorest people cope with the effects of climate change, this crisis will continue to repeat itself	Johnston, 2017
Food prices	The prices for staple food crops like cereals will rise at the detriment of poor consumers	Karugia, 2011
Adaptation to climate risks in East Africa	Adoption of adapted varieties of crops might be successful to enhancing resilience of farming systems in the short-term	Kelvin, 2017
Beekeeping as integrated watershed conservation and climatic change adaptation	Beekeeping is an environmentally sound, and economically feasible income generating activity for poverty alleviation and food security	Kumsa and Gorfu, 2014

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Vulnerability to the health effects of climate variability, Uganda	Vulnerability to the health impacts of climate change will be shaped by existing burdens of ill-health, climate-sensitive infrastructures, limited technological capacity and political inequality and is expected to be highest in socioeconomically marginalized populations	Labbé et al., 2015
Achieving mitigation and adaptation to climate change	Agroforestry systems readily bundle both mitigation and adaptation strategies and provide several pathways to securing food security for poor farmers, while contributing to climate change mitigation	Mbow et al., 2013
Vulnerability to climate change	Higher standards of living in Tibet have enabled herders to deploy the coping strategies of sheltering livestock and feeding them supplements	Yeh et al., 2014
water availability in a changing climate	Changing rainfall patterns affect seasonal availability and manageability of water	Milly, 2005
Migration as an adaptation measure	A model to predict future climate change-migration relationships developed	MCLeman and Smit, 2006
Food relief in North East Uganda	Elderly and children affected by drought. 80 people died because of lack of food in 2 counties	Ojore, 2017
Drought in East Africa	Thousands of animals including cows, goats, and sheep have already been lost	Okoroafor, 2017
climate change and drought in East Africa	Nearly eleven million people in Kenya, Ethiopia and Somalia are dangerously hungry and in need of humanitarian aid	OXFAM, 2017
Temperature rise	Earth's 2016 surface temperatures were the warmest since modern record keeping began in 1880	Potter, 2016
Neglected and underutilized crop	Key to future food and nutrition security may very well lie in the untapped potential of neglected and underutilized crops	Pauline, 2015
Rainfall distribution patterns	Reduced interannual variability occurred, the percentage of dry years did not increase but the percentage of wet and very wet years decreased	Ramos, 2001
Indigenous knowledge related to climate variability	The rich indigenous knowledge can be combined with science to monitor climate change	Speranza et al., 2017
Food security and seasonal climate	Extreme climate events, such as droughts and floods, and lower agricultural productivity contribute to food insecurity	Rarieya and Fortun, 2010
Managing vulnerability to drought and enhancing livelihood resilience: Technological, institutional and policy option	Drought represents one of the most important natural factors contributing to malnutrition and famine that affects the most vulnerable communities, especially women, children and the elderly	Shiferaw et al., 2014
East African precipitation	Precipitation amounts over East Africa have been declining over the last decades	Souverijns, 2016
Perceptions of climate change	There is a decline about climate concerns alongside an increase in skepticism regarding its seriousness and anthropogenic causes in the developed countries	Spence, 2011
Africa's hidden hunger	Many families in East Africa, eat one meal in a day	Stefan, 2012
Attribution of extreme weather and climate	Climate models show an increased occurrence of extreme temperature and rainfall events worldwide which are attributed to anthropogenic activities	Stott et al., 2016

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Floods and landslides	An estimated 400 people were killed by floods and landslides in Eastern Uganda	DREF, 2010
Lake Victoria ecological changes	Decline in fish species is the highest documented loss of biodiversity caused by humans in an ecosystem	Witte, 1999
Climate change and agriculture	Climate change has relationship with agriculture in one or another way	Yohannes, 2016
Climate variability-farmers perception, Kenya	Farmers are aware of climate changes because of decrease in rainfall amount, rainfall variability, erratic rains and prolonged drought periods	Gichangi et al., 2015
Adapting agriculture to climate change-Kenya	Households face considerable challenges in adapting to climate change, need for greater investments to support households to make strategic, long-term decisions	Bryan et al., 2013
Migrations and decision making	Insufficient rainfall drives agro pastoralists to migrate to new areas	Salerno, 2016
Climate change and environmental threats	Increased evaporation rates, will increase levels of salinity in the lake, harm fish stocks worsening food security situation	Human Rights Watch, 2015
Drought tolerant maize for farmer adaptation	Major barriers to crop adoption is unavailability of seeds	Fisher, 2015
African indigenous cattle: Unique genetic resources	African livestock genetic resources are in danger due to uncontrolled crossbreeding	Mwai, 2015

Results

From *Table 1,* six themes have been developed to summarize information published and give suggestions on mitigation and adaptation strategies.

Anthropogenic activities contributing to climate change in East Africa

Anthropogenic activities have caused challenges to mankind (Stott et al., 2016) by greatly contributing to climate change. There is a growing trend in awareness about this in some parts of the world including East Africa although in other areas, there is a decline in knowledge by the public about climate change being caused by human actions (Capstick et al., 2015).

Activities such as forest clearing for charcoal, bush burning as a way of facilitating growth of fresh grass for animals and swamp destruction for agriculture contribute greatly to climate change. They negatively influence biogeochemical cycles of evapotranspiration, hydrological cycles, rainfall distribution and amount, increase flood and drought frequencies (Evans, 1996; Ramos, 2001).

Industrial and agricultural development

Climate change with reduced precipitation levels has characterized most of the areas in East Africa. Over the past several years, Ethiopia has embarked on a massive plan for dams, water-intensive irrigated cotton, sugar plantations, irrigation canals and other infrastructural developments in the Omo River Basin. Predictions indicate that these developments could have a devastating impact on water bodies particularly Lake Turkana, dropping water levels and potentially causing the lake to recede into two small pools. If these predictions are accurate, areas of Lake Turkana with high levels of

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biological productivity that are critical for fish breeding will dry up causing significant effects on fisheries. In addition, dramatic reductions in freshwater from the Omo River into Lake Turkana, will increase salinity and temperature in the lake. This will harm fish stocks worsening the already dire food security situation in the region and affecting the lives of many people (Human Rights Watch, 2015).

In *Figure 2a,* eucalyptus trees were planted by a tea factory in what used to be a wetland, to be used for firewood as a form energy in tea processing and it has drained the water leaving the land dry while in *Figure 2e,* the water level and quantity has dropped substantially due to drainage and destruction of the vegetation. Most ecosystems in Uganda and East Africa as a whole have been destroyed and this hinders carbon sequestration processes, increasing greenhouse gases (GHGs).



Figure 2. *A destroyed ecosystem in Hoima District, Uganda. (Source: Authors)*

Food and water security in East Africa due to climate change

Food and water are inseparable and are useful for sustenance of life. They influence the quality of life people have. Despite having significant land and water resources, East Africa is one of the world's most food-insecure regions. There is water stress despite the

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presence of rivers and lakes. River Nile, Lake Victoria, Lake Tanganyika and Lake Turkana are some of the important water bodies in the region. Current management of these water resources needs improvement as a result of exploitation of fishery resources and pollution of water ecosystems by industrial and agricultural activities. The Nile basin which covers the biggest area in the region is an example of a drainage system that would shock absorb the adverse effects of climate change if managed sustainably by all countries in the Nile Basin Initiative. The technology to draw water for various agricultural operations is still undeveloped. Agro-pastoral groups move across borders instead of tapping such water that is near them. Changing rainfall patterns affect seasonal availability and manageability of water, and by 2070, there will be less water in East Africa and other parts of the world (Milly et al., 2005; FAO, 2011).

More so, the water level in Lake Victoria has dropped and warmed. The surface water has warmed by almost 1.2°C in 82 years, and the temperature has risen by 1.6°C in water greater than 50 m deep over the same time period (FAO, 2016). Whereas global climatic changes may have primarily contributed to these changes, anthropogenic activities like forest and swamp destruction cannot be underestimated. High temperatures will impact fisheries productivity by lowering the quantity and size of fish but may affect water ecology as well. The warm temperatures lead to faster depletion of the limited oxygen supply, negatively impacting fish development. The impact of climate change on commercial fishing in East Africa's water bodies is uncertain, and reductions in water levels and the impacts of extreme climate events resulting from climate change are likely to have significant negative effects on the water bodies. Decline in fish species in Lake Victoria is the highest documented loss of biodiversity caused by humans in an ecosystem (Witte, 1999). This is a big concern as fish is an important source of protein and income to many people.

According to the Food and Agriculture Organization of the United Nations (2001) "Food security is a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life". This means that food should be available, accessible, utilizable and stable. The current food situation in East Africa cannot make it possible for most households to have enough, nutritious and even have preference of certain foods to others. The food insecurity problem is compounded by climatic changes of drought and floods which affect agricultural production in many ways (Rarieya and Fortun, 2010).

Besides, there is another problem of "hidden hunger" that affects many communities. This is caused by low intake of vitamins from vegetables. This is a serious public health concern affecting many families in East African countries (Stefan, 2012). Surprisingly, it is not understood well and for that reason, most people are not aware about it (Francesco et al., 2011). Climate change is a complex global problem involving food constraints, water scarcity even in abundance, movements of people, climate health related illnesses and above all occurrence of unpredictable disasters (*Table 2*).

Low developed countries are the most affected, particularly Sub-Saharan Africa and South-Eastern Asia. People line up early in the morning hours to collect water in some areas in Uganda (*Fig. 3a*) while in *Figure 3b,* women and children trek long distance to get water from a river whose depth has also been reducing due to a long dry spell.

The situation is worse in conflict stricken areas like Somalia where there are internally displaced people (*Figure 3c*). The dwindling water levels in surface water and other water sources is related to anthropogenic activities.

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Table 2. *Undernourishment in different parts of the world, emphasis on East Africa. (Source: Adapted from FAO, IFAD and WFP, 2015)*

	2005-07		2010-12		2014-2016	
	No.	**%**	**No.**	**%**	**No**	**%**
WORLD	942.3	14.3	820.7	11.8	794.6	10.9
Developed regions	15.4	<5.0	15.7	<5.0	14.7	<5.0
Developing regions	926.9	17.3	805.0	14.1	779.9	12.9
Africa	213.0	22.7	218.5	20.7	232.5	20.0
Northern Africa	7.0	<5.0	5.1	<5.0	4.3	<5.0
Sub-Saharan Africa	206.0	26.5	205.7	24.1	220.0	23.2
Eastern Africa	122.5	37.8	118.7	33.7	124.2	31.5
Middle Africa	47.7	43.0	53.0	41.5	58.9	41.3
Southern Africa	3.5	6.2	3.6	6.1	3.2	5.2
Western Africa	32.3	11.8	30.4	9.7	33.7	9.6
Asia	665.5	17.3	546.9	13.5	511.7	12.1
Caucasus and Central Asia	8.4	11.3	7.1	8.9	5.8	7.0
Eastern Asia	217.6	15.2	174.7	11.8	145.1	9.6
South-Eastern Asia	319.1	20.1	274.2	16.1	281.4	15.7
Southern Asia	319.1	20.1	274.2	16.1	281.4	15.7
Western Asia	17.2	9.3	18.4	8.8	18.9	8.4
Latin America and the Caribbean	47.1	8.4	38.3	6.4	34.3	5.5
Caribbean	8.3	23.5	7.3	19.8	7.5	19.8
Latin America	38.8	7.3	31.0	5.5	26.8	<5.0
Central America	11.6	7.6	11.3	6.9	11.4	6.6
South America	27.2	7.2	ns	<5.0	ns	<5.0
Oceania	1.3	15.4	1.3	13.5	1.4	14.2



a **b**

c

Figure 3. *Water shortages in different areas of East Africa. (Source: Authors)*

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Food security and water access in the region remain one of the serious development bottlenecks that impact other key sectors and have led to dismal realization of sustainable development goals.

Misuse of natural resources

In many ways, water, wetlands have either been diverted, drained in expansion of cities or polluted and degraded with permanent horticultural facilities such as greenhouse facilities which discharge effluent (Entebbe in Uganda) and Kisumu in Kenya all located on the shore of Lake Victoria. These activities affect water quantity and quality. When water is displaced from its natural watershed where it sustains healthy ecosystem, due to diversion or expansion of towns, natural hydrological cycle is affected. Agrochemical pollutants from the greenhouse flower farms end up in surface water causing eutrophication. This affects fisheries development. It also creates a need to search for ground water using boreholes which reduce the water cycle. Urbanization, deforestation and wetland destruction greatly destroy water-retentive landscapes and lead to the loss of precipitation over the affected area. If there are no trees to receive the rain, it will evaporate, creating desert-like conditions. An example is Kampala, Uganda's capital city, where Nakivubo wetland covered by natural vegetation has reduced by approximately 14% in 5 years while cultivated area in the lower Nakivubo wetland had increased by more than 350% in the same period. (Isunju, 2016).

In *Figure 4a,* a bulldozer clears the area for a flower farm in Lutembe, Entebbe-Uganda. The area, deemed to be of international importance under an international convention on wetlands was destroyed to set up a flower farm on a bay off the shores of Lake Victoria. In *Figure 4b,* buildings have been constructed there and the wetland is no more while in *Figure 4c,* the residents of Ndemi, Michore, Kieni and Rukuma, destroy pipes that are used to draw water from river Malewa by Mahee Flower farm in Kenya. The residents accuse Mahee Flowers farms of polluting the water with chemical effluents and blocking water movement.

Discussion

As seen in *Table 1,* a number of researches have been done about climate change and people in the region are aware of the problem. Climate change theme is a dominant topic that is discussed and covered in most researches. It is however evident in most papers and reports that the issue of traditional measures and technologies to address food insecurity have not been emphasized in mitigating effect. Most publications have tackled on causes and implications of the problem. It is important that research on non-traditional technologies and modern technologies be emphasized (*Fig. 5*) and if possible be improved for the sake of resource constrained people who are the majority in the region. This can promote sustainable and inclusive development. Policies that encourage farmers and emphasize the re-establishment of such structures should be implemented.

Furthermore, the problem of food insecurity is exacerbated by some farmers who sell food from the garden directly, *Table 1*. This could be due to economic hardships faced by the people, and a weak government policy that does not enforce keeping food in silos to prepare for disasters like droughts. The issue of food security and climate change seem to be intertwined that it is almost impossible to talk about one while leaving the other.

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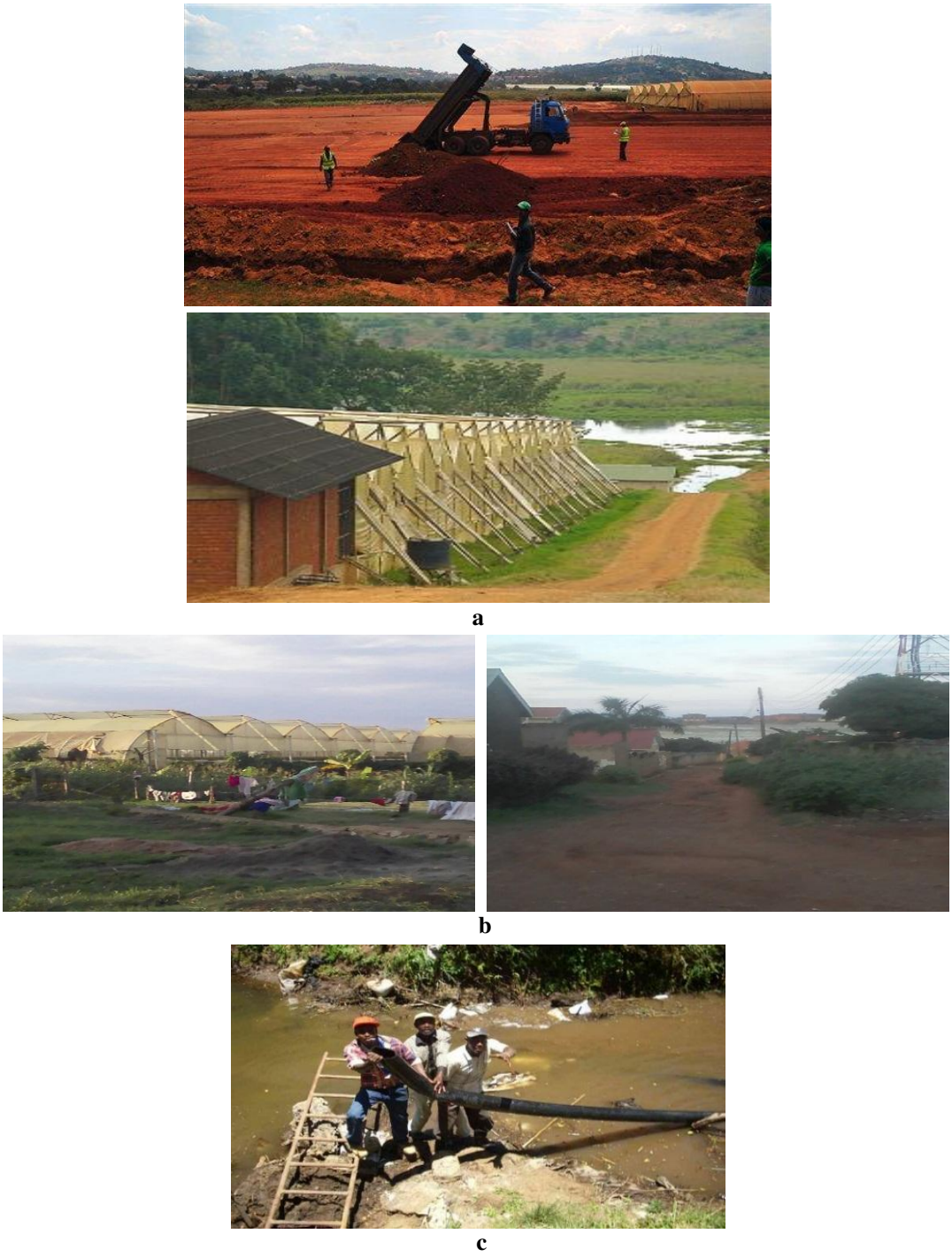


Figure 4. *Destruction of wetland and river ecosystems in Uganda and Kenya. (Source: Authors)*

The destruction of ecosystem for agricultural activities, urban development is one of the reasons that partly explain why climatic disasters continue to affect East Africa. Many fragile ecosystems have been damaged in most areas (*Figs. 2* and *4*). Most agricultural activities take place in areas that act as buffers for carbon sequestration i.e. wetlands and forests. When these are destroyed, there is a disturbance in carbon dioxide flux management, hydrological cycles and species extinction that are interlinked in a complex manner. This situation is intensified by grazing huge numbers of livestock that

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move within countries and across the region as is it the case with almost all pastoral groups in East Africa (Karamajong of Uganda, Turkana of Kenya, Masai of Tanzania and the Ethiopians). This causes soil erosion due to vegetation loss and emission of GHGs, thus global warming. The phenomenon is more complex with other problems of wars in South Sudan, Somalia and general political instabilities in the region which contribute to food insecurity.

It is evident that the number one climatic disaster affecting East Africa is drought (*Table 3*). It causes more misery than all the other disasters combined and is more recurrent. This increasing trend and frequency in drought is a sign of degenerating environmental problem that is brought about by climate change induced by anthropogenic activities. The frequency of droughts is higher than that of any other climatic disaster. This explains the movements of pastoral groups across borders where they end up fighting with the local people hence increasing insecurity.

Table 3. *People affected by climatic disasters in Uganda, East Africa, since 1979. (Source: Adjusted from Climate Policy Paper, 2012)*

Year	Population	Disaster
1979	500000	Drought
1987	600000	Drought
1997	153500	Floods
1997	100000	Epidemic
1998	126000	Drought
1999	700000	Drought
2001	655000	Drought
2005	600000	Drought
2007	718045	Floods
2008	11000000	Drought
2010	338	Landslides
2013	500000	Drought

Hunger and "hidden hunger"

Pregnant women, children and elderly need vegetables regularly. Unfortunately, most of indigenous vegetables are at the verge of extinction in most communities, especially in war torn areas of South Sudan and Somalia. This is because of inadequate conservation measures and insecurity. Those vegetables used to be grown in back yard gardens but in recent times, people live in urban areas and cannot access them. East Africa remains the sub-region with the biggest hunger problem, with over 31% undernourished people (FAO, IFAD, WFP, 2015). This phenomenon is further complicated by inadequate clean water supply in most of the rural areas and some areas in urban centers of East Africa (*Fig. 3*).

Suggested mitigation and adaptation measures to guard against food insecurity

1. Traditional strategies

Traditional ways of guarding against food insecurity in East African communities were very important in preparing people for any future disaster that could occur. These

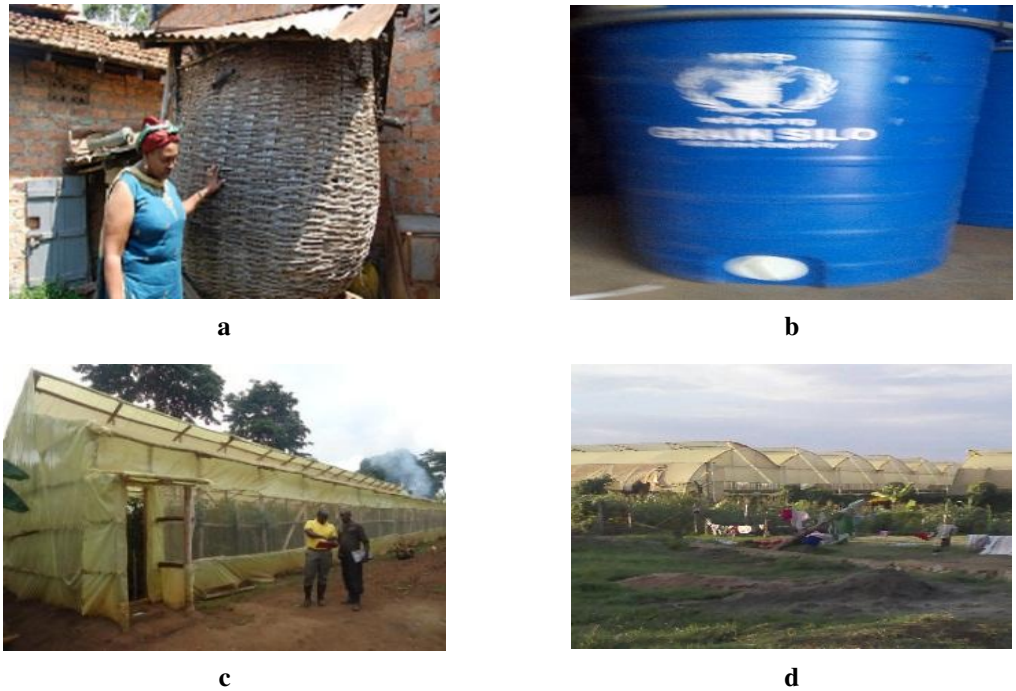
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technologies were cheap and did not need any expertise to harness. In most communities however, these simple technologies phased out. Therefore, communities and policy makers should do the following:

Revive use of silos

Most areas in East Africa used to have silos controlled by government at sub-county level where farmers would collectively store food but also, individual families owned silos for grains (Afedraru, 2013). They played a crucial role in periods of bumper harvests to store what the markets could not absorb at reasonable prices. This practice worked well to safeguard families from future risks of climatic disasters. On the contrary, most of the farmers in most communities nowadays, have no silos and sometimes sell their food crops even when it is still in the garden risking the lives of their members in case of food shortage. The few government silos that are still in existence are empty and in a very bad state. This has been noticed in recent times when most communities in the region, particularly Uganda, have been affected by various disasters, especially drought.

Traditional silos (*Fig. 5a*) are very few and even where they exist, they have no grains to store. Modern silos (hermetic) (*Fig. 5b*) are being promoted by WFP in some parts of East Africa. Improvised greenhouses (*Fig. 5c*) can be useful to resource constrained farmers while commercial farmers can use semi-automated greenhouses (*Fig. 5d*) to address food insecurity and erratic weather. In mitigating impact of climate change on food security, silos are not an option but a requirement (Afedraru, 2013).



a **b**

c **d**

Figure 5. *Technologies that need promotion and adoption. (Source: Authors)*

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Growing indigenous vegetables

This can address the problem of "hidden hunger". Most of the homesteads in East Africa used to have these vegetables in backyard gardens. They are unique and well adopted to the local conditions. They can reduce GHG emissions which come from heavy use of fertilizers and pesticides used in growing introduced varieties. They respond well to organic soil amendment, can grow reasonably well in water scarce conditions (Pauline et al., 2015) and are resilient to climate change (*Fig. 6*).

Most of these vegetables are rich in vitamins. However, most farmers have opted to grow the hybrid varieties because of economic gains leaving indigenous species at the verge of extinction.



Figure 6. *Indigenous vegetables at the verge of extinction in East Africa (a) Lycoperscum esculentum (b) African night shade (c) Solanum anguivi. (Source: Authors)*

Apiary projects

Combined with agroforestry, it is possible to run these profitable enterprises. In a situation where food is scarce, honey can be a good source of carbohydrates, medicine and source of income. Beekeeping is an environmentally sound, technically suitable and economically feasible income generating activity for poverty alleviation and food security (Kumsa and Gorfu, 2014). Beekeeping could be one of the major sources of income, food for people in local communities that are struggling with climate change.

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2. *Non-traditional strategies*

Non-traditional measures are modern ways, services and technologies that can enhance resilience of communities to effects of climate change in enhancing food security. They are more expensive compared to non-traditional measures and require expertise.

Insurance

With climate change continuing to ravage the region, farmers need viable and affordable ways to manage these weather-related risks which threaten food security and economic stability. Most farmers in East Africa lack access to insurance services.

Greenhouse farming

Farmers need to adopt the use of this technology to grow crops. It has many advantages i.e. growing of crops throughout the year, easy management of pests and diseases and efficient water use. Although there are challenges with this type of farming like the cost of the technology, especially to smallholder, financially constrained farmers, there are many benefits if well maintained. Governments needs to draft policies on how to help farmers access these important agricultural technologies cheaply and where possible, establish agricultural financing mechanisms to enable resource constrained but knowledge equipped farmers to have them.

3. *Other strategies*

Acceptance of new foods

In some areas of East Africa, certain foods like insects and worms are not eaten due to negative perceptions yet they are a good source of protein just like fish, chicken and meat. If promoted as food, insects emit fewer GHGs (FAO Forestry Paper, 2013) than livestock. There are diverse types of insects that can play nutritional roles and they are easy to farm compared to traditional sources of animal protein. Incorporation of insect farming in other farming enterprises is a good way of diversifying livelihoods and enhancing resilience to climate change (Chandra et al., 2016).

Good Agricultural Practices (GAPS)

Agroforestry programs

These should be integrated in all aspects of land and natural resources management. Activities such as cutting trees should be prohibited with strictness. As a land use system, agroforestry provides immense benefits such as CO2 sequestration and enhances soil fertility. Most of these functions have direct benefits for local adaptation while contributing to global efforts to control atmospheric GHG concentrations (Mbow et al., 2013). Agroforestry approaches are sustainable and cheaper. Some of the known agroforestry trees that are of use include: mango (Mangifera indica), Paw paw (Carica papaya), mulberry (Morus alba). Agroforestry provides many of the principles for developing climate-smart agricultural systems (FAO, 2013).

New crop varieties and resistant animal breeds

These can be tolerant to drought conditions and survive heat stress respectively. Climate change greatly affects crop yield by limiting crop growth and development

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(Hatfield and Prueger, 2015) and therefore food crops i.e. maize, beans need to be drought resistant and high yielding. Cash crops like coffee can also be of resistant varieties. An example is the Drought Tolerant (DT) maize varieties that have been developed and tested by the Drought Resistant Maize for Africa (DRMA) project in Eastern and Southern Africa, with superior characteristics. The challenge is adoption due to unavailability of improved seeds, high prices and inadequate information (Fisher et al., 2015). As for animal breeds, some local pastoral groups have kept their indigenous breeds that withstand local conditions although others have made steps to cross breed for resistance, milk and beef production. Generally, breeding improvement programs of African indigenous livestock are still few even when the demand of livestock products is continually increasing (Mwai et al., 2015).

Water storage

This is one of the biggest challenges that most smallholder farmers still face. This is because most of the technologies required to harvest and store water for a considerable long time require money. Construction of ponds and other surface water storage methods to facilitate micro irrigation should be enhanced. Water harvesting is an integral part in agricultural production to eradicate hunger. In situations where farmers are unable to harvest and store water using dams, reservoirs and tanks, they should conserve soil moisture by mulching, zero tillage and terraces.

Settled farming

Although migrations are taken as an adaptation strategy to reduce vulnerability to harsh environmental conditions (Ingty, 2017; McLeman and Smit, 2006; Speranza et al., 2009; Yeh et al., 2014), different communities in East Africa have problems relating to land i.e. there is no more land to allow unplanned migrations. This is due to population growth influencing people to protect their land. Governments need to plan and set laws that restrict movement of animals in large numbers to new areas. Farmers should be encouraged to plan agricultural activities which can make individuals develop a sense of responsibility and ownership. Land in East Africa has caused conflicts especially in Uganda.

Maintain and restore degraded ecosystems

Destruction of ecosystems is one of the serious causes of GHGs, which contribute to warming of the Earth. In some communities, forests and swamps have been degraded (*Fig. 2*). Restoration of damaged ecosystems is key in sequestration of carbon.

Policies

Climate change policies and laws need to be harmonized to tackle similar challenges facing the region. Policies on agriculture, water management and movements of people need to be updated to reflect current regional problems (*Fig. 7*).

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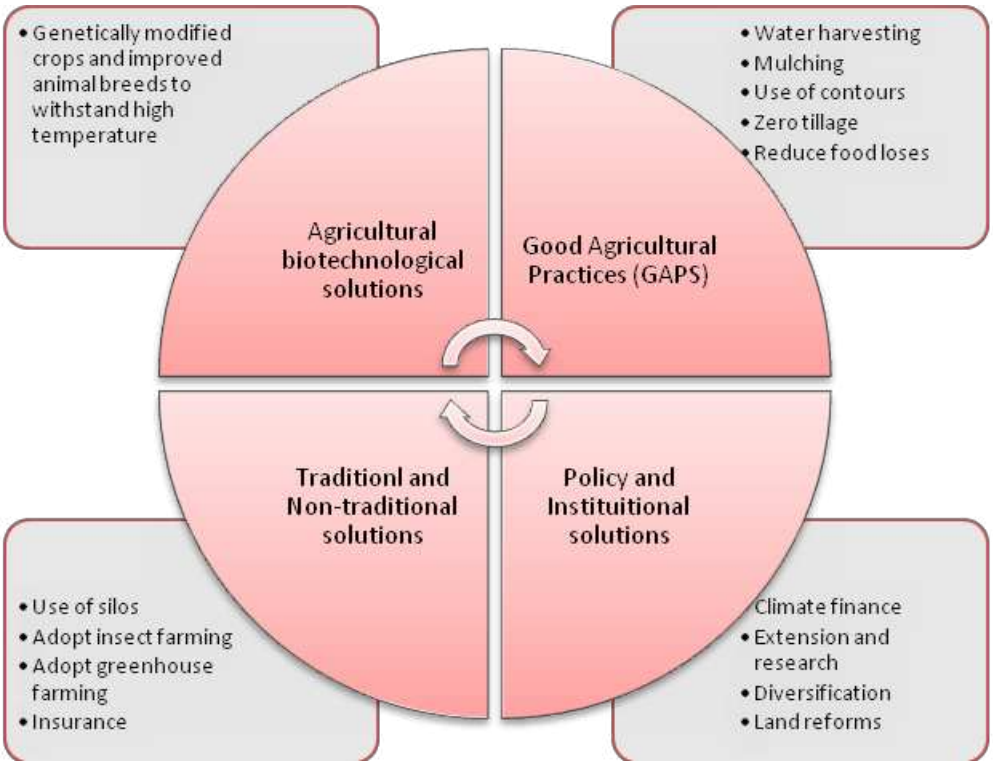


Figure 7. *Mitigation and adaptation strategies. (Source: Authors)*

Countries should try all possible options to streamline the policies to stabilize food security and increase resilience in agricultural systems (Kelvin et al., 2017). Policies that strengthen institutions and agricultural technologies can improve adaptation and resilience of communities (Shiferaw et al., 2014). Policies need to revive traditional and non-traditional options to fully optimize available options of dealing with climate change and food insecurity.

Policies have to be designed in a way that is integrative across sectors and interlinked but avoid duplication. They should be clear and easy to implement, supported by regional governments to improve resilience of communities to climate change shocks.

Conclusions and recommendations

Generally, the results of this paper reveal the following:

The destruction of fragile ecosystems by man's ill-judged activities is greatly responsible for climate change in East Africa that in turn causes food insecurity and other disasters.

Simple but useful technologies like use of silos commonly termed as granaries which used to exist and help in preservation of food have slowly been neglected and have contributed to food insecurity.

Farmers sell from their gardens before crops reach maturity stage for harvesting and this exacerbated food insecurity.

We recommend and emphasize integration of traditional and non-traditional measures to guard against food insecurity and climate change. Governments should focus on working out a "solid" policy that protects natural resources like wetlands,

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promotes both traditional and non-traditional technologies and support farmers to adopt new agricultural technologies. This needs synergy across all the East African countries through investment in research, technologies and collaborations to improve on peoples' livelihoods.

There is need to find out why silos and back yard gardens that characterized rural East African communities have reduced yet they would provide cheap sources of food to fight "hidden hunger".

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EFFECT OF *SILYBUM MARIANUM* (L.) GAERTN. ON GERMINATION, EARLY GROWTH AND NUTRIENT UPTAKE OF *ZEA MAYS* L.

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Abstract. In our experiment, the effect of water extract of *Silybum marianum* (L.) Gaertn. on germination and early growth of *Zea mays* L. was examined in laboratory circumstances. The examined concentrations (m/v%), originated from dried *S. marianum* water extract were the following: 1%, 5%, 7.5% and 10%. The fresh and dry weight of maize, the length of coleoptile and radicle and the nutrient element contents were measured. The results demonstrate that the 1% milk thistle water extract treatment has a significant positive effect on all of the measured parameters of maize. In the case of higher concentrations, the fresh and dry weight of coleoptile significantly decreased compared to the control. The same tendency can be observed for the radicle. N, K, Ca, Mg and Zn concentration of radicle and K, Ca and Zn concentration of coleoptile increased when maize was treated with milk thistle extract, at all kind of concentration levels. The Cu and Fe content of maize seedlings decreased at all applied concentrations. Our results demonstrate the importance of *S. marianum's* appearance in field because it has very strong allelopathic effects. Further examination is needed to determine if the remaining plant residues have an effect on germination and growth of crops.
Keywords: *biomass production, corn, milk thistle, growth inhibition*

Introduction

Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, from the release of biochemicals, known as allelochemicals, to plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems. Allelochemicals are a subset of secondary metabolites not required for metabolism (growth and development) of the allelopathic effects and are important part of plant defence against herbivory (Fraenkel, 1959; Stamp, 2003).

Commonly cited effects of allelopathy include reduced seed germination and seedling growth (Lehoczky et al., 2011a, b, 2014). Inhibition due to allelopathy is complex and can involve the interaction of different chemical classes, like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates and amino acids

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with different compound mixtures sometimes having higher allelopathic effect than individual compounds alone (Győri et al., 2014; Lehoczky et al., 2016; Nwaichi and Ayalogu, 2010).

The basic approach used in allelopathic research for agricultural crops has been to screen both crop plants and natural vegetation for their capacity to suppress weeds (Lehoczky and Gólya, 2013). To demonstrate the phenomenon and mechanism of allelopathy, plant origin, production, and identification of allelochemicals have to be established as well as persistence in the environment over time in concentrations sufficient to affect different plant species. Interactions among allelopathic plants, host crops, and other non-target organisms must also be considered (Ferguson and Rathinasabapathi, 2012).

Many researchers have documented the allelopathic effect of milk thistle on mustard, cucumber, wheat and sorghum (Isam and Hussain, 1988), but there is little knowledge on the effect on maize. Qasem and Foy (2008) established, that milk thistle have the potential to be used in solving problems of other weed species and represents an excellent source of natural chemicals that may be involved in developing natural herbicides.

S. marianum is by far the most widely known species. Milk thistle, also known as the Marian, St. Mary's or Our Lady's thistle, is native to the Mediterranean region of Europe and is grown commercially as a medical plant in Egypt, Tunisia, China and Argentina (Anonymous, 1995; Penksza and Szerdahelyi, 2009) but it has been reported as a noxious weed in many other countries. Otherwise, milk thistle is a serious weed in many areas of North and South America, Africa, Australia and the Middle East (Holm et al., 1997).

Weeds are an important variable in crop production (Bilalis et al., 2010; Efthimiadou et al., 2009; Karkanis et al., 2010; Lehoczky et al., 2013, 2015). Weed science will be advantageously positioned in the future if research focuses on research – decision process, weed biology and ecology, weed control and management practices, herbicide resistance, issues related to transgenic plants, environmental issues, and potential benefits of weeds (Hall et al., 2000).

Weeds have serious effect on crop production. Nowadays, the milk thistle is one of the most prevalent weeds found in the fields in North Africa. This research will be positioned for the future, focusing on interaction, weed biology and ecology, weed control and management practices, herbicide resistance.

The basic aim of the experiment was to make bioassay examination which assesses the allelopathic effects of *Silybum marianum* (L.) Gaertn. on maize seedlings in laboratory circumstances. The test plant's (maize) element composition, germination and growth inhibition were also investigated at the end of the experiment.

Materials and methods

The *Silybum marianum* (L.) Gaertn. leaves were dried on 45 °C. After drying, the sample was chopped than milled. The plant material of *S. marianum* was collected from agricultural area. The element content of milk thistle is shown in *Table 1*.

The first 150 ml of the 10% concentration water extract was made by soaking 15 g powdered plant sample in 150 ml tap water. The mixture was kept at room temperature for 24 h and was mixed occasionally. After 24 h, the extract was filtered through MN 619 G ¼ filter paper. The 10% concentration water extract was diluted to obtain the

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concentrations of 1%, 5% and 7.5%. Tap water was used as a control (Ø). Twenty maize seeds (*Zea mays* L.) were placed separately into 18 cm diameter Petri dishes on MN 640 m filter paper. 15 ml of each concentration of water extracts were added to the Petri dishes in four replications. All of them were placed in a Binder APT Line KBW plant growing chamber at 21 ± 1 °C for 7 days.

Table 1. *Element content of Silybum marianum L. Gaertn. leaves (mg kg^{-1}, N (%)) N = 2 ± SD*

N	P	K	Ca	Mg
2.42 ± 0.09	1764 ± 42	34165 ± 54	35432 ± 2917	5156 ± 59
Fe	**Mn**	**Cu**	**Zn**	**Mo**
321 ± 86	30.4 ± 2.4	14.8 ± 0.7	43.5 ± 0.5	1.07 ± 0.15

The used experimental design and method have already been published by Lehoczky et al., (2011a, b, 2017).

Seven days after the start of the experiment, the shoots and roots of every seedling were measured with millimetre paper. The fresh weight of the shoots and roots was determined at the same time. After drying, the dry weight was also measured.

Determination of the seedling nutrient concentrations for P, K, Ca, Mg, Fe, Mn, Cu, Zn and Mo was carried out with ICP-OES method after microwave Teflon bomb digestion with cc. HNO_3 + H_2O_2. The nitrogen concentration of samples was determined using the Kjeldahl method.

The substances with allelopathic effects of the *Silybum marianum* L. Gaertn. sample have been investigated by HPLC. According to the methanol extract, 1 g plant sample contained 63 µg rutin and 194 µg salicylic acid. Both rutin and salicylic acid have inhibitory effects on germination (Basile et al., 2000; Szepesi, 2009; Szabó, 1997).

ANOVA (Analysis of Variance) was used to evaluate the significant differences. On the figures the error bars indicate the standard deviation and the a–e indicate significant differences within treatments at the 5% level of probability according to Duncan's test. For the statistical analysis Sigma Plot 12.0 was used. Examinations were carried out in three replications and in different cases with four replications. The experiments were conducted in controlled environmental conditions, so there was no difference between the results of three biological replications. The data of three repetitions were summarized and used for statistical analysis – results were calculated from twelve data.

Results

Usually, the effects of allelopathy include reduced seed germination and seedling growth. All maize seeds started to germinate by the end of the seventh day, hence the germination percentage was 100% in all treatments. However, the number of seeds with a length of a few mm shoot have increased in parallel with increasing extract concentration (*Fig. 1a, b*).

Decrease of shoot and radicle length was observed at the 5, 7.5 and 10% extract treatments. The length of shoot increased by 18% in the 1% extract treatment. The increase is significant compared to the control. The length of radicle increased by 18%

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in the 1% milk thistle extract treatment. The decrease is parallel with the increasing concentration of milk thistle extracts.

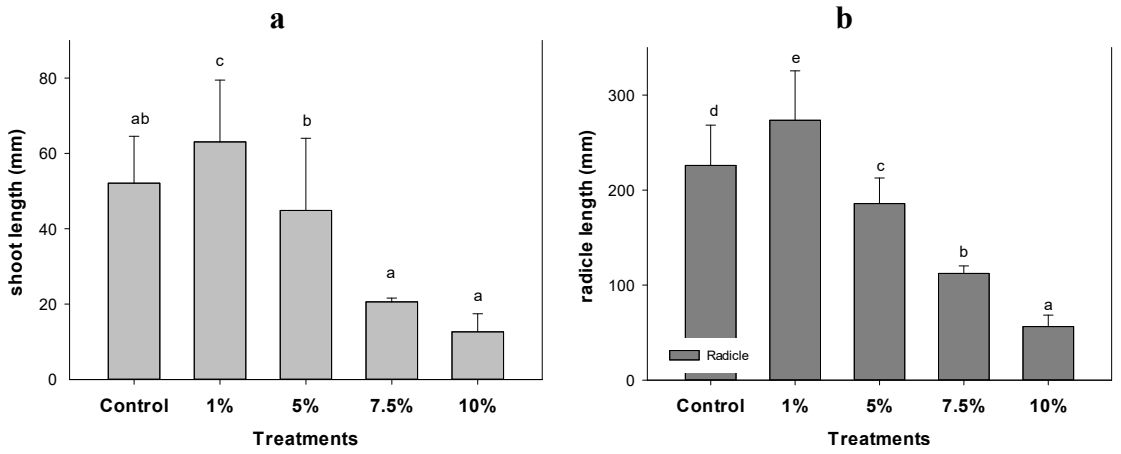


Figure 1. *The effect of Silybum marianum L. Gaertn. water extracts (a) on the length of shoot of maize ($LSD_{5\%}$ 12.36) N = 240 ± SD and (b) on the length of radicle of maize ($LSD_{5\%}$ 49.80) N = 240 ± SD*

Additionally, the effect of *S. marianum* was observed on the fresh weight of shoot (coleoptile) and root (radicle) of maize (*Fig. 2a, b*).

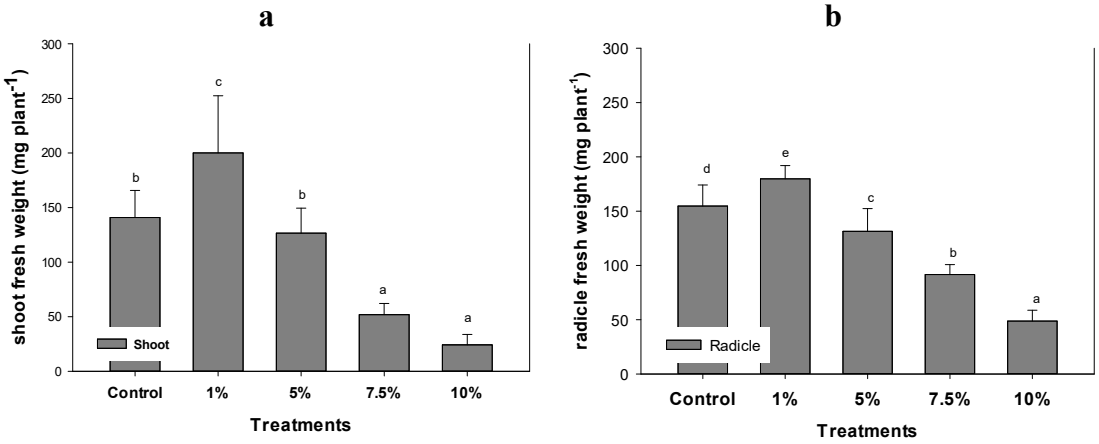


Figure 2. *The effect of Silybum marianum L. Gaertn. water extracts (a) on the fresh shoot weight of maize ($LSD_{5\%}$ 35.11) N = 12 ± SD and (b) on the fresh radicle weight of maize ($LSD_{5\%}$ 18.86) N = 12 ± SD*

The positive effect was observed when 1% extract was applied. The fresh weight of coleoptile was significantly increased by 30% in the 1% treatment. At the higher concentrations, the effect was negative on fresh weight. The fresh weight of shoot decreased by 11%, 64% and 83% in the 5%, 7.5% and 10% treatment, respectively. In the case of the highest concentration the decrease was significant compared to the control. The same tendency can be observed in the case of the fresh weight of radicle (*Fig. 2b*). The fresh weight of radicle increased by 14% in the 1% extract treatments.

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Decrease was observed at the higher concentration. The highest decrease was in the case of the 10% extract treatment. The dry weight of shoots and radicles also were measured (*Fig. 3a, b*).

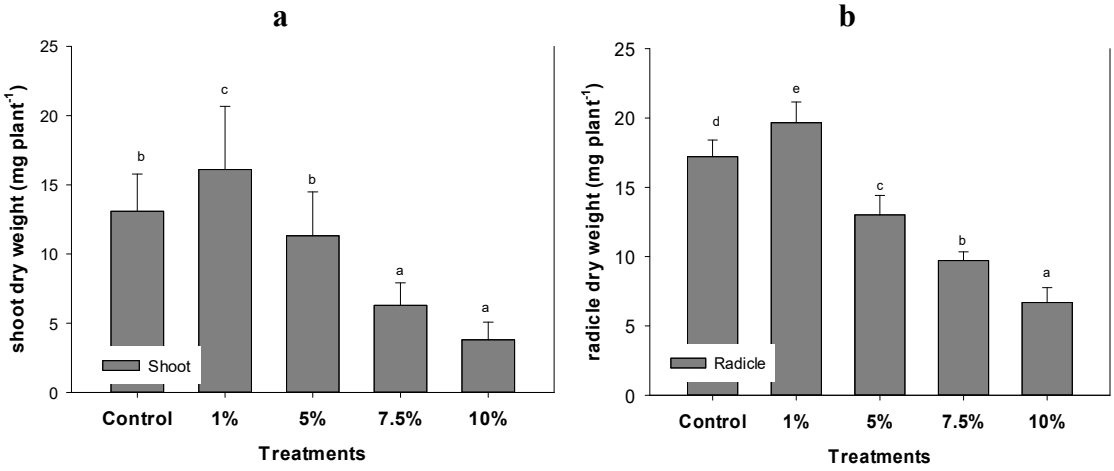


Figure 3. *The effect of Silybum marianum L. Gaertn. water extracts (a) on the dry weight of shoot of maize ($LSD_{5\%}$ 4.06) N = 12 ± SD and (b) on the dry weight of radicle of maize ($LSD_{5\%}$ 1.72) N = 12 ± SD*

The 1% extract treatment had less positive effect on dry weight than on fresh weight of shoot. The dry weight of shoot increased with 19%. The decrease at the higher extract concentrations also had less negative effect on the dry weight of shoots as well as on the fresh weight of shoot. The decrease was mathematically proven in both cases.

Figure 4 shows the different extract treatments on the shoot/root dry weight rate of maize. The shoot/root dry weight ratio of seedlings was higher in the case of 1 and 5% concentrations compared to the control (0.76), which is a trend that is not statistically significant.

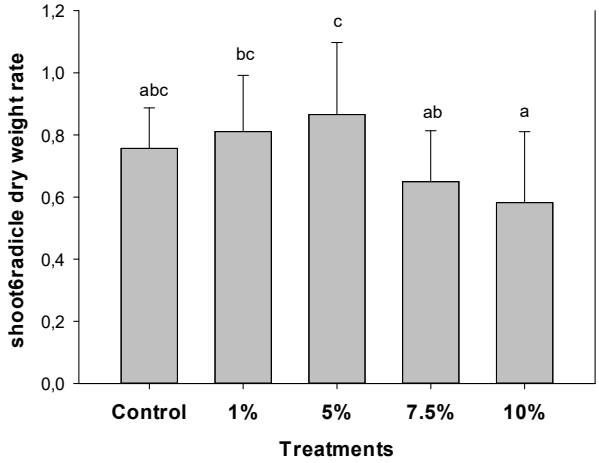


Figure 4. *The effect of Silybum marianum L. Gaertn. water extracts on the shoot/root dry weight rate of maize ($LSD_{5\%}$ 0.33) N = 12 ± SD*

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In the case of the 1% extract treatment, the mass of the root and shoot both increased, but the increase of shoot weight was greater than of root weight. Similar results were observed at 5% concentration level regarding the ratio, however the root and shoot growth was also inhibited (*Fig. 3a, b*). The inhibitory effect was more strongly manifested in the case of roots, which explains the growth of shoot/root dry weight ratio shown in *Figure 4*. In the case of the 7.5 and 10% extract treatments the shoot/root ratio was significantly lower compared to the treatment with 5% concentration. The background in both cases is the strong growth inhibiting effect of the extracts, in which the shoot growth inhibition was stronger than the root growth inhibition, thus the significant reduction of the ratio can be explained.

Tables 2 and *3* show the element concentrations of shoot and radicle of maize. The concentration of N is significantly decreased in shoot in 1%, and increased in 5, 7.5 and 10% treatment in radicle. The concentration of P significantly decreased by 22% in the 10% extract treatment in shoot compared to the control, but there was no significant difference detected in radicle between *S. marianum* treatments and control. The concentration of K is significantly increased in both shoot and radicle in 5%, 7.5% and 10% water extracted milk thistle treatments. The increment is 36%, 32% and 36% in shoot, and 53%, 54% and 56% in radicle, respectively. The concentration of K was not changed significantly parallel with the increasing water extract concentration. The content of calcium significantly increased in all extract treatments; the highest increase could be observed at 7.5% water extract concentration in shoot and at 5% in radicle treatment compared to the control. In order to protect roots against the adverse effects of high concentrations of various other cations in the soil solution, the Ca^{2+} concentrations required for optimal growth have to be substantially higher in soil solution than in balanced flowing nutrient solutions (Asher and Edwards, 1983). Also increment was determined in the Mg content in the radicle of maize when maize was treated with milk thistle extract. Mg is also required for optimal plant growth. At permanently insufficient root supply, remobilization of Mg from mature leaves reduces their longevity (Lange et al., 1987). The content of Cu decreased parallel with the increasing extract concentration. The Fe content decreased in the all treatments. The concentration of molybdenum in shoot was changed significantly in the 10% treatments, while no significantly changes were observed in radicle compared to the control.

Discussion

Allelopathic effect of milk thistle on different crops has been documented sporadically, but there is no literature on its effect on maize.

Therefore, a laboratory based allelopathic extracts bioassay was conducted. During our experimental period the allelopathic effects of donor species on germination and seedling growth of maize was observed. Results has been shown, *Silybum marianum* L. extract did not have any effect on the germination of maize. The maize seedlings growth was inhibited by 5, 7.5 and 10% extract of *Silybum marianum* L. but growth stimulation effect was observed by 1% concentration extract.

Sultana and Asaduzzaman (2012) also observed inhibition effect on the above-mentioned parameters in case of canola and ryegrass. They established that the extracts from fresh water at 100% was more toxic to receiver species followed by 50% concentration of fresh and 100% from hot water extracts. This concentration reduced the root, shoot growth of ryegrass and canola by 84.97%, 84.27% and 89.90%,

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respectively. The result also revealed that the allelopathic pattern of hot water extracts was the same; however, it was less toxic to both receiver species.

Table 2. *Element concentrations of shoot of maize treated with 1, 5, 7.5 and 10% milk thistle water extract treatments (mg kg^{-1}, N (%)) N = 2 ± SD*

	N	P	K	Ca	Mg
Control	5.7 ± 0.2 b	9752 ± 412 b	24458 ± 4353 a	210 ± 42 a	1805 ± 97 a
1%	5.1 ± 0.2 a	9789 ± 209 b	27259 ± 1410 a	623 ± 8 b	1834 ± 21 a
5%	5.6 ± 0.0 b	9551 ± 245 b	38087 ± 1979 b	763 ± 51 b	1834 ± 1 a
7.5%	5.8 ± 0.1 b	9382 ± 345 b	35635 ± 707 b	779 ± 129 b	1881 ± 96 a
10%	5.7 ± 0.1 b	7636 ± 624 a	38043 ± 5139 b	762 ± 196 b	1962 ± 4 a
Mean	*5.6*	*9222*	*32696*	*627*	*1863*
LSD5%	*0.4*	*1056*	*8136*	*304*	*192*
	Fe	**Mn**	**Cu**	**Zn**	**Mo**
Control	84.4 ± 10.7 d	9.4 ± 0.1 b	7.5 ± 1.5 b	35.4 ± 6.3 a	0.43 ± 0.04 b
1%	76.9 ± 11.7cd	9.5 ± 0.8 b	6.7 ± 0.9 ab	35.2 ± 1.7 a	0.38 ± 0.03 b
5%	63.5 ± 0.1 bc	9.1 ± 0.5 b	5.3 ± 0.2 a	45.8 ± 2.3 ab	0.41 ± 0.01 b
7.5%	51.1 ± 5.9 ab	8.5 ± 0.9 b	4.7 ± 0.4 a	45.8 ± 3.4 ab	0.42 ± 0.03 b
10%	39.9 ± 1.4 a	6.1 ± 0.3 a	4.8 ± 0.0 a	51.5 ± 4.5 b	0.23 ± 0.09 a
Mean	*63.1*	*8.5*	*5.8*	*42.8*	*0.37*
LSD5%	*23.0*	*2.0*	*1.7*	*5.1*	*0.15*

Note: different letters indicate significant difference (P < 0.05)

Table 3. *Element concentrations of radicle of maize treated with 1, 5, 7.5 and 10% milk thistle water extract treatments (mg kg^{-1}, N (%)) N = 2 ± SD*

	N	P	K	Ca	Mg
Control	3.6 ± 0.1 a	6894 ± 413 a	22883 ± 2593 a	425 ± 67 a	1362 ± 162 a
1%	3.5 ± 0.2 a	6830 ± 361 a	27693 ± 1699 a	2967 ± 749 b	1908 ± 100 a
5%	4.2 ± 0.2 b	7348 ± 31 a	48743 ± 3604 b	5116 ± 337 c	3972 ± 441 b
7.5%	4.6 ± 0.1 b	7358 ± 270 a	50157 ± 4391 b	4736 ± 671 c	4027 ± 276 b
10%	5.6 ± 0.3 c	7271 ± 332 a	51724 ± 2579 b	3104 ± 864 b	3794 ± 429 b
Mean	*4.3*	*7140*	*40240*	*3270*	*3013*
LSD5%	*0.5*	*898*	*9538*	*1787*	*620*
	Fe	**Mn**	**Cu**	**Zn**	**Mo**
Control	57.3 ± 2.8 c	6.0 ± 2.1 a	24.1 ± 5.1 c	34.6 ± 5.2 ab	0.25 ± 0.06 ab
1%	54.8 ± 1.5 c	5.1 ± 0.7 a	15.9 ± 1.8 b	32.4 ± 3.5 a	0.36 ± 0.06 ab
5%	52.2 ± 2.2 bc	5.4 ± 0.5 a	13.3 ± 0.1 ab	40.1 ± 1.6 ab	0.39 ± 0.02 b
7.5%	47.9 ± 3.4 ab	5.1 ± 0.3 a	11.2 ± 1.6 ab	42.2 ± 3.4 b	0.29 ± 0.12 ab
10%	42.4 ± 2.1 a	5.2 ± 0.4 a	8.8 ± 0.2 a	51.2 ± 1.7 c	0.19 ± 0.02 a
Mean	*50.9*	*5.3*	*14.7*	*40.1*	*0.29*
LSD5%	*2.0*	*2.8*	*7.2*	*8.9*	*0.20*

Note: different letters indicate significant difference (P < 0.05)

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The allelopathic potential of *S. marianum* was investigated by Khan et al. (2011) on kidney bean (*Phaseolus vulgaris* L.), mung bean (*Vigna radiate* L.), chickpea (*Cicer arietinum* L.) and soybean (*Glycine max* L.). The results show that by increasing the leaves extract concentration of *S. marianum*, a significant decrease was noted in the germination percentage, mean germination time, germination index and seed vigor index of test species. The tolerance order of the mung bean and soybean against the extract concentration of *S. marianum* was higher than the other two species, while the chickpea was more susceptible to the toxicity of milk thistle.

Milk thistle is a major weed in sugar beet, wheat and canola causing large yield reductions (Shimi et al., 2006). However, research has documented the allelopathic effect of milk thistle on mustard, cucumber, wheat and sorghum (Inam and Hussain, 1988; Lehoczky et al., 2017).

In our experiment, the uptaken elements, e.g. K, Ca and Zn, were increased both in the shoot and root when maize was treated with *S. marianum*, but the Cu and Fe content decreased as a result of the milk thistle extract treatment.

In summary, the microelement concentrations decreased by the application of milk thistle water extracts. The micronutrients have prominent effect on dry matter, grain and straw yield (Aller et al., 1990). Fe plays role e.g. in nitrogen fixation (Römheld and Marschner, 1991) and it is very essential for normal plant growth (Fageria, 2007). Mn is utilized in enzyme activation (Burnell, 1988), Zn is important to membrane integrity and protein synthesis (Zhao et al., 2012). So, the adequate uptake of micronutrients has an effect on plant growth and development. Otherwise, plant will suffer from growth inhibition.

Seed germination is affected by the nutritional status of the mother plant and therefore by nutrient concentration in the seeds (*Table 1*). P and K concentrations of below 0.3% in seeds negatively affect their total germination (Hrdlicková et al., 2011). Seed germination and the time required for the 50% of the seeds to germinate are significantly affected by nitrogen (Hejcman et al., 2012). There was no detected inhibited germination in our experiment.

In our experiment the inhibition effect of milk thistle on the measured parameters of maize was also observed. Significant decrease was observed at increasing milk thistle extract concentration in the fresh and dry weight of shoot and radicles of maize, length of shoot and radicle. Compared to the stimulating 1% treatment, the 5, 7.5 and 10% extract resulted in significant reduction of the fresh weight of coleoptile, which was 11, 6 and 83%, respectively. The length of shoot deceased by 12% in the 5% extract treatment, 60% in the 7.5 and 76% in the 10% milk thistle extract treatments. But a remarkable stimulating effect was observed in 1% extract treatment on the fresh weight (30%) and length (14%) of coleoptile.

Lehoczky et al. (2017) investigated the allelopathic effect of milk thistle on winter wheat. According to their results, the winter wheat is more sensitive for applied concentrations of *S. marianum* than the maize. The concentrations of 1%, 2%, 3%, 4% and 5% of water extract were used. They found that the allelopathic potential of milk thistle increased with increasing concentration. The fresh and the dry weight and the length of the shoot and radicle of wheat significantly decreased at 5% concentration.

Recent assessment of allelopathic effect of weed on weed has the goal of using naturally produced allelochemicals to reduce reliance on herbicides (Putman and Duke, 1978). Milk thistle is a worldwide known weed. It has a special allelopathic effect, therefore, there is a significant difference when we grow milk thistle as medicinal plant

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or it acts as a weed. In the latter case, *S. marianum* may decrease the yield due to its allelopathic effect. So, special attention should be given to the plant residues of milk thistle to be worked in the soil after the vegetation period.

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IMPACT OF HARVESTING TIMES ON CHEMICAL COMPOSITION AND METHANE PRODUCTIVITY OF SORGHUM (*SORGHUM BICOLOR* MOENCH L.)

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Abstract. A two-year field study was conducted during 2016 and 2017 to determine the influence of harvesting times on biomass yield, quality and bio-methane yield of sorghum. Harvesting times considerably influenced the growth attributes, biomass quality and bio-methane yield. The maximum plant height, and dry matter (DM) yield were recorded 105 DAS, whilst lowest values of these parameters were recorded 60 DAS. Similarly, the maximum protein and sugar concentration were found 60 days after sowing (DAS), after that a substantial reduction in protein and sugar concentration were found with advancing maturity. Moreover, the highest acid and neutral detergent fiber, lignin, cellulose and hemi-cellulose were found 120 DAS whereas; minimum acid detergent fiber, neutral detergent fiber, lignin, cellulose and hemi-cellulose were observed 60 DAS. Likewise, maximum specific methane yield was recorded 60 DAS, while minimum specific methane yield were recorded 120 DAS, conversely, maximum methane yield ha^{-1} were recorded after 105 DAS owing to higher dry matter yield ha^{-1}. Interestingly, we also found strong positive correlation between dry matter yield and methane yield and negative relationship between lignin concentrations and specific methane yield. In conclusion, sorghum crop can be harvested after, 105 DAS owing to high dry matter yield ha^{-1} for maximizing its potential for bio-methane yield.
Keywords: *harvesting time, biomass yield, sorghum, biomass quality, methane yield*

Introduction

Energy is essential for substantial growth and development of nations; in addition, energy security and resource scarcity are key issues globally. The depletion of fossil fuels and increase in the green house gases due to combustion of fossil fuels has promoted the interest of environmentalists and policy makers for alternate energy sources (Monti et al., 2012). Amongst the alternate energy sources plant biomass is considered to be a sustainable and cheap source. Moreover, the plant biomass could cater the above-mentioned problems. Energy crops are imperative biomass sources which can be used for the production of bio-energy. Among energy crops, sorghum is an indispensible crop cultivated globally for the production of bio-methane. Variety of attributes including, low water requirement, salinity and drought tolerance (Vasilakoglou et al., 2011; Hassan et al., 2018a), short growth period and well adoptability in arid and semi-arid regions (Reddy and Sanjana, 2003) makes it a promising bio-energy crop. Moreover, sorghum crop can efficiently transfer the available water into dry matter production than the other C_4 crops (Dercas and Liakatas, 2007).

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Together with productivity, the quality of the biomass is important to bio-fuel conversion production systems. The quality of the produced can be optimized by just selecting the optimum harvesting time. Stage of harvesting is an imperative factor which substantially influences the biomass yield, chemical composition as well as biomass digestibility (Ball et al., 2001). The variations in biomass composition and yield have been reported owing to cultivar, environment and harvesting stage (Pordesimo et al., 2005; Hassan et al., 2018b). Prolonged maturity enhances the structural fiber and lignin concentrations and decreases biomass digestibility (Pordesimo et al., 2005) and consequently the bio-methane yield. The most common changes linked with harvesting time are biomass yield (Ayub et al., 2003), biomass quality i.e. protein concentrations, structural fiber and lignin concentrations and biomass digestibility and its conversion to bio-energy (Schittenhelm, 2008). So far, in Pakistan most of the research on sorghum has been focused on its fodder production aspects. There is no report available regarding the effect of harvesting time on chemical composition and biomass yield in the context of methane production. Therefore, this study was planned to determine the influence of harvesting times on biomass yield, chemical composition and bio-methane yield.

Materials and methods

Study site

A field study was conducted during 2016 and 2017 at Post Graduate Agriculture Research (PARS), University of Agriculture, Faisalabad, Pakistan. The study site comes under a sub-tropical climate, with a mean temperature of 27-39 °C during summer season and 6-21 °C during winter season (Chattha et al., 2017a). Furthermore, the prevailed climatic conditions during both the years are given in *Table 1*. The soil samples were collected before sowing crop in each season, and were analyzed by standard procedures of Homer and Pratt (1961). The soil was sandy loam and averagely the soil had a pH of 7.95, Ec (1.2), organic matter (0.89%), nitrogen (0.03%) phosphorus (6.43 ppm) and potassium (186 ppm) respectively.

Table 1. *Prevailing climatic conditions for the experimental site during year 2016 and 2017*

Months	Monthly mean maximum temperature (°C)		Monthly mean minimum temperature (°C)		Monthly average temperature (°C)		Rainfall (mm)		Relative humidity (%)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
May	39.8	41.1	25.6	26	32.7	33.5	25	10.1	28.8	29.8
June	40.2	39.8	28.5	27.3	34.4	33.5	39.9	41.6	38.9	44.5
July	36.6	38.5	27.4	28.9	32	33.7	193.5	161.4	59.6	70
August	35.7	38.1	26.5	28.6	31.1	33.4	48.1	66	62.2	68.9

Experimental design and crop cultivation

The experiment was composed of five harvesting times i.e. 60, 75, 90, 105 and 120 days of sowing and a randomized complete block design with three replications were used for study. The final seed bed was prepared by ploughing three times followed by planking. The net plot size was 5 m × 3 m. In both years, nitrogen (N) as urea (46% N) 60 kg ha^{-1} was applied, while phosphorus as single super phosphate (21% P)

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40 kg ha^{-1} was applied. The sorghum variety YS-2016 was collected from Fodder Research Institute Faisalabad. The sorghum variety YS-2016 is late maturing. All the P and half of N were applied at the sowing, while rest of the N was applied with first irrigation. The crop was sown on 2nd May and 6th May during the year 2016 and 2017 respectively. In total three irrigations were applied during both the years. First irrigation was applied as soaking irrigation, second irrigation was applied 35 days after sowing and third irrigation was applied 68 days after sowing. All other management practices were kept normal and uniform during both years for good stand establishment.

Sampling and measurements

Ten plants were selected at each harvesting time in order to determine the plant height, leaves per plant, stem diameter, fresh and dry weights per plant. Moreover, the whole plots were harvested and dried to determine the biomass yield and later on mathematically converted to tones per hectare basis.

Biomass analysis

The samples for chemical analysis were oven dried, ground and sieved through 1 mm mesh screen. The concentrations of protein and ash were determined according to the AOAC (1990). Sugar, acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin concentrations (% DM) were determined according to Dubois et al. (1956), Georing and Van-Soest (1970) and Van-Soest et al. (1991). Methane measurement was taken using Bioprocess Control's AMPTS equipment. Liquid manure was used a source of bacteria in order to anaerobically digest the sorghum sample. The 16 g of substrate was used in each digester after that the total volume of digesters were made up to 400 ml. Afterwards, digesters were perched with nitrogen gas in order to create the anaerobic conditions. The temperature of digesters was kept at 37 °C by standing them in water bath. The samples were allowed to digest for 28 days, in laboratory. The methane produced by each sample on every day was recorded from computer operated systems. At the end by using the amount of volatile solids the quantity of specific methane produced by each sorghum sample was calculated. Later on the specific methane produced by each sample was converted into hectare basis mathematically.

Statistical analysis

The collected data were analyzed by computer software Statistix 8.1 and least significant difference test was used to compare the treatments means at 5% probability level (Steel et al., 1997). The graphs were generated by using Sigma plot software 9.

Results

The results revealed that harvesting times had substantiated influence on the growth attributes of sorghum i.e. plant height, stem diameter and leaves per plant (*Table 2*). The plant height ranged between 164-212 cm in 2016 and 161-210 cm in 2017. Plant height increased progressively and reached the maximum extent at 105 days after sowing (DAS), after that there was no increase observed in plant height. Similarly, the maximum stem diameter was observed when crop was harvested 105 DAS, whereas the minimum stem diameter was observed 60 DAS. Likewise, maximum leaves per plant

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was recorded after 60 DAS afterwards, a substantial reduction in leaves per plant were recorded with minimum leaves at 120 DAS (*Table 2*).

There were significant differences among the harvesting times for fresh weight per plant, dry weight per plant and dry matter yield (DM; *Table 2*). The maximum fresh weight and dry weight per plant were recorded 105 DAS, after that both fresh and dry weight per plant started declining, whereas the minimum fresh weight per plant and dry weight were recorded at 60 DAS. Similarly, the crop harvested 105 DAS produced maximum DM yield (16.26 t ha^{-1}, 16 t ha^{-1}), followed by crop harvested after 120 DAS, while the minimum DM yield (10.46 t ha^{-1}, 10.06 t ha^{-1}) were obtained at 60 DAS.

Table 2. *Effect of harvesting times on growth and biomass characteristics of Sorghum bicolor*

Harvesting times	Plant height (cm)		Stem diameter (cm)		Leaves per plant		Fresh weight per plant (g)		Dry weight per plant (g)		DM yield t ha^{-1}	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
60 DAS	164c	161c	1.03c	1.01c	15.13a	14.60a	133.33d	130.00c	27.83e	26.53d	10.46d	10.06d
75 DAS	184b	182b	1.09bc	1.06c	12.53b	12.16b	149.67cd	145.33c	34.66d	32.40c	12.72c	12.36c
90 DAS	197b	199a	1.20ab	1.18b	11.53b	11.16b	166.67bc	163.00b	38.00c	35.90bc	14.73b	14.36b
105DAS	212a	210a	1.33a	1.30a	9.80c	9.56c	185.00a	182.67a	45.06a	43.00a	16.26a	15.96a
120DAS	212a	210a	1.28a	1.26ab	8.03d	7.66d	177.33ab	174.67ab	41.06b	39.10ab	16.13a	15.80a
LSD ($p \leq 0.05$)	13.43	13.22	0.13	0.11	1.21	1.00	18.30	16.83	2.36	4.58	0.64	0.73

Values sharing different letters differed at $p < 0.05$, DAS: days after sowing

There were considerable differences among the harvesting times for qualitative attributes (*Table 3*). The highest protein and sugar concentrations (% DM) were obtained from the earliest harvesting (60 DAS), while lowest protein and sugar was obtained from latest harvesting (120 DAS). Moreover, the harvesting times also had considerable influence on the acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin, cellulose and hemi-cellulose concentrations (% DM) (*Fig. 1*). A linear increase in ADF, NDF, lignin, cellulose and hemi-cellulose were found with advancing maturity. The maximum ADF and NDF concentrations were recorded 120 DAS that was comparable with 105 DAS, whilst lowest ADF and NDF concentrations in plant biomass were recorded at 60 DAS (*Fig. 1a, b*). The highest lignin, cellulose and hemi-cellulose concentrations in plant biomass were recorded at 120 DAS that was comparable with 105 DAS, whilst, lowest lignin, cellulose and hemi-celluloses, were recorded 60 DAS among the harvesting times (*Fig. 1c, d*). Similarly, ash concentrations significantly decreased with maturity, with maximum ash concentration (10.53, 10.76%) were observed 60 DAS, while lowest ash concentration (7.63, 7.80%) were recorded 120 DAS that was comparable with 105 DAS (*Table 3*).

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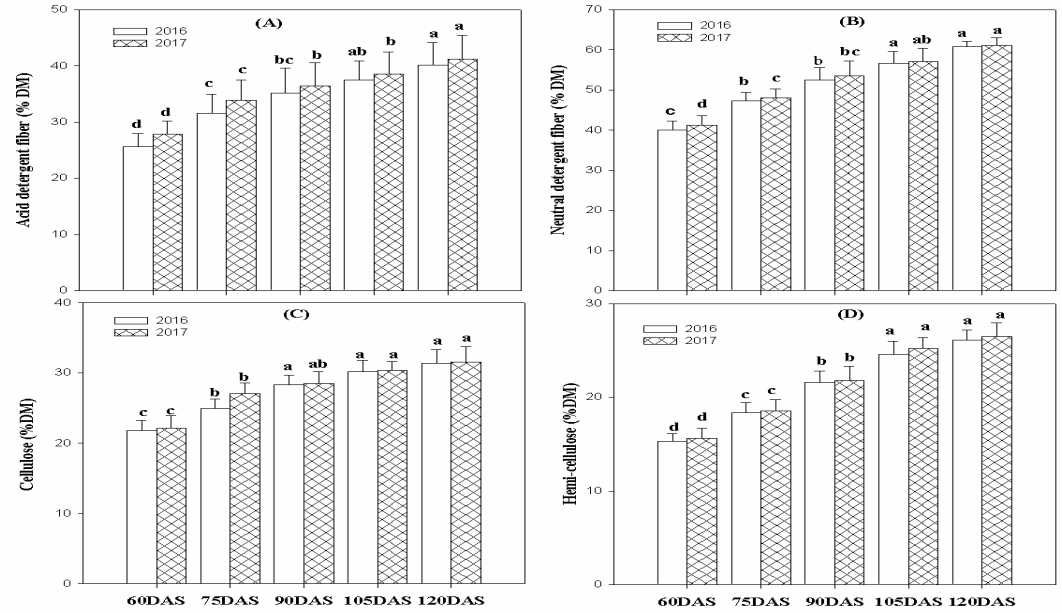


Figure 1. *Influence of harvesting times on acid detergent fiber (A), neutral detergent fiber (B), cellulose (C) and hemi-cellulose (D) concentration during the 2016 and 2017. Values represent means ± S.D. The significant differences were measured by the least significant differences at 0.05 and indicated by different letters*

Table 3. *Effect of harvesting times on protein, sugar, lignin and ash concentration of sorghum bicolor*

Harvesting times	Protein concentration (DM %)		Sugar concentration (DM %)		Lignin concentration (DM %)		Ash concentration (DM %)	
	2016	2017	2016	2017	2016	2017	2016	2017
60 DAS	12.53a	12.12a	11.58a	11.46a	3.11d	3.24d	10.53a	10.76a
75 DAS	11.550b	11.47ab	10.86b	10.14b	4.13c	4.20c	9.53b	9.64b
90 DAS	11.06bc	10.93bc	10.13c	10.10b	5.11b	5.22b	8.57c	8.69c
105DAS	10.33cd	10.26cd	9.96c	9.41bc	5.54ab	5.59ab	7.66d	7.86d
120DAS	10.10d	10.02d	9.22d	9.14c	5.76a	5.85a	7.63d	7.80d
LSD *($p \leq 0.05$)*	0.76	0.71	0.71	0.76	0.46	0.46	0.78	0.74

Values sharing different letters differed at $p < 0.05$, DAS: days after sowing

The results revealed that harvesting times substantially influenced the specific methane yield and methane ha^{-1} basis (*Fig. 2*). The specific methane yield (SMY) considerably decreased with advancing maturity, with maximum SMY were observed at 60 DAS, followed by 75 DAS, whilst lowest SMY were recorded at 120 DAS (*Fig. 2a*). Conversely, methane yield ha^{-1} increased with advancing the maturity up to 105 DAS with maximum methane yield ha^{-1} were recorded after 105 DAS, followed by 120 DAS, while lowest methane yield ha^{-1} were recorded 60 DAS (*Fig. 2b*). We also observed the significant positive correlation between DM yield and methane yield during both the years (*Fig. 3*); an increase in DM yield appreciably increased the methane yield. Interestingly, a negative correlation was observed SMY and lignin concentrations (*Fig. 4*); it was found that increase in lignin concentrations substantially reduced the specific methane yield.

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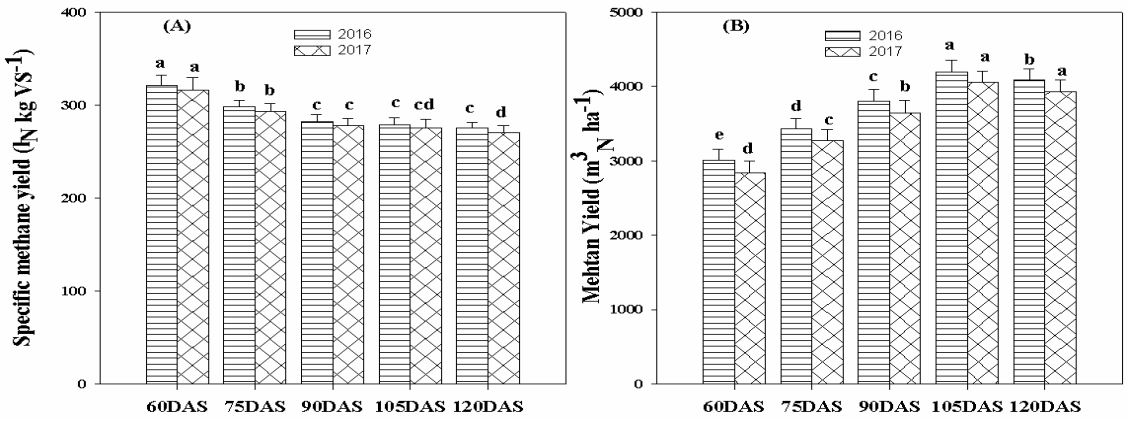


Figure 2. *Influence of harvesting times on specific methane yield (A) and methane yield ha^{-1} basis (B) during the year 2016 and 2017. DAS: DAS: Days after sowing, Values represent means ± S.D. The significant differences were measured by the least significant differences at 0.05 and indicated by different letters*

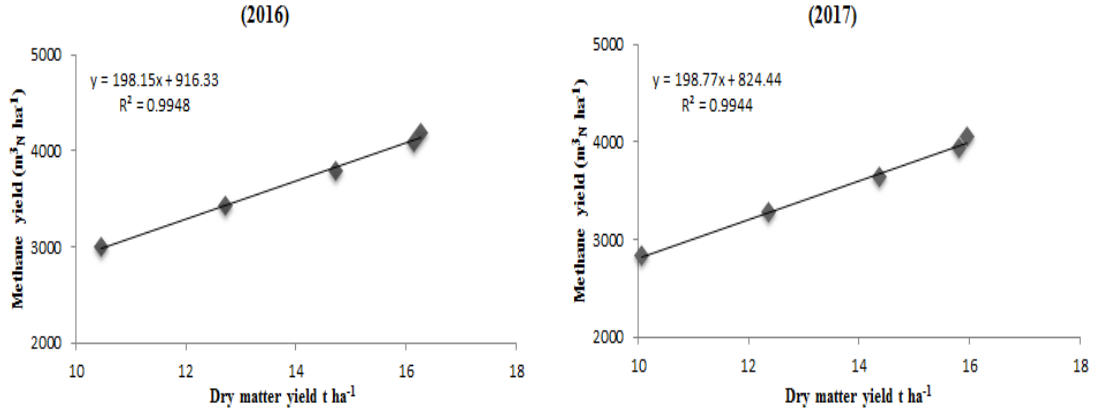


Figure 3. *Relationship between dry matter yield ha^{-1} and methane yield m^3_N ha^{-1} during 2016 and 2017*

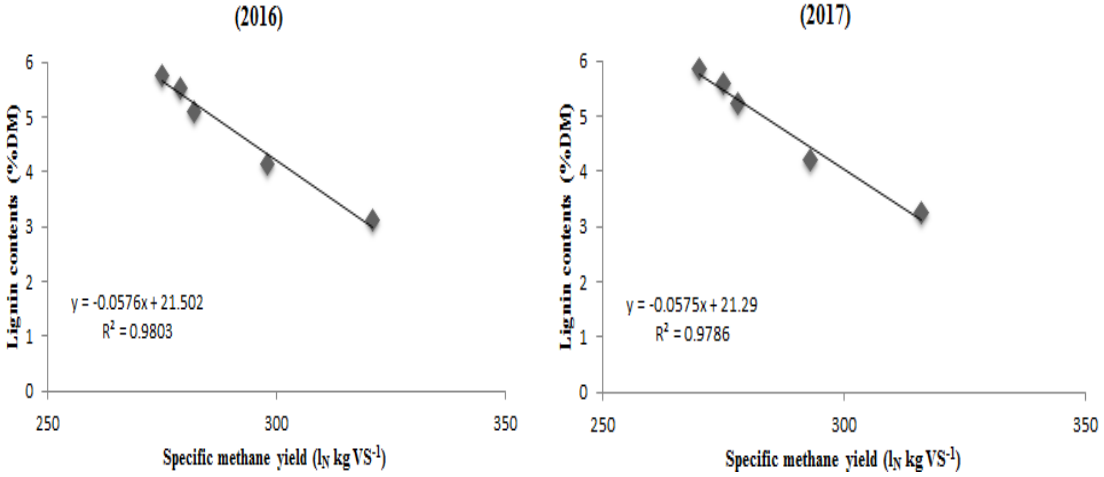


Figure 4. *Relationship between specific methane yield l_N kg VS^{-1} and lignin concentration (%DM) during 2016 and 2017*

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Discussion

Results revealed that harvesting times significantly influenced the dry matter yield, biomass quality and bio-methane yield. The plant height increased with delaying the harvesting up to 105 days after sowing (DAS), afterwards, delay in harvesting had no significant effect on the plant owing to termination of stem elongation. These results are in consistence with earlier finding of Tariq et al. (2011), who also found the appreciable influence of harvesting times on plant height. Similarly, the maximum stem diameter was found 105 DAS that was due to continuous accumulation of fibrous compounds in central stem. Current findings are supported by Naeem et al. (2007) and Amodu et al. (2007) who also proved a significant effect of harvesting time on stem diameter. The DM yield positively increased with delaying maturity up to 105 DAS, afterwards a little bit reduction in DM yield was found. The lower DM yield at early stage can be ascribed to lower plant height and stem diameter, moreover, the maximum DM yield at 105 DAS was possibly due to maximum plant height and stem diameter. These results are in accordance with findings of Chattha et al. (2017b), Ram et al. (2007) and Hussain et al. (2002) who also reported the substantial increase in DM yield with advancing maturity. Nonetheless the reduction in DM yield after 105 DAS might be due to reduction in the availably of water soluble carbohydrates, more falling of leaves and initiation of plant senescence. The protein concentrations decreased positively with the passage of time with minimum protein concentration were found 120 DAS. Moreover, leaves are rich source of protein and stems are considered to be low in protein concentrations. Therefore, the reduction in protein concentrations with advancing maturity can also be due loss of leaves and increase in stem proportion. Other researchers also reported the considerable reduction in protein concentrations with advancing the maturity (Ayub et al., 2002; Butler and Muir, 2003). Similarly, the maximum sugar concentration was found at earlier harvesting and lowest sugar concentrations were found at later harvesting stage. We suppose that younger plants have more physiological activities, thus have more sugar production. These findings are corroborated with earlier results of Umer et al. (2017b) who also reported the considerable influence of harvesting times on the sugar concentrations. Similarly, structural fibers including ADF, NDF, cellulose, hemi-cellulose and lignin concentrations tended to increase, whereas the ash concentrations tended to decrease with advancing the maturity stage. The structural fibers including cellulose, hemi-cellulose and lignin are parts of secondary cell wall, and they appear during the formation and thickening of cell walls, therefore, cell wall thickening increases with maturity which in turn influences the structural fiber and lignin concentrations. Earlier researchers also reported structural fiber and lignin contents considerably increased with the advancing maturity (Filya, 2004; Carmi et al., 2005; Miron et al., 2006). The ash concentrations decreased with delayed harvesting owing to loss of plant leaves and translocation of inorganic nutrients form vegetative plant parts to reproductive parts. The considerable differences in ash concentrations with varying harvesting stages have also been documented by Kitaba and Tamir (2007).

We also reported the considerable influence of harvesting times on the specific methane yield and methane yield per hectare basis. The negative correlation between SMY and lignin concentration might be due to the reason that the higher lignin concentration decrease the digestibility of biomass and consequently decrease SYM and vice versa. The maximum SMY were reported 60 DAS, whereas, the minimum SMY were reported 120 DAS, conversely, maximum methane yield ha^{-1} were found 105 DAS, whereas the minimum were reported at 60 DAS (*Table 2*). The maximum SMY at

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60 DAS can be ascribed to lower lignin concentrations, because lower lignin concentrations resulted in earlier and better digestibly of dry matter which leads to higher specific methane yield. In addition, higher lignin concentrations substantially reduced the DM digestibility (Yosef et al., 2009) and ultimately the specific methane yield. Similarly, despite of the lower SMY at 105 DAS, the methane yield ha^{-1} increased due to higher dry matter production ha^{-1} basis.

Conclusion

Harvesting times had considerable influence on the biomass yield, biomass quality and bio-methane yield. Early harvesting produced the crop significantly lower in DM yield and also lower in structural fiber and lignin concentrations. Moreover, later harvesting produced the crop higher both in DM yield and structural fiber and lignin concentrations. Similarly, maximum SMY were recorded 60 DAS, however, it was over compensated by 105 DAS owing to higher dry yield ha^{-1}. Conclusively, harvesting 105 DAS have advantage over the other harvesting times due to higher DM yield in order to get maximum methane yield ha^{-1}. However, further studies are needed to include more cultivars to explore the effect of different harvesting times and other agronomic factors i.e. sowing times, planting geometry and locations on chemical composition and bio-methane yield of sorghum under climatic conditions of Pakistan.

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ESTIMATION OF WATER PRODUCTIVITY AND CALIBRATION AND VALIDATION OF THE CROPSYST MODEL FOR RICE UNDER NITROGEN AND IRRIGATION MANAGEMENT

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Abstract. Simulation of yield response to water and fertilizer plays a key role in improving the efficiency of agricultural water. In this regard, the purpose of this study is the calibrating and validating of the CropSyst model to evaluate the effect of nitrogen fertilization and irrigation on the growth of rice in the crop field at the Rasht Rice Research Institute between 2013 and 2014. Three irrigation treatments (continuous, around five and eight days) were considered as the main factors and the amount of nitrogen in four concentration (0, 90, 120, and 150 kg N ha^{-1}) as a sub-factor. Based on the evaluation results it can be concluded that the CropSyst model, with the normalized root mean squared error ($RMSE_n$) 7 and 15% with explanation factor, R^2 of 0.73–0.84 for simulating the rice grain yield, and also $RMSE_n$ 9 and 10%, R^2 of 0.77–0.82 for simulating the biological yield, had the appropriate accuracy of the simulations. According to $RMSE_n$ 1 and 0.8%, R^2 of 0.58–0.73 for simulating the leaf area index, suggested a moderate simulation. These results showed that a reasonable estimate of the model as the efficiency of a model for the grain yield, biological yield, and LAI were 0.87, 0.98, and 0.80, respectively. The results of the simulation based on the amount of transpiration water productivity for both years showed that treatment I_3N_2 and I_3N_3had the highest amount. The amount of evaporation in irrigation water management two-year period only 39% of evapotranspiration is evaporated, While the amount of intermittent irrigation management 5 and 8 days, respectively, 35% and 32% respectively. These subjects simulations suggest that, given the good models and maximum data consistency, if management is aimed at maximizing the efficiency of water use can solve these models as a means to support the planning application.
Keywords: *intermittent irrigation, nitrogen, modeling, simulation*

Introduction

Water consumption improvement in agriculture is very important, compared with other sectors, because of the existing complexity in production, and the exploitation process and optimized application of irrigation water and nitrogen, especially in dry and semi-dry areas facing water constraints, is an important goal in this field (Raza et al., 2014). Climate changes, degrading of water resources and continuous drought has influenced agriculture in general and farmers' incomes specifically (Rezaei et al., 2013). As rice receives more irrigation water than other grain crops, developing water-saving

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irrigation approaches for rice re seen as a key component to deal with water shortages (Li and Barker, 2004). In recent years, many efforts have been applied in Iranian rice farms to decrease water consumption, and numerous reports have been published about the effect of low irrigation in decreasing water consumption and increasing rice efficiency (Pirmoradian et al., 2004; Razavipour and Kavosi, 2007). According to these reports, by changing the irrigation method from flood to intermittent irrigation without decreasing yield, or with an acceptable percent in decline, we can economize the use of water and increase the efficiency (Asadi et al., 2003). One of the most important zones of rice production in Iran is Guilan Province. Currently, climate change, reduction of fresh water, inappropriate use of water resources, construction of several dams across the White River basin, and the drought in the agricultural sector have threatened rice production and the income of farmers in the region (Zare et al., 2014)

These reports showed that permanent flood irrigation is not only a necessity but, also in dry and semi-dry areas in which the higher efficiency is important, we need to accept the expense of management to decrease the time and amount of irrigation (Razavipour and Kavosi, 2007). Additionally, in some cases, the average water pressure was recommended for better yield (Asadi et al., 2003). In some physiological periods of rice, intermittent pressure, as compared with permanent flood irrigation, caused an increase in the yield, although increasing the pressure will result in a decreased yield (Asadi et al., 2003).

Due to different intervals of time and place, it is difficult to determine various levels of yield through farm experiments. As such, computer simulation models can be used as appropriate tools for cultivation system studies and prepare optimized consumption patterns for the two inputs. Using simulation models is a way to predict and check water balance, growth process simulation, and the study of different management scenarios (Amiri and Rezaei, 2013a). One of the effective methods to reach these goals is the use of some models for plant growth, such as CropSyst (Crop Simulation System), which is a simulation model for rice growth that enables us to obtain the correct results through calculations of data statistics regarding the situation of water, weather, Earth, management system, and plant genetics *(*Amiri and Rezaei, 2013b). By using this model and its results, we can prevent excess consumption of water and nitrogen fertilizers.

Improvement of the CropSyst model was started by Stokel and colleagues in 1994. CropSyst had been developed during the last 15 years to a multi-crop, multi-year simulation model and can be connected to GIS software, which consist of programs like the CropSyst cultivation simulator system, reconstruction of meteorological data (ClimGen), GIS connection program (ArcCs), watershed models, and several other useful programs (Singh, 2008). This model has been used in various cases and in different areas (Saadati et al., 2012). In Southeast Australia, the CropSyst model could simulate the phenology, dry material, and the yield of wheat, green pea, and mustard (Diaz-Ambrona et al., 2001). Quite well. Since the 1980s, crop modellers have been pointing their attention on rice productions. Part of the APSIM crop models (Confalonieri et al., 2009), and the models of the CERES family basically implement a RUE based approach. Part of the models include crop growth equations based on the transpiration use efficiency (Keating et al., 2003). WARM (Confalonieri et al., 2006) is a novel model for paddy rice simulations developed by an interdisciplinary network of scientists working in different fields of rice research and modelling. The simplified model (CropSyst) may cost less in parameterization terms than the more complex models to achieve a similar level of confidence in the results, but WARM proved more

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accurate than the simplified model. The complexity of WOFOST is required to test hypotheses about processes at a lower level (Confalonieri et al., 2006). Therefore, models that are relatively simple to use with minimal input data that is readily available or easy to obtain may be a more useful analytical tool for technical staff, water managers, policy makers and other end-users in less developed countries (Khov et al., 2017).

Regarding the importance of rice cultivation in Guilan Province, and the necessity to optimize economic use of agriculture inputs, and the need to use simulation models of plant growth in irrigation management, this experiment has been conducted with the aim of gaining the best management for irrigation, nitrogen, and evaluate the application of the CropSyst model for rice yield under various water and nitrogen management scenarios.

Materials and methods

Field experiments

A two-year field experiment was conducted at the experimental farm of the Iranian Rice Research Institute in Rasht (37' 12° N; 49' 38° S; 7 m below sea level) from 2013–2014. The experimental design was a split plot with a complete randomized block and three replicates. The plot size for the subplots was 15 m^2 (3 m × 5 m). In this experiment, the main plots were three irrigation regimes: pond during growth period as a control treatment (I1), 5-day intervals (I2), and 8-day intervals (I3), and subplot treatments of four levels of N (no N application (N1), 90 kg (N2), 120 kg (N3), and 150 kg (N4)); urea was the source of N. A mixed commercial fertilizer was applied at the rate of 25 kg ha^{-1} of phosphorus (P) (in the form of phosphorus pentoxide (P_2O_5)) and 75 kg ha^{-1} of potassium (K) (in the form of dipotassium oxide (K_2O)). The rice variety 'spring' resulted in the highest yield in Guilan Province. Field experiments (*Table 1*) were carried out on a clay soil tissue (9% sand, 44% silt, and 47% clay). For determining the soil characteristics site of this experiment, several random samples of soil were obtained from a depth of 0–30 cm before transplanting and adding fertilizers and, after mixing soil samples, soil was sent to the laboratory for analyzing the soil in terms of physical and chemical properties (the results are shown in *Table 1*). Nitrogen fertilizer was applied three times during bolting, 50% at the time of transplanting to the field, 25% at maximum tillering, and 25% of the land. Irrigation was applied 20 days after transplanting management and to measure how many water counters in each plot were used.

Daily weather data on maximum and minimum temperatures, rainfall, and sunshine hours were collected for the entire growing season from a meteorological station beside the Iranian Rice Research Institute (*Fig. 1*).

Table 1. *Characteristics of the used soil*

pH of dough	Total N (%)	OC (%)	P (ppm)	K (ppm)	CEC (meq/100 g)	Tissue	Depth soil (cm)
7.2	0.13	1.32	11.9	188	31	Clay	30-0

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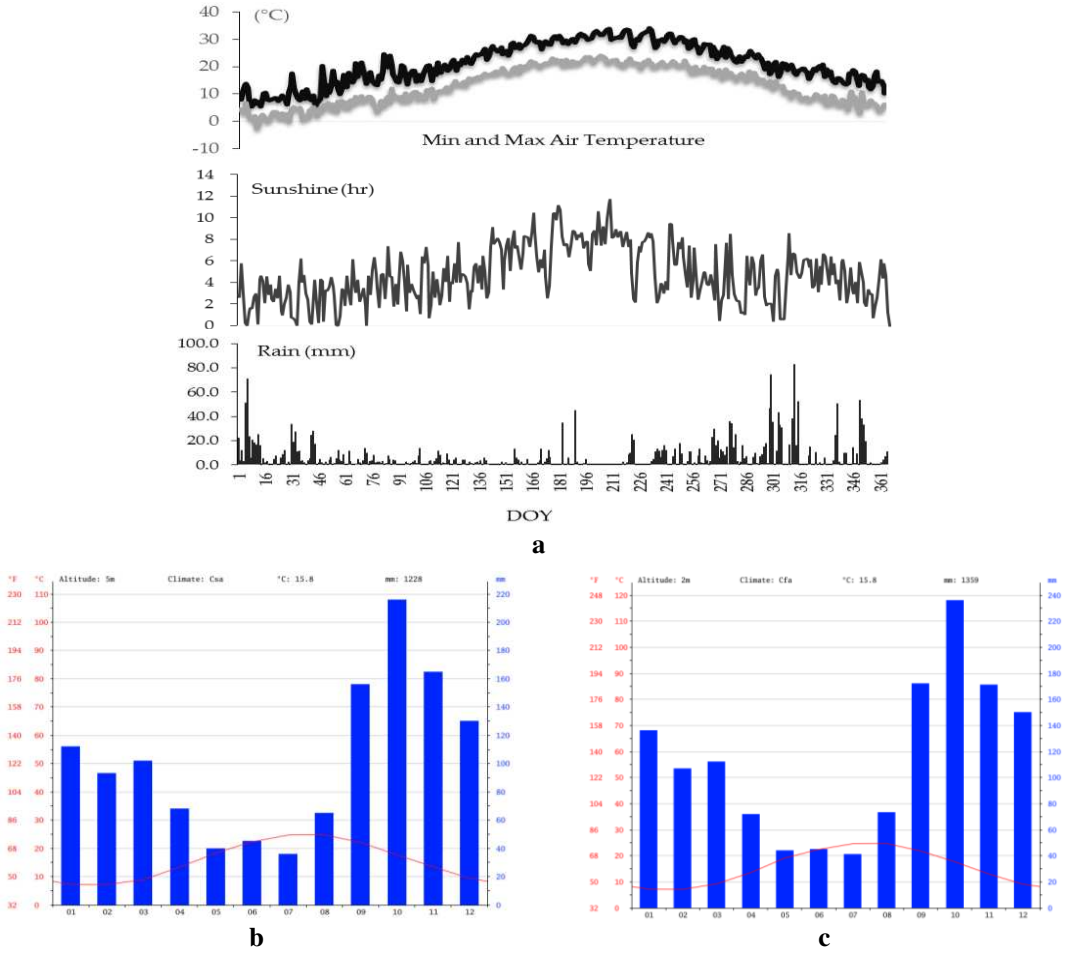


Figure 1. *a: Measured daily meteorological variables at the study site for the two years of the experiment (from 1 January 2013–22 September 2014). DOY is day of year. b, c: Measured daily Temperature and rainfall at the study site for the two years of the experiment (2013, 2014)*

The continuous irrigation amount during the growing season was about 5 cm. Samples of each plot have been studied with respect to the growth using a destructive analysis method. Plant harvesting was on 11–15 August in all years of the experiment. All plots were bonded and separated by 0.5-m-wide strips of bare soil to avoid lateral movement of water and nutrients among treatments. The plots were hydrologically separated by plastic sheets installed 40 cm below the soil surface to restrict water and N flow between adjacent plots. Measurements of samples were collected at the beginning of transplanting in all treatments, Crop samples were taken at regular intervals of 10–15 days to determine leaf area index (LAI) and total and panicle biomass over two years. The LAI of plants was measured by a Model GA-5 manufactured by OSK Japan meter. In other to evaluation of biological yield, 1 m^2 of each plot were harvested (Includes all vegetative and reproductive organs of plants top of the soil) and then placing it in the oven until (Drying machine plant material) dried.

CropSyst model descriptions

Version 3 of the CropSyst model was used in this study. Crop development was simulated on the basis of the accumulated thermal time required to reach each

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phenological stage. In order to evaluate the simulation effects of nitrogen fertilizer and irrigation on the growth yield of rice, some models were used which simulate the plant growth on a day-by-day and phase-by-phase basis to obtain the results. To run the models four sets of data were required as input. File locations, soil, and plant management definition, application, and all required parameters of the model range were stated in the manual model (Stöckle, 2003). Model inputs for the simulation consisted of daily weather information (including maximum and minimum temperature, precipitation, and solar radiation), soil (including physical and chemical properties of each layer), the cultivar characteristics (such as growth factors, growth, yield, and biomass) and crop management practices (including the use of irrigation and fertilizer), and the culture system (Amiri and Rezaei, 2013b).

Simulation model

Yield simulation of the CropSyst model depended on the total biomass, which accumulated at physiological maturity (B_{PM}), and the harvest index (HI = harvestable yield/aboveground biomass; *Eq. 1*):

$$Y = B_{PM}\,HI \qquad \text{(Eq.1)}$$

Where Y is yield (kg m^{-2}) and B_{PM} is also in kg m^{-2}.

To introduce the processes of the CropSyst simulation, some equations are more important in this study, so they are presented here. Crop transpiration is dependent on biomass production (B_{Pt}) and LAI were effective in the output of CropSyst based on calibration results. The third equation is the yield, which was one of the main evaluated outputs. Therefore, these three equations are presented in this paper; also, more information can be found in another study (Stöckle et al., 2003). There is a relationship between crop transpiration and biomass production, which is based on carbon and vapor exchange in leaves. Thus, the potential daily biomass production can be calculated as (*Eq. 2;* Kumar et al., 2006):

$$AGB_{PT} = \frac{k_{BT} T_P}{VPD} \qquad \text{(Eq.2)}$$

Where AGB_{PT} is the crop transpiration-dependent biomass production (kg m^{-2} day^{-1}), T_P is the crop potential transpiration (kg m^{-2} day^{-1}), VPD is Average daily steam pressure loss (kPa), and K_{BT} is a biomass-transpiration ratio (kPa).

Water productivity estimation

In this study, the amount of water productivity was calculated (*Eq. 3*) based on the sum of water and rainfall, with respect to the amount of water productivity WP_{I+R} (kg/m^2), where Y is the yield (kg/h), I is the amount of irrigation (mm), and R is the precipitation (mm). The amount of rainfall, evaporation, average maximum daily temperature, and irrigation in the years studied is shown in *Table 2*.

$$WP = \frac{Y}{I + R} \qquad \text{(Eq.3)}$$

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Table 2. *Amounts of water irrigation, precipitation, and evaporation and maximum temperature*

2014		2013		Treatments	
P	**I**	**P**	**I**	**Nitrogen**	**Irrigation**
59	804	79	744	N_1	I_1
	801		724	N_2	
	789		712	N_3	
	759		732	N_4	
	726		701	N_1	I_2
	741		703	N_2	
	746		701	N_3	
	746		707	N_4	
	419		469	N_1	I_3
	459		459	N_2	
	496		461	N_3	
	466		429	N_4	

Calibration of the models

Initially, soil, weather, and irrigation files were prepared similarly for all models. Then measured and estimated crop parameters were inserted in the models. After calibration of the model and determination of the optimal coefficients, a validation model was performed by using the second year of treatment (*Table 3*). In these models, plant development was measured with respect to temperature time (°C–days). From the start to the increase in the planting stage, temperature time must be specified for each phenological stage. Based on the type of plant phenology, the important and fundamental steps included the emergence, flowering, time of maximum leaf area index, end of flowering, start of grain filling, and physiological maturity.

Table 3. *Some relevant crop parameters used in the CropSyst model for the rice simulation*

Parameters	Amount	Unit
Optimum temperature (T_{opt})	27	°C
Maximum water uptake	10	mm day^{-1}
Maximum rooting depth	1.5	m
Maximum expected leaf area index (LAI)	5.9	$m^2 m^{-2}$
Specific leaf area (SLA)	30	$m^2 kg^{-1}$
Leaf duration	750	°C-days
Extinction coefficient for solar radiation (k)	0.5	-
Emergence Stage	135	°C-days
Peak LAI	1350	°C-days
Begin flowering	1290	°C-days
Begin grain filling	1300	°C-days
Physiological maturity	1900	°C-days
Base temperature (T_{base})	12	°C

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Model evaluation

Several statistical methods were used to compare the simulated and observed results. In this article, we used a combination of graphical analyses and statistical measures to compare the simulated and measured final biomass and yield and LAI graphically. In this article, we evaluated model performance by using the absolute root mean square error (RMSE), normalized root mean square error ($RMSE_n$), coefficient of residual mass (CRM), coefficient of determination (R^2), and effective modeling (EF). RMSE, $RMSE_n$, CRM, and R^2, index of agreement (IOA), and EF characteristics are common tools to test the goodness of fit of simulation models (*Eqs. 4–9;* Pala et al., 1996; Fila et al., 2003):

$$RMSE = \left[\frac{\sum_{i-1}^{n} \left(P_i - O_i \right)^2}{n} \right]^{1/2} \quad \text{(Eq.4)}$$

$$RMSEn = \frac{RMSE}{\overline{O}} \quad \text{(Eq.5)}$$

$$R2 = \frac{\sum_{i-1}^{n} \left(P_i - O_i \right)^2}{\sum_{i-1}^{n} \left(O_i - \overline{O} \right)^2} \quad \text{(Eq.6)}$$

$$EF = \frac{\sum_{i-1}^{n} \left(O_i - \overline{O} \right)^2 - \sum_{i-1}^{n} \left(O_i - \overline{O} \right)^2}{\sum_{i-1}^{n} \left(O_i - \overline{O} \right)^2} \quad \text{(Eq.7)}$$

$$CRM = \frac{\sum_{i-1}^{n} O_i - \sum_{i-1}^{n} P_i}{\sum_{i-1}^{n} O_i} \quad \text{(Eq.8)}$$

$$IOA = \frac{\sum_{i-1}^{n} \left(P_i - O_i \right)^2}{\left| \left(P_i - \overline{P} \right) \right| + \left| \left(O_i - \overline{O} \right) \right|} \quad \text{(Eq.9)}$$

Where Pi is the simulated value, Oi is the measured value, and n is the number of measurements. The simulation was considered excellent if the normalized RMSE was less than 10%, was considered good if the normalized RMSE was greater than 10% and less than 20%, was considered fair if the normalized RMSE was greater than 20% and less than 30%, and was considered poor if the normalized RMSE was greater than 30%. The amounts of RMSE and $RMSE_n$ were at an optimum status if the simulated and observed were the same, and was equal to zero. If the *p*-value (*p*(t)) from the paired *t*-test was greater than 0.05, it was concluded that there were no significant differences existing between the measured and simulated values (Eitzinger et al., 2004). The IOA is also used to evaluate the model; it is a descriptive parameter value between zero and one, indicating how weak the model is in predicting results (Eitzinger et al., 2004).

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Results and discussion

Calibration of the models

Initially, soil, weather, and irrigation files were prepared similarly for all models. Then, measured and estimated crop parameters were inserted in the models. The crop parameters used in this study are presented in *Tables 4–6* for the models. A revalidation of the model depends upon its successful calibration based on field experimental data, and the accurate estimation of the specific model's coefficients in a given environment (Eitzinger et al., 2004).

Validation and evaluation of the models

The model validations were based on the comparison between simulated and observed data for all treatments other than those used in the model calibration. Results showed that the average grain yield of RMSE under calibration and validation conditions was 461 and 847 kg per hectare, respectively. The average $RMSE_n$ of grain yield under calibration and validation conditions were 7% and 15%, respectively. The amounts of the measured grain yield showed a desirable simulation of this parameter in the agriculture season by the model which we could then use in irrigation planning and nitrogen rice fertilizer management (*Tables 4* and *6*). Araya et al. (2010) who report a deviation range of validation data of −13 to 15.1% for grain yield.

Table 4. *Observed and simulated grain yields and relative error for rice in 2013*

Treatment		Relative error (%)	Simulation grain yield (kg/ha)	Observed grain yield (kg/ha)
Irrigation	Nitrogen			
I_1	N_1	15	5053.8	4382.5
	N_2	7	6533.6	6120.1
	N_3	–15	6525.6	7664
	N_4	–2	6953.6	7093
I_2	N_1	3	5273.3	5135
	N_2	–1	6001	6084.4
	N_3	3	6825.9	6648.2
	N_4	10	7163.1	6478
I_3	N_1	9	5024	4596.3
	N_2	2	6405	6302
	N_3	13	7466	6604
	N_4	1	7213	7124

Table 5. *Calibration results of crop parameters in CropSyst model for rice simulation in 2013*

Plant parameters	EF	C_{RM}	IOA	T test	$RMSE_n$ (%)	RMSE	R^2	Number of samples
Biology Yield	0.98	0.06	0.97	0.13	9	1264	0.77	12
Grain Yield	0.89	–0.02	0.99	0.32	7	461	0.73	12
LAI	0.22	0.2	1	0.04	29	1	0.58	12

Notes: $RMSE_n$ (%) is the normalized root mean square error; R^2 is the adjusted linear correlation coefficient between simulated and measured values; EF is the efficiency of model; IOA is the index of agreement; T test: tested the amounts with respect to the mean; CRM is the coefficient of residual mass

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Table 6. *Validation results of crop parameters in the CropSyst model for rice simulation in 2014*

Plant parameters	EF	C_{RM}	IOA	T test	$RMSE_n$ (%)	RMSE	R^2	Number of samples
Biological Yield	0.80	–0.04	0.96	0.27	10	1219	0.82	12
Grain Yield	0.98	–0.14	0.98	0.03	15	847	0.84	12
LAI	0.38	–0. 7	1	0.02	68	82	0.82	12

Notes: $RMSE_n$ (%) is the normalized root mean square error; R^2 is the adjusted linear correlation coefficient between simulated and measured values; EF is the efficiency of model; IOA is the index of agreement; T test: tested the amounts with respect to the mean; CRM is the coefficient of residual mass

The results of treatment evaluations showed that the average RMSE was equal to 847 kg per hectare, relative RMSE was 15%, and CRM was –0.14, which, compared with 386 kg per hectare, 8%, and 0.12, was greater than the calibration phase, and indicates the closeness of the simulation amounts and validation values. For both calibration and validation phases, the amount of CRM that excluded the maximum LAI and dry material in the first year was negative, which showed that the amount in the simulation in most treatments was more than the observation amounts. In other words, in most treatments, the estimated amount of the model was more than the real amounts. Ouda et al. (2015), who reported The RMSE was 8% of the mean observed yield.

The EF under the calibration and evaluation conditions was also 0.89 and 0.87, respectively. Based on the studies, simulation of this model under different levels of nitrogen was performed on corn plants, and the EF of the calibration and evaluation phases were 0.52 and 0.90, respectively (Mohseni, 2008). Additionally, in other studies of the simulation of wheat plant yield by the CropSyst model, the value of the RMSE was equal to 0.21 Mg/ha and the value of the correlation factor was 0.72 (Sadras, 2002). Modeling efficiency (EF) were 0.82 and 0.75 for biomass and soil water content, respectively (Bellocchi et al., 2002; *Tables 5* and *6*)

According to the research on the simulation of rice plant yield in North Italy, using the CropSyst model, the $RMSE_n$ amplitude of dry material simulated measured for the calibration and validation year was 11%–29% and 10%–52%, respectively, and the CRM amplitude of dry material simulated measured for calibration and validation years was –0.03% to 0.17% and –0.02% to 0.17%, respectively (Confalonieri and Bocchi, 2005). Moreover, based on studies of the evaluation of the CropSyst model on the simulation of both the effects of water and nitrogen on wheat plants, the value of the RMSE for the simulation yield in the CropSyst model was 0.36 Mg/ha. The value of the RMSE for the simulation of dry material in CropSyst was 1.27 Mg/ha (Saadati et al., 2012). The comparison of these results with the values gained in this research for rice plants showed that the CropSyst model could also simulate the rice yield well.

Results showed that the average RMSE yield biology under calibration and validation conditions were 1264 and 1219 kg per hectare respectively. The amounts of average $RMSE_n$ yield biology under calibration and validation conditions were also 9% and 10%, respectively. The EF that was obtained under calibration and evaluation conditions is also equal to 0.99 and 0.80, respectively. For performance recognition of the CropSyst model in the simulation of dry material production and the yield of the reaction to the water and nitrogen, researchers studied the separate products in a season under experimental conditions with extensive preparations from dry to full irrigations

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and from low nitrogen existing in the soil to high level conditions. In these evaluations, the reported value of RMSE was 0.443 t/ha (Pala et al., 1996). Simulation results showed satisfactory application by suitable value of RMSE of biomass growth (0.58-3.52 t ha^{-1}) and water content in the soil profile (20.9-50.6 mm) (Raza et al., 2014).

Results showed that the average RMSE under calibration and validation conditions were 1 and 0.80 m^2 of leaf surface in each square meter at ground level. The values of $RMSE_n$ for the maximum LAI under calibration and validation conditions were 29 and 23, respectively. The results showed an acceptable accuracy of this model for simulation of the maximum LAI. The amounts of the measured parameters indicated that the medium simulation of this parameter, as well as the agriculture season by which this model can be used for planning of rice irrigation and nitrogen fertilizer management. The EF under calibration and evaluation conditions obtained were 0.12 and 0.32, respectively.

Our observations showed that relative error of simulation yield in the calibration phase was –15% to 15% and, in the evaluation phase, it was between 2% and 52%. Additionally, the amount of biomass simulation relative error in the calibration phase was –22% to 2% and in the evaluation phase, it was between –10% and 27%. Other research indicated that the amplitude amount of error for the growth simulation of the wheat plants between the simulation and observation yield in this plant was 2.7–37.2% and, for dry material, it was between 2.2–30% (Mohseni et al., 2008). Results showed that R^2 for the calibration model data was 0.84, which means that the model is suitable for grain yield simulation. Additionally, a relatively high R^2 value means low dispersion of the data (Amiri and Rezaei, 2013a).

The amount of IOA (Willmott agreement index) was one of the most important indicators of the plants' assessment modeling. The IOA under calibration (first year) conditions for maximum LAI, grain yield, and dry material was 1, 0.99, and 0.97, respectively. Additionally, the IOA under evaluation conditions for maximum LAI, grain yield, and dry material was 1, 0.98, and 0.96, respectively. Results of the research by Zhang et al. (2011) show that IoA was in the excellent range (Zhang et al., 2011).

Tables 7–11 show the comparisons between the amounts of simulated and measured grain yield.

Table 7. *Observed and simulated grain yields and relative error for rice in 2014*

Treatment		Relative error (%)	Simulation grain yield (kg/ha)	Observed grain yield (kg/ha)
Irrigation	Nitrogen			
I_1	N_1	34	4867	3646.5
	N_2	12	6622.6	5907.1
	N_3	5	6614.6	6307
	N_4	2	6705	6577
I_2	N_1	52	5276.3	3476.3
	N_2	19	7225.1	6097
	N_3	28	7266	5677
	N_4	11	6966.8	6302
I_3	N_1	29	5030.1	3907.3
	N_2	8	6470	5684.9
	N_3	7	7040.4	6610
	N_4	6	7140	6712

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Table 8. *Observed and simulated LAI and relative error for rice in 2013*

Treatment		Relative error (%)	Simulation LAI	Observed LAI
Irrigation	Nitrogen			
I_1	N_1	89	3.19	2.28
	N_2	9	4.31	4.13
	N_3	5	4.25	4.6
	N_4	12	4.87	4.82
I_2	N_1	39	3.55	1.73
	N_2	33	4.69	2.83
	N_3	20	4.69	2.62
	N_4	36	4.19	4.9
I_3	N_1	4	2.4	1.68
	N_2	26	4.52	3.59
	N_3	31	5.3	3.8
	N_4	16	4.8	5

Table 9. *Observed and simulated LAI and relative error for rice in 2014*

Treatment		Relative error (%)	Simulation LAI	Observed LAI
Irrigation	Nitrogen			
I_1	N_1	45	3.3	2.28
	N_2	-3	4.01	4.13
	N_3	-4	4.42	4.6
	N_4	2	4.9	4.82
I_2	N_1	106	3.65	1.73
	N_2	74	4.89	2.83
	N_3	79	4.89	2.62
	N_4	0	4.89	4.9
I_3	N_1	19	3.34	1.68
	N_2	24	4.4	3.59
	N_3	17	4.49	3.8
	N_4	-8	5.32	5

Table 10. *Observed and simulated biomass and relative error for rice in 2013*

Treatment		Relative error (%)	Simulation biomass (kg/ha)	Observed biomass (kg/ha)
Irrigation	Nitrogen			
I_1	N_1	–2	10,107	10,335
	N_2	–2	13,066	13,366
	N_3	–15	13,051	15,397
	N_4	–22	13,720	17,571
I_2	N_1	2	10,549	10,371
	N_2	–1	14,326	14,532
	N_3	1	14,081	13,962
	N_4	–7	14,100	15,100

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I_3	N_1	–3	10,049	10,386
	N_2	–7	12,811	13,772
	N_3	–9	14,221	15,684
	N_4	–6	14,062	14,985

Table 11. *Observed and simulated biomass and relative error for rice in 2014*

Treatment		Relative error (%)	Simulation biomass (kg/ha)	Observed biomass (kg/ha)
Irrigation	Nitrogen			
I_1	N_1	17	9735	10,335
	N_2	18	13,244	13,366
	N_3	–5	13,228	15,397
	N_4	0	13,688	17,571
I_2	N_1	15	10,553	10,371
	N_2	7	14,451	14,532
	N_3	–3	14,532	13,962
	N_4	–4	14,232	15,100
I_3	N_1	26	9661	10,386
	N_2	27	12,940	13,772
	N_3	–4	13,241	15,684
	N_4	–10	13,451	14,985

Results showed a change from flood to intermittent irrigation, the real grain yield decreased, and the increase of nitrogen consumption led to the grain yield increasing. The model also showed a decrease and an increase in simulated grain yield pretty well. The minimum amount of grain yield was under the condition of the non-use of nitrogen fertilizer, and the maximum yield was under the nitrogen treatment condition of 120 kg N per hectare, with intermittent irrigation was every 5–8 days.

It is worth noting that low irrigation is also effective in this matter. In 5-day irrigation management, the model could not simulate the yield well because of incompetent ability to estimate grain yield. The existence of some weather parameters, such as wind speed and carbon dioxide changes in the farm, which were not present in the model (the model anticipates grain yield according to other climate parameters) might also be another reason for the decrease in foresight accuracy by the model (Honar et al., 2010).

In testate fertilizer amounts, the error percent of the model was higher, but it will be decreased by increasing the fertilizer amounts so that after forth fertilizer application the error will be erased completely. Changes in the amount of simulated grain yield by the CropSyst model for the first and second year are shown in *Figure 2*. The minimum amount of grain yield was under the condition of the non-use of nitrogen fertilizer, and maximum yield was under nitrogen treatment condition of 120 kg nitrogen per hectare and intermittent irrigation was every eight days. The results showed that the gain yield would increase by increasing the nitrogen consumption. The model also showed fluctuations in simulated grain yield rather well. The amount of grain yield will increase by adding more nitrogen via irrigation management, but in higher levels of nitrogen (150 and 120 kg ha^{-1}) a slight increase will be shown. According to other indices to

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assess irrigation regimes and fertilizer levels, the most suitable treatments regarding environmental aspect were 5-day irrigation regime and 45 kg N ha^{-1} (Zare et al., 2014).

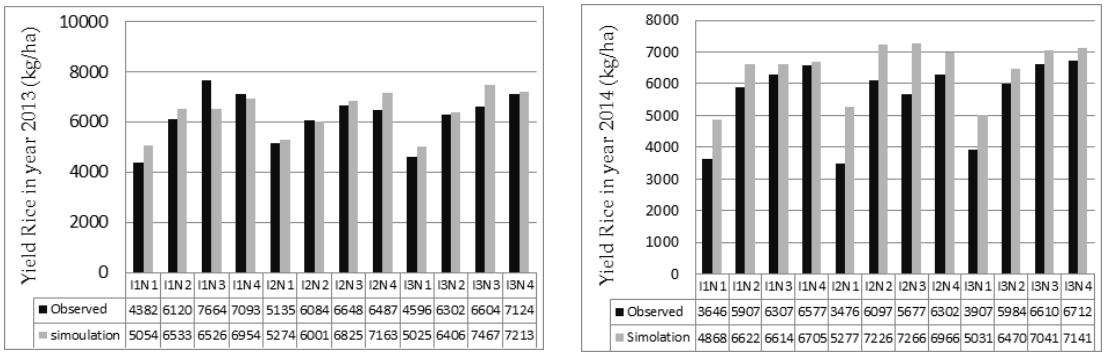


Figure 2. *Observed and simulated grain yields for rice in 2013 and 2014. Irrigation. (I1 = continuously flooded, I2 = 5 per day, I3 = 8 per day) and N fertilizer (N1 = 0, N2 = 90, N3 = 120, N4 = 150 (kg/ha))*

On the other hand, the grain yield will decline under flood irrigation conditions. Additionally, by changing the irrigation model from flood to intermittent treatments of every 5–8 days, simulated grain yield will show a slight increase; this indicates that the amount of nitrogen consumption is more than the nutritional needs of the spring type of rice in this research. Changing of the irrigation from flood to intermittent will reduce hydrostatic pressure at the ground level, which causes a reduction of water loss through leakage and deep percolation (Bouman et al., 2007).

Results of the research showed that the change in flood irrigation management reduced deep losses. Therefore, investigation of results about the amount of leakage and deep percolation in the period of the research showed the minimum and maximum water loss through deep percolation as 117 and 221 mm, respectively, under flood and non-flood management (Amiri and Rezaei, 2013a). Results also indicated that changing from flood to non-flood irrigation reduced irrigation water, which was consistent in other studies (Pirmoradian et al., 2004; Amiri and Rezaei, 2013a).

Other studies showed that changing the irrigation method from flood to non-flood will result in saving irrigation water. Due to the increasing competition for water, water-saving technologies, such as alternate wetting, drying, and aerobic rice are being developed to reduce water consumption while maintaining a high yield. The components of the water balance of these systems need to be disentangled to extrapolate water savings from the field scale to the irrigation system scale (Bouman et al., 2007). As shown in this study, irrigation and N fertilizer management improved the efficiency of water consumption and, thus, reduced the impact of water shortages. The results of this study provided a basic information base for making irrigation and N management decisions in the study area.

Water productivity estimation

The amount of water productivity was calculated based on the sum of water and rainfall, as shown in *Table 12*. The results show that the change in flood irrigation management increased water productivity. The model also showed the simulated

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decrease and increase rather well. Additionally, results from the treatments of I_3N_3 and I_3N_4 in both models showed the greatest amount of water productivity was in the first and second years. This result suggests that the mechanism of the plant's production performance, despite the reduction in available water to the surface, has been successful. Furthermore, the value of the evaporation results in the CropSyst model showed that, in irrigation flood management, up to 39% of evapotranspiration occurs. While the value of intermittent irrigation management of five and eight days, respectively, was 35% and 32%. The amount of water productivity was calculated based on the sum of water and rainfall, with greater reliance on management practices, especially irrigation methods. The results Jalotal et al. (2006) suggest that water productivity in rice can be enhanced mainly by adjusting the transplanting date. The enhanced water productivity in delayed transplanted paddy was due to reduction in ET while the yield remained unaffected (Jalotal et al., 2006). Results of the research by Umair et al. (2017) E was 30% of total ETa and results closely matched the observed data collected during the MLM and isotopes approach (Umair et al., 2017).

Table 12. *Simulated grain yield and water productivity amounts via the CropSyst model*

Year	Irrigation	Nitrogen	P (mm)	I (mm)	E (mm)	ET (mm)	GY (kg/h)	*WP*
2013	I_1	N_1	79	744	330	537	5053/8	0.61
		N_2	79	724	350	586	6533/6	0.81
		N_3	79	712	347	550	6525/6	0.83
		N_4	79	732	363	544	6953/6	0.86
	I_2	N_1	79	701	340	487	5273/3	0.68
		N_2	79	703	329	495	6001	0.77
		N_3	79	701	382	498	6825/9	0.88
		N_4	79	707	385	464	7163/1	0.91
	I_3	N_1	79	469	270	429	5024/1	0.92
		N_2	79	459	273	444	6405	1.19
		N_3	79	461	297	431	7466/4	1.38
		N_4	79	429	331	431	7213	1.42
2014	I_1	N_1	59	804	330	533	4867/8	0.56
		N_2	59	801	407	586	6622/6	0.77
		N_3	59	789	347	550	6614/6	0.78
		N_4	59	795	360	544	6705/6	0.79
	I_2	N_1	59	726	319	550	5276/3	0.67
		N_2	59	741	362	551	7225/1	0.9
		N_3	59	746	382	493	7266/9	0.9
		N_4	59	746	360	502	6966/18	0.87
	I_3	N_1	59	419	271	518	5030/1	1.05
		N_2	59	459	318	436	6470	1.2
		N_3	59	496	251	439	7040/4	1.27
		N_4	59	466	330	429	7140	1.3

I1 = Continuously flooded, I2 = 5 per day, I3 = 8 per day and N fertilizer N1 = 0, N2 = 90, N3 = 120, N4 = 150 (Kg/ha)

P = Precipitation, *I* = Irrigation, *E* = Evaporation, *ET* = Evapotranspiration, *GY* = Grain Yield, *WP* = Water productivity

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Results of the research by Amiri and Rezaei (2013a) show that the change in flood irrigation management reduces deep losses (Amiri and Rezaei, 2013a). So that investigation of results about the amount of leakage and deep percolation in a period of the research show the minimum and maximum of water dissipation through deep percolation as 117 and 221 mm, respectively, in flood and non-flood conditions. Results of that study also indicates that changing from flood to non-flood irrigation reduces irrigation water consumption, which is consistent with the research of Pirmoradian et al. (2004). They showed that changing irrigation methods from flood to non-flood will result in saving irrigation water (Pirmoradian et al., 2004).

Irrigation and N fertilizer management, as shown in this study, improves the efficiency of water use and, thus, reduces the impact of limited water. The results of the study provided an information base for making irrigation and N management decisions in the study area. These results suggest that due to the good simulation models and maximum data consistency that the CropSyst model can be used to support the management and optimization of water and nitrogen fertilizer to cultivate rice.

Conclusions

The CropSyst model was sufficiently accurate in the simulation of yield underwater-saving and crop density conditions for our study site. The results of this study clearly indicate that the CropSyst model can be used with a high degree of accuracy for yield simulation and were similar to those of other researchers (Asadi et al., 2003; Bouman et al., 2007; Amiri and Rezaei, 2013a; Umair et al., 2017). The CropSyst model could also simulate the rice yield rather well. An agreement index (IOA) was also used to evaluate the model. Given the negligible difference between observed and simulated value performance, it can be concluded that this model might be useful as a simulated model of the effect of water and nitrogen management which can be used in yield estimates. The results of this study showed that the model generally predicted grain yield and final biomass fairly satisfactorily across a range of datasets covering levels of irrigation and N conditions within two years in Northern Iran.

Thus, the most optimal conditions to offer to farmers was under nitrogen treatment condition of 120 kg nitrogen per hectare and intermittent irrigation was every eight days.

In the current study, the CropSyst crop simulation model was calibrated, validated, and used as a tool to provide estimates of water productivity of rice under a range of N fertilizer and water regimes in a humid region of Iran. The simplicity of the model in its required minimum input data, which are readily available or can be easily collected, makes it user friendly. The authors suggest that for better validation of models using other models and other concentration Nitrogen and Irrigation Management in Other areas suitable for rice cultivation.

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Author contributions. Farzad Paknejad, Ebrahim Amiri, Bahram Mirshekari, Mojtaba Mirakhori designed and performed the research. Ebrahim Amiri analyzed the data, discussed the results and wrote the paper. Bernd Honermeier, commented and edited the manuscript.

Conflicts of interest. The author claimed no conflict of interest.

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GLYPHOSATE-BASED HERBICIDE ALTERS THE HISTOLOGICAL STRUCTURE OF GILLS OF TWO ECONOMICALLY IMPORTANT CYPRINID SPECIES (COMMON CARP, *CYPRINUS CARPIO* AND BIGHEAD CARP, *ARISTICHTHYS NOBILIS*)

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Abstract. The present study primarily aims to investigate the histopathological effects which a glyphosate based herbicide could cause on the gills of two economically important Cyprinid fish (common carp and bighead carp), and to determine which species is more sensitive in terms of glyphosate contamination. The pesticide concentrations tested in laboratory conditions were decreasing (72 mg/l, 40 mg/l and 20 mg/l) and prepared by dilution of the stock solution of the commercial product used in plant protection. The experiment was short-term of 96 h. In general, the herbicide caused different pathological alterations in the fish gills, such as lamellar lifting, edema, proliferation of the glandular cells and epithelium, covering the gill filament, fusion, vasodilatation of the secondary lamellae and aneurysms. In addition, we observed a tendency towards the enhancement of the gill histological changes which degree of expression was proportional to the increasing pesticide concentrations. However, bighead carp was more sensitive compared to common carp when it comes to the tested chemical and the alterations in the gill histological structure were more pronounced.
Keywords: *histopathology, fish, biomarkers, pesticide, contamination*

Introduction

Pollution of water is mainly due to contamination with hazardous chemicals from agricultural runoff and waste water from household and different industries. One of the major chemicals from agricultural runoff are pesticides which play an important role in the agricultural productivity and have been widely applied to protect agricultural crops. On the other hand, they can cause severe damage to non-target organisms, both in the terrestrial and aquatic environment (Magar and Shaik, 2013; Grung et al., 2015).

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The glyphosate herbicides, post-emergent herbicides with a broad-spectrum and low selectivity, have been intensively applied on numerous field cultures, urban and industrial areas to control unwanted plants, including the macrophytes in aquatic systems (Nedelkoska and Low, 2004; Kolpin et al., 2006; Cąvas and Konen, 2007). In addition, most of the commercial formulations of herbicide-based glyphosate consist of glyphosate isopropylamine salt (IPA) and a surfactant in order to increase the herbicide's efficacy (Tsui and Chu, 2003).

Fish are among the group of non-target aquatic organisms which represent the largest and most diverse group of vertebrates and a number of characteristics make them excellent experimental models for toxicological research (Raisuddin and Lee, 2008). In this sense, fish are often used as indicators of pesticide water contamination due to the fact that they respond to it with physiological and behavioral alterations (Banaee et al., 2011, 2013; Murthy et al., 2013).

Cyprinidae is a family of freshwater fish which consists of 1600 species. This makes it the most diverse family. The most popular species, common carp (*Cyprinus carpio* Linnaeus, 1758) is a wide-spread and econocmically important fish which has immense significance for aquaculture. Carp is also very popular in sports fishing due to its fast growth rate, hardiness and prolific breeding in confined water. Furthermore, it has been proposed as a test organism in toxicological research because it is relatively insensitive and as a consequence, survives and accumulates contaminants even at heavily polluted sites (Snyder et al., 2004; Brumbaugh et al., 2005; Reynaud and Deschaux, 2005; Oruc and Usta, 2007; Reynders et al., 2008; De Boeck et al., 2010; Georgieva et al., 2014; Stoyanova et al., 2015). Bighead carp (*Aristichthys nobilis* Richardson, 1845) is another Cyprinid species which has become popular in aquaculture in recent years. It is usually bred with common carp and silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844) for better results. Similarly to common carp, it grows quite rapidly and its meat has started increasingly to be preferred for consumption in more countries (Čihař, 1975; Karapetkova and Jivkov, 2010). However, it has not been that widely studied in terms of the effects of different pollutants compared to common carp (Li et al., 2005; Hu et al., 2010; Li et al., 2014). Thus, in the present study we propose this Cyprinid species together with common carp as model species for ecotoxicological research.

Viana et al. (2013) suggest that the biomonitoring and risk assessment processes should include analyses at different levels of biological organization, from sub-cellular and cellular analysis of tissues and organs, to those of population and community levels. Therefore, Van der Oost et al. (2003) also suggest the use of biomarkers as biological indicators from an exposure to a stressor responding in various ways which can be successfully applied. In this sense, according to Van Dyk and Pieterse (2008) histology is an important diagnostic and sensitive tool to detect the direct effects of chemical compounds on target organs and can therefore, be used to assess the health of fish populations in contaminated aquatic systems. The advantage of histology as a biomarker lies in its intermediate location with regard to the level of biological organization. Furthermore, the histological changes appear as a medium-term response to sub-lethal stressors, and histology provides a rapid method to detect the effects of irritants, especially chronic ones, in various tissues and organs (Devi and Mishra, 2013; Binukumari and Vasanthi, 2014). Thus, the histopathological changes due to various pesticides have been extensively studied in different fish species (Ullah and Zorriehzahra, 2015).

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The gills are efficient tools for monitoring of aquatic ecosystems because of their large area which is in contact with the water and their high permeability (Oliveira Ribeiro et al., 2005; Evans et al., 2005; Vigliano et al., 2006). They are the main respiratory organ and have a large surface area, a thin water-blood diffusion distance and play an important role in osmoregulation and acid base equilibrium. In fish, the gills are also the main target organs of xenobiotic molecules. The changes in gill tissue affect respiration and ion uptake in freshwater fish (Fernandes and Mazon, 2003; Fanta et al., 2003; Bhuvaneshwari et al., 2015). Moreover, Nowak (1992) states that the gill histological changes, such as hyperplasia and hypertrophy, epithelial lifting, and aneurysms have been more pronounced in this particular organ after pesticide exposure.

The impact of glyphosate on fish has been studied mainly in commercial products which included other active ingredients and not just glyphosate itself (Neškovic et al., 1996; Ramirez-Duarte et al., 2008; Ayoola, 2008). We did not find any data regarding the effects of glyphosate on bighead carp which is economically important species in aquaculture and it is artificially bred together with common carp for better results. Thus, the present study primarily aims to investigate the histological effects which a glyphosate based herbicide could possibly cause on the gills of two economically important Cyprinid freshwater fish (common carp and bighead carp) and to determine which species is more sensitive in terms of pesticide contamination. The second objective aims to establish the degree of expression of each histological alteration which could be observed.

Material and Methods

Chemicals and experimental setup

The test pesticide in the present study was a glyphosate (IUPAC name N-(phosphonomethyl)-glycine) based herbicide which is used to control grasses, herbaceous plants, including deep-rooted perennial weeds, some broadleaf trees, shrubs, and conifers (Standing Committee on Plant Health, 2001). We used decreasing glyphosate concentrations of 72 mg/l, 40 mg/l and 20 mg/l under laboratory conditions for a total acute period of 96 h. All three concentrations were prepared in advance by dilution of the commercial product stock solution used in plant protection, given by the company which produces the chemical. The concentrations we used are lower than those actually applied in agriculture as a particular amount of the chemicals enter the water ecosystems through rainfall and wind drift.

Forty healthy common and bighead carps were obtained from the Institute of Fisheries and Aquaculture, Bulgaria where the fish are reared under strict controlled conditions. The fish were of the same size-group (mean length 17.6 cm ± 2.9 and mean body mass 46.3 g ± 8.4 for common carp and mean length 18.65 cm ± 1.33 and mean body mass 53.02 g ±5.3 for bighead carp) with no observed external pathological abnormalities. After transportation, the fish were moved in 100 l glass aquaria with chlorine-free tap water (by evaporation) to acclimatize for a week. They were randomly divided into four groups, including control (n=10) and not fed during the experiment.

The water physico-chemical characteristics, such as pH, temperature, dissolved oxygen, oxygen saturation and conductivity were measured once per day according to a standard procedure (APHA, 2005). All experiments were conducted in accordance with Directive 2010/63/EU.

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Histopathological analysis

The fish dissection was performed according to the procedures given in the EMERGE Protocol (Rosseland et al., 2003). The samples were placed in vials with 10% neutral buffered formaldehyde solution (pH 7.0). Then they were rinsed in tap water, dehydrated in a graded series of ethanol concentrations, cleared in xylene, embedded in paraffin wax with melting point of 54–56 ºC, sectioned to a thickness of 5–7 μm using a rotary microtome (Leica RM 2125) and mounted on sterilized glass slides. The sections were then deparaffinised, stained with hematoxylin and eosin (H&E) for histological examinations and prepared for light microscopy analysis (Romeis, 1989).

Semi–quantitative scoring

Ten paraffin sections were produced from the gills of each specimen. Each section was taken from a different location from the paraffin block, instead of in series. The histological alterations in the gill structure of each paraffin section were analyzed by observing the whole gill surface. The degree of expression was studied, including the corresponding changes in the gill surface in relation to the normal histological structure. The histological alterations in the gill epithelium were determined semi–quantitatively by using the grading system of Pierce et al. (1978) and Zimmerli et al. (2007) which we combined and modified. Each grade represented specific histological characteristics and was categorized as follows:

(0) – no histological alterations which represented normal histological structure;
(1) – mild histological alterations;
(2) – moderate histological alterations;
(3) – severe histological alterations;
(4) – very severe histological alterations in the gill surface architecture.

Statistical analysis

The statistical program which was used was Graph Pad Prism 7 for Windows. In order to study the differences between the degree of expression of each histological alteration between the control and treated with different glyphosate concentrations groups, *t*-test was applied. *P* value was set at 0.05. This was possible as for each degree of expression a numerical value from 0 to 4 was given. Ten fish from each test group and species were analyzed. The results are presented as average.

Results and Discussion

No histopathological changes were observed in the control fish gills. Gill structural details of the control carps are shown in *Figure 1A* and *2A*. With regard to the grading system, proposed as the control carp histological characteristics, these were evaluated as relatively normal (0). Comparing both Cyprinids exposed to glyphosate, we found in the gill structure proliferative changes (lamellar lifting, edema, proliferation of epithelial covering the filament and glandular cells, proliferation of cartilage, fusion), degenerative changes and changes in the circulatory system (vasodilatation of the primary sinus and blood vessels in the secondary lamellae) which all varied in their degree of expression (*Figure 1, 2*).

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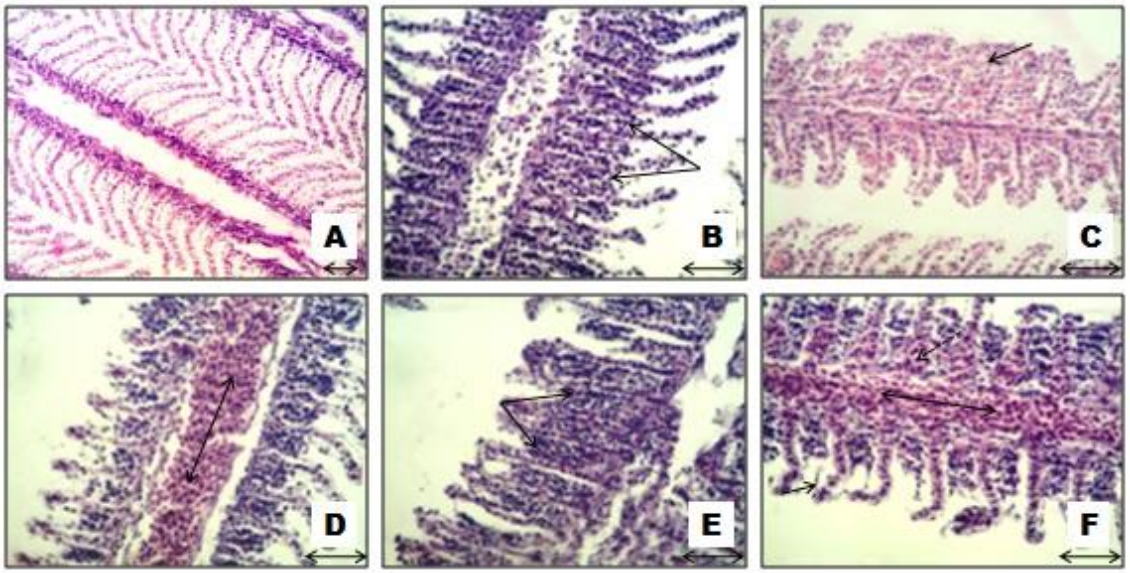


Figure 1. *Histopathological alterations in common carp gills, (H&E): A – control group fish gills, x200; B – proliferation of the gill epithelium, x400; C – fusion of the secondary lamellae, x400; D – vasodilatation of the central venous sinus, x400; E – epithelium proliferation, x400; F – lamellar lifting, vasodilatation of the central venous sinus and the secondary lamellae (dashed arrow), x400*

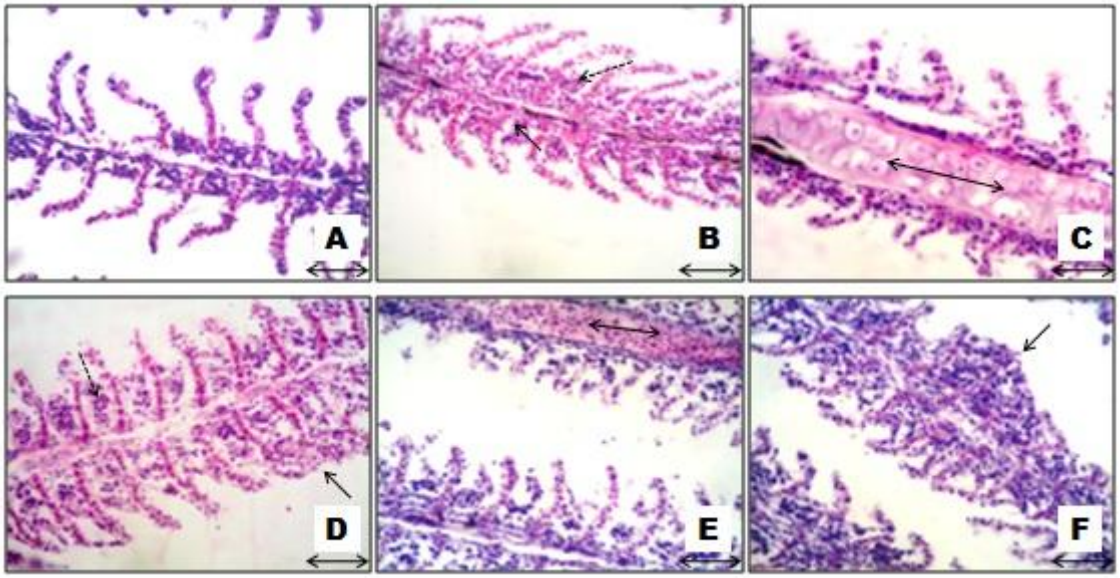


Figure 2. *Histopathological alterations in bighead carp gills, (H&E), x400: A – control group fish gills; B – proliferation of the gill epithelium (dashed arrow) and edema; C – proliferation of the cartilage tissue; D – proliferation of the gill epithelium (dashed arrow) and fusion; E – vasodilatation of the central venous sinus; F – fusion of the secondary lamellae*

For the degree of expression of each alteration the statistical analysis showed that there was a significant difference ($P < 0.05$) between the control for both Cyprinids and all test groups exposed to glyphosate (72 mg/l, 40 mg/l and 20 mg/l).

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Table 1 shows that lamellar lifting and edema were more pronounced – in severe and very severe degree of expression in both tested species, when increasing the concentrations of glyphosate. We consider that lamellar lifting and edema actually represent a compensatory-adaptive mechanism which increased the distance between the epithelium and blood vessels in the secondary lamellae. Hence, the changes found in the fish gills could slow down the oxygen access into the blood circulatory system which in turn would result in a gas exchange limit. As reported by Schwaiger et al. (2004) and Camargo and Martinez (2007) lamellar lifting and edema could lead to oxygen deficiency and subsequent oxidative stress for the fish. In addition, we found proliferation of the filamentous epithelium as probably another type of compensatory-adaptive mechanism in the fish gills exposed at all herbicide concentrations. It increased the thickness and the layers of the different epithelial cells which could likely delay the toxicant access through the gills. Proliferation of the filamentous epithelium was expressed mainly in a severe degree in the gills of common carp, while in the bighead carp gills it was found in a very severe degree of expression. Furthermore, proliferation of the connective cartilage tissue, leading to increased volume of gill filaments, was presented only in the gills of bighead carp which showed specificity in regards to glyphosate contamination. Ramah (2011) also reported cartilage hyperplasia in *Ctenopharyngodan idella* gills under herbicide exposure but in general this histopathological alteration is not often found in fish gills under the influence of different pesticides. Fusion as a more severe form of proliferation of the filamentous stratified epithelium was presented in a similar degree of expression in both tested species. In terms of the proliferative changes, we consider that bighead carp showed higher sensitivity under the influence of glyphosate compared to common carp because we also observed very severe proliferation of the filamentous epithelium and cartilage hyperplasia.

We found changes in the gills circulatory system such as vasodilation in the secondary lamellae and the main venous sinus. Vasodilatation was presented in a moderate degree of expression in the bighead carp gills at all glyphosate concentrations, while this change was in such a degree in the common carp gills only at the highest glyphosate concentration (*Table 1, Figure 1, 2*). Therefore, we consider that bighead carp had higher sensitivity in relation to the pesticide effects which could be found even at the lower concentrations of the toxicant. It also enhanced the blood cell flow and increased the internal pressure of the blood vessels. However, aneurysms in the secondary lamellae were observed only in the common carp gills. Cengiz (2006) also reported aneurysms in the secondary lamellae in common carp gills under the effect of deltamethrin.

We did not found aneurysms in the bighead carp gills in our previous studies (unpublished data) related to pesticides effects, either. Thus, we suggest that the bighead carp blood vessels could have higher resistance to pesticide contamination. Despite of the internal pressure and vasodilatation in blood vessels, aneurysms in the secondary lamellae of the bighead carp gills did not occur (*Table 1*).

Degenerative changes in the gills were also presented in both fish species which were found in a moderate and severe degree in the bighead carp gills at all glyphosate concentrations, while they were presented in a severe degree in the common carp gills only at 72 mg/l glyphosate (*Table 1*).

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Table 1. *Histopathological alterations in common carp and bighead carp gills caused by the test herbicide*

Gills histopathological alterations	Concentration herbicide							
	Common carp				Bighead carp			
	Control	20 mg/l	40 mg/l	72 mg/l	Control	20 mg/l	40 mg/l	72 mg/l
Lamellar lifting	1	3	4	4	1	**2***	4	**4***
Edema	0	2	3	4	0	2	3	4
Proliferation of stratified epithelium	0	*3***	3	*3***	0	*2***	3	*4***
Proliferation of glandular cells	0	**1***	2	**3***	0	1	2	2
Fusion	0	**0***	1	**2***	0	**0***	1	**2***
Proliferation of cartilage tissue	0	*0***	0	*0***	0	*2***	2	*3***
Degeneration of gill epithelium	0	*0***	*1***	3	0	*2***	*3***	3
Vasodilatation of secondary lamellae	0	**0***	1	**2***	0	2	2	2
Vasodilatation of central venous sinus	0	1	1	2	0	2	2	2
Aneurysms	0	0	*2***	*3***	0	0	*0***	*0***

(0) – no histological alterations which represented normal histological structure; (1) – mild histological alterations; (2) – moderate histological alterations; (3) – severe histological alterations; (4) – very severe histological alterations of the gill surface architecture

* **Bold** – Significant differences in the degree of expression of each histological alteration for common carp and bighead carp separately after exposure to 20, 40 and 72 mg/l ($P < 0.05$)

** *Italic* – Significant differences in the degree of expression of each histological alteration between common carp and bighead carp together after exposure to 20, 40 and 72 mg/l ($P < 0.05$)

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The high degree of degenerative changes could lead to thinning of the epithelial filaments and secondary lamellae, which on the other hand could lead to more rapid toxicant penetration. Similarly to us, Santhakumar et al. (2001) reported histopathological effects such as degenerative and necrotic changes in fish gills under the influence of sublethal concentrations of monocrotophos. Ghanbahadur et al. (2015) found degenerative alterations and necrosis in *Rasbora daninonius* gills after acute and chronic endosulfan exposure. Such results were also reported in common carp gills under lindan exposure by Binukumari and Vasanthi (2014) and under deltamethrin exposure by Cengiz (2006), respectively. The observed proliferative and degenerative histopathological alterations in this study suggest the development of a two-way process in the fish gills under the glyphosate influence. On one hand, the toxicant induced a compensatory-adaptive process which was related to transport delay of the toxicant by lamellar lifting and edema, as well as induced process of mitosis in the gill epithelium and cartilage in the bighead carp gill. On the other hand, glyphosate induced degenerative changes which were related with necrotic processes. Regarding the two Cyprinids separately the t-test proved significant differences ($P < 0.05$) for the degree of expression of proliferation of glandular cells between the fish exposed to 20 mg/l and 72 mg/l. Similar differences were also proved for fusion and vasodilatation of secondary lamellae between 20 mg/l and 72 mg/l (*Table 1*). Regarding the degree of expression of the histological alterations in bighead carp, the statistical analysis proved significant differences for lamellar lifting and fusion between the groups treated again with the lowest and highest glyphosate concentrations.

Comparing the two fish species together, statistically significant differences were found in the degree of proliferation of stratified epithelium in common carp and bighead carp between 20 mg/l and 72 mg/l. These were also found for proliferation of cartilage tissue and aneurysms between 20 mg/l and 72 mg/l, and for degeneration of gill epithelium but between 20 mg/l and 40 mg/l ($P < 0.05$).

In sum, we observed proliferative and degenerative changes in the bighead carp gills at all glyphosate concentrations. However, pronounced proliferative histopathological alterations in the common carp gills were found at the highest pesticide concentration. These results showed bighead carp as a more sensitive species in comparison to common carp. Therefore, we believe that bighead carp is an appropriate species for ecotoxicological tests, as well as a good bioindicator for pesticide pollution of water bodies. We consider that the results from such *ex situ* experiments could be applied for better plant protection practices, in the field of water legislation for reducing maximum allowable concentrations of organic pollutants, and the protection of freshwater fauna by using bioindicators and applying histological biomarkers. Last but not least, we suggest that further research in this particular area need to be carried out in order to better study the glyphosate toxicity in different commercial mixtures used in plant protection, and its interactions (antagonistic and synergistic) with other pollutants.

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MEASUREMENT OF TOXIC METAL CONTAMINATION, PHENOLIC AND FLAVONOID CONTENTS, ANTICANCER, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF DIETARY AND MEDICINAL PLANT *JUGLANS REGIA* L.

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Abstract. Medicinal plants have a proven long history for their effective use to prevent and treat diseases. During the recent decades, the modern drug discovery approaches have refocused on traditional medicinal plants because of their higher contribution for providing new drugs, drug candidates, novelty and mechanism insights. *Juglans regia* L. is very famous for providing precious and medicinal dry fruits which are extensively being used in many parts of the world. In this study four parts of *J. regia* i.e. fruits, leaves, stem bark and green husk were investigated for metal contamination by using atomic absorption spectroscopy and found that Cd contamination in its fruits, Cu and Mg concentration in all parts of *J. regia* (fruits, leaves, stem bark and green husk) were critically above the standard permissible limits. Methanolic extracts of various parts for *J. regia* were screened for antimicrobial activities against twelve different human pathogens which indicated that fruits, leaves, stem bark and green husk were proved to be potential source of antimicrobial activities in comparison to the standard clinical drugs i.e. ciprofloxacin and nystatin which was used as positive control. Whereas, *J. regia* fruit had potent antioxidant properties with IC_{50} value of 6.938693 mg mL^{-1}. Total phenolic contents were ranged from 12.6987 to 31.9685 mg of GA g^{-1} whereas, highest flavonoid contents were found in the fruit's extract which was 25.8231 mg g^{-1} and lowest in leaves extract of *J. regia* i.e. 7.2861 mg g^{-1}. Breast cancer is the top most prevalent cancer in women worldwide and is responsible for nearly 58% of all cancer deaths. The *Juglans regia* fruits, green husk, stem bark and leaves showed promising antiproliferative activities against MCF-7 breast cell lines. Fruit of *J. regia* was proved to be more potent as compared to other parts screened with highest antiproliferative activity i.e. GI_{50} 1.581722 at conc.1 mg/mL.
Keywords: *Juglans regia, toxic metals, antimicrobial, antioxidants, anticancer, total phenolic, flavonoids*

Introduction

Therapeutic plants are the manufacturing plants of medicinal phytochemicals which carry the nature designed unmatched structural novelties and properties that bind to bio macromolecules for the critical modulations to regulate disorders. These therapeutic plants are as yet being utilized extensively by rural people as natural remedies to treat diseases without legitimate standardization or regulations and that which poses genuine danger to human health especially, because of unwanted metals defilements and lack of

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dosage standardization that results into various unwanted interactions with bio macromolecules and toxins. Emergence of drug resistance (primarily due to wicked adaptive native nature of microorganisms and unregulated excessive use of anti-infectious drugs), emergence of new diseases, insufficiencies of the existing drugs and poor outcomes of drug discovery programs have created a highly critical emergency for the discovery and development of new antimicrobial drugs, templates and leads (Gutmann et al., 1998; Cohen, 1992). Natural biological processes essentially require energy which comes through oxidation responses but imbalanced and excessive oxidation due to reactive oxygen species (ROS). ROS are highly reactive species which unwantedly and harmfully modulate the structures and hence the functions of bio macromolecules including nucleic acids, proteins and lipids. Subsequently, this give birth to various biological disorders including deadly tumors, neurodegenerative, cardiovascular disorders, cataracts, diabetes, aggravations and atherosclerosis (Aruoma, 1998). Nature has unmatched metabolic designing and manufacturing enzymatic machinery which provide new drugs, leads, knowledge and inspiration for the design and discoveries of the medicinal and organic chemists for their efforts of new drug development as well as synthetic methodologies to fight diseases including caused by the deadly infections and reactive free radicals. Medicinal plants have been used for a long time in the treatment of various diseases. In today's World over 70% of the clinical molecular drugs are either natural products based or derived (Recio et al., 1989; Silver et al., 1993). Pakistan is naturally blessed with diverse climatic ranges that start from northern Himalayas to southern costal condition which lies after middle hot range. This diverse climatic range in turns provides diverse and rich fauna and flora contain numerous medicinal species. These medicinal species are being used by the local are traditional herbal medicines since long time. Pakistan has rich history, society and learning of utilizing these therapeutic species to treat and prevent different diseases. Thus, Pakistani is a major producer, user and supplier of medicinal species (Saeed et al., 2012). Biological screening of these therapeutic plants is the key beginning stage for the discovery of biologically relevant natural products from these medicinal plants. Unwanted and excessive generation of free radicals based diseases poses the urgent needs for the discovery of new antioxidant molecules and plants made, phenols, flavonoids, tannins and anthocyanidins are the most promising skeletons for the new drug discovery and development against these diseases (Saeed et al., 2012; Peschel et al., 2006).

Walnut (*J. regia*) possesses high medicinal properties because of its diverse natural chemical constituents and its mineral contents. Its different parts (leaves, barks and natural products) have been and are being utilized as a part of traditional herbal medicinal products. Leaves of *J. regia* are being used for treating venous inadequacy, hemorrhoids, hypoglycemia, and looseness of the bowels, microbial infections, dental caries contamination and strums bruises. Its leaves also treat skin diseases, purify the blood and helminthic, and are calming, astringent and depurative. They are being used as major herbal component for the treatment of clogging, endless hacks, asthma, looseness of the bowels, dyspepsia astringency, keratosis, diarrhea, hypoglycemia, sinusitis, cool and stomach throb (Mouhajir et al., 2008; Vaidyaratnam, 2005). Green husk of *J. regia* has cancer preventing properties and is antifungal, astringent, wart vendor impacts and treats skin diseases (Noumi et al., 2010; Amaral et al., 2003). Stem bark of *J. regia* is used to treat gastric, liver and lung tumor (Liu et al., 2004; Baytop, 1999). People utilized the fruits of *J. regia* as pharmaceutical to recuperate intestinal

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sickness, enhance memory shortages in transgenic mouse model of Alzheimer's ailment. It is being used as insect lithic, diuretic, anthelmintic and stimulant as well as for the treatment of low back torment, incessant pee, shortcoming of legs, unending hack, asthma and clogging (Tagarelli et al., 2010).

Keeping in view the importance of *J. regia* as dietary and folk remedial source, this study was aimed to assess the toxic metal contaminations, antimicrobial properties, antioxidant potential, phenolic contents and flavonoids contents of locally grown plants.

Material and methods

Plant material collection and preparation

Juglans regia L. is locally known as Akhrot and was collected from Rayala, Dhirkot, Azad Kashmir, Pakistan. Fruits, leaves, green husk and stem bark of *Juglans regia* L. were used to investigate the toxic metal contaminations, antimicrobial, antioxidant, total phenolic and flavonoids contents. The samples were carefully collected, separated and packed into bags and then transported to the laboratory for investigation.

Preparation of extract

Each part of the plant was shade dried for 10 days at room temperature then, were ground into fine powder with the help of electrical grinder and were stored at 4 ºC for further studies. The 100 g dried powder of each part of *J. regia* was separately macerated in 500 mL of methanol and kept at room temperature (25 ± 2 °C) for 10 days. After maceration period, the solvent extracted material was filtered. These extracts of plants portions were dried on rotary evaporator at low temperature (60 °C) and reduced pressure.

Acid digestion, sample preparation for the estimation of metal contaminations

One gram of dry powder of each part of *J. regia* was subjected to acid digestion for the estimation of their metal contaminations. 1 g of powder of each part of *J. regia* was taken in a glass beaker and 6 mL of concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) were added (in 2:1 V V^{-1}). Mixture was stirred slowly at room temperature. The glass beaker was covered with a petri dish and heated at 70 °C for 3 h. Small volumes of HNO_3 and H_2O_2 were added to this mixture along with continuous heating at 70 °C till a clear solution was obtained. The excess of acid was evaporated and a semi dry mass was obtained. This semi dry mass was solubilized in 10 mL HNO_3 (0.1 M) and was transferred to a 50 mL volumetric flask. Total volume of solution was made 50 mL with the addition of distilled water.

Specification of AAS

Operating parameters of Atomic Absorption Spectrophotometer (AAS) AAnalyst^{700} was used in the analysis of trace metals using respective Wavelength in the range of 190-900 nm, Diffraction grating: 1800 lines mm^{-1} blazed at 236 nm and 597 nm, Grating area: 64 × 72 mm, reciprocal linear dispersion: 1.6 nm mm^{-1} (nominal), Focal length: 267 mm, Spectral bandwidths: 0.2, 0.7 and 2.0 nm, dual height, motorized slit drive for automatic slit selection with wide-range segmented solid-state detector.

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AAnalyst[700] used winlab 32 program to automatically calculate the mean of three reading of each samples of *J. regia* for each metal.

Determination of antimicrobial activity

Preparation of sample solutions for anti-microbial activity

10 mg of dried methanolic extract of each part of *J. regia* was dissolved in 1 mL of respective solvent in 5 separate air tight eppendorf tubes to prevent contamination and were kept for four days to ensure solubility.

Test microorganisms

Twelve common human pathogens viz., three gram positive bacteria (Staphylococcus aureus, Proteus vulgaris and Bacillus subtilis), four gram negative bacteria (Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Bordetella bronchiseptica), three fungi (Aspergillus flavus, Fusarium solani, Rhizopus stolonifer) and two yeasts (Saccharomyces cerevisiae, Candida albicans) were used to assess the antimicrobial properties of the selected plant's parts.

Antimicrobial bioassay

In vitro screening of the methanolic extracts of four parts of *J. regia* was performed by disc diffusion method as described by (Scorzoni et al., 2007). Two series of experiments were conducted for testing the antibacterial, antifungal and anti-yeast activities of these extracts. These extracts were tested for their antimicrobial activities with reference to standard reference antibiotics (ciprofloxacin and nystatin). All the steps, inoculums, petri dishes, preparation of dilution were performed under aseptic conditions. The plates containing bacterial culture were incubated for 24 h at 37 °C whereas, fungi and yeast dilution were incubated at 25 °C for 72 h. After incubation period, all plates were examined for the presence of zone of inhibition (mm) to assess their antimicrobial activity. Each experiment was performed in triplicates.

Determination of antioxidant activity

Sample preparation for antioxidant activity

10 mg of dried methanolic extract of each part of *J. regia* was dissolved in 2 mL of methanol in 5 separate test tubes. DPPH radical cation decolorization assay was used to evaluate the antioxidant properties of the methanolic extracts of *J. regia*.

DPPH decolorization assay for antioxidant activities

DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activities were analyzed to evaluate the antioxidant properties of the extracts of four parts of *J. regia* according to the method (Amarowicz et al., 2004). DPPH stock solution was prepared by reacting potassium per sulphate and DPPH (33.75 mg potassium persulphate + 64.8 mg of DPPH in 100 mL of distilled water) and allowed to stand in dark for 16 h to generate DPPH radical acation. Absorbance of the standard solutions was recorded at 734 nm ($Ao_{Control}$) after diluting with water up to respective volume. For percent scavenging activity (% RSC) of each extract, 1 mL of DPPH and 200 µL of each extract concentration (1 mg, 2 mg and 5 mg crude extracts each dissolved in per mL MeOH) were mixed and then

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followed by the addition of 1.8 mL water to make the volume of reaction mixture up to 3 mL. The absorbance (Ai) of each reaction was recorded for each sample at 734 nm and the blank used for each sample contained 2.8 mL of distilled water and 200 µL of extract solutions. All measurements were taken in triplicates. The percentage radical scavenging activities (% RSC) were calculated using the formula:

$$\% \text{ RSC} = \frac{\left[\text{Ao}_{\text{Control}} - \text{Ai}_{\text{Sample}}\right]}{\text{Ao}_{\text{Control}}} \times 100$$

Total phenolic and flavonoid contents

Total phenolic contents in four parts of *J. regia* were determined by Folin–Ciocalteu method (Jimoh et al., 2007). Phenolic contents were expressed as gallic acid (GA) equivalents (mg of GA g^{-1} of extract). Total flavonoid contents in four parts of *J. regia* were measured as described previously by Piccolella et al. (2008). The total flavonoid contents were calculated as rutin equivalents (mg of Rutin g^{-1} of extract).

Anticancer activity

Cell lines and cell viability assay

Human breast disease (MCF-7) cells were obtained from ATCC (Rockville, MD). All cells were cultured in a humidified 5% CO_2 environment at 37 °C in an incubator and refined in RPMI-1640 medium supplemented with 10% warm inactivated infant calf serum, 100 IU/mL penicillin and 100 µg/mL streptomycin. Restraint of cell multiplication by the walnut extracts was measured by the MTT test. Rapidly, PC3 cells were plated in 96-well culture plates (1 × 105 cells/well) independently. After 24 h incubation, cells were treated with *Juglans regia* crude extracts (0.5 mg/mL and 1 mg/mL; eight wells for every focus) for 24 h; MTT concentration (5 mg/mL) was then poured to each well. After 4 h incubation, the formazan particles were kept in dimethyl sulfoxide (100 µL) and afterward the absorbance was measured in an ELISA at 570 nm. Cell reasonability proportion was computed by following formula:

$$\text{Cell viability } (\%) = \frac{\left[\text{Ac} - \text{At}\right]}{\text{Ac}} \times 100$$

where Ac is the absorbance of control cell and At absorbance of treated cells, respectively.

Cytotoxicity was expressed as the concentration of the extracts in growth inhibition of cell by 50% (GI_{50} value).

Statistical analysis

All values were expressed as mean ± standard error of means. By using one-way analysis of variance (ANOVA) technique (Steel et al., 1980). The mean was compared by using LSD at 5% (0.05) probability level by using statistic 8.1 software.

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Results and discussion

Metal contaminations of J. regia

During current study the contaminations of six metals (Cd, Cr, Cu, Mn, Mg and Zn) in four parts of *J. regia* extracts using Atomic Absorption Spectrophotometry (AAS) were investigated. The wave length were taken to measure the concentrations of each metal in each extract and the mean concentration of each metal in each extract (in mg L^{-1}) are given in *Table 1*.

Table 1. *Metal concentration in J. regia for the estimation of metal contamination (mg/L)*

#	Part used (Analyte absorbance)	Cd (228.8 nm)	Cr (357.9 nm)	Cu (324.8 nm)	Mn (279.5 nm)	Mg (285.2 nm)	Zn (213.9 nm)
1	Fruits	4.872	0.156	19.84	0.469	105.5	1.139
2	Leaves	0.323	0.047	4.555	0.313	124.9	0.417
3	Stem bark	0.037	0.815	3.612	0.260	113.0	0
4	Green husk	0.037	0.079	4.175	0.110	94.0	0

The present study reports the estimation of metal contaminations of six metals, namely; Cd, Cr, Cu, Mn, Mg and Zn in selected parts of *J. regia* and were compared with the standard permissible limits (SPLs) by the FAO/WHO to evaluate their potential impact on the health. These studies indicated that Cd concentration in fruits and leaves, Cr concentration in fruits, stem bark and green husk, Cu and Mg concentration in all parts of *J. regia* (fruits, leaves, stem bark and green husk) were above the standard permissible limits, Mn concentration in fruits and leaves and Zn concentration in all plant's parts of *J. regia* were below the standard permissible limits, Zn was not found in stem bark and green husk of *J. regia*. The concentration of each metal in all four tested parts were also represented in *Fig. 1*.

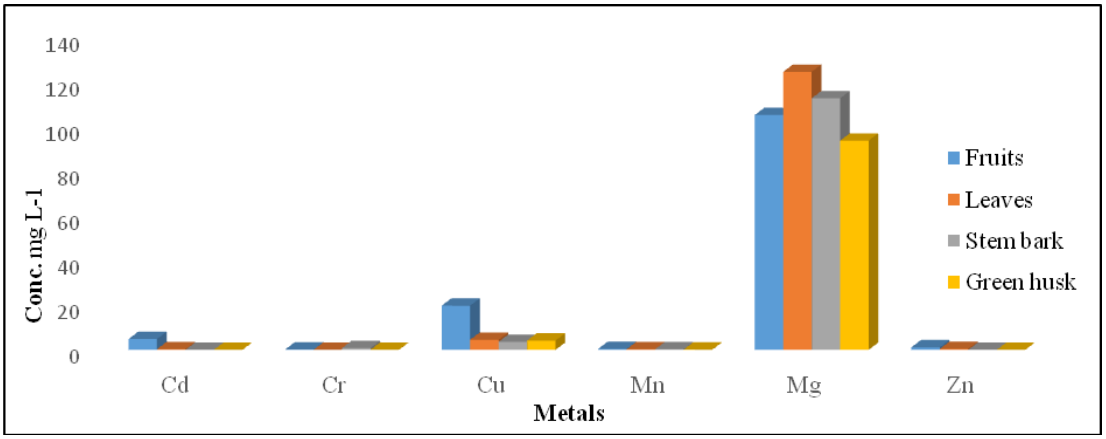


Figure 1. *Metal contaminations in four parts of J. regia*

Higher Cd concentration in fruits and leaves of *J. regia* was the major contributing reason to the potential health risk in Nanning, China (Cui et al., 2004) and in Boolaroo, Australian (Kachenko and Singh, 2006). The main contaminations of Cd were reported in grape, spinach and celery. Therefore, these results should be used as important

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guidelines for the producers, traders, consumers and regulatory authorities. Unfortunately, in these areas there are no regulatory practices for the assessment of these walnut fruits which are extensively used by the local inhabitants and sold in the market throughout the country. The amount of Cu was critically above the standard permitted limit which indicate the high composition of the Cu in these selected area that ultimately accumulate in the flora, fruits and vegetables. It also worth noticing, that Zn was also omitted in the samples of *J. regia.* The standard acceptable limits (SPLs) of metal contamination (in dietary and medicinal species) are: Cd = 0.06 mg L^{-1}, Cr = 0.05 mg L^{-1}, Cu = 0.1 mg L^{-1}, Mn = 0.26 mg L^{-1}, Mg = 50 mg L^{-1} and Zn = 5.0 mg L^{-1} (Singh et al., 2010).

Antimicrobial activities of J. regia

Antibacterial activities of the methanolic extracts were assessed by the disc diffusion method against twelve common human pathogens. Zone of inhibition (mm) of all these extracts were mentioned in *Table 2*.

Table 2. *Anti-microbial activity of methanolic extracts of four parts J. regia against tested human pathogens*

Mean diameter of zones of inhibition (mm) ± standard error of means (S.E.M)							
#	**Pathogens**	**Fruits**	**Stem bark**	**Green husk**	**Leaves**	**Ciprofloxacin**	**Nystatin**
1	*S. aureus*	$14.33_{a} \pm 0.33$	$13.33_{b} \pm 0.67$	$11.67_{d} \pm 0.33$	$10.33_{b} \pm 0.33$	$33.33_{a} \pm 0.33$	---
2	*P. aeruginosa*	$13.67_{a} \pm 0.33$	$12.67_{b} \pm 0.33$	$18.16_{a} \pm 0.28$	$10.67_{b} \pm 0.33$	$33.33_{a} \pm 0.33$	---
3	*K. pneumonia*	$10.67_{bc} \pm 0.33$	$10.33_{c} \pm 0.33$	$13.33_{bc} \pm 0.67$	$8.67_{c} \pm 0.33$	$29.67_{b} \pm 0.33$	---
4	*P. vulgaris*	$8.67_{de} \pm 0.33$	$15.16_{a} \pm 0.28$	$12.67_{bcd} \pm 0.3$	$7.67_{c} \pm 0.33$	$32.33_{a} \pm 0.67$	---
5	*E. coli*	$13.33_{a} \pm 0.67$	$11.33_{c} \pm 0.33$	$12.33_{cd} \pm 0.67$	$19.33_{a} \pm 0.57$	$20.67_{e} \pm 0.33$	---
6	*B. subtilis*	$7.67_{e} \pm 0.33$	$16.33_{a} \pm 0.57$	$0.00_{f} \pm 0.00$	$0.00_{d} \pm 0.00$	$25.33_{c} \pm 0.57$	---
7	*B. bronchiseptica*	$9.67_{cd} \pm 0.00$	$13.67_{b} \pm 0.33$	$0.00_{f} \pm 0.00$	$0.00_{d} \pm 0.00$	$23.16_{d} \pm 0.28$	---
8	*A. flavus*	$0.00_{f} \pm 0.00$	$0.00_{d} \pm 0.00$	$13.67_{b} \pm 0.33$	$0.00_{d} \pm 0.00$	---	$20.67_{d} \pm 0.33$
9	*F. solani*	$0.00_{f} \pm 0.00$	$0.00_{d} \pm 0.00$	$0.00_{f} \pm 0.00$	$0.00_{d} \pm 0.00$	---	$28.33_{b} \pm 0.33$
10	*R. stolonifer*	$11.67_{b} \pm 0.33$	$0.00_{d} \pm 0.00$	$0.00_{f} \pm 0.00$	$0.00_{d} \pm 0.00$	---	$39.67_{a} \pm 0.33$
11	*S. cerevisiae*	$0.00_{f} \pm 0.00$	$0.00_{d} \pm 0.00$	$0.00_{f} \pm 0.00$	$8.67_{c} \pm 0.33$	---	$23.33_{c} \pm 0.33$
12	*C. albicans*	$0.00_{f} \pm 0.00$	$10.66_{c} \pm 0.57$	$0.00_{f} \pm 0.00$	$0.00_{d} \pm 0.00$	---	$11.67_{e} \pm 0.33$

Note: the means followed by same letters are non-significant

Results of the sensitivity tests were expressed as (0) for no sensitivity, (7-10 mm) for low sensitivity, (11-18 mm) for moderate sensitivity and (> 19 mm) for high sensitivity. Fruit's extract of *J. regia* exhibited significant zone of inhibitory activities (11-18 mm); 14.33 ± 0.33, 13.67 ± 0.3, 13.33 ± 0.67 and 11.67 ± 0.33 against *S. aureus, P. aeruginosa, E. coli* and *R. stolonifer*, while it showed marginal activity against *K. pneumonia, P. vulgaris, B. subtilis and B. bronchiseptica* i.e. 8.67 ± 0.33, 8.67 ± 0.33, 7.67 ± 0.33 and 9.67 ± 0.00, respectively. The low sensitivity conferred by gram negative bacteria was due to composition of their cell wall as previous study of Parekh and Chanda (2007) depicted that gram negative bacteria were more resistant than gram positive bacteria against all the drugs tested. Fruit extract showed no activity against other tested microorganisms whereas, the extract of stem bark exhibited significant activity; 13.33 ± 0.67, 12.67 ± 0.33, 15.16 ± 0.28, 11.33 ± 0.33, 16.33 ± 0.57 and 13.67 ± 0.33 against *S. aureus, P. aeruginosa, P. vulgaris, E. coli, B. subtilis* and *B.*

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bronchiseptica, respectively, while other strains of tested microorganism were found to be resistant to stem bark extract of *J. regia*. The finding of Salar and Suchitra (2009) suggested that polar extract showed higher antimicrobial properties against tested microorganisms which are in line with the current study. Methanolic extract of the green husk of *J. regia* showed considerable activity against *S. aureus* (11.67 ± 0.33), *P. aeruginosa* (18.16 ± 0.28), *K. pneumonia* (13.33 ± 0.67), *P. vulgaris* (12.67 ± 0.33), *E. coli* (12.33 ± 0.67) and *A. flavus* (13.67 ± 0.33) whereas, *B. subtilis, B. bronchiseptica, F. solani, R. stolonifer, S. cerevisiae* and *C. albicans* were found to be resistant to green husk extract of *J. regia. A. flavus* was also inhibited by the extract used however all other fungal and yeast strains were found resistant as shown in *Figures 2 and 3*.

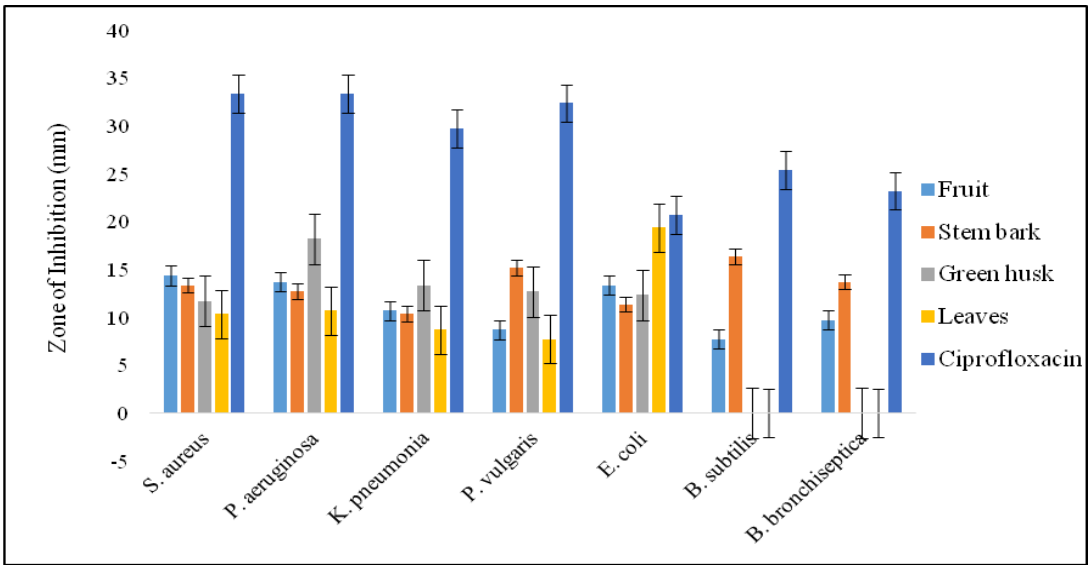


Figure 2. *Antibacterial activities of the methanolic extract of J. regia*

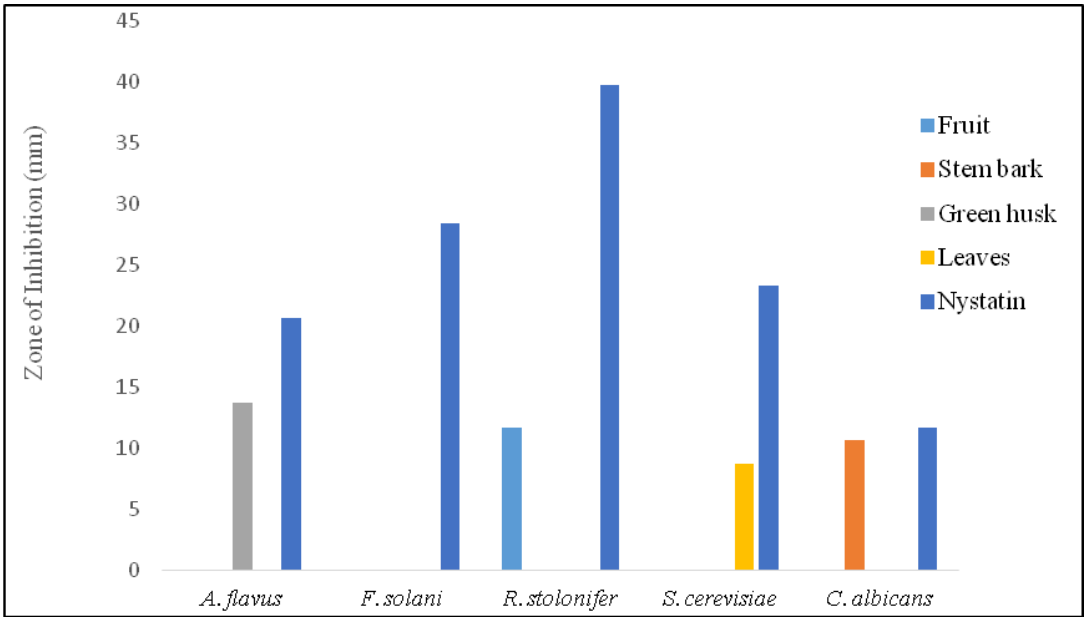


Figure 3. *Antifungal activities of the methanolic extract of J. regia*

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These results were in accordance with the previous studies which also deduced that plant extracts were more effective against bacteria than fungi (Mehmood et al., 2013). Methanolic extract of leaves of *J. regia* conferred high activity i.e. 19.33±0.57 mm against *E. coli* while, it showed low activity (10.33 ± 0.33, 10.67 ± 0.33, 8.67 ± 0.33, 7.67 ± 0.33 and 8.67 ± 0.33) against *S. aureus, P. aeruginosa, K. pneumonia*, *P. vulgaris* and *S. cerevisiae,* respectively. Extract of its leaves was found inactive against other bacterial and fungal strains. These results were in harmony with the previous investigations of Naika et al. (2012). Moreover, the current study revealed that plant parts extracts were more active against gram positive bacteria as compared to gram negative bacteria tested due the fact that the outer membrane of the cell wall of gram negative bacteria acts as a barrier to many synthetic and natural antibiotics (Tortora and Funke, 2001).

Antioxidant activities of J. regia

Antioxidant properties of the methanolic extracts of four parts of *J. regia* under discussion were measured by DPPH radical decolorization assay. Three concordant readings were taken for each extract at 734 nm and mean (Ai) was calculated. Value of control (Ao) at 734 nm was 0.1. The percentage radical scavenging activity (% RSC) was calculated using the formula: % RSC = $[(Ao_{Control} - Ai_{Sample})] / Ao_{Control} \times 100\%$. All results for these methanolic extracts are summarized in *Table 3*. It was observed that the methanolic extract of *J. regia* fruit showed stronger free radical scavenging ability with 6.938693 mg mL^{-1} IC_{50} value. Similarly, methanolic extracts of leaves, stem bark and green husk showed 9.224862 mg mL^{-1}, 8.901780 mg mL^{-1} and 13.862772 mg mL^{-1} IC_{50}, respectively. Methanol extracts are generally found to possess higher antioxidant activity because the polar solvents in extraction of all the phenolic compounds and that might be carrying the most of the bioactive compounds from plants (Fatima et al., 2007). Our results are graphically shown in *Figure 4* including the IC_{50} values of all of methanolic extracts.

Table 3. *Antioxidant activity of the methanolic extracts of four parts of J. regia*

#	Part used	1 mg mL^{-1}	3 mg mL^{-1}	5 mg mL^{-1}	IC_{50}
1	Fruits	35.26	39.29	42.13	6.938693
2	Leaves	21.13	27.07	30.02	9.224862
3	Stem bark	18.26	22.61	27.16	8.901780
4	Green husk	10.16	17.24	24.02	13.862772

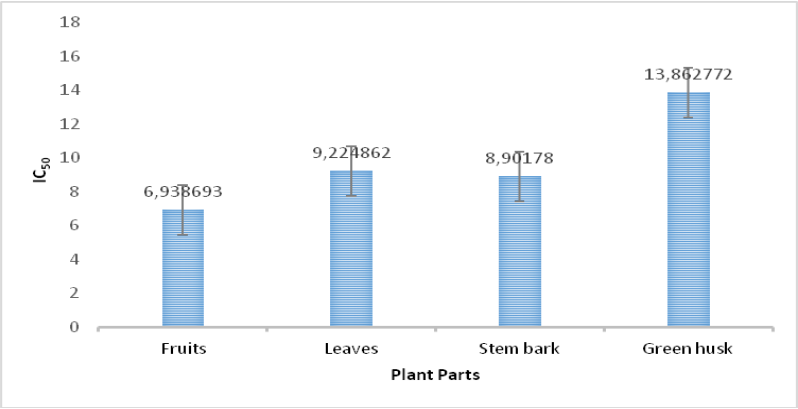


Figure 4. *IC_{50} values of the methanolic extracts of four parts of J. regia*

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Phenolic contents of four parts of J. regia

Total phenolic contents of methanolic extract of four parts of *J. regia* were assessed by using Folin-Ciocalteu phenol reagent and results are tabulated in *Table 4*. The values ranged from 12.6987 to 31.9685 mg of GA g^{-1} of extract. The highest value was recorded in the stem bark extract which was 31.9685 mg GA g^{-1} and lowest value in green husk which was 12.6987 mg GA g^{-1} (*Fig. 5*). The phenolic contents estimated in this studies were probably, responsible for the free radical-scavenging activity of *J. regia*. The phenolic compounds were attributed to the overall antioxidant activities, it is important to point out that during present study studies it was recorded that the relationship between antioxidant activities and amount of phenolic contents in all extracts which are also in agreement with the previous investigations (Anwar et al., 2013).

Table 4. *Total phenolic contents of J. regia (in methanolic extracts of fruits, leaves, stem bark and green husk)*

#	Part used	Conc. Used	Absorbance	mg of GA g^{-1} of extract
1	Fruits	2.5 µL	70.90	$30.2689_a \pm 3.46a$
2	Leaves	2.5 µL	80.75	$19.8762_b \pm 2.32b$
3	Stem bark	2.5 µL	79.25	$31.9685_a \pm 4.33a$
4	Green husk	2.5 µL	22.32	$12.6987_b \pm 1.74_b$

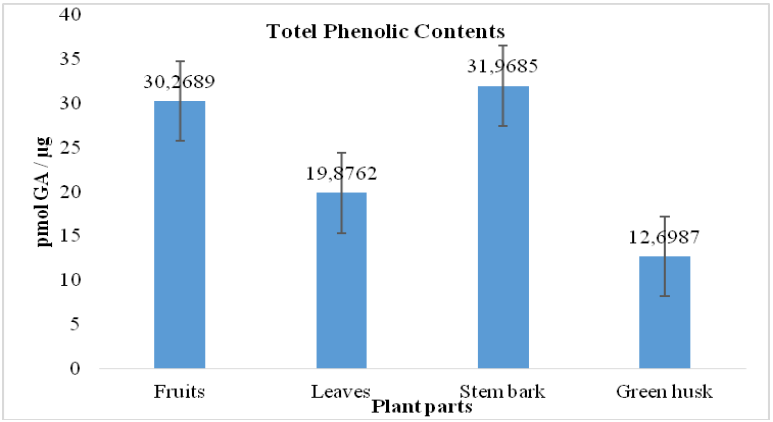


Figure 5. *Total phenolic contents of fruits, leaves, stem bark and green husk of J. regia*

Flavonoid contents in the methanolic extracts of four parts of Juglans regia

The total flavonoid contents in the methanolic extracts of the all selected parts of *J. regia* are tabulated in *Table 5*.

Table 5. *Total flavonoid contents of J. regia (methanolic extracts of fruits, leaves, stem bark and green husk)*

#	Part used	Conc. Used	Absorbance	(mg of Rutin g^{-1} of extract)
1	Fruits	10 µL	0.49	$25.8231_a \pm 2.32$
2	Leaves	10 µL	0.170	$7.2861_c \pm 1.15$
3	Stem bark	10 µL	0.315	$17.2896_b \pm 2.88$
4	Green husk	10 µL	0.298	$15.9864_b \pm 2.33$

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The highest contents were found in the fruit's extract 25.8231 mg g^{-1} and lowest value in leave's extract of *J. regia* i.e. 7.2861 mg g^{-1} (*Fig. 6*). The plant extract usually containing flavonoids and phenols has already been reported to possess promising biological activities (Maria et al., 2014).

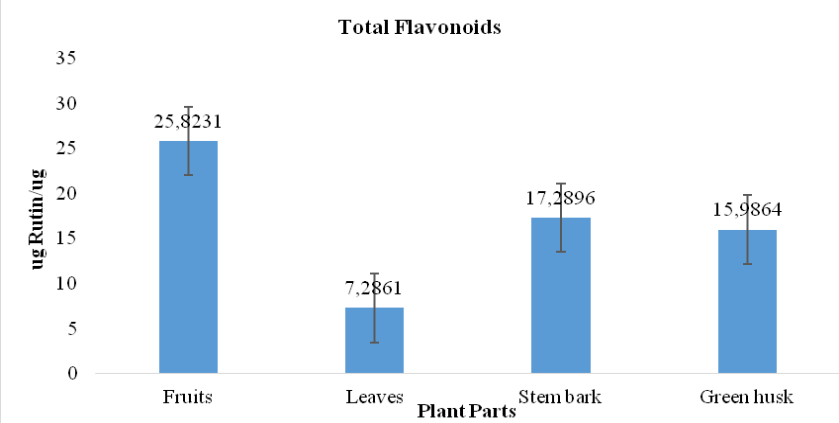


Figure 6. *Total flavonoid contents of fruits, leaves, stem bark and green husk of J. regia*

Anticancer activity of four parts of J. regia

Juglans regia fruits, leaves, stem bark and green husk extracts were evaluated for anti-cancer activity in MCF-7 cell line using the MTT assay against the human breast cancer. The (Growth inhibition) GI_{50} value of all four extracts of *Juglans* were calculated in 0.5 mg mL^{-1} and 1 mg mL^{-1} concentrations. GI_{50} value gave an indication of the potency of each plant extracts as shown in *Table 6* and *Figure 7*.

Juglans green husk extract at concentrations of 0.5 mg/mL and 1 mg/mL exhibited a strong anticancer effect which depicted that only 7% and 14% of the cells were alive and 93% and 86% cells were damaged, respectively.

J. regia green husk proved valuable potential that could enhance cytotoxicity against MCF-7 breast cancer and could be used as therapy against breast cancer. Tested crude extract had medicinal advantages not only for MCF-7 cell line but found to be potent against other type of cancer also. Similar studies have been carried out by Hasan et al. (2011) and Alshatwi et al. (2012) which suggested that green husks and root bark extracts of walnut brought apoptosis in MDA-ME231 human breast cancer and prostate cancer cell lines PC-3 by up-regulating bax gene, caspase 8 and caspase 3 expression and down-controlling Bcl-2 gene expression. Methanolic extract of Stem bark of *J. regia* conferred sensitivity as 10% and 25% against tested cell lines at concentration of 0.5 mg mL^{-1} and 1 mg mL^{-1} whereas 90% and 75% alive cells were recorded. Study of Sanchez-Gonzalez et al. (2014) also suggested that consumption of walnuts could help to reduce the risk of developing cancer. The extract of walnut fruits exhibited significant activity at concentration in 0.5 mg mL^{-1} that were 33% cancer cell were alive and 67% cell damaged, at 1 mg mL^{-1} concentration only 2% cancer cell were observed alive and 98% were damaged.

The walnut fruit extract was assessed against MCF-7 cells line. The extract demonstrated a dosage dependent inhibition effect. 1 mg/mL concentration conferred best anti-proliferative property as compared to 0.5 mg/mL concentration of the extract used. Oguzhan and Zeynep (2016) also reported anticancer activity of walnut fruit

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against MCF-7 cells. Anticancer potential of leaves extract of *Juglans regia* at concentration 0.5 mg/mL recorded as 24% cells were alive and 76% cells were damaged, at 1 mg/mL concentration only 12% cancer cell were observed alive and 88% were damaged.

The leaves crude extract of *J. regia* was found to be more powerful against tested cancer cells that may reduce malignancy cells by means of suppression of the p65 phosphorylation, which is supposed to be safe component to reduce malignant disease in cells. Early finding of Taghizadeh et al. (2011) also suggested that plants extract showed higher cytotoxicity potential against MCF-7 cells which was in harmony with current study.

Table 6. *Anticancer activity of J. regia (methanolic extracts of fruits, leaves, stem bark and green husk)*

#	Part used	GI_{50} at 0.5 mg/mL	GI_{50} at 1 mg/mL
1	Fruits	32.51318	1.581722
2	Leaves	23.6417	11.89427
3	Stem bark	90.15817	74.69244
4	Green husk	6.821963	13.47754

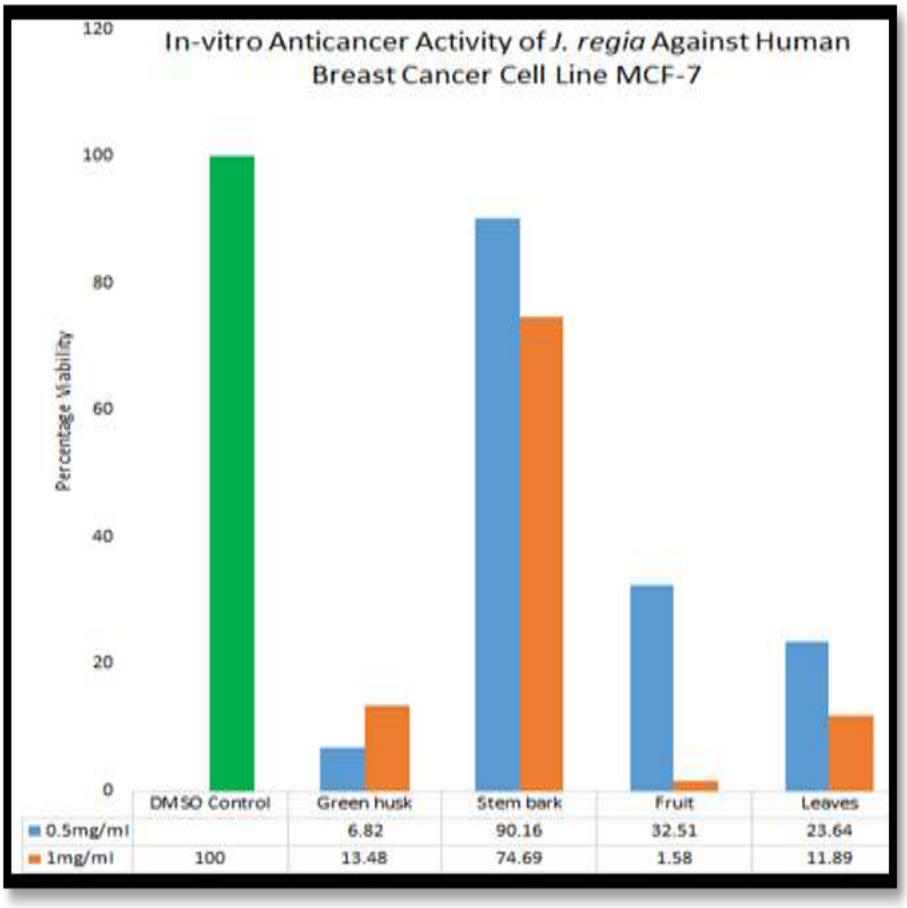


Figure 7. *Anticancer activity of J. regia methanolic extracts of fruits, leaves, stem bark and green husk*

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Conclusion

Present investigations concluded that dietary and medicinal plants should be analyzed for metal contamination before use. The results of toxic metal contamination in *J. regia* indicated that the contaminations of Cd were above SPLs in fruit of *J. regia* whereas Zn was below the SPLs. Therefore, these results should be used as important guidelines for the producers, traders, consumers and regulatory authorities to assure the recommended amount of metals in this important dietary fruit by focusing on soil conditions of walnut growing area. Moreover this medicinal plant was proved as potential source of anti-cancer, anti-microbial, anti-oxidant activity. The compounds like phenolic and flavonoids found in different parts of walnut may be used as food supplement and in pharmaceutical industry to cure fatal ailments after detail study both in vivo and in vitro.

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SUSTAINABLE WATER RESOURCES MANAGEMENT AND POTENTIAL DEVELOPMENT OF MULTI-PURPOSE DAM: THE CASE OF MALAYSIA

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Abstract. As water scarcity increases globally due to rapid population growth, unsustainable use, water degradation and climate change, water security is increasingly becoming a serious and growing concern. Sustainable water resources management and development are central to addressing this pressing concern. One of the ways forward is through the development of multi-purpose dams. However, dam development is often mired with controversies over its environmental and social impacts. It is anticipated that multi-purpose dam development is going to gain attention in Malaysia; the question at issue is not only about the multi-purpose dam development per se, but the sustainable water resources management. This article attempts to discuss water security issues in the Malaysian context, the needs for sustainable water resources management and environmentally sustainable multi-purpose dam development.
Keywords: *water security, sustainable water resources management, multi-purpose dam, environment, social, economy, climate change*

Introduction

By 2025, it is projected that about 1.8 billion people will experience absolute water scarcity. Severe water stress affecting 1/3 of the world's population is expected to double to 2/3, and water withdrawals are predicted to increase by 50% in developing countries and 18% in developed countries. (BERNAMA, 2012).

Water scarcity may be defined as when the issue of water insecurity persists for a significant period of time, that is, when a large number of people in a region do not have access to safe and affordable water to satisfy their daily needs or livelihoods. Water security is defined as the capacity of a population to safeguard sustainable access to adequate quantities of acceptable quality water for sustaining livelihoods, human well-being, and socio-economic development. Many factors contribute to water security, ranging from biophysical and infrastructural, institutional, political, social, financial – many of which lie outside the water realm. Addressing this goal therefore require interdisciplinary collaboration across sectors, communities and political borders.

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The American Society of Civil Engineers gives a holistic definition of sustainable water resources systems. Water resources systems should be designed and managed to fully contribute to the objectives of society, now and in the future, while maintaining their ecological, environmental, and hydrological integrity (Petersson and Ostrowski, 2003). Providing water for drinking and irrigation, producing hydropower and flood protection are by all means functions that contribute to the objectives of society. Water shortage will not be possible to solve without dams, although solving it only by means of dams will clearly not be preferable solution. Many collateral impacts of dams are running contradictory to society's objectives, or either the ecological or the economic integrity of the water resources system. To ensure the identification and implementation of widely accepted and sustainable solutions for water and energy development, it will be of major importance to implement well-structured and transparent planning processes for multi-purpose dam from the very beginning (Petersson and Ostrowski, 2003). This article attempts to discuss the current water security issues in the Malaysian context, the needs for sustainable water resources management and environmentally sustainable multi-purpose dam development.

Water security issues: The Malaysian context

Malaysia is a tropical country, having an average annual rainfall of 2400 mm and relatively rich in water resources. In the early decades of independence, Malaysia never experienced any serious water crisis. However, Malaysia is facing water shortage and having frequent water rationing nowadays, especially in Kuala Lumpur, Selangor and Putrajaya. The situation is exacerbated by the *El Nino* phenomenon which is anticipated to further worsen the water crisis in Malaysia (Raja Zainal Abidin, 2004). Such a paradox of plenty is due to the complexity of water security issues and must be sustainably addressed (Chan et al., 2003).

Over the past decades, Malaysia was lacking of a central agency entrusted with the overall responsibility of holistic planning and management of the overall aspects of water resources. There are too many agencies having jurisdiction over different aspects of water resources management either direct or indirectly. *Table 1* shows the responsibilities of the Malaysian water related agencies.

Table 1. *Malaysia's water related agencies*

Agency	Area of responsibility	Description
National Water Resources Council (NWRC)	Water resources matters – cross boundaries/interstate/issues of national interest	Ensure coordination between various State Governments in the management of river basins
Ministry of Natural Resources and Environment	Policy matters	Formulates policies/guidelines/regulations for the national water resources
Ministry of Energy, Green Technology and Water (KeTTHA)	Policy matters	Formulates the policies and strategies for national water supply and sanitation
Ministry of Finance – Water Asset Management Inc, PAAB	Asset management	Acquire and manage all state owned water related assets
National Water Services Commission (SPAN)	Regulatory matters	Regulate water service industry (water and sewerage services)
State and Territory Governments (13 states and three territories)	Raw water matters	Regulates raw water abstraction and catchment management

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Conflicts in water resources management such as allocation of water rights, flood management, pollution control, environmental protection, etc are resolved through inter-agency coordination and consultation. Such a mechanism has led to sectoral management of water, conflicting/competing objectives and disputes between agencies.

Malaysia also suffers from a plethora of sector-based water laws, both at federal and state levels, and from the lack of a comprehensive water law. *Table 2* shows legislations that relevant to Malaysian water resources management. All these legislations are contained within the laws that are enforced by the various water-related government agencies, and many of these laws are outdated, redundant or ambiguous and need to be reviewed in today's context. These diversified water legislations are also not comprehensive enough and do not deal directly with water issues, thus the government agencies are having difficulty to enforce effectively.

Table 2. *Legislation that relevant to Malaysian water management*

Purpose	**Laws and regulations**
Parliamentary acts	• National Policies of Government of Malaysia - Third National Agricultural Policy (1998–2010) • Federal Constitutions • Ministerial Functions Act 1969 (Perintah Menteri-Menteri Persekutuan 2004): DID Role In On River Management in Flood Mitigation and River Conservancy • Water Act 1920 (Revised 1989)
Land acts	• National Land Code 1965 And Waters • Land Conservation Act 1960
Flood mitigation	• Drainage Works Act 1954 (1988) • Local Government Act (1976): (Peninsular) • Earthworks Bylaws • Street, Drainage and Building Act 1974 (1994) • Town and Country Planning Act 1976
River management	• Waters Act 1920 (Act 418) & Water Supply (Federal Territory of Kuala Lumpur) Act 1998 (Act 581) • Mining Enactment 1962 (F.M.S. Cap 147) • Environmental Quality Act 1974 (Act 127) & Subsidiary Legislation • Ministerial Functions Act 1969 (Act 2) • National Forestry Act 1984 (Act 313) & Wood-Based Industries (State Legislatures Competency) Act 1984 (Act 314) • Fisheries Act 1985 (Act 317) and Regulations • Water Act 1989 - Chapter 15 • Sewerage Services Act 1993 (Act 508) & Regulations And Orders
Hydrological & water resources	• Drainage Works Act 1954 (1988) • Waters Act 1920 (Cap 146) (1989)
Urban stormwater	• Street, Drainage and Building 1974 (Act 133) - Section 70A(9) • Street, Drainage and Building 1974 (Act 133) - Section 71
Agriculture drainage	• Third National Agricultural Policy (1998–2010) • Irrigation Areas Act 1953 (1989) • Drainage Works Act 1954 (1988) • Waters Act 1920 (Cap 146) (1989) • Fisheries Act 1963 (1985)/Inland Fisheries Act

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Malaysia is still practising the traditional water management system which employs and depends heavily on the water supply management approaches – cater to demand. *Table 3* shows the water production and consumption in Malaysia where production increases to cater the increasing water consumption. With the increasing demand and consumption, more water has to be produced and supplied. Thus, more structures like dams, water treatment plants and water distribution pipes need to be built. This approach is unsustainable in the long run as water demand may eventually surpass water supply. There are plans by the government to privatize water supply in almost every state. However, several water privatization schemes have not produced desirable results. The water privatization process is still lacking transparency and accountability. Unsuccessful privatization leads to unsustainable water management.

Table 3. *Water production and consumption in Malaysia 2009–2012 (MLD)*

	2009	**2010**	**2011**	**2012**
Production	13,495	14,065	14,564	15,145
Consumption	8,551	8,972	9,164	9,532
Buffer	4,944	5,093	5,400	5,613

The average water tariff of Malaysia is 0.39 USD/m^3. Comparing to that of Indonesia (0.77 USD/m^3) and Singapore (1.88 USD/m^3), water tariffs in Malaysia can be deemed as one of the lowest in the world. Such a situation does not encourage water conservation but instead leads to water wastage and overuse of water, both of which undermine the sustainability of water. *Table 4* shows the average urban water use (litre/capita/day) in Asian countries. Malaysian average urban water use (litre/capita/day) is 344, which is much higher than that recommended by UN (165) and other Asian countries, i.e. Japan, China, Singapore and Indonesia. Agriculture accounts for 68% of total water consumption in Malaysia. However, the irrigation efficiency is 50% at best in the larger irrigation schemes and less than 40% in the smaller ones. There is also no recycling of irrigated water being practised and these factors have contributed to high rate of water wastage.

Table 4. *Average urban water use (litre/capita/day) in Asian countries*

Country	**Average urban water use (litre/capita/day)**
Malaysia	344
Japan	314
China	204
Singapore	153
Indonesia	130

The national average of non-revenue water (NRW) is 36% (36 l loss out of every 100 l) which is way too high. *Table 5* shows the NRW in all states in Malaysia 2013. The high level of NRW in Malaysia is caused by aged pipe network, poor maintenance of pipe network, illegal connections and absence of coherent implementation for active leakage control. It is estimated that 40% or 50,900 km of the pipe network was laid 40 to 60 years ago or longer. However, the lacking of funding for asset replacement and

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poor construction resulted in increasing leakage in the pipe network. Such a situation is further exacerbated by illegal connections which significantly contribute to commercial losses and revenue loss to the utilities. Thus, all these have now become the focus of regulatory oversight under the new licensing regime and areas to be improved (SPAN, 2012).

Table 5. *Non-revenue water (NRW) in all states in Malaysia 2013 (SPAN, 2012)*

State	System input volume	Billed authorized consumption	NRW	NRW (%)
Johor	1,580	1,163	417	26.4
Kedah	1,326	651	675	50.9
Kelantan	430	202	228	53.1
Labuan	63	46	16	25.9
Melaka	482	375	107	22.1
Negeri Sembilan	734	468	267	36.3
Pulau Pinang	988	809	180	18.2
Pahang	1,065	504	561	52.7
Perak	1,200	835	365	30.4
Perlis	211	80	132	62.4
Sabah	1,132	530	602	53.2
Sarawak	1,150	790	359	31.3
Selangor	4,564	2,989	1,575	34.5
Terengganu	623	413	210	33.8
MALAYSIA	15,549	9,855	5,694	36.6

Climate change and water pollution issues also threaten the water security. The changing weather pattern is not adequately taken into account of the water resources planning. The weather and climate change, locally and globally, affect water resources in Malaysia. For example, *El Nino* phenomenon that occurred in 1997/98 has brought severe drought resulting in water crises in many parts of Malaysia. Many water catchments in Malaysia have yet to be gazetted and legally protected. Water catchments are exposed to incompatible development resulting in adverse environmental effects, which ultimately make water resources degraded and unsustainable. Water pollution reduces total water availability considerably as the increasing cost involved in treating polluted will not be economically viable and in some instances, polluted water are not treatable for consumption. Almost half of the rivers in Malaysia are getting polluted nowadays. There are 1,064 monitoring stations in Malaysia, out of this number, 619 (58%) were found to be clean, 359 (34%) were slightly polluted and 86 (8%) were polluted (WEPA, 2006).

Sustainable water resources management

Without the conscious efforts in addressing water resources management issues, it would not able to safeguard quantity and quality of water for sustaining livelihoods, human well-being, and socio-economic development (Cosgrove and Rijsberman, 2000).

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Robust water policy

Efforts to develop, conserve, utilize and manage water resources have to be guided by a robust national water policy to ensure water resources in the country are managed sustainably and holistically. The re-structuring of water services industry which was initiated by the federal government has established a holistic water policy which covers not only water supply but also sewerage services to improve the efficiency of the water services sector for the benefit of the people as well as to maintain the sustainability of water resources. The national water policy is outlined in the National Policy Objectives for the Water Supply and Sewerage Services Industry. The policy aims to establish a transparent and integrated structure for water supply and sewerage services that delivers effective and efficient services to consumers. The policy provides a regulatory environment which facilitates financial self-sustainability amongst the operators in water services industry in the long term and an effective system accountability and governance between operators in water services industry. It also aims to regulate tariffs and ensure the provision of affordable water services on an equitable basis; the safety and security of water services systems and the long-term benefits of the consumers. The policy ensures long-term availability and sustainability of water supply including the conservation of water and contributes to the sustainability of watercourses and water catchments. The policy also facilitates the development of competition to promote economy and efficiency in water services industry and improve the quality of life and environment through the effective and efficient management of water services (PAAB, 2013). Also, the water policy should always keep up to the pace with climate change in view of climate change is also a challenging issue in managing water resources whereby the dynamic climatic condition could affect the availability and sustainability of water resources.

Effective governance and institutional matters

The government is primarily responsible for the planning, development and management of water resources projects in the country. As discussed in the previous section, there is no central agency entrusted with the overall responsibility of holistic planning and management of water to manage the overall aspects of water resources. To establish an effective governance structure for water resources in the country, it is necessary to recognize and understand the close relationship between land use and water resources management issues. The National Water Resources Council (NWRC) which was established in 1997 is a step forward towards integrated approach in managing water resources. Besides coordinating function and ensuring uniformity in policies, standards and priorities, being the forum where the federal and state governments link water and land strategy can be formulated resulting in optimal utilization and conservation of the nation's water resources, the NWRC should be provided with legal mandate to plan, coordinate and execute Integrated Water Resources Management (IWRM) in which it balances the conditions for sustainable development (national interest) and desirable socio-economic development (public interest) with ecological conditions (environmental integrity) (Azhar, 2000).

Water economic efficiency

Due to the increasing water shortage issues, water has become an economic good that has its own economic value. In Malaysia, government subsidies are common and

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tariff is practically very low because water is still regarded as a social good where the concept of full cost recovery is not observed because of sociopolitical considerations. In addition, the absence of established economic regulation complicates the problem. Increasing tariff to cost recovery levels often faced with resistance resulting water utilities are not able to comply with its service obligations. In view of the highly capital-intensive nature of the water service industry, more private investment is required to lighten the fiscal burden of the government. There is also a need to revise the tariffs which balance full cost recovery and consumer's ability to pay and it can be achieved if the capital investment cost can be kept as low as possible and the tariff set at an economic value. The tariff for domestic and non-domestic water supplies are expected to move towards those of developed nations whereby gradually tariff revision is needed to reflect closely the actual costs of developing water resources facilities. Also, to ensure the efficiency of the water services, specific actions need to be taken particularly in reducing leakages and non-revenue water. With the effective addressing non-revenue water issues and raising public awareness on water saving, hence the water resources can be conserved (Salleh, 2011).

Sustainable water resources development

In line with the increasing water demand, water resources development have to meet the rapid growth in population, industries, tourism and other economic activities. We also recognize the challenges in water shortage, rising water pollution and other environmental pressures. Hence, it is necessary to ensure sustainable water resources development. Integrated and holistic approaches which require engagement and close partnership between stakeholders, including federal and states governments, regulatory authorities, environmental agencies, health agencies, water service providers, contractors, land users, consumers, etc are needed especially in conducting a detailed environmental impact assessment prior to the initiation of new water resource development projects. In addition, the identified sites for dam development should be conserved and gazetted as water catchment areas to ensure project can be implemented as planned because there may not be any other suitable alternative sites available for dam construction (Salleh, 2011).

Effective construction, operation and maintenance of water structures

Apart from proper planning of water structures, safety is particularly concern in ensuring effective water structure construction, operation and maintenance. An Inter-Departmental Committee on dam safety was established in May 1986 which consists of Drainage and Irrigation Department Malaysia, National Electricity Board, Penang Water Authority, Malacca Water Board, Muda Agricultural Development Authority, Sabah Electricity Board and Sarawak Electricity Supply Corporation under the stewardship of Public Works Department Malaysia. The committee has launched the Guidelines for Operation, Maintenance and Surveillance of Dams in October 1989 to provide specific procedures and practice to suit site and management conditions of individual dams. The guidelines are applicable to all dams (except tailings dams). The guideline not only prepares an operation and maintenance manual for the dam, it also stipulates the responsibilities for operation and maintenance activities, routine inspections as well as adequate instructions and arrangements for emergency actions. Continuous examination of the condition of a dam and its appurtenant structures and the

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review of operation, maintenance and monitoring procedures and results are needed in order to determine whether a hazardous trend is developing or appears likely to develop. These surveillance activities should be conducted throughout its active life by trained and competent personnel and the published guidelines should be diligently followed and reviewed periodically by the dam owners to ensure the sustainability of multi-purpose dam (Othman, 2006).

Potentials of multi-purpose dam development in Malaysia

International Commission on Large Dams defined large dam – more than 15 m in height, while including smaller dams up to 10 m height as well, if they are otherwise significant with respect to storage volume, density of population, etc. Dam is an artificial barrier together with appurtenant works constructed for the purpose of holding water or any other fluid or silt across a natural water course or on the periphery of reservoir to control the flow or the level of the water (DID, 2009). By proper altering the spatial and temporal distribution of runoff, dam could serve several functions (Zhou and Guo, 2013).

With the rapid economic development, the role of dam has become more and more important to meet society's water requirements as the dam is the cornerstone in the development and management of water resources of a river basin. The International Commission on Large Dams (ICOLD) has been promoting the theme of a water-food-energy nexus and integrated solution whereby one problem cannot be solved without thinking of the other sides of the problem. This has highlighted the importance of multi-purpose dams in resolving the issues of water, food and energy. However, most of the existing dams in the world are single-purpose dams (49.2%). Among the single-purpose dams, 48% are for irrigation, 20% for hydropower (production of electricity), 13% for water supply, 9% for flood control, 5% for recreation and 5% for navigation, fish farming and others. ICOLD recorded only 16.8% of the worldwide dams can be categorized as multi-purpose dams where they are mostly built for irrigation purposes (24%), followed by flood control (19%), water supply (17%), hydropower (16%), recreation and navigation/fish farming (19%) and others (5%) (ICOLD, 2014). A multi-purpose dam is gaining importance as it fits well into regional development programs, such as improving food production, electricity supply, and the general physical and social infrastructure in rural areas. In addition, multi-purpose dams can complement strategies for climate change adaptation. For example, hydropower generation can be combined with increased water storage or flood regulation.

To date, there are 65 major dams that have been developed in Malaysia. *Table 6* shows the major dam development in Malaysia from 1950s to 2000s. Most of them were constructed for water supply. There were also dams built for hydropower, irrigation, flood mitigation, environmental and recreation purposes. Since 1980s, massive development has been devoted for water supply dams considering the increasing water demand due to population growth and country transformation from an agriculture-based economy into an emerging industrial manufacturing entity. It is noted that only 9 out of 65 dams in Malaysia serve more than one purpose in their function. Thus, it can be affirmed that the multi-purpose dam concept is still not very popular in the water resources planning and development of Malaysia.

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Table 6. *List of dams in Malaysia*

No	Purpose	Before 1950s	1950s	1960s	1970s	1980s	1990s	2000s	Total
A	Water supply	Gunung Pulai Scheme (Johor) 1. Pulai I + II dams 2. Pontian Kechil dam	Klang Gates dam[2] (Kuala Lumpur)	Air Hitam dam (Penang) Congok dam (Johor)	Lebam dam (Johor) Durian Tunggal dam (Melaka) Langat dam (Selangor) Pinangsoo dam (Sabah)	Semenyih dam (Selangor) Terip dam (Negeri Sembilan) Malut dam (Kedah) Ahning dam (Kedah) Mengkuang dam (Penang) Layang Upper dam (Johor) Layang Lower dam (Johor) Timbangan dam (Sabah) Sika dam (Sarawak) Bukit Kuda (Labuan)	Sungai Tinggi dam (Selangor) Linggiu dam (Johor) Air Kuning dam, (Perak) Pedas dam (Negeri Sembilan) Juaseh dam (Negeri Sembilan) Upper Muar dam (Negeri Sembilan) Gemencheh dam, (Negeri Sembilan) Kelinchi dam (Negeri Sembilan) Babagon Dam (Sabah)	Sungai Selangor dam (Selangor) Chereh dam (Pahang) Jus dam (Melaka) Betotan dam (Sabah) Milau dam (Sabah) Gerugu dam, (Sarawak) Labu dam (Selangor) Teluk Bahang dam (Penang) Kelalang dam (Sarawak) Telibung dam (Sabah)	
		3	1	2	4	10	9	10	39
B	Hydropower	Chenderoh dam (Perak)		Cameron Highland Scheme (Pahang) 1. Ringlet Falls dam 2. Batang Padang Scheme Jor and Mahang (Perak)	Temenggor dam (Perak)	Kenyir dam (Terengganu) Batang Air (Sarawak) Bersia dam (Perak) Kenering dam (Perak)[5]	Pergau dam (Kelantan)[5]	Bakun dam (Sarawak)	
		1		3	1	4	1	1	11
C	Irrigation	Bukit Merah dam(Perak)[1] Labong dam (Johor)		Muda Agricultural Development Authority Scheme (Kedah) 1. Pedu and Muda dams	Bukit Kwang dam (Kelantan)	Anak Endau dam (Pahang)[1] Pontian dam (Pahang)[1] Machap dam (Johor)	Timah Tasoh dam (Perlis)[3]	Beris dam (Kedah)	
		2		2	1	3	1	1	10
D	Flood mitigation					Sg Batu dam (Selangor)[4] Sembrong dam (Johor)	Bekok dam (Johor)[4]		
						2	1		3
E	Environmental			Silt retention Repas baru dam, (Pahang)					
				1					1
F	Recreation							Putrajaya dam, (Wilayah Persekutuan)	
								1	1
Total		6	1	8	6	19	12	13	65

Large dams defined as >15m and/or storage volume >3 million m^3
[1]Water supply/irrigation
[2]Water supply/flood mitigation
[3]Water supply/irrigation/flood mitigation
[4]Flood mitigation/water supply
[5]Hydropower/flood mitigation

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The growing of water demand requires either better water supply management or additional supplies. Dam management plays a vital role in ensuring equitable water supplies. The management of dams in Malaysia is divided into four groups, i.e. flood mitigation, hydroelectric, water supply and agriculture as shown in *Table 7*. Generally, the water supply dams in Malaysia are managed and operated by private and water board of respective states. Drainage & Irrigation Department (DID) is presently managing 15 dams within Malaysia and providing adequate irrigation water, flood mitigation and silt retention. More dams are scheduled for implementation in the future to meet the ever increasing demands and social expectations of the public. Tenaga Nasional Berhad, the main Malaysian energy provider, is operating 3 hydroelectric schemes in the peninsular Malaysia with an installed generating capacity of 1,911 MW. They are the Sungai Perak, Terengganu and Cameron Highlands hydroelectric schemes with 21 dams in operation. Despite having most of the dams for water supply purpose, Malaysia is still facing water shortage crisis. Water supply is relatively unstable in some parts of the country, particularly in Klang Valley. The development and population growth is occurring at a robust pace, and even now the residents are starting to experience water shortage and water rationing, which can be more acute in the next decade. Syarikat Bekalan Air Selangor Sdn Bhd (SYABAS) had issued a warning that the Klang Valley will face a water crisis unless the authorities implement three water resource projects by 2014. The projects are the Sungai Langat 2 water treatment plant, the Pahang-Selangor raw water tunnel and the Salak Tinggi dam projects, which have not taken off so far because of the differences between the federal and state government, particularly over land acquisition and water price (Bedi and Avineshwaran, 2013). According to SYABAS, these three water resource projects must be expedited to meet the rising demand for water supply in Selangor, Kuala Lumpur and Putrajaya, which is growing by 3.5% yearly. Such a situation has triggered the need to investigate the feasibility of multi-purpose dam development in Malaysia. With the increasing water demand in Malaysia, multi-purpose dam is likely to become relevant in ensuring equitable water demand management. The multi-purpose dam is not only accelerating socio-economic growth but also mitigating the miseries of the population from suffering of the vagaries of floods and droughts.

Table 7. *Dams management in Malaysia (as of 2006)*

No	Purpose	Dam operator
1	Water supply	Private & Water Board
2	Flood mitigation/environment	Drainage & Irrigation Department
3	Hydroelectric	Tenaga National Berhad
4	Agriculture	Others

Aspects to be considered in developing multi-purpose dam

There are generally five interconnected aspects, namely legal, technical, economic, social and environment in developing a dam. These aspects need to be considered in the planning, funding, constructing, operating and maintaining of a multi-purpose dam to ensure the sustainability of water resources.

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Legal aspects

Existing legal framework and institutional matters

Legal and regulatory issue is one of the areas concerned in managing water resources. However, there is no specific act available for dam development and management, particularly multi-purpose dam in Malaysia. The only dam development relevant legal requirements are shown in *Table 8* which are covered under Environmental Quality (Prescribed Activities) (Environmental Impact Assessment) Order 1987 for the construction of dams of drainage and irrigation, power generation and transmission as well as water supply (CIDB, 2008).

Table 8. *Environmental Quality (Prescribed Activities) (Environmental Impact Assessment) Order 1987 for dam construction*

Schedule	Activities	
3 (a)	Drainage and irrigation	Construction of dams and man-made lakes and artificial enlargement of lakes with surface areas of 200 ha or more
13 (b)	Power generation and transmission	Dams and hydro-electric power schemes with either or both of the following: (i) Dams over 15 m high and ancillary structures covering a total area in excess of 40 ha (ii) Reservoirs with a surface area in excess of 400 ha
19 (a)	Water supply	Construction of dams or impounding reservoirs with a surface area of 200 ha or more

Taking other countries as benchmark, Japan enacted "Specified Multipurpose Dam Law" in 1957 to centralize the construction and administration of dams under the control of river administrators (Musiake and Koike, 2009) and South Korea enacted "Specific Multi-Purpose Dam Law" in 1966 to address various issues including financing of the construction of dams, support for flood victims, rational use and development of water resources to promote economic development and enhance the quality of life (Ministry of Strategy and Finance, Republic of Korea (2013). An adequate legal planning in the construction of multi-purpose dam is necessary. In 1966–1975, the South Korea's government has established master plans to construct multi-purpose dams for water supply and control. After 1990's, the scope of multi-purpose dams was expanded to cover water supply, water control, and environmental preservation (Moon and Kwon, 2003). Under Water Resources Bureau of Ministry of Construction and Transportation, the entities namely Water Resources Policy Team and Water Resources Development Team are responsible to dam related policy making, research and development, dam related public relation, dam related National Assembly matters and budget, operation of dam related acts and system improvement, handling of property rights of dams and water facilities, planning and coordination, budget and setting and supervision of multi-purpose dam construction. However, in the Malaysia's context, as mentioned earlier, the government is still practicing the traditional water resources management system which employs and depends heavily on the water supply management approaches – cater to demand. Besides, Malaysia is also lacking a regulatory framework to govern the development and management of dam, particularly multi-purpose dam, as compared to other advanced countries.

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Prior to 2005, water supply was under the purview of respective state government. The state governments mostly own the water infrastructures and operate the water services. There are also some states, such as Selangor and Johor, where the water services are privatized and responsible for the treating and supplying of water to consumers. Therefore, institutional issues such as the water industry were not centrally regulated, causing inconsistency in service delivery. The varying degree of quality of water supply and services, different rules, procedures, key performance indexes (KPIs) and level of services, non-uniform in legislation and tariff setting principles have become challenges for the development of water industry. In the states where water services are privatized, operations are fragmented with several private operators involved in providing water supply to the states which is highly inefficient (PAAB, 2013). The federal government takes the initiative to reform the industry for the benefits of all stakeholders including federal government, state governments and consumers in the early of 2003. It was an extensive process which included amendments to the constitution and passing of new legislations to enable the government to mobilize the reformation. An amendment to federal constitution pertaining to jurisdiction on water supply was gazetted on 10 February 2005 whereby water services and supplies are listed under concurrent list between state governments and federal government. The amendment to the ninth schedule involves the transfer of water supplies and services from the state list to the concurrent list whereby the water supplies and services are shared responsibility between the state and the federal governments. It was a pertinent move which gave the federal government authority over the water services in the states. The tenth schedule was also amended. The revenue from water supplies and services (previously assigned to the states) is now under the federal government. *Figure 1* shows the water governance before and after the federal constitution amendment in 2005.

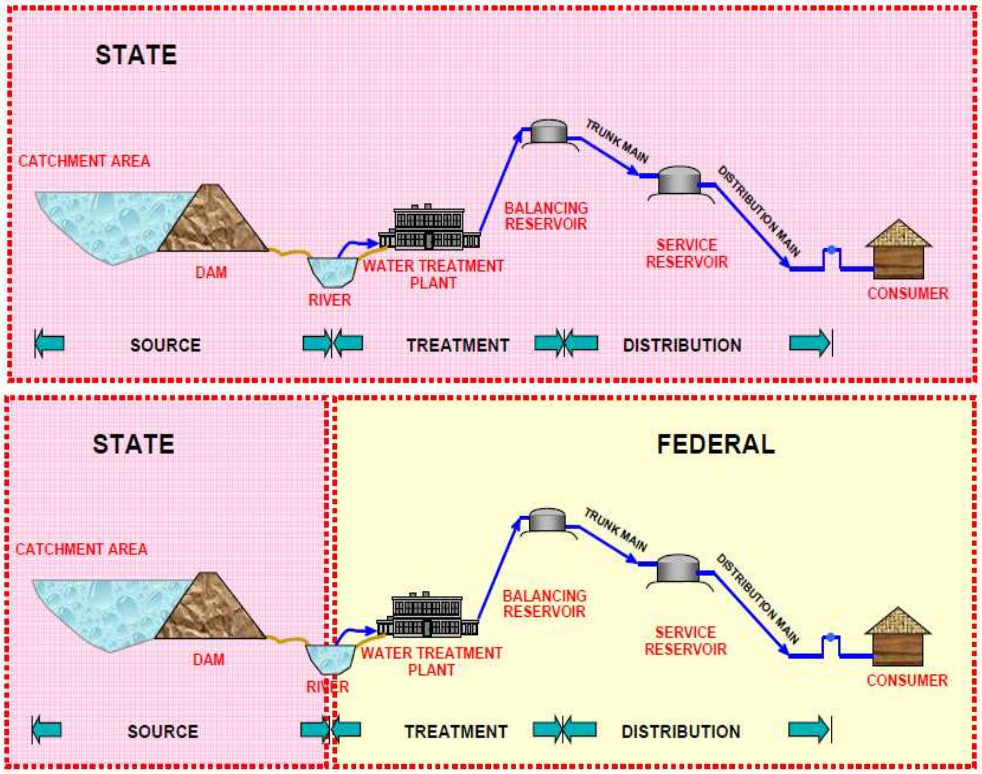


Figure 1. *Water governance after federal constitution amendment in 2005 (Wahab, 2011)*

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With effect from 21 March 2005, the federal government holds the right in regulating water treatment, distribution until the delivery to consumer whereas the ownership and control of rivers, canals, dam and water catchment remains under the state governments. The state governments retain the power to declare and regulate water catchment areas, water sources and river basins (Wahab, 2011). *Figure 2* shows the roadmap of acts amendments. Further amendments were carried out in July 2006 whereby parliament passed two new legislations namely the National Water Services Commission Act 2006 and Water Service Industry Act 2006 (WSIA). *Table 9* shows the policy and institutional framework on water services reform. National Water Services Commission Act provides for the establishment of National Water Services Commission (SPAN) as the technical and economic regulator and set out the function and power of SPAN. Water Service Industry Act provides the legal framework required for the regulation of the water and sewerage service industry. The Acts were enforced on 1st February 2007 and 1st January 2008, respectively.



Figure 2. *Roadmap of water services acts amendments (PAAB, 2013)*

Table 9. *Policy and institutional framework on water services reform (Wahab, 2011)*

Body	Area of responsibility	Description
Federal Government	Policy matters	Development of a holistic water policy for the country by setting policy directions
State Government	Raw water matters	Regulate raw water abstraction and catchment management
National Water Resource Council (NWRC) – chaired by Prime Minister	Water resources matters – cross boundaries/inter state/issue of national interest	Ensures coordination with the various State Governments in the management of the water basins
National Water Services Commission (SPAN)	Regulatory matters	Regulate the Water Services industry (Water and Sewerage services)

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Impact assessments and monitoring

Under Environmental Quality (Prescribed Activities) (Environmental Impact Assessment) Order 1987, the construction of dams requires obtaining the following approvals from the Director-General of Department of Environment prior to project commencement:

i. Environmental impact assessment for prescribed activities
ii. Site suitability evaluation
iii. Written notification or permission to construct
iv. Written approval for installation of equipment
v. License to occupy and operate prescribed premises and prescribed conveyances

However, the schedules only cover drainage and irrigation; power generation and transmission and water supply. There is no specific schedule for multi-purpose dam construction. Considering constructing a multi-purpose dam is a complex process as it needs to take into account of the challenges of social, environment and its sustainability and it would be a challenge for the proposal to construct a multi-purpose dam.

Constructing a dam represents a potential water resource which is tantamount to potential hazard to downstream life, property and natural resources. Failure of any type of dam structure can result in loss of life, property and natural resources. Thus, a dam structure needs to be planned, constructed, and kept under proper surveillance, maintained to keep them in safe serviceable condition. A manual on Dam Safety, Inspection and Monitoring has been developed by Department of Irrigation and Drainage (DID) of Malaysia specifies the dam safety, inspections and monitoring throughout the lifecycle of the dam. *Figure 3* shows the lifecycle of a dam where it starts from planning, design, construction, commissioning, reservoir filling, operation, maintenance and surveillance to abandonment or rehabilitation. Each stages, the roles of government and consultants in monitoring the lifecycle of the dam is shown in *Table 10* (DID, 2009).

Lawsuits and dispute settlement

Lawsuits and dispute settlement is another legal and regulatory aspect to be taken into account in constructing a multi-purpose dam, particularly over environmental impact assessments (EIA) and community relocation. According to Sovacool and Bulan (2011), critiques related to reliance EIA on inadequate data, poor or incomplete hydrological analysis, and predictions of sediment delivery incorrect by more than one order of magnitude, as well as flawed projections about the effect on biodiversity and displaced communities have led to lawsuits filed against the dam construction projects in Malaysia. Non-governmental organization (NGOs) banded together to oppose dam construction projects, where they argued that consultation with the displaced communities was prone to multiple errors related to surveys of land, compensation, choice of contractors and consultants for the site, and the logistics of the relocation itself. The ineffective executions of the environmental and social impact assessment for the dam construction planning have resulted dispute and questioned on the legitimacy of parts of EIAs.

In South Korea, the "Act on Dam Construction and Assistance, Etc. To Neighbourhood Area" has been enforced in 10th Dec 2009 to give a guideline for construction, management of dams and support for residents of dam construction area.

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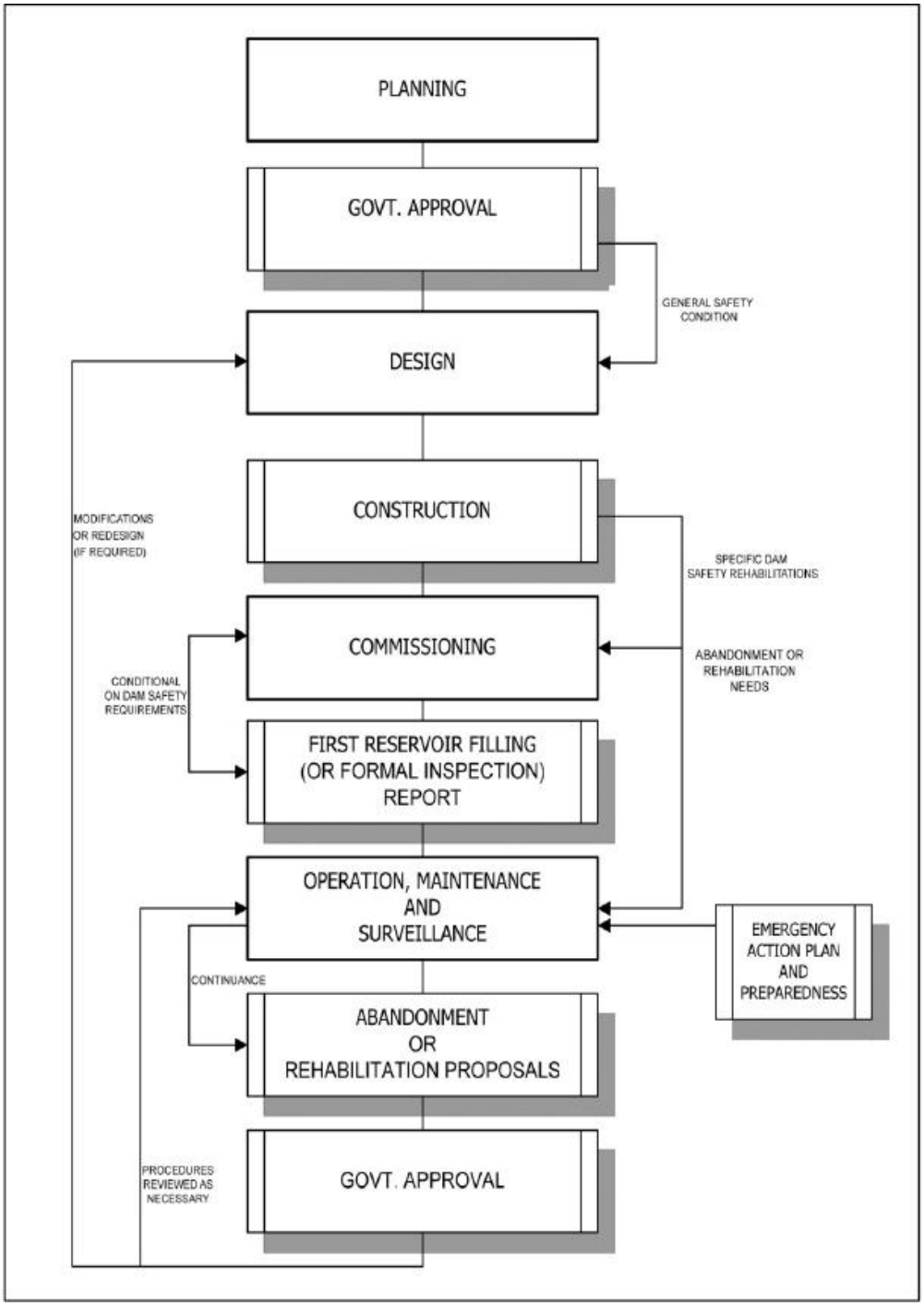


Figure 3. *Lifecycle of a dam (DID, 2009)*

The purpose of the Act is to contribute to the development of the national economy through the rational development and utilization of water resources by providing for the construction and management of dams, the reuse of construction costs, environment countermeasures according to the construction of dams and assistance to the residents of areas adjacent to dams (Ministry of Land, Transport and Maritime Affairs Korea 2009). Malaysia is still lacking an Act to be enacted specifically for dam development and management which is able to provide a guideline for developing environmentally and

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sustainable sound dams. In the Act, a holistic dam planning, development and management mechanism should be expounded where a pre-survey, social and environmental impact assessment shall be carried out effectively together with the local residents' councils and civil organization to avoid dispute and ensure construction of dams in harmony with local environment and communities.

Table 10. *Roles of government and consultants in dam's life cycle (DID, 2009)*

Stage	Activity	Role of government/DID	Consultants
Planning	• Concept	- Ascertain need - Reconnaissance	
	• Prefeasibility study	- Prepare TOR/Invite proposals - Appoint consultants/Finance	- Carry out investigations and prepare report
	• Feasibility study	- Review report of consultants - Prepare TOR/Invite proposals - Appoint consultants/Finance - Review report of consultants	- Carry out investigations and prepare report, including EIA, EMP and socio-economic study of the people affected by the project
Design	• Detailed design	- Prepare TOR/Invite proposals - Appoint consultants/Finance - Review report of consultants - Review consultants production	- Carry out investigations and prepare report including design and O&M manual prepare tender documents including drawings specifications, etc
Construction	• Tendering	- Prequalify/Call for tenders	- Prepare criteria/NIT - Assist government/DID in evaluation
	• Construction	- Evaluate tenders/Award - Supervise construction, executed by constructors - Make payment to the contractors as per contracts	- Assist DID in supervision of construction of works, including evaluation of the work executed, preparation of payment to the contractor and evaluation of contractual claims, if any
First reservoir filling	• Vigilance	- Exercise vigilance till reservoir is full, and after	- Assist government/DID in exercising vigilance
		- To keep liaison with relevant authorities for emergency preparedness	- Prepare First Reservoir Filling Report, including inspection and monitoring and assessment of dam's performance
Operation and maintenance	• Surveillance • Monitoring • Inspection • Operation • Maintenance	- Execute - Execute - Execute - Execute	
Aging	• Rehabilitation	- Seek assistance of consultants preferably designers	- Design and supervise construction executed by contractors
Abandoning	• Decommissioning	- Execute, get structures dismantled as and if required	- Advise/Design dismantling, if required

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Technical aspects

Hydrology, rainfall and sedimentation

The complexity in planning, constructing, operating and maintaining of multi-purpose dams is a major challenge in its realization. These activities involve sophisticated technical requirements. In the planning and constructing of a multi-purpose dam, hydrology of the water catchment is one of the main challenges to be overcome. The dam should be designed to cater the rainfall volume. Learning from the experience of Bakun (Sovacool and Bulan, 2011) whereby Bakun area was prone to lots of rainfall, more than 4500 mm per year in some years. Building a dam in that type of weather proved difficult. The underestimation of the rainfall has caused unsuitable design and led to the redesign of Bakun dam. Such a flawed dam design has caused a big lost to the investment. In addition, sedimentation is also a challenge, in which logging, land clearing and unsustainable agricultural practices have caused the severe deforestation. High rainfall will cause the runoff of large amounts of sediment, silt, clay and gravel into the water body. Such a high sedimentation rate may impede the dam construction, operation and maintenance whereby extra technical considerations need to be taken into account to avoid damage to the dam.

Geographical isolation and provision of infrastructure

Due to the geographical and hydrological needs, the location of the dam construction is usually planned in an isolated area whereby no access roads and other infrastructure around the construction site. Transporting the construction materials, turbine and transformer components to the dam site is a challenge as the items are way too heavy to be transported. Therefore, a massive infrastructure development from scratch is required to take place to bring in construction materials and generators. Despite the said necessity for the dam construction, there are also needs to supply accommodation, medical service, food, clean water, electricity to the construction staffs before construction could commence. In other words, building a dam is not solely concerning about the reservoir only, but it needs a holistic planning for the adjacent development to ensure the sustainability of a dam (Sovacool and Bulan, 2011).

Dam excavation, construction, coordination and human resources

In addition to the provision of infrastructure, the dam excavation and construction are of the main technical challenges whereby each dam needs river diversion, tunnelling, channelling and access to the dam to transport machines and turbines (Sovacool and Bulan, 2012). The complexity of the project hence required coordination and human resources for the excavation and construction. Over thousands of skilled workers, engineers and contractors are needed in the construction of dam in building spillways, powerhouses, transformers, turbines, and the dam face. In developing country like Malaysia, there is lack of technical capacity to build dam, hence thousands of foreign workers need to be imported to participate in the dam construction. Besides that, building a dam also needs joint venture with many sub-contractors in which the selection of the competent sub-contractors is particularly important. The coordination of these workers and sub-contractors is formidable because any flaw in coordination will cause a major disaster to the project.

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Infrastructure operation and maintenance

The challenge of operating a multi-purpose dam is the balancing of competing demands. For example, the water level often needs to be as high as possible in view of the stored water in the dam serves as the stored energy for hydroelectric power generation purpose. However, for flood control purpose, the water level needs to be as low as possible to provide the storage space for rainwater runoff. Operators need to release enough water for downstream navigation, but store enough water to have a safety margin for electricity generation. River flow in a tropical rainfall country can rise as much as 10 m per day, 20 m when raining or flooding (Sovacool and Bulan, 2011). Such a contrast makes the operation of multi-purpose dam critical and challenging in the management of water level, mismanagement and misoperation might cause catastrophic damage in the downstream area with a clean sweep of the life and property that comes in its way. For instance, a tragedy happened in Cameron Highlands in 2013, whereby at least three people are dead and dozens of houses and cars were damaged after flood water was released from the dam. The operator claimed that the water was released "as a measure to avoid flooding the entire Bertam Valley", following heavy rains and flash flooding that "caused an overflow into Lake Ringlet that surpassed maximum levels". Local sources reported that one of the dam's four gates was opened to help lower the water level, and that if it had not been opened, all four dam gates might have opened automatically which potential to cause even more damage (Hydroworld.com, 2013). The dam maintenance is another challenge for the dam due to the huge sheer size and volume, underwater maintenance of tunnels, intake structures and the reservoir, completely shut down for 80–90 days is required for the turbines maintenance service to take place every 10–15 years (Sovacool and Bulan, 2011). With the high rainfall around the reservoir makes the service maintenance work difficult to be carried out.

Economical aspects

Future water demand

The population of Malaysia is continuing to grow where the population of Peninsular Malaysia will double its size from 18.1 million in 2000 to 38 million in 2050. The future water demand is depending on the population growth and socio-economic development. Urbanization and migration from rural to urban area is also evident where a relative number of the population concentrated in urban areas where high economic activities exist. The increasing population associated with the increasing economic development gives improvements in the standard of living results in the increased demand for water supply. Thus, it has given a pressure on the water resources. The challenge is not only to provide adequate water for the increasing demand but also stimulate the development in rural areas to relieve migration and reduce the pressure in the urban areas. The domestic and industrial water demand for Peninsular Malaysia is projected to increase 3-fold from 9543 MLD (3483 million m^3/yr) in 2000 to 31,628 MLD (11,543 million m^3/yr) in 2050. On a Peninsular Malaysia-wide basis, during periods of severe drought, the average natural or unregulated flows can barely meet the projected demand in 2050 of 31,628 MLD. Therefore, new dams or water reservoirs are needed as new sources to augment water supply over the next 50 years (Mohamad Salleh, 2011).

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Capital intensity, financing and returns on investment

Constructing a dam is a huge investment in the water infrastructure and its affiliated infrastructure. Financing remains a big challenge relating to where the money will come to meet the project plan. Building a dam is normally financed by public borrowing. Any cost overruns in building a dam can increase the stock of debt. The recurring financing cost can further escalate if interest rates go up. For example, the actual cost of Pakistan's Tarbela dam, the majority of which was borrowed from external sources, amounted to 23% of the increase in Pakistan's external public debt stock between 1968 and 1984 (Flyvbjerg et al., 2014). In addition, the cost analysis of the dam construction often omits the effects of inflation and the risks of unanticipated inflation which resulting financed with additional debt. For example, the hyperinflation in Argentina, Brazil, Turkey and Yugoslavia caused staggering nominal cost overruns, e.g. 7-times initial budget for Brazil's Estreito dam (1965–1974), or 110-times initial budget for Yugoslavia's Visegrad dam (1985–1990). Also, the effects of unanticipated inflation exacerbate when the dam takes longer than planned time to complete a project. For example, during the planning stage of Pakistan's Tarbela dam, it was assumed that inflation would not have a significant impact on the project's costs. A general contingency of 7.5% has been added in accordance with normal practice for the project of this size and duration. However, the project which was launched in 1968, was meant to commence in 1976, but the opening was delayed until 1984. Such a delay has caused the actual cost of the dam in nominal terms nearly four times the initial budget due to the actual cumulative inflation in Pakistan during 1968–1984 was 380%. It is about 8 out of 10 large dams delay of 2.3 years in completion whereby the expected annual inflation rate to be 2.5% but it turned out to be 18.9%. Large dams particularly have a high tendency to face unanticipated inflation. Thus, the outsides view suggests that in most cases dam will be too challenging in absolute terms and take too long time to build and deliver a positive return on investment (Flyvbjerg et al., 2014).

Social aspects

Community relocation, resettlement and compensation

The construction of dams often, if not always, involves relocation of communities and livelihoods. When relocation and resettlement take place, compensation is recognized as providing support to displaced people and to the maintenance of the legitimacy of socio-political structures and the modernization project (Wang et al., 2013). Affected community have to make the difficult decision of abandoning their homes and livelihoods, causing emotional distress. Therefore, adequate compensation is needed to compensate their loss of natural resources, social heritage and land which cause misery to their distressed livelihood. Without adequate compensation, resettlement of people into a new area will not improve the lives of the community and this has failed to accomplish the purpose of resettlement (Sovacool and Bulan, 2011). Thus, the relocation, resettlement and compensation of the community are of the most challenging social barrier to be taken into account prior to the construction of dam.

Community livelihood and welfare

Social impacts on community livelihood and welfare caused by the relocation and resettlement are diverse. For example, the relocation of residents due to Manwan Dam

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China has caused the diversified income sources, widened income gaps and gave an overall adverse impact on standard of living (Fu and He, 2003). Also, the displacement of the entire indigenous population (around 10,000 people) in Bakun dam region has caused a drastic process of socio-economic disintegration and cultural extinction of the indigenous communities. Such a displacement has also caused the abandonment of traditional livelihood and economic activities which were adapted to local ecosystem and the complete loss of knowledge of these practices within the local population (Choy, 2005). It is also argued that the construction of Three Gorges Dam has impacted the cultural heritage whereby it has eradicated some historical heritage sites and unique sources of knowledge due to dam inundation (Jackson and Sleigh, 2000).

Environmental aspects

Climate change

The future climate change is going to impact the hydrological regime and water resources in Malaysia (Wong et al., 2009). *Table 11* shows the projected rainfall for 2025–2050 for Malaysia. This hydroclimate projection study shows a projection increase in average annual precipitation (mm) in Perak, Kedah, Terengganu, Kelantan and Pahang. However, a decrease in average annual precipitation (mm) is projected in developed urban areas like Klang, Selangor and Johor (Kavvas et al., 2007). Higher maximum and lower minimum rainfall observed could result in more extreme hydrological conditions. These changes in rainfall have led to changes in the river flows. The flood flow changes range from +6% to +47%, while the low flow changes range from -35% to -93%, in which more frequent floods and droughts are projected in future (Zakaria and Shaaban, 2007). Climate change is a challenging factor in the planning and constructing of a multi-purpose dam in a tropical country like Malaysia as the dynamic condition of the local region could change the climatic condition as well as the level of hydrological regime.

Table 11. *Average annual rainfall (Kavvas et al., 2007)*

Regions	**Sub regions**	**Average annual precipitation (mm)**		
		1984–1993 (1)	**2041–2050 (2)**	**(2)–(1)**
North West Region	West Coast	2151.0	2099.3	-51.7
	Perak	2314.8	2398.0	83.1
	Kedah	2082.9	2163.0	80.1
North East Region	Northeast Cost	3114.3	3292.2	177.8
	Terengganu	3467.8	3497.4	29.5
	Kelantan	2661.8	2865.0	203.2
Central Region	Klang	2281.5	2158.9	-122.6
	Selangor	2282.3	2176.9	-105.4
	Pahang	2381.8	2523.1	141.3
Southern Region	Johor	2247.8	2139.4	-108.4
	Southern Peninsular	2324.5	2298.4	-26.1

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Land clearing, deforestation, degradation, impoundment and greenhouse gas emission

The most significant environmental challenge is the land clearing, deforestation, land degradation, impoundment of reservoir and greenhouse gas emission (Sovacool and Bulan, 2011). A huge area of bare land is needed for water impoundment. Therefore, extensive logging is the only way to acquire land within the catchment area. It is noted by Choy (2005) that the deforestation and submerging of the catchment area will destroy biomass home to rare and endangered species, protected bird species, protected mammals, protected plants including herons, eagles, woodpeckers, silvered leaf monkeys, Bornean gibbons, langurs, and flying squirrels. To accelerate the logging process, access roads is built to the dam and further worsen the deforestation and lead to the degradation of the exposed bare land. It is also noted that impoundment process during the dam construction will become a significant source of greenhouse gas emissions, especially carbon dioxide and methane arising from microbial decomposition of the submerged forest, vegetation, wildlife and soil. *Figure 4* shows microbial and methanogenesis and denitrification of impoundment process. The inundated forest biomass will be transformed into carbon dioxide (CO_2) and methane (CH_4) and nitrous oxide (N_3O) through decomposition, microbial methanogenesis and denitrification. The anoxic condition in the deep reservoir will trigger the conversion of organic carbon into CH_4 instead of CO_2. CH_4 in shallow waters may form bubbles and release to the atmosphere. The emission of these greenhouse gases may be built up in the atmosphere as a heat trapping layer. Carbon emission is primarily contributed by the flooded biomass. Other sources of carbon emission include (i) carbon from the soil and peat of the reservoir; (ii) dissolved organic carbon/particulate organic carbon; (iii) organic debris from the catchment and; (iv) decomposition of animals and algae in the reservoir following their death.

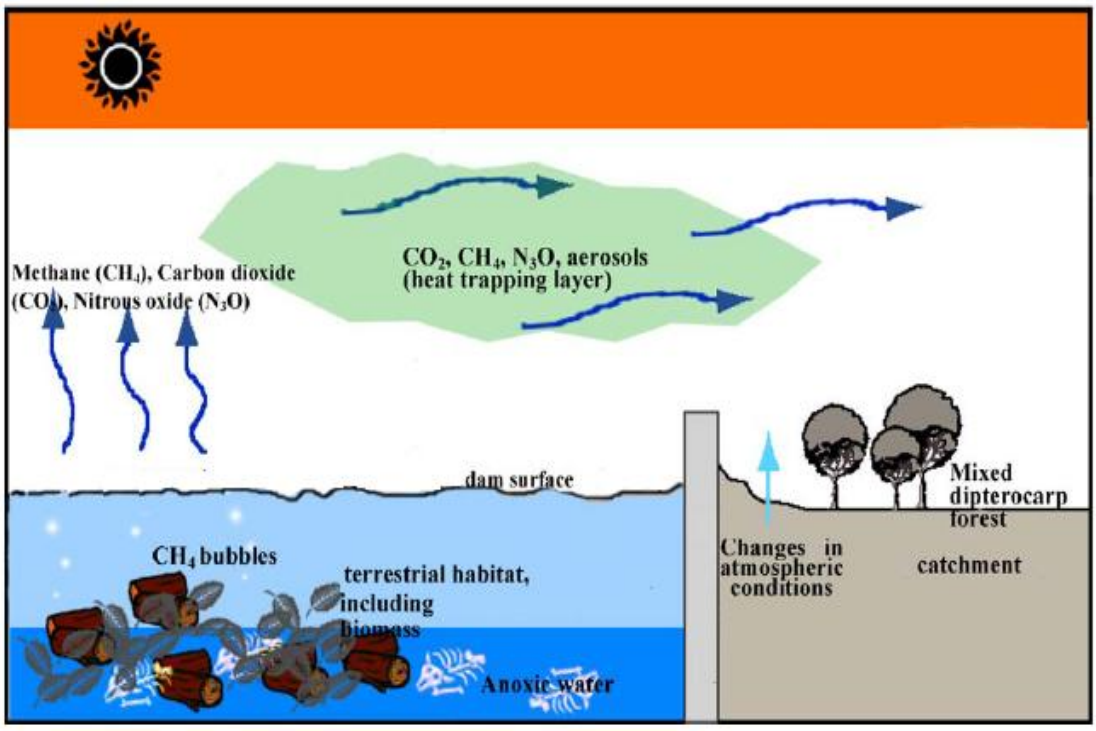


Figure 4. *Microbial methanogenesis and denitrification of the impoundment process (Choy, 2005)*

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The warming effect is also associated with greenhouse gas emission when the extra energy attempts to leave the earth surface in the form of infrared radiation, but much of it will be absorbed in the atmosphere by the principal heat-trapping gases such as water vapour, CO_2 and CH_4. This turn of event will not only contribute to global warming, but also affects the sink capacity for atmospheric pollution and hydrological conditions in the surrounding area of dam. This sequence of climatic change will pose a severe ecological threat on forest ecosystem in the catchment area. As illustrated by Choy (2005) in *Figure 5*, the microclimate change in the surrounding area of dam is mainly attributed to the complete exposure of the dam surface to direct solar irradiance. Some of the heat is reflected to the space immediately from the top of the clouds (17%) while adsorbing about 4% of it. The rest is absorbed by the atmosphere (23%) or the surface (46%). Respectively, the aerosols and terrestrial surface reflect about 8% and 6% of the incoming radiation in the form of infrared radiation but much of it is trapped in the atmosphere by the heat trapping gases (CO_2, CH_4, N_2O, and water vapour). This serves to worsen the warming effect.

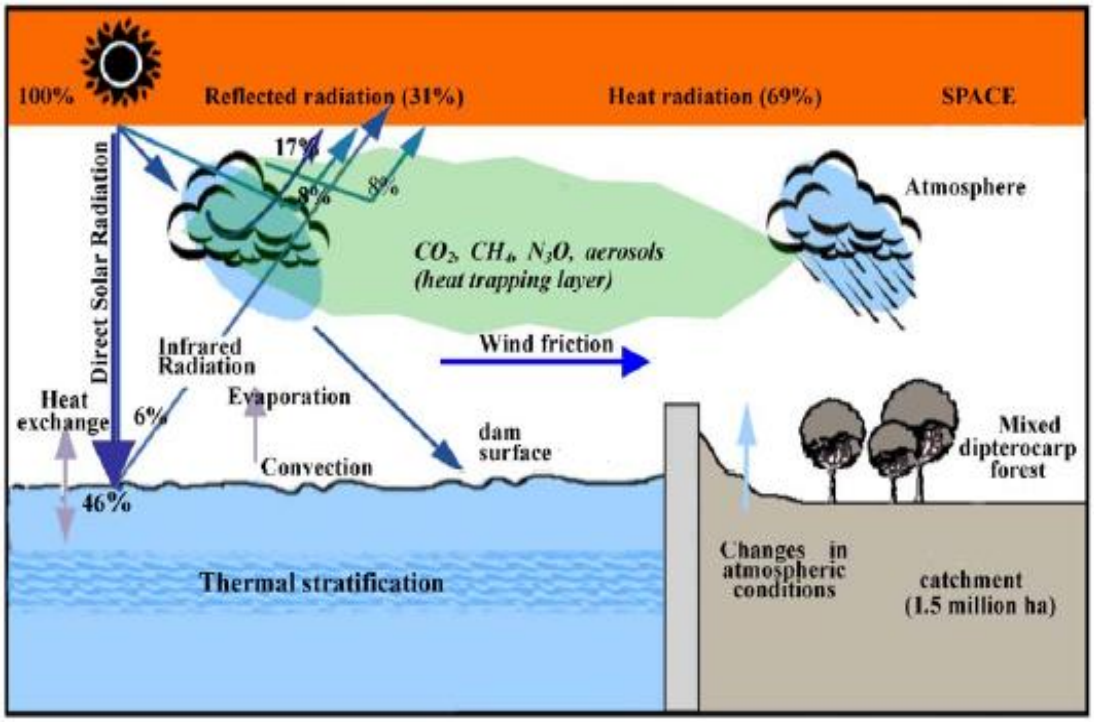


Figure 5. *Warming effect and seasonal microclimate change at the surrounding area of dam (Choy, 2005)*

Changes to water quality and river flow

Another environmental challenge is noted by Sovacool and Bulan (2011) where the construction, impoundment and operation of dam will also affect the water quality, hydrology as well as river flow. The development project will negatively influence the water quality of the catchment area through changes in the concentration of dissolved oxygen, nutrient loads, suspended sediments, and that tidal encroachment could aggravate bank erosion. During the land clearing and construction stage, due to the extensive logging and construction activities, the water body of catchment is affected by

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the increased turbidity, loss of pool nursery habitat, reduced organic input and impaired the migration of fish. The environment would be further impacted during impoundment where river flow and organic input will be reduced and resulting in loss of habitat due to reduced water depth. The impoundment also results in increased phytoplankton production. Fish migration will be impaired due to the replacement of natural riverine habitat with artificial deep lake habitat. During the operation of the dam, the rapid water fluctuations resulting from the operation of the spillway would also increase downstream riverbank erosion, wash away trees, and prevent the spawning of fish. It would negatively influence the population of freshwater fish which become easily disoriented in slow moving waters, and fish living near the dam would be susceptible to pulverization from passing through the turbines or super-saturation and excess nitrogen from the water around the concrete face. It would also alter upstream species composition and replace native riverine species with lake-adapted species in reservoir.

Concluding remarks

Water security has become a serious issue due to rapid population growth, unsustainable use, water degradation and climate change. Sustainable water resources management and development is needed to overcome the water security issue. Therefore, multi-purpose dam has been proposed to provide affordable water and energy for agriculture, industrial and domestic sectors and ensure essential irrigation water supplies for food security. No doubt that water resources are necessary for sustainable economic development of a country. However, building a dam should not primarily be considered as an economic decision, which ascertains that the total benefit obtained from the dam operation exceeds the total cost incurred by building and maintaining the dam. Dam has to be understood as an anthropogenic interference at a central position of a complex natural system, having multiple impacts, mainly on the downstream drainage basin. Having only proper development of multi-purpose dam for management of river waters can improve economic viability, preserve ecosystem and enhance social justice of the country in the coming years.

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REQUIREMENTS FOR ESTABLISHING INNOVATIVE RURAL INSTITUTIONS TO PROMOTE THE FOOD SECURITY OF RICE GROWERS IN MAZANDARAN, IRAN

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Abstract. The issue of food security is considered an inseparable part of international development policies, so creating a nationally effective system to ensure food security, in addition to supporting the nutrition and health of the community, improves food industry status and increases production and income levels. To this end, the present study was carried out to identify the essential requirements for establishing innovative rural institutions to improve the food security of rice growers in Mazandaran, Iran. Economic, socio-cultural, infrastructure, policy-making, educational, and technical requirements were investigated, with each considered an observed variable with some items for each and to survey the impact of them on dimensions of food security and finally food security itself. The dependent variable was food security, measured by the five dimensions of availability, access, use, sustainability and safety. From a total of 113,141 rice growers in Mazandaran Province, Iran 216 were selected as the sample according to the Cochran method. Structural equation modelling and Amos 22 software were used in this study. The results showed that all six requirements mentioned above have an effect on food security, with technical requirements exhibiting the greatest effect, with a coefficient of 0.914. Suggestions are proposed for implementing these requirements.
Keywords: *innovative organizations, rural institutions, food security, rice growers, small-scale farmers, requirements*

Introduction

Food security is a great challenge, and due to food prices and global economic crises, the number of malnourished people has meteorically increased (Herbel et al., 2012). The Food and Agriculture Organization (FAO, 2010) has expressed that "Food security exists when all people at all times have physical and economic access to healthy, adequate and nutritious food to meet dietary requirements and food preferences for an active and healthy life." Food security is important because the goal of ensuring food security addresses all aspects of development. Food security is a complex concept with three basic pillars: 1) the availability of food, which means a sufficient amount of food based on a stable diet; 2) access to food, which means the ability to obtain adequate and nutritious food; and 3) the use of food, which means meeting food needs and cultural preferences (Asian Development Bank, 2013).

Finally, according to various researchers, food security can be divided into five elements: sustainability, availability, accessibility, utility, and safety. To conduct a comprehensive analysis and discussion, identifying the relationship among all five elements is necessary (Pereira et al., 2014).

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To reduce future food insecurity, the International Food Policy Research Institute (IFPRI) recommends a revision of the global food system focusing more on quality (nutrition) and safety and diversity than on expertise; a reduction in food and waste losses; and gender issues. Increasing sustainable products should focus on nutrition. To achieve this goal, approaches have been suggested that include investing in agricultural research and development to produce more nutritious food, leveraging more small-scale agricultural production for the value chain with a greater sensitivity to food quality (being nutritious), empowering women to link farming with nutrition, supporting policies and institutions for better nutrition and ensuring security in food systems (European Commission, 2015).

The latest estimates of the FAO show that a reduction in global hunger is continuing; from 2012 to 2014, approximately 805 million people in the world suffered from chronic malnutrition, a decrease of 100 million over the last decade of approximately 209 million people compared to 1990–1992. Since 1990, the prevalence of malnutrition in the world has fallen from 18.7% to 11.3%, and in the developing world, it has dropped from 23.4% to 13.5%. The FAO developed the Food Insecurity Experience Scale (FIES) to fill the global gap of food security monitoring, especially for assessing access levels at the individual and household levels. The FIES measures the severity of food insecurity, which is described as the number of people experiencing problems in obtaining food. The measurement of food insecurity through experience-centric scales is not an entirely new approach. These types of scales have been widely used to assess food insecurity in the United States since 1995. Similar tools such as the Household Food Insecurity Access Scale, Food and Nutrition Technical Assistance (FANTA) and the FAO Food Security Scale in the Caribbean and Latin America are currently being accepted at national, regional and project levels (FAO, IFAD and WFP, 2014).

In Iran, as in many countries, food security has been addressed through agricultural and rural development approaches, and there have been ongoing efforts to increase food availability in rural and urban communities (Hamidifar, 2012). According to Bakhshi et al. (2008), Iran's ranking in Southwest Asia based on indices of vulnerability and food security showed that Iran has high nutritional energy consumption, ranked sixth. In terms of the combined index of nutrition and food security, Iran is the twelfth-ranked country in Southwest Asia. In the economic access index, Iran is ranked fourteenth; in the agricultural macroeconomic index, fifth; in food availability index, fifteenth; in the cultural-health index, tenth; and in the total index of vulnerability, ninth. Moreover, Iran ranked first among the least vulnerable countries among the twenty countries in the region. Food security studies have identified food insecurity as a major problem in most societies with respect to the amount of food intake and nutritional, economic, social and cultural status (Rostami et al., 2014).

Rice is one of the cheapest and most effective foods available to eliminate acute malnutrition. Many studies show great potential for increasing rice production, especially in Asia. Food security in Asia has traditionally been defined as maintaining basic prices for rice in the main rural markets in the country, which provide the main food supply to more than 50% of the population. Thus, reaching self-sufficiency in terms of rice production is an effective method to promote food security at the national level. Sustainable production of rice in South Asia is the main source of food security in this region. Rice paddies have full potential for self-sufficiency in rice production, as is currently occurring in India and Pakistan. Since poverty is one of the factors directly affecting food insecurity, through employment in agricultural activities and projects

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currently underway in many countries in this area, people can escape extreme poverty. A survey conducted in South Asia shows that the easiest way to promote national food security is obtaining self-sufficiency in rice production (Bishwajit et al., 2013).

A study by John and Fielding (2014) in South Asia indicates that the most important limitation in rice production in this region is the socio-economic constraint of production, which was examined according to seven factors:

1. Difficult access to water for irrigation
2. Lack of quality grain
3. High cost of irrigation
4. Expensive nitrogen fertilizer of limited supply
5. Insufficient farmer knowledge
6. Lack of access to agricultural information
7. High prices of input

Given the above-mentioned issues, one can categorize the major issues and problems that have arisen due to the lack of attention to rural innovative institutions in achieving food security as follows:

1. Failure to achieve food security due to problems in the five aspects of availability, accessibility, utility, sustainability and safety (Herbel et al., 2012)
2. Failure to achieve food security due to the lack of access of small farmers to natural resources, productive assets, markets, and knowledge and information (Herbel et al., 2012)
3. Failure to achieve food security due to inappropriate management models in rural areas (Moradi and Popzan, 2014)

Among the most important benefits and achievements of this research is help in creating effective production organizations to improve food security. Overall, productive and sustainable production organizations and institutional arrangements with market agents and policymakers are also dependent on the three types of relationships developed by small-scale producers (Herbel et al., 2012):

1. Connection or linkage or intergroup relationships among small-scale producers within organizations
2. Establishing communication or inter-group relationships between small producer organizations to create reference organizations
3. Establishing communication or inter-group relationships between small producer organizations and market agents and policymakers

The main goal of this study is to finding the set of requirements that important for establishing rural innovative institutions to improve food security from rice grower's perspective in Mazandaran Province, Iran. In this regard, the following objectives were extracted:

- Recognizing the economic requirements to establishing rural innovative institutions for improving food security of rice growers in Mazandaran Province
- Recognizing the socio-cultural requirements to establishing rural innovative institutions for improving food security of rice growers in Mazandaran Province

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- Recognizing the infrastructural requirements to establishing rural innovative institutions for improving food security of rice growers in Mazandaran Province
- Recognizing the policy-making requirements to establishing rural innovative institutions for improving food security of rice growers in Mazandaran Province
- Recognizing the educational requirements to establishing rural innovative institutions for improving food security of rice growers in Mazandaran Province
- Recognizing the technical requirements to establishing rural innovative institutions for improving food security of rice growers in Mazandaran Province

Theoretical framework

Governments and international organizations are seeking effective institutional frameworks for managing and helping rural and agricultural development to address the challenges of increasing farmers' integration in local and regional markets and value chains, increasing public-private investment in agriculture, increasing concern regarding food security in the world, rural poverty, regional disparities, flexibility, and climate adaptability in land use. The overall challenge is to strengthen rural development processes in government to ensure positive effects and new opportunities for micro-farmers, rural communities, local economies and communities and nations as a whole. The expected outcomes are to reduce vulnerability and increase social capacity, flexibility and sustainability in agricultural systems on a larger scale than exists today. New institutional arrangements and partnerships create a link between the private and public sectors needed to strengthen the governance of natural resources and land; improve information, consultation, and technical development; and support the families of farmers, communities, and rural areas to achieve sustainability (Natural Resources Institute, 2016).

Allameh (2012) described the following factors affecting food security:

- Political, social and economic stability
- Type of resources available to community producers
- Conservation and sustainable use of sustainable resources
- Different consumption cultures
- Level of income and financial ability of households
- Demographic indicators
- Price policies
- Rural and agricultural development

Allameh showed the aspects and components of food and nutrition security as presented in *Figure 1*.

Keshavarz and Karami (2014) investigated the causes and factors affecting food insecurity and provided recommendations for policymaking in the field of extension to improve the food security of rural households. The authors used the Sen (1981) perspective and the study by him as shown in *Figure 2* to explain the framework for their research.

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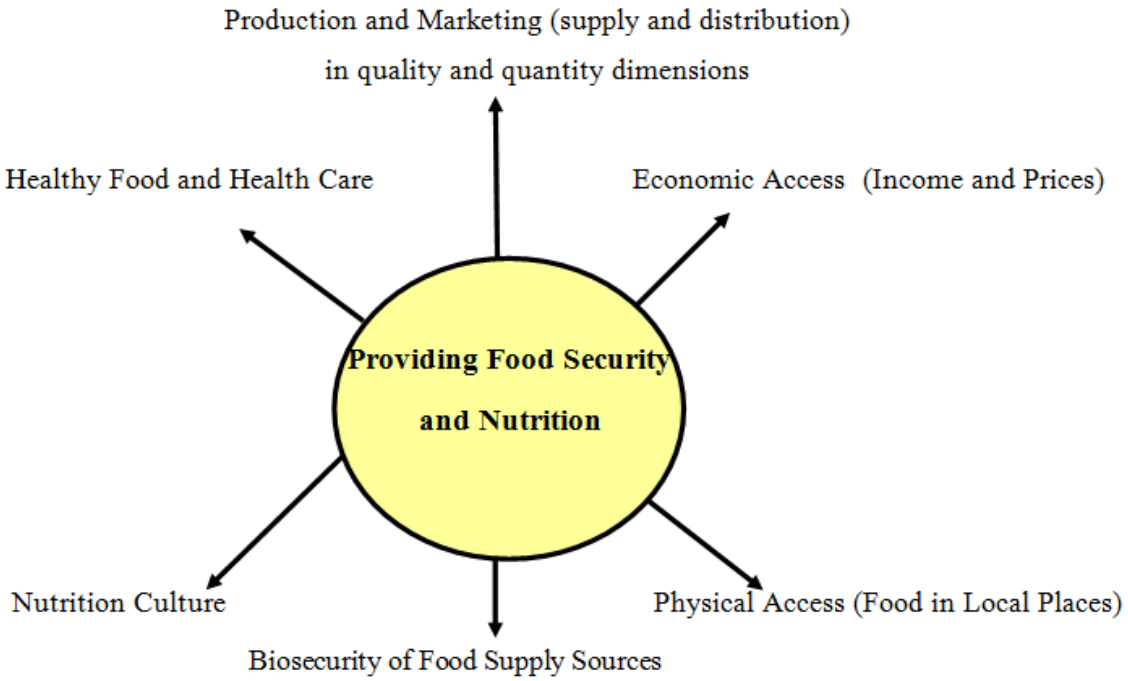


Figure 1. *Main components of food and nutrition security. (Source: Allameh, 2012)*

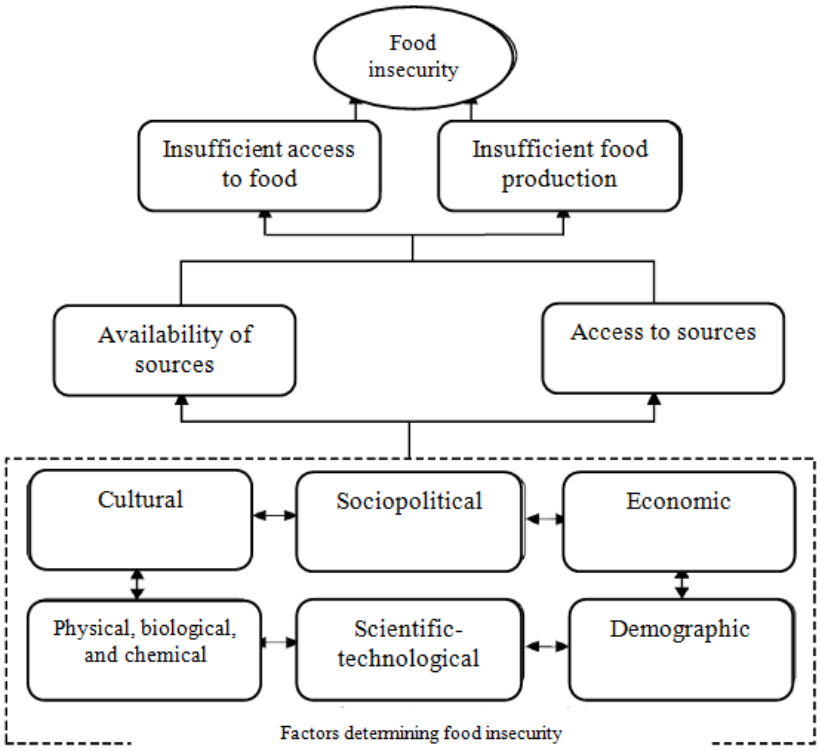


Figure 2. *The conceptual framework of the food insecurity process of rural households*

Based on the local of our research, *Table 1* shows the GPS coordinates for Mazandaran Province (Survey Locality).

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Table 1. *The GPS coordinates for Mazandaran Province, Iran*

Specifications	Coordinates
Latitude	37°7'18.04"N
Longitude	49°56'30.20"E

Figure 3 shows the map of Mazandaran Province's cities and roads include 22 cities and suburban that represent the selected statistical population in this research.

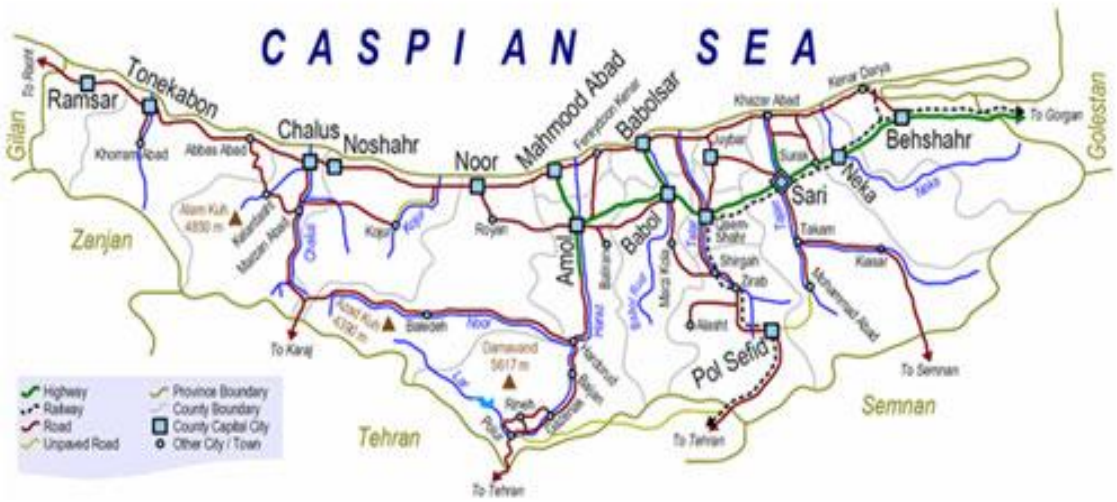


Figure 3. *The map of Mazandaran Province and the cities*

Materials and methods

The above-described principles and techniques were used in this study with the aim of solving executive problems and applying practical knowledge in Mazandaran province of Iran. The research process was both qualitative and quantitative and cross-sectional in terms of the timeline. The research approach was quasi-experimental, and the researcher had some control over the variables. The study involved a survey considering data collection. The population consisted of 113,141 rice growers in Mazandaran and used a stratified random sampling method with proportional allocation. The original sample volume was determined using the Cochran formula (*Eq. 1*) for 216 people (Cronbach, 1951).

$$n = \frac{Nt^2 s^2}{Nd^2 + t^2 s^2} \qquad \text{(Eq.1)}$$

The tool used in this study for data collection was a questionnaire. The questionnaire consisted of eight sections:

- Section 1: Economic requirements (11 items)
- Section 2: Social-cultural requirements (10 items)
- Section 3: Infrastructure requirements (6 items)
- Section 4: Policymaking requirements (9 items)

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- Section 5: Educational requirements (7 items)
- Section 6: Technical requirements (6 items)
- Section 7: Personal treatments (15 items)
- Section 8: Food security dimensions (Availability (7 items), Accessibility (7 items), Utility (5 items), Sustainability (5 items), Safety (4 items))

To evaluate the content validity of the questionnaire, portions were provided to professors of agricultural promotion and development as well as to experts and specialists in the field of rice and members of the rice community. Cronbach's alpha method was used to calculate reliability. A pre-test was used, and 30 questionnaires were distributed among the rice growers to calculate Cronbach's alpha. Structural equation modelling using a classical approach was used, and SPSS 16 and Amos 22 software were used for analysis.

Results

Describing the data

For a better understanding of the nature of the population studied in the research and to become more familiar with the research variables, it is necessary to describe these data before the analysis of statistical data (*Table 2*).

Table 2. *Demographic characteristics of the subjects (n = 216)*

Items	Mean	Median	Mode	Min	Max	SE
Age (year)	51.68	50	40–50	25	90	0.85
Gender	-	-	Male			
Level of education	-	-	Diploma			
Family members	4 persons		-			
Land size (m^2)	7752	7000	8000	2000	50000	4.72
Amount of yearly rice production (ton/year)	3.35	3	2.4	0.50	25	0.23
Experience in agriculture	27.27 years	25 years	30 years	2	70	1.19
Ownership	-	-	Personal			
Familiar with Rice Institution	-	-	No			
Membership in Mazandaran's Rice Institution	-	-	No			

Table 2 shows that the average age of respondents was over 51 and that most of them were males with a high school diploma. Moreover, the average number of people in households was 4, their average agricultural land area was less than 1 hectare, and they produced more than 3 tons of rice per year. A significant percentage of respondents were not only not members of their province's rice association but were not familiar with the association.

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Inferential statistics

To determine the estimation method for unknowns in the equation of structural equations, the contribution of each unknown to the parameter volume is first calculated. Given the sample size (n = 216) and the number of unknown parameters of the model (NPar = 119), the contribution of each unknown parameter was calculated to be 1.81 (*Eq. 2*).

$$\frac{n}{NPar} = 1.81 \qquad \text{(Eq.2)}$$

However, given that all research questions had a 5-point Likert scale, the normal assumption of multiple-variable observations was not confirmed. There are two approaches for solving this problem: 1) using the Bayesian approach or 2) using the latent variable assignment approach to each question that has a Likert scale. In this research, we chose the second approach for the following reasons.

The measures to check goodness of fit using the Bayesian approach are mostly graphical, while in the classical approach, all criteria are quantitative and easy to compute and compare.

To use the Bayesian approach, it is better to have the statistical information of an expert population available to provide a useful analysis. If this is not the case, there are no significant differences between the Bayesian and classical approaches (Lee, 2007).

After assigning a latent variable with a normal distribution to items with Likert scales, using the AMOS22-embedded equation approach with ten times the original sample size, we generated 2160 random numbers from the normal multivariate distribution. The generated data with multivariate normal distribution were the basis of our calculation. As the share of each unknown parameter of 2160 generated randomized samples was 10.8 and there was a multivariate normal assumption, using the maximum likelihood method in the classical approach, the unknown parameters were estimated.

After charting using AMOS22 software, the software outputs are presented in tables as well as a graph of the structural equation model as shown in *Figure 4*.

We present some of the goodness-of-fit criteria of the model in *Table 3*.

Table 3. *Some of the goodness-of-fit criteria of the model*

Model	X^2 Value	df	p-value	X^2/df	RMSEA	Pr (RMSEA≤0.05)
Fitted model	2974.055	2914	0.214	1.020	0/019	0.999
Saturated model	0.000	0			Uncountable	Uncountable
Dependence model	4592.630	2926	0.000	1.569	0.103	0.000

Given the goodness-of-fit criteria presented in *Table 3,* the p-value in the fitted model is 0.214, which is greater than 0.05. Thus, it is at an acceptable level. The RMSEA value is 0.019, which is less than 0.05, and the chi square over the degree of freedom ($1 < x^2 / df < 3$) is calculated as 1.020, both of which are acceptable. Therefore, the fitted model is a suitable and dependable model in terms of all criteria for goodness of fit.

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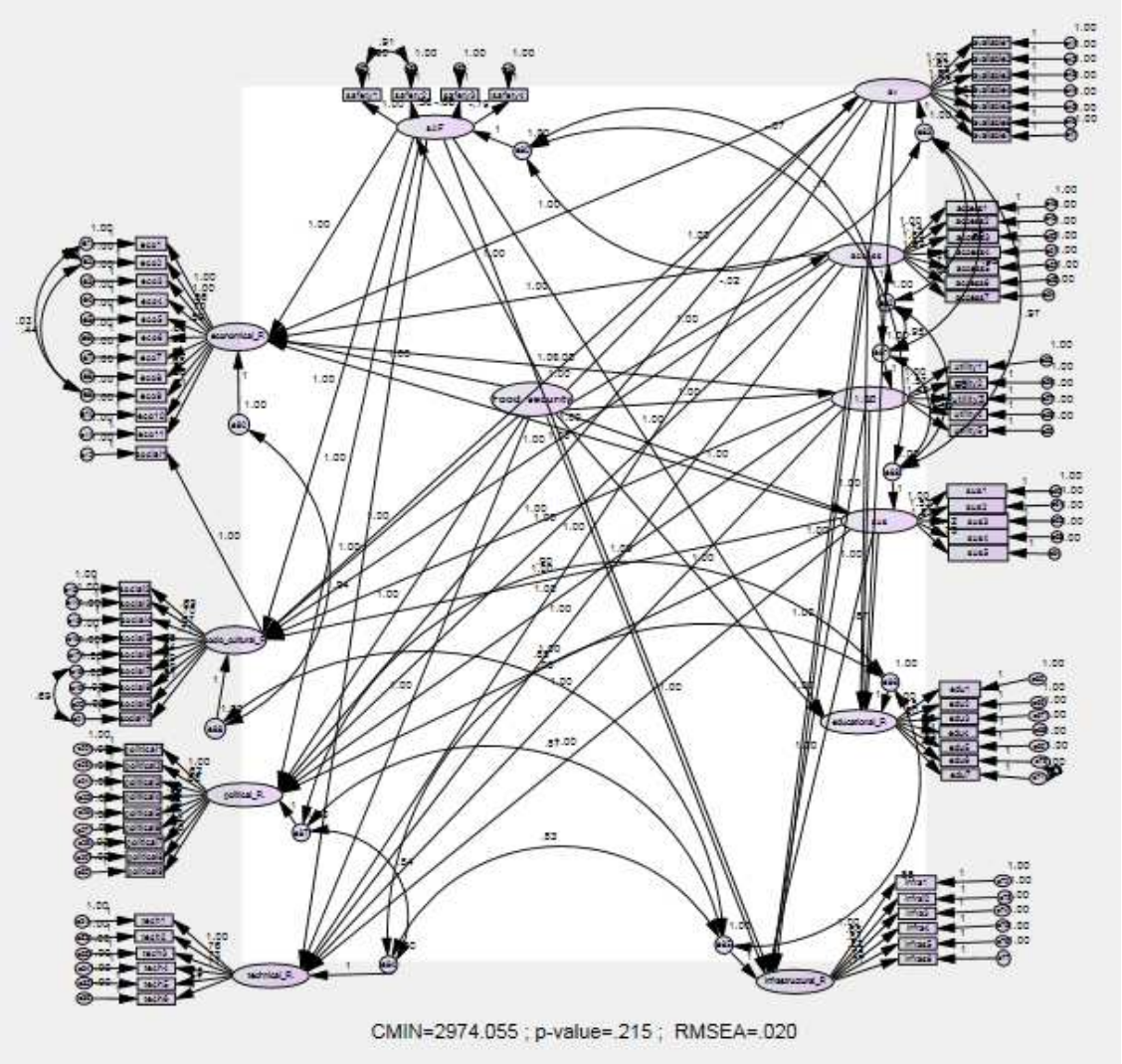


Figure 4. *Model-fitted data model diagram*

To determine the effect of each independent variable on the other dependent variables, we calculated the total effect (standardized) of all the paths (*Table 4*).

Table 4. *Total effect level (standardized) of the research requirements on food security dimensions*

Independent variables	Dependent variable (dimensions)					
	Sustainability	Utility	Safety	Accessibility	Availability	Food security
Technical requirements	0.416	1.115	1.560	2.344	0.242	0.919
Educational requirements	0.080	0.137	0.699	1.196	0.312	0.914
Social-cultural requirements	0.165	0.376	1.068	1.456	0.630	0.862
Infrastructure requirements	0.292	0.630	1.035	1.789	0.701	0.844
Policymaking requirements	0.025	0.892	1.013	1.999	0.304	0.771
Economic requirements	0.181	0.765	1.339	2.113	0.923	0.757

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According to *Table 4,* the technical requirements had the greatest impact on food security, with a coefficient of 0.919, followed by educational requirements with a path coefficient of 0.914, socio-cultural requirements with a path coefficient 0.862, infrastructure requirements with a coefficient of 0.844, policymaking requirements with a path coefficient of 0.771 and, finally, economic requirements with a path coefficient of 0.775. Moreover, by examining the effect of each of the requirements on the five aspects of food security, one can see that the technical, educational, socio-cultural, infrastructure, policymaking and economic requirements with path coefficients of 2.344, 1.196, 1.465, 1.789, 1.999 and 2.113, respectively, had the greatest impact on access to food security.

Table 5 summarizes the impact of each of the five dimensions of availability, access, use, sustainability and safety on food security.

Table 5. *Total effect (standardized) of each dimension of food security on the dependent variable (food security)*

Independent variables (observed variables)	Dependent variable (food security)
Availability	0.672
Accessibility	0.642
Sustainability	0.632
Utility	0.629
Safety	0.521

Table 5 shows the impact of each of the five aspects of food security on the dependent variable (food security), which, according to the results presented, shows that the dimension of availability is the most effective, with a coefficient of 0.672, followed by access, sustainability, use and safety. The effect of each of the observed (independent) variables on food security (dependent variable) is presented in *Table 6*.

Table 6. *The total effect of standardized variables on the dependent variable (food security)*

Independent variables (observed variables)	Dependent variable (food security)
Educational Requirements	
• Launching publications and journals on rice crop operations	0.808
• Conducting workshops on rice crop operations	0.811
• Increasing human capital through training an expert force	0.810
• Using the FFS method and holding a rice farm day	0.809
• Organizing various local classes, such as development classes, etc.	0.810
• Strengthening the proper use of indigenous and local knowledge	0.808
• Strengthening access to knowledge and information	0.814
Technical Requirements	
• Using new technologies in agriculture, such as nanotechnology, nuclear technology, etc.	0.812
• Using proper technology in planting, preservation and harvesting	0.807
• Using new methods in irrigation	0.806

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• Making multifunctional use of rice cultivation such as rice cultivation with breeding of ducks, fish, etc.	0.805
• Using hybrid seeds and modified varieties	0.807
• Using machines in transplantation	0.804
Infrastructure Requirements	
• Creating industries and conversion and complementary enterprises in villages	0.807
• Improving access to information and communication technology (ICT)	0.807
• Improving the access of farmers to the proper equipment for the preparation and renovation of arable land	0.809
• Improving access of skilled rice growers to a proper transportation system	0.809
• Improving access of farmers to suitable arable lands for cultivation	0.804
• Strengthening the access of rice growers to water resources and optimal management	0.812
Policy-making Requirements	
• Ensuring that the voices of small-scale producers are heard by the authorities to participate in the policymaking and decision-making process	812/0
• Promoting local governance and decentralization in rural management programs	0.801
• Integrating land under rice growth	0.804
• Adopting appropriate rules to reduce conflicts on land	0.801
• Creating a targeted, coordinated and coherent planning system in villages	0.808
• Improving product pricing policies	0.808
• Improving lending policies for subsidizing loans to rice growers	0.808
• Improving supportive policies for saving and securing energy	0.806
• Improving the import and export policies of rice crops	0.807
Sustainability (Food security)	
• Managing and conserving resources, especially water resources, for sustainable livelihoods	0.654
• Managing risk in rice production (use of inputs, weather conditions, etc.)	0.633
• Confronting the structural causes of food insecurity	0.658
• Monitoring food security and finding effective factors in the vulnerability of rice growers	0.641
• Creating diversity in agriculture and employment	0.577
Utility (Food Security)	
• Improving food security policies to improve food patterns and cultural patterns	0.646
• Teaching the correct use of natural resources and adapting to climate change	0.643
• Teaching the proper use of water resources and new methods of irrigation	0.638
• Improving the diagnosis of quality product	0.644
• Increasing the knowledge and information of skilled workers in using the product	0.577
Safety (Food Security)	
• Improving the quality of rice grains through training new cultivation practices	0.526
• Using mechanisms to ensure the health of the food	0.546
• Improving the quality of food	0.438

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• Accessing healthy and nutritious food	0.577
Accessibility (Food Security)	
• Improving access to credit and savings systems	0.651
• Improving access to input and outlet markets	0.653
• Strengthening access to knowledge and information	0.656
• Empowering women and improving their access to resources	0.652
• Improving access to natural resources (land, water)	0.651
• Increasing revenue for microprocessors	0.655
• Improving the supply of food for the vulnerable majority	0.577
Availability (Food Security)	
• Improving and restoring resources, especially land	0.655
• Investing in new and consistent technologies	0.656
• Investing in rural markets	0.654
• Investing in infrastructure in villages	0.657
• Improving consumption patterns through a variety of training programs	0.651
• Improving research and innovation in agriculture	0.648
• Possibly improving food production in the countryside, especially through small-scale farmers	0.577
Social-Cultural Requirements	
• Ensuring the effectiveness of institutions in improving local innovation capacity through the creation of craft exhibitions, food industry, holding rice festivals, etc.	0.812
• Empowering small-scale producers to participate in the policymaking and decision-making process	0.804
• Reducing poverty reduction through self-sufficiency in rice production	0.805
• Improving gender equality through empowerment of rural women	0.803
• Promoting institutional networking and interaction between institutions (collaboration among cooperatives, etc.) in sharing information	0.805
• Strengthening social capital (trust, honesty, goodwill, self-healing, hedonism, friendship, solidarity, sacrifice, etc.)	0.803
• Building capacity and empowerment of local people	0.795
• Improving nutritional patterns and food preferences through culture	0.799
• Increasing flexibility in vulnerable populations through increased awareness	0.804
• Improving nutritional quality and food security	0.800
Economic Requirements	
• Improving access (physical and financial) to imported markets (seeds, fertilizers, poisons, etc.)	0.812
• Improving access (physical and financial) to outbound markets (product)	0.812
• Determining guaranteed purchase price	0.797
• Strengthening access for financial assistance to financial services (insurance, etc.)	0.805
• Improving collective investment by fundraisers and community members	0.799
• Creating sustainable livelihoods through the development of tourism and home care	0.804
• Reducing the cost of merchant transactions (such as shipping costs, marketing, etc.)	0.801

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• Increasing reliance on non-agricultural economies (agriculture)	0.800
• Ensuring the effectiveness of rural institutions in improving government finance and credit, such as loans, subsidies, etc.	0.804
• Creating job opportunities for farmers	0.803
• Increasing income of rice growers	0.800

According to the results presented in *Table 6,* we conclude the following:

Among the observed variables, the greatest impact is related to the following variables in order of importance:

- "Strengthening access to knowledge and information" is related to educational requirements with a path coefficient of 0.814.
- "Using new technologies in agriculture, such as nanotechnology, nuclear technology, etc." is related to technical requirements with a path coefficient of 0.812.
- "Strengthening the access of rice growers to water resources and optimizing their management" is related to infrastructure requirements with a coefficient of 0.812.
- "Ensuring that small farmers' voices are heard by the authorities to participate in the policy and decision-making process" is related to policy requirements with a path coefficient of 0.812.
- "Ensuring effectiveness of institutions in improving local innovation capacity through the creation of handicraft exhibitions, food industry, holding rice festivals, etc." is related to socio-cultural needs with a path coefficient of 0.812.
- Improving access (physical and financial) to imported markets (seeds, fertilizers, pesticides, etc.) and "improving access (physical and financial) to outsourced markets (product)" related to economic requirements with a path coefficient of 0.812.

Moreover, all of these variables were the most important variables according to the research background and experts' opinions.

Discussion

Ciheam (2013) asserted that by providing a consistent set of services, rural innovative manufacturing organizations can increase farmers' incomes and improve food security. These services include improving access to and management of natural resources, information and technology and facilitating participation in policymaking processes.

- In the present research, economic requirements have a positive impact on improving food security, which include the following:
  - Improving the financial and physical access of farmers to input markets (seed, fertilizer, poison, etc.) and improving the financial and physical access of outsiders to outsourced markets (the product), which are consistent with the findings of the studies by CIHEAM (2013), Mumuni and Oladele (2016),

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IFAD (2008), Silvestri et al. (2015), Olayemi and McIntyre (2014) and Herbel et al. (2012).
- Strengthening access of rice growers to financial services (insurance, etc.), which is in line with the findings of CIHEAM (2013), FAO (2016) and NRI (2016).
- Reducing transaction costs for traders (such as product cost, marketing, etc.), which is consistent with the findings of Biénabe and Sautier (2005).
- Improving collective investment of rice growers and members of the association and institution, which is in line with the findings of Mumuni and Oladele (2016) and Herbel et al. (2015).
- Increasing income and creating job opportunities for rice growers, which is in line with the findings of Keshavarz and Karami (2014), IFAD (2003) and Wabwoba and Wakhungu (2013).
- Improving government finance and credit, such as loans, input subsidies, etc., which is in line with the findings of CIHEAM (2013) and Keshavarz and Karami (2014).
- Creating sustainable livelihoods through the development of tourism and home care, which is consistent with the findings of Uphoff and Buck (2006).
- Determining the guaranteed purchase price, a variable that has been extracted from the results of interviews with experts in the Delphi qualitative section.

- The results showed that socio-cultural requirements have a positive effect on improving food security, which include the following:
  - Empowering small-scale producers to participate in policymaking and decision-making processes, which is in line with the findings of Herbel et al. (2012), FAO (2016) and IFAD (2003).
  - Reducing poverty through self-sufficiency in rice crops, which is consistent with the findings of Bishwajit et al. (2013).
  - Strengthening social capital (trust, honesty, good luck, self-esteem, hedonism, friendship, solidarity, sacrifice, etc.), which is consistent with the findings of Nejatian et al. (2014).
  - Building capacity and localization of locals, which is in line with the findings of Waters-bayer et al. (2015) and IFAD (2003).
  - Promoting institutional networking and inter-institutional interaction (collaboration between institutions) in sharing information, which is consistent with the findings of Agrawal and Perrin (2009) and NRI (2016).
  - Improving nutritional patterns and food preferences through culture, which is consistent with the findings of Saadi et al. (2014) and Guyomard et al. (2012).
  - Improving the quality of nutrition and food security, which is consistent with the findings of Mcinerney (2014), European Commission (2015), Waters-bayer et al. (2015) and Balie (2009).
  - Ensuring the effectiveness of institutions in enhancing local innovation capacity through the creation of handicraft exhibitions, food industry, rice festivals, etc., which is consistent with the findings of Waters-bayer et al. (2015).

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- Increasing flexibility in vulnerable populations by raising awareness, which is consistent with the findings of CIHEAM (2013), Mumuni and Olade (2016), NRI (2016), European Commission (2015) and Waters-bayer et al. (2015).
- Improving gender equality through empowerment of rural women, which is consistent with the findings of Movahedi and Samian (2014) and Mcinerney (2014).

- In this study, infrastructure requirements had a positive effect on improving food security, which include the following:
  - Strengthening access to water resources and optimal management, which is consistent with the findings of John and Fielding (2014), Mcinerney (2014), CIHEAM (2013), Herbel et al. (2012) and FAO (2016).
  - Improving the access of rice growers to cultivated land suitable for cultivation, which is consistent with the findings of Keshavarz and Karami (2014) and Wabwoba and Wakhungu (2013).
  - Improving the access of rice growers to appropriate equipment for the preparation and modernization of agricultural land, improving the access of rice growers to the appropriate transportation system, improving access of rice growers to ICT, and creating conversion and supplementary factories in villages, all of which were extracted through interviews with experts and using the Delphi qualitative method.

- Moreover, according to the results, policy requirements for improving food security have a positive effect on food security. These requirements are as follows:
  - Ensuring that the voice of small-scale producers heard by the authorities to participate in the policymaking and decision-making processes, which is consistent with the findings of Herbel et al. (2012), FAO (2016) and IFAD (2003).
  - Promoting local governance and decentralization in rural management programs, which is consistent with the findings of Moradi and Papzan (2014), NRI (2016), Agrawal and Perrin (2009) and Briner and Wittmer (2000).
  - Establishing appropriate rules to reduce conflicts over land, which is consistent with the results of NRI (2016).
  - Establishing a targeted, coordinated and coherent planning system in villages, which is in line with the findings of Wabwoba and Wakhungu (2013) and the European Commission (2015).
  - Improving product pricing policies, which is consistent with the results of Keshavarz and Karami (2014) and Balié (2009).
  - Improving the policy of subsidizing loans to farmers, improving support policies for energy saving and energy, improving the policies for the import and export of rice products, and integrating land slabs, all of which are extracted from the results of interviews with experts using the qualitative Delphi method.

- The results of the research show that educational requirements have a positive effect on the improvement of food security, which include the following:

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- Strengthening access to knowledge and information, which is in line with the findings of Mumuni and Oladele (2016), John and Fielding (2014), Silvestri et al. (2015) and Herbel et al. (2012).
- Holding a variety of local classes, such as developmental classes, etc., which is in line with the findings of McInerney (2014), CIHEAM (2013), Herbel et al. (2012) and Saadi et al. (2014).
- Increasing human capital through specialist training, which is in line with the findings of Keshavarz and Karami (2014).
- Using the FFS method and organizing a rice farm day, holding workshops on rice crop operations, organizing publications and journals on rice crops, and enhancing the proper use of indigenous and local knowledge, which are derived from expert interviews using the Delphi qualitative approach.
- Finally, based on the results of the research, technical requirements affect the improvement of food security, including the following:
- Using modern technologies in agriculture such as nanotechnology; using appropriate technology in planting, preserving and harvesting; using modern methods in irrigation; using hybrid seeds and modified varieties; using machines for transplanting; and multipurpose use of rice cultivation such as rice cultivation with duck breeding, fish, etc., all of which are in line with the findings of Herbel et al. (2015), John and Fielding (2014), Mbaga (2013), Keshavarz and Karami (2014) and Uphoff and Buck (2006).

Conclusion

Poverty and food insecurity are closely interrelated; poor food and nutrition have a major effect on human health, reduce human development and, in the long-run, reduce labor productivity. Food security is an integral part of international development policies. Creating a nationally effective system for ensuring food security, in addition to helping to improve nutrition and community health, will improve food industry and increase production and income levels. Thus, improving the infrastructure and existing policies is important in creating a nationally effective food control system (Pereira et al., 2014).

Innovative institutions, as living structures and organizational dynamics, have a positive effect on creativity and innovation as these structures are less formalized and focused. With their greater flexibility, these institutions can adapt to the needs of the environment to facilitate creativity and innovation. The environment is constantly evolving; therefore, organizations, including cooperatives, must adapt to new conditions to survive and even to maintain the status quo. Such adaptations require factors such as innovation and creativity in the organization. In fact, innovation is a response by organizations to the transformation of the market to compete with other companies to ensure their survival and growth and to improve management considering the innovations of the day. Certain principles and frameworks applied by companies in a competitive environment are applicable for transforming organizations in terms of structure, workflow, the identification of the main and secondary processes, organizational culture, etc. Improving the adaptability of organizations has attracted the attention of many experts, and adaptability can make organizations more effective and efficient in the turbulent and uncertain world of today (Moosavi et al., 2014). One of the main functions of innovative rural organizations is to improve food security. The

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definition of household food security is the physical, social and economic access of members of the family to healthy, complete and sufficient diets all the time that satisfy dietary requirements and their willingness to eat to have a healthy life. Access to healthy and nutritious foods, receiving adequate food and being free from hunger are essential. In addition to having food available to the household, there must be the financial ability to buy food to have "food security."

Findings of this research show that the Technical Requirements have the most impact on Food Security so the rural innovative institutions can train and educate the new technologies to the small- scale farmers such as Nano technologies and Nuclear technologies. For example, Silver Nano particles that could raise the efficiency of rice fields and production of rice varieties that resistant to the drought and salinity through nuclear technology.

Also, one of the important and innovative matters in these results that should considered is the impact of all the requirements on accessibility in food security, because of geographic condition and lack of water resources the rural innovative institutions could work on some practical ways for these problems, include the improving access to the small dams and reduce the water consumption with the new methods such as System of Rice Intensification (SRI) and Rice Dry-Soil.

At the end, the following suggestions are made to improve food security through the creation of rural innovative institutions:

- Acquiring indispensable materials at the local level: For the development of agriculture in developing countries, access to five essential resources is essential: capital (land, machinery, and renewable resources such as water), markets (for example, infrastructure adequacy, communication networks that provide farmers with access to the latest prices and the ability to meet the supply standards in supermarkets), credit (preventing early product sales by small farmers or improving access to basic facilities such as fertilizers), knowledge (in cases where there is an urgent need to invest in agricultural subsidiary services to help research be transformed to the work environment) and risk management tools.
- Improving access to social support: Support networks in the country can be created by rural cooperatives in different rural areas, making food products locally much cheaper to the people of the same area. Agricultural policies, which generally take into account macroeconomic interests, should pay attention to the food security of people in deprived areas at a micro level, as the development of these areas will play a major role in the development of the country in the future. The most important point is preventing the compound burden of malnutrition, hunger and poverty, and all the social disadvantages of poverty, which will extend into other areas of the country. Providing food security in remote rural areas is only possible through social and agricultural support.
- Improving access to land and other assets by implementing programs based on social risk management and the empowerment of poor rural households. The strengthening of social capital and the use of collective action and sharing scarce resources can help improve the livelihoods of small-scale farmers and poor households.
- Promoting the culture of nutrition in rural areas by conducting educational courses and developing radio and television programs.

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- Holding vocational relearning courses to increase the level of education of the head of household to increase human capital.
- Policy-makers and development practitioners need to improve their understanding of institutional change.
- Innovative partnerships between small producers, governments and the private sector must be considered and built.

Recommendation for future studies:

- In this research, innovative organization was studied for rice growers we recommend that other researcher study on wheat grower.
- The requirements of establishing rural innovative institution in this research were surveyed in six variables as independent (observe) variables and the dependent variable is food security. So, we recommend that other researchers work on other factors such as environmental requirements.

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ASSESSMENT OF THE EFFECTS OF LAND USE SCENARIOS ON WATERSHED SURFACE RUNOFF USING HYDROLOGICAL MODELLING

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Abstract. To manage watersheds, simulations of hydrological phenomena are very important. This paper investigates hydrological modelling of Taleqan basin in Iran, and studies the effects of land use scenarios on the model runoff using Soil and Water Assessment Tool (SWAT). For this purpose, hydro-meteorological data, soil map, land use maps, and digital elevation model of the area were prepared and used for model parameters' sensitivity analysis, model calibration, and validation. Sensitivity analysis was performed using SUFI-2 algorithm. Model performance was evaluated by using time series visual comparison plots and statistical indicators of Nash-Sutcliffe (NS) and the coefficient of determination (R^2). It was shown that the model has high potential to predict monthly runoff. Then, the effects of land use changes on surface runoff in the basin were studied. For this purpose, practicable land use options in the watershed such as agroforestry, afforestation, expansion of agricultural land, and agriculture on terrace were considered. Hence, various management scenarios resulted from combinations of land use activities. They were applied to the calibrated model, and their effects on surface runoff were studied. The results show that management scenarios may have significant effects on runoff control.
Keywords: *hydrological simulation, land use change, management scenario, model calibration, sensitivity analysis, SWAT*

Introduction

Surface runoff in a watershed is a major issue in hydrological watershed studies, because most hydrological processes are associated with it directly or indirectly and are somehow affected by it. Understanding hydrological processes and their simulations in a watershed scale and predicting changes of these processes in the future have been perpetual challenges for hydrologists.

Thus, hydrological models are widely used by managers and hydrologists as a tool for understanding natural and human activities affecting watershed hydrology and its planning and management in recent years (Ouyang et al., 2014). These models can provide a precise scientific framework for analysing the water movement in the watershed, and can create a reliable database on the system behaviour. Developments of hydrological models largely depend on geographic information system that made it possible to simulate large areas (Afzal et al., 2015; Jung and Kim, 2017).

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Soil and Water Assessment Tool (SWAT) is commonly used for watershed modelling. The good performance of this model to assess the effects of land management practices on water, sediment, and agricultural products in large and complex watersheds with varying soils, land use, various management conditions during different periods of time and topography has been proved (Kim, 2015).

However, along with successful applications of the mentioned models due to some shortcomings, some numerical processing such as parameters calibration and sensitivity analysis are also essential (Romagnoli et al., 2017). Through sensitivity analysis of model parameters, identifying sensitive parameters based on their effects on the output of the model is possible. Identifying sensitive parameters lead to uncertainty reduction and better simulation (Shahoei et al., 2017).

Ghoraba (2015) conducted a study with the aim of watershed modelling in Simly dam, Pakistan. He used SWAT to estimate monthly volume of water entering the reservoir and determined the water balance to help administrators to plan and manage these important water reservoirs.

Vilaysane et al. (2015) studied the feasibility and performance of the SWAT model to predict river flow in the Xedone river basin, in the southern part of Laos.

Gosain et al. (2006) successfully used the SWAT model to predict the impacts of climate changes on river basin hydrology in 12 areas in India.

Setegn et al. (2008) conducted hydrologic modelling of Lake Tana basin and Pereira et al. (2014) studied the effects of deforestation on water balance components in the watershed located in the eastern coast of Brazil using SWAT model. Li et al. (2014) introduced some approaches for evaluating the effects of land use changes and biophysical conditions on the discharge. Santos et al. (2014) conducted a study on the effects of climate change, human interventions, and modelling uncertainties on the estimation of river basin and its components' characteristics.

In this paper, SWAT was used for modelling the basin that is located in the Northwest of Karaj, Alborz Province, Iran. This watershed is one of the most important sources of water supply for the province of Tehran. The main objectives of this study are to simulate surface runoff and identify sensitive hydrological parameters associated with it, and study the effects of different management scenarios on watershed stream flow. Sensitive parameters were determined by applying sensitivity analysis tool using SWAT-CUP software. More sensitivity analysis of the three most sensitive parameters and their effects on the normalized surface runoff changes is performed. The SWAT model was calibrated according to the changes in the values of the sensitive parameters within authorized ranges. Model calibration and validation were done by using SWAT-CUP software and SUFI2 algorithm. Then, simulation results with measured values of runoff in watershed outlets were compared. Then, the effects of applying different management scenarios on surface runoff were investigated. For this purpose, possible land use options in the watershed such as agroforestry, afforestation, expansion of agricultural land, and agriculture on terrace and their combinations were considered. Therefore, sixteen management scenarios were created. They were applied to the calibrated model, and the results were studied. Considering all possible combinations of management options to obtain all practical solutions could be very interesting not only for experts and decision makers but also for the general public who are following these issues seriously. Modelling these scenarios helps local and national managers to make the best decisions considering efficiency, condition, time, and cost. This may prevent environmental and social impacts, and waste of time and money.

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Study area

The Taleqan watershed is located on the southern slopes of the Alborz Mountains, at 120 km northwest of Tehran, Iran. Most of the area is mountainous with steep slopes with annual average precipitation of 632 mm. Annual average temperature is 10.5 °C and due to Meteorological Organization reports the area prevailing climate type is cold semi-wet. The study area average elevation above sea level is 2735 m. The most precipitation in the region is in the form of snow. Prevailing watershed hydrology system is based on high volume of runoff in the spring due to melting snow and considerable spring rains. *Figure 1* shows the location of studied area located in the upstream of Taleqan dam basin, which is located at 36° 04' to 36° 21' of northern latitude and 50° 38' to 51° 12' eastern longitude. Most land use in this area is pasture. Part of the land is used by irrigated and rain-fed agriculture. Soil of the basin is mainly loamy and silt loamy.

The main gauging station is Gelinak Station. Two other gauging stations, Joestan (middle of the basin) and Gatehdeh (beginning of the basin) stations, were selected to compare the results with the results of the Gelinak findings.

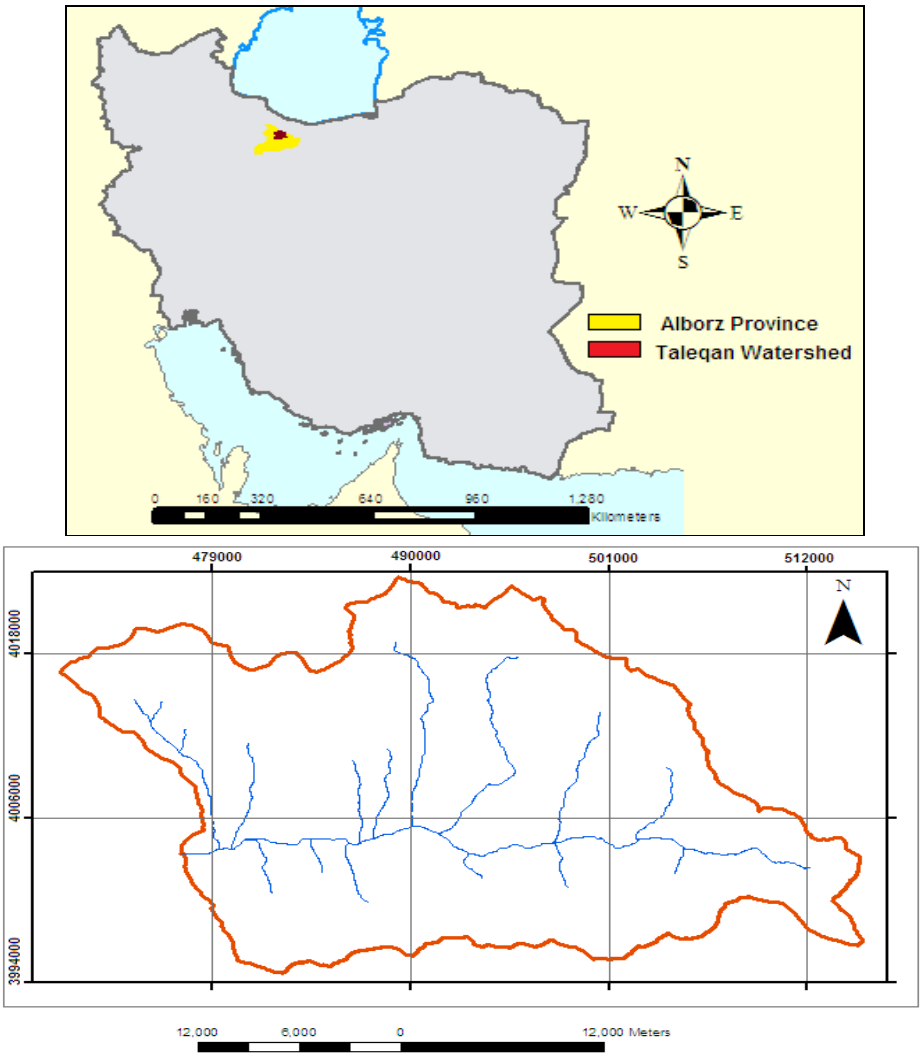


Figure 1. *The study area in Alborz Province in Northern Iran and the watershed geographical location*

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Materials and methods

SWAT model and performance evaluation

In this study, the SWAT model was used to simulate Taleqan watershed hydrological response. SWAT is a semi-distributed and continuous mathematical model that needs various information to run. This model first used in Agricultural Research Service of United States of America, in order to simulate the hydrological processes and predict the impact of management practices on water, sediment and agricultural chemical balance in the large and complex basins. (Azzellino et al., 2015; Bonansea et al., 2015).

In the SWAT model, watershed is divided into subbasins according to DEM input map, which are linked with each other through a network of streams. Then, on the basis of soil and land use in the subbasin, they are divided into smaller units known as HRUs. For each hydrological unit, water and sediment and nutrient and losses cycles are determined and weighted average is calculated for each subbasin. Then, after the accumulation of these quantities of the river network to the basin outlet, the final amount is determined for the basin (Neitsch et al., 2011).

This model allows the modelling of the basin without monitoring data, as well as providing predictive scenarios based on input changes such as land cover, land use operations, climate on nutrient cycling, water quality and quantity for users.

To assess the qualitative and quantitative performance of the model, visual comparison of the time series plots, and statistical indicators such as Nash–Sutcliffe index (NS) and coefficient of determination (R^2) are addressed (Rahman et al., 2013).

The coefficient of determination R^2 is defined as the squared value of the coefficient of correlation. It is calculated as (*Eq. 1*):

$$R^2 = \left(\frac{\sum_{i=1}^{n} (O_i - \overline{O})(P_i - \overline{P})}{\sqrt{\sum_{i=1}^{n} (O_i - \overline{O})^2} \sqrt{\sum_{i=1}^{n} (P_i - \overline{P})^2}} \right)^2 \quad \text{(Eq.1)}$$

with O and P indicate observed and predicted values, respectively. R^2 can also be expressed as the squared ratio between the covariance and the multiplied standard deviations of the observed and predicted values. (Krause et al., 2005)

The efficiency index NS proposed by Nash and Sutcliffe (1970) is defined as one minus the sum of the absolute squared differences between the predicted and observed values normalized by the variance of the observed values during the period under investigation. It is calculated as (*Eq. 2*):

$$NS = 1 - \frac{\sum_{i=1}^{n} (O_i - P_i)^2}{\sum_{i=1}^{n} (O_i - \overline{O})^2} \quad \text{(Eq.2)}$$

According to performance rating (Cho and Olivera, 2009), Nash–Sutcliffe Efficiency values greater than 0.75 are very good and greater than 0.65 are good. Moreover, the coefficient of determination (R^2) should be greater than 0.60 (Santhi et al., 2001).

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Model input data

The input required for the SWAT model includes digital elevation maps, soil data, precipitation data, temperature, meteorology, soil map, and land use map (Arnold et al., 1998; Neitsch et al., 2011).

-*Meteorological data:* In this study, monthly and daily meteorological data for the 15-year period from 2000 to 2014 were received from the Meteorological Organization and Iran Water Resources Management Company. Daily data include maximum and minimum daily temperature and precipitation. The climatologic stations are listed in *Table 1*. Moreover, monthly data such as average solar radiation, dew point temperature, and average wind speed of the study area were introduced to the model.

Table 1. *Rainfall and temperature stations*

Station name	X (UTM)	Y (UTM)	Elevation (m)
Hasanjun	477266	4009639	1990
Joestan	490956	4004551	2220
Nesa	4900835	3998736	2310
Dehdar	505796	4004490	2400

-*Digital Elevation Map (DEM):* To prepare basin DEM map, digital topographic map of 2013 with a scale of 1:25000, provided by the National Cartographic Centre, was used.

-*Soil map:* In order to provide digital soil map and soil information of the basin, soil maps and reports of the Institute of Soil and Water Research (2001) was used.

-*Land use map:* To prepare the land use map (*Fig. 2*), interpretation of aerial photographs, updated digital maps of land use by satellite images ETM, and written reports related to Taleqan Basin, provided by Soil and Water Conservation Research Centre, were used.



Figure 2. *Watershed land use map*

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Results and discussion

Model implementation

Hence, to run the model, the data layers described in previous sections are presented as input to the model and the basin is divided into 35 subbasins and each subbasin is divided into hydrological response units (HRU). *Figure 3* shows the study area subbasins, stream flow, weather stations, and hydrometric stations.

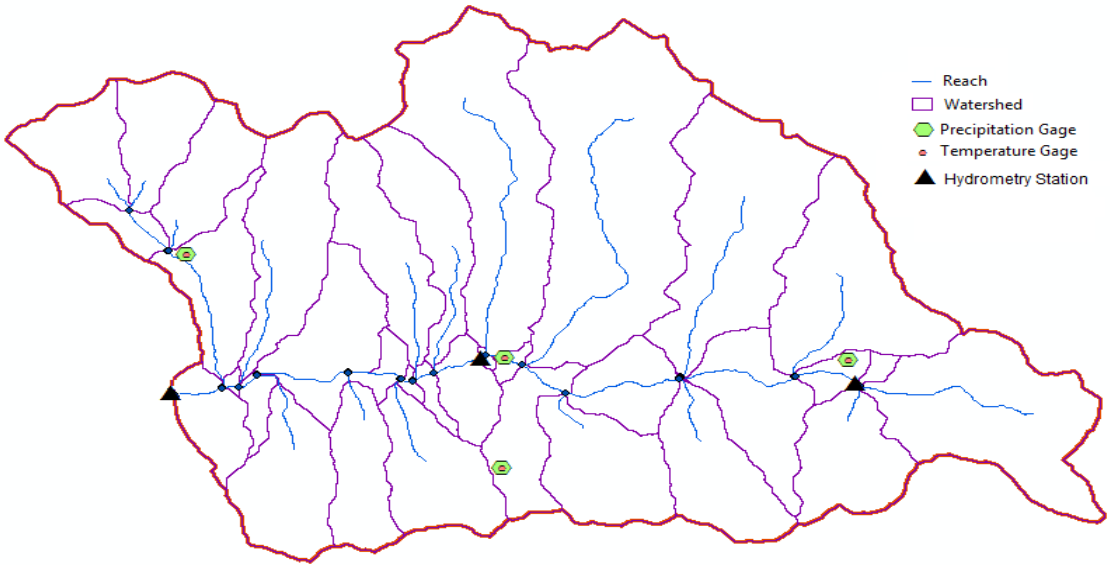


Figure 3. *Taleqan watershed, stream flow, weather stations and hydrometric stations*

Sensitivity analysis

The relative importance of input parameters with respect to its output is evaluated typically by using a sensitivity analysis. Parameters represent processes, and sensitivity analysis provides information on the most important processes in the study region. Many parameters are involved in the SWAT model to simulate rainfall-runoff. Thus, reducing the number of parameters by neglecting less effective parameters seems necessary (Zhang et al., 2009; Malago et al., 2017). The sensitivity analysis is performed by means of SWATCUP software, SUFI-2 algorithm. In this algorithm, parameters are changed, and a large number of runs are performed in order to see the impact of each parameter on the objective function. The advantage is that this produces more reliable results. This method uses a multiple regression approach to quantify sensitivity of each parameter (*Eq. 3*):

$$g = \alpha + \sum_{i=1}^{n} \beta_i b_i \qquad \text{(Eq.3)}$$

Where g is the objective function value, α is the regression constant, β is the coefficient of parameters, and b is the relative significance of each parameter (Abbaspour et al., 2018).

After sensitivity analysis, 18 parameters were determined as parameters to which the model is more sensitive. Sensitivity analysis showed that the parameters of the curve

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number (CN) is the most important factor, and soil evaporation compensation factor (ESCO) and soil available water capacity (SOL_AWC) are among the most important factors controlling the flow in the basin, respectively. The parameters' sensitivity ranking is given in *Table 2*.

Table 2. *Sensitive parameters description and ranking*

Sensitivity order	Parameter	Description	Domain	Fitted value
1	CN2	Curve number	.mgt	-0.291
2	ESCO	Soil evaporation compensation factor	.hru	0.965
3	SOL_AWC	Available soil water capacity	.sol	0.379
4	GW_DELAY	Time required for water leaving the bottom of the root zone to reach the shallow aquifer	.gw	56.213
5	SNO50COV		.bsn	0.578
6	ALPHA_BF	Baseflow alpha factor, lower number means a slower response	.gw	0.142
7	SNOCOVMX	Minimum snow water content that corresponds to 100% snow cover	.bsn	446.116
8	SMTMP	Snow melt base temperature	.bsn	-6.297
9	SNO_SUB	Initial snow water content	.sub	96.040
10	SFTMP	Snowfall temperature	.bsn	2.902
11	ALPHA_BNK	Baseflow alpha factor for bank storage	.rte	0.078
12	GWQMN	Threshold depth of water in the shallow aquifer required for return flow to occur	.gw	0.451
13	EPCO	Plant uptake compensation factor	.hru	0.387
14	SOL_CBN	Organic carbon content (% soil weight)	.sol	13.160
15	SURLAG	Surface lag coefficient; controls fraction of water entering reach in one day	.bsn	16.062
16	SOL_BD	Soil bulk density	.sol	0.879
17	GW_REVAP	Rate of transfer from shallow aquifer to root zone	.gw	0.317
18	SOL_K	Saturated hydraulic conductivity	.sol	0.376

More sensitivity analysis of the three most sensitive parameters and their effects on the normalized surface runoff changes is done. The results are shown in *Figure 4*. The runoff shows the greatest sensitivity to CN parameter. CN is a dimensionless parameter, which is linked to land use and soil type. Hence, by 10% positive and negative changes, runoff changes become very significant.

The second parameter in terms of sensitivity is ESCO. ESCO value reduction causes more water transfer to the upper layers of the soil, leading to an increase in the surface runoff. The third sensitive parameter is SOL_AWC. By reducing the amount of this parameter, surface runoff increases. As the empty spaces to hold water decrease, the amount of surface runoff increases.

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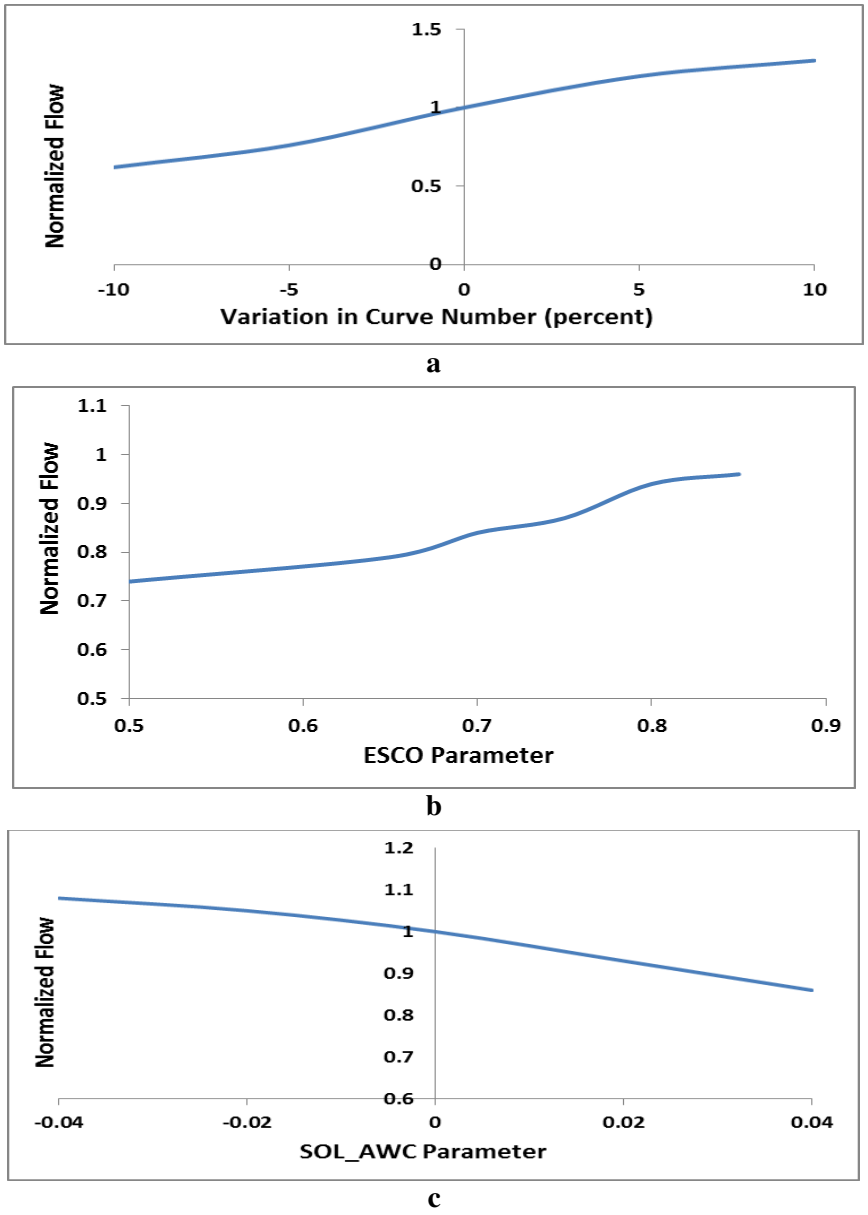


Figure 4. *Normalized flow changes due to (a) curve number changes; (b) ESCO parameter changes; (c) SOL_AWC parameter changes*

Calibration and validation

After determining the most sensitive model parameters, the model was calibrated using SUFI-2 algorithm (Abbaspour, 2008) for the years 2000 to 2010, using observed flow data by three hydrometric stations in which characteristics are shown in *Table 3*. Daily runoff data from these three stations in Iran is collected by the Water Research Institute of Iran. The optimal values of the parameters after calibration are presented in *Table 2*.

Runoff calibration results in these three stations are shown in the *Figure 5*. As can be seen, simulated runoff follows observed runoff curve in terms of both the values and the peak values occurrence time. Nash–Sutcliffe coefficient values of statistical indicators (NS) and R^2 before and after calibration are shown in *Table 4*. The values of these

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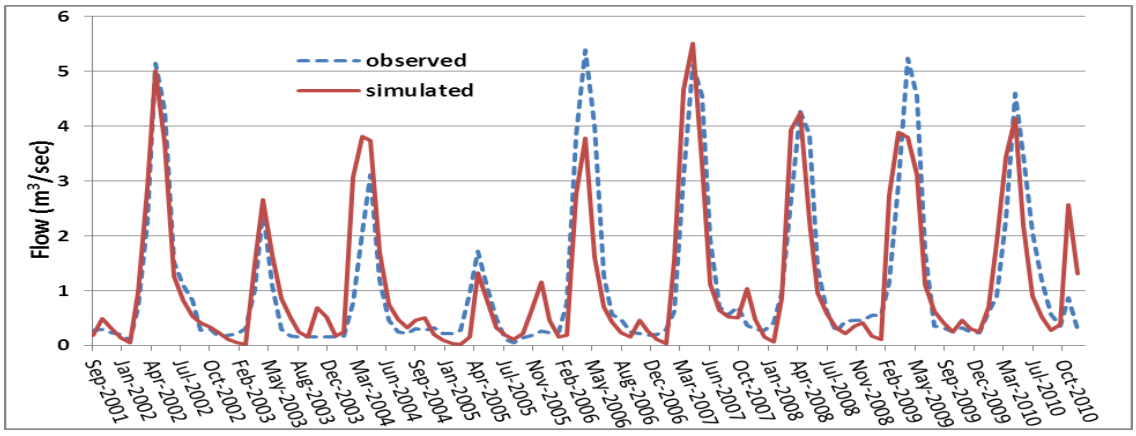
coefficients after calibration compared to the pre-calibration values are very different. NS coefficients in Gatehdeh, Joestan, and Gelinak are respectively, 0.8, 0.78 and 0.84 and R^2 values for these three stations had been estimated 0.86, 0.81 and 0.87, respectively. The values of these coefficients indicate that the simulation of basin is of good quality. In *Figure 6,* scatter chart of observed runoff due to simulated runoff values for Gelinak station is shown. It shows high correlation between the observed and simulation values.

Table 3. *Watershed hydrometric stations*

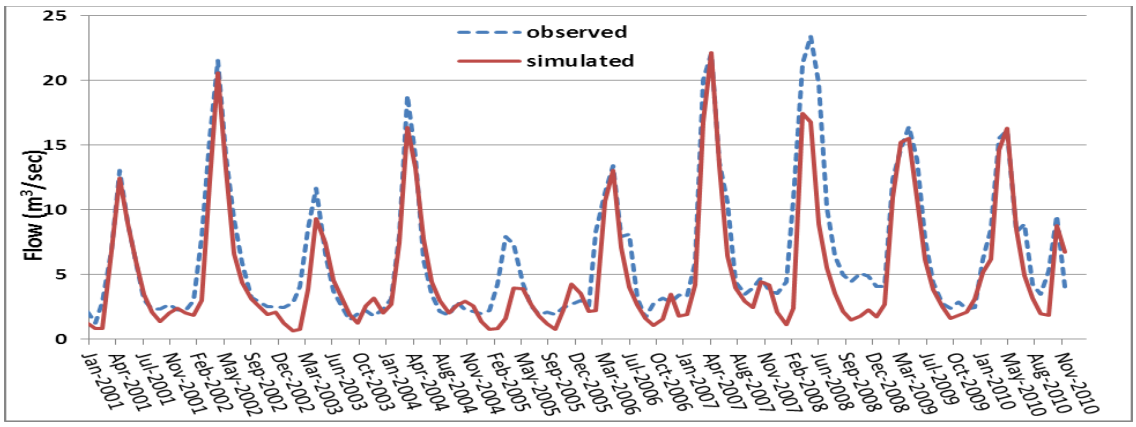
Station name	X (UTM)	Y (UTM)	Elevation (m)
Gatedeh	506183	4003119	2320
Joestan	489962	4004430	1930
Gelinak	476567	4002534	1780

Table 4. *Nash–Sutcliffe and R^2 statistical indexes before and after calibration interval*

Station name	Before calibration		After calibration	
	NS	R^2	NS	R^2
Gatedeh	0.13	0.25	0.8	0.86
Joestan	0.27	0.46	0.78	0.81
Gelinak	0.46	0.54	0.84	0.87

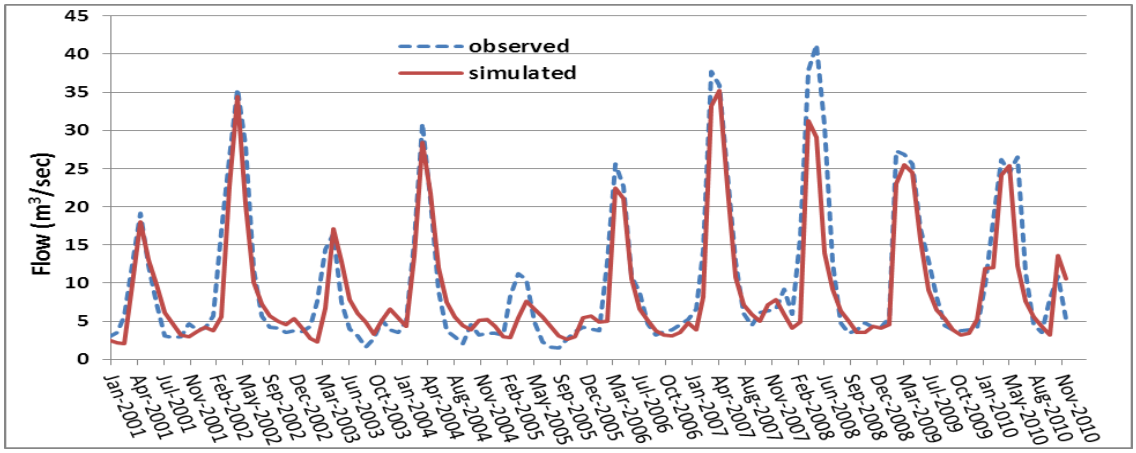


a



b

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c

Figure 5. *Monthly observed and simulated surface runoff in calibration period in (a) Gatehdeh station; (b) Joestan station; (c) Gelinak station*

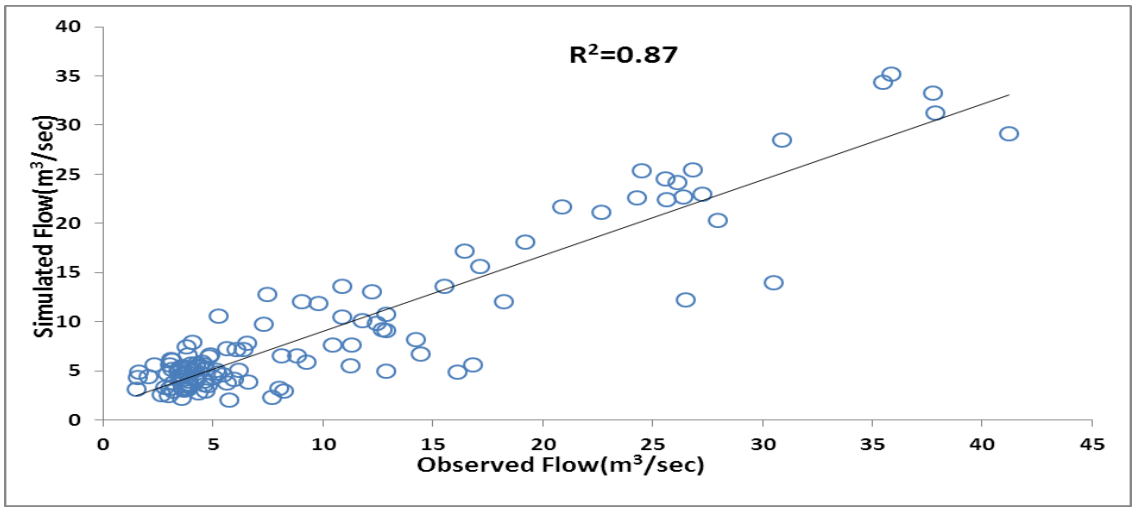


Figure 6. *Observed runoff due to simulated runoff values in calibration period for Gelinak station*

After model calibration, validation of the model is done using observed data from 2011 to 2014. The results of the model validation in Gelinak station is shown in *Figure 7*. The values of statistical indicators of Nash–Sutcliffe (NS) and R^2 coefficients during this period for three stations are shown in *Table 5*. Regarding the values of statistical indicators, it shows goodness of fit of the model.

Table 5. *Nash–Sutcliffe and R^2 statistical indexes for validation interval*

Station name	Validation period	
	NS	R^2
Gatedeh	0.76	0.85
Joestan	0.73	0.75
Gelinak	0.79	0.84

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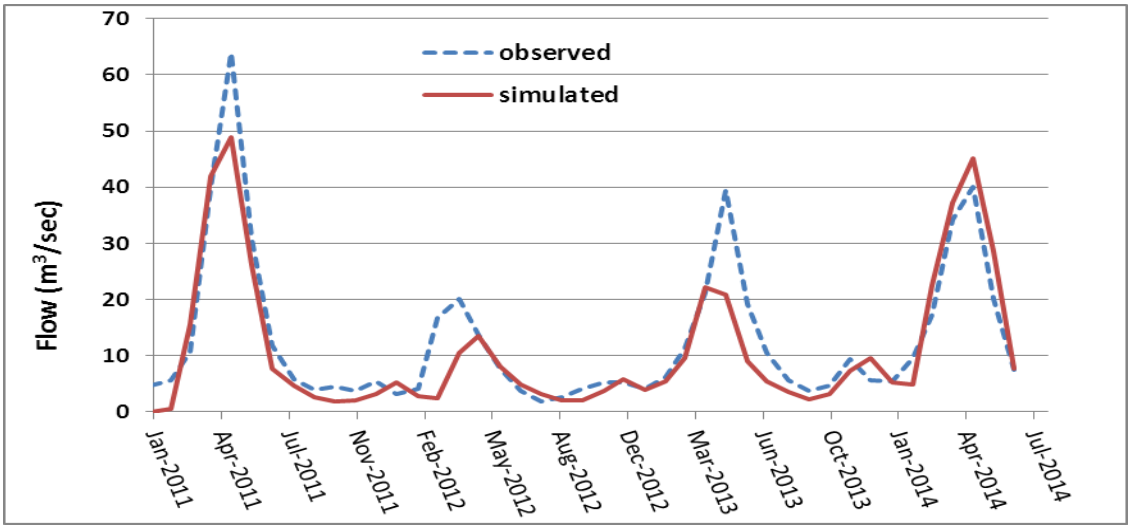


Figure 7. *Monthly observed and simulated surface runoff in validation period in Gelinak station*

For example, for Gelinak station which is the main basin outlet, the coefficients are 0.79 and 0.84 respectively, which shows the quality of modelling and suitable parameters setting in calibration period.

In *Figure 8,* a scatter chart of simulated and observed runoff values for the Gelinak station is shown during validation, which indicates acceptable correlation between observed and simulated values

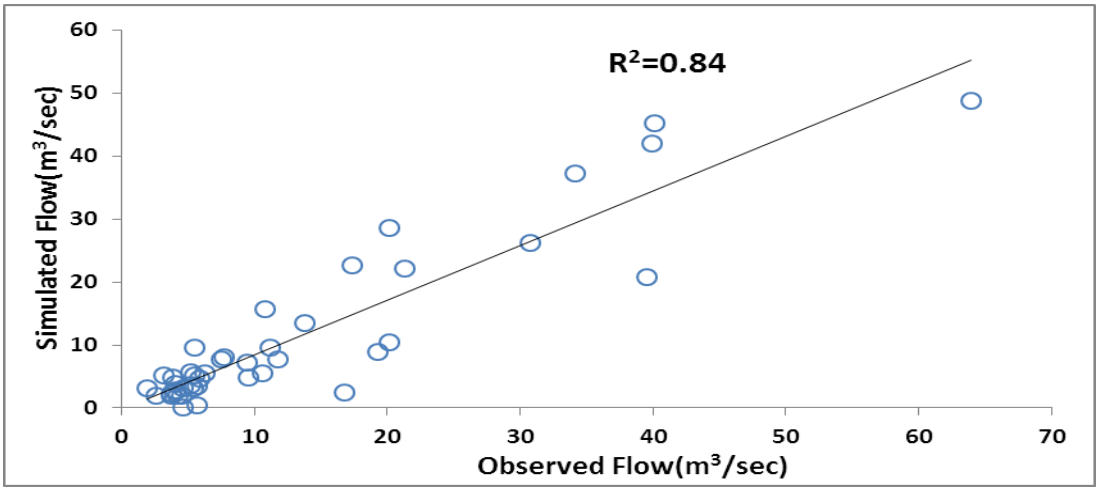


Figure 8. *Scatter chart of observed runoff due to simulated runoff values in validation period in Gelinak station*

Effects of land use scenarios on surface runoff

After watershed modelling, the effects of land use changes on surface runoff are investigated. For this purpose, practicable management activities in the watershed are listed in *Table 6* by considering existing restrictions. These management options and the

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required conditions are based on the studies performed in Agriculture Office of Alborz Province. The areas in which each management activity can be applied, considering *Table 6* conditions, are specified in *Figure 9*.

Table 6. *Management activities and specifications*

Management activity	Executable areas specifications
Afforestation	Lands with semi-deep or deep soil, low density land cover, max. slope 45% and max. elevation 2600 m
Agroforestry	Agricultural lands with slope of 15% to 30% with semi-deep or deep soil
Expansion of agricultural land	Agricultural lands with max. slope of 12% with deep soil and max. elevation 2400 m
Agriculture on terrace	Agricultural lands with slope of 12% to 15% with deep soil

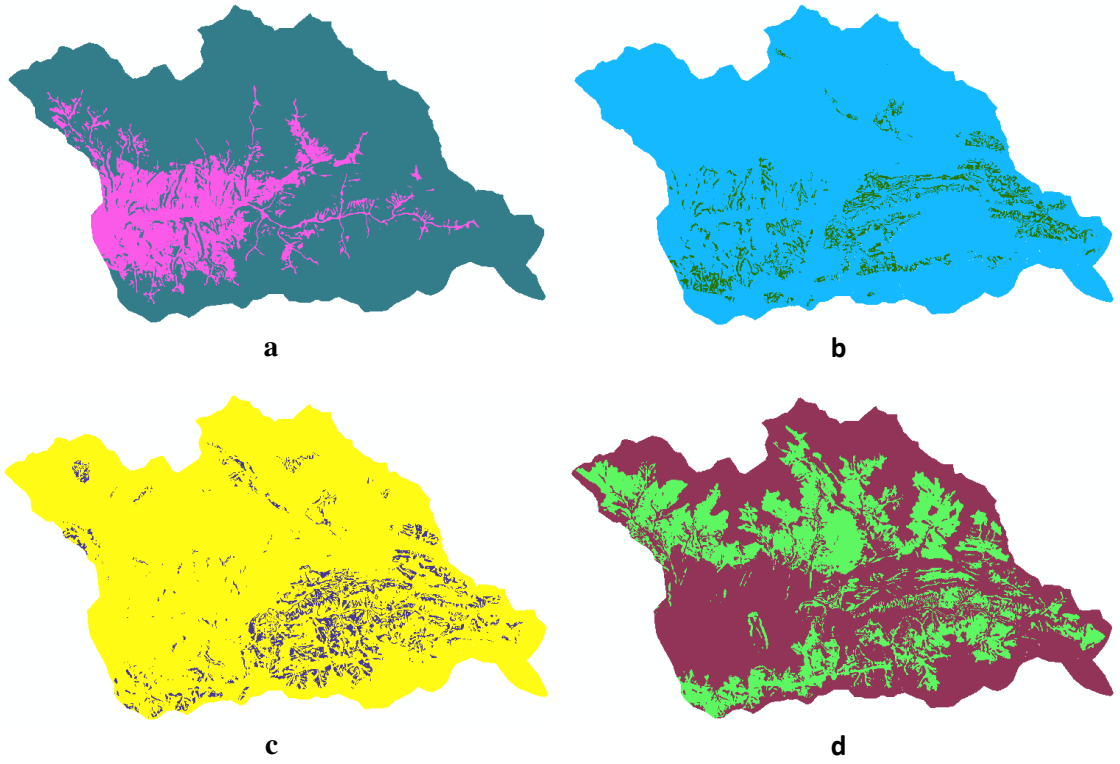


Figure 9. *Regions in which each management activity can be applied (a) agricultural land; (b) agriculture on terrace lands; (c) agroforestry lands; (d) forest lands*

So, combinations of 2^n possible management activities are obtained. Where n is the number of distinct management activities. These management scenarios are shown in *Table 7,* where scenario sn1 represents the current land use. *Table 8* expresses each scenario land use details in percent. *Figure 10* shows each scenario information, including different types of land uses and corresponding areas.

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Table 7. *Possible management scenarios*

Management activity	sn1	sn2	sn3	sn4	sn5	sn6	sn7	sn8	sn9	sn10	sn11	sn12	sn13	sn14	sn15	sn16
Agriculture on terrace	0	1	0	0	0	1	0	0	1	1	0	1	0	1	1	1
Afforestation	0	0	1	0	0	1	1	0	0	0	1	1	1	1	0	1
Expansion of agricultural land	0	0	0	1	0	0	1	1	1	0	0	1	1	0	1	1
Agroforestry	0	0	0	0	1	0	0	1	0	1	1	0	1	1	1	1

'1' represents for application of the activity
'0' represents for not applying the activity

Table 8. *Each scenario land use details (in percent)*

Land use	sn1	sn2	sn3	sn4	sn5	sn6	sn7	sn8	sn9	sn10	sn11	sn12	sn13	sn14	sn15	sn16
Agriculture on terrace	0	6.3	0	0	0	6.3	0	0	6.3	6.3	0	6.3	0	6.3	6.3	6.3
Forest	0	0	34.2	0	0	34.2	34.2	0	0	0	32.1	34.2	32.1	32.1	0	32.1
Agricultural land	6.5	6.5	6.5	23.4	6.5	6.5	23.4	23.4	23.4	6.5	6.5	23.4	23.4	6.5	23.4	23.4
Agroforestry land	0	0	0	0	8.7	0	0	8.7	0	8.7	8.7	0	8.7	8.7	8.7	8.7
Pasture	89.4	83.1	55.2	72.5	80.7	48.9	38.3	63.8	66.2	74.4	48.6	32	31.7	42.3	57.5	25.4
Rock out-crop	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1

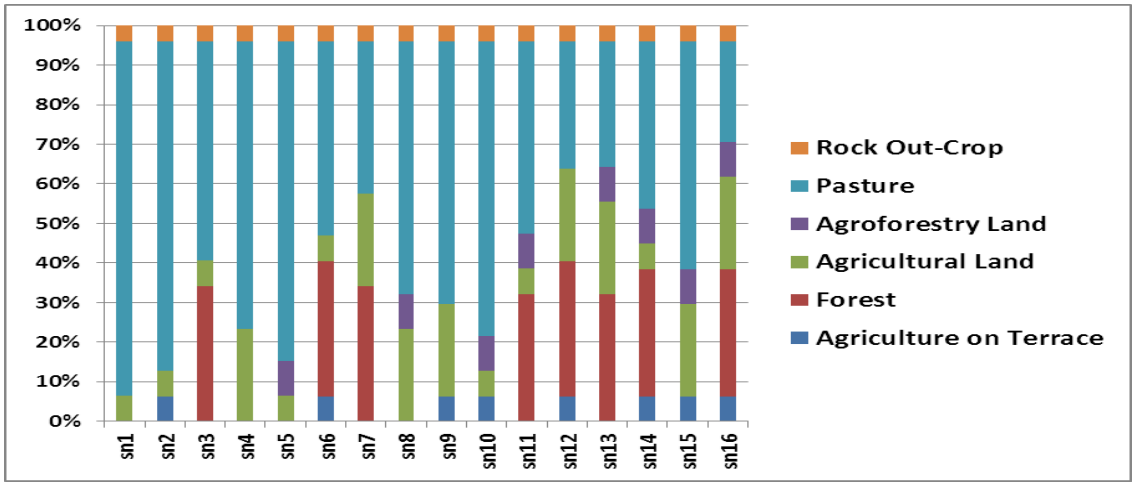


Figure 10. *Types of land uses and corresponding areas for each scenario (in percent)*

To estimate surface runoff from changes in land use scenarios, the SWAT model was run in the range of calibrated parameters. *Table 9* shows changes in average seasonal and annual surface runoff (in percent) for each management scenario. According to the results, applying management scenarios to the watershed leads to changes in runoff compared to current condition. It can be seen that applying scenario sn2 (agriculture on

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terrace) reduces average annual surface runoff less than 2%. However, sn3 (afforestation) decreases average annual runoff about 5%. Expansion of agricultural lands and agroforestry activities change runoff by 3.15% and 3.62%, respectively. Among individual scenarios, sn3 is the most effective management activity for runoff control due to good potential of this watershed for afforestation.

Each of scenarios sn6 to sn11 is obtained by combining two management activities. Application of sn6 (combination of agriculture on terrace and afforestation) reduces average runoff by 8%. Scenario sn7 (combination of expansion of agricultural land and afforestation) affects average annual runoff about 10%. Scenarios sn8 (combination of expansion of agricultural land and agroforestry), sn9 (combination of expansion of agricultural land and agriculture on terrace) and sn10(combination of agroforestry and agriculture on terrace) reduce surface runoff less than 7%. However, by applying sn11 (combination of agroforestry and afforestation activities), the average runoff is decreased more than 12% compared to current land use condition. Considering combinations of two management options (scenario sn6 to scenario sn11), it can be observed that the most effective scenario for runoff management is sn11.

Considering combinations of three management options (scenario sn12 to scenario sn15), sn13 (combination of afforestation, expansion of agricultural land and agroforestry activities) have the greatest impact on reducing runoff. By applying scenario sn13, average annual surface runoff is reduced by 19%. The second effective scenario in this category is sn14, which reduces runoff more than 16%. This scenario is the combination of afforestation, agriculture on terrace and agroforestry activities. Sn12 (combination of afforestation, agriculture on terrace and expansion of agricultural land activities) and sn15 (combination of expansion of agricultural land, agriculture on terrace and agroforestry activities) affects watershed average runoff by 14.43% and 13.48%, respectively.

Table 9. *Average seasonal and annual runoff changes (in percent) for each management scenario*

Seasonal/ annual interval	Management scenarios															
	sn1	**sn2**	**sn3**	**sn4**	**sn5**	**sn6**	**sn7**	**sn8**	**sn9**	**sn10**	**sn11**	**sn12**	**sn13**	**sn14**	**sn15**	**sn16**
Spring	0	-1.64	-4.71	-3.14	-3.32	-7.44	-8.66	-6.53	-5.81	-6.61	-12.02	-14.06	-18.29	-16.18	-13.12	-21.88
Summer	0	-1.86	-5.10	-3.40	-4.17	-9.83	-11.34	-7.84	-6.72	-7.11	-13.33	-15.22	-19.85	-17.47	-14.24	-23.52
Autumn	0	-1.78	-4.92	-3.28	-3.87	-8.12	-10.08	-7.21	-6.36	-6.89	-12.55	-14.71	-18.94	-16.88	-13.75	-22.69
Winter	0	-1.70	-4.17	-2.78	-3.12	-6.93	-9.58	-6.21	-5.44	-6.73	-11.68	-13.73	-18.68	-15.55	-12.82	-21.16
Annual	0	-1.75	-4.68	-3.15	-3.62	-8.08	-9.92	-6.95	-6.08	-6.84	-12.40	-14.43	-18.94	-16.52	-13.48	-22.31

The last management scenario is the combination of all land use options. Scenario sn16 land use map is shown in *Figure 11*. It can be observed that by applying this scenario, surface runoff decreases more than 22% compared to the current condition. This scenario makes the most significant effect on the average runoff compared to the other management scenarios. Moreover, by considering average seasonal runoff changes, it can be seen that the most decrease in surface runoff occurs in summer in each scenario. On the other hand, the least changes occur in winter.

Figure 12 shows average annual surface runoff resulted from each scenario application. Terrace agriculture (sn2) does not affect surface runoff considerably. However, from scenario sn6 (combination of management options) onwards, decrease

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in surface runoff is noticeable. It can be seen that average annual runoff is 126 m^3/s considering current land use. However, it is reduced to 108 m^3/s by applying sn11 (combination of agroforestry and afforestation activities) and to 100 m^3/s by applying sn13 (combination of afforestation, expansion of agricultural land and agroforestry activities). The main change occurs when sn16 land use scenario is applied to the model and the average annual runoff is reduced to about 95 m^3/s.

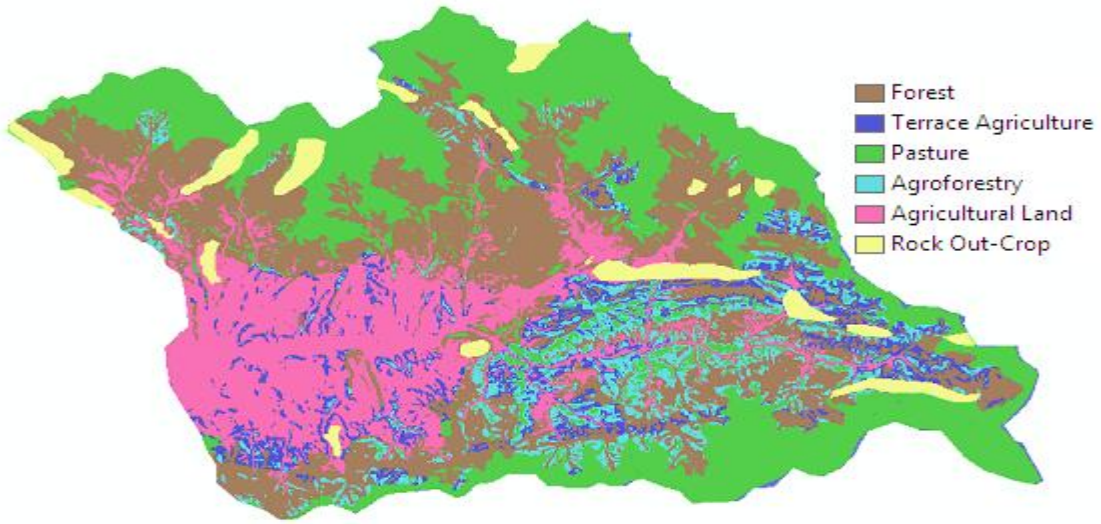


Figure 11. *Scenario sn16 land use map*

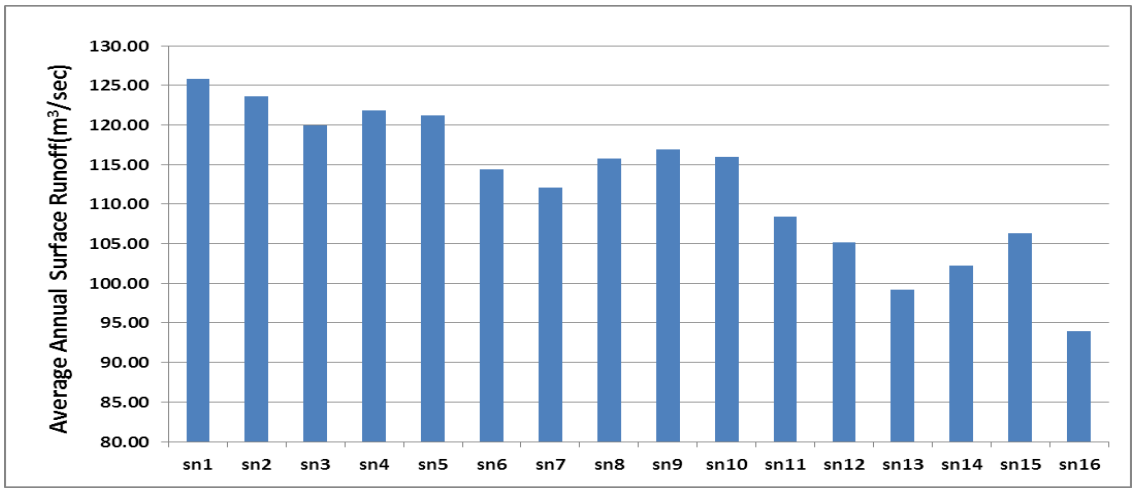


Figure 12. *Average annual surface runoff resulted from each scenario application*

In recent years, some researches were performed to study the impacts of land use changes on the study area flow. In a study by Kazemi and Bayat (2017), by using topographical maps and aerial photographs, land use maps of 1971, 1988 and 2003 were prepared. Then land use change was calculated. Then relationship between low flow indices and land use in the period of study were investigated. The results showed that the low flow indices have experienced an upward trend. Increasing rangeland coverage during these years was in accordance with the increasing of indices in the period

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studied. It was concluded that land use changes due to human intervention has a direct impact on the trend of low flow indices.

Hosseini et al. (2012) studied the effects of land use changes on water balance of the Taleqan catchment before and after the dam construction by using SWAT. The results indicated progressive increase in surface runoff and decline in interflow and groundwater flow. Therefore, one of the main challenges facing development planners is the control of the accelerated degradation of the natural resources.

In these researches, the impacts of land use changes on the watershed in the past time periods were studied. However, in the current work all possible land use scenarios were introduced to the model to study the corresponding future land use scenarios effects on the watershed. Since, decision makers can make the best decisions due to environmental consequences by assessing the results of each land use scenario simulation before implementation.

Conclusions

In this study, Taleqan watershed modelling was done using the Soil and Water Assessment Tool (SWAT). In order to do this, different kinds of information such as digital elevation model (DEM), soil data, temperature and precipitation data, and soil and land use maps in standard formats were provided as inputs for the model.

The relative importance of input parameters with respect to its output was evaluated using sensitivity analysis. Sensitivity analysis showed that the curve number (CN) parameter is the most important factor and Soil Evaporation Compensation Factor (ESCO) and soil available water capacity (SOL_AWC) are among the most important factors affecting the flow in the basin, respectively.

Calibration was performed by SUFI-2 algorithm, using river flow observation of three hydrometric stations within the basin.

It was observed that simulated values follow observed values of both the amplitude and the time of couriers. Nash-Sutcliffe coefficients in Gatehdeh, Joestan, and Gelinak stations are respectively 0.8, 0.78, and 0.84 and the R^2 values for these three stations were 0.86, 0.81, and 0.87, respectively. It was shown that the values of these statistical indicators after calibration compared to the pre-calibration values are very different. The statistical indicators values indicate that the basin modelling and simulations were performed with good quality.

After model calibration, it is necessary to ensure the quality of the modelling by means of validation process. Validation results in hydrometric stations show watershed modelling quality and suitable parameter settings in calibration period. NS and R^2 coefficients values were convenient. For example, in Gelinak station, which is the main outlet of the watershed, these indexes are 0.79 and 0.84. Moreover, scatter charts of observed and simulated surface runoff showed good correlation between these values in both calibration and validation intervals.

The effects of land use changes on the basin surface runoff were studied. It was seen that by applying scenario sn16, the most reduction in surface runoff was obtained compared to the other scenarios. Application of this scenario leads to 22.31% reduction in runoff compared to current condition. Moreover, in all scenarios, effects of management activities in the summer are the most. This can be due to the evolution of land cover and canopy, which in addition to significant evapotranspiration, makes runoff to decrease. According to results, management options combinations reduce

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surface runoff considerably compared to individual activities. Afforestation is the most effective activity among single options. Considering combinations of two management options, the most effective scenario is sn11 (a combination of afforestation and agroforestry activities), which changes surface runoff about 12.4% compared to current land use condition. On the other hand, sn9 has the least impact on runoff (about 6% reduction). However, in this condition, runoff decreases more than scenario sn5 (the most effective singular activity).

Considering combinations of three management options, sn13 (a combination of afforestation, expansion of agricultural land, and agroforestry activities) have the greatest impact on reducing runoff. By applying scenario sn13, surface runoff is reduced by 18.94% compared to the current condition, which is considered a significant change. Moreover, scenario sn14 reduces surface runoff by 16.52%. This scenario applies a combination of afforestation, agriculture on terrace, and agroforestry activities.

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APPENDIX

Appendix 1. *The study area summarised data used for modelling*

Parameter	Description	Value
TMPMX1	Average daily maximum air temperature for January (°C)	2.58
TMPMX2	Average daily maximum air temperature for February (°C)	4.45
TMPMX3	Average daily maximum air temperature for March (°C)	11.12
TMPMX4	Average daily maximum air temperature for April (°C)	17.94
TMPMX5	Average daily maximum air temperature for May (°C)	24.43
TMPMX6	Average daily maximum air temperature for June (°C)	30.52
TMPMX7	Average daily maximum air temperature for July (°C)	33.66
TMPMX8	Average daily maximum air temperature for August (°C)	32.29
TMPMX9	Average daily maximum air temperature for September (°C)	27.37
TMPMX10	Average daily maximum air temperature for October (°C)	20.22
TMPMX11	Average daily maximum air temperature for November (°C)	10.51
TMPMX12	Average daily maximum air temperature for December (°C)	4.75
TMPMN1	Average daily minimum air temperature for January (°C)	-9.20
TMPMN2	Average daily minimum air temperature for February (°C)	-6.52
TMPMN3	Average daily minimum air temperature for March (°C)	-1.62
TMPMN4	Average daily minimum air temperature for April (°C)	3.06
TMPMN5	Average daily minimum air temperature for May (°C)	7.32
TMPMN6	Average daily minimum air temperature for June (°C)	12.00
TMPMN7	Average daily minimum air temperature for July (°C)	15.47
TMPMN8	Average daily minimum air temperature for August (°C)	14.59
TMPMN9	Average daily minimum air temperature for September (°C)	10.74
TMPMN10	Average daily minimum air temperature for October (°C)	5.41
TMPMN11	Average daily minimum air temperature for November (°C)	-0.07
TMPMN12	Average daily minimum air temperature for December (°C)	-4.68
TMPSTDMX1	Average total monthly precipitation in January (°C)	3.54
TMPSTDMX2	Average total monthly precipitation in February (°C)	3.81
TMPSTDMX3	Average total monthly precipitation in March (°C)	4.99
TMPSTDMX4	Average total monthly precipitation in April (°C)	4.58
TMPSTDMX5	Average total monthly precipitation in May (°C)	3.59
TMPSTDMX6	Average total monthly precipitation in June (°C)	3.31
TMPSTDMX7	Average total monthly precipitation in July (°C)	2.45
TMPSTDMX8	Average total monthly precipitation in August (°C)	2.51
TMPSTDMX9	Average total monthly precipitation in September (°C)	3.10
TMPSTDMX10	Average total monthly precipitation in October (°C)	3.77
TMPSTDMX11	Average total monthly precipitation in November (°C)	3.93
TMPSTDMX12	Average total monthly precipitation in December (°C)	3.40
TMPSTDMN1	Standard deviation for daily minimum air temperature in January (°C)	6.68
TMPSTDMN2	Standard deviation for daily minimum air temperature in February (°C)	5.71
TMPSTDMN3	Standard deviation for daily minimum air temperature in March (°C)	4.27
TMPSTDMN4	Standard deviation for daily minimum air temperature in April (°C)	3.51

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TMPSTDMN5	Standard deviation for daily minimum air temperature in May (°C)	3.01
TMPSTDMN6	Standard deviation for daily minimum air temperature in June (°C)	2.76
TMPSTDMN7	Standard deviation for daily minimum air temperature in July (°C)	2.23
TMPSTDMN8	Standard deviation for daily minimum air temperature in August (°C)	2.23
TMPSTDMN9	Standard deviation for daily minimum air temperature in September (°C)	2.73
TMPSTDMN10	Standard deviation for daily minimum air temperature in October (°C)	2.87
TMPSTDMN11	Standard deviation for daily minimum air temperature in November (°C)	2.87
TMPSTDMN12	Standard deviation for daily minimum air temperature in December (°C)	4.50
PCPMM1	Average total monthly precipitation in January (mm)	210.50
PCPMM2	Average total monthly precipitation in February (mm)	268.54
PCPMM3	Average total monthly precipitation in March (mm)	328.70
PCPMM4	Average total monthly precipitation in April (mm)	357.50
PCPMM5	Average total monthly precipitation in May (mm)	201.30
PCPMM6	Average total monthly precipitation in June (mm)	54.20
PCPMM7	Average total monthly precipitation in July (mm)	33.04
PCPMM8	Average total monthly precipitation in August (mm)	29.25
PCPMM9	Average total monthly precipitation in September (mm)	35.14
PCPMM10	Average total monthly precipitation in October (mm)	141.43
PCPMM11	Average total monthly precipitation in November (mm)	243.96
PCPMM12	Average total monthly precipitation in December (mm)	210.32
PCPD1	Average number of days of precipitation in January	20.80
PCPD2	Average number of days of precipitation in February	19.41
PCPD3	Average number of days of precipitation in March	18.89
PCPD4	Average number of days of precipitation in April	17.07
PCPD5	Average number of days of precipitation in May	13.69
PCPD6	Average number of days of precipitation in June	5.98
PCPD7	Average number of days of precipitation in July	4.07
PCPD8	Average number of days of precipitation in August	4.36
PCPD9	Average number of days of precipitation in September	4.83
PCPD10	Average number of days of precipitation in October	9.56
PCPD11	Average number of days of precipitation in November	15.60
PCPD12	Average number of days of precipitation in December	19.87
SOLARAV1	Average daily solar radiation for January (MJ/m2/day)	11.50
SOLARAV2	Average daily solar radiation for February (MJ/m2/day)	15.42
SOLARAV3	Average daily solar radiation for March (MJ/m2/day)	20.83
SOLARAV4	Average daily solar radiation for April (MJ/m2/day)	25.04
SOLARAV5	Average daily solar radiation for May (MJ/m2/day)	28.95
SOLARAV6	Average daily solar radiation for June (MJ/m2/day)	32.08
SOLARAV7	Average daily solar radiation for July (MJ/m2/day)	30.93
SOLARAV8	Average daily solar radiation for August (MJ/m2/day)	28.12
SOLARAV9	Average daily solar radiation for September (MJ/m2/day)	23.99
SOLARAV10	Average daily solar radiation for October (MJ/m2/day)	17.73
SOLARAV11	Average daily solar radiation for November (MJ/m2/day)	12.37
SOLARAV12	Average daily solar radiation for December (MJ/m2/day)	10.14
DEWPT1	Average daily dew point temperature for January (°C)	-8.69

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DEWPT2	Average daily dew point temperature for February (°C)	-6.53
DEWPT3	Average daily dew point temperature for March (°C)	-5.16
DEWPT4	Average daily dew point temperature for April (°C)	-2.08
DEWPT5	Average daily dew point temperature for May (°C)	-0.60
DEWPT6	Average daily dew point temperature for June (°C)	0.65
DEWPT7	Average daily dew point temperature for July (°C)	4.47
DEWPT8	Average daily dew point temperature for August (°C)	3.64
DEWPT9	Average daily dew point temperature for September (°C)	-0.09
DEWPT10	Average daily dew point temperature for October (°C)	-1.68
DEWPT11	Average daily dew point temperature for November (°C)	-3.92
DEWPT12	Average daily dew point temperature for December (°C)	-6.88
WNDAV1	Average daily wind speed in January (m/s)	2.30
WNDAV2	Average daily wind speed in February (m/s)	2.65
WNDAV3	Average daily wind speed in March (m/s)	3.01
WNDAV4	Average daily wind speed in April (m/s)	3.23
WNDAV5	Average daily wind speed in May (m/s)	3.31
WNDAV6	Average daily wind speed in June (m/s)	3.22
WNDAV7	Average daily wind speed in July (m/s)	2.98
WNDAV8	Average daily wind speed in August (m/s)	2.74
WNDAV9	Average daily wind speed in September (m/s)	2.70
WNDAV10	Average daily wind speed in October (m/s)	2.57
WNDAV11	Average daily wind speed in November (m/s)	2.31
WNDAV12	Average daily wind speed in December (m/s)	2.33

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ANATOMICAL ASSESSMENT OF MAIZE (*ZEA MAYS* L.) SEEDLINGS EXPOSED TO COLLOIDAL SILVER NANOPARTICLES

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Abstract. Due to the widespread use of silver nanoparticles (nAg) in different products, the possibility of their leakage into the environment has been increasing. It is, therefore, crucial to study the possible effects of nAg on plants as the key components of ecosystems. In the present study, the effects of different concentrations of colloidal nAg on sweet maize were investigated at the anatomic level. Using dynamic light scattering and electron microscopy, the size of the nAg was estimated less than 80 nm. After one week of treatment, thin cross sections of roots and shoots were prepared and studied by light and fluorescence microscopes. Results showed that cortex and vascular cylinder area of roots and shoots of treated plants significantly ($P<0.05$) decreased versus control. Significant ($P<0.05$) reduction in diameter of metaxylem vessels was also identified. In addition, deform cells and Ag accumulations in root tissues of treated seedlings were observed. The results of this study indicate that nAg can enter into plant root and make considerable adverse effects on the plant root anatomy suggesting that nAg can become a future emerging pollutant.
Keywords: *auto fluorescent, bioaccumulation, metaxylem, micrograph, uptake*

Introduction

Due to their small size (1-100 nm) and the increase in their surface-to-volume ratio, nanoparticles have special properties making them suitable for various practical applications. Nowadays, variety of engineered nanoparticles are being used to make a wide range of products including personal care products, next-generatin batteries and solar cells, and environmental remediation of water, sediments, and soil (Buzea et al., 2017). Therefore, the particle's exposure to the environment and plants has been drastically increasing. Researchers are trying to carefully assess the risks and potential effects of nanoparticles on plants and humans and animls health and to propose methods to prevent or reduce the contamination of the environment with nanoparticles as emerging pollutants (Ghorbanpour et al., 2017).

Among various engineered nanoparticles, silver nanoparticles (nAg) due to their special properties (antimicrobial, electrical, optical and catalytic), have found applications in industries (Sintubin et al., 2012). Nowadays, engineered silver nanoparticles are used to make products such as food packages, textiles, electronic devices, home appliances, cosmetics, medical devices, water disinfectants, sprays (Varner et al., 2010), and also to produce pesticides (Thul et al., 2013). Increasing the

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production and use of products containing nAg will eventually lead to an increase in the probable release of these particles to the environment from production to disposal/reuse precedures; so there is an urgent need to study the effects of engineered nanoparticles on plants which are the important members of ecosystems and food chain.

The effects of nanoparticles can be investigated at different levels in plants. These effects can vary according to size, shape, and concentration of nanoparticles, and age, and species of plants (Rico et al., 2011). In recent years, numerous research studies have been carried out on the effects of nanoparticles on plants and toxic effects of nAg have been reported. For example, cytotoxicity and genotoxicity in onion (*Allium cepa* L.) and broad bean (*Vicia faba* L.) roots treated with nAg have been observed by decreasing in mitotic index and chromosomal aberrations (Kumari et al., 2009; Patlolla et al., 2012). Mustante and White (2012) reported a decrease in biomass of pumpkin (*Cucurbita pepo* L.) plants treated with nAg. Wang et al. (2013) reported toxic effects of nAg accumulated in poplar (*Populus deltoides* × *nigra*) and arabidopsis (*Arabidopsis thaliana* (L.) Heynh.) on evapotranspiration. Also, uptake of nAg by roots and transport to the shoots was observed in arabidopsis (Geisler-Lee et al., 2014). However, the mechanisms of the nAg impacts and their targets in plants are not adequately studied in the literature hence more research needs to be done at different levels such as anatomical, cytological, cytogenetical and molecular.

At anatomical level there are only few research studies on the impacts of nanoparticles. For instance, Thuesombat et al. (2014) reported low impact on the leaf anatomy of rice (*Oryza sativa* L.) plants treated with nAg (20 nm; 10 ppm), while a significant change was observed at 100 ppm concentrations in the anatomical traits. This study demonstrated that the effects of nanoparticle were concentration dependent. Pokhrel and Dubey (2013) also studied the number of metaxylem vessels (a type of transport tissue in vascular plants) in the primary root of maize exposed to 75 ppm of citrate coated nAg. They reported an increase in the number of metaxylem vessels in treated plants. In the study of Mousavi Kouhi et al. (2015), significant changes were observed in the anatomy of leaf and root of canola (*Brassica napus* L.) treated with zinc oxide nanoparticles.

Due to the paucity of the research on anatomical responses of plants exposed to nanoparticles, in this paper, the effects of nAg on maize seedlings at anatomical level were investigated. In addition to being an important crop, maize is considered to be a model plant in the environmental and genetic studies (Strable and Scanlon, 2009). It is worth noting that silver is an unnecessary element with unknown physiologically functions in plants. However increasing the concentration of the element to a certain extent can be fatal for plants.

Materials and Methods

Preparing suspensions and solutions

Coloidal nAg with a concentration of 4000 ppm (NanoSilver-W4000) GEBER was purchased fromBaran Chemistry Pasargad Company. The size of the nanoparticles was measured with the dynamic light scattering (DLS) method (*Figure 1A*) and with a particle size analyser device (Particle Metrix GmbH, Germany) less than 80 nm. Also, the shape and size of the nanoparticles were examined by a high resolution field emission scanning electron microscope (FE-SEM) (MIRA3 TE SCAN, Czech

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Republic) (*Figure 1B*). Using distilled water, concentrations of 300, 200, and 100 ppm were prepared from a homogenous suspension of 4000 ppm. The homogeneity of the suspensions was also maintained after dilution.

Since studies have shown that part of the nAg effects is due to the release of silver ions from them (Mcshan et al., 2014), in this study, silver nitrate was used as silver ion and positive control for comparison. The silver nitrate solution, as a positive control, was made from white powder of silver nitrate (Sigma-Aldrich) and distilled water at concentrations of 300, 200, and 100 ppm. For the sake of brevity, in the rest of the paper, nAg1, nAg2, nAg3, AgN1, AgN2, and AgN3 have been used correspond to the treatments with nAg of 100, 200, and 300 ppm and with silver nitrate of the same concentrations, respectively.

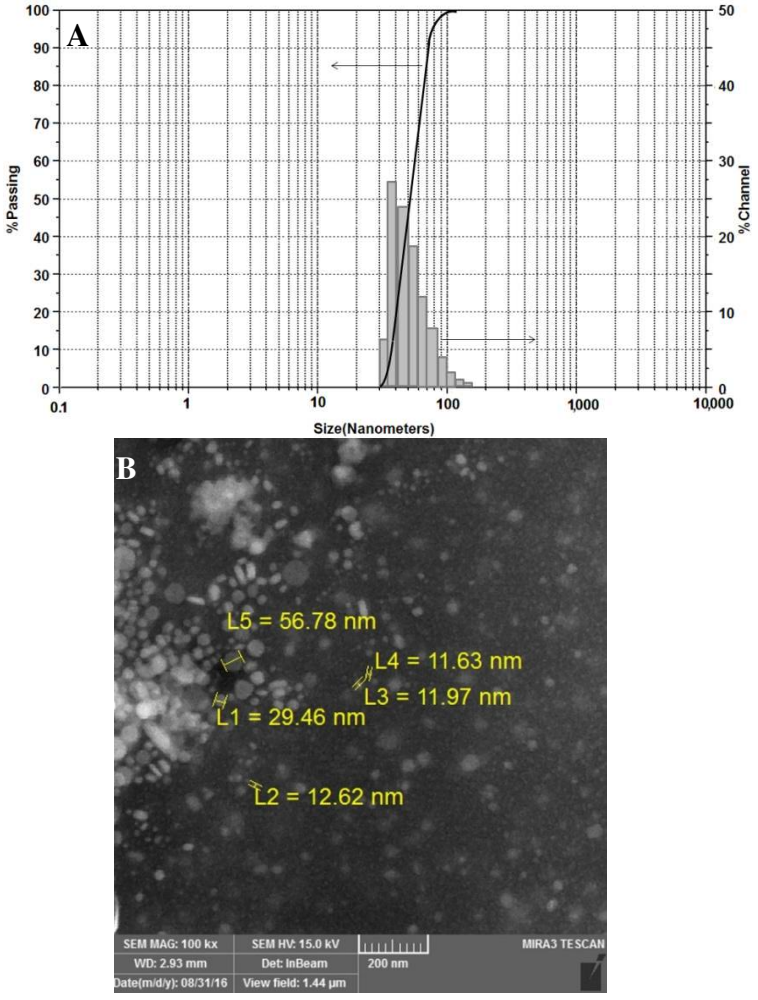


Figure 1. *A) The dynamic light scattering graph and B) the field emission scanning electron (FE-SEM) micrograph of the colloidal nAg (NanoSilver-W4000) GEBER show that the size of these spherical particles is less than 80 nm.*

Seeds

The sweet maize (*Zea mays* L. var. *Saccharata* 'Ksc 403') seeds were obtained from the Seed and Plant Improvement Research Institute, Karaj, Iran. In order to reduce the probability of contamination, the seeds were first put in ethanol 70% for 30 seconds and

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then rinsed three times with sterile distilled water. Thereafter they were immersed in sodium hypochlorite 5% solution for 10 minutes. Next, they were rinsed three times with sterile distilled water (Rao et al., 2006). Decontaminated seeds were laid in Petri dishes on sterile filter papers. Petri dishes containing seeds and 15 mL distilled water were incubated in dark at 25 °C for one week.

Seedling's treatments

Triplicates of seven-day-old seedlings with equal size were selected for a completely random design experiment. nAg suspension and silver nitrate solution were poured into the 15 mL tubes. Distilled water was used as the negative control. The entrance of the tubes was covered by a selefon with a small hole in its center so that only the seedling's root could be passed through it. In this condition, the seed and shoot were not directly in contact with the contents of the tube. In order to prevent the effect of light exposure, the tubes were covered by aluminum foil. The seedlings were exposed to daily photoperiod of 16 hours of light and eight hours of darkness at 25 °C for one week.

Anatomical analyses

For anatomical studies, the root and shoot were isolated separately and fix in the ethanol 70%:glycerol. Using regular flat shaving razor blades, thin cross sections of roots and shoots were obtained from the root cellular maturation zone and 2 cm above the crown of shoot, respectively. For staining, the sections were immersed in toluidine blue 0.5% (in phosphate buffer) for 20 seconds and then rinsed with tap water. The specimens were studied with an optical microscope (Olympus). Due to the fact that some of the cell wall componds (e.g. phenolic compounds), are auto-fluorescent (Mylle et al., 2013), some specimens were studied without staining to evaluate the possible changes in their fluorescence properties using a fluorescent microscope (E800-Nikon, Japan). Digital images were made from the slides and the cortex area, vascular cylinder area, the ratio of cortex area to the vascular cylinder area, and the diameter of openings of metaxylems were calculated using Image Tool software V. 3.0 calibrated by a micrometer slide.

Data analysis

Analysis of variance (ANOVA)($P<0.05$) was performed using SPSS ver.18 software and the Duncan test was used to diagnose the different means. Charts were plotted using MATLAB software.

Results

Figure 2 shows non-stained transverse sections of the primary roots for controls and treatments.The cross-section of negative control seedling's root demonstrates that, from outside to inside, there are root hairs, epidermis, cortex parenchyma, endodermis, a layer of pericycle cells, bundles of phloem, protoxylems, metaxylems, and the pith. As is seen in this figure, the pith cells are spherical and the walls of endodermal cells are thicker on the three sides (U thickening). Also, the number of metaxylam cells in the majority of the sections was counted 6 and 7, the xylems and phloems are in alternative order, the type of stele is medullated protostele, and the arrangement of xylems is

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exarch. In the cross-sections of the roots treated with nAg100, black areas in the root hairs and epidermis are observed. The black region in the corresponding positive control sections (AgN100) are visible from the epidermis to endodermis. In the cross sections of roots treated with nAg200, black areas are visible in the cortical parenchyma and from the sites of emergence of lateral root primordia to the pricycle cells. In its corresponding positive control (AgN200) sections, in addition to the cortex, the black areas are visible around the metaxylems. In the transverse sections of primary roots at the highest concentration of the study (nAg300), black accumulations are visible in the vascular cylinder cells and the protoxylem cells and the bundles of phloem vessels are deformed and distorted. Also, the pith cells are darkened and deformed. The primary root sections of its corresponding positive control (AgN300), exhibit greater amounts of silver penetration. The black areas and accumulations from the epidermis to the pith cells are visible. Here, the shape of the cells is normal in bundles of phloem vessels.

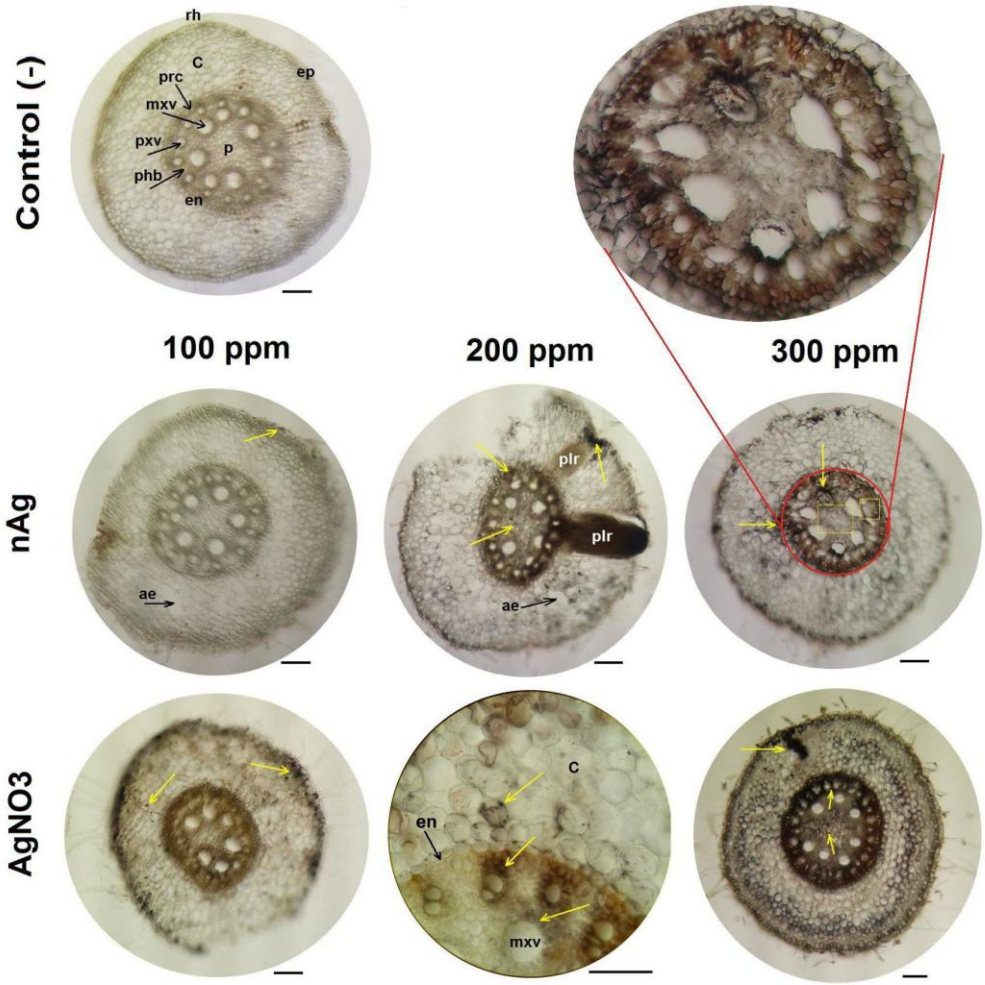


Figure 2. *Light micrographs represent impacts of silver treatments on maize primary root in non stained transverse sections. Light arrows indicate selected dark region of silver accumulation. ae: aeranchyma, c: cortex, en: endodermis, ep: epidermis, mxv: metaxylem vessel, p: pith, phb: phloem bundle, plr: primordium of lateral root, pxv: protoxylem vessel, prc: pericycle, rh: root hair. The labels in nAg and $AgNo_3$ treatments are the same as the control labels. Bars = 100µm. All photos are portions of mature zone of maize primary roots. Notice the irregular shape of pith cells and xylem and phloem vessels in magnified image of vascular cylinder of nAg treatment (300 ppm).*

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The fluorescent micrographs of *Figure 3* demonstrate the auto-fluorescent properties of the cell walls of the control and treated roots. In these images the walls of endodermal cells with U-shaped thickening are more reflective and distinguishable from the pericycle and cortical cells. As seen, in nAg300, the accumulation of silver prevents the reflection of light from the thick walls of endodermal cells. Furthermore these micrographs show that by increasing the concentration there is more penetration of Ag into root tissues resulting in less light reflection.

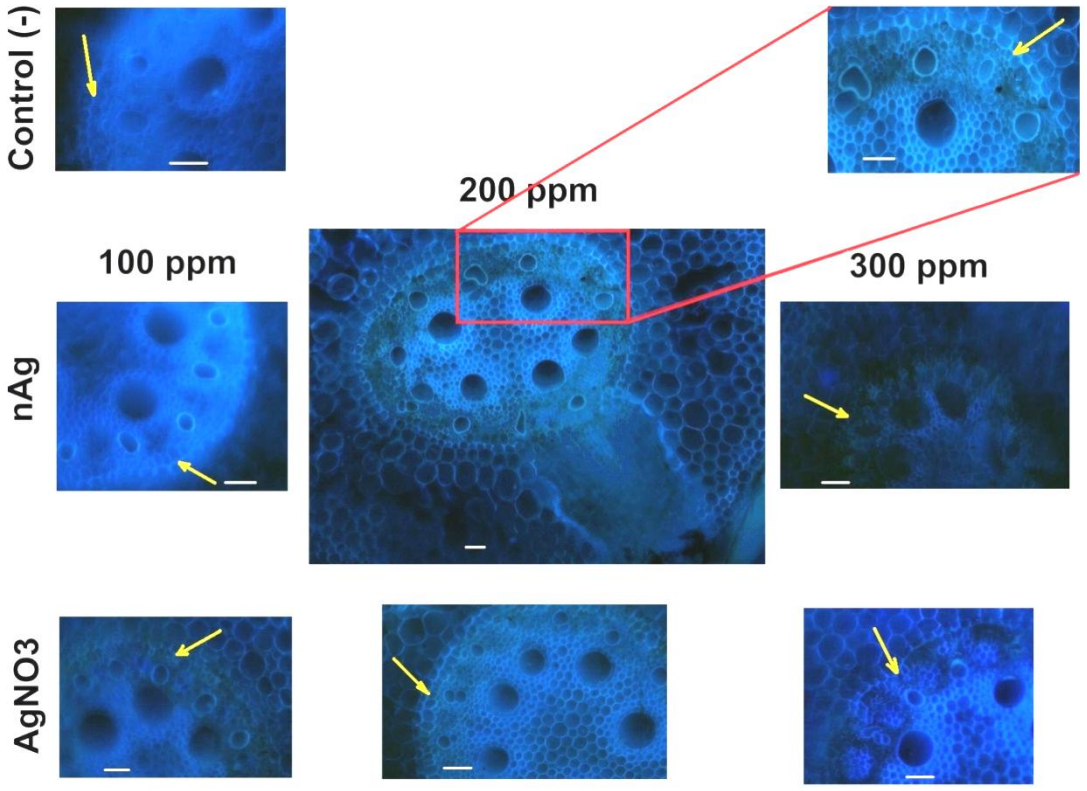


Figure 3. *The Fluorescent micrographs of non stained transverse sections of maize primary roots exposed to silver treatments. Arrows indicate U thickening of endodermis cell walls. In nAg 300 ppm treatment, silver accumulation reduced light reflection; Bars= 50 µm*

As seen in *Figure 4* in the cross section prepared from mesocotyls of shoots, from outside to inside, there are epidermis, cortical parenchyma, endodermis, and vascular cylinder. The vascular cylinder here, in contrast to the transverse section of roots, is not completely circular. Also, adventitious roots are observed in some sections (*Figure 4A*). There is no significant morphological difference in the mesocotyl sections of nAg100 treated seedlings compared to negative and positive controls (*Figure 4B, C*). Also, in the nAg200 and nAg300 treatments, and in their corresponding positive control, there was no significant morphological difference in the shoot transverse sections to negative control.

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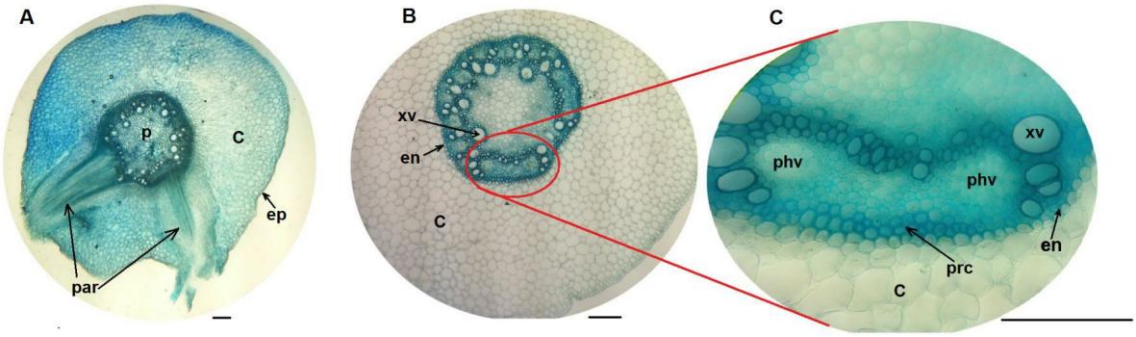


Figure 4. *The light micrographs represent transverse sections of two weeks old maize shoots(mesocotyl). Sections were prepared from two centimeters above the crown. A) Negative control, B) nAg 100 ppm treatment, C) magnification of one part of the vascular cylinder.c: cortex, en: endoderm, ep: epiderm, p: pith, par: primordium of adventitious root, phv: phloem vessel, prc: pericycle, xv: xylem vessel. Bars = 100µm*

As *Figure 5A* shows, there is not significant difference (P <0.05) between the means of cortex area of negative control and nAg100 treatment, however there is 88% significant increase versus its corresponding positive control. Also, there is no significant difference between the means of vascular cylinder and the ratio of cortex to the vascular cylinder area with negative control (P <0.05) (*Figure 5B, C*). The number of metaxylem in this treatment and in the corresponding positive control were counted 6. In the case of shoots, the means of the cortex and the the vascular cylinder area relative to the negative control is reduced 19 and 47%, respectively (*Figure5A, B*). However, the ratio of cortex to vascular cylinders area is 53% higher than negative control (*Figure 5C*).

According to *Figure 5A, B,* the means of the cortex and the vascular cylinder area of the root treated with nAg200 versus negative control is respectively 35 and 29% higher. In the later treatment there is no significant difference between the means of ratio of cortex to vascular cylinder area. Compared to the corresponding positive control, the means of the cortex and vascular cylinder area is respectively 53 and 41%, higher in nAg200 treatment (*Figure A, B5*). The number of metaxylem vessels in this treatment are 5, 6 and 7, and in the corresponding positive control, 6. The shoot cortex area and the vascular cylinder area were 64% and 27%, higher than negative control respectively, and 66% and 86% less than the corresponding control, respectively (*Figure 5A, B*). The ratio of areas versus negative and positive controls is reduced 30% and 10%, respectively (*Figure 5C*).

Figure 5A, B shows that the average of the root cortex and vascular cylinder area in nAg300 treatment is 46% and 39% lower than the negative control, respectively. In this treatment, the mean area of the vascular cylinder is 32% less than the corresponding positive control (AgN300). There is no significant difference between means of the ratio of cortex area to vascular cylinder area (*Figure 5C*). The number of metaxylem vessels in this treatment was 6 and in the corresponding positive control, 7 and 8. The mean of shoot cortex area and vascular cylinder area in this treatment is 29 and 26% less than negative control respectively (*Figure A, B5*). The ratio of areas is 23% less than the corresponding positive control, and there is no significant difference with the negative control (*Figure C5*).

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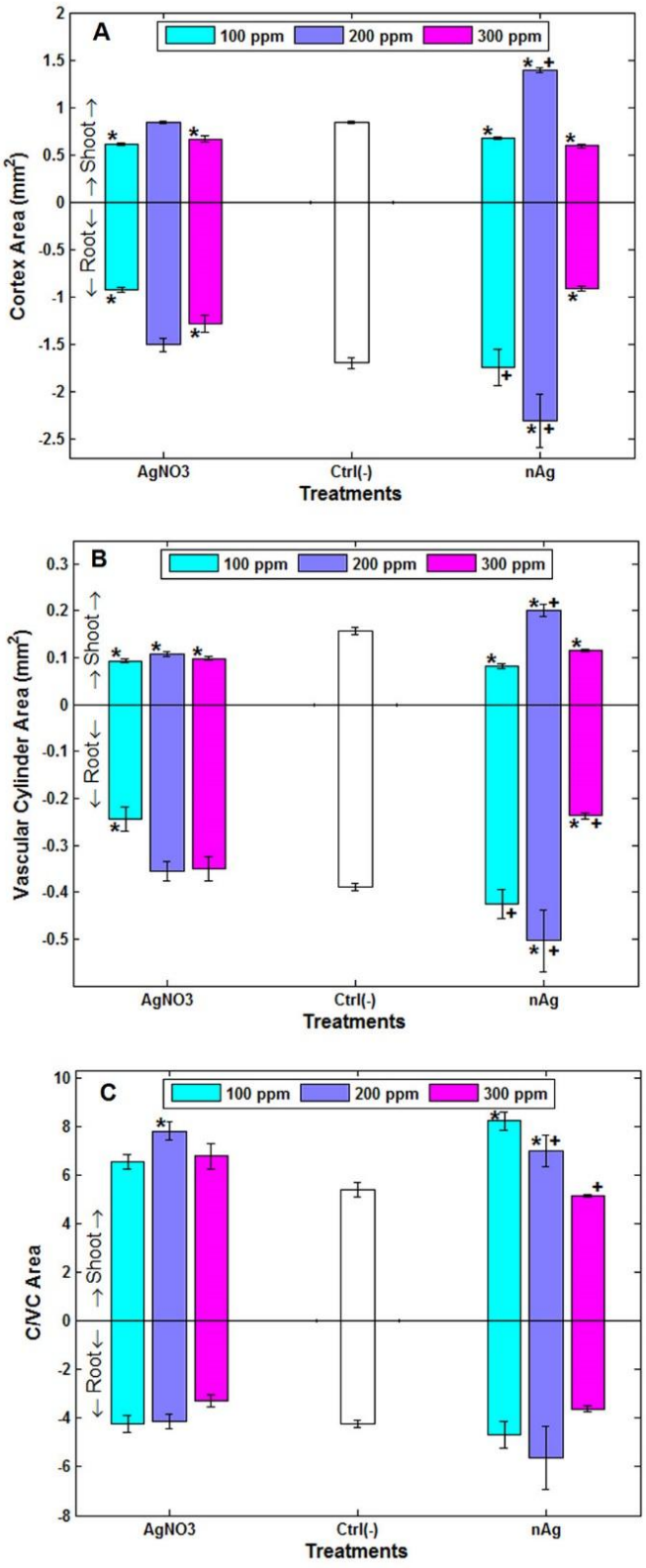


Figure 5. *The means ± SE of shoot and root A)cortex area, B) vascular cylinder area and C) cortex to vascular cylinder area of the controls and treated seedlings. Significant differences ($p < 0.05$) have been shown according to Duncan's test *:versus negative control and +: versus corresponding positive control. C: cortex, Ctrl(-): negative control (distilled water), VC: vascular cylider*

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The means of metaxylem opening diameter are provided in *Table 1*. As presented in the nAg100 treatment xylem diameter is less than the negative control and more than the corresponding positive control. The mean of metaxylem's diameter in the nAg200 treatment is less than negative control and does not have a significant difference with the corresponding positive control. The diameter of the metaxylem vessels is significantly less than the negative control and is more than the corresponding positive control in nAg300 treatment.

Table 1. *The means ± SE (in micrometer) of metaxylem diameter of the controls and treated 14-day-old maize seedling roots. Significant differences ($p < 0.05$) have been shown according to Duncan's test *: versus negative control (distilled water) and +: versus correspondence positive control (silver nitrate). Numbers in parentheses indicate concentrations in ppm*

$AgNO_3$ (100)	$AgNO_3$ (200)	$AgNO_3$ (300)	Control (-)	nAg (100)	nAg (200)	nAg (300)
79.70 ± 1.97*	78.57 ± 2.15*	64.97 ± 3.79*	109.65 ± 5.65	94.15 ± 2.20+*	78.57 ± 2.63*	89.43 ± 2.25+*

Values are mean ± SE

Discussion

The results of this study indicated that Ag^0 / Ag^+ was uptaken by the maize primary roots. Observation of black aggregates in micrographs of transverse sections of seedling's roots treated with nAg and silver nitrate showed that silver could enter into the roots. According to the observations, nAg entered more through the apoplastic pathway and intercellular free spaces and less through symplastic pathway (across cytoplasm) to the root tissues and were accumulated there or entered the emptied cellular elements of vessels via water uptake . It should be noted that as described by Zhao et al. (2012) nanoparticles may enter through natural nano or micro-size pores of plant cell walls. However, the size of the particles used in this study was greater than the size of the cell wall pores of maize primary root which are about 6.6 nm as estimated by Asli and Neumann (2009). The study of transverse sections with fluorescence microscopy showed that the accumulation of silver or the combination of silver with other molecules in endodermal cells at the highest concentration of the present study reduced light reflection.

Qualitative traits, for example, the shape of cells at the highest concentration used in this study (nAg300), have undergone dramatic changes in the vascular cylinder of the root so that the cells of the phloem vessels became distorted (*Figure 2*). Also, the opening of the xylems in the transverse sections are not circular anymore. Silver aggregates appear to have a mechanical pressure on the vascular cylinder cells. These changes can also be due to the interaction of nAg with cell wall components. Rico et al. and Dietz and Herth (2011) also noted that nanoparticles due to their small size and high surface activity can interact with cell structures and components. Not observing these changes in silver nitrate treatments with a corresponding concentration reflects the different effects of nAg.

Micrometrics measurements showed significant reduction in root and shoot cortex area and the vascular cylinder area versus negative control in all treatments except for nAg200. However, the ratio of areas in the root has no significant difference with negative control. The observed differences in the measured traits in the nAg200

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treatment as well as the study of non-stained cross sections suggests that it is due to the absorption of different amounts of silver. In other words, silver absorption was not linear as many factors could influence it. For example, nanoparticles are more likely to penetrate from the sites of lateral root emergence as in these sites the integrity of root tissues is lost.

The number of metaxylems in the root transverse sections of all treatments was similar to the negative control and was 6 or 7. Only in nAg300 treatments 8 metaxylems were observed. Furthermore, a significant reduction of xylem's opening diameter was observed in all treated roots versus control. These results suggest that silver could affect the anatomical traits of root tissues specially the transport tissues. The small proportion of the root vascular cylinder to the cortex area indicates that the Ag^0/ Ag^+ uptaken by the roots cause interference in water and nutrients transportation and as shown in *Figures 2* and *3*, lead to death of many root cells. However, there were no significant difference in the anatomical traits of the shoots of treated seedlings. According to the results the behavior of nAg is relatively similar to that of silver nitrate suggesting that part of the nAg impacts is by releasing of silver ions from them.

Conclusion

The results of this study show that nAg can be absorbed by maize root and subsequently affect the growth of the shoots. Accumulation, mechanical stress, and interaction with cell wall components are the possible mechanisms for the effect of nanoparticles used in this study. Moreover, the findings of this research demonstrate the usefulness of anatomical traits (alongside the study of effects at other levels) for evaluating the effects of nanomaterials in plants. The results of this reseach could raise concerns over possible nAg leakage into the environment and human health risks. The authors plan to explore other stages of maize life in treatment with different size and concentrations of nAg.

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MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF PLANT GROWTH REGULATORS ON *MEDICAGO* X *VARIA* T. MARTYN

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Abstract. The aim of the experiment was to evaluate the effects of a growth regulator on alfalfa (*Medicago x varia* T. Martyn). The authors of the experiments had had the regulator patented in Poland. There were following experimental variants with different application times: Control – application of distilled water both during the six-true-leaf stage and during the early-bud stage, AF_1 – application of the regulator during the six-true-leaf stage and distilled water during the early-bud stage, AF_2 – application of distilled water during the six-true-leaf stage and the regulator during the early-bud stage, $AF_{1,2}$ – application of the regulator both during the six-true-leaf stage and during the early-bud stage. The results were processed statistically with the analysis of variance, while differences between means were verified with Tukey's test ($p \leq 0.05$). It was found that the growth regulator increased stomatal width and the length of the stomatal pore in the stomata of the upper epidermis of the leaf blade. The application positively affected the length and mass of shoots, the number of shoots, but also the mass of inflorescence and roots. In the aboveground biomass there was a rise in the content of protein, phosphorus, calcium, potassium, magnesium, and titanium.
Keywords: *auxin, cytokinin, alfalfa, titanium chelate, stomata, biometrics, phytochemistry*

Introduction

Flow of hormones, organic substances secreted by plants, regulates physiological processes, stimulating, among other things, cell division and plant growth (Kocira et al., 2016; Sosnowski et al., 2016a, b, 2017a, b). Some exogenous plant hormones, like auxin and cytokinin, affect the function and growth of stomata (Dodd, 2003; Saibo et al., 2003). In an experiment with pea plants Gupta et al. (1999) and Pospišilovă (2003) found that synthetic hormones regulate the opening and closing of stomata and, at the same time, the intensity of photosynthesis and transpiration. According to Schroeder et al. (2001) red and blue parts of the light spectrum cause stomatal opening, probably with auxin and cytokinin playing an active role in activating proton pump H+-ATPase. There have not been many publications about the effects of synthetic hormones on the changes in the number of stomata and changes in their sizes or about the relationship between those features.

The relationship between the number of stomata per unit area and gas exchange has been proven in experiments with such plants as soya, strawberry (Klamkowski et al., 2008). It was also hypothesised that stomatal reaction to exogenous hormones might be dependent on hormone concentration (Pospišilovă, 2003). A concentration which is too high causes blocking of stomatal pore opening and hinders gas exchange (Ruclová and Pospišilovă, 2001; Dodd, 2003). One of the features of plant hormones is their ability to interact with other hormones, for example, when synthetic auxin and cytokinin, in

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higher amounts**,** both stimulate ethylene biosynthesis in plant tissue (Lorteau et al., 2001; Khan et al., 2002). However, many supposed reactions of the plants to those hormones may be simply reactions to a high level of ethylene. Another example of interaction is again that between cytokinin and auxin, with them both stimulating cell division in the cambium and vascular system formation. It results in a more effective transport of photosynthetic products throughout the plant and increases plant resistance to stress (Aldesuquy, 2000).

During the last few years an interest in the effects of chemical compounds having hormone-like properties on plants has been growing. Some of the most important exogenous synthetic auxins are: Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), Beta-naphtoxy acetic acid (NOA), 1-Naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D). The best known exogenous synthetic cytokinins are: 6-benzylaminopurine (BAP) and kinetin also called N6-furfuryladenines (Scacchi et al., 2009). The prospect of using other bio stimulants, one of which is titanium in the form of mineral compounds diluted in water, is also of growing importance. Titanium application can regulate biochemical processes, increasing and speeding up biomass growth. Moreover, it stimulates pollination, fertilization, fruit and seed formation, and increases resistance to bacterial and fungal diseases (Carvajal and Alcaraz, 1998). Titanium speeds up growth and development of leaves and is a catalyst making it easier for a plant to take up and process nutrients from soil and from foliar sprays more efficiently (Carvajal and Alcaraz, 1998).

The aim of the experiment was to evaluate the effects of a growth regulator on *Medicago* x *varia* T. Martyn cv. Kometa. An assumption was that its application would affect vegetative and generative development of selected morphological features, influencing the concentration of biomass minerals, such as P, K, Ca, Mg, Mn, Zn, Mo, Cu, Ti and organic compounds, like protein, crude fibre, Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), cellulose, and hemicellulose. Furthermore, the ratios of macro elements Ca:P and K:(Ca+Mg) were calculated.

Material and methods

Design of the experiment

The experiment was carried out in the plant breeding room of the University of Natural Sciences and Humanities in Siedlce (Poland) in 2014, in controlled conditions with the temperature of 24±2/16±2 °C, humidity of 40%, high pressure sodium lamps used to insure photoperiod of 16/8 and light intensity at 200 µmol m^{-2} s^{-1}. *Medicago* x *varia* T. Martyn cv. Kometa, the plant to be tested, was grown in pots. The experiment was set out in a randomised design, with a control unit, and was replicated four times. The pots were filled with 5 kg of soil, which was loamy medium sand taken from the plough layer of the 3d class according to the Polish classification system. The main characteristics of the soil were as follows: phosphorus - 0.48 g kg^{-1} DM, magnesium - 0.10 g kg^{-1} DM; potassium - 0.13 g kg^{-1} DM, copper - 8.7 mg kg^{-1} DM, and zinc - 22.3 mg kg^{-1} DM; boron - 2.3 mg kg^{-1} DM), manganese - 191 mg kg^{-1} DM and iron - 1570 mg^{-1} DM. Additionally, soil pH measured in KCl was 6.3, N-NO_3 concentration was 1.4 mg kg^{-1} DM, N-NH_4 concentration was 60.9 mg kg^{-1} DM, and C_{org} (organic carbon) concentration was 17.1 g kg^{-1}.

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The alfalfa seeds were sown in mid-March at a depth of 2-3 cm in pots (h = 300 mm, ∅ = 200 mm). When the seeds germinated, three representative seedlings in each pot were left to grow and to be used in the experiment later. The growth regulator applied and exclusively used for the experiment was given a patent by the Patent Office of the Republic of Poland (P/407/790). The regulator contained synthetic auxin (concentration of 35 mg dm^{-3}), cytokinin (concentration of 35 mg dm^{-3}) and titanium chelate (concentration of 1 mg dm^{-3}). The plants were sprayed until they were thoroughly wet, using 0.20 dm^3 of fluid per pot (0.067 dm^3 per plant). The plants were treated twice with different experimental combinations, at the six-true-leaf stage, i.e. during the vegetative stage of *Medicago* x *varia* T. Martyn and at the early-bud stage, i.e. during the generative stage of *Medicago* x *varia* T. Martyn.

Experimental treatment

- Control – distilled water application during the sixth-leaf stage and during the early-bud stage,
- AF_1 – regulator application during the sixth-leaf stage, distilled water application during the early-bud stage,
- AF_2 –distilled water application during the sixth-leaf stage, regulator application during the early-bud stage,
- $AF_{1,2}$ - regulator application during the sixth-leaf stage and during the early-bud stage.

All experimental variants were replicated four times.

Analysis of selected morphological features

The following measurements were recorded**:** the number of stomata per 1 mm^2 of the upper epidermis of the leaf blade, stomatal length and breadth (μm), the length of the stomatal pore (μm), stem length (cm), the number of shoots per plant, the number of leaves per shoot, root collar diameter (mm), the total mass of the plant (g DM), the total mass of shoots (g DM), the mass of leaves (g DM) the mass of inflorescence (g DM), the mass of roots (g DM), the mass of the aboveground part together with the mass of roots per plant (g DM).

The biometric measurements were taken after the plants were harvested at the end of flowering. Earlier than that, from the beginning till the end of flowering, each newly bloomed flower was counted. Stomatal parameters were determined with the microscope camera (HDCE Delta Optical). The material to be viewed under the microscope was taken from the central part of upper leaf blade surface, with five leaves of each experimental variant used. The dark-field microscope with a magnification of 400 times was applied. Microscope camera images were sent to the computer and all measurements were taken with microscopic imaging software ScopeImage 9.0.

The methods of chemicals estimation

The plants were harvested, dried and ground to determine the concentration of the following chemical elements: potassium, calcium, magnesium, phosphorus, manganese, zinc, copper, molybdenum, and titanium. Shoots, leaves, and roots were used to determine titanium concentration, but in the case of potassium, calcium, magnesium, potassium, manganese, zinc, copper, and molybdenum a mixture of shoots and leaves

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was used to analyse concentration of those metals. The mineral analyses were reduplicated three times for each experimental variant. Total concentration of potassium, calcium, magnesium, phosphorus, manganese, zinc, copper, and molybdenum was determined with ICP – AES, after dry mineralization in a muffle furnace at 450 °C. Then, 5 ml of a 50% solution of HCl was added to the crucible and evaporated in a sand bath to dissolve carbonates and separate silica. Next, 10 ml of a 10% solution of HCl was added and the contents were poured into a 100 ml volumetric flask.

Furthermore, the ratios of macro elements Ca:P and K:(Ca+Mg) were calculated. Then, the concentration of protein, simple sugars, Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), cellulose, and hemicellulose was measured (g kg^{-1} DM). The air-dry matter was shredded and ground, and the obtained material was subjected to chemical analysis to determine protein, simple sugars, and dry matter concentration, the latter by determining moisture content. The method of determination was near infrared spectroscopy (NIRS), using the NIRFlex N-500 spectrometer and ready to use INGOT calibration applications.

The results physiological and biometric parameters, the concentration of chemical elements and chemical compounds in the biomass, were statistically processed, using analysis of variance. Tukey's test was used to determine $HSD_{0.05}$ for means that were significantly different, and standard error was calculated to find out how the values spread. The Statistica 6 programme was used for all data analysis.

Results and discussion

The results of the experiment showed that regulator application to alfalfa plants did not affect the number and the length of stomata in the epidermis of the leaf blade (*Table 1*). However, the regulator increased the breadth of stomata, with the widest ones being in variants sprayed one time at the six-true-leaf stage (13.7 μm) and in plants treated twice during the six-real-leaf stage and during the early-bud stage (13.9 μm). There was a similar effect in the case of stomatal pore length, where the highest increase, compared to the control, was also in plants treated twice (8.5%). Additionally, treating plants during their early-bud stage, or, broadly speaking, during their generative stage, did not affect their stomata in any way.

Table 1. *The effects on growth regulator applied at different times on parameters of stomata in the upper epidermis of the leaf blade*

Variants	Stomatal number [mm^2]		Stomatal length [μm]		Stomatal breadth [μm]		Stomatal pore length [μm]	
	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]
Control	73 ±10.7	-	25.1 ±8.17	-	11.9 ±7.11	-	13.0 ± 5.12	-
AF_1	76 ±12.4	+4.11	24.9 ±9.15	-0.80	13.7 ± 8.51	+15.1	13.9 ± 3.52	+6.9
AF_2	73 ±14.6	0	24.7 ±7.67	-1.6	12.1 ±7.26	+1.7	13.2 ± 4.41	+4.6
$AF_{1,2}$	75 ±13.9	+2.74	25.6 ±8.94	+2.0	13.9 ±6.75	+16.8	14.1 ±3.97	+8.5
$HSD_{P=0.05}$	n.s.	-	n.s.	-	1.5	-	0.67	-

Mean value ± standard error (SE), +, -[%] − increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage, n.s. – not significant

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The data in *Tables 2* and *3* show that as a result of regulator application there were considerable differences between alfalfa plants it terms of the development of their parts and the growing of their mass. In plants treated twice the shoots, in a statistically significant way, were 27.2% longer than in control plants.

Table 2. *The effects of growth regulator applied at different times on biometry of selected morphological features*

Variants	Stem length [cm]		Number of shoots per plant		Number of leaves per shoot		Root collar diameter [mm]	
	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]
Control	42.3 ±14.2	-	11.5 ±2.7	-	35.0 ±9.7	-	3.7 ± 1.8	-
AF_1	44.2 ± 17.1	+4.5	11.0 ±3.8	-4.3	34.4 ±8.5	-1.7	4.1 ± 2.0	+10.8
AF_2	42.4 ±15.4	+0.4	9.90 ±4.2	-13.9	34.2 ±6.9	-2.3	3.9 ±1.8	+5.4
$AF_{1,2}$	53.8 ±13.5	+27.2	14.0 ±6.7	+21.7	35.1 ±7.7	+0.3	4.3 ±1.7	+16.2
$HSD_{P=0.05}$	9.4	-	2.7	-	n.s.	-	n.s.	-

Mean value ± standard error (SE), +, - [%] − increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage, n.s. – not significant

There were similar results when it comes to the number of shoots. In pots where plants were treated twice there were 21.7% more shoots than in control plants treated with distilled water. As a result, the mass of shoots was 25% higher than the mass in the control. Generally, compared to the control, there was a statistically significant increase in the mass of shoots, inflorescence, and roots, both in plants growing in pots where the regulator was applied during the growing stage (AF_1) and in plants treated twice ($AF_{1,2}$). However, statistical analysis did not show any increase in the number of leaves per shoot, root collar diameter, or leaf mass per plant.

Table 3. *The effects of growth regulator applied at different times on the mass of selected plant parts (g DM)*

Variants	Total		Shoots		Plants		Inflorescence		Roots	
	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]
Control	32.2±10.2	-	11.0 ±2.8	-	16.0 ±4.2	-	1.45 ±0.78	-	3.70 ±1.8	-
AF_1	38.9±10.7	+20	15.9 ±3.1	+44	15.9 ±3.3	-0.6	2.18 ±1.1	+50	5.00 ± 2.0	+35
AF_2	32.1 ±9.9	-0.3	11.4 ±2.7	+3.6	15.8 ±3.0	-1.3	1.38 ±0.96	-4.8	3.50 ± 1.7	-5.4
$AF_{1,2}$	40.4±11.1	+25	16.1 ±3.8	+46	16.9 ±3.9	+5.6	2.23 ±1.3	+53	5.15 ±2.2	+39
$HSD_{P=0.05}$	6.4	-	4.1		n.s.	-	0.61	-	0.99	-

Mean value ± standard error (SE), +, -[%] − increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage, n.s. – not significant

Application of synthetic growth regulators affects nutrient absorption and, at the same time, regulates accumulation and distribution of nutrients. Additionally, there is dependence between application of such regulators and the content of macro and microelements in the aboveground vegetative parts. In the experiment described here (*Tables 4* and *5*), in the plants treated with the regulator there was an increase only in

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the content of phosphorus, manganese, and potassium, with the latter having the highest increase. In the pots with plants treated once, during the vegetative stage (variant AF_1), potassium content, compared to the control, grew by 32%, while for plants treated twice the accumulation of this metal was 28.6% higher.

Table 4. *The effects of growth regulator applied at different times on selected macro elements concentration in the above-grant part of plants [g·kg^{-1} DM]*

Variants	Phosphorus		Potassium		Magnesium		Calcium	
	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]
Control	2.76 ±1.08	-	14.7 ±8.17	-	2.79 ±0.97	-	13.9 ±7.38	-
AF_1	3.45 ±0.87	+25	19.4 ±9.35	+32	3.01 ±1.1	+7.9	14.0 ±5.54	+0.7
AF_2	2.99 ±1.07	+8.3	16.8 ±7.75	+14.3	2.60 ±0.69	-6.8	14.1 ±4.89	+1.4
$AF_{1,2}$	3.36 ±1.11	+21.7	18.9 ±9.19	+28.6	2.93 ±0.87	+5.0	14.3 ±9.11	+2.9
$HSD_{P=0.05}$	0.31	-	1.9	-	n.s.		n.s.	-

Mean value ± standard error (SE), +, - [%] − increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage, n.s. – not significant

Table 5. *The effects of growth regulator applied at different times on selected microelements concentration in the above ground part [mg·kg^{-1} DM]*

Variants	Manganese		Zinc		Copper		Molybdenum	
	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]
Control	45.5 ±9.12	-	22.4 ±6.17	-	5.83 ±0.78	-	0.48 ±0.23	-
AF_1	57.7 ±7.36	+26.8	28.9 ±5.98	+29.0	5.79 ±1.08	-0.6	0.49 ±0.19	+2.1
AF_2	47.8 ± 8.07	+5.05	27.5 ±7.91	+22.8	6.06 ±0.97	-1.3	0.51 ±0.31	+6.3
$AF_{1,2}$	58.1 ±6.12	+27.7	29.0 ±6.79	+29.5	6.04 ±1.11	+5.6	0.47 ±0.29	-2.1
$HSD_{P=0.05}$	9.1	-	n.s.	-	n.s.	-	n.s.	-

Mean value ± standard error (SE), +, -[%] − increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage, n.s. – not significant

The application of the regulator once, during the vegetative stage, and twice, during both vegetative and generative stages, increased phosphorus and manganese content by over 20%. Additionally, there was a considerable change in titanium concentration in many parts of plants (*Table 6*). The highest concentration of titanium was in leaves (from 3.24 to 4.01 mg kg^{-1} DM), with a relatively high concentration in shoots (from 2.01 to 2.89 mg kg^{-1} DM), and the lowest amounts in roots (from 0.99 to 1.67 mg kg^{-1} DM). Treating plants with the regulator increased the total amount of titanium in biomass, with the highest increase in plants treated twice (variant $AF_{1,2}$). Compared to the control, the content of titanium in shoots increased by 43.8%, in leaves by 23.8%, and in roots by as much as 68.7%. Regulator application during the vegetative stage in the strongest way affected the content of titanium in the biomass of roots, by 54.5%. In turn, treating plants in the generative stage resulted in a slight change in the amount of the above metals in plants only by several percent, and the change was statistically

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insignificant. A higher amount of those chemical elements affected ion ratios between some macro elements (*Table 7*).

Table 6. *The effects of growth regulator applied at different times on titanium concentration in shoots, leaves and roots [mg·kg^{-1} DM]*

Variants	Shoots		Leaves		Roots	
	Mean± SE	+, - [%]	Mean± SE	+, - [%]	Mean± SE	+, - [%]
Control	2.01 ±0.72	-	3.24 ±1.35	-	0.99 ±0.74	-
AF_1	2.65 ±0.41	+31.8	3.82 ±0.91	+8.64	1.53 ±0.63	+54.5
AF_2	2.13 ±0.81	+6.01	3.38 ±1.21	+4.32	1.04 ±0.95	+5.05
$AF_{1,2}$	2.89 ±0.53	+43.8	4.01 ±1.12	+23.8	1.67 ±0.81	+68.7
$HSD_{P=0.05}$	0.49	-	0.41	-	0.32	-

Mean value ± standard error (SE), +, -,% − increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage

Table 7. *The effects of growth regulator applied at different times on the ratio of some macroelements to others in the above ground part*

Variants	Ratio			
	Ca:P Mean ± SE	+, - [%]	K:(Ca + Mg) Mean ± SE	+, - [%]
Control	5.04 ±1.21	-	0.88 ±0.71	-
AF_1	4.05 ±0.93	-19.6	1.14 ±0.84	+29.5
AF_2	4.99 ±1.12	-1.0	1.01 ±0.62	+14.8
$AF_{1,2}$	3.36 ±1.02	-33.3	1.09 ±0.48	+23.9
$HSD_{P=0.05}$	0.61	-	0.11	-

Mean value ± standard error (SE), +, -,% − increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage

The lowest Ca: P ratio was in plants treated twice (3.36), but when the regulator was applied during the six-real-leaf stage the ratio was 4.05, while in the control it was wider, with the value of 5.04. Application during the generative stage did not affect the ratio of Ca: P. The ratio of K: (Ca + Mg) was in a statistically significant way higher for plants treated with the regulator in all variants of the experiments and ranged from 1.01 to 1.14.

In the biomass, regulator application affected the amount of organic compounds, like protein, fibre, and structural carbohydrates making up the skeleton of the plant cell wall. The effect of regulator application was dependent on the plant development stage at the time of application (*Table 8*). When the plants were treated during the six-true-leaf stage, a part of the vegetative stage, the content of protein in the biomass increased by 14.5%, while crude fibre decreased by 7.0%. There was a similar effect in plants treated with the regulator twice (variant $AF_{1,2}$). However, compared to the control, regulator application during the early-bud stage, or generative stage, did not affect in a statistically significant way the content of protein and crude fibre. The regulator influence was particularly noticeable in the changing content of cell wall fractions:

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Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), cellulose, and hemicellulose (*Table 9*).

Table 8. *The effects of growth regulator applied at different times on total protein and crude fibre concentration in the above-ground parts [g kg^{-1} DM]*

Variants	Total protein		Crude fibre	
	Mean ± SE	+, - [%]	Mean ± SE	+, - [%]
Control	193 ±12	-	301 ±17	-
AF_1	221 ±13	+14.5	280 ±16	-7.0
AF_2	191 ±11	-1.03	297 ±19	-1.3
$AF_{1,2}$	227 ±17	+17.6	278 ±18	-7.6
$HSD_{P=0.05}$	21.5	-	16.4	-

Mean value ± standard error (SE), +, - [%] - increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage

Additionally, the changes in the content of the above fractions, like in the case of protein and fibre, showed that there was a relationship between the amount of those compounds and the time when the regulator was applied. Acid Detergent Fibre was an exception, with its concentration ranging from 324 to 341 g kg^{-1} DM, without any statistically significant differences between plants of different experimental variants. As regards NDF, ADL, cellulose, and hemicellulose, regulator application resulted in lower content of those compounds in the dry matter of plants treated during the vegetative stage (variants AF_1 and $AF_{1,2}$).

One of the characteristics of hormones is their interaction with other hormones, an example of which is exogenous cytokinin and auxin, in particular in high doses, stimulating ethylene biosynthesis in plant tissue (Lorteau et al., 2001; Khan et al., 2002). Due to the effect of cytokinin and auxin application, different responses of plants treated may be an effect of a greater ethylene concentration, not a reaction to those hormones themselves. Some of such responses may be earlier flowering and fruiting in some plants (Pandey et al., 2003). Another example is an interaction of cytokinin with auxin in stimulating cambium growth and in the forming of vascular tissue, which results in a better transport of photosynthesis products and, in consequence, in a higher yield (Aldesuquy, 2000). In the past synthetic hormones were used for flower thinning to regulate the fruit number. By dropping off some buds and flowers, the plant regulates the number of fruit (Bangerth, 1989).

However, it happens that too many buds or flowers fall off and then the number of fruits is smaller and so is the yield. Some plants, including soya, bloom profusely but young flowers and pods, contrary to fully grown and ripe fruits, drop off in a considerable number (Cho et al., 2002). As a result, the production potential of such plants is smaller (Rylott and Smith, 1990). Generally, there are differences between plants when it comes to the process of flowering, and those differences are also caused by different hormone activity (Galoch et al., 1996). There is relationship between the amount of endogenous cytokinin and the number of fallen flowers and pods from soybean plants (Noden et al., 1990). According to another study, treating plants with synthetic cytokinin prevents this process, balancing hormone level and activity (Nagel et al., 2001). Some researchers have found that auxin is especially important, in

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particular in legumes, with their yield potential not fully achieved despite the fact that those plants can produce large quantities of organic matter (Reinecke et al., 1999). In the Polish climate soybean is one of plants in which the level of auxin in young flowers is too low, which again may be the cause of flowers and pods falling off, resulting in lower yield. Auxin application may change this process if only because there are some enzymes in plants decomposing natural auxin relatively quickly (Rylott and Smith, 1990).

Table 9. *The effect of growth regulator applied at different times on concentration of cell wall fibre fractions: NDF, ADF, ADL, cellulose and hemicellulose [g kg^{-1} DM]*

Variants	NDF - Neutral Detergent Fibre		ADF - Acid Detergent Fibre		ADL - Acid Detergent Lignin		Cellulose		Hemicellulose	
	Mean ± SE	+, - [%]	Mean ± SE	+, - [%]	Mean ± SE	+, - [%]	Mean ± SE	+, - [%]	Mean ± SE	+, - [%]
Control	539 ±21	-	334 ±19	-	52.8±9.6	-	288±12	-	217± 14	-
AF_1	488±20	-9.3	329±17	-1.5	40.3± 7.8	-20.7	249±16	-13.5	188± 13	-13.4
AF_2	528±17	-2.0	324±18	-3.0	50.8±8.5	-3.8	284±15	-1.4	208±19	-4.1
$AF_{1,2}$	471±18	-12.6	341±16	+2.1	41.7±6.7	-21.0	259±17	-10.1	191±13	-12.8
$HSD_{P=0.05}$	37.2	-	n.s.	-	9.8	-	27.6	-	19.3	-

Mean value ± standard error (SE), +, -[%] − increase, decrease to the control, A – regulator, F_1 – sixth-leaf stage, F_2 – early-bud stage, n.s. – not significant

The seed yield depends on pod formation and, to a large extend, on the amount of photosynthetic products and other nutrients transported to pods. Plant hormones, like auxin and cytokinin, take part in distribution of photosynthetic products, stimulating their transport and storage (Rodrigo et al., 1997). Hormones also take part in regulating transport and distribution of nutrients by affecting growth of recipient sink sells and the activity of enzymes dealing with phloem loading and unloading, but they also function as signal substances indicating that sink cells need to be filled with photo assimilates. Plant hormones affect flower stalk thickening by regulating vascular tissue development, at the same time making transport of photo assimilates from vegetative to generative parts more efficient, which, in turn, increases seed yield (Nagel et al., 2001; Aldesuquy, 2000).

Hormones, apart from regulating transport of nutrients, also affect their uptake by plants and their remobilisation during seed formation, at the same time influencing the yield (Czapla et al., 2005). The growth of aboveground mass and of mineral content in plants as a result of regulator application is in fact an effect of a bigger growth of the root system, in particular the lengthening of the root hair zone (Bertell and Eliasson, 1992; Metwally et al., 1997). In consequence, the absorption of water and dissolved mineral nutrients is more intensive. Auxin is the most important factor in this process because it sends out a signal about the demand of sink sells for nutrients and about their state (Haiser and Jönsson, 2006).

Rylott and Smith (1990) found that in plants treated with synthetic auxin, there may be a growing dominance of generative parts over vegetative parts. It was also confirmed by the research of Pandey et al. (2003), who treated cotton plants with IAA and found increased flower sizes. In turn Nagel et al. (2001) discovered that synthetic cytokinin stimulates vascular tissue development and improves transport of bio assimilates from

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vegetative to generative parts, increasing their content in conducting tissue of plants. Finally, Barcley and McDavid (1998) found that pigeon pea plants developed much bigger pods with bigger mass if they were treated with 6-benzylaminopurine (BAP).

Recently there has been a growing interest in foliar treatment with mineral growth regulators in the form of titanium chelate. It has turned out that this chemical compound increases chlorophyll content in leaves, improves immunity to fungal and bacterial diseases, fosters development and growth of leaves, increasing plant yield (Pais, 1993; Prusiński and Kaszkowiak, 2005). Titanium chelate is predominantly used in growing vegetables. Cucumber plants treated three times with a 0.02% solution of this chelate had up to 100% more seeds. Dressing pea seeds with titanium chelate increased the number of pods and resulted in a 60% growth in their yield. In an experiment with bean plants the weight of seeds was also higher but the yield increased by several percent only (Nalborczyk, 1993).

In an experiment carried out by Prusiński and Kaszkowiak (2005) on the effect of titanium in a form of Tytanit, a foliar fertilizer, on yellow lupine, there was a significant increase of the yield, by 7.4-18%. In fact titanium did not significantly increase the seed yield from the main stem, but its application resulted in a significant rise in the number of pods and seeds from branches. However, protein content and its structure in seeds were more affected by the hydro-thermal conditions than by the application of titanium. Similarly, titanium did not affect seed thickness, which was closely related to growth conditions rather than titanium application. Even if titanium affects the activity of photosynthesis (Pais, 1993), decreasing symbiosis efficiency after plant blossom fall (Ruszkowska, 1991), its effect on the supply of assimilates to seeds is still too low to diversify seed plumpness. However, a better formation of seeds in the pods on the main stem and a significantly higher yield of seeds from branches show a substantial role of titanium in the processes of photosynthesis and accumulation. A double application of a 0.02-0.04% solution of titanium straight after plant blossom fall and 7-10 days later resulted in the highest lupine yield (Prusiński and Kaszkowiak, 2005). Titanium applied to legume plants increases intensity of photosynthesis and the mass of the aboveground parts. As a result of an experiment on the effect of titanium application on tomato plants grown on rockwool it was found that the application of titanium at an annual dose of 960 g $Ti \cdot ha^{-1}$ significantly increased total and marketable yields (Kleiber and Markiewicz, 2013). Plant nutrition with titanium resulted in a significant increase in the yield of fruit with the greatest diameters in comparison with other tested combinations. Titanium application positively affected the content of the following nutrients: nitrogen, phosphorus, calcium, magnesium, and potassium.

Conclusion

1. Treating *Medicago* x *varia* T. Martyn plants with a mixture of synthetic growth hormones and titanium chelate increased the breadth of stomata and the length of the stomatal pore. The same treatment affected the length of plant shoots, the number of shoots, but also the mass of shoots, flowers, and roots. In consequence, the total mass of plants was higher.
2. There was a change in plant phytochemistry, with an increase in the content of protein, phosphorus, potassium, manganese, and titanium in aboveground parts. What is more, plants treated with this substance had a lower

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concentration of crude fibre, NDF and ADL fractions, cellulose, and hemicellulose.

3. Furthermore, it should be noted that the best results were achieved after the plants were treated in the vegetative period, during the six-true-leaf stage (variant AF_1) and when the plants were treated twice (variant $AF_{1,2}$).

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EFFECTS OF FEEDING HYDROPONICS MAIZE FODDER ON PERFORMANCE AND NUTRIENT DIGESTIBILITY OF WEANED PIGS

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Abstract. A study was conducted to investigate the effects of feeding hydroponics maize fodder on performance and nutrient digestibility of weaned pigs. A total of 36 pigs were allocated in a completely randomized design to 3 treatments. Treatment 1 contained 100% concentrate (Con_{100}); Treatment 2 contained 50% concentrate and 50% hydroponics maize fodder ($Con_{50}HM_{50}$) while Treatment 3 had 100% hydroponics maize fodder diet (HM_{100}). Each treatment had 12 weaned pigs with three replicates of four pigs per replicate. The experiment lasted for six weeks. Dietary treatments had significant effects ($P < 0.05$) on final weight, weight gain and feed conversion ratio of the pigs. Pigs fed Con_{100} had the highest ($P < 0.05$) final weight while the lowest weight was recorded in pigs on diet HM_{100}. Feed intake and weight gain were highest ($P < 0.05$) in pigs fed concentrate diet (Con_{100}) while the lowest intake was in animals fed HM_{100}. Feed conversion ratio (FCR) was improved ($P < 0.05$) in pigs fed Con_{100} and $Con_{50}HM_{50}$ respectively. Crude protein, crude fibre and ether extract digestibilities were improved ($P < 0.05$) in animals fed Con_{100}. Pigs fed dietary mixtures of concentrate and hydroponics maize fodder ($Con_{50}HM_{50}$) had better ($P < 0.05$) CP and CF digestibility as compared to those on HM_{100}. Inclusion of hydroponics maize fodder in pig nutrition improved performance and nutrient digestibility of weaned pigs.
Keywords: *sprouted, monogastric, technology, diet, concentrate*

Introduction

Pig production is one of the fastest growing livestock sector in developing countries like Nigeria (Imonikebe and Kperegbeyi, 2014). Compared to ruminants, pigs are prolific, have high feed conversion efficiency, early maturing, require small space and easy to manage (Ouma et al., 2014). According to Tewe and Egbunike (1998), pig production represents the cheapest means of correcting animal protein shortage among the impoverished people in Nigeria. However, their production is facing tremendous set back and on the verge of collapse due to unavailability of feed, which accounts for 70-80% of the total cost of production (Olomu and Oboh, 1995). The major factors responsible for the shortage of green fodder are scarcity of land due to small land holding size, water shortage and labour (Naik et al., 2015).

A possible way of solving this problem of feed scarcity in pig industry is through the use of hydroponic farming systems. Fodder produced by growing plants in water or nutrient rich solution but without using any soil is known as hydroponics fodder, sprouted grains or sprouted fodder (Dung et al., 2010a). Production of hydroponics fodder involves

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growing of plants without soil but in water or nutrient rich solution in a greenhouse (hi-tech or low cost devices) for a short duration - approx. 7 days - (Naik et al., 2015).

Hydroponics technology has been recognized as a viable method of producing vegetables (tomatoes, lettuce, cucumbers and peppers) as well as ornamental crops such as herbs, roses, freesia and foliage plants. Different types of fodder crops such as barley (Reddy et al., 1988), oats, wheat (Snow et al., 2008); sorghum, alfalfa, cowpea (AI-Karaki and AI-Hashimi, 2012) and maize (Naik et al., 2012) can be produced by hydroponics technology. Hydroponics fodder is more palatable, digestible and nutritious while imparting other health benefits to the animals (Suraj et al., 2016). Naik et al. (2015) reported yields of 5-6 folds of fresh hydroponics maize fodder in 7 days. Supplementation of sprouted fodder in the ration of pigs is a viable possible alternative technology to conventional green fodder (Naik et al., 2015). However, there is paucity of information on the use of hydroponically sprouted maize fodder in weaned pigs. Therefore, the objective of this study was to determine the effect of feeding hydroponics maize fodder on the growth performance and nutrient digestibility of weaned pigs.

Materials and methods

Study site and experimental design

This study was carried out at the Piggery Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The farm is situated in Southern Nigeria at $7^{0}20^{1}$N, $3^{0}50^{1}$E at an altitude of 200-300 m above sea level. A total of 36 weaned pigs were purchased from a reputable farm in Ibadan, Oyo state, Nigeria. The pigs were fed *ad libitum* and cool clean water was provided. The pigs were allotted into three groups consisting of 12 animals per group, replicated three times with four pigs per replicate in a completely randomized design. The groups were allocated into 3 treatments as follows- Pigs fed 100% concentrate diet (Con_{100}), pigs fed 50% concentrate diet and 50% hydroponics maize fodder ($Con_{50}HM_{50}$) and pigs fed 100% hydroponics maize fodder (HM_{100}). The concentrate composition is presented in *Table 1*. The experiment lasted for six weeks. Pigs were housed in properly disinfected pens and all routine management practices were strictly observed.

Table 1. *Gross composition of concentrate fed to weaned pigs*

Ingredient	Percentage (%)
Maize	43.00
Soyabean meal	15.00
Wheat offal	15.00
Groundnut cake	7.00
Palm kernel cake	15.00
Palm oil	3.00
Limestone	1.25
Salt	0.50
Premix	0.25
Total	100.00
Calculated nutrient	
Crude protein (%)	19.03
Metabolizable energy (kcal/kg)	2905.20

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The hydroponic system

The production of hydroponic maize fodder, that is, sprouted maize grown in a nutrient solution was conducted under natural illumination in growth chamber as described below. The maize used was grown for 7 days.

Planting material

Maize grains were obtained from a local market. The seeds were cleaned from debris and other foreign materials and were subjected to a germination test to check for viability. Clean seeds were washed, sterilized in Hydrogen Peroxide (H_2O_2) solution and soaked in tap water for 24 h before distribution in the trays.

Seed planting and irrigation

Seeds of maize were sown in the planting trays. The seedling rate used was 500 g of maize grain per tray. Trays were irrigated manually with organic hydroponics nutrient solution twice a day (07:30 and 17:30 hr) at a fixed rate of 250 ml/tray/day using a spray gun for 20 s. Drained water were collected in plastic containers which were placed under each planting tray and measured. The seeds were scattered uniformly within the tray. The tray was kept in cool and well illuminated environment.

Green fodder harvesting

The sprouted seeds were grown in the greenhouse for a period of 7 days. The fully grown fodder (*Fig. 1*) was then given to the pigs as whole feed.



Figure 1. *Hydroponic maize fodder (Source: Adebiyi et al., 2018)*

Fodder yield

Samples of the green fodder were taken weekly to determine the dry matter and nutrient contents. The quantity of hydroponics fodder and biomass production was recorded daily by weighing the seeds before planting and weighing the fodder produced.

Growth performance

Growth parameters of the pigs were observed and recorded throughout the experiment. Feed intake was obtained by subtracting the leftover feed from the total quantity of feed served. Weight gain was determined by subtracting the initial live

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weight from the final live weight. Feed conversion ratio was defined as the quantity of feed (kg) consumed to gain a unit of live weight (kg):

$$FCR = \frac{\text{Feed Intake}}{\text{Weight Gain}}$$

Nutrient digestibility

At day 37, three animals were randomly selected from each treatment. The selected pigs were kept in metabolic cages for 5 days. Feed intake and total faecal collection from individual animals were recorded. The experimental feed and faecal samples were dried. The feed and faecal samples were further analyzed for crude protein (CP), crude fibre (CF), ether extract (EE) and ash contents using the procedure of AOAC (2000). The nitrogen free extract (NFE) contents of the samples were obtained using the equation:

$$NFE = 100 - (CF + CP + EE + Ash)$$

Statistical analysis

Data on performance and nutrient digestibility were analysed using the General Linear Model (GLM) procedures of the statistical analysis of variance (SAS, 2010). Duncan Multiple Range Test was applied for mean separation where there were significant differences ($P < 0.05$).

Results and discussion

The results of the proximate composition of the hydroponics maize fodder are presented in *Table 2*. There are changes in the nutrient content of the maize grains and hydroponics fodder. The average dry matter (DM) content of the maize seed was 95.08% whereas the hydroponic maize fodder was 25.00%. The decrease observed in the DM may be due to the decrease in the starch content of the hydroponics fodder. During sprouting, starch is catabolized to soluble sugars for supporting the metabolism of energy requirement of the growing plants for respiration and cell wall synthesis, so any decrease in the amount of starch causes a corresponding decrease in DM (Naik et al., 2015). This result is similar to the findings of Thadchanamoorthy et al. (2012), who reported a DM content of 26.07% for sprouted maize fodder. The crude protein content in the present study showed that hydroponic maize fodder contained 13.75% CP as compared to 8.7% in maize seed. This observation has been reported by other authors (Dung et al., 2010a; Naik et al., 2015). According to Dung et al. (2010a), the use of nutrient solution enhances the CP content of the hydroponics fodder which is higher than the tap water, thus leading to the uptake of nitrogenous compounds. Additionally, sprouting has been reported to alter the amino acid profile of maize seeds and increases the crude protein content of hydroponic fodder (Morsy et al., 2013). In the present study, ether extract of the hydroponics maize fodder was 3.55%. The value reported in the current study was slightly higher than the range of 3.27-3.49% obtained by Singh (2011) and Naik et al. (2015). The increase in the EE content of the hydroponics fodder

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may be due to the increase in the structural lipids and production of chlorophyll associated with the plant growth (Naik et al., 2015).

The CF content of 14.77% was within the range of 7.35-21.20% reported by Naik et al. (2015). Increase in CF contents of hydroponics maize fodder may be attributed to the build-up of cellulose, hemicelluloses and lignin (Cuddeford, 1989). The value of total ash (3.33%) observed in the hydroponic maize fodder in the current study is within the range of 1.75-3.80% reported by Naik et al. (2014). During the sprouting process, the total ash content increases due to the absorption of minerals by the root (Dung et al., 2010b). The NFE content observed (60.72%) was higher than that of hydroponically sprouted grains reported by earlier workers - 1.56-3.64% (Naik et al., 2015).

Table 2. *Proximate composition of hydroponics maize fodder*

Parameter	Percentage (%)
Dry matter	25.00
Crude protein	13.75
Ether extract	3.55
Crude fibre	14.77
Ash	3.33
Nitrogen free extract	60.72

The results of hydroponics maize fodder on the performance of pigs are shown in *Table 3*. Dietary treatments had significant effects ($P < 0.05$) on final weight, weight gain and feed conversion ratio of the pigs. The final weight ranges from 9.04-17.08 kg. Pigs fed Con_{100} had the highest ($P < 0.05$) final weight while the lowest weight was recorded in pigs fed HM_{100}. Feed intake and weight gain were highest ($P < 0.05$) in pigs fed concentrate diet (Con_{100}) while the lowest intake was in animals fed HM_{100}. Feed conversion ratio (FCR) was improved ($P < 0.05$) in pigs fed Con_{100} and $Con_{50}HM_{50}$ respectively. In the present trial, pigs fed dietary mixture of concentrate and hydroponics maize fodder ($Con_{50}HM_{50}$) performed better than those fed solely on sprouted fodder (HM_{100}). Helal (2015) reported higher dry matter intake, final body weight and improved FCR in goats fed dietary mixture of sprouted barley grains and barley straw. Similar results were reported by Fayed (2011) and Helal (2012). Hydroponic sprouts are rich sources of bioactive enzymes and contain grass juice ingredients that improve the performance of livestock (Naik et al., 2013). The increase in weight gain of pigs may be attributed to enhancement of microbial activity in the gut. According to Kruglyakov (1989), hydroponics fodder has simpler forms of vitamin, starch, protein and lipids which have positive effect on the performance of the animals. Nutritional value of sprouted fodder improves due to the modification of heterogeneous compounds into essential form (Chavan et al., 1989). Sprouting of grains has resulted into increase in quantity and quality of protein, sugars, minerals and vitamin (Naik et al., 2015). Weight loss was recorded in pigs fed solely on hydroponics fodder (HM_{100}). This observation may be due to the low DM intake of the pigs. Additionally, pigs being monogastric cannot thrive solely on fodder-based diet.

Feeding hydroponics maize fodder to pigs led to a decrease in the total daily feeding costs of experimental rations, $Con_{50}HM_{50}$ (#61.96) and HM_{100} (#53.97) as compared to Con_{100} (#70.00) which was mainly a concentrate diet (*Table 3*). Feed costs (#/kg)

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significantly reduce as the inclusion of the maize fodder increases. This result is similar to the findings of Fayed (2011) and Helal (2012) who found the lowest feed cost and highest profit in lambs fed dietary mixture of sprouted barley grains and *Tamarix mannifera*. In terms of economic efficiency, it is more profitable (feed cost per weight gain) to feed pigs on $Con_{50}HM_{50}$ (#228.01) as compared to Con_{100} (#316.4) and HM_{100} (#-446.91).

Table 3. *Performance and cost benefit of feeding hydroponics maize fodder to pigs*

Parameters	Con_{100}	$Con_{50}HM_{50}$	HM_{100}	SEM	P-value
Initial BW (Kg)	9.90	10.00	9.97	0.009	0.6094
Final BW (Kg)	17.08^{a}	13.35^{b}	9.04^{c}	0.775	0.0026
Feed intake (DM Kg)	30.89^{a}	12.72^{b}	8.00^{c}	1.334	0.0001
Weight gain	7.18^{a}	3.46^{b}	-0.93^{c}	0.783	0.0021
FCR	4.52^{a}	3.68^{a}	-8.69^{b}	1.425	<0.0001
*Feed cost/kg (#)	70.00	61.96	53.73		
Feed cost/weight gain (#/kg)	316.4^{a}	228.01^{b}	-466.91^{c}		

SEM: Standard error of the mean
^{a,b,c}Means within the same row with different letters are significantly different ($P < 0.05$)
BW: body weight, DM: dry matter, FCR: feed conversion ratio
*Calculated by multiplying the cost/kg of each ingredient used to formulate the diet by the quantity of the ingredient

Nutrient digestibility of pigs fed hydroponic maize fodder is presented in *Table 4*. Crude protein, crude fibre and ether extract digestibilities were improved ($P < 0.05$) in animals fed Con_{100}. However, animals fed dietary mixtures of concentrate and hydroponics maize fodder had better ($P < 0.05$) CP and CF digestibility as compared to those on HM_{100}. Helal (2015) recorded highest digestibility coefficients of CP, CF, EE, NDF and hemicellulose in goats fed sprouted barley. Similar results were reported by Fayed (2011) and Naik et al. (2015). Feeding of hydroponics fodder increased the digestibility of the nutrients which could be attributed to the tenderness of the fodder (Naik et al., 2014). The digestibility of the nutrients of the hydroponics fodder was comparable with the highly digestible legumes like berseem and clovers (Pandey and Pathak, 1991). According to Chung et al. (1989), highly soluble protein and amino acids in response to the early plant growth and enzymatic transformations of sprouted grains are responsible for improved digestibility in animals. The low CF digestibility reported in HM_{100} may be attributed to the minimal crude fibre utilization in monogastrics.

Table 4. *Nutrient digestibility of pigs fed hydroponics maize fodder*

Parameter	Con_{100}	$Con_{50}HM_{50}$	HM_{100}	SEM	P-value
CP	70.51^{a}	66.27^{b}	55.41^{c}	1.50	<0.0001
EE	72.98^{a}	67.37^{c}	68.43^{b}	0.57	<0.0001
CF	50.91^{a}	48.77^{b}	34.82^{c}	1.68	<0.0001
Ash	39.45^{b}	43.52^{a}	42.63^{a}	0.41	0.0001

SEM: standard error of the mean
^{a,b,c}Means within the same row with different letters are significantly different ($P < 0.05$)
CP: crude protein, EE: ether extract, CF: crude fibre

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Conclusion

Inclusion of hydroponic maize fodder in pig nutrition improved performance and nutrient digestibility of weaned pigs. Thus, there is great potential for developing hydroponic technology for fodder production in pig farming. Sole feeding of hydroponic maize fodder exerted negative effects on the performance of the animals. Further research is needed to establish the potential health benefits of hydroponic fodder in monogastrics.

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ASSESSMENT OF ECONOMICAL BIOGAS PRODUCTION FROM CHILEAN MUNICIPAL SOLID WASTE IN A DECENTRALIZED OFF-GRID STRATEGY

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Abstract. The management of municipal solid waste (MSW) is one of biggest challenges that developing countries are currently facing. Despite several environmental friendly alternatives being available, in Chile 90 % of MSW is disposed of in landfills, which produces large amounts of greenhouse gases (GHG) by the uncontrolled anaerobic digestion of its organic fraction. In addition, the way in which MSW is collected and transported increases the negative impact to the environment, especially when wastes classification is not properly performed at the generation point. On the other hand, the use of resulting biogas, in a decentralized off-grid form, at the generation point, could directly reward to waste producers with economic saving. This strategy will reinforce population motivation and an important reduction of the GHG emissions can be expected. Thus, this paper explores the biogas potential of food wastes by using reactors with no heating and stirring devices, with the aim of assessing the minimum expectable energy and $CO_{2,eq}$ savings. Based on laboratory tests at batch and semi-continuous mode and Chilean historical data, it is concluded that more than 1,704 Gg of $CO_{2,eq}$ can be avoided per year and an average of 425 GWh primary energy may be saved by Chilean householders.
Keywords: *CO_2 emission, psychrophilic, food waste, household scale, waste management*

Introduction

Municipal solid waste (MSW) is currently a great concern for modern societies. A sustainable coping strategy is required in order to protect not only environment but human health as well. The increase in the urban population growth rate has led to increased issues related to MSW management, especially in developing countries where traditional landfill disposal is the most common method of MSW management (Guerrero et al., 2013). It is obvious, in accordance with the residues management hierarchy that the most favorable option to deal with MSW is prevention. However, the reduction of the current levels of MSW must be accompanied by better management of the waste since and it may be highlighted that some parts are inedible and will unavoidably become a waste stream.

There are countless alternatives to manage the organic fraction of MSW which is mainly composed by food waste (FW) and garden waste. However, anaerobic digestion (AD) has been highlighted as the most reliable and efficient technology for minimizing the environmental impact of FW waste disposal in terms of greenhouse gas (GHG) emissions, which are generated during landfilling. In addition, AD of FW offers an

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opportunity to produce a large energy yield of biogas and it has been demonstrated rapid public health improvements with important reductions in pathogens (Remais et al., 2009).

The AD process is well known, and related technology has already been successfully tested although MSW collection and separation still remain as key unknown factors for determining the feasibility of AD plants (Olsson and Fallde, 2015). Different systems for FW separation and collection at the generation point are successfully implemented in developed countries, in decentralized or centralized form. However, all these alternatives were supported by several subsystems, which are not yet extended in Chile, such as: the existence of a formal recycling industry, optimal distribution of curbside services and the development of policies for raising awareness, in terms of wastes classification, etc. Several countries have lately developed micro-scale applications to reduce transport requirements by means of creating a "biorefinery" that would dispose of local waste and produce energy (Walker et al., 2017). These micro-scale applications were developed by specific consortium of companies but general public was not included as an active stakeholder. In other words, the main goal was the enhancement of some industrial process rather than the minimization of urban waste management issue. These examples incorporated several complex devices such as; mixers, filters, feeding pumps, water heat exchangers, etc. that increase the installation and operation costs which have been highlighted as the most critical barrier for the popularization of biogas technology (Walekhwa et al., 2009).

On the contrary, small fixed dome digesters that work without stirring or heating have been successfully operated on the long term, mainly in developing countries with installation costs that range from 80 to 150 USD/m^3 (Buysman and Mol, 2013). These AD reactors show very low energy performance since the more complex the reactor configuration is, the more biogas will be produced and fewer impurities will be contained. Moreover, these simple AD plants are designed without cleaning and upgrading biogas stages and consequently power generation or methane grid injection are rarely considered. In spite of such limitations, household scale biogas reactors contribute to reduce $CO_{2,eq}$ emissions, soil and water pollution as well as improve sanitation by reducing pathogenic content of substrate materials. For instance, it has been showed that AD at 20°C reduced indigenous populations of total coliforms by 97.9 to 100 %, indigenous populations of E. coli by 99.6 to 100 %, and provided undetectable levels of indigenous strains of Salmonella, Cryptosporidium, and Giardia (Massé et al., 2010). Besides, the use of biogas represents an opportunity for lowering family expenditures cooking and/or heating fuels which ensure the participation, ownership and responsibility of the final users (Bond and Templeton, 2011), otherwise the lack of proper awareness among user (i.e. householders) inhibits the adaptation of technology.

The proposal

MSW management is nowadays especially worldwide discussed but specially into the Chilean context due to the reviewed national strategies regarding the environmental sustainability politics.

The first step for designing a properly strategy must be always the collection of characteristic data. However, the first national report was published in 2010 and till nowadays no further official update has been provided. Furthermore, Chilean statistics,

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regarding MSW management, show important differences, depending on whether data are provided by public services (CONAMA, 2009; OECD, 2005) or by previous research (Perez, 2010). Besides, some data regarding the organic fraction amount of MSW are missed (see *Fig. 1*). Nevertheless, by accepting data provided by this first national report, Chile is ranked as the major MSW producer in Latin America, with an annual average of 382.4 kg per capita and the organic fraction of Chilean MSW accounts for up to 60 % (90 % FW and 10 % garden wastes) (CONAMA, 2010a).

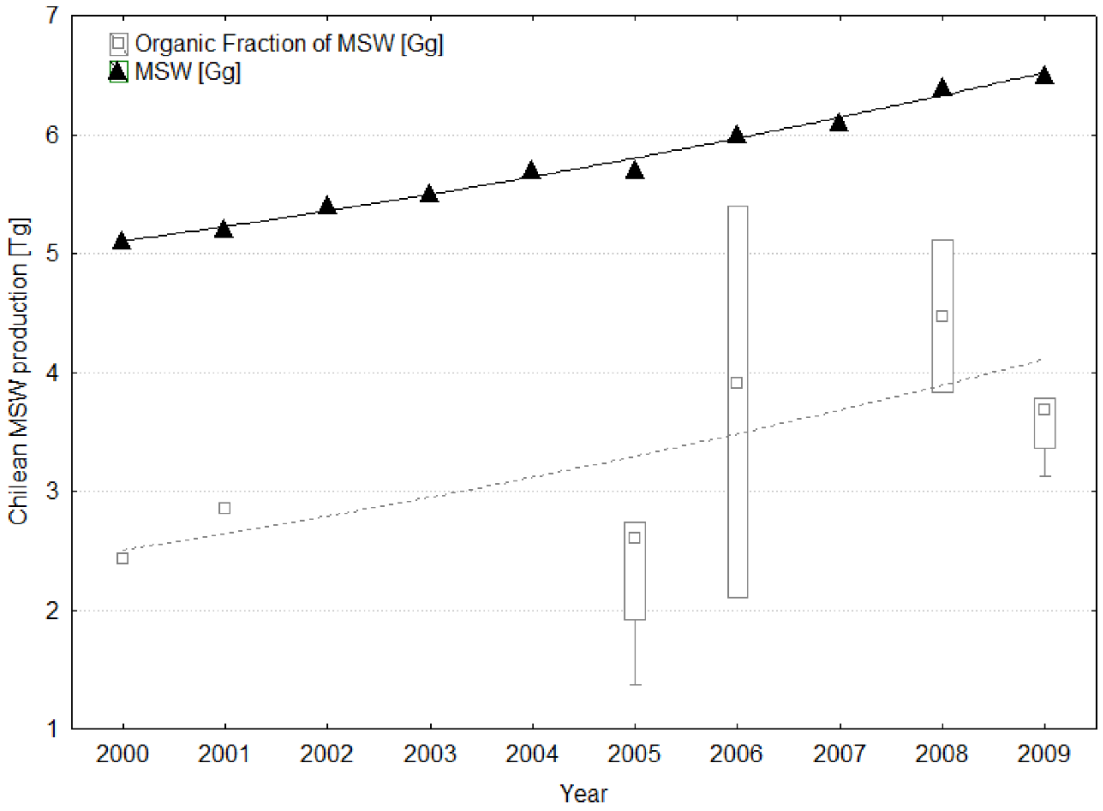


Figure 1. *Statistics of the Chilean MSW production and the amount of organic matter contained by the MSW*

Regarding the available MSW treatment facilities, Chile owns approximately 120 recycling facilities focused on plastics, cartons, oils and metals. These installations are able to recycle approximately 61 % of metals (including aluminium), 55 % of oils, 41 % of papers and cartons, 29 % of glass and 4 % of plastics. However, only 10 % of the organic fraction is currently treated. This organic matter is composed of 90 % food waste (FW) and 10 % gardening waste. Hence, there are 13 installations designed for composting and four that are based on co-incineration. The remaining organic waste is disposed of in 25 sanitary landfills and 139 uncontrolled waste dumping sites which receive MSW from approx. 239 cities. Distances between cities and these places range between 5 and 30 km (CONAMA, 2010b). Despite annual FW generation exhibiting increasing trends, there are no planned future investments, in the short-term, in terms of revalorization or recycling facilities.

MSW is not only an economic and social concern but also an environmental issue, since it is one of the major carbon emission sources from city operations (Galina, 2008).

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According to the Chilean Ministry of Environment (2011), landfills gas emissions account for approximately 2,300 Gg of $CO_{2,eq}$ and these emissions are mainly produced by the uncontrolled AD of the organic matter. From this point of view, it is clear that a reduction of organic matter content leads to a decrease in GHG emissions. Since there is no gas collection and flaring systems in most of Chilean landfills, at present, landfills are the highest contributors to GHG emissions. In addition, MSW introduces another source of environmental impact, the so-called horizontal $CO_{2,eq}$ flows. These refer to the cumulative gases emission produced by the collection (i.e. from single households or other waste generation units) and transportation of collected MSW to landfills. In Chile, collection and transportation is performed by collection trucks that are fuelled by fossil fuels (i.e. diesel).

On the other hand, fossil fuels consumption highly impacts on the national economy since Chile does not own fossil fuels. In spite of Chile lacks of fossil fuel resources, more than 60 % of the energy, in terms of primary energy, is provided by imported fossil fuel. As result, the highest percentage of Chilean household expenses corresponds to energy supplies and this cost is increased year by year (e.g. from 2008 to 2012 natural and liquefied gas consumption was increased by 720 %) according to the Ministry of Economy, Promotion and Tourism (SERNAC, 2015).

As it has been previously stated, AD may address all these aspects by reducing the amount $CO_{2,eq}$ emissions and the household expenses related to the energy consumption. However, the key factor for a successful strategy, regarding MSW, is always related to the waste generation point (i.e. householders). Hence, it has been demonstrated that any strategy for MSW management should be well supported by citizen participation and therefore participation and motivation of population is always required to ensure the success of any waste management policy (Wen et al., 2016). At this point, any proposal must consider the waste producer (i.e. the householder) as the most important player.

It is well understood, that a decentralized strategy highly reduces the transportation of waste materials, which represents the main source of pollution regarding the MSW management chain (Alfonso et al., 2009). Besides, AD of FW contributes to reduce energy demand, and the aforementioned MSW impacts. However, one of the main difficulties of sustaining decentralized facilities is the organic waste availability for feeding the anaerobic digester (Righi et al., 2013) (i.e. the cooperation of waste producers). Therefore, in accordance with similar experiences in other countries (Reddy, 2004), this paper assesses the use of FW, generated by households, for biogas production by using household scale fixed dome reactors which will be daily loaded with this FW. However, the proposal cannot be based on expensive units but on simple small reactors working at psychrophilic range by means of simple fixed dome reactors.

The proposal considers that each building would incorporate a single stage reactor and biogas would be directly used on site by means of boilers or cooking stoves. With the aim of lowering the costs the proposal does not considered the use of biogas for electrical power generation or its injection to the natural gas grid. Consequently, complex cleaning biogas systems are not required and the corresponding installation costs are highly reduced. The FW generated would be disposed of in this AD unit and the effluent would be direct discharged as wastewater in sewers (see *Fig. 2*).

Despite previous authors have estimated a decade ago that biogas might contribute up to 45 % to reduce natural gas consumption in Chile (Seiffert et al., 2009; Bidart et al., 2013), currently AD provides approx. 0.03 % out of total primary energy

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consumption. This unexpected result is explained by two main reasons. On the one hand, authors assumed the expansion of the existing gas pipeline, but no relevant changes have been performed during last decade and this highly impacts economic feasibility. On the other hand, calculations were mainly based on residues provided by forestry industry, wood processing and energy crops which are already being used by other markets (Ignaciuk et al., 2006). In addition, it must be mentioned that co-digestion of FW with other household waste streams has not been considered. In spite of literature has demonstrated that specific biogas yield (SBY) is certainly improved by co-digestion (Iqbal et al., 2014) the potential inhibition of AD (e.g. detergents or papers towels) would make the proposal less reliable (Chen et al., 2008).

The proposal was assessed by determining the technical feasibility of the AD at psychrophilic temperature and by estimating the impact in terms of $CO_{2,eq}$. AD tests were performed in two phases: firstly batch and then semi-continuous operation mode, both at laboratory scale. This, since the batch test is of necessity performed with different substrate mass fractions than the semi-continuous test, and the substrate/inoculum ratio affects the kinetics. Finally, the obtained results were used for calculating the amount of $CO_{2,eq}$ that might be avoided.

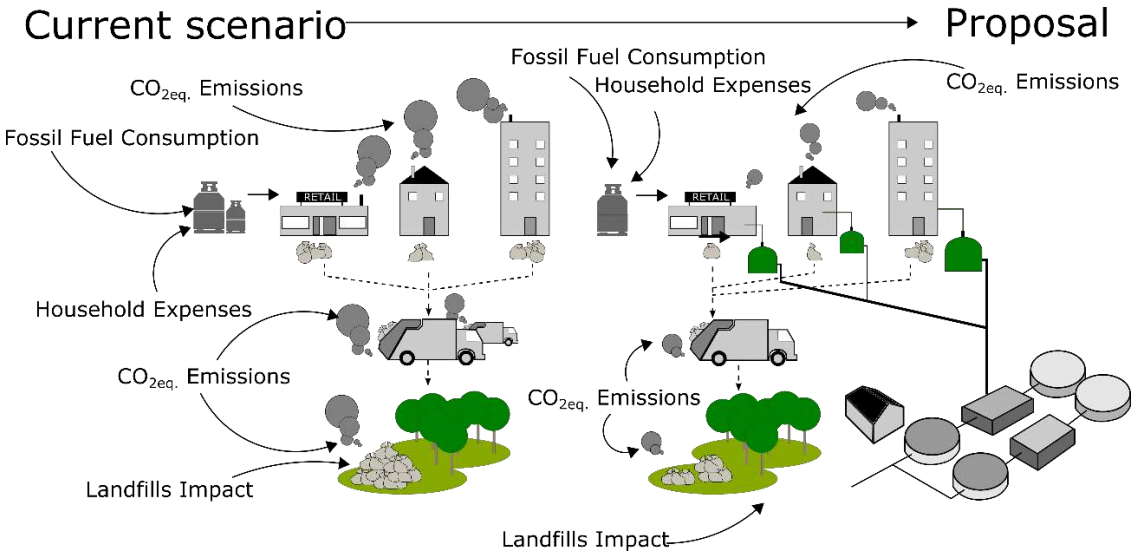


Figure 2. *Diagram of the decentralized AD approach proposed for reducing CO_{2eq} emissions by saving fossil fuels and fugitive emissions*

Materials and methods

AD tests

The AD test was divided into three stages. In the first step, FW and inoculum were characterized. Then, these values were used for setting the biochemical methane potential (BMP) tests. Finally, a semi-continuous experiment with lab-scale digester was carried out, along 90 days, maintaining a fixed organic load rate. These last two stages were performed in accordance with VDI 4630:2016-11.

The canteen, at the Autonomous University of Chile, provided FW on a weekly basis. This included animal and fish bones, fruits and vegetable peelings, seeds and any other organic matter. FW was blended with an industrial blender in order to permit comparison between the results of different fermentation tests (particle sizes of less than

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10 mm) (Omniblend TM767, Chile) and homogenized. Then, 250 g of FW (approx. 250 g) was used for determining FW characteristics and 250 g of FW was frozen (-20 °C) till being used for loading reactors. An industrial partner, who operates a nearby AD plant, provided the inoculum. This AD plant works on mono-digestion, based on pig manure, at psychrophilic range. Inoculum was sieved throughout a 5 mm mesh for removing the death matter.

Regarding the FW and inoculum characteristics, total solids, (TS) and volatile solids (VS) were determined using an electric furnace set at 105 °C and 600 °C, respectively. Total soluble solids (TSS) and Total Dissolved Solids (TDS) were determined according to 2450-D and 2450-C methodology of the Standard Methods (APHA, AWWA, and WEF, 2005). Soluble nitrogen was measured using HANNA nitrogen determination (chromotropic acid method; range: 10 to 150 mg/L N at 420 nm; accuracy: ± 3 mg/L or ± 4 % of reading at 25 °C). The pH and Oxide Reduction Potential (ORP) were determined using a WTW pH 3110-2. Chemical Oxygen Demand (COD) was analyzed by COD Cell Test (Spectroquant®, Merck KGaA, 64271 Darmstadt, Germany) and the applied method corresponds to DIN ISO 15705, which is analogous to EPA 410.4, APHA 5220 D and ASTM D1252-06B. C:N ratio was determined following the standard methodology proposed by The U.S. Department of Agriculture and the U.S. Composting Council (TMECC, 2002). Total carbohydrates were quantified by following the visible range method using phenol-sulfuric. Total oils and fats were determined in accordance with the method extraction Soxhlet (APHA, AWWA, and WEF, 2005) and protein quantification was determined by means of the Pierce BCA Protein Assay Kit (Thermo scientific).

BMP was determined in triplicate (Ra, Rb, Rc) at laboratory scale (5 L batch reactors) (see *Fig. 3*). In spite of containers used for the fermentation test use to be 0.5 L, 1 L or 2 L flasks, in case of inhomogeneous substrate standards recommend to have larger fermentation volumes as this makes it easier to obtain representative samples. These reactors were equally filled with 150 g. of FW (i.e. 7.5 g VS/L), 1000 mL of inoculum and 3000 mL of fresh water. Hence, headspace volume was set at approx. 1000 mL. It must be highlighted that the organic total matter added from inoculum is quite lower than the recommended by standards and therefore the ratio substrate to inoculum rate (SIR) is higher. The main reason for using a high SIR is explained by two considerations. On the one hand, the results, provided by the semi-continuous test, in the long-term are not influenced by the inoculum used in the batch tests. On the other hand, further assays will be focused on the analysis of SIR in order to assess FW buffering capacity, which are not included within the present paper.

Reactors were kept at room temperature without stirring in order to simulate the conditions of the proposed household scale reactors by following VDI 4630:2016-11. The substrate temperature has been continuously measured and recorded by using a stainless steel temperature probe and data logger (Testo, Germany).

In spite of the initial pH adjustment is considered in different way, depending on the followed standards, previous authors have demonstrated that below an initial pH of 6.0, AD of FW is inhibited (Zhai et al., 2015). Thus, the pH was adjusted to 7.0, by means of NaOH, in all batches. Reactors were sealed and remaining air was removed by nitrogen gas flow.

According to VDI 4630:2016-11 guidelines when the new biogas production was found below 0.5 % after three days, the AD batch was ended and the semi-continuous phase took place.

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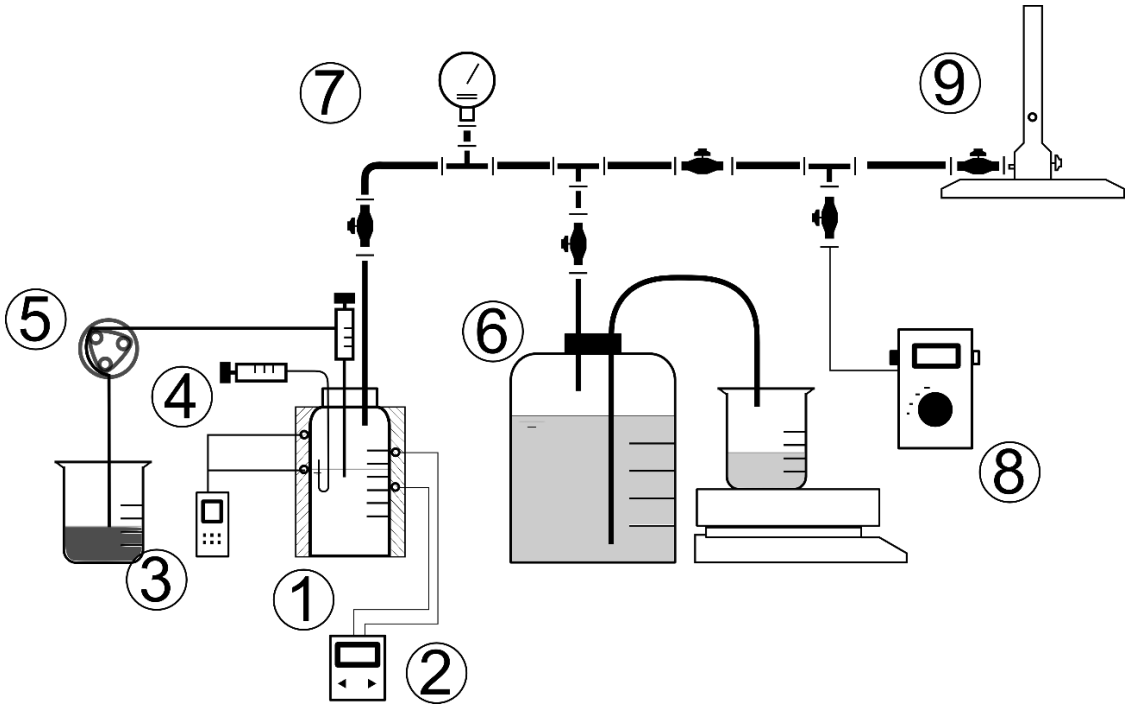


Figure 3. *The schematic representation of experimental laboratory scale biogas test showing (1) 5 L reactors; (2) Temperature probes and data logger; (3) pH and ORP probes; (4) overflow collection; (5) peristaltic pump for loading FW; (6) gas meter; (7) manometer; (8) gas analyzer; (9) burner*

Same reactors were used at semi-continuous stage and running without stirring and without temperature control. AD of FW is prone to instability at high organic load rate and because of that OLR was set at 1 g-VS/L day (i.e. 1-day-portions of 20 g FW) with a volumetric FW to water dilution of 1:2. During the loading operation, reactors were not de-areated. Feeding and digestate removal ware carried out automatically once every day by means of a peristaltic pump, during 90 days and removed digestate included solids and liquid.

The overflowed digestate was analyzed with the aim of determining whether this effluent meet with the requirements set by the national policies and guidelines on wastewater disposal systems. Therefore, parameters such as salinity, electrolytic conductivity (EC), ORP and TDS were measured by using a HI9828 probe made by Hanna.

The measured wet biogas and methane volumes were measured by water displacement and adjusted to the volumes at standard temperature (0 ºC) and pressure (1 atm). The effect of the vapor pressure of water was corrected by using the Antoine equation and the average methane content during the semi-continuous phase was showed (Angelidaki et al., 2009).

Calculations

Energy calculations have been calculated based on the primary energy carried by methane. Since statistics are developed at a regional scale, energy production is

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calculated as the sum of every regional contribution, in accordance with similar previous studies (Moreda, 2016; *Eq. 1*):

$$E = VS \cdot Q_{CH_4} \cdot H_{CH_4} \cdot \sum_{i}^{n} q_i \cdot f_i \quad \text{(Eq.1)}$$

where VS is the average volatile solids content of MSW (g VS/Mg); Q_{CH4} is specific biomethane yield (m^3/g_VS); H_{CH4} is volumetric heating value (kWh/m^3) of biomethane at standard conditions; q is regional MSW and fi is the percentage of FW within MSW (%).

Regarding the $CO_{2,eq}$, calculations have taken into account two main aspects: On the one hand, the $CO_{2,eq}$ emissions avoided by both the reduction of organic waste disposed of at landfills and the corresponding horizontal flows, (i.e. those produced by collection and transportation). On the other hand, the impact of the substitution of fossil fuels (i.e. natural and liquefied gas) by biogas has been quantified.

Despite AD mainly produces CO_2 and CH_4, only CH_4 emissions have been accounted. This because of CO_2 release is only a partial "return" to the atmosphere of the same gas used by photosynthetic organisms to build their biomass (Calabrò, 2009). In addition, for the purpose of estimation, it is assumed that there is no methane oxidation in landfills cover soil and 100 % is released into the atmosphere. Hence, it is possible to estimate the avoided equivalent carbon emissions, based on the conversion factor of methane, in accordance with previous authors (Uusitalo et al., 2014; Friedrich and Toris, 2016; Liamsanguan and Gheewala, 2008). The system boundaries also include collection and transportation of FW from its source to landfills, the so-called horizontal $CO_{2,eq}$ flows. These refer to the cumulative effort of loading waste from single households or other waste generation units from the first stop to the last stop on the collection route and include driving the full truck to the point of unloading. In the estimation, only the energy used for operating the collection trucks is considered. GHG emissions created in the production of collection bags, bins and containers or the manufacture and maintenance of collection vehicles themselves are not considered (Eisted et al., 2009). Due to the on-site AD operation, the overall MSW weight requiring transportation is reduced which highly impacts on $CO_{2,eq}$ emissions produced by collection system (Lee et al., 2007; Lundie and Peters, 2005). In Chile, MSW transportation is performed by diesel-motor trucks. Hence, its contribution may be calculated by the following expression (*Eq. 2;* Zhou et al., 2014):

$$Q = M \cdot P \cdot \mathrm{C} \cdot \mathrm{D} \cdot \rho \quad \text{(Eq.2)}$$

where M is the mass of FW per annum (Mg/y); P: is the average diesel consumption for transportation of FW (0.022 L/(Mg km)); C is the carbon content of the diesel (86.86 %); D is the average transportation distance of the MSW from city to landfill (approx. 15 km) and ρ is the density of the diesel (0.85 kg/L).

It can be noted that these estimations account only for the collection and transportation of the FW fraction since the proposal does not avoid the use of trucks for collecting the rest of waste produced by households. Therefore, values provided by other authors (Larsen et al., 2009) that include diesel consumption for driving empty truck from garage to collection area and from point of unloading to collection area or garage have been not considered. Furthermore, produced biogas is substituted for fossil fuels and consequently another reduction of GHG emissions must be considered. The

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avoided impacts due to the use of AD effluent as fertilizer have been excluded since compost may not comply with specific legislative requirements. Despite the remaining digestate may substitute nitrogen, phosphorous and potassium fertilizers with an efficiency of 34.5 %, 46 % and 60 %, respectively (Salemdeeb et al., 2017), effluent should be dewatered and refined, which leads to a more complex design. On the contrary, if the effluent is directly used as fertilizer, without further treatments, some problems such as pathogens, antibiotics and/or heavy metals, among others, might be expected in the long term.

Results and discussion

AD tests

It must be highlighted that it is particularly difficult to obtain representative FW samples and, therefore, an approach appropriate based in statistical methods is required. Despite the randomly strategy for collecting samples and the long term at it was performed, the results might show some scattering when they are comparing, mainly, due to the different eating habits. However, results (summarized in *Table 1*) were found within the range showed by previous authors (Lie et al., 2009; Yirong et al., 2015; Li et al., 2013). Only, carbohydrate and proteins contents were highlighted lower than average values 55.2 %, 15 % and 23.9 %, respectively in dry basis (Zhang et al., 2014).

Table 1. *Food waste (FW) and inoculum characteristics*

Characteristics	Units	FW	Inoculum
Moisture Content[1]	%	78.6 (3.8)	98.81 (0.1)
TS[1]	%	21.4 (3.8)	1.19 (0.8)
VS[1]	%	19.8 (3.7)	0.48 (0.2)
TSS[1]	g/L	13.8 (0.3)	2.02 (0.1)
pH[1]	-	4.42 (0.4)	6.97 (0.2)
ORP[1]	mV	192.5 (35.8)	-219 (17.2)
Total carbohydrates[2]	%	27.8 (4.8)	-
Proteins[2]	%	6.05 (0.64)	-
Oil and grease[2]	%	22.84 (0.1)	-
Soluble Nitrogen[1]	mgN/L	86.2 (26.0)	-
Ash[2]	%	5.48 (0.8)	-
C:N	-	21.49 (4.21)	-
COD[1]	mg O_2/L	33,488 (575)	6,213 (0.1)

[1]Wet basis; [2]dry basis; (-) values between parenthesis show standard deviation

These differences in FW composition highly influence biogas production since substrates poor in easily degradable carbohydrates yield the lowest methane potential. For instance, the aforementioned average values (Zhang et al., 2014) might produce a theoretical SBY of approx. 864 mL/g-VS while the theoretical biogas in case of used FW is approx. 573 mL/g-VS (Drosg et al., 2013). However, there are some other parameters that highly influence the biogas production, such as temperature and waste to inoculum ratio, among others (Liu et al., 2009; Deepanraj et al., 2015).

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Batch test clearly shows three different steps (see *Fig. 4a*) and the shape of the obtained curve corresponds to the so-called retarded degradation. The first phase is provided by the substrate itself and the inoculum as the carrier of the methanogenic bacteria consortium. Then, there is an intermediate lag phase, in which hydrolysis is developed and pH drops from 7.00 to 6.00 because of the formed acids. This stage is followed by an exponential growth in biogas production that ends when most of the organic matter is transformed (Córdoba et al., 2016). At this point, the average biogas production was 150±13 mL/g-VS and at the end of the test pH value is set above 6.8 which indicates that the fatty acid concentration does not exceed 200 mg/L and therefore the measurement is accepted. Nevertheless, SBY is lower than values provided by previous BMP tests which are commonly performed at higher temperatures. Temperature plays an important role in terms of SBY regarding the anaerobic biodegradation of the complex organic matter. Thus, several authors have showed a reduction on biogas yield that varies from 31 to 60 % when AD temperature decreases from 30 °C to 15 °C and 55 °C to 20 °C, respectively (Kumar et al., 2016; Bouallagui et al., 2004).

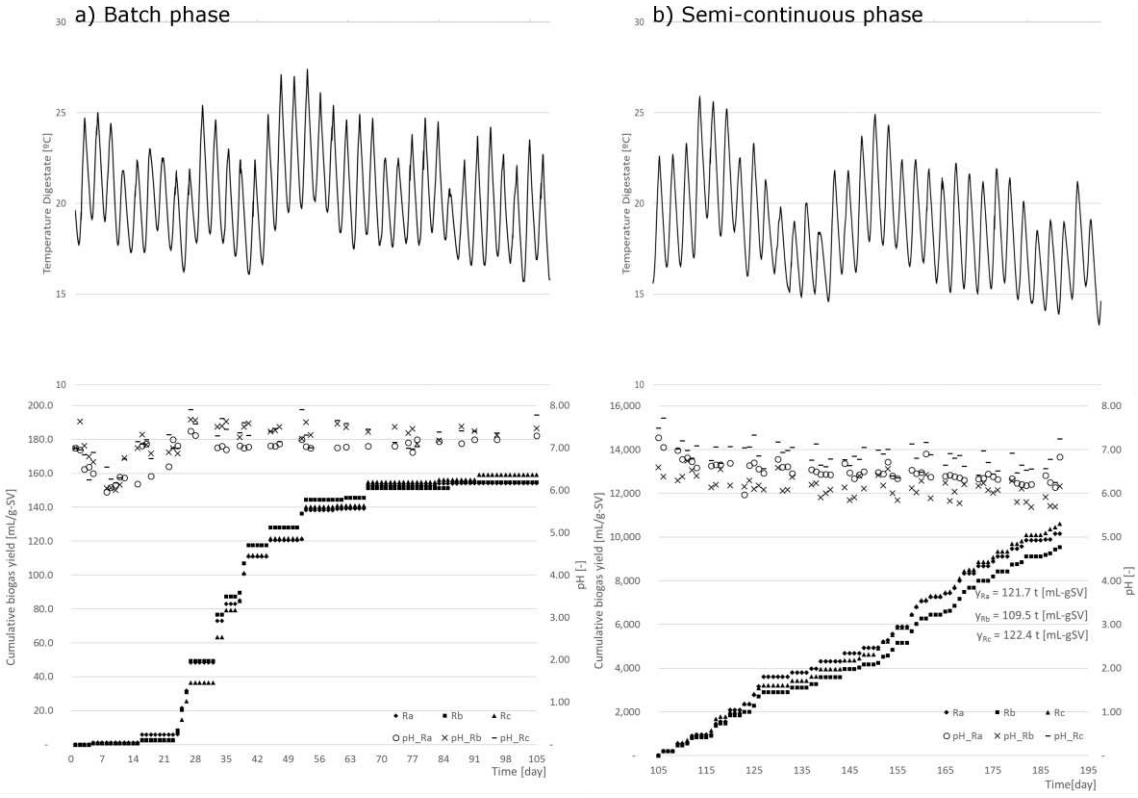


Figure 4. *SBY, temperature and pH during a) batch assays and b) semi-continuous assays*

Although latest reviews have demonstrated the lack of enough researches, regarding AD of FW at psychrophilic temperature, for comparing results (Komilis et al., 2017; Leung and Wang, 2016) the obtained values are still lower than the ones reported by some authors (Bernstad et al., 2013). Comparing SBY can prove difficult as different methods and protocols can all differ from one test to another (Angelidaki, 2009). For instance, the lower SBY, obtained in this research, may be explained due to the SIR. This ratio highly

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affects AD kinetics and SBY. Thus, SBY may be reduced up to 55 % when SIR is increased from 1.6 to 5.0 (Liu et al., 2009). Other authors, showed even higher variations (approx. 80 %) when SIR is increased from 1.3 to 4.0 (Kumar et al., 2016).

Regarding the semi-continuous phase (see *Fig. 4b*), the temperature lowered, compared to the batch assays from an average of 20.26 ± 2.33 °C to 18.66 ± 2.53 °C. In addition, pH slightly decreased. Hence, batch assays finished with a pH of 7.51 while the semi-continuous operation mode showed a pH of 6.75 after 90 days. During three months, the SBY was approx. constant with an average slope of 118 ± 7 mL/g-VS day. Related to the semi-continuous operation mode there are rare reports about successfully operating single-stage anaerobic digestion of FW at psychrophilic temperature. Besides, to the best of our knowledge, AD of FW without stirring has not been addressed. Stirrer devices are commonly used, at any scale, for avoiding foaming and floating layers. An appropriate agitation technology is essential since stirring has a crucial influence on the microbial community structure and the SBY. Thus, it has been highlighted by previous authors a SBY reduction between 30 % to 40 % when the digestate is unmixed (Rusin et al., 2017; Rojas et al., 2010).

From this point of view, it may be argued whether the effectiveness of a more complex reactor since it has been demonstrated the more complex the reactor, the higher the SBY (Rajagopal et al., 2017). However, complex reactors lead to increase the installation costs and the economic issue surely reduce the number of installed reactors and consequently the available amount of FW treated might be highly reduced. Nevertheless, when similar reactors were operated at similar operation conditions, (i.e. HRT, OLR) other authors found similar biogas production and methane content (Khalil et al., 2016; Lou et al., 2013).

Biogas contained an average of 57.7 % (3 %) of CH_4. H_2S was not detected and a minimum amount of CO (approx. 4 ppm) was measured. Based on SBY and its composition it can be concluded that the expectable amount of methane, in the semi-continuous running mode is approx. 14 L of methane per kg of FW. The biogas composition has been kept quite similar which is a good indicator of the AD stability. Despite volatile organic acids and total inorganic carbonate have not been measured, the pH has not set below 6.8 and the methane content has not decreased. These two latest indicators suggest there was not an ongoing rise in acid concentration.

It is clear that the biogas production is actually low but also clear that the system is very cheap to install and operate. Regarding the organic load rate (OLR) that should be considered for the proposed reactor design, it must be noted that mono-digestion of FW might lead to acidification of the fermentation process via an increasing of OLR (Kastner et al., 2012).

For this reason, it is important to consider the size of the proposed small household reactors according to the estimated daily FW produced by a typical Chilean household (approx. 1.5 kg of FW per day). Thus, cases reported by literature (Khalil et al., 2016; Lou et al., 2013) showed a continuous operation for basic small reactors when OLR varied between 0.6 and 1.0 kg VS/(m^3 day). This suggests a reactor volume from 500 m^3 up to 2000 m^3, depending on the building size as long as the HRT which should be set at approx. 60 days.

COD removal is also a key factor for promoting this decentralized off-grid system, since effluents must abide by Chilean laws (NCh 1333) prior to being discarded to the drainage lines. After the laboratory tests, the substrate showed a final COD value of 269 mg O_2/L. It can be observed that COD was reduced by 97 %, which is in concordance

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with the ranges shown in the literature of 80 to 95 % (De Mes et al., 2003). The final obtained COD is even lower than the concentrations of most typical sanitary effluents from human manure, which range between 260 and 480 mg O_2/L (Suarez and Puertas, 2005). In addition, other values related to this effluent quality (see *Table 2*) also meet regulatory standards for being discharged into the municipal sewer system. Hence, it is concluded that the effluent can be directly emptied into the general drainage line without further treatment.

Table 2. *Effluent characteristics*

Characteristics	Value
ORP [mV]	-25.6
pH [-]	9.7
Salinity [%]	0.94
EC [uS/cm]	1,840.0
TDS [ppm]	919

Energy savings and avoided $CO_{2,eq}$

Chile produces yearly more than 3.5 Gg of FW suitable for AD. Assuming that the difference between the FW production from single- and multi-family households is not statistically significant (Schott et al., 2013), the total average amount of FW per household generated in Chile per year can be estimated at 510 kg per household, while in the European Union or India only 190 kg and 319 kg of FW are produced per annum and household, respectively (Bernstad et al., 2012; Rocky et al., 2014).

Results, categorized by region, are summarized in *Table 3*. Despite the metropolitan region (RM) is the largest contributor with more than 1.5 Gg, regions of Arica-Parinacota (XV), Araucania (IX) and Aysen (XI) showed the highest potential methane production due the amount of FW produced per household. In addition, Arica and Parinacota (XV) is one of the smallest regions, in terms of households, which means that could be considered for testing the proposal. On the contrary, the regions of B. O'Higgins (VI), Atacama (III) and Antofogasta (II) show the lowest potential savings, as less FW is produced and more energy is demanded per household each year. It must be noted that the energy consumed by each household, used for estimating potential savings, is based on the substitution of biogas for natural and/or liquefied gas.

Despite the theoretical and economic potential, differences of up to 30 % may be observed due to the reliable amount of FW that can actually be used, in case of decentralized off-grid system, both values may be assumed to be similar (Rios et al., 2016).

Hence, the overall Chilean potential primary energy generation from semi-continuous operation accounts for nearly 425 GWh per year, which represents approximately 0.2 % of the total primary energy consumption (approx. 419.8 TWh). Comparing these results with other values from similar research studies, similar outcomes were found in terms of the percentage of domestic energy consumption: Sweden (0.2 %) (Schott et al., 2013), Mexico (0.8 %) (Rios et al., 2016), Brazil (0.26 %) (Salomon and Lora, 2009) and Uruguay (0.2 %) (Moreda, 2016). From this point of view, AD of FW at a decentralized scale does not seem to be the best option for adding renewable energy to the Chilean energy supply.

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Table 3. *Summary of FW produced, energy consumption and potential energy generation by AD of FW categorized by household and region*

Region	Total FW produced [t]	FW produced per household [kg]	Potential energy from AD of FW per household [kWh]
I	105,266.4	1,141.5	195.9
II	108,861.9	685.7	117.7
III	57,363.9	684.1	117.4
IV	122,489.0	541.3	92.9
V	325,883.0	585.5	100.5
VI	133,011.4	484.0	83.1
VII	199,579.5	633.2	108.7
VIII	358,202.3	581.8	99.8
IX	235,839.8	795.5	136.5
X	205,160.4	802.4	137.7
XI	24,911.5	751.4	128.9
XII	35,785.0	721.1	123.7
XIV	81,838.4	697.9	119.8
XV	63,495.6	1,200.2	206.0
RM	1,556,899.0	738.6	126.8

However, households may achieve an average saving of 1.5 % in terms of primary energy consumption, which reinforces the separation of FW at the generation point and the corresponding reduction of organic matter disposal. In spite of relatively low potential energy savings, there is an important impact in terms of avoided GHG emissions. These can be divided into two main categories: horizontal flows, produced by the MSW transport systems and the direct GHG emissions from landfills and fossil fuels replacement. Then, approximately 713 Gg $CO_{2,eq}$ can be avoided yearly from landfills emissions and 878.6 Gg $CO_{2,eq}$ from the reduction of fuel consumption in MSW transportation services. In addition, taking into account that biogas has a neutral carbon cycle and considering EPA emissions factors, which report an average of 2.15 kg $CO_{2,eq}$ per cubic meter of natural gas (measured at normal conditions), it can be estimated that around 112.8 Gg $CO_{2,eq}$ might be avoided when this fuel is replaced by methane.

Conclusions

The results suggest that AD of FW in Chile might incorporate approx. 425 GWh per annum (in terms of primary energy) by using household scale reactors in a decentralized off-grid system. Hence, firewood and natural or liquefied gas households can be partially replaced which impacts on the reduction of household expenses.

Despite the energy provided is quite lower than other alternatives based on high rate anaerobic reactors at psychrophilic temperature, depending on the corresponding installation costs of, it may be desirable to operate economical and simpler versions while these have shown a positive impact in terms of environmental protection.

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Hence, due to the reduction of the amount of MSW that requires collection, transportation and disposal and the partial substitution of natural gas, it is estimated that 1,704 Gg $CO_{2,eq}$ emissions can be avoided annually.

This positive effect on environment may be used by Chilean authorities in order to support the required financial policies which have been highlighted as an encouraging key factor in other countries (mostly in China, India and EU). Results must be considered as a basic framework that may orient the decision-making process for the implementation of short-term environmental policies. At this point, XV region is suggested for testing this proposal at pilot scale due to the high estimated savings. Further investigations should be developed regarding the operational aspects with the aim of improving the technical design of reactors or the maintenance operations, among others.

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NON-RANDOM DISTRIBUTION OF BIOCRUST IN A NATURAL ARID ENVIRONMENT IN THE NORTHERN MEXICAN PLATEAU

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Abstract. Biocrust, as photosynthetic organisms, has been considered to show an inversely proportional relationship with vascular plant cover. However, some studies report association and facilitation relationships between them. This research explores whether i) biocrust is associated with shrub species, ii) biocrust cover is higher in undershrub microenvironments than in open areas between plants and, iii) soil moisture and solar radiation affect biocrust distribution. Biocrust cover was analyzed by morphotype in microenvironments under the canopy of two dominant shrub species (*Larrea tridentata* and *Parthenium incanum*) and in open areas. Soil moisture and solar radiation were recorded at each microenvironment. Thirty-three interception lines (975 points) were used to analyze the association between vascular plants and biocrust and 20 sampling plots to estimate morphotype cover. Results reveal a positive association between biocrust and plant species compared to open areas. Lichens and cyanobacteria showed a higher cover under *L. tridentata* than under *P. incanum* and in open areas, suggesting a species-specific facilitative association with *L. tridentata*, maybe due to a higher soil water content in this microenvironment. Mosses had lower cover in the open area where the highest solar radiation was recorded. Lichens and cyanobacteria distribution appeared to be related to soil moisture.
Keywords: *creosote bush, cyanobacteria, lichens, mosses, northeastern Mexico*

Introduction

Biological soil crusts are made up of extremely long-lived organisms, they provide continuous ecosystem services (Castillo-Monroy and Maestre, 2011) and have been considered as long term environmental indicators (Belnap et al., 2001) that provide structure to the soil (Richer et al., 2012), contribute to water balance, erosion control and nutrient cycling (Castillo-Monroy and Maestre, 2011). Furthermore, the presence of lichen crusts improves soil resistance to erosion and increases its water holding capacity (Abed et al., 2013).

Vegetation dynamics and the microenvironments that plants create affect biological crust distribution (Zhao et al., 2010) particularly in arid environments where plant species are arranged in patches on a bare soil matrix (Puigdefábregas and Sánchez, 1996) creating islands of resources (Reynolds, 1999). However, the way vascular plant cover affects the biological soil crust (BSC) distribution remains controversial; some authors have found a positive relation between shrub cover and total BSC cover, but a negative relation with lichen cover (Ochoa-Hueso et al., 2011; Root and McCune, 2012). Others report that BSC cover and species richness was higher in microsites

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without vegetation, either grazed or cultivated than in vegetated sites (Eldridge et al., 2000). The relationship between cover of biological crusts and vascular plants has been found to be inversely proportional (Belnap et al., 2001).

Vascular plants are successful competitors for resources such as light, nutrients, and water (Löbel et al., 2006) and this may result in a disadvantage for BSC growing under them. However, microenvironments under shrubs are likely to have more nutrients and higher water holding capacity, thus favoring the BSC development (Bowker et al., 2005); creating facilitation relationships between vascular plants and BSC as has been shown for *Stipa tenacissima* and *Cladonia convoluta* in Spain (Maestre, 2003).

Biological soil crusts dominated by mosses are usually more abundant in moist microenvironments of arid and semiarid environments (Maestre et al., 2001). In these environments, the presence of vascular vegetation may favor the occurrence of some components of BSC (mainly bryophytes), since it may provide moister microsites protected from direct solar radiation (Concostrina-Zubiri et al., 2014; Núñez and Toledo, 2013; Haughian and Burton, 2015). Contrarily, crusts constituted mainly by lichens are related to the areas most exposed to solar radiation and heat, with low moisture and nutrient availability (Haughian and Burton op cit.), even though the water availability has been considered critical for the establishment and development of lichens (Gignac, 2001). Open areas with the dominance of BSC have been found to have the higher cover of cyanobacteria and lichens and less of mosses compared with microsites under the canopy of plants (Mendoza-Aguilar et al., 2014).

However, Gutiérrez et al. (2017) found a higher cover of lichens, cyanobacteria, and mosses underneath vascular plants than in open areas and considered that this might be due to a lower competition for space between the BSC components than in other areas that have higher densities of these components. Because BSC components do not have roots to uptake water and nutrients, these resources are likely to be captured from the atmosphere or through direct contact with the soil (St. Clair et al., 2002). Thus, competition for space becomes very important for BSC components, because it secures access to more resources (Bowker et al., 2010).

These contrasting results highlight the relevance of continuous evaluation of the BSC component distribution and their relation to vascular plant species, exploring association, facilitation and competition relationships between them. This, in turn, may have an impact on nutrient and water flux dynamics and in the overall functioning of the ecosystem. Hence, this research is aimed at determining whether there is a positive or negative association between BSC and vascular plants or they are randomly distributed and whether this distribution pattern is equal for all the components of the BSC in the area.

The Mexican Plateau floristic Province (Rzedowski, 2006) is dominated by arid and very arid climates which favored the development of xerophilous shrublands (63%) and pastures (28%) (Espinosa et al., 2008). In the low and flat areas, the most abundant shrub species is creosote bush (*Larrea tridentata* (Moç. & Seseé ex DC.) Coville) which grows alone or mixed with other drought tolerant species. In addition to the vascular vegetation cover, the area also includes communities of biological soil crusts (BSC). These communities consist of soil particles in association with cyanobacteria, green algae, lichens, fungus, and bryophytes. These biocrusts have physiological qualities such as resistance to desiccation and temporary suspension of breathing, which allow them to develop in environments with scarce water availability (Belnap et al., 2001).

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Several authors have stated that the composition of biological crusts differs depending on whether they are under vascular plants or in open areas (Maestre et al., 2001; Maestre, 2003; Mendoza-Aguilar et al., 2014) and that they might also differ depending on the plant species that they grow under, due to differences in microenvironments. Hence, in this study, the biological crust cover was analyzed in microenvironments under the canopy of the two dominant shrub species (*Larrea tridentata* (Moç. & Seseé ex DC.) Coville and *Parthenium incanum* Kunth) and in open areas. Soil moisture and solar light intensity were recorded for each microenvironment. We hypothesized that i) BSC is positively associated with shrub species, ii) Cover of BSC components is higher in the undershrub microenvironments than in the open area between shrubs, iii) Cover of BSC components differs between the vascular species under which they grow, and iv) solar radiation is more closely related to BSC distribution than soil moisture.

Materials and methods

Study area

This research was carried out in an area of xerophilous scrub in the Mexican Plateau floristic Province (Rzedowski, 2006) in the southern region of the Chihuahuan Desert (24˚47'11" N; 100˚41'54"W) in northeastern Mexico at 1900 m above sea level. The climate of the area is BS_0k (x'): arid, temperate (García, 1981); its mean annual temperature is 15 °C; with an average maximum monthly temperature of 36.6 °C in April and a minimum of -4.9 °C in January (CONABIO, 2016; CNA, 2016). Mean annual rainfall is 429 mm, with a maximum in January (318 mm) (CNA, 2016). Soils are haplic and calcic xerosols with petrocalcic physical phase. These soils support microphyllous desert scrub in the alluvial areas (CONABIO, 2016). This vegetation type, which covers about 49% of the soil surface, is mainly distributed in flat or very gentle slope areas and it is formed by species that support high concentrations of salts with *Larrea tridentata* ((Moç. & Seseé ex DC.) Coville) as the most common species mixed with *Fouquieria splendens* Engelm, *Flourensia cernua* DC., *Zinnia acerosa* DC., *Parthenium incanum* Kunth, *Jatropha dioica* Cerv., *Koeberlinia spinosa* Zucc. and some spiny shrubs (Granados-Sánchez et al., 2011). There is no agriculture in the area, but there is evidence of light cattle and equine grazing.

Methods

Association analysis

Plant species abundance and frequency were previously evaluated in the area. We selected the two most abundant species to analyze their association to biological soil crusts. These species were *Larrea tridentata* (density by m^2 = 0.806; relative frequency = 13.52%) and *Parthenium incanum* (density by m^2 = 0.521; relative frequency = 8.74%). Thirty-three interception lines (Canfield, 1941) of 30 m long were used to record the presence of BSC underneath the two most abundant plant species and in the spaces between them. Lines were established 0.5 km apart with sampling points every 0.5 m. We analyzed the association between plant species and BSC with a total of 975 points: 537 open area points (105 with BSC; 432 without BSC), 266 under *Larrea tridentata* (234 with BSC; 32 without BSC), and 172 under *Parthenium incanum* (161

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with BSC; 11 without BSC). The remaining points fell either on other plant species or on litter.

We tested four microenvironments: i) underneath *Larrea tridentata* (ULt), ii) underneath *Parthenium incanum* (UPi), iii) open area (OA) and (iv) underneath vascular plant (Uvp), defined by adding the data of microenvironments i) and ii). Biological soil crusts communities were classified according to the morphology of their components (Eldridge and Rosentreter, 1999) and the following categories were established: lichens, mosses, cyanobacteria, and hepatics, named herein as morphotypes. *Endocarpon pusillum* (Hedw.) and *Collema coccophorum* (Tuck.) are the most common species of the lichen morphotype, with frequencies of 70 and 46% out of the total of lichens. Cyanobacteria morphotype is mainly composed of species of Scytonema and Microcoleus genera. Liverworts are characterized by species of Riccia and Oximitra genera. Mosses were not identified.

Relationships between BSC morphotype cover and plant species at each microenvironment

Twenty sampling plots (20 m x 20 m) were randomly selected in the area. At each plot, one individual of *Larrea tridentata* and one of *Parthenium incanum* with BSC under them were selected and crusts sampled. Also, biological crusts of corresponding open areas were sampled. Open area sites were sampled at a distance shorter than one meter from the limit of the plant canopy projection to reduce soil variability (Bowker et al., 2006). Six samples were collected from under each plant and six in their corresponding open area; having 20 replicates of each microenvironment with 120 samples each.

Before sampling, the soil surface was sprayed with distilled water to avoid breaking off the crusts, and a sample of the topsoil layer was collected using a Petri dish (9 cm in diameter by 1 cm in depth) taking care that the soil surface remained unaltered. Biological soil crust collected samples were taken to the laboratory to estimate cover by morphotype using a template with 8 radial divisions, stereoscope, microscope, and identification guides (Brodo et al., 2001; Barreno and Pérez-Ortega, 2003; Rosentreter et al., 2007). Percentage cover was visually estimated within each radial division; percentages were then added for each Petri dish and extrapolated to square meter. The soil moisture and solar radiation were recorded at each microenvironment (ULt, UPi and OA). Soil moisture was determined by the gravimetric method and solar radiation was recorded using a luxometer (Extech Instruments, model 401025). Sampling was done from May to December 2014 at different times of the day due to the time required to reach all sampling quadrats; hence, extreme caution was taken to record at virtually the same time all the microenvironments within the same sampling quadrat and data were analyzed as paired t tests (Dytham, 2011).

Chi-square tests (χ^2) were used to determine association (Dytham, 2011) between BSC and vascular plants. To measure the degree of association, contingency tables 2 x 2 were used and the strength of association was determined using *Equation 1* (Badii et al., 2008):

$$V = \left(\frac{ad - bc}{\sqrt{mnrs}} \right) \quad \text{(Eq.1)}$$

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where V = Association coefficient; a = points where both, the plant and the BSC, are present; b = points where only the BSC is present; c = points where only the plant is present; d = points where plants and BSC are absent; m = a + b; n = c + d; r = a + c and s = b + d.

The data were further analyzed using not only BSC presence-absence data but the percentage cover of each BSC morphotype in each microenvironment. These analyses were carried out for three conditions: i) under *Larrea tridentata* (ULt), ii) under *Parthenium incanum* (UPi) and iii) in open areas (OA). The data of the BSC morphotypes cover, solar radiation, and soil moisture were analyzed for normality (Shapiro-Wilk test) and homoscedasticity (Levene test). When data did not meet the former assumptions, a non-parametric analysis was carried out. The comparison of BSC morphotypes cover among the microenvironments was done through the non-parametric Kruskal-Wallis analysis. When significant differences were detected, Mann-Whitney U tests were performed. Solar radiation and soil moisture were analyzed by the Wilcoxon´s test as a non-parametric alternative to the Student's t-test for related samples (Dytham, 2011). All statistical analysis was performed using the SPSS® Statistics software version 19.

Results

Chi-square results showed an association between BSC and microenvironment under vascular plants (Uvp) (χ^2 = 483.012, df = 2, p = 0.001); with a positive association intensity of 0.715. Both plant species showed an association with BSC (χ^2 = 338.535, df = 1, p = 0.001; χ^2 = 313.676, df = 1, p = 0.001) for *Larrea tridentata* and *Parthenium incanum* respectively. Association intensity was positive and similar for both plant species (0.652 for *Larrea tridentata* and 0.655 *Parthenium incanum*).

Cover of lichens (χ^2 = 31.76, df = 2, p = 0.001) cyanobacteria (χ^2 = 7.14, df = 2, p = 0.028) and mosses (χ^2 = 27.28, df = 2, p = 0.001) differed among conditions or microenvironments. Liverwort cover was not included in the analysis due to their very low surface cover (< 0.3%) across samples.

Larrea tridentata microenvironment (ULt) showed a higher cover of lichens and cyanobacteria than *Parthenium incanum* (UPi) (p = 0.001; 0.031) and open areas (OA) (p = 0.001; 0.015) microenvironments. The moss morphotype had similar cover in ULt and in UPi and this was higher than in OA (p = 0.001) (*Fig. 1*).

Water content in the soil was low across sampling dates (< 9.8%) for all microenvironments and samples. Soil moisture content differed between microenvironments; the highest content was recorded in the ULt microenvironment (6.36%). Soil water content for UPi and OA microenvironments were similar (p = 0.911) (*Table 1*).

Solar radiation values ranged from 2,400 to 95,300 lux. The three microenvironments had statistically different solar radiation values (ULt vs. OA p = 0.001; UPi vs. OA p = 0.001; ULt vs. UPi p = 0.002). Solar radiation was higher for the OA microenvironment, followed by ULt and then UPi (*Table 1*).

Cover of mosses was lower in the OA microenvironment which showed the highest solar radiation. Lichens and cyanobacteria cover were not related to solar radiation since their highest cover was in the ULt microenvironment that has an intermediate solar radiation.

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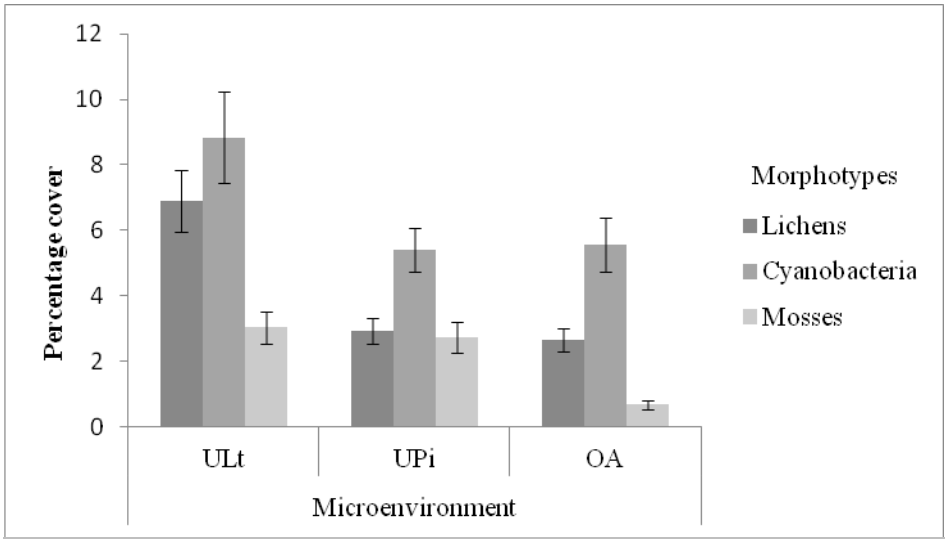


Figure 1. *Percentage cover of BSC morphotypes for microenvironment. Error bars are standard errors. ULt = under Larrea tridentata, UPi = under Parthenium incanum, and OA = open areas*

Table 1. *Solar radiation and soil moisture (mean ± standard error) for each microenvironment. Different letters in columns are for statistical differences ($p < 0.05$)*

Microenvironments	Solar radiation (lux)	Soil moisture (%)
Underneath *Parthenium incanum* (UPi)	16253 ± 24.36 a	5.49 ± 0.31 a
Underneath *Larrea tridentata* (ULt)	29143 ± 38.61 b	6.36 ± 0.39 b
Open area (OA)	41018 ± 40.45 c	5.48 ± 0.32 a

Discussion

The results of this study showed an association between BSC and microenvironment under vascular plants (Uvp). These findings are consistent with observations of Maestre and Cortina (2002), who found a positive association between spatial patterns of BSC, composed of cyanobacteria and lichens, and cover patterns of *Stipa tenacissima*; a dominant plant species in the semi-arid southeast of Spain. Other studies have also shown a facilitative relationship between BSC and plant species, specifically *Stipa tenacissim* and *Cladonia convoluta* (Maestre, 2003). Here, both plant species (*Larrea tridentata* and *Parthenium incanum*) showed a positive and similar association intensity with BSC.

There seems to be a consensus that vascular plant-biocrust interactions are highly species specific, dependent on the BSC dominating organism's traits and plant characteristics (Rivera-Aguilar et al., 2005; Serpe et al., 2006; Deines et al., 2007). BSC dominated by *Diploschistes* sp. reduced plant emergence (Escudero et al., 2007; Serpe et al., 2008) maybe due to the sealing of the soil pores by the biocrust which might reduce water availability and thus inhibit germination (Eldridge et al., 2010). St. Clair et al. (1984) found higher germination for three grass species on algal crusts compared with mixed crusts of lichens, mosses and algae.

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Interactions between biocrust and vascular plants could have multiple effects, either facilitative (more soil moisture for the biocrust, more nutrient availability for the vascular plant and biocrust) or inhibitory (less water availability for the vascular plant, lack of sunlight for the photosynthesis of the BSC's photobiont). For instance, biocrust had a negative effect on water availability in an area covered with microbiotic crust in southern China, as rainfall infiltration was about half of that recorded in the area without crust (Uchida et al., 2000). However, in an independent study in the same area, Tateno et al. (2003) found a dependence of *Pinus massoniana* Lamb. on N fixed by the microbiotic crust, which improved tree productivity, despite the fact that biocrust decreased the amount of available rainfall.

In contrast, Eldridge et al. (2000) found a significantly higher BSC cover in unvegetated microsites in grazed and ungrazed grasslands in Southeastern Australia than in those with vascular plants. Núñez and Toledo (2013) found that although areas under the shade of vascular plants favor the growth of microorganisms present in the BSC when BSC were analyzed by morphotype, the highest cover of lichens and cyanobacteria was recorded in open areas with higher exposure to solar radiation. Similarly, Mendoza-Aguilar et al. (2014) found a higher cover of cyanobacteria and lichens in the open areas than under plants, which differs to our findings.

We found a higher cover of lichen and cyanobacteria under *L. tridentata* than in open areas. Even though several authors have found that when the BSC were composed mainly of lichens there is an inversely proportional relationship between them and vascular plants (Belnap et al., 2001; Ochoa-Hueso et al., 2011; Núñez and Toledo, 2013) and that lichens are likely to occupy open areas due to a lower competitive ability with respect to vascular plants in obtaining resources (Löbel et al., 2006), in contrast to our results. Such differences in lichens and cyanobacteria distribution may be due to lower competition for space in our study area, where total cover of lichens, cyanobacteria and mosses under *L. tridentata* was 18.8% compared to 54.9% found by Mendoza-Aguilar et al. (2014) for a semiarid area in Alicante, Spain or 85.34% in Valle de Quibor, in Venezuela (Núñez and Toledo, 2013). At high densities, competition for space is frequent between components of the BSC (Castillo-Monroy and Maestre, 2011).

Very little has been studied about the effect of competition among the components of BSC. Lichens and bryophytes have chemical compounds that play an important role in nutrient intake (Hauck et al., 2009), so that species with different chemical compounds could benefit greatly if they are associated (Castillo-Monroy and Maestre, 2011), but there can also be competition between them. After studying BSC lichen communities in a gypsum outcrops area in central Spain, Maestre et al. (2008) found that there were fewer species combinations and less co-occurrence of those lichens than expected by chance. These results were in agreement with their hypothesis that BSC lichen communities are mainly structured by competitive interactions.

Competitive interactions between BSC components have been documented, both at the pair and species level, and have concluded that the intensity of competition at the community level increases as aridity increases (Bowker et al., 2010). It is most likely that, in more arid conditions, competition would increase and the lichens that are now distributed under the shrubs, would occupy the open areas. Probably, aridity in regions where lichens are mainly distributed in the inter-shrub spaces is more severe than in our study area.

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Vascular plant canopy tends to favor certain biological soil crust morphotypes over others and may enhance biocrust formation; however, it can also create unfavorable conditions such as depriving crusts of adequate light for photosynthesis or bury the biocrusts with heavy litterfall (Zhang et al., 2016). These, among other factors, may cause vascular species to facilitate or inhibit BSC components.

In this study, we found that cover of lichen and cyanobacteria was higher under *L. tridentata* that under *P. incanum* indicating an association that could be the result of facilitation. This could be due to the higher soil water content recorded for ULt microenvironment and the fact that *P. incanum* has a denser canopy which prevents lichens and cyanobacteria from receiving enough sunlight for photosynthetic processes. *P. incanum* also accumulates more litterfall which may difficult the establishment of lichens and cyanobacteria. A facilitative association was established by Bu et al. (2016) who found that BSC cover exhibited a clear positive correlation with a cover of *Artemisia desertorum* (Spreng), and a negative correlation with *Caragana korshinskii* (Kom) and *Stipa bungeana* (Trin) cover in a temperate, semi-arid Province of Shaanxi in China. Similar results, although studying bryophytes have been reported by Ingerpuu et al. (2005) who found a species-specific facilitative effect between two out of three tested vascular species (a legume: *Trifolium pratense* L., a grass: *Festuca pratensis* Huds. and a forb: *Prunella vulgaris* L.) and two bryophyte species (*Brachythecium rutabulum* (Hedw.) B.S.G. and *Rhytidiadelphus squarrosus* (Hedw.) Warnst.). Only the first two vascular plants had a significant effect on bryophytes.

In our study, the moss morphotype had a higher cover in the two microenvironments, with lower solar radiation (ULt and UPi) than in the open inter-shrub spaces (OA). Mosses covered, as an average, four times more surface in the ULt and UPi microenvironments than in the open area. Mosses seem to be related to conditions of lower solar radiation and to tolerate competition from vascular plants better than lichens (Löbel et al., 2006). These results are consistent with those of other authors (Maestre et al., 2001; Ingerpuu et al., 2005; Castillo-Monroy et al., 2010; Mendoza-Aguilar et al., 2014; Haughian and Burton, 2015) who found higher cover of mosses under vascular plants than in open areas.

Conclusions

The results presented here reveal a positive association between BSC and the plant species *Larrea tridentata* and *Parthenium incanum*, compared to open areas; thus confirming our first hypothesis.

The lichen and cyanobacteria morphotypes showed a higher cover under *Larrea tridentata* (ULt) than under *Parthenium incanum* (UPi) and in open areas (OA) suggesting a species-specific facilitative association with *L. tridentata*. The moss morphotype had a higher cover in ULt and UPi microenvironments which is perhaps a result of lower solar radiation than in OA.

Distribution of BSC components can be potentially relevant for ecosystem functioning (e.g. hydrophobicity and water re-distribution, C sequestration and N fixation). Hence, studies aimed at determining how microclimate could modify BSC distribution would improve our understanding of the functioning of ecosystems. The results reported here make an important contribution to the knowledge of the interactions between vascular plants and biocrust components.

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VARIABILITY OF CHEMICAL WEATHERING INDICES IN MODERN SEDIMENTS OF THE VISTULA AND ODRA RIVERS (POLAND)

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Abstract. The survey was conducted to determine chemical weathering indices in modern river sediments in Poland. Weathering Index of Parker (WIP), Chemical Index of Alteration (CIA), Vogt's Residual Index (V), Harnois's Chemical Index of Weathering (CIW), and Plagioclase Index of Alteration (PIA) was calculated for 178 sediment samples taken from the Vistula River and its tributaries as well as for 114 samples from the Odra River and its tributaries. The indices values were in the ranges: WIP 0.5–20; CIA 4–80; V 0.1–2.8; CIW 4–82; and PIA 3–81.7. The observed variation of values resulted from a varied sample lithology and varied climatic conditions in the Poland area. Sediments from the upper parts of the Odra and the Vistula drainage basins (southwestern part of the country), where pre-Quaternary rocks are exposed, are more resistant to chemical weathering and characteristic for lower susceptibility to leaching of elements. The highest elements losses due to leaching are characteristic for samples taken from the lower parts of the Odra and the Vistula drainage basins, dominated by Quaternary deposits. Additionally, it was found that larger indices values, reflecting effects of stronger weathering, are observed in the southwestern part of the country (with the warmer and more humid climate) comparing to the northeastern part (with prevailed colder and drier climatic conditions).
Keywords: *river sediments, indices of chemical weathering, drainage basins, Poland, moderate climate, lithology*

Introduction

The chemical composition of modern river sediments is affected by many factors, primarily the lithology of river basins and climatic conditions. However, there are also other important factors, including topography of the terrain, vegetation type, and land use of the drainage area (Bahlburg and Dobrzinski, 2011).

Sediments that accumulate on the river bottom are derived from material originating from erosion and the weathering of rocks occurring within the drainage area (e.g. quartz grains, feldspars, carbonates, clay minerals and heavy metals) and from in-situ material (debris of dead plants and animals, and minerals precipitated from water, e.g. goethite, fluoroapatite and makinavite). In industrialised, urbanised and agricultural areas, the sediment is also enriched in organic and inorganic contaminants contained in wastewater discharged into the surface water, as well as those transported by surface runoff (Bojakowska et al., 2006; Ciszewski, 1997, 2002, 2005; Cempiel et al., 2014).

Under surface conditions, clay minerals (notably illite and kaolinite) and iron hydroxides, e.g. goethite, form within weathering covers due to chemical weathering of unstable minerals, especially feldspars. The weathering covers are also depleted in Ca^{2+}, K^{+}, Na^{+} and Mg^{2+} ions as a consequence of their leaching (Fedo et al., 1995; Bland and

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Rolls, 1998; Bahlburg and Dobrzinski, 2011). As a result of erosion, material from the weathering covers and soils is transferred to river channels and partly deposited there.

To reflect the intensity of chemical weathering processes and to determine the degree of depletion of soils and river sediments in mobile components relative to immobile ones due to the weathering process, the chemical weathering indices are used (Price and Velbel, 2003). They are widely employed in research of both modern and fossil weathering profiles and marine and lacustrine sediments (Harnois, 1988; Nesbitt and Young, 1982; Bäumler and Zech, 2000). The weathering indices are also useful for monitoring the variability of components in longitudinal river profiles, and for evaluating soil fertility and development (Chetelat et al., 2013; Li and Yang, 2010; Bilinski, 2008; Shao et al., 2012; Price and Velbel, 2003 after Delvaux et al., 1989). These indices reflect the effects of climate on the weathering of parent rocks, characterise neotectonic-related transformation, quantify the properties of the regolith, and allow for a better explanation of elemental mobility during weathering processes (Neall, 1977; Fedo et al., 1996; Sharma and Rajamani, 2000). The ideal index should allow for a comparison of studies carried out at different locations, on various materials, and in weathering profiles of different ages. The index should also be easy to apply and based on elements commonly found in surface environments (Harnois, 1998).

The purpose of the study was to determine the factors affecting significantly the weathering of sediments based on variations in the values of weathering indices in the Vistula and Odra river basins. The results of geochemical studies carried out within the framework of the National Environmental Monitoring (PMS) were used.

Weathering indices

The most frequently used weathering indices are: Weathering Index of Parker (WIP), Chemical Index of Alteration (CIA), Chemical Index of Weathering (CIW), Vogt's Residual Index (V), and Plagioclase Index of Alteration (PIA) (*Table 1*). They are sensitive even to minor geochemical changes such as hydrothermal alterations along fault lines, and groundwater level fluctuations.

Table 1. *Chemical weathering indices*

Index	Formula	Reference
Weathering Index of Parker (WIP)	$WIP = \left(\frac{2Na_2O}{0.35} + \frac{MgO}{0.9} + \frac{2K_2O}{0.25} + \frac{CaO}{0.7} \right) \times 100$	Parker (1970)
Vogt's Residual Index (V)	$V = \frac{Al_2O_3 + K_2O}{MgO + CaO + Na_2O}$	Vogt (1927)
Chemical Index of Alteration (CIA)	$CIA = \frac{Al_2O_3}{Al_2O_3 + CaO + Na_2O + K_2O} \times 100$	Nesbitt and Young (1982)
Chemical Index of Weathering (CIW)	$CIW = \frac{Al_2O_3}{Al_2O_3 + CaO + Na_2O} \times 100$	Harnois (1988)
Plagioclase Index of Alteration (PIA)	$PIA = \frac{Al_2O_3 - K_2O}{Al_2O3 + CaO + Na_2O - K_2O} \times 100$	Fedo et al. (1995)

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Weathering Index of Parker **(WIP)** involves the mobility of sodium, potassium, magnesium and calcium during weathering processes. These are the most mobile major elements and therefore the index is not recommended for the analysis of strongly weathered material (Eswaran et al., 1973; Price and Velbel, 2003). The index of Parker is most useful for reflecting changes in the amount of Na^+, K^+, Ca^{2+} and Mg^{2+} cations, if the alteration of feldspars into clay minerals is the major chemical (hydrolytic) weathering process, and there are similar mobilities between the major cations (Parker, 1970). The WIP is the appropriate index for the assessment of weathering of rocks where hydrolysis is the main weathering process (Gupta and Rao, 2001). The WIP value is in the range from 0 to 100, and its maxima correspond to the least weathered rocks.

The source of inaccuracy in calculating the index is the assumed simplification that the total amount of calcium in the rock is contained in silicate minerals. This simplification is particularly disadvantageous if the rock contains large amounts of detritus or carbonate cement. Doubts also arise as to not involving relatively immobile phases, such as Al_2O_3, in the formula, which makes it impossible to observe changes in the composition of essential minerals (Bahlburg and Dobrzinski, 2011). The disadvantages of the WIP are eliminable using the Chemical Index of Alteration (CIA) that involves all geochemical data of the rock (Nesbitt and Young, 1982).

Vogt's Residual Index **(V)** is defined by the ratio of the amount of oxides of immobile cations (mainly Al) to mobile cations (magnesium, calcium and sodium) with the assumption that the potassium content remains constant during the weathering process (Haskins, 2006). This index is an attempt to determine the maturity of residual sediments, and its greater value indicates higher intensity of weathering processes (Price and Velbel, 2003; Haskins, 2006). Vogt's index was used to compare the chemistry of glacial tills and marine clay deposits, showing that glacial tills are much more heavily weathered than marine clays. This suggests that the weathering process ended before deposition of marine sediments (Roaldset, 1972).

Chemical Index of Alteration **(CIA)** is the most commonly used proxy in research of fluvial deposits. Its value clearly reflects an increase in weathering intensity of silicates from the river's lower to upper reach. Evaluation of CIA values combined with a comprehensive facies analysis allows for a precise approximation of chemical and physical weathering conditions in the past (Shao et al., 2012).

The CIA illustrates the ratio of Al_2O_3, mostly immobile during weathering, to the mobile cations Na^+, K^+ and Ca^{2+} (Bahlburg and Dobrzinski, 2011). High CIA values indicate washing out of the mobile Ca^{2+}, Na^+ and K^+ cations during weathering in relation to immobile components (Al^{3+}, Ti^{4+}) (Nesbitt and Young, 1982). Its low values suggest lack of chemical alterations and cool and/or arid climate conditions (Fedo et al., 1995). The greatest CIA value (up to 100) is typical of kaolinite weathering. For illite the figures vary between 75 and 90, for muscovite it is 75, and for feldspars 50. Fresh basalts show CIA values in the range of 30–45, and fresh granites and granodiorites 45–50 (Nesbitt and Young, 1982; Fedo et al., 1995).

Harnois's Chemical Index of Weathering **(CIW)** is calculated similarly to the CIA, eliminating only the K_2O contribution. In the calculations, aluminium, which is associated with potassium feldspars, is not separated, thus the CIW can reach very high values in the case of rocks rich in these minerals, no matter whether the rocks are chemically weathered or not (Fedo et al., 1995).

Plagioclase Index of Alteration **(PIA)** has been proposed as a substitute for the CIW. It is particularly applicable for the separate monitoring of weathering of plagioclases.

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The PIA attains values of 50 in non-weathered rocks and close to 100 in clay minerals such as kaolinite, illite and gibbsite, in accordance with the values derived from the CIA formula.

Study area and research methods

Characteristics of the study area

The Vistula River basin covers an area of 183,176 km^2, which is ca. 59% of the total land area of Poland. According to the physic-geographical regionalization, the Vistula River basin is located within the following subprovinces: Central Western Carpathians, Outer Western Carpathians, Eastern Beskids, Eastern Subcarpathia, Northern Subcarpathia, Silesian–Cracow Upland, Małopolska Upland, Lublin–Lviv Upland, Polesie, Central Polish Lowlands, Podlasie–Belarus Uplands, South Baltic Lakelands, East Baltic Lakelands, Southern Baltic Coast, and Eastern Baltic Coast (Kondracki, 2009).

In the Upper Vistula River basin, which is a very diverse area in terms of geology, there are pre-Quaternary deposits. The most elevated part of the basin, the Tatra Mountains, is composed partly of crystalline rocks (gneisses, schists and granites) and sedimentary rocks (conglomerates, quartzites, clays, limestones, and marls). The Pieniny Klippen Belt is composed of Jurassic–Cretaceous and Paleogene carbonates. In Podhale, there are Paleogene sandstones and shales, while the Outer Carpathians are built of sandstones and shales, the so called flysch. Outcrops of pre-Quaternary rocks are also found in the Lublin Upland (Cretaceous limestones and marls), Małopolska Upland (limestone and marls in the Nida Trough), Holy Cross Mountains (limestones, dolomites, sandstones and quartzites) and Silesian–Cracow Upland (conglomerates, limestones, dolomites, clays and sandstones) (Marks et al., 2006).

Most of the Lower Vistula River basin area is covered by Quaternary deposits. These are Pleistocene glacial tills, ice-dammed lake clays, glaciofluvial and fluvial sands, fluvial gravels and sands, and peats and muds. Some areas of the Małopolska Upland and Lublin Upland are covered by loess and loess-like deposits. The river valleys are filled with Holocene alluvial and fluvial muds, sands and gravels (Marks et al., 2006).

The Odra River basin covers an area of 118,861 km² (of which 106,056 km² in Poland), including the Warta River basin of 54,529 km². In the physic-geographical regionalization of Poland, the Odra River basin is located within the area of the following subprovinces: the Sudetes and Sudetes Foothills, Central Polish Lowlands, Silesian–Lusatian Lowlands, South Baltic Lakelands, and Southern Baltic Coast (Kondracki, 2009). Almost the entire area of the Odra River basin is covered by Quaternary glacial and fluvial deposits (tills, sands, muds, peats) (Kondracki, 2009). In some places, for example in the Opawa River basin, Quaternary loess is found. In the Sudetes area, situated in the southwestern part of the drainage basin (upper reaches of the Odra River's left tributaries: the Kwisa, Bóbr and Orlica), pre-Quaternary magmatic, metamorphic and sedimentary rocks are exposed on the surface. The oldest rocks include gneisses, granitogneisses and migmatites of the Sudetes Mountains. In the Kaczawskie Mountains, Kłodzko region, and north of Strzegom, in the Sudetes Foreland, there are rocks metamorphosed in the much shallower, so called greenschist zone. These are various types of phyllites (which are metamorphosed mudstones and clay shales), metasandstones, metavolcanites, and crystalline limestones.

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Sedimentary rocks of the Sudetes are represented by conglomerates, sandstones, greywackes, mudstones and clay shales. In the Paleozoic, two basic groups of igneous rocks were formed: (1) gabbro, peridotites and serpentinites (Nowa Ruda–Słupca, Ząbkowice Śląskie (Szklary) region in the Sudetes Foreland, Ślęża and its vicinity and (2) granites (Karkonosze Ridge, the massifs between Złoty Stok and Kłodzko, Kudowa region). Younger sedimentary formations are represented in the Sudetes and their foreland by the Upper Permian limestones, marls and gypsum, Triassic red sandstones and mudstones, as well as limestones and marls, and Upper Cretaceous sandstones and marls (Table Mountains, and the vicinity of Lwówek Śląski, Wleń, Bystrzyca Kłodzka and Międzylesie). This area was covered by Quaternary ice sheets, resulting in the accumulation of glacial, fluvial, eolian and slope deposits: various types of clays, sands, gravels, loesses and alluvial muds (Cwojdziński and Kozdrój, 2007).

Fluvial sediments in the area of Poland are dominated by quartz; carbonates and feldspars are present in smaller quantities. These components are represented by grain fractions >0.06 mm, which usually account for more than 90% of the sediment. This material originates mainly from the erosion of rocks occurring in the drainage basin. Fine-grained fractions (<0.06 mm) contain mostly minerals of the mica/illite group, and quartz, kaolinite and chlorite. Carbonates, feldspars, pyroxenes and amphiboles are less frequent. Significant amounts of organic matter and hydrated iron and manganese oxides are also present (Bojakowska and Sokołowska, 1998).

The area of Poland lies within the transitional climate zone. Change of isotherms system depending on season from latitudinal to longitudinal is a characteristic feature of Poland's climate. Summer temperature drop from south to north is connected with the increase of latitude. Winter temperature drop from west to east is in contrast conditioned by atmospheric circulation. In spatial distribution of temperatures Baltic coast is the warmest in the coldest months, whilst mountain peaks are the coldest. In July predominant part of Poland has the average temperature about 17.0–18.0 °C (Richling and Ostaszewska, 2005). The lowest average annual air temperatures are characteristic for the highest areas. Generally, besides mountain areas average annual air temperatures lower than 7 °C are distinctive for eastern part of Masurian Lake District and Region of Podlasie (Woś, 2010).

In Poland, similarly like in western and northern Europe, south-westerly, westerly and north-westerly winds are dominant. Wind field is deformed by Baltic Sea, lakes and land relief (mountains) (Richling and Ostaszewska, 2005).

"Zoning" is the most significant feature of spatial distribution of total annual precipitation on the area of Poland. Lowland zone receives below 600 mm of precipitation per year, so less than the average annual rainfall. Especially small rainfalls occur in the central Poland (about 500 mm). In the north of the country rainfall is larger, but precipitation level does not achieve there 700 mm. Precipitation increases together with elevation above sea level towards south from lowlands zone in central Poland. And so, Małopolska and Lubelska Upland receive on average over 550 mm of precipitation, and Holy Cross Mountains zone over 650 mm. Precipitation amounts quickly increase southwards and in the highest parts of the Tatra Mountains elevate even 1600–1700 mm. In the Sudetes precipitation levels are sometimes higher than at the same heights in the Carpathians (1200–1300 mm). Sudeten Foreland receives in turn less rainfalls than the Carpathian Foothills, what is associated with different land relief (Richling and Ostaszewska, 2005).

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Research methods

The research is based on results of analyses of 178 sediment samples collected from the Vistula River and its tributaries (Bug, Dunajec, San, Pilica, Bzura and Narew rivers) and 114 sediment samples from the Odra and Warta rivers and their tributaries (Nysa Łużycka, Bóbr, Kwisa, Kaczawa, Bystrzyca, Barycz, Nysa Kłodzka and Mała Panew rivers). The sediments were analysed within the framework of the National Environmental Monitoring (PMŚ) in 2015. Sampling sites are shown in the sketch map (*Fig. 1*). Sediment samples were collected at sites located along the rivers, downstream of the mouths of tributary rivers and other watercourses longer than 50 km, and downstream of major cities or towns where industrial plants are located.

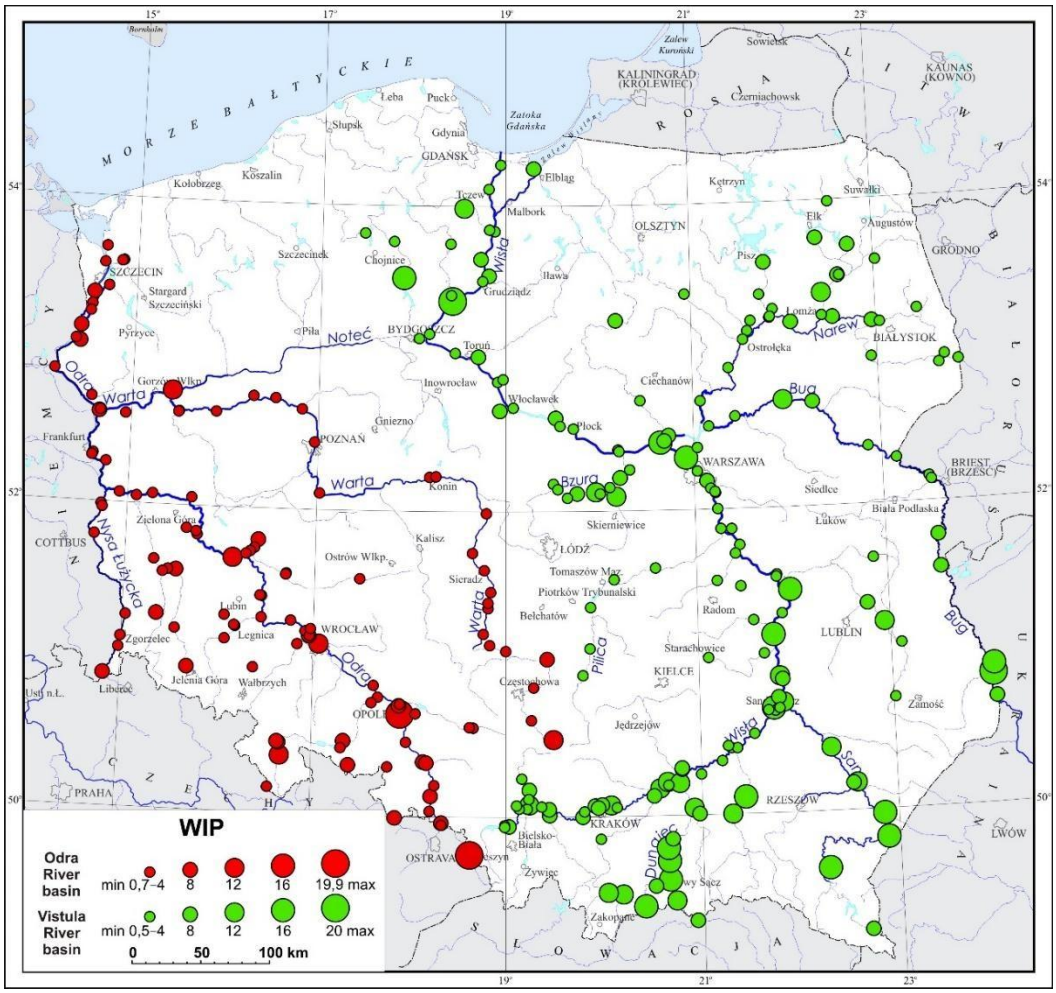


Figure 1. *Sampling sites and Weathering Index of Parker values*

Sediment samples were taken with an aluminium scoop from river banks, where the accumulating sediment is characterised by the highest content of the mud and clay fraction (*Fig. 2*). Each sample was an averaged sample of four or five samplings from a 5-cm thick surface sediment layer over a stretch of about 50 m. The sediment samples were dried at room temperature and then sieved through nylon sieves with a mesh size of 0.2 mm. The Al, Ca, Mg, K and Na concentrations were determined using atomic

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plasma emission spectrometry (ICP–OES) after digestion of the samples in aqua regia (*Fig. 3*). The detection limits were 0.01% for Al, Ca, Mg and K, and 0.001% for Na.

Results of the Al, Ca, Mg, K and Na determinations were used to calculate WIP, V, CIA, CIW and PIA values according to the formulas shown in *Table 1* (molecular proportions of oxides).

Statistical parameters (the minimum, maximum, median, mean and geometric mean) for the contents of elements and the calculated weathering indices were determined using the Statistica program. Maps of the variability of weathering indices in sediments were compiled using ArcGIS software.



Figure 2. *River sediment sampling in the Vistula River basin. (Photo: A. Dusza-Dobek)*



Figure 3. *iCAP 6500 duo Thermo Scientific ICP–OES instrument used for determination of analysed chemical elements. (Photo: I. Jaroń)*

Research results

The Al content in sediments of the Vistula River basin ranges from 0.08 to 1.95%, and its mean, geometric mean and median values are 0.39%, 0.31% and 0.28%, respectively (*Table 2*). The calcium contents vary in the range of 0.04–4.22%, and the

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mean, geometric mean and median values are 0.93%, 0.61% and 0.64%, respectively. The magnesium content varies from 0.01 to 0.92%, and its mean, geometric mean and median values are 0.19%, 0.12% and 0.12%, respectively. The sodium content in sediments of the Vistula River basin ranges from 0.003 to 0.103%; the mean value is 0.014%, the geometric mean 0.012%, and the median 0.011%. The potassium contents range between 0.02 and 0.40%, and its mean, geometric mean and median values are 0.07%, 0.06% and 0.06%, respectively. Standard deviation values for all elements of the Vistula River basin were in a range between 0.01 and 0.84, whereas coefficient of variation (CV) values were between 71 and 100 (*Table 2*). Because of the clear variability in the contents of individual elements in the Vistula River basin, the drainage basins of the Dunajec, San, Pilica, Bzura, Bug and Narew rivers have also been discriminated, for which the content ranges of the elements and the mean, geometric mean and median values are determined (*Table 2*).

Table 2. *Statistical parameters of elements content in river sediments*

Element	Mean	Geometric mean	Median	Minimum	Maximum	Standard deviation	Coefficient of variation
				%			
Vistula River basin (n=178)							
Al	0.39	0.31	0.28	0.08	1.95	0.31	79
Ca	0.93	0.61	0.64	0.04	4.22	0.84	90
Mg	0.19	0.12	0.12	0.01	0.92	0.19	100
Na	0.014	0.012	0.011	0.003	0.103	0.01	71
K	0.07	0.06	0.06	0.02	0.40	0.05	71
Bug (n=12)							
Al	0.28	0.25	0.32	0.09	0.48	0.12	43
Ca	1.52	1.05	1.26	0.21	4.22	1.28	84
Mg	0.10	0.08	0.09	0.03	0.24	0.07	70
Na	0.009	0.008	0.009	0.004	0.015	0.003	33
K	0.06	0.05	0.05	0.02	0.11	0.03	50
Dunajec (n=10)							
Al	0.83	0.81	0.77	0.60	1.13	0.19	23
Ca	1.55	1.41	1.42	0.61	2.52	0.67	43
Mg	0.55	0.52	0.53	0.25	0.87	0.19	35
Na	0.016	0.016	0.017	0.009	0.021	0.003	19
K	0.13	0.12	0.13	0.09	0.19	0.03	23
San (n=7)							
Al	0.59	0.58	0.56	0.42	0.78	0.12	20
Ca	2.10	1.99	2.24	1.02	2.84	0.68	32
Mg	0.51	0.50	0.49	0.34	0.77	0.14	27
Na	0.016	0.015	0.016	0.008	0.021	0.005	31
K	0.12	0.12	0.13	0.07	0.15	0.03	25
Bzura (n=11)							
Al	0.32	0.27	0.24	0.11	0.70	0.19	59
Ca	0.86	0.67	0.79	0.19	2.08	0.60	70
Mg	0.13	0.10	0.11	0.04	0.31	0.09	69

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Na	0.017	0.012	0.011	0.004	0.073	0.02	118
K	0.06	0.05	0.05	0.02	0.11	0.03	50
Narew (n=30)							
Al	0.19	0.19	0.19	0.11	0.42	0.06	32
Ca	0.73	0.61	0.66	0.15	2.18	0.44	60
Mg	0.12	0.10	0.11	0.03	0.47	0.09	75
Na	0.011	0.011	0.011	0.007	0.019	0.002	18
K	0.05	0.05	0.05	0.03	0.07	0.01	20
Pilica (n=13)							
Al	0.25	0.21	0.16	0.10	0.60	0.16	64
Ca	0.37	0.22	0.27	0.04	1.52	0.42	114
Mg	0.06	0.04	0.03	0.02	0.25	0.07	117
Na	0.007	0.007	0.007	0.003	0.012	0.003	43
K	0.04	0.03	0.03	0.02	0.09	0.03	75
Odra River basin (n=114)							
Al	0.44	0.34	0.29	0.12	1.52	0.34	77
Ca	0.43	0.26	0.25	0.04	4.54	0.61	142
Mg	0.13	0.08	0.06	0.01	0.69	0.14	108
Na	0.024	0.019	0.018	0.006	0.172	0.02	83
K	0.08	0.07	0.06	0.03	0.28	0.05	63
Warta (n=26)							
Al	0.22	0.21	0.21	0.12	0.48	0.08	36
Ca	0.41	0.23	0.22	0.04	2.41	0.62	151
Mg	0.07	0.05	0.04	0.01	0.58	0.11	157
Na	0.016	0.015	0.015	0.009	0.029	0.005	31
K	0.06	0.05	0.05	0.03	0.09	0.02	33

The Al content in sediments of the Odra River basin is in the range of 0.12–1.52%, and its mean, geometric mean and median values are similar to those determined in the Vistula River basin (*Table 2*). The calcium content varies between 0.04 and 4.54%, which is close to the values recorded for sediments from the Vistula River basin, but the mean and median values were below 0.5%. The magnesium content varies in the range of 0.01–0.69% (the median 0.06%), the sodium content is 0.006–0.172% (the median 0.018%), and the potassium content is 0.03–0.28% (the median 0.06%). The mean, geometric mean and median values for magnesium are much higher in the Vistula basin sediments, for sodium in the Odra basin, whereas for potassium the values are very similar (*Table 2*). Standard deviation values for the elements in the Odra basin varied between 0.02 and 0.61 and coefficient of variation values between 63 and 142. Like with the case of the Vistula and Odra river basins, separate statistical parameters have also been determined for the Warta River basin (*Table 2*).

The WIP values in sediments of the Vistula River basin varied between 0.5 and 20, and the mean, geometric mean and median values were 5.1, 3.7 and 3.8, respectively, whereas standard deviation and coefficient of variation values were 4.05 and 79 respectively (*Table 3, Fig. 1*). The highest value of this index was recorded in sediments of the Fryba River in Chełmno, where the bedrock is represented by limestone, opoka

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(polish name of siliceous-carbonate rock), mudstone and claystone. Values ranging from 17.1 to 13.5 are reported from sediments of the Bug River near the border with Ukraine (limestones and dolomites), and of the Vistula River in Potrawin (limestones, opokas and marls) and Sandomierz (sands, gravels, alluvial muds, Pleistocene peats and muds, with the bedrock composed of loess, limestone, marl and opoka). The lowest WIP value was measured in sediments of the Kamienna River in Starachowice (the bedrock is composed of conglomerates, sandstones and mudstones). Low WIP values are typical of sediments of the Radomka River in Ryczywół, and of the Pilica River in Mniszew (alluvial sands, gravels and muds).

The V index values in the Vistula drainage basin were in the range between 0.1 (Brda River in Tuchola) and 2.8 (Pszczynka River in Jedlina), with the mean of 0.4, geometric mean 0.3, median 0.3, standard deviation 0.32 and coefficient of variation 80. In the Vistula basin, the highest levels of the chemical weathering index V were found in the tributaries of the Upper Vistula River (Pszczynka, Soła and Iłownica rivers) and in the middle course of the Pilica River (in Spała) (*Table 3, Fig. 4*). In turn, the lowest values were reported from sediments of the Brda River, middle course of the Wieprz River, Bug River, and the Narew River and its tributaries.

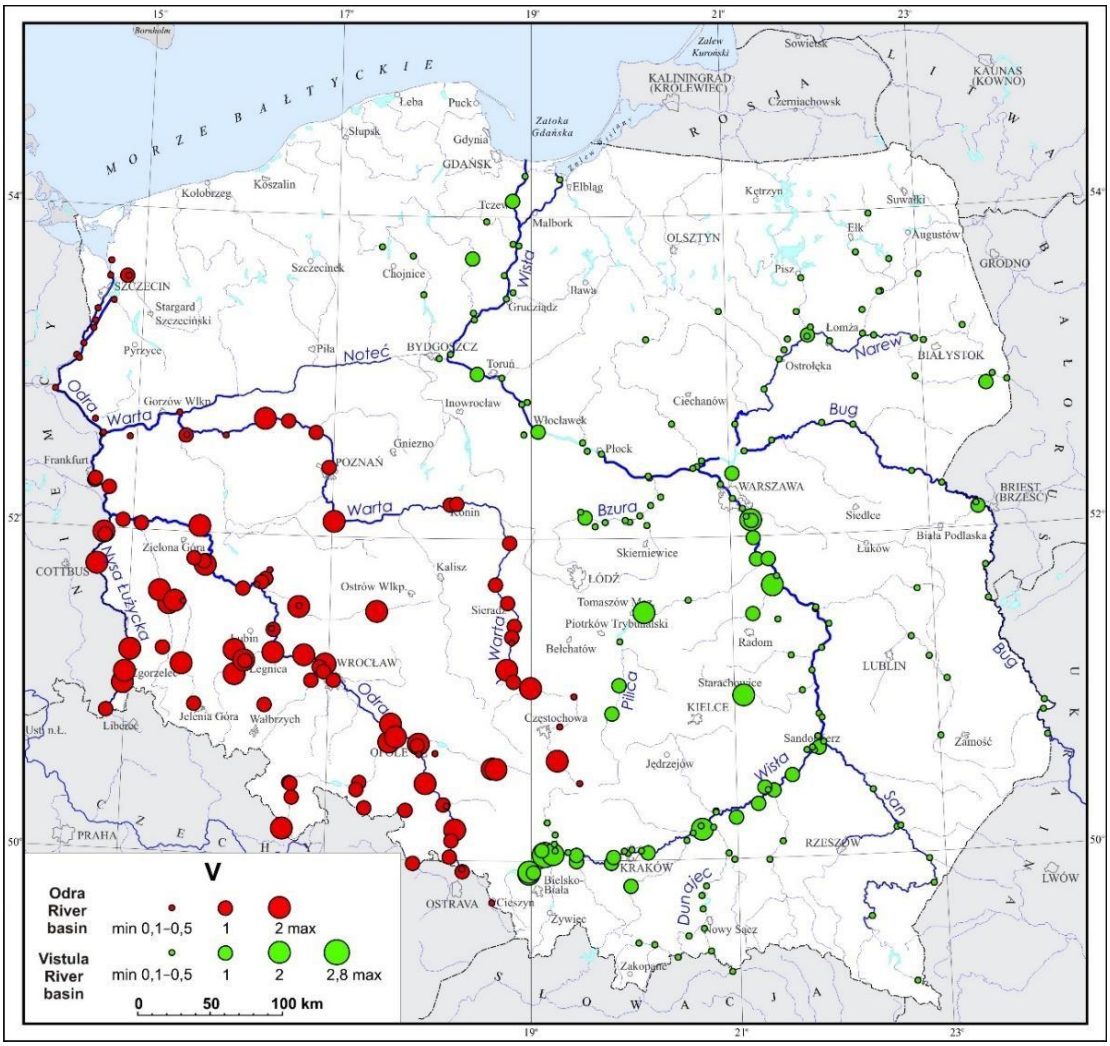


Figure 4. *Vogt's Residual Index values*

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Table 3. *Statistical parameters of chemical weathering indices*

Chemical weathering index	Mean	Geometric mean	Median	Minimum	Maximum	Standard deviation	Coefficient of variation
				%			
Vistula River basin (n=178)							
WIP	5.1	3.7	3.8	0.5	20	4.05	79
V	0.4	0.3	0.3	0.1	2.8	0.32	80
CIA	27.6	24.3	24.2	4	80	14.07	51
CIW	28.9	25.3	25	4	82	15.20	53
PIA	26.2	22.5	22.2	3	81.7	14.79	56
Bug (n=12)							
WIP	6.6	4.8	5.5	1.3	17.1	148.58	2251
V	0.2	0.2	0.2	0.1	0.3	0.11	55
CIA	15.4	14.2	14	6.6	24.3	5.45	35
CIW	15.8	14.6	14.2	6.7	25.4	6.03	38
PIA	13.8	12.8	12.7	5.9	22	4.27	31
Dunajec (n=10)							
WIP	10	9.4	9	4.5	14.7	94.47	945
V	0.3	0.3	0.2	0.2	0.6	0.16	53
CIA	28.4	27.6	25	21	44	6.85	24
CIW	29.3	28.5	25.7	21.4	45.8	7.75	26
PIA	27.1	26.3	23.6	19.8	43.5	7.17	26
San (n=7)							
WIP	11.2	11	11.7	7.7	14.4	67.70	604
V	0.2	0.2	0.2	0.1	0.4	0.14	70
CIA	18.2	16.9	15.2	10.5	34.2	7.50	41
CIW	18.7	17.3	15.6	10.7	35.8	8.78	47
PIA	16.6	15.3	13.6	9.5	32.6	7.09	43
Bzura (n=11)							
WIP	4.4	3.4	3.8	1.1	10.1	91.21	2073
V	0.3	0.3	0.3	0.2	0.6	0.17	57
CIA	24	22.9	22.9	15	41.9	7.55	31
CIW	24.7	23.6	23.5	15.3	43.4	8.65	35
PIA	22.5	21.3	21.5	13.5	41.2	8.04	36
Narew (n=30)							
WIP	3.8	3.3	3.4	1.1	10.8	55.67	1465
V	0.2	0.2	0.2	0.1	0.7	0.21	105
CIA	18.9	17.3	17.8	6.8	43	7.27	38
CIW	19.6	17.9	18.4	6.9	46	8.71	44
PIA	16.8	15.1	16.1	5.5	41.9	6.82	41
Pilica (n=13)							
WIP	2.1	1.5	1.6	0.5	7.6	61.03	2906
V	0.7	0.6	0.6	0.2	1.8	0.67	96
CIA	38.4	35.5	36.2	18.1	67.7	13.62	35

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CIW	40.6	37.2	38	18.6	72.9	17.29	43
PIA	37.9	34.4	34.9	16.5	70.6	15.79	42
Odra River basin(n=114)							
WIP	3.2	2.5	2.3	0.7	19.9	2.95	92
V	0.8	0.7	0.8	0.1	2	0.46	58
CIA	45	40.8	48.9	8	72	16.73	37
CIW	48.4	43.7	52.9	8.9	76.7	18.57	38
PIA	44.9	39.9	48.5	6.8	75	18.47	41
Warta (n=26)							
WIP	2.5	1.9	1.8	0.8	11.8	2.70	108
V	0.7	0.6	0.7	0.1	2	0.47	67
CIA	37.3	34.2	37.8	8.9	67.4	13.82	37
CIW	40.6	36.8	40.8	8.9	74.7	15.93	39
PIA	36.1	32.6	35.6	8.4	71.6	15.06	42

The CIA values in sediments of the Vistula River basin are in the range of 4–80; the mean is 27.6, geometric mean 24.3, and median 24.2. Standard deviation value is 14.07 and coefficient of variation is 51 (*Table 3, Fig. 5*).

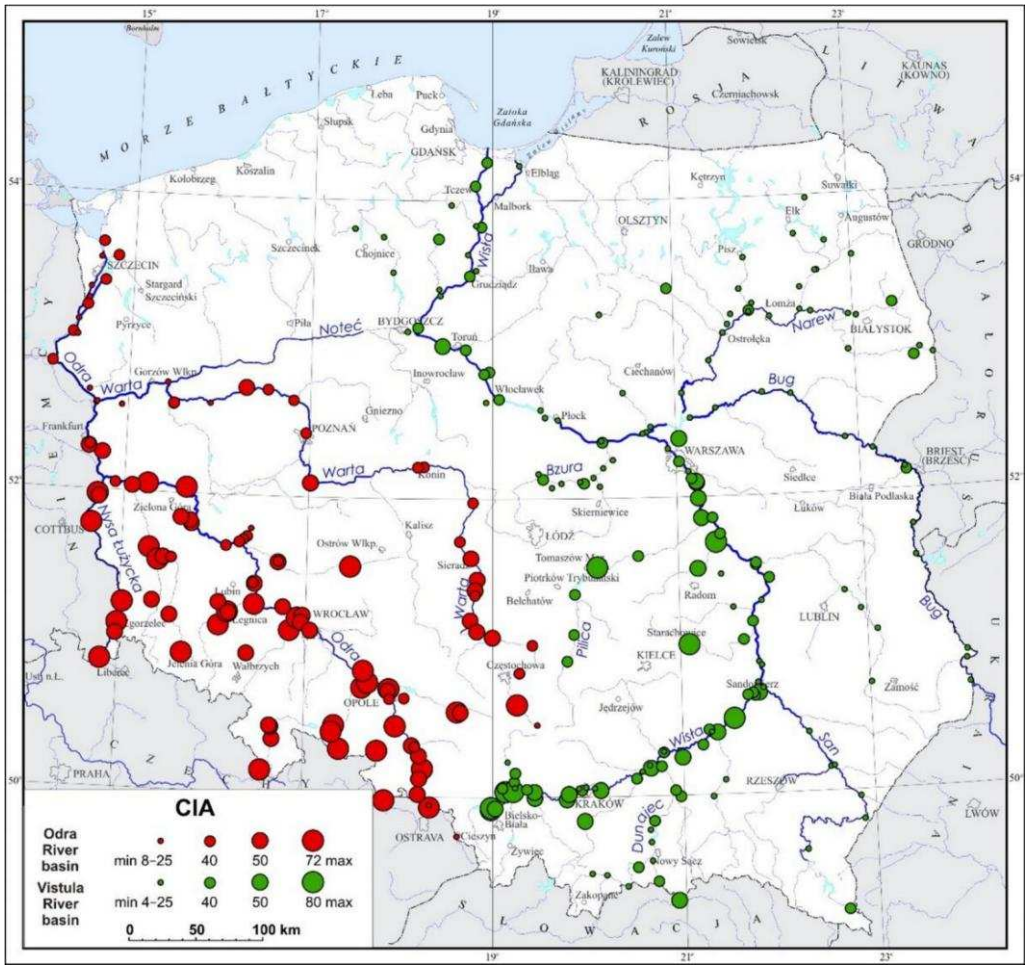


Figure 5. *Chemical Index of Alteration values*

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The highest values are found in sediments of the Pszczynka River in Jedlina and the Soła River (sands, gravels, alluvial muds and peats), while the lowest ones are observed in sediments of the Brda River in Tuchola (upon the bedrock composed of limestones, opokas, marls, phosphorites and cherts) and of the Wieprz River in Jaszczów (Cretaceous limestones, opokas and marls).

In sediments of the Vistula River basin, the CIW values varied from 4 (Brda River in Tuchola) to 82 (Pszczynka River in Jedlina), and the mean, geometric mean and median values were 28.9, 25.3 and 25, respectively. Standard deviation amounted 15.20 and coefficient of variation 53 (*Table 3, Fig. 6*).

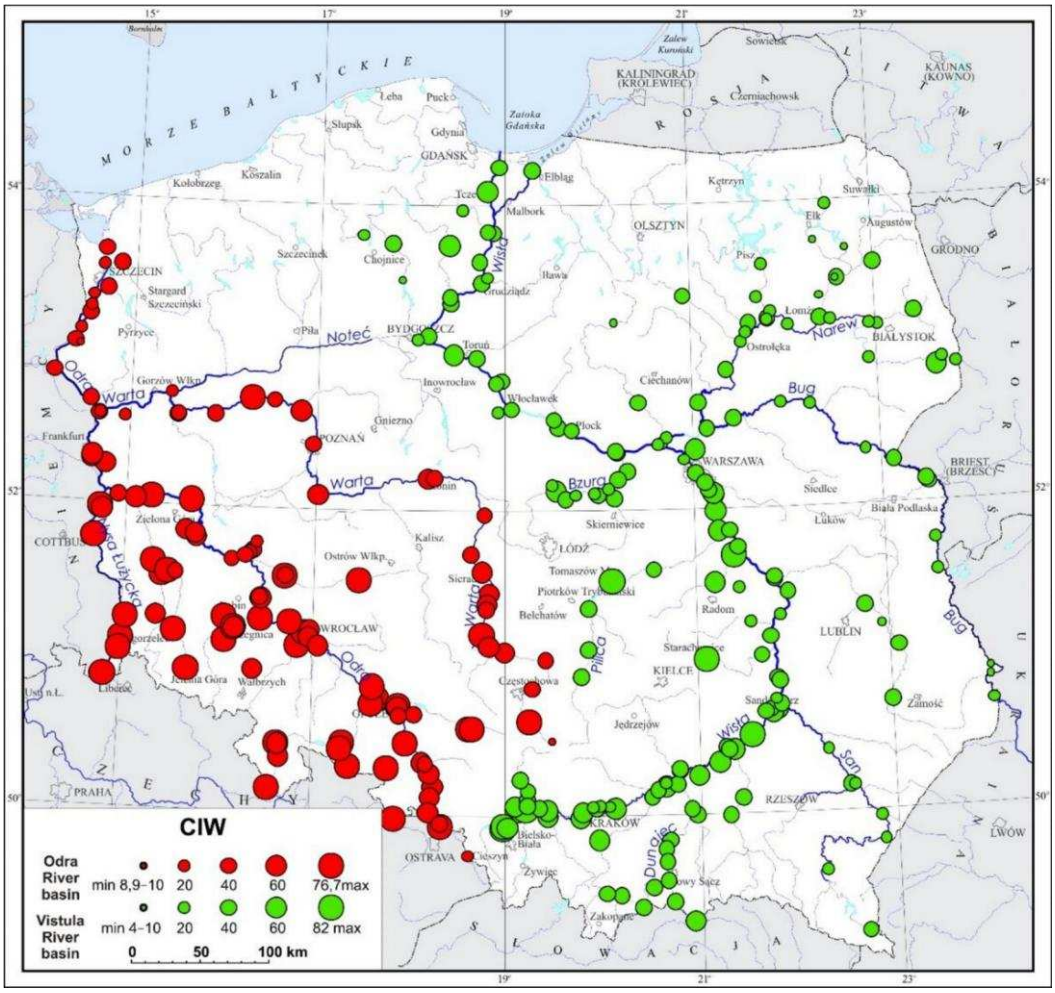


Figure 6. *Chemical Index of Weathering values*

The PIA values ranged from 3 (Brda River in Tuchola) to 81.7 (Pszczynka River in Jedlina); the mean was 26.2, geometric mean 22.5, and median 22.2, standard deviation 14.79 and coefficient of variation 56 (*Table 3, Fig. 7*).

In sediments of the Odra River basin, the Parker index values ranged from 0.7 to 19.9, and the mean, geometric mean and median values were 3.2, 2.5 and 2.3, respectively. Standard deviation and coefficient of variation values were 2.95 and 92 (*Table 3, Fig. 1*). The highest WIP value was found in sediments of the Olza River in Ropice (draining the Cretaceous carbonates), and the lowest one is reported from the

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Czerna Wielka River with the bedrock composed of sands, gravels and muds. The values of 17.1–10.1 were found in sediments of the Olszanka and Warta rivers in Kromołów and Santok.

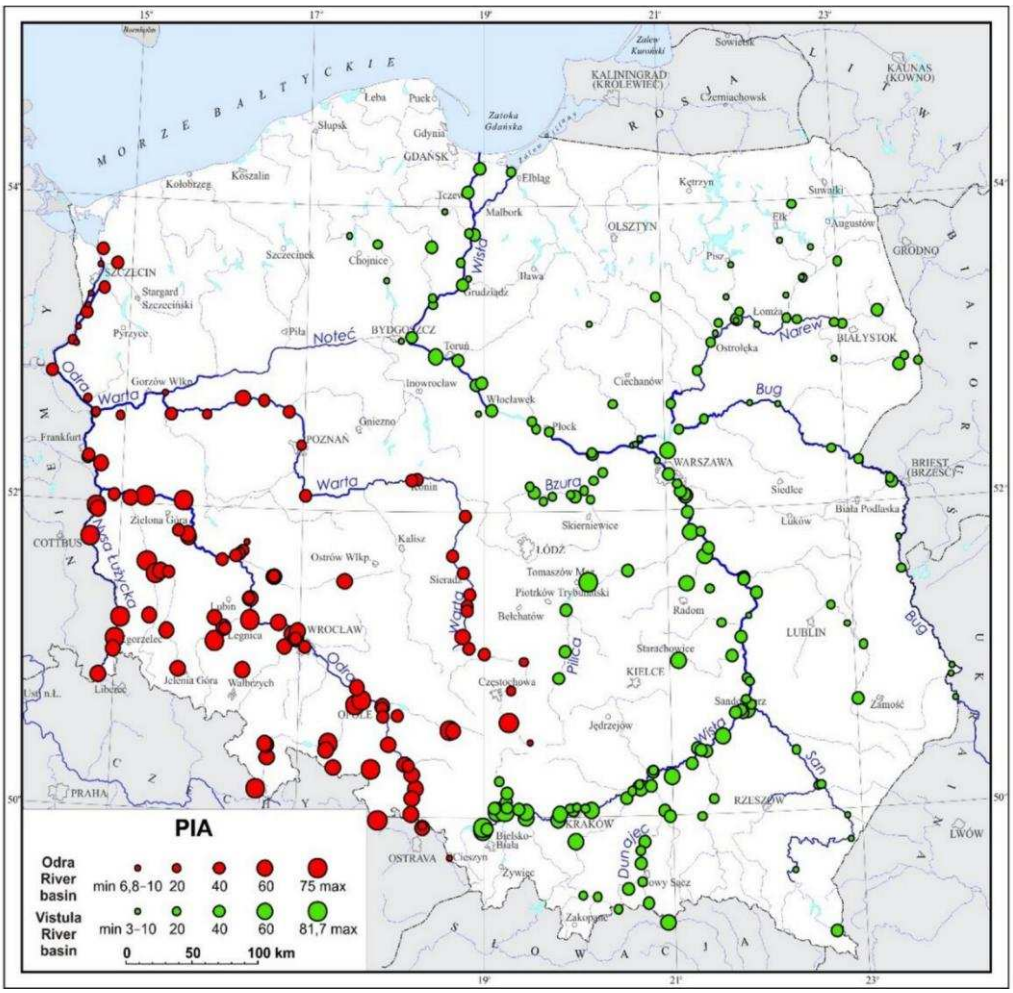


Figure 7. *Plagioclase Index of Alteration values*

The V index values in sediments of the Odra River basin are in the range from 0.1 (Warta River in Kromołów) to 2 (Zimny Potok stream in Ciemnice); the mean was 0.8, geometric mean 0.7, and median 0.8, standard deviation 0.46 and coefficient of variation 58 (*Table 3, Fig. 4*). The Vogt's index values are very high in the upper course of the Nysa Łużycka River, at its confluence with the Odra River, and at the confluence of the Kwisa and Bóbr rivers. The lowest values were recorded in the lower reach of the Odra River, at its mouth to the Baltic Sea, and near the confluence with the Warta River in the middle course of the Odra.

The CIA values varied between 8 and 72, and the mean, geometric mean and median values were 45, 40.8 and 48.9, respectively. Standard deviation and coefficient of variation values were 16.73 and 37, respectively (*Table 3, Fig. 5*). The highest levels were observed in sediments of the Nysa Łużycka River in Zasieki (sands, gravels, alluvial muds and peats), and the lowest values were recorded in sediments of the Rurzyca River near its confluence with the Odra (glacial tills in the bedrock). Very high

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values were also found in the Zimny Potok stream in Ciemnice (fluvial clays, muds and sands) and in the Nysa Łużycka River in Zgorzelec. Low values are reported from the Warta River in Kromołów – a district of Zawiercie (Jurassic limestones) – and from the Olza River (Cretaceous limestones and shales).

The CIW values ranged from 8.9 (Rurzyca River in Nawodna) to 76.7 (Nysa Łużycka River in Zasieki), and the mean, geometric mean and median values were 48.4, 43.7 and 52.9, respectively. Standard deviation amounted 18.57 and coefficient of variation was 38 (*Table 3, Fig. 6*).

The PIA values varied between 6.8 (Rurzyca River in Nawodna) and 75 (Nysa Łużycka in Zasieki), and the mean, geometric mean and median values were 44.9, 39.9 and 48.5, respectively. Standard deviation value was 18.47 and coefficient of variation value was 41 (*Table 3, Fig. 7*).

Graphical representation of the distribution of chemical weathering indices shows that they distinctly differ between the Odra basin and the Vistula basins, and that the WIP index reveals the lowest variability (*Fig. 8*).

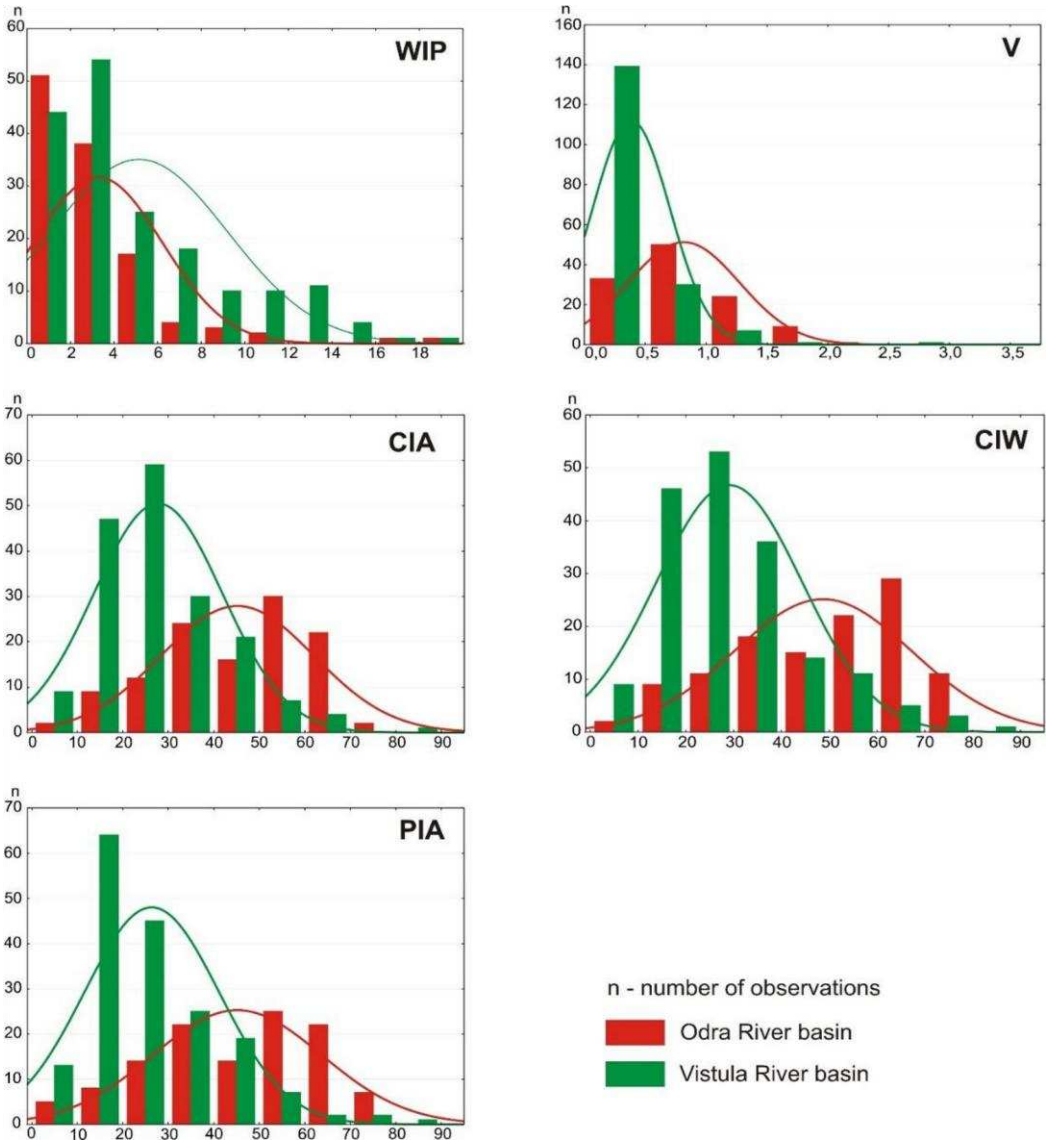


Figure 8. *Histograms of chemical weathering indices*

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The dominant WIP values in sediments of both basins are from 0 to 4. In the case of the Odra basin sediments, the WIP values are usually in the range of 0–2, and less frequently of 2–4. In the Vistula basin, the most common values vary from 2 to 4, and less frequently from 0 to 2. The CIA values in the Odra basin are generally in the range of 30–70, while in the Vistula basin they vary between 10 and 40 (*Fig. 8*). The distribution of the values of the plagioclase index of alteration is similar. The most common PIA values in the Odra basin sediments are in the range of 30–70, while in the Vistula basin they are between 10 and 50 (*Fig. 8*). In the Vistula basin, the dominant Vogt's index values are up to 0.5, while in the Odra basin between 0.5 and 1.0. The CIW values also differ between both basins. In the Vistula basin, the dominant values are in the range of 10–40, while in the Odra basin they vary from 30 to 70. In general, fluvial sediments in the Odra River basin are characterised by a greater number of samples with low values of the weathering indices than those in the Vistula basin, except for the WIP.

The most common values of the analysed indices (the percentile range is 25–75) in sediments of the drainage basins are well illustrated in the box plots (*Fig. 9*).

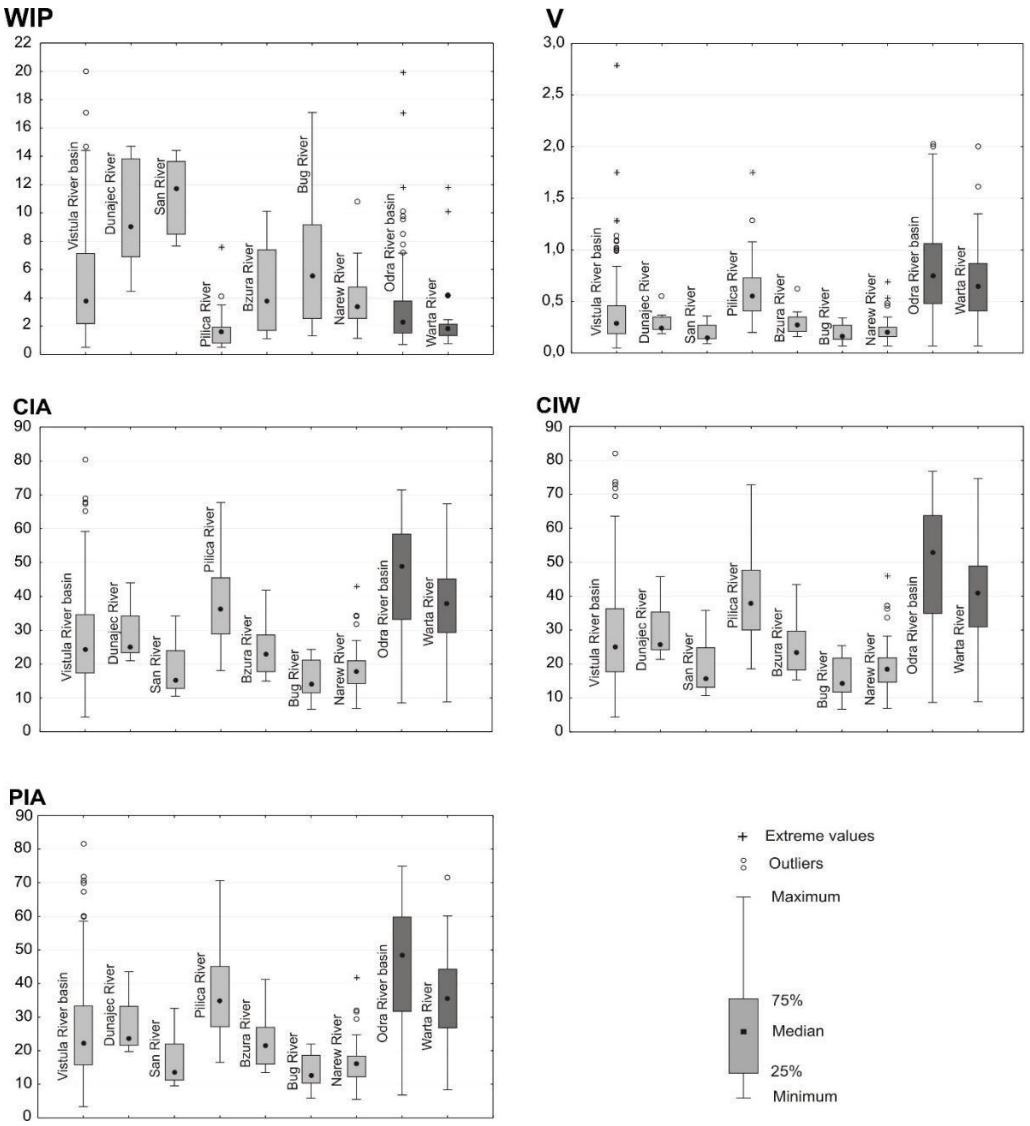


Figure 9. *Boxplots of chemical weathering indices in sediments of selected rivers*

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The range of the most common WIP values for sediments of the Odra basin (1.5–3.8) is much narrower than for those of the Vistula River system (2.2–7.1). For the Bug, Dunajec and Bzura rivers, the range is very wide, for the San River it is narrower, and the narrowest is for lowland rivers: the Narew, Pilica and Warta. The highest WIP values are observed in sediments of the drainage basins of the Dunajec and San rivers that drain the Carpathians and Subcarpathia. The lowest values are recorded in sediments of the Warta and Pilica basins (*Fig. 9*).

Discussion

The calculated indices are complementary and show similar trends. Sediments supplied into the Lower Odra River basin were subjected to stronger chemical weathering during transport (because they carry away more mobile elements) and mixing with the sediments of the Warta River.

High values of standard deviation and coefficient of variation indicate on a very extensive dispersion of data in relation to average values. Variability of data in the case of majority of elements for both Vistula and Odra River basins and their tributaries is below 100, what gives information about moderate differentiation of elements content. Much higher values than 100 in the case of Ca and Mg were present for Pilica, Odra and Warta River basins and they inform about huge results variability. It is worth highlighting that Warta and Pilica have their sources on Małopolska Upland area, which is built of carbonate rocks. Coefficient of variation values for V, CIA, CIW and PIA indices point to moderate diversification of results. WIP is an exception among them and its values vary from 604 (San) to 2906 (Pilica). These values show extremely huge variability of obtained results. CV values below 200 suggest that these elements distribution in sediments is moderately homogenous and mainly involved with their natural (lithogenic) origin. CV values above 200 indicate on uneven distribution of data set and suggest that the part of elements was artificially provided to the environment. It is clearly visible in *Table 3*.

The WIP values in sediments of the Odra and Vistula basins are much lower as compared with those from other regions of the world. The WIP value for samples of suspended fine particles in Chinese rivers varies from 24.7 to 58.3, and the average is 38.8. In samples from floodplain areas the values range from 26.4 to 59.4, and the mean value is 44.4 (Shao et al., 2012). This index value in fluvial sands of Taiwan is in the range of 18–65 (Garzanti and Resentini, 2016), and it varies between 40.8 and 47.6 in stream sediments of the Alaknanda River basin (India) (Panwar and Chakrapani, 2016).

The range of the most frequent CIA values in the Odra basin (33.2–58.4) is wider than in the Vistula basin (17.4–34.6). The greatest variability of CIA values is observed in the case of sediments of the Pilica and Warta rivers. For the Bzura, Dunajec, San and Bug rivers, the variability is moderate, and for the Narew River it is the lowest.

The average CIA values calculated for the rivers of Poland are much lower than the average values for the rivers in the world, Asia and North America, although high levels have been sporadically recorded in some samples. The CIA value in sediments of various rivers across the world varies between 48.2 and 89.9, and the average is 72, with the highest levels in the African rivers (83.4 on average) as compared with the other continents. The lowest value observed in the North American rivers is 66.0. In the Asian rivers, the average value of this index is 71.7, which is close to the world's average. The highest CIA values are found in sediments of the Congo River (89.9), and the lowest

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ones are recorded in watercourses of the Kola Peninsula in the Arctic area (48.2) (Li and Yang, 2010). The CIA values in suspended matter of the Chinese rivers were between 58.8 and 91.3, with the average of 71.4, which is close to the world's average. In floodplain areas, the values varied from 44.5 to 84.3, with the average of 61.3. For comparison, sands of the Taiwan rivers show the CIA values in the range of 46–77. The values in the Chinese rivers are 72–88, and in the Alaknanda River basin (India) the average value is 56 (Garzanti and Resentini, 2016; Shao et al., 2012; Selvaraj and Chen, 2006; Panwar and Chakrapani, 2016). The highest levels of this index (88.9–94.5) were recorded in stream sediments of southwestern Nigeria (Madukwe et al., 2016).

The compilation of curves for V, CIA, CIW and PIA values for the individual river subbasins shows that they are comparable and the highest levels are observed in sediments of the Warta and Pilica river basins. In the remaining subbasins they are lower (*Fig. 7*). The similarity is undoubtedly due to the similar lithologies of the underlying rocks. The neighbouring drainage basins drain the area of the Mid-Polish Lowlands, and the rivers' headwaters are located within the Małopolska Upland, where limestones and marls are the important lithologic components.

The lowest V, CIA, CIW and PIA values are found in sediments of the drainage basins of the Narew, Bug and San rivers of eastern Poland, which drain areas of diverse geological structures. In the San drainage basin area, there are mainly flysch deposits. In the Bug River basin, carbonate-rich rocks (limestones, marls and loess) account for a considerable proportion, whereas in the Narew basin area there are only glacial deposits.

The V and CIW values in sediments of the Vistula and Odra river basins are definitely lower than in other regions of the world. The V index levels in weathering profiles of the Coweeta Basin, North Carolina, USA, are 1.9–16.2 (Price and Velbel, 2003). In sands of the Taiwan rivers, the CIW values are in the range of 47–95 (Garzanti and Resentini, 2016), and in stream sediments of Nigeria they vary between 89 and 98, locally reaching 95.3–98.2 (Gajere et al., 2015; Madukwe et al., 2016).

Similarly, the PIA values in the rivers of Poland are lower than the values observed in rivers of other climatic zones. In the fluvial sands of Taiwan, the PIA values vary from 46 to 94, and in sediments of the Kaoping River the average is 66 (Garzanti and Resentini, 2016; Selvaraj and Chen, 2006). The highest values of this index (94–98) were recorded in stream sediments of SW Nigeria (Madukwe et al., 2016).

In Poland, the length of sediment transport by rivers is likely to have little effect on the value of chemical weathering indices. Their decreasing values from the south towards the north of Poland in the analysed sediments of the whole study area indicate the dominant role of the chemical composition of bedrocks in the drainage basins.

This observation clearly confirms the different range and values of WIP, which are significantly smaller in the Odra River sediments than in the Vistula River sediments. The greatest variability of the WIP in the upper parts of the river basins (*Fig. 1*) is related to the drainage of igneous, metamorphic and sedimentary rocks of the Sudetes in the upper Odra River basin, and of the Carpathian flysch in the upper Vistula River basin. In the lower reaches of the rivers, the WIP, CIA, V and PIA values are less varied, indicating that their values are influenced mainly by the geological structure of those parts of the drainage basins where Quaternary deposits are exposed: glacial tills, clays, sands, gravels, peats and alluvial muds.

The greatest effect on the values of weathering indices in particular river subbasins can also be attributed to the lithologies.

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In the Bug River basin, the high WIP value and the low values of other indices are due to high calcium contents in sediments (derived from the drainage of loess, limestones and marls). In contrast, the Warta and Pilica river basins, which drain Quaternary deposits over almost the entire drainage basins, the WIP values are the lowest, as opposed to the values of the other indices (*Fig. 7*).

CIA values are used to assess the effect of climate on the rate and mode of weathering more often than the other indices are. At lower geographical latitudes, the average annual temperatures are higher and the intensity of chemical weathering decreases. In the present research, the CIA values show a dependence on the average annual temperature and precipitation. A moderate level of chemical weathering dominates in central Poland (Wielkopolska Lake District, Wielkopolska Lowland and Mazovian Lowland), in the area of the lowest annual precipitation. Fluvial sediments in northern Poland (Lower Odra and Lower Vistula drainage basins), with elevated annual precipitations (600–700 mm), are characterised by the highest degree of weathering. High weathering levels are also typical of sediments in the Sudetes and the Carpathians, where the annual precipitation is 800–1200 mm. The high intensity of chemical weathering in the Carpathians is best illustrated by the CIA values, while in the Sudetes by the WIP values.

Conclusions

1. The values of weathering indices in river sediments of Poland vary in a wide range, but they are lower than the values found in other warmer and humid climates of the world.
2. The values of weathering indices in river sediments are affected mainly by the chemical composition of bedrocks of the drainage basins. In those parts of the drainage basins, which are underlain by pre-Quaternary rocks, the sediments show greater resistance to chemical weathering and lower susceptibility to leaching of elements. The highest losses of elements due to leaching are characteristic of the lower parts of the Odra and Vistula drainage basins composed of Quaternary deposits.
3. The degree of chemical weathering of sediments of the analysed rivers of Poland varies according to climatic conditions that differ between the north and the south of the country. The highest degree of weathering is observed in the lower drainage basins of the Odra and Vistula rivers and in mountainous areas (the Sudetes and the Carpathians). In central Poland, the moderate degree of chemical weathering is dominant.
4. Comparison of chemical weathering indices variability in river sediments in southern Poland will be very worthwhile, especially in the Carpathians e.g. Dunajec and Wisłoka rivers and in the Sudetes (Nysa Łużycka and Bóbr).

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SOIL PHYSICO-CHEMICAL PROPERTIES IN LAPALALA WILDERNESS OLD AGRICULTURAL FIELDS, LIMPOPO PROVINCE OF SOUTH AFRICA

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Abstract. Natural recovery of abandoned agricultural fields take decades due to biotic and abiotic constraints. A key question is how does soil physico-chemical properties prevent the transition from degraded to restored state. This study examined changes in soil physico-chemical properties between old field sites that were abandoned 35 year ago and adjacent natural sites in Lapalala Wilderness, located in the Limpopo Province of South Africa. Top soils were collected from three paired sites (old field and natural sites) in five different locations, over three months and were quantified for soil moisture, total soil macro elements (N, C and P), pH, exchangeable cations (K, Ca, Mg, Na), resistivity, penetration resistance, cumulative infiltration and hydraulic conductivity. Results show that soils in old field sites had lower pH, total N and C, K, Mg, gravimetric soil moisture and were compact compared to soils in adjacent natural sites. Soil resistivity, total P, Na, Ca, cumulative infiltration and hydraulic conductivity showed no significant ($p > 0.05$) differences between the two sites. It appears that the soils in old field sites have poor properties, and indication that soil manipulation techniques e.g. fertilizer addition, top soil removal and soil transfer must be considered for vegetation recovery to take place.
Keywords: *land abandonment, soil reconstruction, soil fertility, soil nutrient, litter*

Introduction

Land abandonment commonly referred to as the termination of crop cultivation or livestock grazing is a widespread phenomenon in South Africa (Andrew et al., 2003). Social (rural depopulation, poor land use practices), economic (globalization of agricultural markets, shortage of capital) and ecological (declining soil fertility) reasons have been highlighted as causes for land abandonment (Andrew et al., 2003; Kakembo and Rowntree, 2003). The abandonment of land has a wide range of ecological consequences e.g. soil erosion, competitive exclusion, increased wildfires and invasion by invasive plants. All these consequences are linked to habitat loss and decline in ecosystem service provision (Plieninger et al., 2014). To restore abandoned agricultural lands (also referred to as old fields), models of ecological restoration (successional and alternative state) have been tried and they have produced mixed results (Hobbs and Suding, 2008). The broader objective of these models is to restore natural ecosystem processes, functions, and structure (Hobbs and Suding, 2008). For example, soil manipulation in old fields has aimed to improve soil properties with the view that improved soils will facilitate native vegetation recovery (Blumenthal et al., 2003; Ruwanza et al., 2012). Recent studies have shown that soil transfer from intact natural

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areas to old fields has the potential to recover both soils and plants in old fields (Wubs et al., 2016; Bulot et al., 2017).

The effect of land abandonment on soils has been varied. Some studies have shown that soils in old fields have high chemical properties compared to natural areas, this a result of past cultivation (Hooker and Compton, 2003; Gabarrón-Galeote et al., 2015). Additionally, some studies have shown that vegetation recolonization in old fields can influence soil changes (Bonet and Pausas, 2004), although the duration change is unclear. Since vegetation is known to be a driver of soil changes, variations in soil properties tend to be highly dependent on plant colonization (Gabarrón-Galeote et al., 2015). For example, secondary succession in Mediterranean environments usually starts with grasses and herbs, with a gradual increase in woody species over time (Rey Benayas, 2005; Cuesta et al., 2012). The above-mentioned changes in vegetation type contributes to changes in soil properties due to litter deposition and decomposition. Other studies have shown that the persistence of grassland cover combined with the slow recovery of woody species in semi-arid old fields result in the slow recovery of soil properties (Rey Benayas, 2005; Ruiz-Sinoga and Martínez-Murillo, 2009).

An understanding of changes in soil properties within old fields has the potential to improve our knowledge of ecological restoration in these areas. For instance, understanding the changes in soil properties in old fields has contributed to the development of new soil manipulation techniques (e.g. top soil removal and soil transfers) that are yielding successful plant and soil recovery (Wubs et al., 2016; Bulot et al., 2017). Besides that, insights into soil properties in old fields has helped our general understanding of bacterial and fungal activities, nutrient uptake and plant-soil interactions (McKinley et al., 2005). Therefore, the objective of this study was to assess the differences in soil physico-chemical properties between old field sites and adjacent natural sites in Lapalala Wilderness, South Africa. The research question was whether soil properties, namely soil physico-chemical properties, resistivity, penetration resistance, infiltration and hydraulic conductivity are different between old field sites and adjacent natural sites. Results of this study are envisaged to guide future restoration initiatives in Lapalala Wilderness old fields.

Materials and methods

Study sites and soil collection

Soils were collected at three different sites that were located at Lapalala Wilderness in the Limpopo province of South Africa (*Table 1* and *Fig. 1A*). The three sites were approximately 200 m apart to provide a measure of independence (Galatowitsch and Richardson, 2005), and each old field site was paired with a natural site, approximately 10 m from each other (road demarcation). The mean annual rainfall in this area is approximately 500 mm, which falls in the summer months between October and February. Temperatures in the area are cold in winter and hot in summer with mean daily maximum and minimum temperatures of 30 °C in January and 14 °C in June. Vegetation in the area is classified as Waterberg Mountain Bushveld, which is found in the savanna biome (Mucina and Rutherford, 2006). Soils in the area are nutrient poor sandy soils derived from the Kransberg subgroup of the Waterberg groups (Mucina and Rutherford, 2006). However, the central sections of Lapalala Wilderness are dominated by nutrient rich clay soils derived from norite/epidiorite intrusions. The dominant plant species in the natural areas include *Dichrostachys cinerea*, *Sclerocarya birrea*,

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Vachellia tortilis, *Ziziphus mucronata*, *Grewia* spp, *Arisitida congesta*, *Panicum maximum*, *Eragrostis rigidior* and *Aristida adscensionis.* The old field sites were previously used for tobacco and cattle farming approximately 35 years ago. Currently, they are used for animal grazing and are dominated by few scattered woody pioneer species (*V. karroo*) and low grass cover.

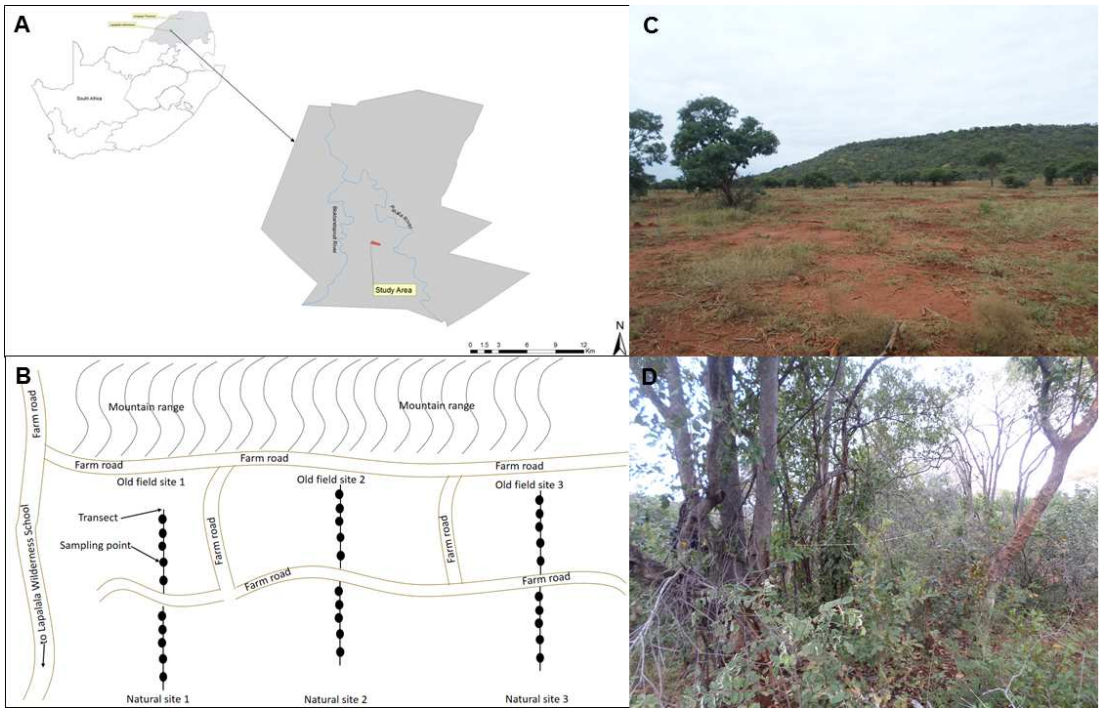


Figure 1. *Map showing (A) the location of the Lapalala Wilderness study area in the Limpopo Province, South Africa and (B) the experimental setup in the old fields, (C) photo of old field, and (D) photo of natural site. Location details of the sites are presented in Table 1. Photos taken by Sheunesu Ruwanza*

At each site, a 40 m transect (from west to east) running into the site, with the first point being 2 m from the road demarcating paired sites, was established. The above-mentioned transect comprised five soil collecting points that were 10 m apart (*Fig 1B*). Therefore, a total of 30 soil collecting points were marked, thus five soil samples points per site from six sites (three old field sites and three natural sites). In April 2017, two soil cores (30 cm apart) were collected at each collecting point for soil physico-chemical and moisture analysis. The soil physico-chemical analysis was done once due to limited finance. Besides, the expectation that repeated monthly measurements on soil physico-chemical properties would yield variations within three months was less. During the months of May and June, 30 soil cores per month were collected for soil moisture measurements only. Therefore, a total of 60 soil sample were collected in April (30 for physico-chemical analysis and 30 soil moisture measurements) and 30 soil cores per month for the months of May and June for soil moisture measurements. The soils were collected using a soil core measuring 8 cm in diameter and 8 cm depth after the hand removal of overlying debris. After soil collection, both soil physico-chemical properties and soil moisture measurements were assessed under laboratory conditions at the University of Venda in Thohoyandou, South Africa. Measurements on soil

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penetration resistance level and infiltration rate were conducted under field conditions at each of the soil collecting points for all the three months.

Table 1. *Characteristics of the three paired study sites located in Lapalala Wilderness in the Limpopo Province of South Africa. Old filed sites and natural sites were adjacent to each other*

Site number	Site identification	Site status	Coordinates	Soil texture*
Site 1	Site 1a	Old field site	23°51'53.83"S, 28°18'09.55"E	Loam
	Site 1b	Adjacent natural site	23°51'48.91"S, 28°18'12.36"E	Loam and clay
Site 2	Site 2a	Old field site	23°51'52.15"S, 28°17'51.58"E	Clay
	Site 2b	Adjacent natural site	23°51'42.56"S, 28°17'53.53"E	Clay
Site 3	Site 3a	Old field site	23°51'48.12"S, 28°17'35.04"E	Loam and clay
	Site 3b	Adjacent natural site	23°51'37.83"S, 28°17'41.09"E	Loam and clay

*Textural classes according to handbook of standard soil testing methods (1990) for advisory purposes. Compiled by the non-affiliated soil analysis work committee, Soil Science Society of South Africa

Soil chemical properties and pH

Soil pH was measured in 1:5 soil:KCl extract (Rhoades, 1982). Soil total P was analyzed using Bray-II extract method (Bray and Krutz, 1945). Soil total N was analyzed using the complete combustion method using an elemental analyzer (Euro EA; Eurovector, Milan, Italy). Soil total C was analyzed using the modified Walkley-Black method (Chan et al., 2001). Exchangeable cations were extracted in a 1:10 ammonium acetate solution using the centrifuge procedure (Thomas, 1982), filtered and analyzed by atomic absorption spectrometry (SP428; LECO Corporation, St. Joseph, Michigan, USA).

Soil physical properties

Soil resistivity, gravimetric soil moisture, and soil penetration resistance levels

Soil resistivity was measured using a resistivity meter. Gravimetric soil moisture, which calculates soil moisture using the difference between the fresh and dry weight, was calculated by first weighing wet soils, drying them in an oven at 105 °C for 72 h, then reweighing to obtain the water content (Black, 1965). Gravimetric soil measurements were expressed as a percentage. Soil penetration resistance levels were measured using a pocket penetrometer (SOILTEST, Inc., Evanston, Illinois, USA). When the penetrometer is pushed into the soil, it pushes a metal ring to scale, marking the penetration resistance value in kg cm^{-2} (Leung and Meyer, 2003).

Soil infiltration and hydraulic conductivity

Soil infiltration rate and hydraulic conductivity were measured using a mini disk infiltrometer (Decagon Devices, Pullman, WA, USA). The infiltrometer, an acrylic tube with a semipermeable plastic disk and a rubber stopper, consists of two chambers which were all filled with water. The upper chamber controls suction, whilst the lower chamber allows infiltration and a small tube inside the disk regulates the suction rate, which was set at 2.0 cm in this experiment (Latorre et al., 2013). Measurements were

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taken on the soil surface after hand removal of litter. The water infiltration rate was measured at every 30 s intervals for 5 min from the drop-in water level of the lower chamber in mL. The infiltration rate was determined from the measured cumulative infiltration rate over time using the method of Zhang (1997). The level of soil hydraulic conductivity was calculated from the infiltration data using the van Genuchten-Zhang method (Zhang, 1997, 1998). For more information regards the method of calculating hydraulic conductivity and the equation used see mini disk infiltrometer manual at Decagon Devices (2014).

Statistical analysis

Data were analyzed using STATISTICA version 13 (StatSoft, 2015). Proof of normality and homogeneity of variance were assessed on all measurements using Kolmogorov-Smirnov tests and Levene's test respectively and data were normally distributed. Comparisons on soil Ph, resistivity, total nutrient concentrations (P, N and C) and exchangeable cations (K, Na, Ca and Mg) between old field sites and natural sites were analyzed using a *t*-test since data were collected once in April. Gravimetric soil moisture content, soil penetration resistance levels, and soil hydraulic conductivity were analyzed using a repeated measures ANOVA since data were collected over 3 months on the same transect. Where ANOVAs were significant, the Tukey's honestly significant difference Unequal *n* test was used to determine the differences between site conditions and months at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

Soil chemical properties and pH

Soils in both old field sites and natural sites were clay (57%) and loam (43%). The soils in old field sites were very acidic with an average pH of 4.73 ± 0.15 and those in natural soils were strongly acidic with an average pH of 5.13 ± 0.20, with no significant ($p > 0.05$) difference between the two sites (*Table 2*). Similarly, soil P showed no significant ($p > 0.05$) difference between old field sites and natural sites (*Table 2*). Both soil total N and C were significantly ($p < 0.001$) higher in natural sites than in old field sites. The exchangeable cations of K and Mg were significantly ($p < 0.01$) higher in natural sites than in old field sites (*Table 2*). In contrast, the exchangeable cations of Na and Ca showed no significant ($p > 0.05$) difference between old field sites and natural sites (*Table 2*).

Soil physical properties

Soil resistivity, gravimetric soil moisture, and soil penetration resistance levels

Soil resistivity showed no significant ($p > 0.05$) difference between old field sites and natural sites (*Table 2*). The gravimetric soil moisture content varied significantly between the two sites ($p < 0.001$) and the three months ($p < 0.001$). Gravimetric soil moisture content was significantly ($p < 0.001$) higher in natural sites (8.13 ± 0.63) compared to old field sites (5.94 ± 0.31). The observed above-mentioned significant ($p < 0.001$) differences were recorded in April and June, but not in May (*Fig. 2A*). There was significant interaction ($p < 0.01$) between the site conditions and the months for gravimetric soil moisture content (*Fig. 2A*). Soil penetration resistance levels also

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varied significantly between the two sites ($p < 0.001$) and the three months ($p < 0.001$). Significantly ($p < 0.001$) higher soil penetration resistance levels were recorded in old field sites (3.87 ± 0.07) than in natural sites (1.43 ± 0.08). These above-mentioned significant ($p < 0.001$) differences were observed in all the three months (*Fig. 2B*). Interactions between site conditions and months in relation to soil penetration resistance levels showed significant ($p < 0.001$) difference (*Fig. 2B*).

Table 2. *Soil physico-chemical properties of soil samples taken from old field sites and adjacent natural sites. Data are means ± se and t-test results are shown ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). NS = not significant and $p > 0.05$*

Soil properties	Old field sites (n=15)	Natural sites (n=15)	T test values
pH	4.73 ± 0.15	5.13 ± 0.20	2.35*
Soil Resistivity (Ohm)	1733.33 ± 272.02	1284.67 ± 188.15	1.36ns
Total nutrient concentrations (mg/kg)			
P Bray II	2.84 ± 0.34	3.17 ± 0.33	0.70ns
Total N	373.33 ± 78.96	2166.67 ± 304.36	5.70***
Total C	8113.33 ± 652.82	19540.00 ± 1308.54	7.81***
Exchangeable cations (mg/kg)			
K	108.96 ± 17.47	217.66 ± 29.33	3.18**
Na	17.79 ± 0.85	19.17 ±1.57	0.77ns
Ca	924.00 ± 160.96	1380.40 ± 202.43	1.76ns
Mg	248.56 ± 37.47	411.68 ± 39.82	2.98**

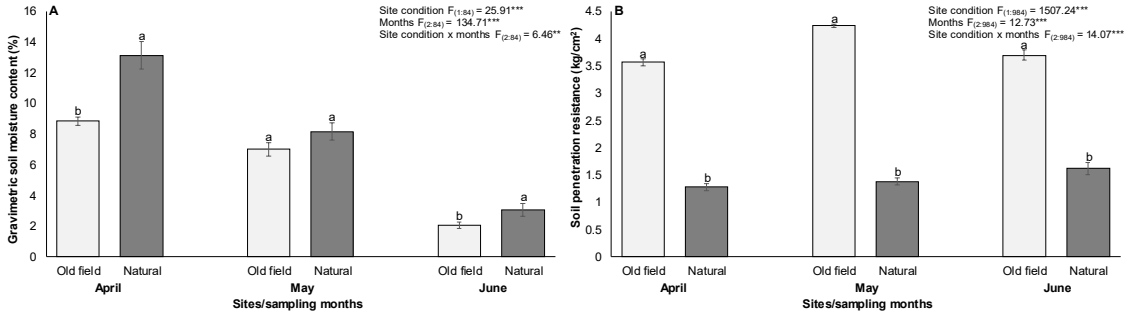


Figure 2. *Results show (A) gravimetric soil moisture content (%) and (B) soil penetration resistance levels (kg/cm^2) in soil samples taken from old field sites and adjacent natural sites. Bars represent mean ± se and results of repeated measures ANOVAs are shown ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Bars with different superscripts are significantly different at $p < 0.05$. NS = not significant and $p > 0.05$*

Soil infiltration and hydraulic conductivity

Cumulative infiltration rates showed no significant ($p > 0.05$) difference between old field sites and natural sites (*Fig. 3A, B, C*). The average cumulative infiltration rates for all the three months in the old field sites was 23.78 ± 1.5 cm, compared to 17.31 ± 1.1 cm in the natural sites after 5 min, and these showed no significant ($p > 0.05$) difference between the three months (*Fig. 3A, B, C*). Contrary, interactions between site conditions and months in relation to cumulative infiltration rates showed significant ($p < 0.01$)

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difference (*Fig. 3A, B, C*). Soil hydraulic conductivity levels varied significantly between the two sites ($p < 0.05$), but not between the three months ($p > 0.05$). The observed above-mentioned significant ($p < 0.05$) differences between sites showed higher soil hydraulic conductivity levels in natural sites (12.99 ± 2.67) than in old field sites (8.75 ± 2.04) (*Fig. 4*). Monthly differences in soil hydraulic conductivity levels were only observed in June. Interactions between site conditions and the three months in relation to soil hydraulic conductivity levels showed no significant ($p > 0.05$) difference (*Fig. 4*).

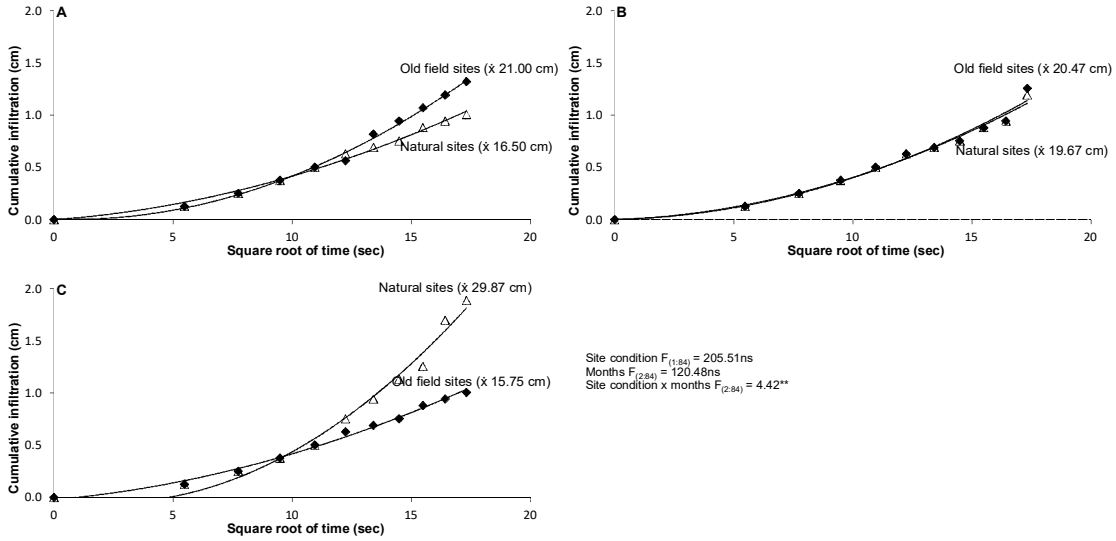


Figure 3. *Cumulative infiltration levels in soil samples taken from old field sites and adjacent natural sites for the months (A) April, (B) May, and (C) June. Results of repeated measures ANOVAs (based on average infiltration rate after five minutes) are shown ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). NS = not significant and $p > 0.05$*

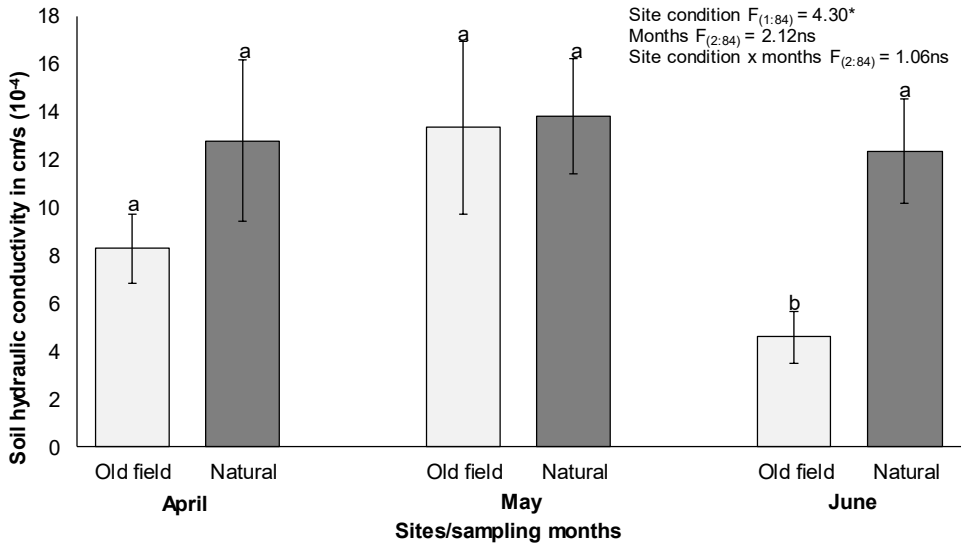


Figure 4. *Soil hydraulic conductivity levels in soil samples taken from old field sites and adjacent natural sites. Bars represent mean ± se and results of repeated measures ANOVAs are shown. Bars with similar letter superscripts are not significantly different at $p > 0.05$. NS = not significant and $p > 0.05$*

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Discussion

Results of this study showed that soil properties differ between old field sites and adjacent natural sites. Soils under natural sites had higher pH, total N, total C, K, Mg, gravimetric soil moisture and were less compact compared to soils in old field sites. Contrary, soil resistivity levels, total P, Na, Ca, cumulative infiltration and soil hydraulic conductivity (except in June) showed no significant differences between the two sites. These results (for some soil chemical properties) concur with previous studies that have shown reduced soil properties in old fields with such reductions linked to years since abandonment (Odum et al., 1984; Feng et al., 2007; Cuesta et al., 2012). Odum et al. (1984) showed that soil P and Ca declined in top soils 10 years after land abandonment. Feng et al. (2007) showed mixed results, a decrease in available P and an increase in total and available N, K and total P with increase in field abandonment age. Cuesta et al. (2012) reported higher soil C, NH_4N, and available N in reforested areas than in old fields, however, they also showed no changes in soil pH, total N, P, K, NO_3N and P between the two sites. These above-mentioned studies point to current environmental conditions (e.g. type of plant species present in both old fields and natural sites) as the driver to changes in soil properties (Odum et al., 1984; Feng et al., 2007; Cuesta et al., 2012). For example, different plant communities differ in their capacity to modify soil properties, given that plant functional traits such as growth form, biomass allocation, and tissue chemistry can affect organic matter decomposition and nutrient dynamics (Carrera et al., 2009).

Changes in soil properties in our old fields could be a result of several factors that include past fertilization, vegetation recovery stage and the type of plant species present in the old fields (Feng et al., 2007; Cramer et al., 2008; Cuesta et al., 2012). Theories on soil recovery following land abandonment posit that soil nutrients especially N, C and P will be high soon after land abandonment because of previous agricultural practices (McLauchlan, 2006: Cramer et al., 2008). As the recovery process progresses and plant communities begin to occupy old fields, some soil nutrients tend to decrease due to utilization by plants (Feng et al., 2007). However, as vegetation cover and organic matter content increase, soil nutrients are likely to increase with time, although this is dependent on the type of colonizing plants. Contrary, soil nutrient losses during early vegetation recovery stages are exacerbated by soil erosion and nutrient leaching which are known to be high in less vegetated old fields (Shen and Hong, 2003).

Based on above-mentioned vegetation recovery theories in old field, we anticipated that soil properties in our 35-year old fields should be similar to those in natural sites. Contrary, we found the opposite for some soil properties. We suggest that the lack of vegetation, which is linked to increased soil erosion, could explain the observed reduced soil nutrient in old field sites. Only a few scattered pioneer trees (e.g. *Vachellia karoo* and *Senegalia nigrescens*) and grass (e.g. *Cynodon dactylon* and *Panicum maximum*) species were observed in old fields (Sheunesu Ruwanza, personal observation; see photo on Fig. 1C). Previous studies have shown that the absence of vegetation in old fields is associated with low soil nutrient levels (McLauchlan, 2006: Cramer et al., 2008), this linked to reduced litter content, organic matter, and decomposition rate. Plant litter accumulation is known to improve soil nutrients especially soil N, C, and P, this because of decomposing residual (McLauchlan, 2006; Austin and Ballaré, 2010; Bansal et al., 2014). There is considerable evidence that litter plays an important role in soil carbon storage and nutrient cycle which in turn affect soil nutrients (Aerts and de Caluwe, 1997; Marínez-Yrzar et al., 2007), this likely to be the case in reported high

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soil nutrient content in natural sites which are vegetated. It is well reported that soil pH influences solubility of soil nutrients, making them available in insufficient quantities in acidic soils (Kidd and Proctor, 2001), as are the soils in Lapalala Wilderness old field sites. Besides soil pH being a factor affecting soil nutrient availability, the reported soil compaction and low soil moisture levels in old field sites can explain soil nutrient reductions. Moist and less compact soils are known to promote decomposition and release of soil nutrients as compared to dry and compact soils (Wolf et al., 2013; Van Horn et al., 2014), as are the soils at Lapalala Wilderness old field sites.

Low vegetation cover in old field sites, which created barren soil surfaces, has been reported to promote soil erosion and nutrient leaching (Lesschen et al., 2008). Loss of soils due to erosion is directly linked to a decrease in soil nutrients (Xue et al., 2011). Also, lack of vegetation which is linked to low above-ground biomass and litter can reduce C input into soils, which then influences soil micro-aggregate formation leading to accelerated soil erosion (Wan and Luo, 2003). This likely to take place in less vegetated old field sites than in vegetated natural sites. The reported compact soils in the old field sites could also facilitate erosion and nutrient leaching due to low infiltration capacity which promotes runoff. Indeed, previous studies have reported that soil compaction reduces soil porosity (Silva et al., 2008) thus causing low soil water infiltration (Nawaz et al., 2013) which subsequently increase soil erosion.

Conclusions

This study provides evidence of the poor state of soils in Lapalala Wilderness old fields, several years after land abandonment. The persistence of these soils in a poor state may hamper both soil and vegetation recovery. Therefore, active soil manipulations techniques should be considered if soils in Lapalala Wilderness old fields are to be improved. Different techniques to improve soil properties in old fields can be used e.g. soil nutrient manipulation (fertilizers or organic matter addition), top soil removal and soil transfer (Blumenthal et al., 2003; Ruwanza et al., 2012). The above-mentioned approaches have been shown to improve soil properties in old fields (Holmes, 2008; Blumenthal et al., 2003; Wubs et al., 2016; Bulot et al., 2017). Not only do they improve soil properties, they also steer plant community recovery (Wubs et al., 2016). However, the feasibility of these above-mentioned techniques in Lapalala Wilderness old fields needs to be tested.

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A GEOCHEMICAL INVESTIGATION OF PADDY (P) AND NON-PADDY (NP) SOILS ON A CATENA: A CASE STUDY OF GHAEMSHAHR IN MAZANDARAN PROVINCE, IRAN

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Abstract. Paddy soils are a main soil resource for food production and they cover a large area of the world's surface. A geochemistry study was performed on a catenary sequence of Paddy and Non-Paddy soils in the lowland and upland areas of Ghaemshahr City in Mazandaran Province, Iran. 10 soil profiles were investigated and then, a comparison was made between the Paddy and Non-Paddy soils based on their geochemical compositions and developments. Soil clay mineralogical analysis showed that the Non-Paddy soils had a large amount of vermiculite, whereas smectite was the dominant clay mineral of the Paddy soils. The cation exchange capacity, clay content, and bulk density are higher in the Paddy soils. Rice cultivation increases the amount of amorphous Fe and decreases the amount of free Fe-oxide. The results of free Fe-oxide and amorphous Fe assessments, revealed that the Non-Paddy and upland soils were more developed than the Paddy and lowland soils. The mean concentrations of the major elements did not show any significant differences between the two types of soils. The mean values of all the heavy metals, except for Mn and As, in the Paddy soils were lower than those of the Non-Paddy soils. The reason for the reduction of heavy metals in the P compared to NP soils was their probable relationships with Fe/Mn oxide minerals and subsequent leaching. Based on principal component analysis, the differences between the Paddy and Non-Paddy soils were related to Mn and Fe group elements. The results indicated that the concentrations of the elements and other properties of the Paddy soils were dependent on the soil parent materials, especially on the textures of the sedimentary layers.
Keywords: *geochemistry, heavy metal, principal component analysis, soil development, smectite*

Introduction

The chemical composition of soil in primary stage of pedogenesis, will be reflect the chemical composition of the parent material, whereas the chemical composition of adult soils highly controls by the factors of weathering environment (Thanachit et al., 2006). Rice is the main source of food for more than half of the world's population and is cultivated on almost 155 million hectares in 114 countries (Kögel-Knabner et al., 2010). Paddy soils are anthropogenic soils widely distributed in eastern Asia and some other parts of the world (Huang et al., 2015). The formation of P soil, which is hydromorphic, is severely influenced by anthropogenic factors (Gong, 1983). It is not a natural soil and can be strongly affected by such management techniques as leveling, tillage, puddling,

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submerging (Wissing et al., 2013), and the use of fertilizers. Its formation is also enforced by a special management, which can lead to its characteristic redoximorphic features (Kölbl et al., 2014). Frequent flooding leads to anoxic situation in the soil and decrease rates of organic matter decomposition (Sahrawat, 2005), while it exacerbates mineral weathering (Nanzyo et al., 1999) and leaching process.

In paddy rice management, soils are held submerged for several months of the year and as a result, their geochemical, micromorphological, and mineralogical features undergo some changes triggered by periodic submergence and drainage. Moreover, P soils grow from various parent materials and may be extremely altered by their chemical compositions, mineralogies, and other properties, such as texture (Kyuma, 2004; Chandrajid, 2005; Prakongkep et al., 2008). Hasannezhad et al. (2008) and Sano et al. (2006) reported that clay mineralogy is further influenced by its parent material rather than by redoximorphic conditions. In contrast, Hasannezhad et al. (2008) found an enhanced formation of smectite that was caused by the hydromorphic conditions of the P soils developed on alluvial materials. Elements concentrations in P soils have been studied by Chandrajith et al. (2005), Prakongkep et al. (2008), and Halim et al. (2015). Prakongkep et al. (2008) discovered that the concentrations of the elements and other properties of Thai P soils frequently depended on their parent materials, especially on the textures of the alluvial layers.

Many P soils become contaminated with heavy metals (Wong et al., 2002; Kyuma, 2004), while some useful trace elements become deficient due to geochemical variations or crop harvest (Kon et al., 2001). The affluence of trace elements in tropical Asian P soils is significantly diverse. Trace elements in agronomical soils mainly depend on the geochemical compositions of their parent materials during the primary stage of tillage (De Temmerman et al., 2003). Mitchell (1974) reported that the total amounts of trace elements in soils basically depend on the types of their parent materials and on which pedogenic processes occur.

Halim et al. (2015) studied the heavy metals of P soils in Bangladesh and reported that the average amounts of Zn, As, Mn, Ni, Cu, Pb, and Cr found in the P soils and sub-soils were less than those causing the mean and moderate pollutions of the selected soils of the world. Domingo and Kyuma (1983), Wang et al. (2003), and Wong et al. (2002) investigated the concentrations of elements in the P soils of many tropical Asian countries and found significantly different abundances of trace elements in them. Each region was rich in some particular elements. Although the concentrations of trace elements in different soils and plants have been measured in numerous studies in several parts of the world (Kabata-pendias, 2011), little research has been conducted in this field in Iran.

Therefore, this paper aimed to firstly evaluate the concentrations of the major and trace elements in the studied P soils and secondly compare the P and NP soils under study based on geochemistry and soil development.

Material and methods

Study area and sampling

The study area was located in Ghaemshahr, Mazandaran Province in northern Iran (*Fig. 1*). Its average annual temperature and precipitation are 16.1°C and 734 mm, respectively. The soils were developed under xeric and thermic soil moisture and temperature regimes, respectively (Banaei, 1998). The parent materials in the studied

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area were derived from alluviums altered from primary rocks content calcite, dolomite and loess moved to this flatland. 10 representative profiles were selected along a catena based on their topography for this research: 5 pedons for P and 5 others for NP soils. Soil colors were determined by Munsell color system (Munsell, 2014). The soils were defined and classified based on Soil Taxonomy (Soil Survey Staff, 2014; *Table 1*).

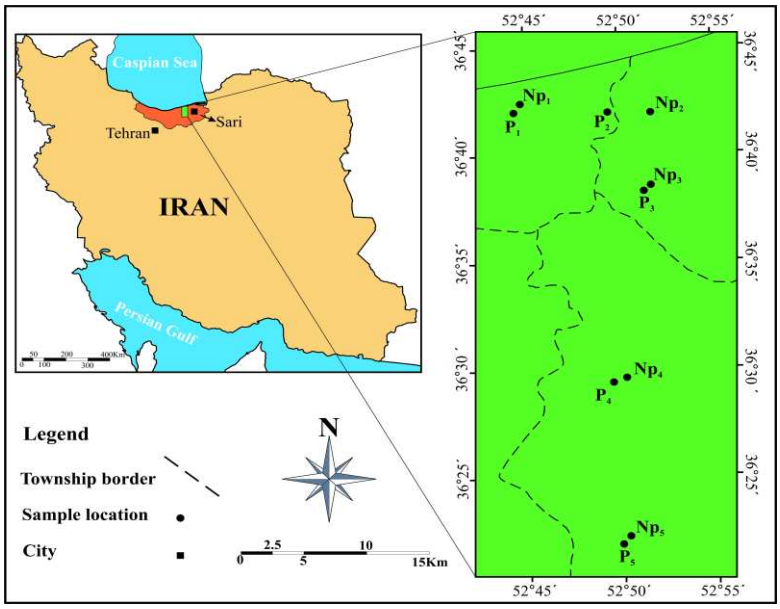


Figure 1. *Location of the study area and sampling sites along the 10 Pedons are indicated*

Physico-chemical analysis

Particle-size distribution was defined by the method of Bouyoucos hydrometer (Bouyoucos, 1962). The organic carbon contents of the soils were obtained through wet oxidation by using chromic acid and via return titration by using ferrous ammonium sulphate based on the method described by Nelson (1982). The pH of the soil samples was measured with a pH electrode in a suspension of 1:1 (soil: H_2O). Sodium acetate (NaOAc) was applied at pH 8.2 to evaluate CEC (Chapman, 1965). A titration method was employed to assess carbonate content (US Salinity Laboratory Staff, 1954). In addition, the method of Mehra and Jackson (1960) was used to evaluate dithionite-extractable Fe (Fe_d) for the bulk soil samples of < 2 mm. The taken samples were dried at 105°C for 24 h in an oven and weighted to determine Bulk Density (BD) through the method of paraffin sections (Blake and Hartge, 1986). Amorphous Fe (Fe_o) was extracted by using 0.3 M of ammonium oxalate solution (pH 3) in a dark place (McKeague and Day, 1966).

Mineralogical analysis

The method of Mehra and Jackson (1960) was utilized to eliminate the attaching chemical factors and dissociate the clay fractions (Kittrick and Hope, 1963). For clay discretion (< 2 μm), centrifugation of the iron-lacking soil samples at 750 rpm was done for 5.4 min (Kittrick and Hope, 1963). A D8 ADVANCE X-ray (Bruker) diffractometer was used to perform an X-ray analysis on the fine and coarse clay fractions (Jackson, 1975). To make a credible comparison between the Relative Peak Intensities (RPIs) of

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the different samples, we made use of equal concentrations of the clay suspensions. 001 reflections were considered after Mg and K saturations and Ethylene Glycol (EG) salvation. Perusing of the K-saturated samples was followed after performing the drying and heating processes at 330 °C and 550 °C for 4 h. An overnight treatment of the samples with 1 N HCl solution at 80 °C was carried out for kaolinite identification in the presence of trioctahedral chlorite. A semi-quantitative assessment of the clay minerals was conducted based on the respective peak areas obtained from X-Ray Diffraction (XRD) in the glycol-treated samples (Johns and Grim, 1954).

Geochemical analysis

The geochemical analyses of the soil samples were done via X-Ray Fluorescence (XRF) spectroscopy of the compacted powder/pellets (Nisbet et al., 1979) and aqua regia digestion with a blend of $HClO_4$, HNO_3, HCl, and HF at 200 °C followed by ICP/OES and ICP/MS for the major and trace elements, respectively. Also, many trace elements were analyzed through Instrumental Neutron Activation Analysis (INAA) (Hoffman, 1992). The geochemical analyses were performed at the Actlabs laboratory (Ontario, Canada).

Statistical analysis

The concentration of elements and physicochemical properties including texture and CEC of P and NP soils were statistically analyzed using Principle Component Analysis (PCA) with the SPSS (version 21). Also Independent Samples T-test was applied to compare the concentration of elements in P and NP soils and if there were significant differences between two land uses based on the concentration of elements by the SPSS (version 21).

Results and discussion

Physico-chemical properties

The major physicochemical and morphological properties of the studied soils are presented in *Table 1*. As depicted in this table, the soils have Munsell colors ranging from 2.5Y to 10YR. The P soils were poorly drained since displaying a 2.5Y hue resulted from the annual redoximorphic conditions over many years. The evidence showed that endosaturation was responsible for the redoximorphic properties of the soils. A considerable diversity was observed between the textures of the P and NP soils, from clay to silty clay loam. A higher amount of clay was found in the lowland area (*Table 1*). This could be explained by the depositions of the clay-size particles originated from the upland area (Khormali et al., 2009). Wilding et al. (1982) reported that soil texture in a lowland area is finer than in any other locations. According to *Table 1,* Profile NP_5 has a coarser texture compared with the other profiles (SiCL). The other soils have a relatively heavy texture (silty clay to clay). $CaCO_3$ percentage ranges from 2% in the C horizon of NP_4 to 27% in the A horizon of P_4. BD showed considerable differences in the 2 selected land uses. BD was higher in the P than in the NP soils. P_2 and NP_2 had the highest and lowest contents of BD, respectively. Soil compaction caused by tillage processes had increased BD in the P soils. According to Dang et al. (2002), tillage practices decrease soil Organic Matter (OM) and result in a higher BD and lower total porosity.

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Table 1. *Morphological and physicochemical properties of the studied soils*

Horizon	Depth (cm)	Color (moist)	pH	CEC[a] ($cmol(+)kg^{-1}$)	Sand (%)	Silt (%)	Clay (%)	Textural class[b]	CCE[c] (%)	OC[d] (%)	BD[e]
NP_1, Haplaquolls											
A	0-20	10YR3/2	7.76	32	4	44	52	SiC	15	1.26	1.78
AB	20-40	2.5Y3/2	7.80	34.1	8	44	50	SiC	16	1.19	1.81
B_g	40-60	2.5Y5/3	8.02	34.1	6	48	46	SiC	14	0.86	1.84
BC_g	60-100	2.5Y4.5/3	8.22	34.5	6	44	50	SiC	18	2.15	1.80
P_1, Haplaquepts											
A_p	0-15	2.5Y4.5/4	7.79	41.8	10	32	58	C	19	1.02	1.89
AB_g	15-40	2.5Y4.5/4	7.89	38	8	33	59	C	18	1.06	1.96
B_g	40-70	2.5Y4.5/3	7.82	37	7	37	56	C	23	0.16	1.95
BC_g	70-100	2.5Y4/2	7.93	32	8	42	50	SiC	20	0.62	1.94
NP_2, Calciaquepts											
A	0-10	10YR4/4	6.04	38.3	14	40	46	SiC	4	3.06	1.83
AB	10-35	2.5Y3/2	7.65	35.8	10	41	49	SiC	7	1.17	1.82
B_{kg1}	35-70	2.5Y4/2	7.57	30.0	14	42	44	SiC	18	0.66	1.80
B_{kg2}	70-100	2.5Y4/1.5	7.50	34.9	13	42	45	SiC	14	0.64	1.81
C_g	100-130	2.5Y4/1.5	7.47	21.6	12	40	48	SiC	12	0.46	1.78
P_2, Haplaquepts											
A	0-20	10YR4/3	8.04	45	10	36	54	C	13	2.03	1.99
B_{wg1}	20-50	2.5Y3/4	7.83	35	12	38	58	C	5	0.47	1.99
B_{wg2}	50-70	2.5Y3/4	7.78	28.3	14	40	56	C	5	1.40	1.91
C_g	70-90	2.5Y3/5	7.70	31	8	42	50	SiC	10	1.63	1.89
NP_3, Calciaquolls											
A	0-25	10YR3/2	7.24	38	9	42	49	SiC	11	3.54	1.78
B_g	25-55	2.5Y3.5/2	7.80	33	11	41	48	SiC	13	0.78	1.72
C_{kg}	55-80	10YR4/2	7.99	32	14	34	52	C	18	0.51	1.70
P_3, Calciaquepts											
A_{pg}	0-20	2.5Y4/1	7.75	41	12	36	52	C	13	1.62	1.90
B_g	20-45	2.5Y4/1	7.70	42	12	34	54	C	12	1.24	1.92
B_{kg}	45-65	2.5Y4/2	7.81	44.1	10	34	56	C	13	0.62	1.91
C_g	65-90	2.5Y4/2	8.01	40	18	32	50	C	9	0.64	1.89
NP_4, Haploxerolls											
A	0-19	10YR3/2	7.67	30	17	41	42	SiC	9	1.48	1.70
B_{w1}	19-45	10YR3/3	7.78	28.7	17	43	40	SiC	10	0.78	1.68
B_{w2}	45-65	10YR3/4	7.84	27.9	19	42	39	SiCL	5	0.55	1.72
C	65-100	10YR3/3.5	7.69	31.2	23	35	42	C	2	0.4	1.71
P_4, Endoaquepts											
A_g	0-18	2.5Y4/1	7.65	40	20	29	51	C	27	1.87	1.97
CB_g	18-45	2.5Y4/4	7.85	32	17	30	53	C	24	0.66	1.95
C_g	45-90	10YR4/6	7.77	30	26	34	40	C	25	0.35	1.92
NP_5, Calcixerolls											
A	0-27	10YR3/2	7.75	32.4	16	44	40	SiCL	9	1.37	1.67
AB	27-44	10YR3/3	7.81	29.9	18	43	39	SiCL	3	0.73	1.65
B_K	44-66	10YR3/4	7.77	32	20	41	39	SiCL	12	0.8	1.68
CB	66-90	10YR3/3.5	7.65	22.8	21	37	42	C	13	1.0	1.63

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P_5, Haplaquepts											
A_{pg}	0-21	2.5Y3.5/2	7.28	37	18	32	50	C	16	1.84	1.92
B_{g1}	21-47	2.5Y3/3	7.82	32	15	35	50	C	18	1.18	1.93
B_{g2}	47-65	2.5YR4/1	7.81	30	19	34	47	C	17	1.08	1.90
C_g	65-95	2.5YR3/1	7.92	30	20	35	45	C	10	0.62	1.91

[a]CEC: cation exchange capacity
[b]C: clay, si: silty, L: loam, S: sandy
[c]CCE: cation exchange capacity
[d]OC: organic carbon
[e]BD: bulk density

Bar graphs of mean values of Fe_d and Fe_o for surface horizon of soil profiles are shown in *Figure 2*. In most soils, based on morphological properties, pedogenic Fe and clay content, soil age is estimated (Costantini and Damiani, 2004). Dethier et al. (2012), reported that, the Dominant forms of Fe in soils, are Fe_t, Fe_d, Fe_o. Therefore, (Fe_d-Fe_o) indicates crystalline form of Fe, Fe_o/Fe_d represents the degree of crystallinity of Fe oxides, and finally (Fe_d-Fe_o)/Fe_t indicates the ratio of crystalline to total Fe (Schaetzl, 2005). Rezapoor et al. (2009) studied different forms of Fe oxides and their distribution in calcareous soils in Northwest of Iran, and reported that, with increasing weathering, Fe_d increases and Fe_o/Fe_d decreases. In hydromorphic condition and deficiency of oxygen, Fe is converted to Fe (II), and in this condition, the possibility of forming a crystalline Fe oxides is lower, resulting in a higher Fe_o/Fe_d ratio. In this study, paddy soils have a higher amount of Fe_o compared to non-paddy soils, whereas, non-paddy have a higher content of Fe_d. B_k horizon of NP_5 has a higher content of Fe_d and lower content of Fe_o, whereas, C_g horizon of P_1 has a lower content of Fe_d and a higher content of Fe_o. The studied paddy soil samples have higher contents of Fe_o results in higher ratios of Feo/Fed compared to non-paddy soils. Along the catena, from lowland to upland, this ratio always decreases, which is probably an indication of higher weathering in upland soils compared to the lowland soils.

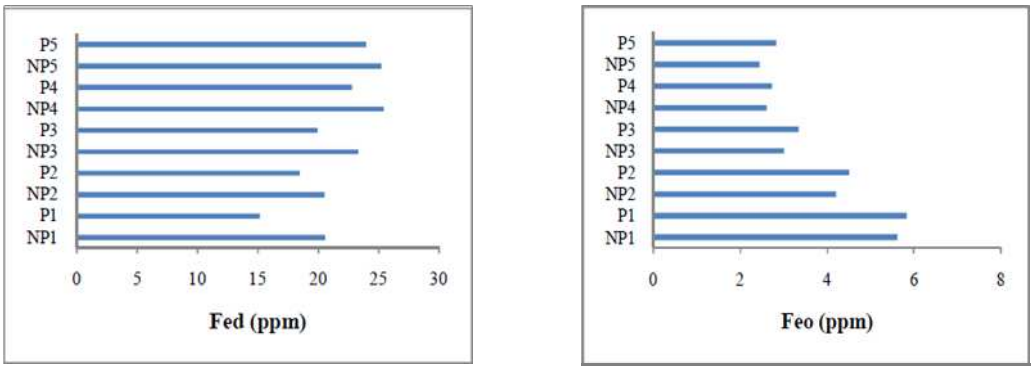


Figure 2. *Bar graphs of mean values of Fe_d and Fe_o for surface horizon of soil profiles*

Mineralogical characteristics

Figure 3 demonstrates the results of X-ray diffractograms recorded in the selected P and NP soils. Two cases of peaks were shown for almost identical results. As can be observed, illite, chlorite, kaolinite, smectite, and vermiculite exist in the clay fraction.

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The main mineral found in the P soils was smectite whereas in NP soils was illite. Illite and chlorite as clay minerals are mostly seen in areas with a limited process of soil formation (Wilson, 1999). Their existence in parent materials mostly determines their abundance in soils. The P soils in this study displayed a higher amount of smectite due to their several months of poor drainage and saturation each year (*Fig. 3b*). In these conditions, smectite neo-formation had occurred due to the reduction of some iron in the presence of some soluble ions like Al, Mg, and Si coming from the adjacent uplands (Borchardt, 1989). Small amounts of kaolinite were also found in all the studied soil profiles. In the NP soils, vermiculite was present in large amounts (*Fig. 3a*). Vermiculite occurrence in these soils was mainly caused by high leaching conditions and K removal from mica (Douglas, 1989).

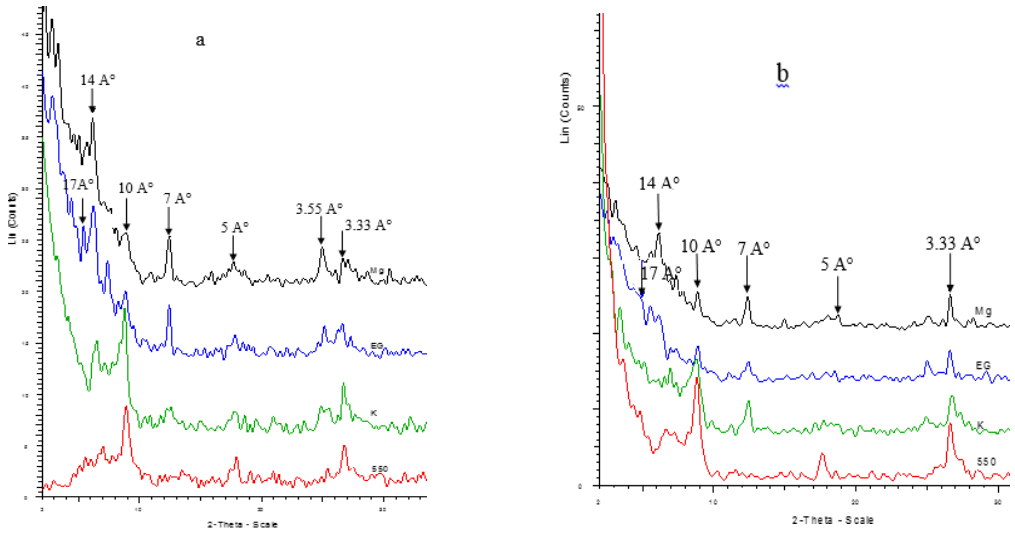


Figure 3. *X-Ray diffraction patterns of clay fraction for the representative soil profiles. (a) Bkg_1 horizon of NP_2 and (b) Bwg horizon of P_2*

Geochemical analysis

The full geochemical analyses of the soils are not demonstrated because of the large data set obtained, but the mean concentrations of the P and NP soils are exhibited in *Table 2*. Si, Al, Ca, Fe, Mg, and K were the most elements found in both P and NP soils. None of the major element concentrations showed any significant differences between the P and NP soils. The contents of Ba, Cs, S, and Zr in the P soils were higher, but Br and Rb amounts were lower than those of the NP soils. The mean values of all the heavy metals in the P soils, except for Mn and As, were lower than those of the NP soils.

Strong associations were found between Fe/Mn oxides and other heavy metals in the P soils. In paddy rice management, soils are commonly submerged and thus, excessive soil water may elevate leaching and migration of soluble heavy metals. Talyor and Mckenzie (1966) discovered that Co content was dependent on Mn oxide minerals. Therefore, the lower heavy metal contents in the P compared to the NP soils could be ascribed to Fe/Mn oxide minerals.

The mean concentrations of Si, Mn, Mg, As, Co, Cr, Cs, Cu, Ni, and Rb in the studied soils were higher than the amounts reported for normal surface soils worldwide

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(Essington, 2004; Kabata-Pendias, 2011), whereas the mean values of Mn, Ti, As, Co, Cr, Cu, Ni and Zn were higher than the amounts reported for the other contaminated P soils (Wong et al., 2002; Wang et al., 2003; Chandrajith et al., 2005). Only Pb concentration was lower than those of the global and other unpolluted P soils. The amounts of other elements in the studied P soils were comparable to the values obtained for the normal surface soils and P soils in the world. Pb concentration is lower than both worldwide and other unpolluted paddy soils. In comparison to the critical soil concentration values established, only Cr values were higher (Alloway, 1995).

Table 2. *The mean values of some oxides and elements in studied P and NP soil samples, mean values for uncontaminated P soils, mean values of normal surface soils worldwide and critical soil concentration for contaminated soils*

Element	Mean values for non-paddy soils	Mean values for paddy soils	Mean values for uncontaminated paddy soils[a]	Mean values for normal surface soils worldwide[b]	Critical soil concentration[c]
			(g kg^{-1})		
SiO2	509.6	507.75		250-410	
Al2O3	148.7	137.48		10-300	
Fe2O3	62.9	60.32		2-550	
MnO	1.08	1.21	0.39	0.27-0.53	1.5-3
MgO	23.4	23.46		0.4-9	
CaO	66.7	78.9		0.7-500	
Na2O	8.7	9.4		0.15-25	
K2O	24.9	23.31		0.08-37	
TiO2	8.3	8.3	5.4	2.3-26	
P2O5	1.9	2.13		0.035-5.3	
			(mg kg^{-1})		
As	12.55	13.33	1.7	4.4-9.3	
Ba	403.33	416.11		175-520	
Be	2.11	2.11		0.35-3.52	
Br	14.11	11.11		1-110	
Co	21.66	21.33	10.2	4.5-12	25-50
Cr	112.44	108.33	64	12-83	75-100
Cs	9.22	9.55		5.06	
Cu	45.88	40.88	20.7	13-24	60-125
Ni	61.77	57.44	25.5	13-34	100
Pb	20.11	17.88	23.3	22-44	100-400
Rb	160	158.88		30-120	70-400
S	340	400		30-1600	
Sr	209	254.11		87-210	
Zn	101.11	93.55	61	45-100	
Zr	152.8	170.11		60-2000	

[a]Data from Wong et al. (2002); Wang et al. (2003); Chandrajith et al. (2005)
[b]Data from Essington (2004); Kabata-Pendias (2011)
[c]Data from Alloway (1995)

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Independent Samples T-test

An Independent Samples T-test was applied to compare the concentration of elements in P and NP soils and if there were significant differences between two land uses based on the concentration of elements. Levene's test was conducted to evaluate homogeneity of variance. Results are presented in *Table 3*. As seen in *Table 3,* Result of Levene and T-test of all elements were not statistically significant ($p > 0.05$). The results indicated that there was no significant difference between the mean (M) and standard deviation (SD) of concentration of elements in P and NP soils.

Table 3. *Independent Samples T-test of some oxides and elements between studied P and NP soil samples*

Element	P soils		NP soils		*df*	*t*	*p*
	M[a]	*SD*[b]	*M*	*SD*			
SiO2	507.75	41.40	509.60	57.20	16	0.080	0.937
Al2O3	137.48	14.80	148.70	10.80	16	-1.840	0.084
Fe2O3	60.32	4.70	62.90	6.00	16	-1.010	0.326
MnO	1.21	0.23	1.08	0.20	16	1.160	0.263
MgO	23.46	3.40	23.40	2.20	16	0.490	0.961
CaO	78.90	20.00	66.70	23.60	16	1.170	0.256
Na2O	9.40	1.90	8.70	3.00	16	0.615	0.547
K2O	23.31	2.90	24.90	2.60	16	-1.240	0.233
TiO2	8.30	0.49	8.30	0.52	16	-0.138	0.892
P2O5	2.13	0.35	1.90	0.25	16	1.150	0.265
As	13.33	2.23	12.55	3.04	16	0.618	0.546
Ba	416.11	53.14	403.33	43.28	16	0.559	0.584
Be	2.11	0.33	2.11	0.33	16	0.000	1.000
Br	11.11	4.70	14.11	4.25	16	-1.41	0.175
Co	21.33	2.59	21.66	2.17	16	0.295	0.772
Cr	108.33	6.51	112.44	6.26	16	-1.364	0.191
Cs	9.55	1.50	9.22	2.04	16	0.393	0.699
Cu	40.88	5.62	45.88	9.95	16	-1.312	0.208
Ni	57.44	5.29	61.77	6.18	16	-1.690	0.130
Pb	17.88	3.01	20.11	2.52	16	0.080	0.109
Rb	158.88	24.72	160.00	46.36	16	0.063	0.950
S	400.00	16.00	340.00	15.00	16	0.919	0.372
Sr	254.11	51.20	209.00	40.15	16	2.080	0.054
Zn	93.55	10.00	101.11	13.05	16	-1.370	0.187
Zr	170.11	41.78	152.80	21.46	16	1.107	0.285

[a]Mean
[b]Standard deviation

Parent material uniformity

The relationship between the total amounts of Ti and Zr was applied as an index of uniformity for soil parent materials (Alonso et al., 1991). As shown in *Figure 4,* P_1 and NP_1 have the Ti/Zr ratios of about 56-58 and 59-60 through their profiles in the lowland

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area, respectively. In P_3 and NP_3, Ti/Zr ratios are relatively constant around 52-53 and 56-59, respectively. Finally, in the upland region, Ti/Zr ratios in P_5 and NP_5 are nearly 47-54 and 48-51, respectively. Few differences were observed in Ti/Zr ratios in the C compared to the other horizons, which demonstrated uniformity of the parent materials. Nable et al. (1999) and Marques et al. (2004) reported that the slight variations of 5 and 16 units respectively in Ti/Zr ratios in the profiles indicate the uniformity of parent materials. Therefore, the studied soil parent materials were uniform.

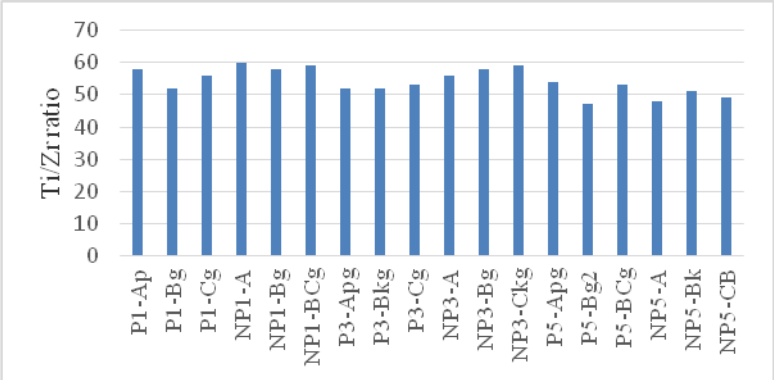


Figure 4. *Bar gragh of Ti/Zr ratio of studied paddy and non-paddy soils*

Principal component analysis

Principal component analysis (PCA) was applied to assess the elements under similar geochemical treatments and group the soil samples according to their geochemical associations (Weber and Davis, 1990; Bellehumeur et al., 1994). PCA of the concentrations of the introduced elements and physicochemical properties, including texture and CEC, were applied for all the P and NP soils. The PCA results of the concentrations of the elements and physicochemical properties of the NP soils are displayed in *Figure 5b*. 2 factors illustrated only 72% of variation in the data. Finally, 4 affinity groups were identified. The first group was composed of Si, Zr, and Na relevant to the textures of the soils. Quartz (SiO_2) was the basic mineral in the sand fraction. Zr was a residual element in the soils, the main (Stiles et al., 2003; Kabata-Pendias, 2011) and abundant (Lukashev and Pietukhova, 1974) mineral of which is zircon ($ZrSiO_4$) in sandy soils. The second group contained Ca, Co, and Fe. The reasons for the presence of Ca in this group were unknown. The third group included CEC, Zn, Cs, and Br and the last group was comprised of clay, Al, K, Ni, Mg, Cr, Sr, and Ba. This group was associated with clay content since the mentioned elements were parts of oxides and clay minerals (Kabata-Pendias, 2011; Sterckeman et al., 2006).

The PCA results of the concentrations of the elements and physicochemical properties of the P soils are exhibited in *Figure 5b*. 68% of variation in the data for these soils was illustrated by 2 factors. 3 groups of properties were distinguished. The first group contained sand, Na, Zr, and Si, which were linked to the textures of the soils. The second group consisted of CEC, clay, Al, Fe, K, Ni, As, Cs, Co, Pb, Zn, Rb, Ba, Mn, and Mg. Al, K, Rb, and Cs were related to feldspar and clay contents (Sterckeman et al., 2006; Kabata-Pendias, 2011). Being parts of the group of subordinate oxides, Mn, Pb, Zn, Ni, Zn, Ni, Co, As, and Cu were linked with Fe since they can be exchanged with Fe in the oxide structure (Kabata-Pendias, 2011). Manganese is responsible for the

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great degree of assembly of Mn concretion with some trace elements, especially with Pb, Cr, and As. Moreover, the oxidation of these metals by Mn oxides probably controls their redox behaviors in soils (Bartlett, 1986). The third group included Ca, Sr, Br, and S. Strontium belongs to the alkaline earth metals and has the same behavior as Ca. Its physical properties, particularly the size of its ionic radius, are partly similar to those of Ca, indicating their possibility of being replaced with each other (Kabata-Pendias, 2011).

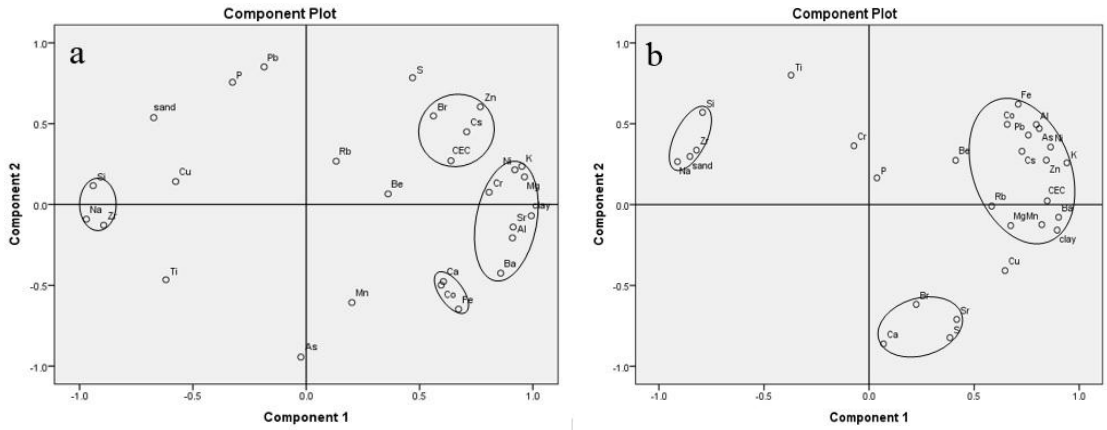


Figure 5. *PCA results for the chemical composition and physicochemical properties of studied soils: (a) distribution of elements and soil properties of non-paddy soils (b) distribution of elements and soil properties of paddy soils*

Based on PCA, the difference between the P and NP soils was related to the group elements of Mn and Fe. In the P soils, most of the heavy metals were gathered in one group and linked with Fe and Mn, while they were diffused in the NP soils. Mn and Fe are the most frequent metals in the lithosphere and thus, their oxides and hydroxides cause the solubility or alluviation of heavy metals in soils (Jounes and Jacobson, 2009). These oxides and hydroxides also play a main role in As, Pb, Cu, Zn, Cr, and Ni preservations in soils (Aydinalp and Marinova, 2003).

Conclusions

This research revealed that the concentrations of the elements and other properties of P soils depend on soil parent materials, especially the textures of sedimentary layers. Ti/Zr ratio confirmed uniformity of the parent materials along the catena and within the profiles. Profiles P_5 and NP_5 with a deep water table were the most weathered soils based on Fe_d and Fe_o values. The studied profiles had higher Si amounts and lower Ca, Mg, and K contents compared with the other pedons of the catena; however, Al and Fe amounts were uniform. Profiles P_1 and NP_1 were less weathered because they had the lowest positions in the landscape with shallower water tables. In the collation between the P and NP soils, the Ca, S, Sr, Ba, and Zr contents of the former soils were found to be higher than those of the latter soils. Contrarily, the amounts of Cr, V, and some heavy metals, including Pb, Ni, Cu, and Zn were higher in the latter soils compared to those of the former soils. The reason for the reduction of heavy metals in the P compared to NP soils was their probable relationships with Fe/Mn oxide minerals and

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subsequent leaching. Clay mineralogy revealed that Smectite amount was higher in the P soils because of its poor annual drainage and saturation for a long period of time. Vermiculite was the dominant clay mineral under well-drained conditions. There were two reasons for finding no significant differences between the P and NP soils under study based on geochemical properties: 1) short period of rice cultivation; and 2) lack of any significant differences between them in terms of drainage.

We suggested that complementary studies, including microprobe analysis, be performed on thin sections of the studied soils in order to determine if the smectite in clay fraction of soils is formed from other minerals deformation or in situ formation.

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DETERMINATION OF THE THERMAL DIFFUSIVITY OF VERMICOMPOST AND THE EFFECT OF MOISTURE AND TEMPERATURE ON IT USING THE 1D FOURIER CYLINDRICAL SOLUTION

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Abstract. Earthworm is the crucial actor in vermicompost production. Separating the worms with the least harm will decrease the costs of the vermicompost production. One of the key factors in using heat for separation of the worms is the determination of the thermal diffusivity of vermicompost. The thermal diffusivity of vermicompost has been determined by using a one-dimensional (1D) Fourier equation applied to a cylinder. The measurements have been carried out at 40, 50, 60, 70 ºC temperature levels and moisture contents of 10, 20, 30, 40, 50 % (wet base). The experiments have been conducted by heating vermicompost in an insulated box. The thermal diffusivity is estimated by keeping time–temperature records. The values of the thermal diffusivity of vermicompost ranged from 1.3973×10^{-7} to 2.861×10^{-7} m^2 s^{-1}. By increasing the moisture content of vermicompost from 10% to 50%, the thermal diffusivity of vermicompost is decreased. The thermal diffusivity of vermicompost increases with increases in the temperature from 40 ºC to 70 ºC. The relationship between thermal diffusivity (α), moisture content (MC), and temperature (T) is described using a polynomial model, with the coefficient of determination being 0.99.
Keywords: *Fourier's equation, moisture content, polynomial model, temperature, cooling time*

Introduction

One of the most important methods for processing municipal wastes is recycling them to vermicompost. Because of this process, in addition to reducing health and environmental problems, significant quantities of organic fertilizers, known as vermicompost, are produced. Vermicompost is a biological and organic fertilizer, which is produced by means of constant and calm passing of decaying organic matter from the digestive tract of earthworms and the excretion of these substances from the worm body (Ismail et al., 2003). These materials will be smeared with mucus, vitamins, and enzymes while passing through the body of the worm and will be ultimately enriched as an organic fertilizer. This type of fertilizer can be produced and used for building and improving soil nutrients. Therefore, vermicompost is a worm extract with a percentage of organic matter and food and carbohydrate substrates (Sathe, 2005). Farmers use this type of compost, which is a mixture of processed organic matter, due to its unique and

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specific modification effects on the physical, chemical, and biological properties of soil and consequently growth and increase of the product (Manna et al., 2003). Effective separation of earthworms from the organic media after completing a vermicomposting batch process could increase the rate of waste bioprocessing by earthworms and minimize the monetary investment in earthworms for such operations.

There are various methods for separating worms from vermicompost. Among them are mechanical separation, reducing the moisture of the vermicompost, using infra-red rays and using electric shock (Chaoui and Keener, 2008). The mechanical separation is of little efficiency and kills a large number of worms. Moisture Reduction is time-consuming and needs large space(Fieldson, 1988). Using infra-red rays and electric shock kills too many worms.(Chaoui and Keener, 2008)

In the controlled-heat and layered method of separation the worms are made to move to layers with lower temperature and with their arrival on the surface they can be separated from the vermicompost. The thermal properties of vermicompost are needed to predict heat transfer and increasing temperature in vermicompost for separating earthworms by heating from the organic media after completing vermicomposting.

The physical significance of thermal diffusivity is associated with the propagation of heat into the medium during temperature changes with time. The higher the thermal diffusivity, the faster the propagation of heat into the medium (Jain and Pathare, 2007). In any constant temperature of the thermal source, the more the value of the thermal diffusivity coefficient, the faster the object gets into thermal stability. Thus, there will be fewer temperature differences in the object (Bairi et al., 2007).

The measurement of thermal diffusivity can be divided into two groups: direct measurement and measurement by the values of thermal conductivity, namely specific heat and density, in an experimental manner (indirect prediction). The best method is the direct measurement of thermal diffusivity (Dickerson, 1965). There are four common methods to determine the thermal diffusivity experimentally: least squares estimation, use of heat penetration data, use of time–temperature charts, and use of analytical solutions (Singh, 1982). The thermal diffusivity of cheddar cheese was determined by using the analytical solution for an infinite cylinder with experimentally obtained time–temperature data (Marschoun et al., 2001). The thermal diffusivity of pet food was measured by center temperature data (Kee et al., 2002). The thermal diffusivity of Marquis wheat seed was studied in bulk at 9.2% (dry base) of the moisture content, and the value of thermal diffusivity was determined by indirect method (Babbit, 1945). The thermal diffusivity of rough rice was obtained in the moisture range of 12% to 20% (d.b) (Wratten et al., 1969). The thermal diffusivity of dairy cattle manure was measured in different moisture and temperature range (Amin-nayyeri et al., 2009). The thermal diffusivity of the sawdust was determined by using heat conduction and specific heat capacity in an indirect way (Ahn et al., 2009).

The objectives of this study were to determine the thermal diffusivity of vermicompost and to develop a mathematical model of the thermal diffusivity of vermicompost within the experimented temperature and moisture content limits. The research results have potential applications in separating earthworms by heating from the organic media after completion of vermicomposting.

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Material and Methods

Sample preparation

The vermicompost used in this study produced at the Vermicompost production unit located at the Vermicompost Production Industrial Center in Tehran, Iran.

The particle size of the tested vermicompost was 38.9% with a size of 0.3, 44.85% with a size of 0.6 and 16.25% with a size of 1.18 mm and was determined as powder particles.

To determine the initial moisture content of the vermicompost, 100 g of vermicompost according to ASAE S358.2 standard was placed in the oven at 103 ± 3 ° C for 48 hours. (ASAE Standards S269.4, 1998).

Experimental setup

The vermicompost sample was put in an aluminum cylinder with a length of 150 mm and diameter of 7.5 mm. The very thin wall of the cylinder had very low thermal resistance to conduction. A K-type thermocouple was fixed on the central axis and at the middle length of the sample-holder cylinder to sense the central temperature of the sample.

The cylinder was put into water with a certain temperature until the sample temperature was changed by the equilibration on the water. Next, the sample-holder cylinder was cooled by a water bath at temperatures of 30, 40, 50, 60 ºC for the initial temperature of the sample (T_0) 50, 60, 70, 80 ºC, respectively. The temperature of the sample center was measured and recorded in a data logger (CHY 502 A, Taiwan) at an interval time of 2 min. *Figure 1* shows the schematic of the apparatus used for measuring the thermal diffusivity of the vermicompost sample.

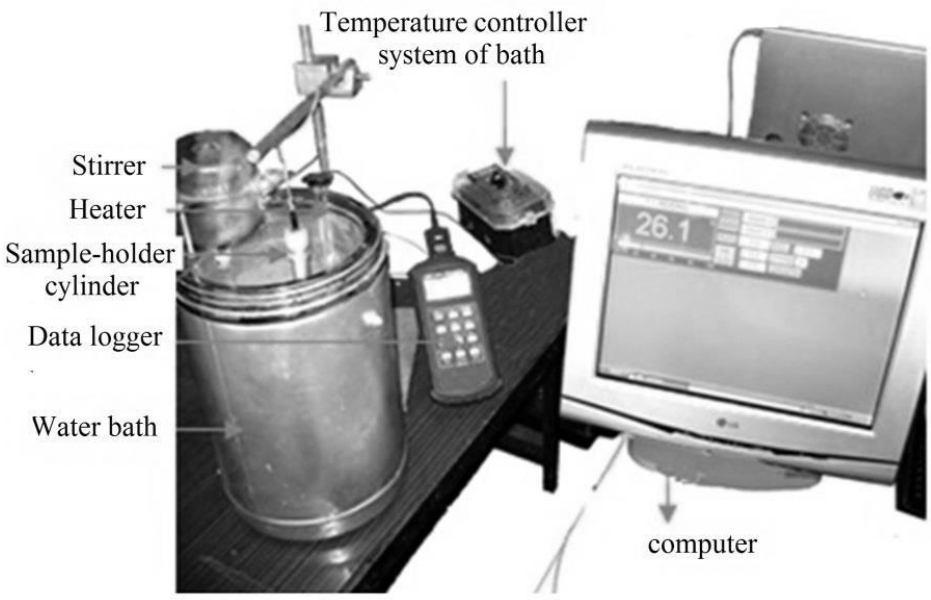


Figure 1. *Schematic of the apparatus used for measuring the thermal diffusivity of vermicompost*

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Theoretical principle

Thermal diffusivity includes the effects of properties like mass density (ρ), thermal conductivity (k), and specific heat capacity (C_p) this is described by Equation 1 (Mohsenin, 1980).

$$\alpha = \frac{k}{\rho C_p} \quad \text{(Eq.1)}$$

When heat is transferred by conduction through solid material it generates a temperature field can be described by Fourier equation in cylindrical coordinates:

$$\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T}{\partial r}\right) + \frac{\partial^2 T}{\partial x^2} = \frac{1}{\propto}\frac{\partial T}{\partial t} \quad \text{(Eq. 2)}$$

Where: T is temperature in ^{o}C; r is radius in m; x is axial coordinate in m; a thermal diffusivity in $m^2\ s^{-1}$; and t is time in s.

Considering the very small internal temperature gradient, the derivative $\frac{\partial^2 T}{\partial x^2}$ is ignored. As the cylinder is long enough to consider that the heat exchanges are made quasi-exclusively through the lateral surface, the problem is simplified and becomes one-dimensional (1D). Thus, the Equation 2 can be rewritten as Equation 3:

$$\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T}{\partial r}\right) = \frac{1}{\propto}\frac{\partial T}{\partial t} \quad \text{(Eq. 3)}$$

Equation 3 can be solved as Equation 4 (Bairi and Laraqi, 2003):

$$\theta = \frac{T_m - T_e}{T_0 - T_e} = 2\sum_{n=1}^{\infty} exp(-\varphi_n^2 Fo_c)\frac{1}{\varphi_n}\frac{J_1(\varphi_n)}{J_0^2(\varphi_n) + J_1^2(\varphi_n)}J_0(\varphi_n r^*) \quad \text{(Eq. 4)}$$

Equation 4 can become linear after a long time and then only the first term of the series solution can be acceptable to solve it. Thus, Equation 4 can be rewritten as Equation 5:

$$\theta = \frac{T_m - T_e}{T_0 - T_e} = \beta\ \exp\left(-\varphi_1^2 F_0\right) \quad \text{(Eq. 5)}$$

Where the constant β is given by Equation 6:

$$\beta = 2\frac{1}{\varphi_n}\frac{J_1(\varphi_1)}{J_0^2(\varphi_1) + J_1^2(\varphi_1)}J_0(\varphi_n r) \quad \text{(Eq. 6)}$$

The Fourier number is given by Equation 7:

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$$Fo_c = \frac{\alpha t}{r^2} \quad \text{(Eq. 7)}$$

Therefore, Equation 5 can be rewritten as Equation 8:

$$\theta = \frac{T_m - T_e}{T_0 - T_e} = \beta \exp\left(-\varphi_1^2 \frac{\alpha t}{r^2}\right) \quad \text{(Eq. 8)}$$

The parameters are defined below:

θ: dimensionless temperature ratio, T_0: initial temperature of the vermicompost in ºC, T_m: temperature of the center of vermicompost in ºC, T_e: temperature of the environment in ºC, Fo_c: Fourier number in dimensionless, r: radial cylindrical coordinate, φ_n, φ_1: positive roots of the Bessel's equation, J_0 and J_1: the Bessel function of first kind of order 0 and 1, r^*: the reduced radius ($r^* = r/R$), and t: time in s.

The value for the first root φ_1 is 2.405. Thus, thermal diffusivity can be calculated by measuring the slope of Equation 9 (Bairi et al., 2007):

$$\alpha = \frac{1}{\tau}\left(\frac{R}{2.405}\right)^2 \quad \text{(Eq. 9)}$$

Where τ is the time constant.

Results and Discussion

The logarithmic dimensionless temperature ($Ln\theta$) was plotted against the cooling time for the vermicompost sample with four variable temperatures with the moisture content of 40% (*Fig. 2*). *Figure 2* represents the linear relationship with a negative slope between the logarithmic dimensionless temperature and the cooling time for four temperature levels. Moreover, the values of slope ($/A/$) increased with decreases in the temperature.

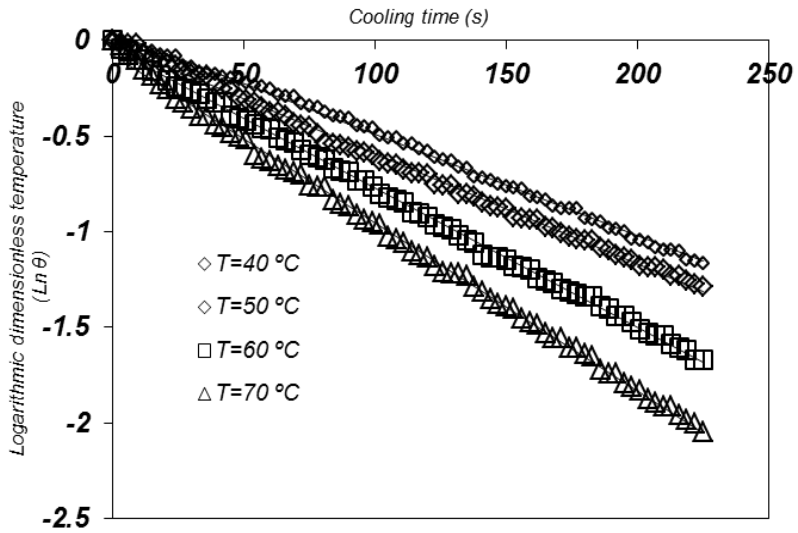


Figure 2. *Evaluation of dimensionless temperature (Lnθ) during the cooling time for vermicompost at 40% and different temperatures*

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A regression analysis was performed to show the linear relationship between the cooling time and the logarithmic dimensionless temperature. *Table 1* illustrates the parameters of the linear relationship and correlation coefficients between logarithmic dimensionless temperature and cooling time. The coefficient of determination, R^2, ranged from 0.9924 to 0.9996, and the standard error was found to vary from 0.01821 to 0.09386. The higher R^2 and lower standard error represent the higher accuracy of the computation of thermal diffusivity. According to *Table 1*, the value of slope (*|A|*) increases with increases in temperature, while it decreases with increases in the moisture content from 10% to 50% on wet base (w.b).

Table 1. *Relationship between the dimensionless temperature ln(θ) and the cooling time t for vermicompost [ln (θ) =At+B]*

Moisture content %	Temperature (°C)	Coefficient		R^2	es
		A	B		
10	40	-0.01720	-0.0675	0.9949	0.04452
	50	-0.02124	0.0503	0.9973	0.07146
	60	-0.02428	-0.02731	0.9933	0.05863
	70	-0.02942	-0.2581	0.9924	0.08509
20	40	-0.01637	-0.0885	0.9946	0.05698
	50	-0.02022	-0.04070	0.9994	0.02730
	60	-0.02326	-0.0242	0.9992	0.03646
	70	-0.02824	-0.024	0.9981	0.05575
30	40	-0.01564	-0.1373	0.9949	0.04563
	50	-0.01898	0.02858	0.9935	0.07743
	60	-0.02218	-0.2677	0.9970	0.06243
	70	-0.02621	0.3522	0.9957	0.08282
40	40	-0.01501	-0.0640	0.9992	0.01947
	50	-0.01790	0.09460	0.9936	0.05205
	60	-0.02144	-0.05820	0.9996	0.01821
	70	-0.02533	0.01810	0.9938	0.09386
50	40	-0.01437	0.06290	0.9994	0.01947
	50	-0.01731	0.06460	0.9936	0.05205
	60	-0.02070	-0.05666	0.9996	0.01821
	70	-0.02366	0.06410	0.9938	0.09386

The thermal diffusivity was calculated by using Equation 9. The value of thermal diffusivities, α, in $m^2\ s^{-1}$ ranged from 1.3973×10^{-7} to 2.861×10^{-7} $m^2\ s^{-1}$. These values are within the general range of the thermal diffusivity of agricultural materials. The thermal diffusivity of Marquis wheat seed was studied in bulk at 9.2% (d.b) of the moisture content, and the value of thermal diffusivity was equal to 1.15×10^{-7} $m^2\ s^{-1}$ (Babbit, 1945). The thermal diffusivity of rough rice was obtained from 0.856×10^{-7} to 1.05×10^{-7} $m^2\ s^{-1}$ in the moisture range of 12% to 20% (d.b) (Wratten et al., 1969). The value of thermal diffusivity of manure was reported to be 1.65×10^{-7} $m^2\ s^{-1}$ (Houkom et al., 1974). The thermal diffusivity of dairy cattle manure was measured from 0.904×10^{-7} to 2.11×10^{-7} $m^2\ s^{-1}$ (Amin-nayyeri et al., 2009). *Table 2* presents the variance analysis results of the thermal diffusivity of the vermicopost sample that include the effects of temperature and moisture parameters as well as of their interactions. As presented in *Table 2*, the temperature and moisture parameters have become significant ($P < 0.001$), but their interaction happens to be non-significant.

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Table 2. *The variance analysis results of the thermal diffusivity of vermicopost*

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	Fcal-value	Probability
Treatment	5.69E-15	19	2.99E-16	2133.05***	0.0000
T	5.52E-15	3	1.84E-15	13111.03***	0.0000
MC	1.67E-16	4	4.20E-17	298.75***	0.0000
MC×T	1.22E-21	12	1.02E-22	0.0007ns	0.0000
Residual	5.62E-18	40	1.41E-19		
Total	5.70E-15	78			

*** Highly significant; ns non-significant

Figure 3 presents the effect of moisture content on the thermal diffusivity of vermicompost. By increasing the moisture content of vermicompost from 10% to 50% (w.b), thermal diffusivity decreases from 2.861×10^{-7} to 1.3973×10^{-7} m^2 s^{-1}. The results for thermal diffusivity of vermicompost are in general agreement with the findings of some previous researchers. The thermal diffusivity of guna seed decreased from 9.311×10^{-8} m^2 s^{-1} to 8.5×10^{-8} m^2 s^{-1} as the moisture content increased (Avira et al., 2008). The investigation showed that the thermal diffusivity of millet grains decreased from 2.03×10^{-7} to 1.52×10^{-7} m^2 s^{-1}. (Subramanian and Viswanathan, 2003).

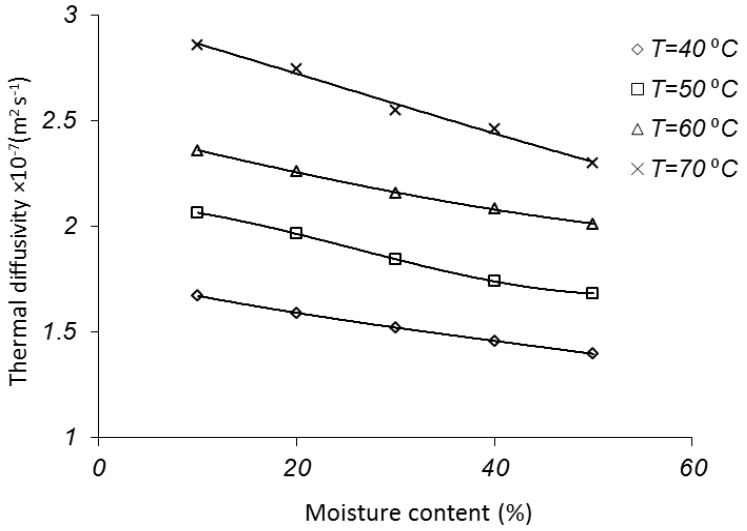


Figure 3. *Thermal diffusivity of vermicompost versus moisture content at different temperatures*

Figure 4 shows the effect of temperature on the thermal diffusivity of vermicompost. The thermal diffusivity of vermicompost increased with rising temperatures (*Fig. 4*). Moreover, the line of 10% moisture content is higher than other moisture content levels. The thermal diffusivity of solid food increased almost linearly with increases in the temperature (Fito et al., 1984). The investigation showed that the thermal diffusivity of cumin seed increased from 0.653×10^{-7} to 16.64×10^{-7} m^2 s^{-1} (Singh and Goswami, 2000).

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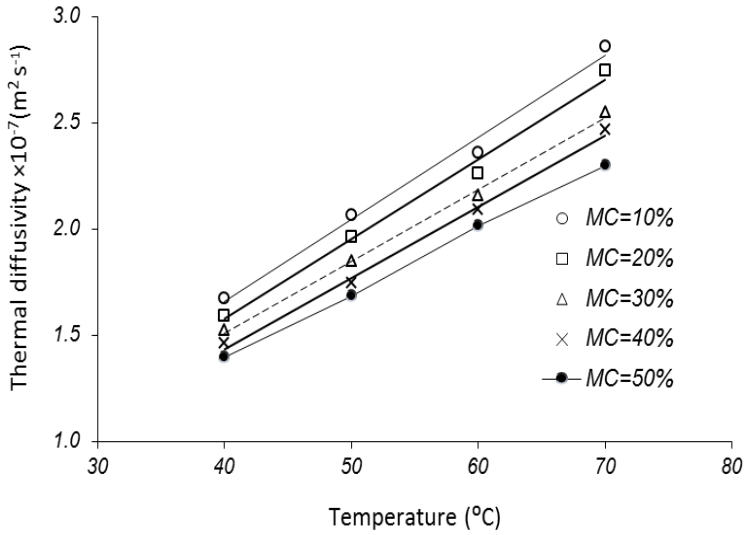


Figure 4. *Thermal diffusivity of vermicompost versus temperature at different moisture contents*

A polynomial relationship among the thermal diffusivity (α), temperature (T), and moisture content (MC) was obtained by multiple regression analysis with a value of 0.99 for the coefficient of determination as Equation 10:

$$\alpha = 1.01\times10^{-7} + 1.35\times10^{-9}T + 1.93\times10^{-11}T^2 - 1.3\times10^{-9}MC + 4.57\times10^{-}12\ MC^2 \quad \text{(Eq. 10)}$$

Table 3 shows that the effect of temperature (high F-value) on the thermal diffusivity is more than that of moisture content. The significance of respective regression coefficients in Equation 10 also confirms the results.

Table 3. *Analysis of variance (ANOVA) for effect of parameters in Equation 10 on thermal diffusivity of vermicompost*

Source of variation	Sum of squares	Degree of freedom	Mean sum of squares	Fcal-value	Probability
Regression	3.47E-14	4	8.67E-15	38403.03***	0.0000
T	2.55E-16	1	2.55E-16	1130.22***	0.0000
T^2	7.48E-17	1	7.48E-17	331.05***	0.0000
MC	3.02E-17	1	3.02E-17	134.02***	0.0000
MC^2	3.43E-17	1	3.43E-17	151.97***	0.0000
Residual	3.38E-18	15	2.25E-19		
Total	3.47E-14	19			

*** Highly significant

Any comparison between the measured and estimated values of the thermal diffusivity (*Fig. 5*) showed that the maximum differences were within $\pm0.01\times10^{-7}$ m^2s^{-1}, showing reasonable accuracy for estimating the thermal diffusivity.

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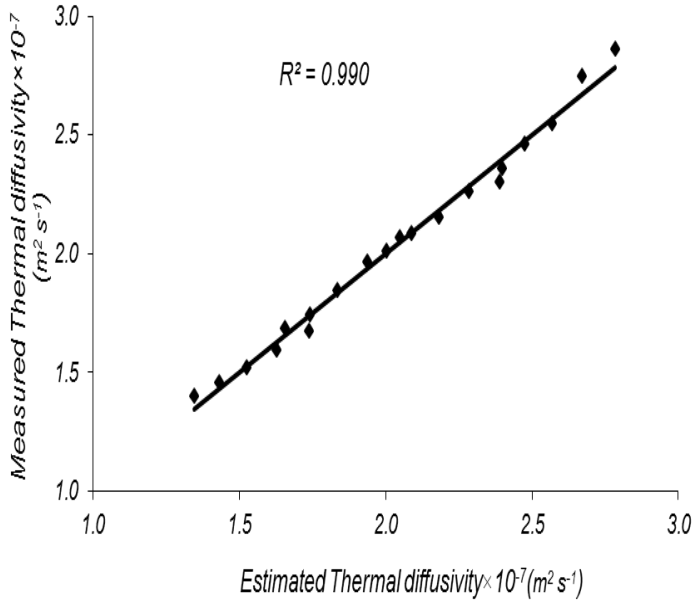


Figure 5. *Estimated versus measured values of the thermal diffusivity of vermicompost*

Conclusions

The thermal diffusivity of vermicompost was determined using a *1D* solution of the Fourier equation of heat. The thermal diffusivity of vermicompost is dependent on the latter's moisture content and temperature. The effect of temperature on the thermal diffusivity was more than that of moisture content. By increasing the moisture content of the vermicompost sample from 10% to 50%, its thermal diffusivity decreased from 2.861×10^{-7} to 1.3973×10^{-7} m^2 s^{-1}. The thermal diffusivity of vermicompost increases with rising temperature. The developed model can also be used acceptably within the range of variables studied in this research where the thermal diffusivity of the vermicompost ranged from 1.3973×10^{-7} to 2.861×10^{-7} m^2 s^{-1}. The resulting thermal diffusivity of vermicompost can be used to develop heat transport models for the design of more optimal temperature control in composting systems.

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K-MEDOIDS AND FUZZY C-MEANS ALGORITHMS FOR CLUSTERING CO_2 EMISSIONS OF TURKEY AND OTHER OECD COUNTRIES

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Abstract. One of the most fundamental problems of societies is environmental problems that have emerged as the result of human behaviors which can be summarized as "dominating nature" and "using nature carelessly". The main causes of climate change and greenhouse gas emissions, which are considered to be most important environmental problem nowadays, are used of fossil fuel resources intensively in order to overcome the energy requirement. Carbon dioxide (CO_2) is the largest share of greenhouse gas emissions. To reduce CO_2 emissions, significant studies have been done in the world. In this study, we tried to classify member countries of OECD according to CO_2 emissions indicators from fossil fuel consumption. For the clustering, both the classical cluster analysis and the fuzzy cluster analysis were implemented. The clusters obtained from both methods are simply interpreted.
Keywords: *fuzzy clustering, Paris climate agreement, Kyoto Protocol, climate change, greenhouse gas emission*

Introduction

Climate change can be described in a very general way in the form of "regardless of their cause, long-term and slow-growing changes in climate conditions" (Türkeş, 2003).

However; with the industrial revolution, in addition to natural climate variability, it has entered a new era of human activities that affect the climate. Since the industrial revolution, the accumulation of greenhouse gases in the atmosphere has been rapidly increasing, especially; as a result of human activities such as burning of fossil fuels, land use changes, deforestation and industrial processes. With the understanding that these increases in the concentration of greenhouse gases are beginning to pose serious threats, and that this problem can be resolved with global efforts, global scale co-operation and organizations have been started to form. In this process, The "Framework Convention on Climate Change" signed at the United Nations Conference on Environment and Development held in Rio de Janeiro in June 1992 under the auspices of the United Nations and The "Kyoto Protocol" formed as a result of the "Third Party Conference" held in Japan in December 1997 are special important development (Dam, 2014).

One of the main reasons why the Kyoto Protocol is so important is that it obligates the parties with greenhouse gas reductions on certain dates and at certain rates. In addition, the Kyoto Protocol has introduced three new mechanisms that provide flexibility for greenhouse gas reduction (Gençer, 2008). These mechanisms called the Kyoto Protocol Flexibility Mechanisms, allow countries for greenhouse gas reduction to undertake joint activities outside their own borders. Turkey, in 2004, has became a party to the United Nations Framework Convention on Climate Change, signed the protocol

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in 2009 and has been the 178th of countries that ratified the Kyoto Protocol. Between 2008 and 2012, Turkey had no obligation to any greenhouse gas emission reduction. On the other hand; according to the protocol, Turkey aims to reduce at least 20% reduction in carbon emissions until 2020.

The Kyoto Protocol is the first step in the climate protocol. However, this protocol is not a result. Because, only developed countries took on the greenhouse gas reduction obligation in the Kyoto Protocol. The world was trying to establish a new agreement on the global scale since Kyoto Protocol. In Paris on December 12, 2015, countries adopted an international agreement to address climate change that requires deeper emissions reduction commitments from all countries—developed and developing. The Paris Climate Agreement, in which 196 countries have reached consensus and 187 countries have proposed a reduction plan (INDC), includes countries that account for more than 96% of total global emissions. The objective of the agreement is to maintain the increase in global temperatures well below 2 degrees Celsius above pre-industrial levels, whilst making efforts to limit the increase to 1.5 degrees (Savaşan, 2017).

The agreement aims to ensure global greenhouse gas emissions peak as soon as possible, and to balance emissions and removals of greenhouse gases in the second half of this century. Furthermore, the agreement addresses adaptation to climate change, financial and other support for developing countries, technology transfer and capacity building, as well as loss and damage (Savaşan, 2017).

This new agreement has set countries' minimum obligations, implemented mechanisms to spur additional action in developing countries, supported the most vulnerable countries in addressing climate change, and established systems to hold countries to their commitments. The Paris Agreement will be strengthened over time using its solid framework.

Turkey, in the context of the struggle against climate change, presented the Intended Nationally Determined Contribution (INDC) plan as a volunteer to the UN Secretariat before the Climate Summit in Paris. Turkey stated that aims to increase greenhouse gas emissions less than 21% from the reference scenario until 2030 in this declaration. Turkey which refers to special conditions in INDC Plan, set targets for renewable energy use and the transition to nuclear energy. Also, Turkey declared that greenhouse gas emissions will be reduced by low-carbon investments that will provide energy efficiency in urbanization, transport and industry sector (Karakaya, 2015).

The atmosphere, which is of vital importance to the living creatures in the world, contains many gases in it. Nitrogen (78.08%) and oxygen (20.95%) in the atmosphere form 99% of the volume of clean and dry air. The remaining dry air section consists of argon (0.93%), an inert gas, and some trace gases, which are very small in quantity. Although its deposition in the atmosphere is very small, the CO_2, which is an important greenhouse gas, is in fourth place with 0.03% (Türkeş, 2008). Carbon dioxide (CO_2) is the most important greenhouse gas that is effective on global warming. Although it is at a very low volumetric rate in the atmosphere, its share in total greenhouse gases exceeds 80% (Koçak, 2006). As a result of from decay of plants, volcanic activity, human and animal breath and also combustion of fossil fuels such as coal, oil and natural gas, CO_2 accumulates in the atmosphere. Because of burning fossil fuels, forest fires, etc., the CO_2 concentration has been increasing rapidly since the beginning of this century. While CO_2 concentration is 290 ppm in the early 20th century, it is estimated that CO_2 concentration will increase to 500 ppm at the end of the 21st century. CO_2 absorbs some of the infrared heat energy released from the earth. Therefore, an increase in CO_2

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concentration will cause an increase in the average surface temperature of the world scale (Koçak, 2006).

Turkey's carbon dioxide emissions data table and chart are given in *Table 1* and *Figure 1* between 1990 and 2015.

Table 1. *Greenhouse gas emissions by sectors (CO_2 equivalent), 1990-2015. (Source: TUİK (2015): Seragazı emisyon Envanteri)*

Year	Total	Change compared to 1990 (%)	Energy	Industrial processes and product use	Agriculture	Waste
1990	214.0	-	134.4	23.7	44.8	11.1
1991	221.1	3.3	138.5	25.4	45.8	11.3
1992	227.4	6.3	144.7	25.1	46.1	11.5
1993	236.7	10.6	152.2	26.0	46.8	11.8
1994	230.3	7.6	148.9	25.3	44.0	12.0
1995	246.6	15.2	163.5	27.3	43.4	12.4
1996	264.2	23.5	179.2	28.1	44.2	12.7
1997	275.6	28.8	191.2	29.0	42.1	13.2
1998	277.6	29.7	191.0	29.3	43.7	13.5
1999	276.4	29.2	190.2	27.8	44.4	14.0
2000	296.5	38.6	211.7	27.8	42.5	14.5
2001	277.7	29.8	195.0	27.9	39.8	15.0
2002	284.6	33.0	201.9	29.3	38.0	15.4
2003	304.1	42.1	216.6	30.5	41.2	15.9
2004	315.1	47.3	223.3	33.1	42.2	16.5
2005	337.2	57.6	241.0	35.9	43.3	16.9
2006	361.7	69.0	260.5	39.0	44.8	17.5
2007	395.0	84.6	291.4	41.5	44.4	17.7
2008	391.8	83.1	288.5	43.4	42.1	17.8
2009	400.9	87.4	294.6	45.1	43.4	17.9
2010	406.8	90.1	291.8	51.0	45.8	18.2
2011	436.4	103.9	313.9	55.8	48.1	18.5
2012	448.9	109.8	319.3	57.7	53.8	18.1
2013	442.2	106.6	308.3	60.2	57.2	16.5
2014	455.6	112.9	321.2	60.8	57.2	16.4
2015	475.1	122.0	340.0	60.7	57.4	16.9

According to the *Table 1,* the total greenhouse emissions of 2015 as CO_2 equivalents increased 122% compared to 1990. Total greenhouse gas emissions in 2015 were calculated as 475.1 million tonnes (Mt) CO_2 equivalent. The largest share of the CO_2 equivalent in 2015 emissions was energy-related emissions with 71.6% followed by industrial operations and product use by 12.8%, agricultural activities by 12.1% and waste by 3.5%. There are many studies on climate change and greenhouse gas emissions. In this study, we tried to classify in terms of CO_2 emissions the Organisation for Economic Co-operation and Development (OECD) countries which also Turkey is a member. For this purpose, we used fuzzy c-means algorithm which is most commonly

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known in fuzzy partition. Then, in order to make a comparison, analyzes were made by classical k-medoid algorithm. For analysis of greenhouse gas emissions, which is one of the important ecological issues, it is aimed to be shown that besides classical techniques, fuzzy techniques which are more flexible can be used.

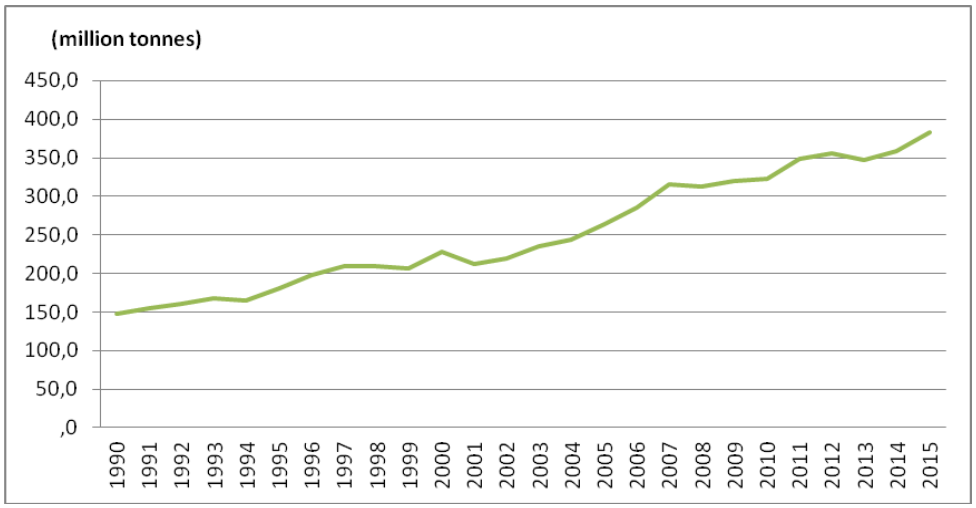


Figure 1. *CO_2 emissions in Turkey between 1990 and 2015*

Methods and materials

Clustering

Clustering analysis or simply clustering is one of the multivariate statistical methods helps to divide data labels of which are not exactly known to subgroups according to similarities and explore different correlation and structures in large data sets. The desired goal in this analysis is to divide the data set so that the elements assigned to the same cluster are as similar as possible but that the two objects in different clusters should not be as similar as possible. Hence, clustering analysis tries to model the ability in clustering similar objects into homogeneous classes or categories. In clustering, initially the number of clusters and number of object that assigned to clusters is not certain. By collecting similar objects into clusters, clustering analysis tries to recreate unknown clusters, hoping that each cluster found represents an actual object category or category. Classifying datasets, clustering analysis only uses some mathematical criteria on the composition of clusters. For this reason, clustering algorithms are equipped with distance measures to measure the similarity of the presented samples.

Clustering techniques are distinguished according to how assignments are made, in other words, according to their assignment patterns (Hartigan, 1975). In classical cluster analysis, each datum must be exactly assigned to a cluster. These classical methods convert non-empty partitions of the data into non-empty and double disjoint subclasses. Such hard data aggregation way can be insufficient when data is equal distance to two or more clusters. Such special data points may represent similar hybrid or mixed objects at a rate equal to two or more species. Classical (hard) partition forces these data points to be fully assigned to one of the clusters; but they should belong to all at equal rates. Hard partition is defined as below;

Let $X = \{x_1, x_2, \ldots x_n\}$ be a set of n vectors in R^d, representing the data. For an integer $c > 2$, a hard clustering of X into c clusters consists of c disjoint subsets of X', $S_1, \ldots, S_c$

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whose union is X. An equivalent way of defining clusters is achieved by using functions. For each $i = 1, \dots, c$ define $u_i: X \to \{0,1\}$ by $u_i(x) = 1$ if $x \in S_i$ and $u_i(x) = 0$ if $x \notin S_i$ (Bezdek, 1973).

These functions are called membership functions. Because it determines each data point belongs a specific cluster. In hard clustering, membership value is equal to 1 if the data belongs to the cluster, and 0 otherwise. As can be seen, in classical clustering theory, boundaries of clusters and adjectives of clusters are definite. In real life, however, the boundaries of each cluster and the attributes of each element belonging to these clusters are not so certain. Thus, it can be seen that the concept of classical clustering is inadequate in some situations encountered in real life.

Classical clustering analysis is organized under hierarchical and non-hierarchical headings. Hierarchical methods generate arrays of clusters usually starting from clusters that contain a single unit, by performing concatenation until all units are aggregated in a cluster or they generate clusters by starting from cluster that contain all units and separating units sequentially. Non-hierarchical techniques are methods which aim to divide units into clusters that are homogeneous in themselves and heterogeneous among themselves and estimate the parameters of sub-populations (group or cluster mean vectors and covariance matrices) through prototype sets. The most common non-hierarchical methods are k-means clustering, k-medoid clustering and fuzzy clustering. In this study we will study k-medoid and fuzzy methods.

K-medoids algorithms

It attempts to reduce the distance between a point labeled on the cluster and center of cluster. The method selects medoid, which means point the closest to center of cluster, instead of the average of data as a cluster center. Medoids for each cluster are calculated by using the following formula (*Eq. 1*) by finding the object i within the cluster that minimizes

$$\sum_{j \in C_i} d(i, j) \qquad \text{(Eq.1)}$$

where c_i is the cluster containing object i and $d(i,j)$ is the distance between objects i and j.

There are two advantages of using existing rules as the centers of the clusters. First, a medoid rule serves to usefully describe the cluster. Second, there is no need for repeated calculation of distances at each iteration, since the k-medoids algorithm can simply look up distances from a distance matrix. The most common realization of *k*-medoid clustering is the partitioning around medoids algorithm and is as follows (Kaufman and Rousseeuw, 1990):

- Initialize: randomly select *k* of the *n* data points as the medoids.
- Assignment step: associate each data point to the closest medoid.
- Update step: for each medoid *m* and each data point *o* associated to *m* swap *m* and *o* and compute the total cost of the configuration (that is, the average dissimilarity of *o* to all the data points associated to *m*). Select the medoid *o* with the lowest cost of the configuration. Repeat alternating steps 2 and 3 until there is no change in the assignments.

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Kaufman and Rousseeuw suggested the use of silhouettes both to determine which objects lie well within their clusters and which do not and also to judge the quality of the clustering obtained. For each object i, let $a(i)$ be the average distance of i from all other objects in cluster c_i . For every other cluster $c \neq c_i$, let $d(i,c)$ be the average distance of i from the objects in c. After computing $d(i,c)$ for all clusters $c \neq c_i$, let $b(i)$ be the smallest. The cluster for which this minimum is attained is called the neighbour of i. The number s(i) is given by using *Equation 2:*

$$s(i) = \frac{b(i) - a(i)}{\max(a(i), b(i))} \quad \text{(Eq.2)}$$

If $s(i)$ is close to 1, object i can be said to be 'well classified'. If $s(i)$ is close to 0, it is unclear whether i should belong to cluster $c_i$or to its neighbor. A negative value suggests that i has been misclassified. The following summary values are also defined:

- The mean of $s(i)$ for all objects i in a cluster is called the average silhouette width of that cluster.
- The mean of $s(i)$ for all the objects is called the average silhouette width for the entire data set and is denoted by $\bar{s}(c)$, where c is the number of clusters.

Kaufman and Rousseeuw suggest that $\bar{s}(c)$, can be used for the selection of a best value of c, by choosing that k for which $\bar{s}(c)$, is maximal (Richards and Rayward-Smith, 2001).

Fuzzy clustering

Fuzzy clustering is the generalization of this point of view. In fuzzy set theory, an element belongs to a cluster with membership values between 0 and 1, including 0 and 1. In other words, in a fuzzy set, a fuzzy set element belongs partially to a cluster.

A fuzzy clustering of X into c clusters consist of functions $u_1, \ldots, u_c$ where $u_i: X \to [0,1]$ and $X_i u_i(x) = 1$, for all $x \in X$. Formally, given a set of objects, $\{x_1, x_2, \ldots x_n\}$a fuzzy clustering of k fuzzy clusters, $c_1, \ldots, c_k$, can be represented using a partition matrix, $U = [u_{ij}].(1 \leq i \leq n, 1 \leq j \leq k)$, where u_{ij} is the membership degree of x_i in fuzzy cluster c_j. The partition matrix should satisfy the following three requirements:

- For each object, x_i, and cluster, c_j, $0 \leq u_{ij} \leq 1$. This requirement enforces that a fuzzy cluster is a fuzzy set.
- For each object, x_i, $\sum_{j=1}^{k} u_{ij} = 1$. This requirement ensures that every object participates in the clustering equivalently.
- For each cluster, c_j, $0 < \sum_{i=1}^{n} u_{ij} < n$. This requirement ensures that for every cluster, there is at least one object for which the membership value is nonzero.

Fuzzy cluster analysis therefore allows gradual memberships of data points to clusters in [0, 1]. This gives the flexibility to express that data points belong to more than one cluster at the same time. Furthermore, these membership degrees offer a much finer degree of detail of the data model. Aside from assigning a data point to clusters in (equal) shares, membership degrees can also express how ambiguously or definitely a data point should belong to a cluster.

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The first use of this method based on fuzzy set theory in clustering analysis was carried out by Bellman et al. (1966). Many fuzzy clustering algorithms are based on the optimization of the objective function. The most widely used algorithm is the fuzzy c-means algorithm (Ruspini, 1970).

Fuzzy c-means algorithms

The fuzzy c-means algorithm is presented in its initial form by Dunn (1974) as an alternative to the classical k-means clusters and is completed by Bezdek (1974). The fuzzy c-means algorithm is based on the objective function. A large family of fuzzy clustering algorithms have been created to attempt to minimize the objective function of these basic c-means algorithms. In short, the most basic algorithm for fuzzy clustering analysis is the fuzzy c-means algorithm. The mentioned objective function is defined as *Equation 3* (Bezdek, 1981):

$$J(X;U,V)=\sum_{j=1}^{c}\sum_{i=1}^{n}\left(u_{ji}\right)^{m}\left\|x_i-v_j\right\|^2 \qquad \text{(Eq.3)}$$

where n is the total number of patterns in a given data set and c is the number of clusters;$X=\{x_1,\ldots x_n\}\subset R$ and $V=\{v_1,\ldots,v_c\}\subset R$ are the feature data and cluster centroids; $U=[u_{ji}]$ and $c\times n$ is a fuzzy partition matrix composed of the membership grade of pattern xi to each cluster j. value is the total of pattern measurement of all weighted least square errors. The weighting exponent m is called the being effective on the clustering performance of FCM (Şahinli, 1999).

Unfortunately, the object function $J(X;U,V)$ can not be directly minimized. Therefore, an iterative algorithm is used, which alternately optimizes the membership degrees and the cluster parameters. That is, first, the membership degrees are optimized by using *Equaition 4* for fixed cluster parameters, then the cluster parameters are optimized by using *Equation 5* for fixed membership degrees:

$$U_t=J_U\left(V_{t-1}\right) \qquad \text{(Eq.4)}$$

and

$$V_t=J_V\left(U_t\right) \qquad \text{(Eq.5)}$$

The main advantage of this scheme is that the optimum can be computed directly in each of two steps. By iterating the two steps, the joint optimum is approached (although it cannot be guaranteed that the global optimum will be reached—the algorithm may get stuck in a local minimum of the objective function *J*).

The update formulae J_U and J_V are derived by simply setting the derivative of the objective function J w.r.t. the parameters to optimize equal to zero (Höppner et al., 1999). Independent of the chosen distance measure the following update formula (*Eq. 6*) for the membership degree and following update formula (*Eq. 7*) for prototype is obtained from J(X;U,V):

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$$u_{ji} = \left[\frac{1}{\sum_{k=1}^{c} \left[\frac{\left\| x_i - v_j \right\|}{\left\| x_i - v_k \right\|} \right]^{2/(m-1)}} \right], 1 \le j \le c, 1 \le i \le n \qquad \text{(Eq.6)}$$

$$v_j = \frac{\sum_{i=1}^{n} \left(u_{ji} \right)^m x_i}{\sum_{i=1}^{n} \left(u_{ji} \right)^m}, \quad 1 \le j \le c \qquad \text{(Eq.7)}$$

In *Table 2* a brief description of the fuzzy *c*-means algorithm is given. Algorithm can be terminated when the relative change in the cluster centers gets very small or the objective function *J* cannot be minimized anymore (Bezdek, 1974).

Table 2. *FCM algorithm steps*

- Select the fuzzifier exponent *m* (*m* > 1) and initialize the fuzzy partition matrix $U = (u_{ji})$ randomly
- **while** termination conditions not met **do**
1. Compute the cluster centers v_j
2. Update the fuzzy partition matrix $U = (u_{ji})$
end while

The fuzzy c-means algorithm detects clusters in the form of dots. It is not effective in finding other cluster shapes. Also, the fuzzy c-means algorithm has some advantages and disadvantages. Sensitivity to noise points, adherence to initial values, long-running computation time are the disadvantages of the algorithm. On the other hand, unsupervised and constant convergence are the main advantages of the algorithm.

As seen in the first step of the fuzzy c-means algorithms, determining of the number of initial clusters is an important task. The fact that the number of the initial cluster is not known in advance makes it necessary to test the validity of the clusters. The validity indices are used to determine which set of clusters is appropriate clustering from the clusters in the fuzzy clusters. There are many validity indices in the literature. The most commonly used index in fuzzy clustering analysis is the Xie-Beni index. The Xie-Beni index is defined as follows in *Equation 8* (Xie and Beni, 1991):

$$XB(c) = \frac{\sum_{j=1}^{c} \sum_{i=1}^{n} \left(u_{ji} \right)^m \left\| x_i - v_j \right\|^2}{N \min_{ji} \left\| x_i - v_j \right\|^2} \qquad \text{(Eq.8)}$$

Xie-Beni index is also known as the density and separation index. It is based on two basic features; compactness and separation. In *Equation 8,* the numerator shows the closeness of the fuzzy partition, i.e. the closeness of the elements in the cluster, and the denominator shows the strong separation between the clusters, i.e. the distance between

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the clusters (Xie and Beni, 1991). It is seen in empirical studies that this index should take small values.

Results and discussion

In this study, it is aimed to determine the classification of the OECD countries by fuzzy clustering and classical clustering analysis in terms of the indicators from fossil fuel consumption and detect the cluster Turkey belongs, and other countries in this cluster, and evaluate whether it shows similar functions between Turkey and these countries or not. For the analysis, we used CO_2 emissions data from fuel combustion by sector from open access platform -International Energy Agency (IEA). 32 countries and five variables are used. The variables used are shown in *Table 3*.

Table 3. *Used variables*

Variables ID	Variables
X_1	Electricity and heat production
X_2	Other energy industry own use
X_3	Manuf. industries and construction
X_4	Transport
X_5	Other sectors

For the application, R studio 3.4.0 which is an environment for statistical computing program and "fclust" package was used. In order to apply the algorithm, the initial parameters (excluding the number of cluster) were selected randomly. The Xie-Beni results were calculated to determine the number of clusters.

As seen in *Table 4* and *Figure 2,* the experimental results revealed that for fuzzy c - mean algorithms, the elbow was located at c = 4. On the other hand, the smallest value of Xie-Beni index is the number of cluster is 4.

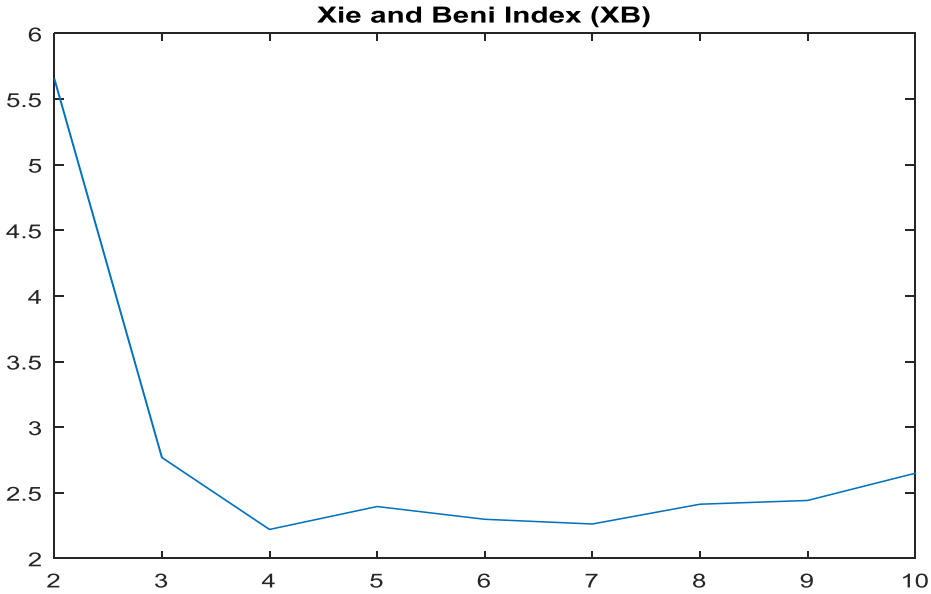


Figure 2. *Emissions cluster for OECD Countries, Xie- Beni graphic for number of cluster*

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Table 4. *Results of Xie-Beni index*

c	2	3	4	5	6	7	8	9	10
XB	***5.657***	***2.769***	***2.220***	***2.395***	***2.298***	***2.262***	***2.413***	***2.441***	***2.649***

Scattering of the clusters which are obtained from result of fuzzy c- means algorithm in Rstudio package program are shown as in *Figure 3*.

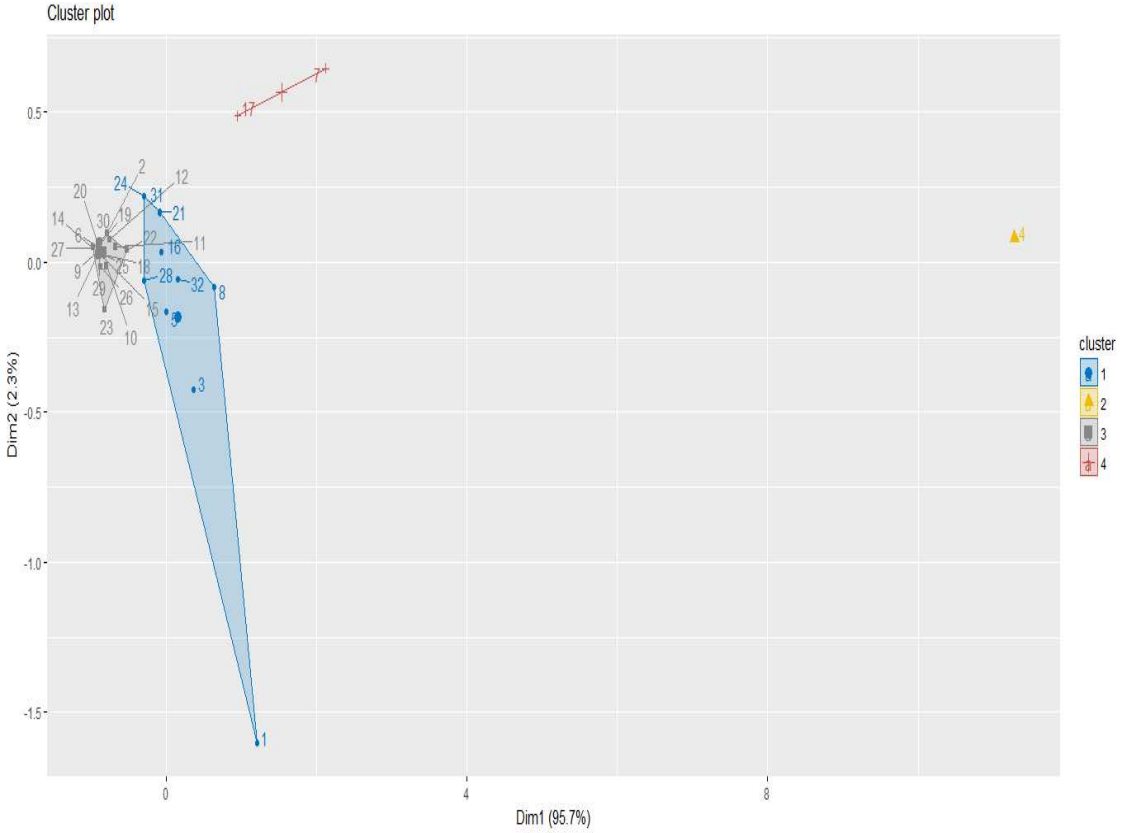


Figure 3. *Emissions cluster for OECD countries, c = 4*

Membership values of OECD countries are given in *Table 5*. If a unit has the largest membership value in a cluster, then it is assigned to this cluster.

Table 5. *Cluster membership degrees for OECD member countries*

Countries	**Cluster 1**	**Cluster 2**	**Cluster 3**	**Cluster 4**
CND (Canada)	0.001	0.255	0.611	0.133
CL (Chile)	0.999	0.000	0.000	0.001
MEX (Mexico)	0.000	0.005	0.974	0.021
USA (United States)	0.000	1.000	0.000	0.000
AUS (Australia)	0.000	0.002	0.935	0.063
ISR (Israel)	0.999	0.000	0.000	0.001

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J (Japan)	0.000	0.000	0.001	0.999
KOR (South Korea)	0.000	0.019	0.962	0.019
NZ (New Zealand)	0.999	0.000	0.000	0.001
A (Austria)	0.999	0.000	0.000	0.001
B (Belgium)	0.998	0.000	0.000	0.002
CS (Czech Republic)	0.999	0.000	0.000	0.001
DK (Denmark)	0.999	0.000	0.001	0.000
EST (Estonia)	0.996	0.001	0.000	0.003
FIN (Finland)	0.999	0.001	0.000	0.000
F (France)	0.000	0.002	0.849	0.149
G (Germany)	0.001	0.156	0.054	0.789
GR (Greece)	0.999	0.000	0.000	0.001
H (Hungary)	0.999	0.000	0.000	0.001
IRL (Ireland)	0.990	0.000	0.001	0.009
I (Italy)	0.000	0.002	0.788	0.209
NL (Netherlands)	0.982	0.000	0.000	0.018
N (Norway)	0.998	0.000	0.000	0.002
PL (Poland)	0.000	0.002	0.694	0.304
P (Portugal)	0.990	0.003	0.001	0.006
SK (Slovakia)	0.983	0.002	0.000	0.015
SVN (Slovenia)	0.996	0.002	0.000	0.002
E (Spain)	0.000	0.001	0.651	0.348
S (Sweden)	0.999	0.000	0.000	0.001
CH (Switzerland)	0.999	0.000	0.000	0.001
TR (Turkey)	0.000	0.002	0.872	0.126
GB (Great Britain)	0.000	0.001	0.986	0.013

As a result of the analysis, with the help of fuzzy c-means clustering, the number of cluster has been determined as 4. As can be seen in *Table 6,* the first cluster was determined as New Zealand, Chile, Austria, Belgium, Czech Republic, Denmark, Israel, Finland, Greece, Hungary, Ireland, Netherlands, Norway, Poland, Portugal, Slovakia, Sweden, Switzerland. While United States took place in the second cluster, the third cluster were identified as Canada, Mexico, Australia, South Korea, France, Italy, Poland, Spain, Turkey and Great Britain. The countries in the last group were designated as Japan and Germany.

In order to compare the results of fuzzy clustering, the k-medoid clustering method of classical clustering methods is also used. NCSS program was used for analysis. After data entry in NCSS for k-medoid application, "Medoid Partitioning" was selected from the "Clustering" section in the analysis section and the number of clusters was entered as minimum 2 and maximum 10 in order to determine the optimal number of clusters. The results are shown in *Table 7*.

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Table 6. *Clusters according to the countries(FCM)*

Countries	Clusters
ISR CL NZ A B CS DK EST FIN GR H IRL NL N P SK SVN S CH	1
USA	2
CND MEX AUS KOR F I PL E TR GB	3
J G	4

Table 7. *Silhouette statistics according to the number of cluster*

Number Clusters	Minimize This	Adjusted	Maximize This
	Average Distance	Average Distance	Average Silhouette
2	**189.034**	**11.815**	**0.890**
3	48.701	4.566	0.530
4	24.173	3.022	0.543
5	27.745	4.335	0.568
6	18.111	3.396	0.563
7	15.991	3.498	0.570
8	12.068	3.017	0.507
9	9.822	2.762	0.439
10	9.581	2.994	0.429

Based on the average silhouette statistics obtained for the clusters, the number of clusters with which the average silhouette statistics value is maximized as k = 2.

According to k-medoid clustering, OECD countries were divided into 2 clusters in points of carbon dioxide emissions. As seen in *Table 8,* the classification was made in the United States as a cluster, the remaining 31 OECD countries as the second cluster.

Table 8. *Clusters according to the countries(K-medoid)*

Cluster 1	**USA**
Cluster 2	**CND CL MEX AUS ISR J KOR NZ A B CS DK EST FIN F G GR H NL N PL P SK SVN E S CH TR GB**

Conclusions

Societies are increasingly fragile against atmosphere-related natural disasters. As a result of increasing greenhouse gas emissions due to human activities, the link between industry-climate change is getting stronger. Although the kyoto protocol is the basic protocol, the results have not been effective. For this reason, the Paris Climate Agreement, which is one of the important protocols on fighting climate change, was organized in 2015. The main objective of the protocol is to reduce the emissions of all greenhouse gases, primarily carbon dioxide. For each country, the measures are clearly stated in the protocol.

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The changes in the atmosphere of the earth largely took place in the years following the industrial revolution and especially in the 20th century. In the 21st century, it is estimated that greenhouse gas emissions will further increase the global average temperature. Carbon dioxide (CO_2), the most important of the six greenhouse gases, its emission have increased especially during the past century. In this study, CO_2 emissions resulting from the fuel combustion of 32 countries were classified by using fuzzy c-means algorithm - based on fuzzy logic-, and classical k-medoid algorithm. As a result of the analysis, in the fuzzy clustering analysis, the cluster number is 4; whereas in classical clustering analysis, the number of clusters was found to 2.

It can be said that the k-medoid clustering analysis is created an artificial cluster because the first cluster is only formed by the USA. It can also be said that the classification based on the fuzzy c-means algorithm reflects the natural classification resulting from the combination of the variables in the data matrix. Since the data used in the study show a global structure, the fuzzy c-means method of conventional fuzzy clustering methods was used and produced useful results.

Japan and Germany, being in fourth cluster in the clustering result, are ranked 2nd and 3rd, respectively, according to CO_2 emission values for 2015. According to the year 2015 statistics, the total CO_2 emission was 32.294 million metric tons in the world, while the emission value of the US was 4997 million metric tons. As for the analyzed CO_2 emission variables of the USA, clustering of the US in a single cluster is an important and verifiable result in this matter.

According to the 2015 greenhouse emission inventory report, published by the Turkish Statistical Institute, in Turkey, total greenhouse gas emissions in 2015 were 475.1 million tons (mt), equivalent to CO_2. In this inventory, the largest share of CO_2 emissions for 2015 created energy-driven emissions.

CO_2 emissions in the world has being estimated to increase by an average of 2% each year since 1971 and by 45% until 2030. With this study, it is aimed to shed light on researchers working on environment and climate change. However, classification for carbon dioxide emission can be enriched by adding other countries besides OECD countries or different greenhouse gas statistics can be clustered for countries. Thus, greenhouse gas emissions of countries can be scrutinized from a wider perspective.

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MAXIMUM ENTROPY MODELING (MAXENT) OF CURRENT AND FUTURE DISTRIBUTIONS OF *IPS MANNSFELDI* (WACHTL, 1879) (CURCULIONIDAE: SCOLYTINAE) IN TURKEY

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Abstract. Climate change has reached the highest rates of the last 1.400 years. This has begun to attract the attention of researchers, and the number of studies on estimation of the environmental impacts of climate change on species has also begun to increase. *Ips mannsfeldi* (Wachtl, 1879) is a bark beetle (Curculionidae: Scolytinae) species spreading on black pine (*Pinus nigra*) fields in Turkey. This study aims to model the current and future (2070) distribution of the species according to two different global climate change scenarios. Locations of *I. mannsfeldi* specimens that were collected in different stands were recorded with GPS. Current and future potential distribution areas of the species have been determined using maximum entropy modeling. RCP4.5 and RCP8.5 emission scenarios reported in IPCC5 were used to estimate the future potential distribution of the species. As a result of the study, it is determined that the species distributes on the Black pine (*Pinus nigra*) forests throughout the Mediterranean part of Turkey at the present time. According to projections to 2070 the distribution area will expand towards the Aegean region. Depending on the changing climatic conditions, it can be seen that this species expands its distribution area and may be a potential pest that causes economic damage to black pine fields in the coming period.
Keywords: *Ips mannsfeldi, MaxEnt, Pinus nigra, climate change, future prediction, modeling*

Introduction

The last three decades have been successively warmer periods of the last 1.400 years in the Northern Hemisphere. Evidence regarding impacts of observed climate change is the strongest and most comprehensive for natural systems. In addition to these impacts, many terrestrial, freshwater and marine species have to shift their geographic ranges, seasonal activities, migration patterns, abundances and species interactions in response to ongoing climate change (IPCC, 2014). Especially, insect communities may severely be impacted by increasing temperature which will affect almost all life history parameters, such as emergence, growth rate, and voltinism (Bale et al., 2002; Cornelissen, 2011). One of the important insect assemblages suffering from climate change is inhabitants of the forest ecosystems because plants and their associated phytophagous insects comprise a major proportion of terrestrial biodiversity (Strong et al., 1984; Wilson, 1988; Chapman, 2009). So, understanding the potential impacts of climate change on insects, especially forest dependent species, is essential for

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management strategies (Met Office, 2011). In this context, bark beetles are among the most serious forest pests world-wide (Epanchin-Niell et al., 2014). One of these beetles is *Ips mannsfeldi* (Wachtl, 1879) which distributes only in black pine and scots pine forests of Austria, Corsica, Romania, Croatia, Bosnia Herzegovina, Macedonia and Turkey (Pfeffer, 1995; Stauffer et al., 1997; Selmi, 1998). The distribution of *I. mannsfeldi* is congruent with the natural range of its host trees (Pfeffer, 1995).

Species distribution models estimate the distribution of species by examining the relationships between the occurrence records of species and the environmental characteristics of the occurrence sites (Elith, 2011). MaxEnt is a software for species distribution modeling which estimates the distribution of species using presence-only records and maximum entropy algorithm (Phillips, 2017).

The aims of the study were to model the current and future (2070) distributions of *Ips mannsfeldi* in Turkey according to RCP4.5 and RCP8.5 (Representational Concentration Pathways) climate change scenarios reported in The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC5).

Materials and Methods

Occurrence Data

Specimens were collected from trees weakened by other insects or drought, trunks in forest stands and trap woods that were placed by Forestry Administration. Specimens were collected coincidentally during field works (*Figure 1*).

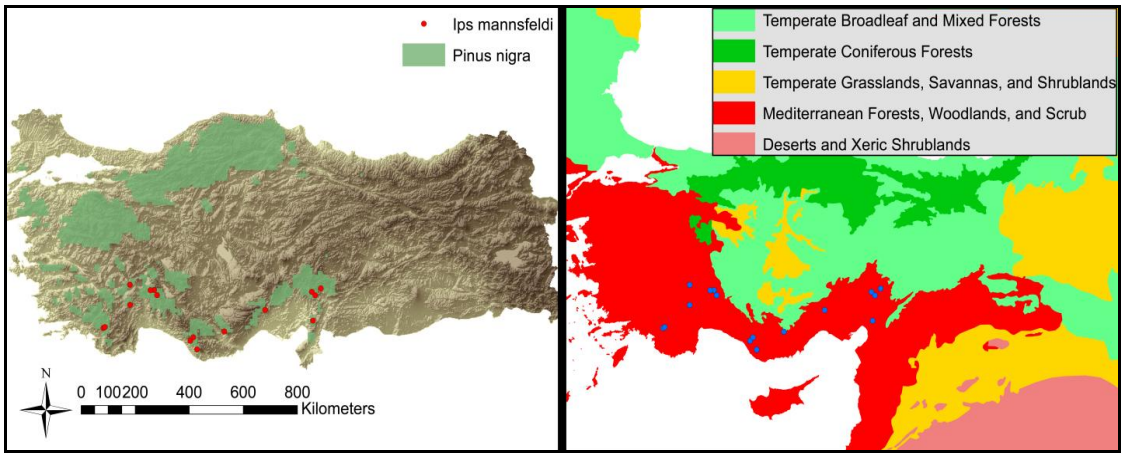


Figure 1. *Distribution of the host tree Pinus nigra in Turkey and the occurrence localities (left). Biome map of Turkey (right)*

The study extent was limited to the part of the Mediterranean biome in Turkey (Olson, 2001) where the host tree *Pinus nigra* is distributed and 10.000 background points also called the "pseudo-absences" (Merow et al., 2013) were selected from this area (*Figure 1*). This approach is ideal as limiting the study extent to areas that are accessible to the species being studied improves the model performance and prevents over-inflation of evaluation metrics (Barve et al., 2011; Soberon and Peterson, 2005; Jimenez-Valverde et al., 2008).

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Environmental Data

SRTM30 digital elevation model (Farr and Kobrick, 2000) and 19 Bioclim variables of the WorldClim database (Hijmans et al., 2005) were used as the environmental variables. Variables showing strong correlation were identified using Spearman's rank correlation coefficient (rs > 0.7). Among the correlated variables the ones that are expected to contribute to the distribution of the species were selected. Five environmental variables were used in the final model (*Table 1*). Bioclim variables for 2070 (CCSM4) and two greenhouse gas emission scenarios RCP4.5 and RCP8.5 were used to project the model. All variable rasters were at 30 arc-seconds resolution.

Table 1. *Environmental variables used in the study*

Variable	Description
Elevation*	-
WC1	Annual Mean Temperature
WC2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
WC3*	Isothermality (WC2/WC7) (* 100)
WC4	Temperature Seasonality (standard deviation *100)
WC5	Max Temperature of Warmest Month
WC6	Min Temperature of Coldest Month
WC7*	Temperature Annual Range (WC5-WC6)
WC8	Mean Temperature of Wettest Quarter
WC9	Mean Temperature of Driest Quarter
WC10	Mean Temperature of Warmest Quarter
WC11	Mean Temperature of Coldest Quarter
WC12	Annual Precipitation
WC13*	Precipitation of Wettest Month
WC14*	Precipitation of Driest Month
WC15	Precipitation Seasonality (Coefficient of Variation)
WC16	Precipitation of Wettest Quarter
WC17	Precipitation of Driest Quarter
WC18	Precipitation of Warmest Quarter
WC19	Precipitation of Coldest Quarter

* indicates the variables used in the final model

Distribution Modeling

MaxEnt version 3.4.1 (Phillips et al., 2006; Phillips et al., 2017) was used through the R package "dismo" (Hijmans et al., 2017) to model the distribution of the species. The model was fine-tuned using the R package "ENMeval" (Muscarella et al., 2014) with trying all combinations of feature types and regularization multiplier values 0.5, 1-10. Since the sample size is low (n=11) k-1 jackknife method described in Pearson et al. (2007) was used to validate the model. Akaike Information Criterion corrected for small samples sizes (AICc) was used as the model selection criterion (Warren et al., 2011). Final binary distribution maps were generated using the threshold value which maximized sensitivity (true positive rate) plus specificity (true negative rate) since this value also maximizes the True Skill Statistic and is not affected by pseudo-absences and prevalence (Liu et al., 2005; Allouche, 2006). Multivariate environmental surface similarity (MESS) plots (Elith et al., 2010) were created using the R package "Ecospat" (Broennimann et al., 2016). Finally, the limiting factor surface map (Elith et al., 2010) was calculated using the R package "rmaxent" (Baumgartner et al., 2017).

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Results

The final model was selected based on the fine tuning using the AICc value and the best model only used Linear and Quadrat features with a regularization multiplier value of 0.5 (*Figure 2*). The ROC curve of the final model shows a high predictive power with an AUC score of 0.88 (*Figure 3*).

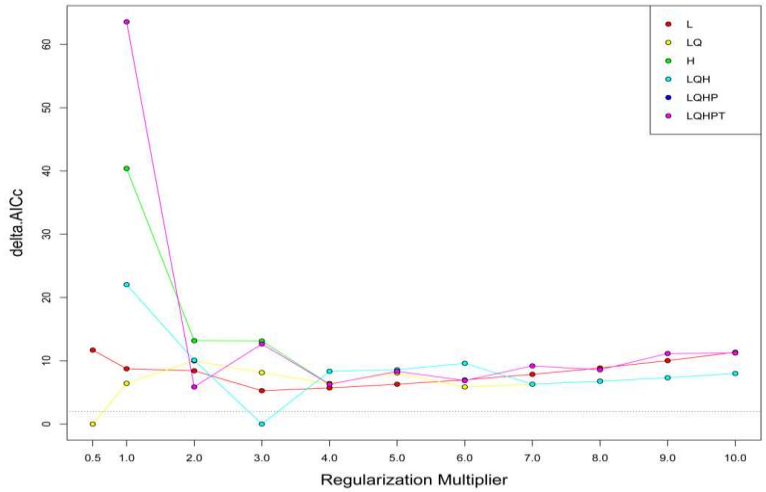


Figure 2. *Results of the fine tuning. X axis corresponds to the beta regularization value of that model. Y axis corresponds to the difference between the smallest AICc value and the AICc value of that model. Legend shows six different combinations of the feature types*

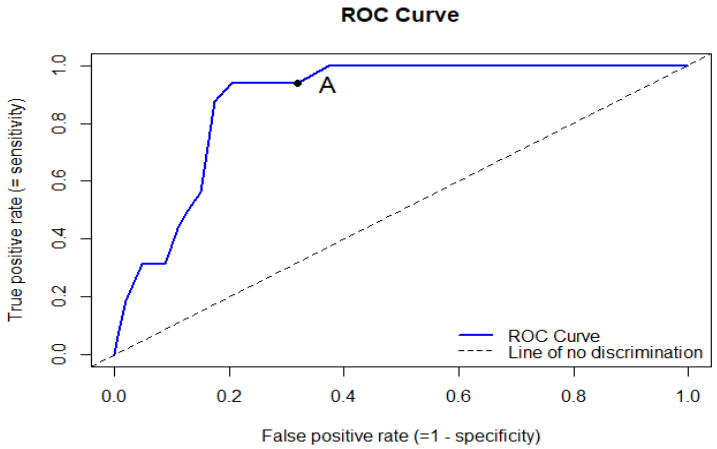


Figure 3. *The Receiver Operating Characteristic curve of the final model. The AUC score is the area under this curve and the AUC score of this model is 0.88. The AUC score of the line of no discrimination is 0.5 which means no predictive power. The point A corresponds to the threshold value of 0.58 used in producing the binary maps*

The most important variable for the model was elevation (*Figure 4*). It appears to have the most useful information as well as more unique information then other variables.

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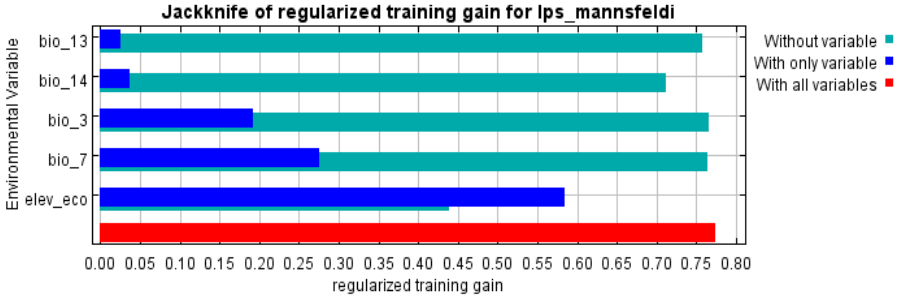


Figure 4. *Results of jackknife test of variable importance (Light blue: without variable, Blue: with only variable, Red: with all variables)*

The threshold value that maximized the sum of true positive rate and true negative rate was 0.58 (*Figure 3*). This value was used to generate the binary maps of current and future distributions (*Figure 5*). Projections predict an expansion of the distribution area towards north-western part of Turkey in both climate change scenarios.

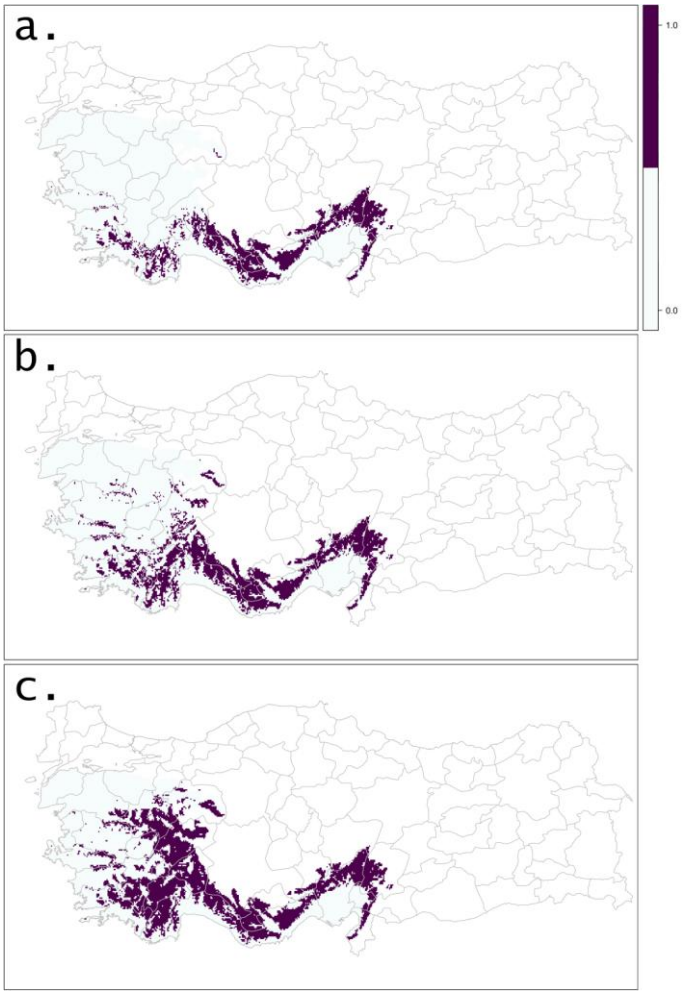


Figure 5. *Distribution maps of Ips mannsfeldi according to the model (a: current distribution, b: Distribution in 2070 according to RCP4.5 climate change scenario, c: Distribution in 2070 according to RCP8.5 scenario)*

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MESS plots show how much the model has to extrapolate in a given region (*Figure 6*). While the areas with negative values are extrapolated into, the areas with positive values require no extrapolation.

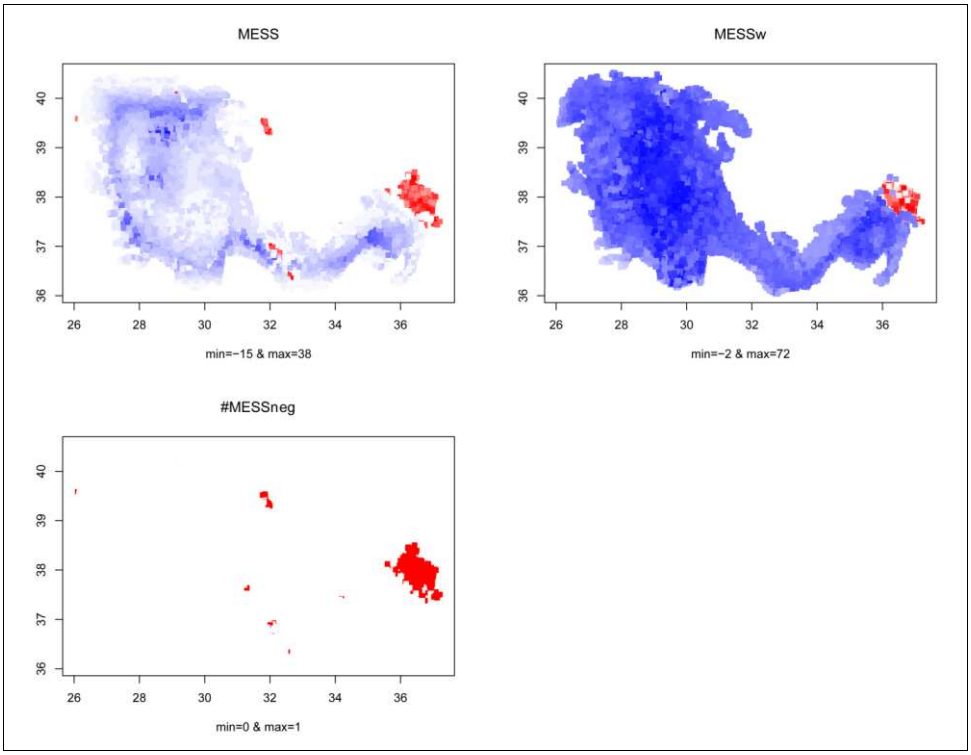


Figure 6. *MESS plots of the model projection to 2070 according to RCP8.5. Pixels in red indicate sites of extrapolation. MESSw plot is the MESS plot weighted by the number of extrapolating predictors. MESSneg plot shows how many predictors are extrapolating in each site*

Predictions into negative MESS areas should be approached carefully. Limiting factors map is shown in *Figure 7*.

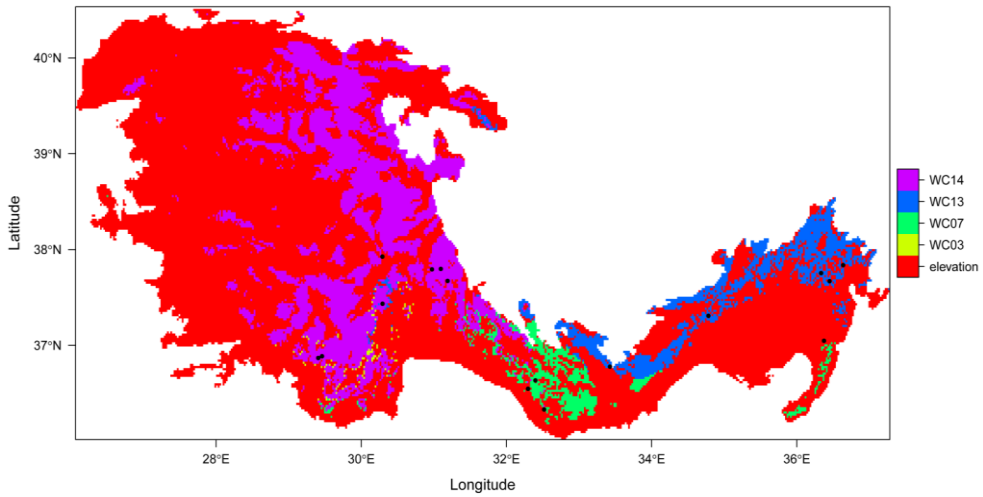


Figure 7. *Limiting factors map. This map indicates the most limiting factor in each grid cell*

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Discussion

Our results show that *Ips mannsfeldi* will expand its range towards north-western part of Turkey (*Figure 5*). The most important factor for the distribution of the species is elevation. However, it's important to note that elevation showed strong correlation with most of the bioclim variables regarding temperature in our case. We chose to keep elevation as a predictor because of its relevance to the distribution of the host tree *Pinus nigra* which occurs between 1000-1800m along the Taurus Mountains in the Mediterranean region and in the inner Anatolian region (Atalay and Efe, 2012). Elevation is also the most limiting factor for the distribution of the species (*Figure 7*). Another curious result is that the second most limiting factor which also prevents the distribution towards north-west part of Turkey is the precipitation of the driest month (WC14). A map of ΔWC14 shows that according to RCP8.5 climate change scenario precipitation of the driest month will drop in those regions (*Figure 8*). Precipitation is known to be important in the distribution of other Scolytinae species. For example, in their work Mendoza et al. (2011) found that *Dendroctonus rhizophagus* had a narrow ecological niche in terms of precipitation. Furthermore, in their ecological niche factor analysis with 19 bioclim variables the species was "essentially linked to the precipitation of the driest month". According to our model the decrease in this variable will make the Aegean region (north-western area of Turkey) available to the species by 2070.

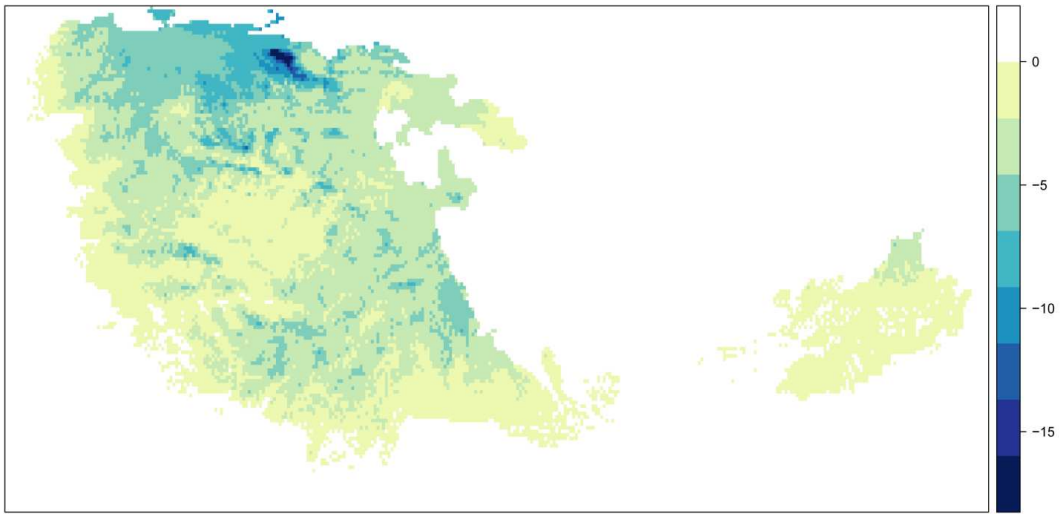


Figure 8. *The map showing the difference between the precipitation of the driest month value (WC14) of current and 2070 RCP8.5 data of each grid cell*

Conclusion

In light of this data, the species has the potential to be an important pest species in the future. The data on the biology and the distribution of *Ips mannsfeldi* is very limited at the moment. Further studies are needed to explore the specific geographical distribution of *Ips mannsfeldi* which depends on forest characteristics across its potential distribution area.

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EFFECTS OF ABIOTIC FACTORS ON INTERNAL HOMEOSTASIS OF *MENTHA SPICATA* LEAVES

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Abstract. *Mentha spicata* plants were subjected to various abiotic stresses in order to evaluate the triggering of signals and the phenolic compound levels of leaves. Total phenolic compound levels exhibited specific responses to a particular abiotic stress factor that responded rapidly to drought exposure, light intensity, and with a slight change in response to salt stress and heat stress. A parallel change was found in an elevation in hydrogen peroxide (H_2O_2) contents. Change in anthocyanin and carotenoid contents in response to drought, different light intensities, salinity and temperature stress was encountered with a change in H_2O_2. A change in the malondialdehyde level was also exhibited, depending on the levels of oxidant and antioxidant availability during particular abiotic stress factors. This risk was accomplished by the regulation of phenylalanine ammonia lyase activity. Prolonged exposure showed a reduction in chlorophyll content. Higher anthocyanin, carotenoid, H_2O_2 and total phenolic compound levels were expressed in the plasticity of plant acclimation at different levels. The tolerance of abiotic stress factors is apparently closely associated with the non-enzymatic antioxidant system via metabolome readjustment. We suggest that biosynthesis of these compounds is tightly regulated, reflecting plant plasticity when acclimating to different types of abiotic stress.

Keywords: *acclimation, climate changes, phenylalanine ammonia lyase, signaling molecules, stresses*

Introduction

Spearmint (*Mentha spicata* L.) belongs to the Lamiaceae family. It is an aromatic plant cultivated as a crop for their secondary metabolites products. Hence, is widely used in the food products, cosmetics and pharmaceuticals industries (Elmastas et al., 2005; Snoussi et al., 2015). In addition, spearmint leaf and oil are used for folk medicine (Nozhat et al., 2014; Snoussi et al., 2015).

Spearmint leaf contains high contents of phenolic compounds and hence could possess strong antioxidant activities among other different antioxidant compounds such as anthocyanin, carotenoids, and ascorbic acids (Kaur and Kapoor, 2002; Qader et al., 2011; Chon et al., 2012; Cao et al., 2015). Moreover, the high phenolic contents consider a promising source of bioactive secondary metabolites in the food industry and in human health (Kaur and Kapoor, 2002; Mimica-Dukic and Bozin, 2008; Soni et al., 2015).

Environmental factors including abiotic and biotic are key to spearmint growth due to the perennial nature of the crop. These variables have a direct impact on biochemical pathways, accordingly affecting the metabolism of secondary metabolites products (Muniram et al., 1992; Edwards et al., 2000; Fletcher et al., 2010). Furthermore, plant tolerance and minimizing the damage caused by stress are based upon stress perception,

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activation of complex signaling pathways in order to respond in a rapid and efficient manner for coping effectively with abiotic and biotic stimuli (Jones and Dangl, 2006; Rejeb et al., 2014). Signaling pathways induced by stress involve the accumulation of metabolites and positively affects a plant's response to stress. These metabolites include reactive oxygen species (ROS), phytohormones, phenolic compounds, ion flux (like $Ca^{2+)}$, activation of mitogen-activated protein kinases (MAPKs), protein phosphorylation and others (Dixon and Paiva, 1995; Lattanzio et al., 2006; Arbona et al., 2013; Baxter et al., 2014; Rejeb et al., 2014).

Review of literature

Abiotic environmental stresses activate a multiple response resulting in changes in primary and secondary metabolites production. Accumulation levels of secondary metabolites including phenolic compounds are controlled in response to environmental cues (Dixon and Paiva, 1995; Payyavula et al., 2012). These compounds are synthesized through derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants and are stress induced (Randhir et al., 2004). Phenylalanine ammonia lyase (PAL) is the key enzyme in the biosynthesis of phenylpropanoid compounds (Solecka, 1997). These include wide ranges of secondary metabolites (Dixon and Paiva, 1995; LaCamera et al., 2004). Phenylpropanoids have important functions in several different pathways in: plant defense against pathogens and predators; protection from UV irradiation; signal transduction and communication with other organisms; and as regulatory molecules (Dixon and Paiva, 1995; Ferrer et al., 2008; Vogt, 2010). Phenylalanine ammonia lyase regulates entry into the phenylpropanoid pathway and is influenced by environmental stimuli, including pathogens, UV radiation, light intensity, temperature and drought, among others (Dixon and Paiva, 1995; Yoshioka et al., 1996; Rivero et al., 2001; MacDonald and D'Cunha, 2007).

Abiotic environmental stresses particularly drought and salinity have the most effect on spearmint growth and production. Moreover, it had shown is sensitive to water stress (Delfine et al., 2005; Telci et al., 2010; Okwany et al., 2012). Furthermore, this sensitivity was experienced through photosynthetic performance changes included stomatal conductance, chlorophyll fluorescence, and chlorophyll content. In addition, all of which affects photosynthetic capacity and eventually productivity in general (Delfine et al., 2005). Hence, the biosynthesis of secondary plant metabolites significantly increases within a plant species under abiotic stressful conditions (Selmar and Kleinwachter, 2013; Stavroula and Rahul, 2017). For that, spearmint has significant antioxidant activity (Elmastas et al., 2005).

M. spicata has been reported to have high phenolic contents among other studied vegetables (Kaur and Kapoor, 2002). The aim of the present work was the examine the biochemical changes of spearmint leaf tissue due to environmental effects. To achieve this aim, the assessment of the alterations in anthocyanins, carotenoids, H_2O_2, MDA, total phenolic compounds, chlorophyll contents and PAL activity of *M. spicata* leaves due to the insults of abiotic environmental factors (drought, salinity, heat shock and light quantity) in time dependent manner to visualize the attempt of these biochemical changes to protect leaves tissues against these insults.

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Materials and methods

Plant materials and growth conditions

Spearmint (*M. spicata* L.) was grown under controlled conditions (14 h light with~ 54 µE, and 21 °C/10 h dark and 20 °C, 55-60% relative humidity) in 2:1:1 peat moss, perlite and vermiculite. Trays filled with cultured pots were transferred to a growth chamber with controlled conditions and after 6 weeks of the process of germination, the plants were exposed to one of the following types of abiotic stress, i.e., light, salinity, temperature, and drought, with different lengths of exposure, relative to the abiotic stress type, or kept in growth chamber conditions without any changes. After the specified time periods of exposure to specific abiotic stress conditions the samples were analyzed in order to investigate the physiological changes in response to the type, duration and intensity of abiotic stress.

Abiotic stress treatments

For drought treatment, plants were irrigated with tap water three times a week for a period of six weeks of growth under controlled conditions. Then the plants were introduced to a water deficit continuing on for three time periods (3 days, 6 days or 9 days), withholding water. At the end of each specific drought exposure plant sample leaves were collected directly in liquid nitrogen in plant growth chamber and stored at -80 °C until further analysis (Egert et al., 2013). Controlled samples were irrigated continually three times a week for a further period (3, 6 or 9 days) and again after the plant reached a period of six weeks' growth under controlled conditions. Salinity treatment was of 6-week-old plants were irrigated with NaCl solution (100 mM) three times per week for up to 14 days. Samples collected in interleaves started at 2 days, 6 days, 10 days and 14 days in a growth chamber (Nishizawa et al., 2008). Controlled samples continued to be irrigated three times a week for a further 2 days, 6 days, 10 days and 14 days with tap water under controlled growth conditions. Heat shock was imposed by transferring 6-week-old plants to grow at 4 °C (cold), 25 °C, 30 °C and 35 °C. Samples collected in interleaves started after 2 h, 4 h and 6 h of incubation at the desired temperature (Egert et al., 2013). Finally, 6-week-old plants were grown at light quantity ≈54 µmol photon/m^2s (control), then light quantity treatment plants were then transferred to grow under 10 µmol photon/m^2s, 100 µmol photon/m^2s, or 1000 µmol photon/m^2s. Samples collected in interleaves started after 2 h, 6 h, 12 h and 24 h of growth under the desired light quantity described by Oelze et al. (2011).

Quantification of chlorophylls, anthocyanins and carotenoids

Chlorophylls quantification was performed according to Porra (2002). In briefly, 20 mg of the tissue samples were ground in 1 ml cold 80% acetone followed by 1 h darkness incubation then centrifugation at 13,000 rpm, 4 °C for 10 min. The supernatant was read on 646.6 and 663.6 nm.

Cold methanol/HCl/water (90:1:1, vol:vol:vol) was used as extraction solution of anthocyanins and carotenoids. Sims and Gamon (2002) was used for quantification of anthocyanins and carotenoids contents.

Measurement of H_2O_2

The leaf hydrogen peroxide content was analyzed as described by Christou et al. (2013) with minor modification. 0.1 g frozen leaf material was thoroughly mixed on ice with 0.1%

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(w/v) trichloroacetic acid. The homogenate was then spinning at 15,000 *g* for 15 min at 4 °C. In a new tube, 500 µl of the supernatant was collected to 500 µl of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI and mixed gently. The absorbance of the assay mixture was read at 390 nm and the content of H_2O_2 was calculated based on a standard curve of known concentrations of H_2O_2.

Lipid peroxidation

The level of lipid peroxidation was analyzed to determine malondialdehyde (Hodges et al., 1999). Briefly, 50 mg of aerial tissue was homogenized in 1 mL of 80% (v/v) ethanol on ice. The homogenate was centrifuged at 16,000 g for 20 min at 4 °C and 0.5 mL of supernatant was mixed together with 0.5 mL of 20% (w/v) trichloroacetic acid containing 0.65% (w/v) thiobarbituric acid. The mixture was incubated at 95 °C for 30 min and then immediately cooled in an ice bath. After centrifugation at 10,000 g for 10 min, the absorbance of the supernatant was measured at 532 nm, subtracting the value for nonspecific absorption at 600 nm. The MDA concentration was calculated from the extinction coefficient 155 mM^{-1} cm^{-1}.

Protein quantification

Protein extracts were determined based on the Bradford method (1976). Briefly, 50 mg of aerial tissue was homogenized in 1 mL of 100 mM HEPS buffer (pH 7.6) on ice. The homogenate was centrifuged at 13,000 rpm for 10 min at 4 °C. 0.2 mL aliquot of supernatant was mixed with 1 ml Bradford reagent, then the absorbance of the supernatant being measured at 595 nm. The content of protein was calculated based on a standard curve of known concentrations of BSA.

Determination of total phenolic content

Total phenolics were determined using the Folin-Ciocalteu (Singleton and Rossi, 1965) reagent and as described by Kaur and Kapoor (2002). 2 g Tissue samples were homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10,000 g for 15 min after which the supernatant was saved. The residue was re-extracted twice with 80% ethanol and the supernatants were pooled, put into evaporating dishes, and evaporated to dryness at room temperature. The residue was dissolved in 5 mL of distilled water. One-hundred micro liters of this extract were diluted to 3 mL with water and 0.5 mL of Folin-Ciocalteu reagent was added.

After 3 min, 2 mL of 20% of sodium carbonate was added and the contents were mixed thoroughly. The absorbance measured at 650 nm with UV/VIS split beam spectrophotometer (SPUV-16, SCO, Germany) for 60 min using catechol as a standard. The results were expressed as mg catechol/100 g of fresh weight material.

Assay of PAL activity

PAL activity in the leaf extract was determined according to Campos et al. (2004). Briefly, 0.2 ml of the protein extract was incubated at 40 °C for 30 min with 2 ml of 50 mM borate buffer (pH 8.5) and 110 µl of 100 mM l-phenylalanine (pre-dissolved in 50 mM borate buffer pH 8.5). Before doing the absorbance readings, the proteins were precipitated by adding TCA at 4% (w/v). The samples with TCA were incubated for 5 min at room temperature and then centrifuged at 10,000 g for 5 min. The absorbance

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of the supernatant was read at 290 nm at the beginning and after the incubation time. PAL activity was calculated as µmol of cinnamic acid per min and mg protein under the specified conditions.

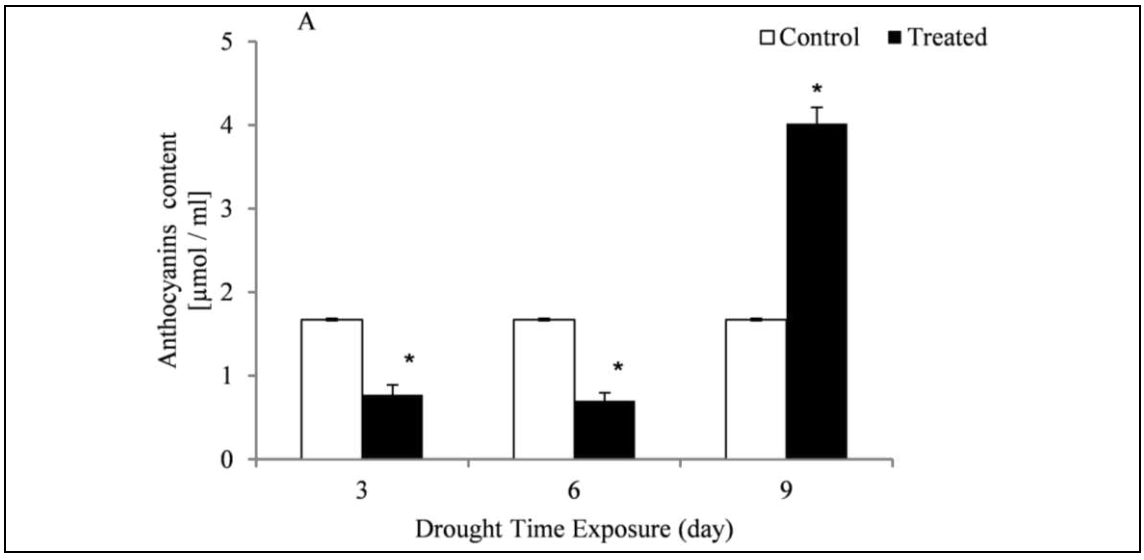
Statistical analysis

For all experiment, samples were analyzed and all the assays were carried out in triplicate. Results are expressed as mean± SD. The comparison between two samples was performed by student's *t*-test and the *P* value of ≤ 0.05 was considered as significant.

Results

Effects on anthocyanin and carotenoid contents

Anthocyanin contents increased significantly 2.3 fold over the control in plants harvested only after nine days of drought treatment. The contents increased significantly in plants exposed to different light regimes (10 µE, 100 µE and 1000 µE) compared with the control (54 µE). However, the rising in content depends on the light quantity and duration of exposure. A significant 4 folds increase in contents was detected in plants exposed to 1000 µE among all time points. 100 µE light exposure showed two points of increments; first after 2 h and the second rising which was mostly constant after the next time points of detections. The behavior of anthocyanins content after 10 µE light exposure showed high elevation after 2 h reaching 7 folds of increment then the rest time points showed around 3 folds of increment. The highest contribution to salinity treatment showed significantly increment reached 1.7 and 1.5 fold after ten and fourteen days of exposure, respectively when compared with the control. Anthocyanin contents were significantly increased after 4 °C treatment at $t = 4$ and after 35 °C at $t = 2$ when compared with the control (25 °C; *Fig. 1*).



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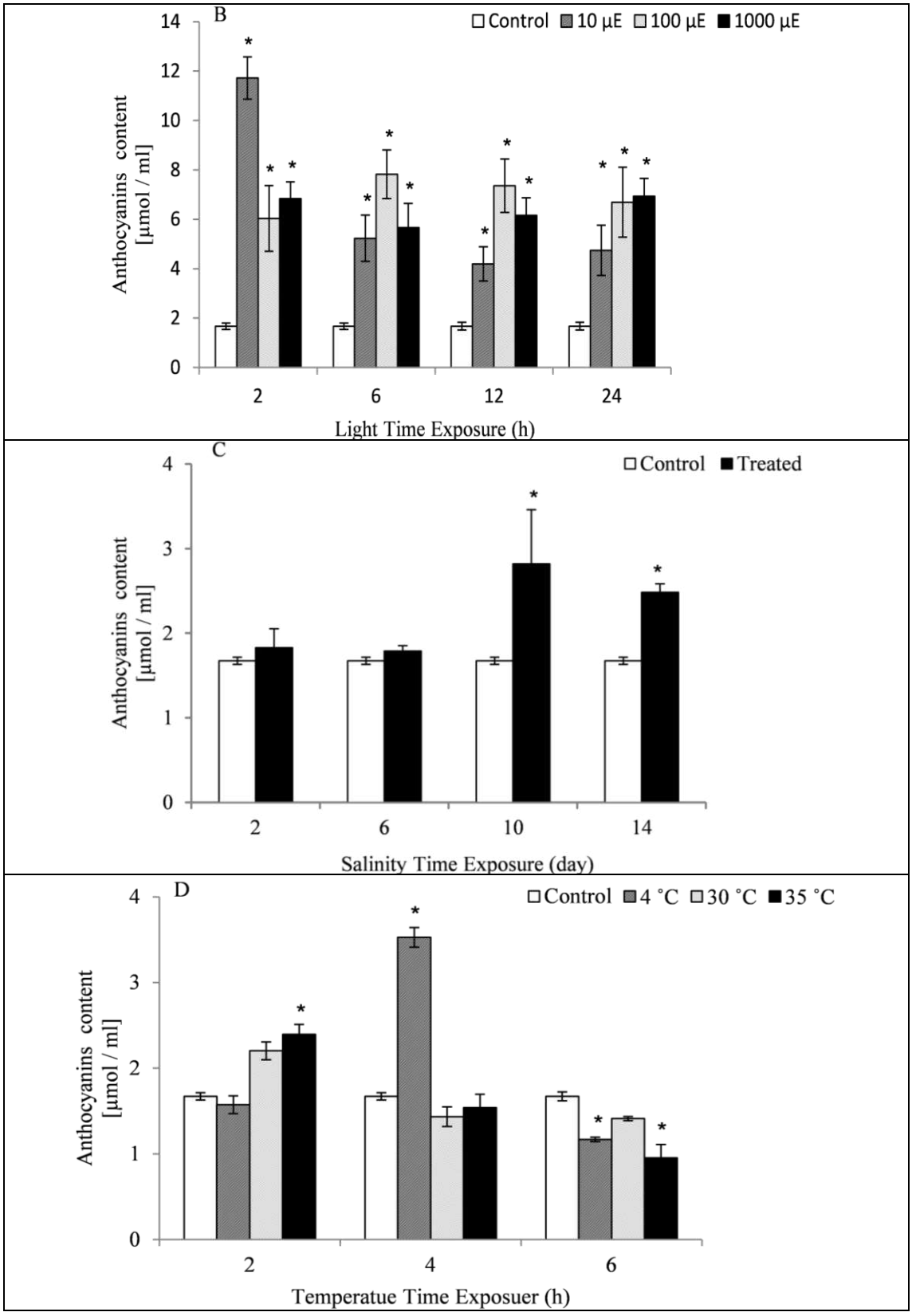
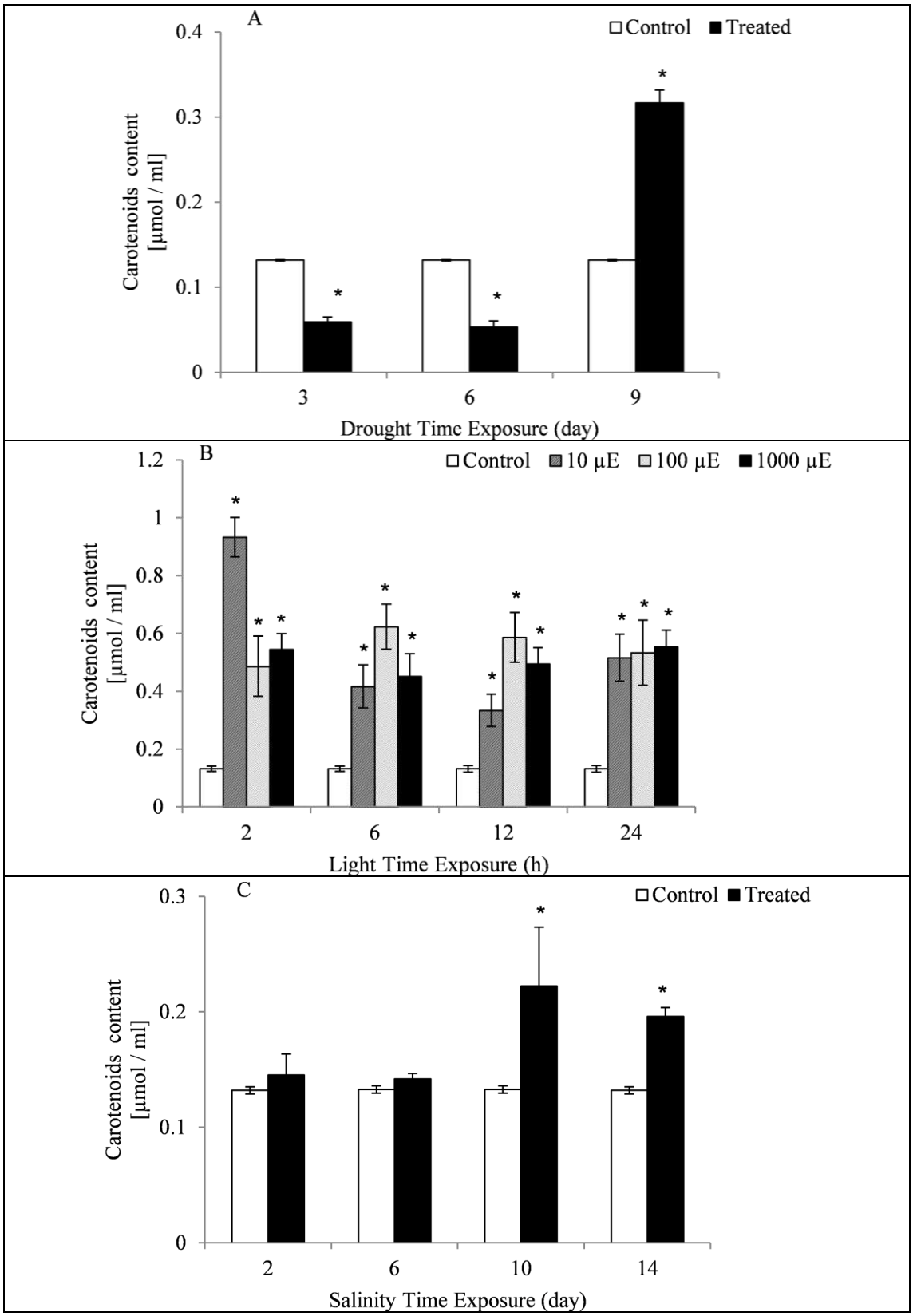


Figure 1. *Anthocyanins contents in leaves of M. spicata subjected to various abiotic stresses. A- Drought. B- Light intensity. C- Salinity. D- Heat. The effect with different time points in comparison with control. Data represent mean values ± SD of n = 3. The significance of difference was calculated using the Student's t test with P ≤ 0.05*

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Carotenoid content elevations exhibited the same pattern of change as that of anthocyanin to various abiotic stresses for a different duration as shown in *Figure 2*.



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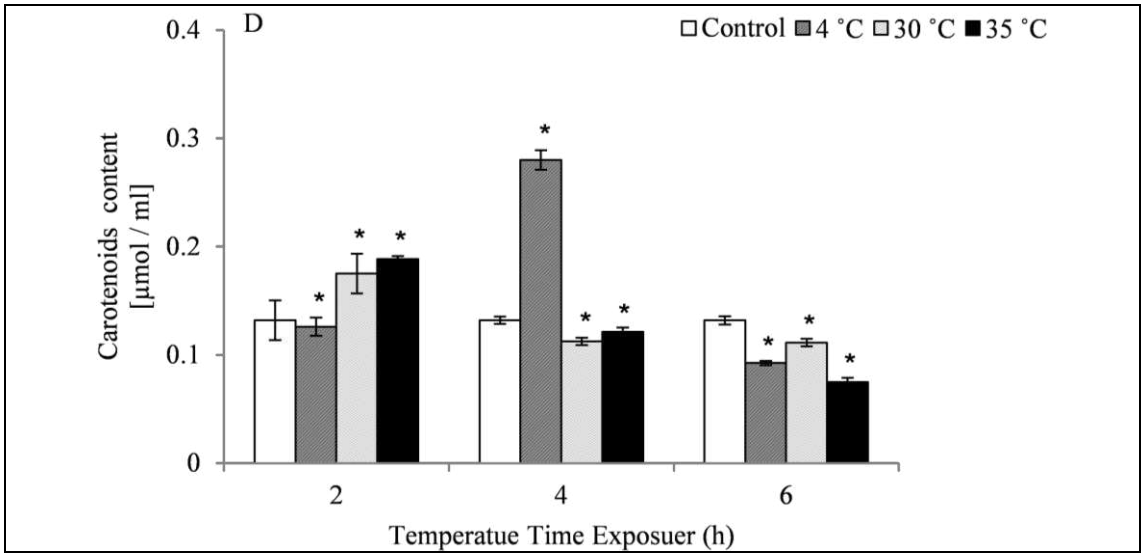
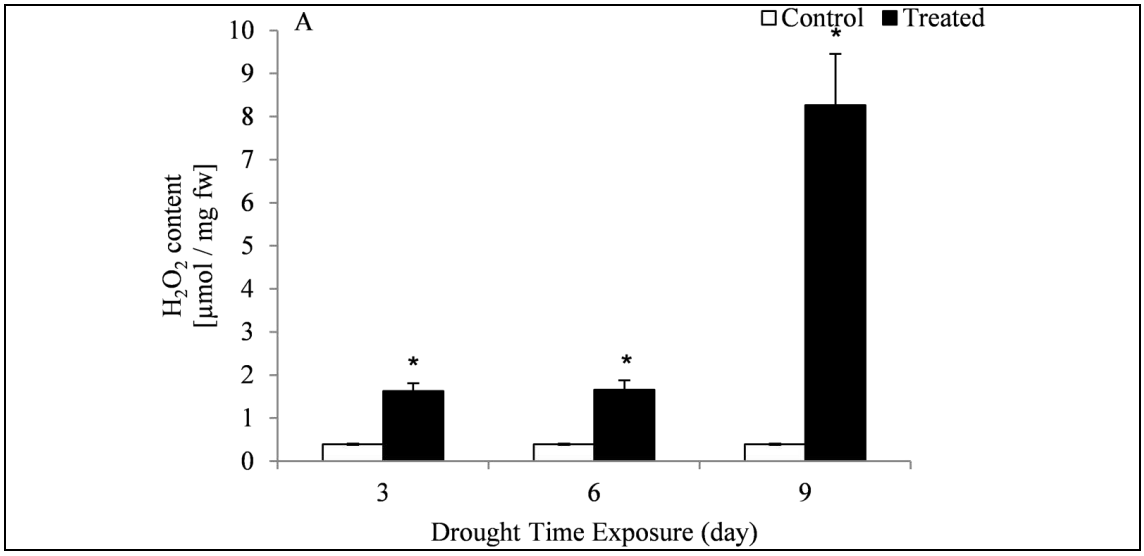


Figure 2. *Carotenoids contents in leaves of M. spicata subjected to various abiotic stresses. A- Drought. B- Light intensity. C- Salinity. D- Heat. The effect with different time points in comparison with control. Data represent mean values ± SD of n = 3. The significance of difference was calculated using the Student's t test with P ≤ 0.05*

Prolonged exposure to drought also salinity and at whatever specific light amount deviation starting with those ordinary light. Furthermore, affectability for temperature variances developed then afterward short or moderate-term exposure (*Fig. 2*).

H_2O_2 levels

H_2O_2 levels were increased significantly and linearly in all treated plant leaves according to duration of exposure compared with plants maintained in control conditions (*Fig. 3*).



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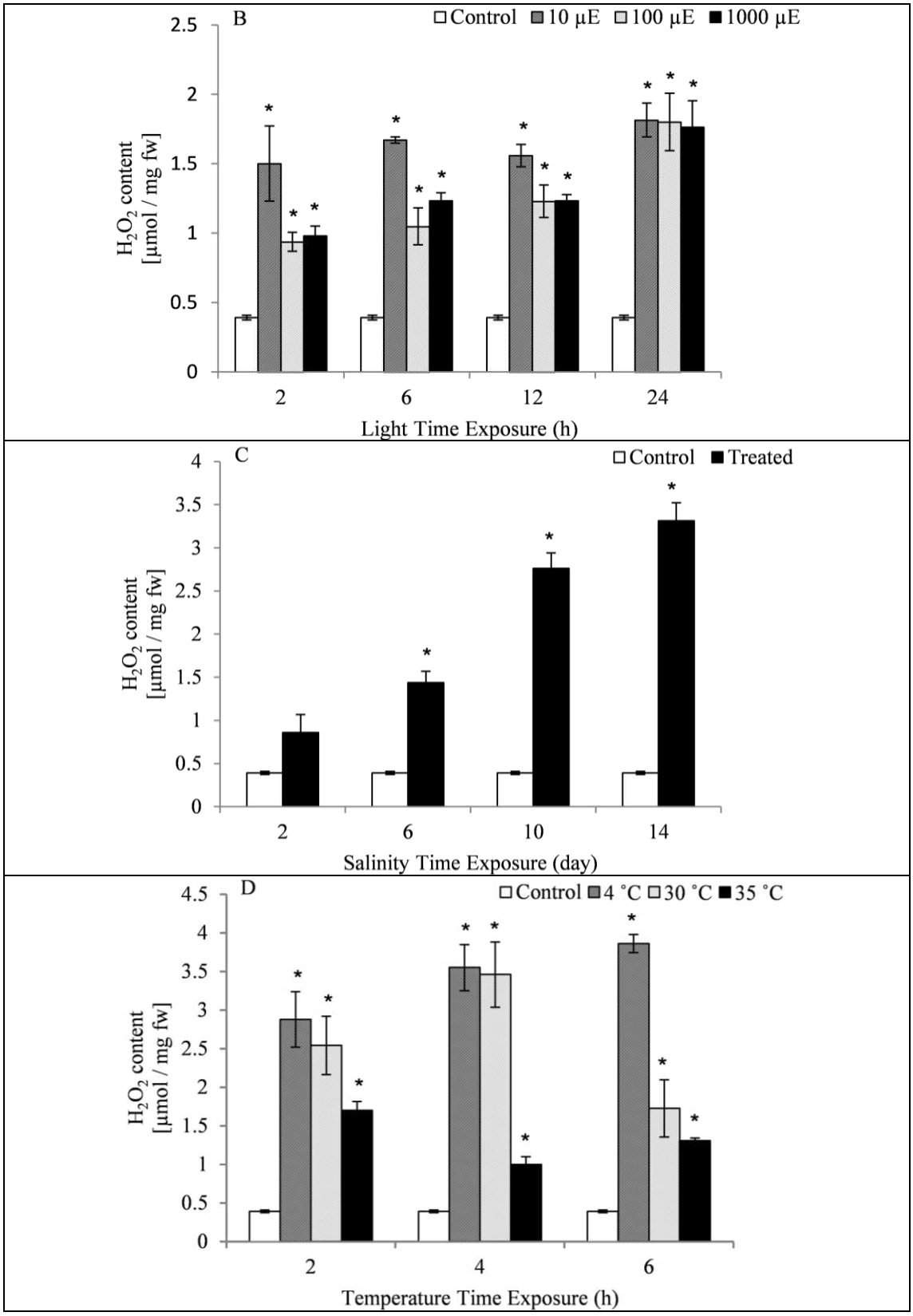


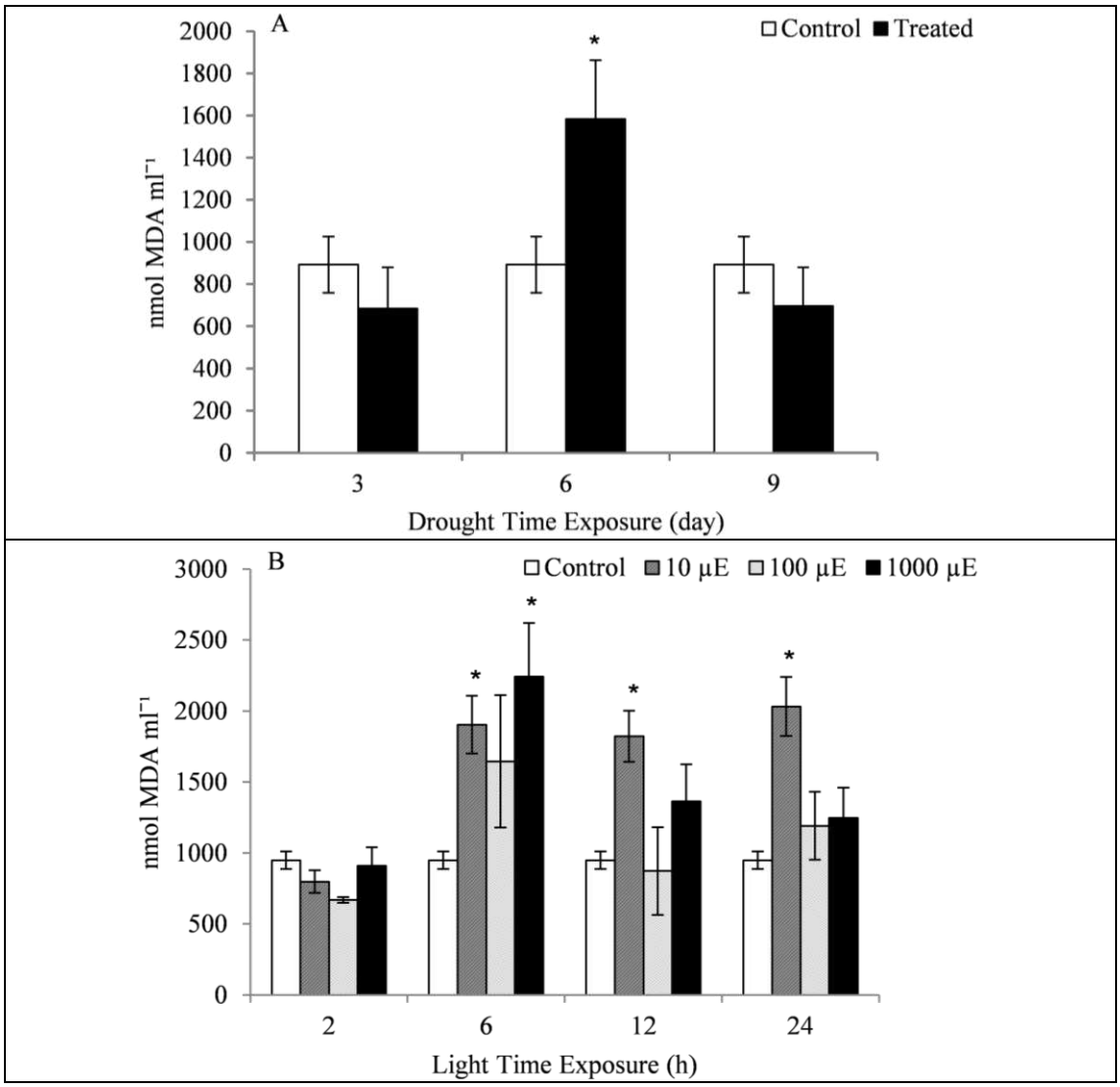
Figure 3. *H_2O_2 contents in leaves of M. spicata subjected to various abiotic stresses. A- Drought. B- Light intensity. C- Salinity. D- Heat. The effect with different time points in comparison with control. Data represent mean values ± SD of n = 6. The significance of difference was calculated using the Student's t test with $P \leq 0.05$*

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Continuous for long duration of drought (9 d) and salinity (14 d) showed the highest H_2O_2 levels reaching 21 and 9 folds when compared with the control, respectively. The basal steady state level of H_2O_2 was 0.4 µmol/g FW instantly raised after short-term (2 h) of different light intensities as well as temperature fluctuations exposure. After 24 h of sustained light intensity exposure, the content was constant despite the light dose (10 µE, 100 µE and 1000 µE compared to growth condition) which reached the maximal level of 4.6 folds compared with the control. The maximal level of H_2O_2 at 4 °C, 30 °C and 35 °C was 10, 9 and 4 folds after 6 h, 4 h and 2 h of exposure, respectively.

Lipid peroxidation changes with abiotic stressors

MDA was analyzed as measuring the end products of lipid peroxidation caused by ROS triggered by various abiotic stresses. The results showed that the lipid peroxidation level significantly increased and reached 1.7 fold after six days of drought treatment, around 2 folds after 6 h of 10 µE exposure and remain constant, 1.7 folds after 6 h of 1000 µE exposure and 2.2 folds after 4 h of 4 °C exposure when compared with the control conditions, as shown in *Figure 4*.



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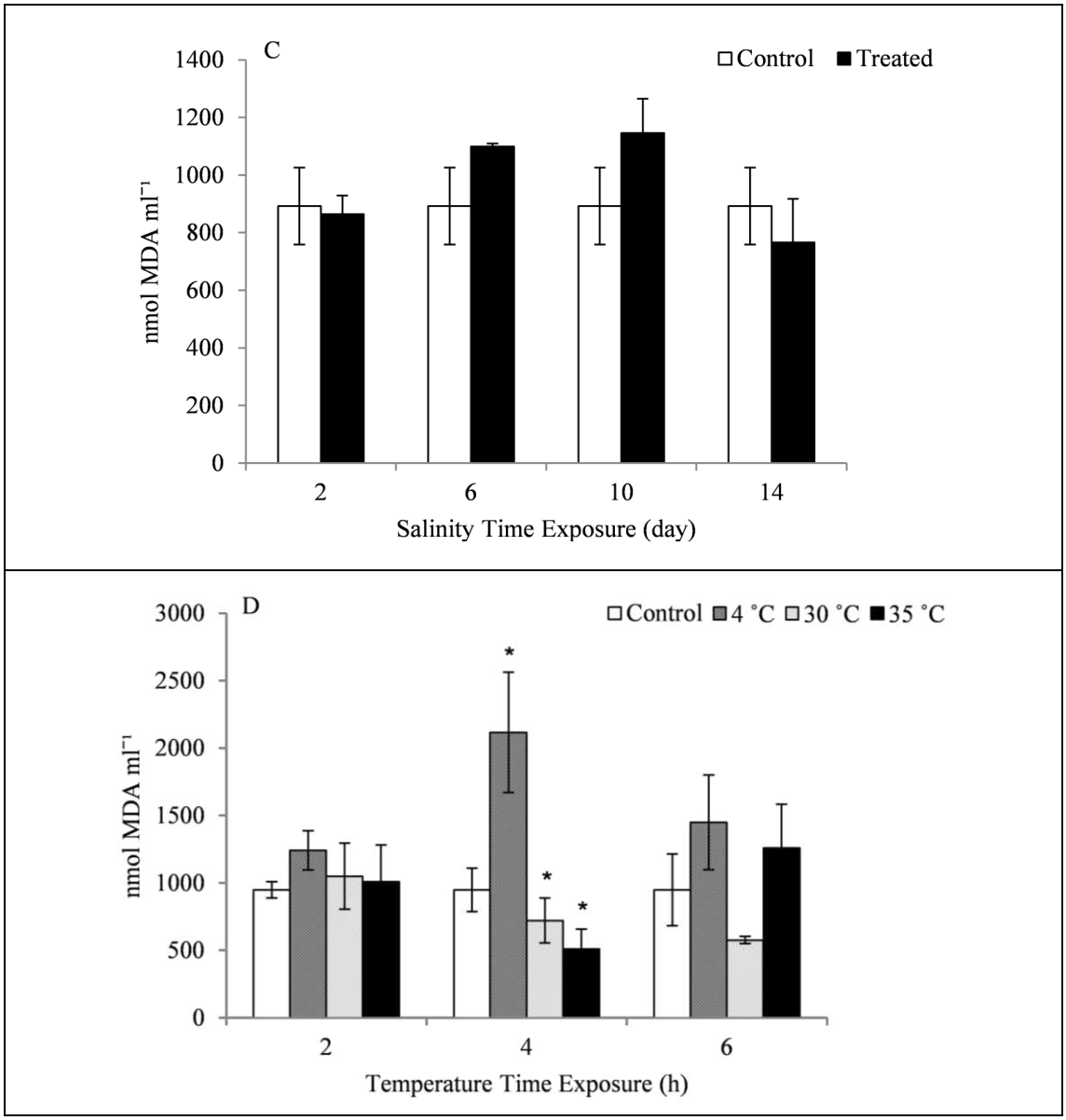
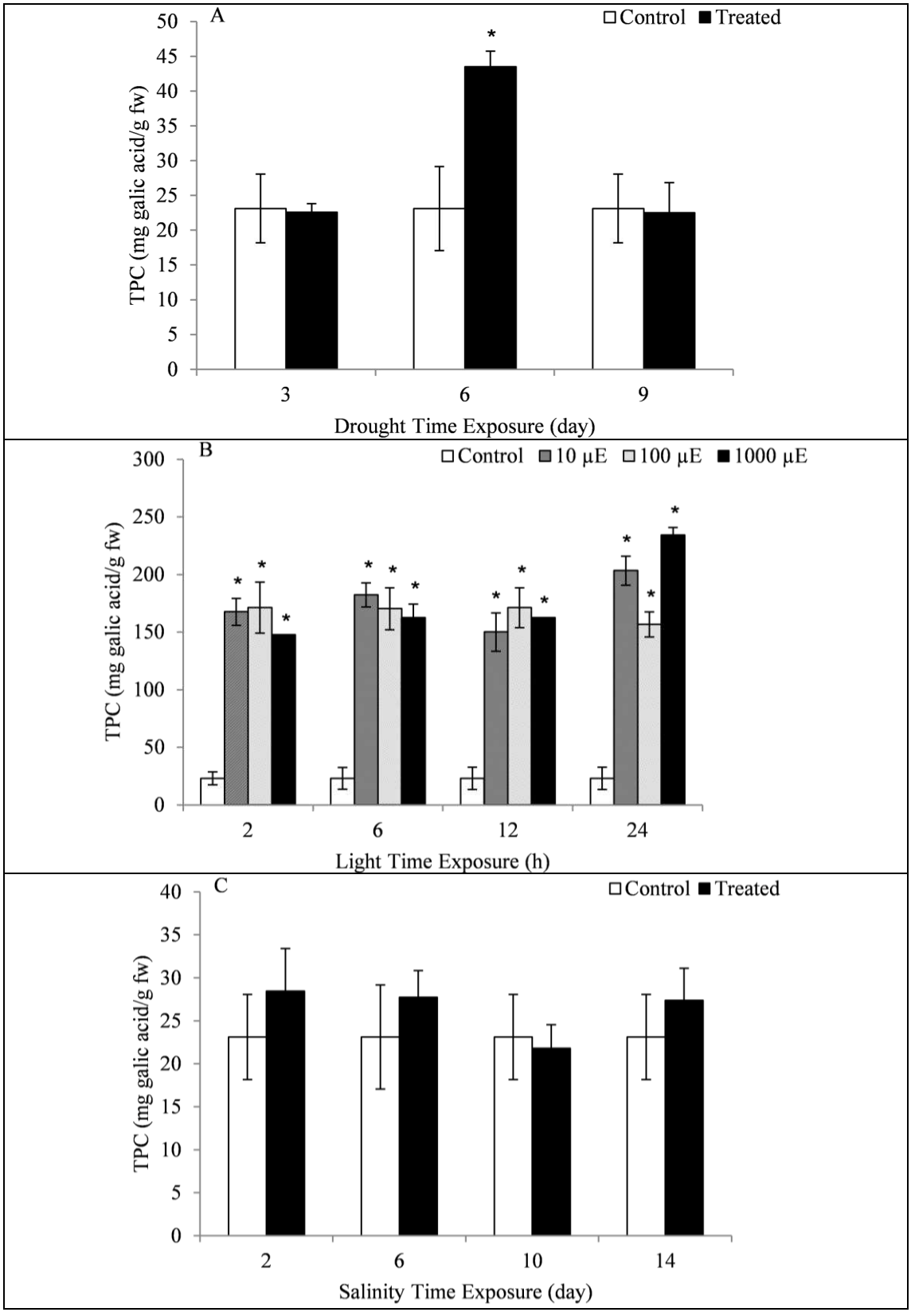


Figure 4. *Lipid peroxidation level (MDA) in leaves of M. spicata subjected to various abiotic stresses. A- Drought. B- Light intensity. C- Salinity. D- Heat. The effect with different time points in comparison with control. Data represent mean values ± SD of n = 3. The significance of difference was calculated using the Student's t test with P ≤ 0.05*

Total phenolic contents

In this study, the amount of phenolic compounds (TPC) as example of nonenzymatic antioxidants (*Fig. 5*). A significant increase in TPC levels was detected in plants of moderate duration drought exposure reached 1.9 fold, strongly related to any change in light intensity regardless to the period of exposure (> 7 folds); the shifting from normal light to low light, moderate light and high light intensities and finally for moderately high temperatures intensity regardless to the period of exposure; 30 °C reached more than 1.7 fold versus the control conditions.

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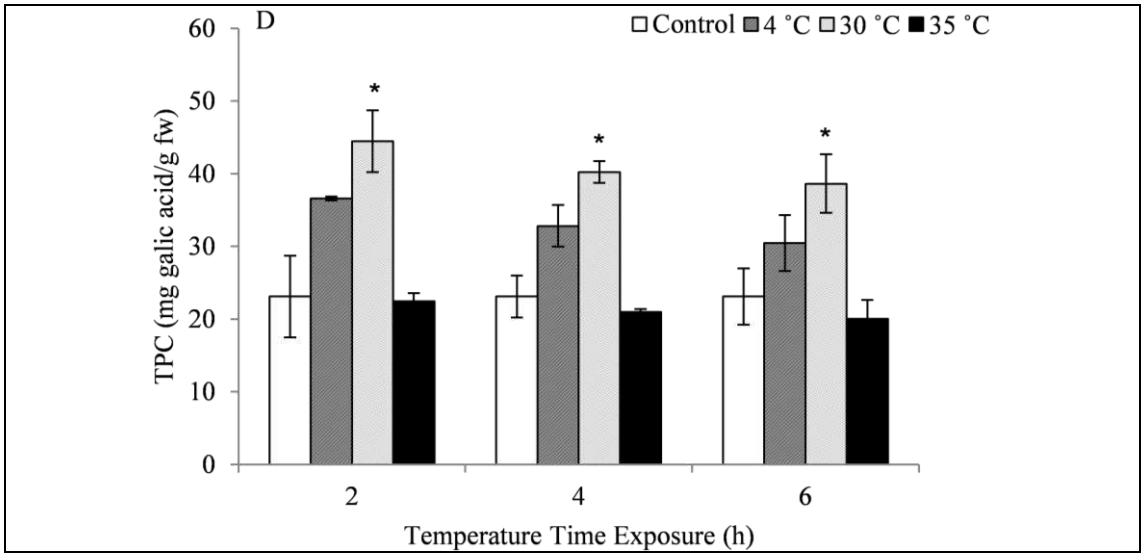
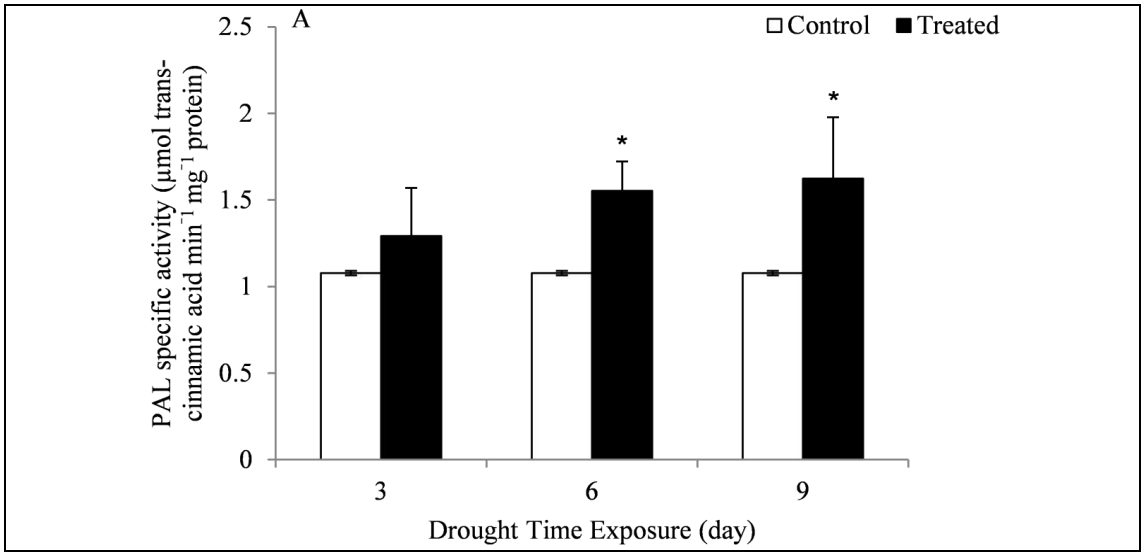


Figure 5. *Total phenol content (TPC) contents in leaves of M. spicata subjected to various abiotic stresses. A- Drought. B- Light intensity. C- Salinity. D- Heat. The effect with different time points in comparison with control. Data represent mean values ± SD of n = 3. The significance of difference was calculated using the Student's t test with P ≤ 0.05*

Effects on PAL-specific activity

Activation of PAL enzyme is also a key component of the antioxidative system of abiotic-challenged spearmint leaves. The results showed that activity of PAL was induced significantly to prolonged exposure to drought, light intensity, salinity and high temperature stresses (*Fig. 6*). PAL-specific activity in leaves of *M. spicata* significantly increased more than 1.4 fold after 6 d of drought treatment, moderate and high light caused more than 1.5 fold activity increments after 24 h of treatment, salinity was caused 1.5 fold increments and 35 °C elevated the activity around 1.4 fold after 6 h of exposure when compared with the control conditions. The overall pattern in the PAL enzyme activity showed similarity to the anthocyanin, H_2O_2 and TPC pattern in plants under prolonged abiotic stress conditional shift.



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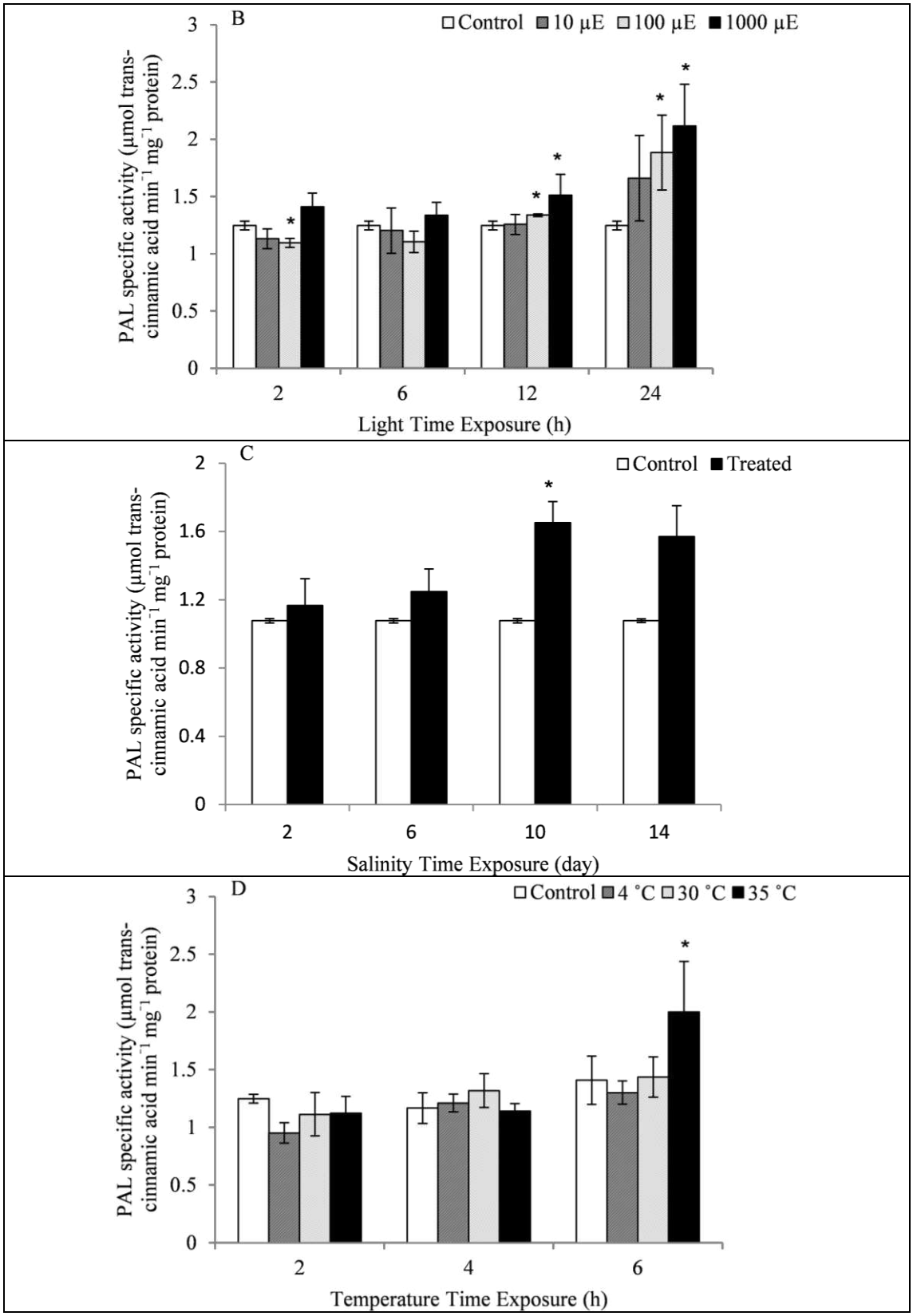
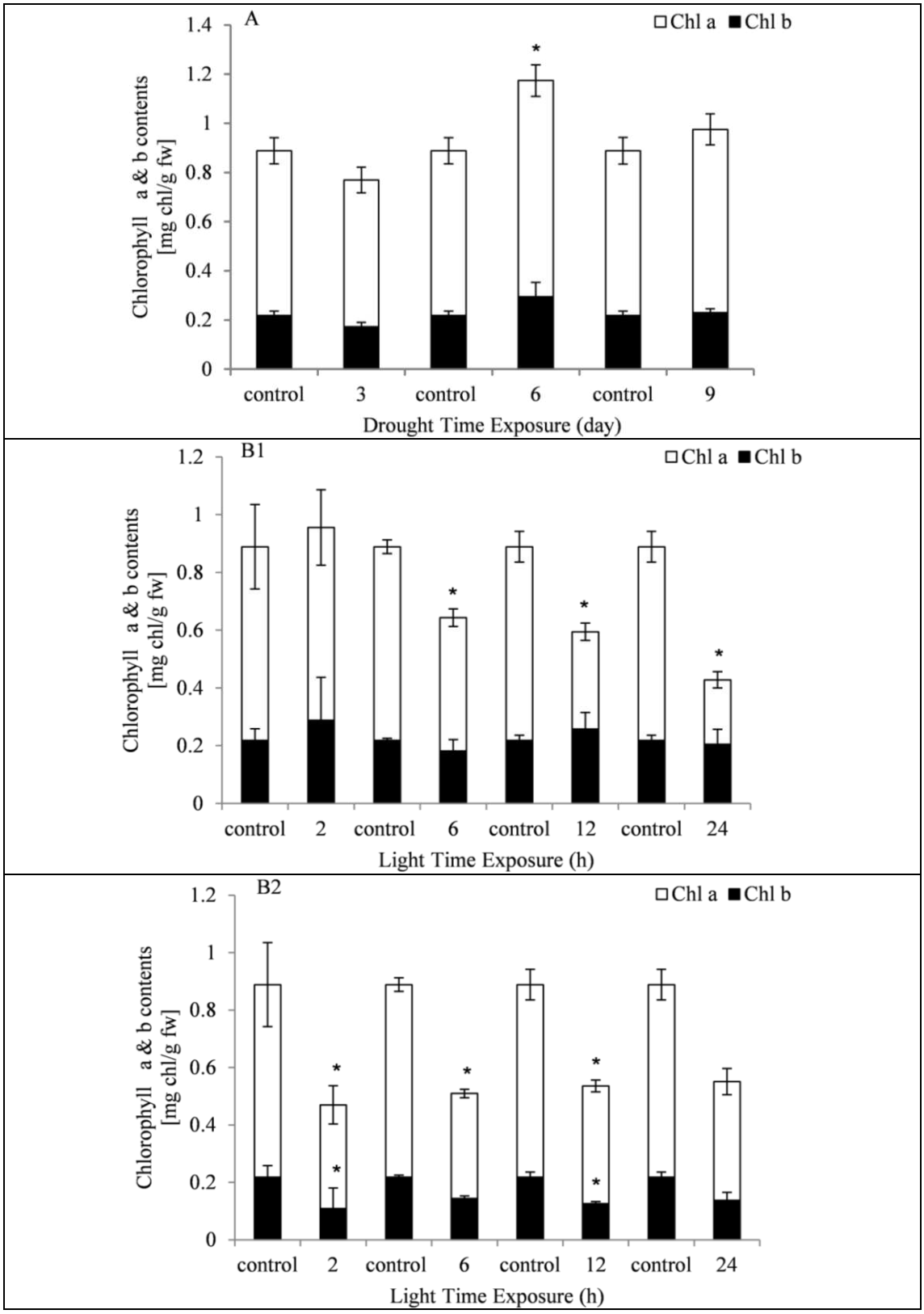


Figure 6. *Phenylalanine Ammonia Lyase (PAL) specific activity of leaves of M. spicata subjected to various abiotic stresses. A- Drought. B- Light intensity. C- Salinity. D- Heat. The effect with different time points in comparison with control. Data represent mean values ± SD of n = 3. The significance of difference was calculated using the Student's t test with P ≤ 0.05*

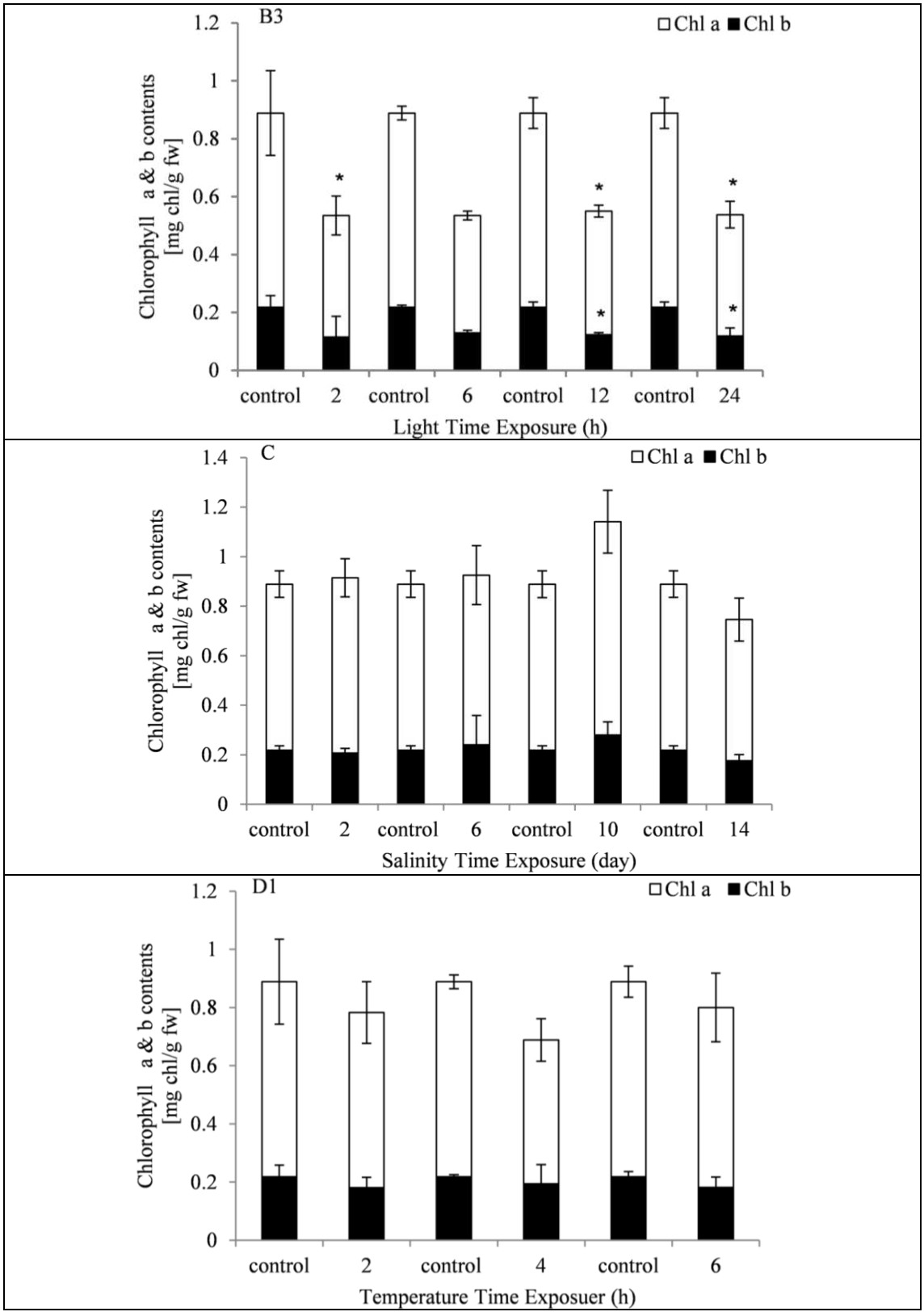
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Effects on chlorophyll contents

Abiotic stresses imposed at chlorophyll *a*, chlorophyll *b* and total chlorophyll contents of *M. spicata* leaves (*Fig. 7*).



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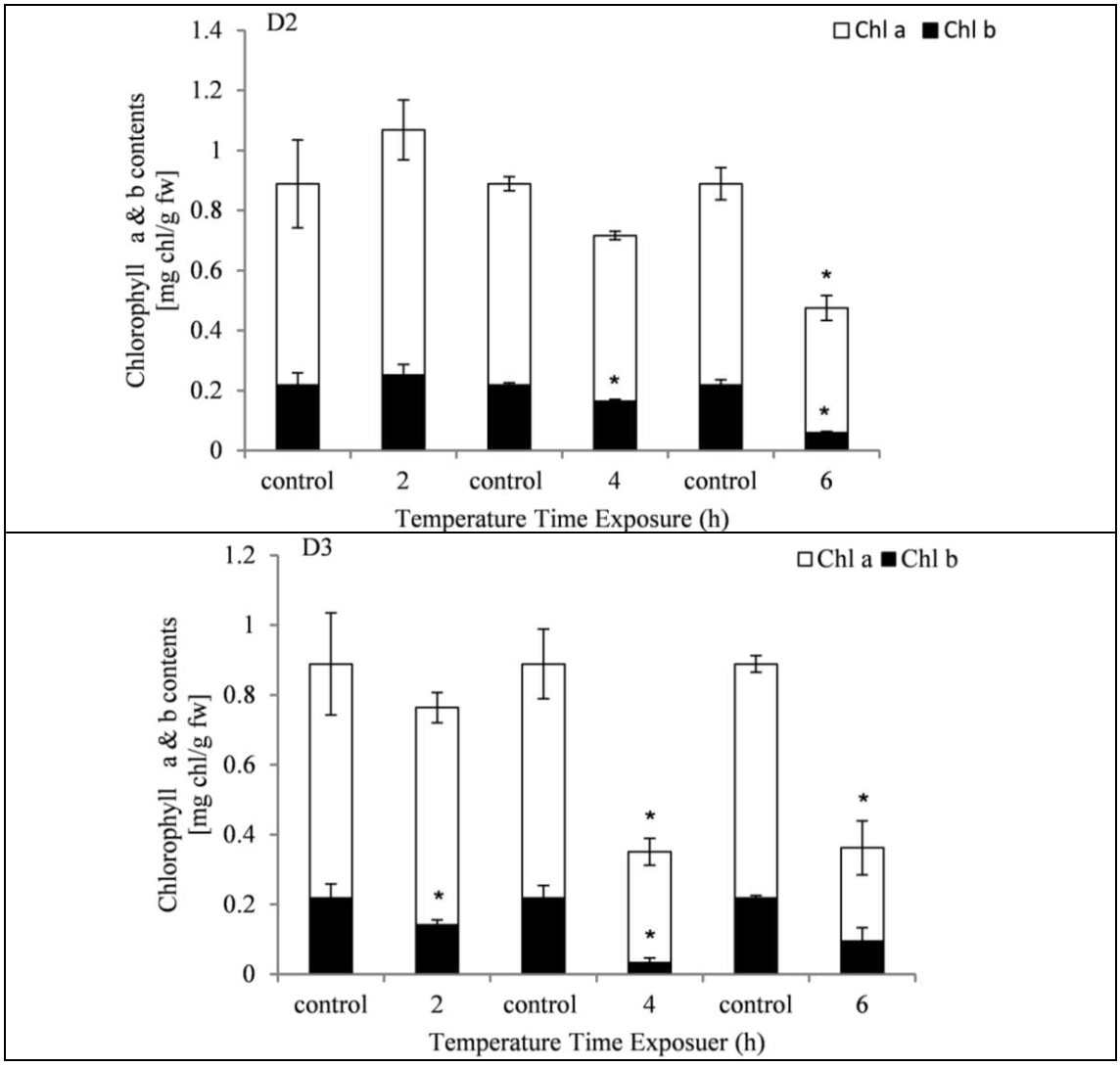


Figure 7. *Chlorophyll a and b contents in leaves M. spicata subjected to various abiotic stresses. A- Drought. B- Light intensity (B1- 10 µE, B2- 100 µE and B3- 1000 µE). C- Salinity. D- Heat (D1- 4 °C, D2- 30 °C and D3- 35 °C). The effect with different time points in comparison with control. Data represent mean values ± SD of n = 3. The significance of difference was calculated using the Student's t test with P ≤ 0.05*

Chlorophyll *a* contents decreased significantly after different light intensities and moderate and high temperatures exposure. While chlorophyll *b* contents decreased significantly after moderate and high light intensities and moderate and high temperatures exposure. These reductions caused strongly reduction in total chlorophyll content.

Discussion

The accumulation of secondary metabolites occurs in plants naturally and under adverse kinds of environmental stress (Akula and Ravishankar, 2011). However, such metabolites play a major role in the adaptation of plants to the environment and in

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acclimatizing to stress conditions. Such accumulation is dependent upon the type, severity and the period of exposure to abiotic stress factors.

Anthocyanin in foliage is stimulated by different abiotic and biotic factors (Chalker-Scott, 1999; Winkel-Shirley, 2001). Accumulation there is influenced by the type of abiotic stress and greatly increased in light (Chalker-Scott, 1999) in order to ensure that the leaf tissue has acclimated to the experimental conditions.

Anthocyanin accumulation, known as signalling, is marked by or linked to the browsing of plant stress (Close and Beadle, 2003; Eryılmaz, 2006) and supposedly has a role in the screening of underlying photosynthetic tissues, increasing light saturation, and light compensation points (Albert et al., 2009) protecting against photoinhibition, and serving as an osmotically active solute and an antioxidant of free-radicals, in addition to functioning as a UV screen (Close and Beadle, 2003; Neill and Gould, 2003; Hughes et al., 2005). However, it must be said that stress tolerance may be a consequence of an improved resistance to oxidative stress (Eryılmaz, 2006). The evidence for these changes in anthocyanin accumulation occurred in line with H_2O_2 accumulation during exposure to different light intensities, to drought for long periods, and to low temperature for short periods of exposure. Consequentially, PAL-specific activity increased with drought and long term light and salinity exposure. In addition, its protection and tolerance roles were apparent when the chlorophyll content was affected by prolonged exposure to abiotic stress factors, such as different light intensities, and different temperatures exposure.

Carotenoids act as light-harvesting pigments and protect photosynthetic systems like nonenzymatic antioxidant compounds against reactive oxygen species generated during stress (Loggini et al., 1999; Chu et al., 2010). Thus, maintaining a higher or constant level of total carotenoids during a stressful condition allows the plant to tolerate this and to cope with oxidative stress (Munné-Bosch and Alegre, 2000). Among the key biological functions of carotenoids are the sensing and signalling of oxidative stress conditions through nonenzymatic oxidation by ROS (Ramel et al., 2012).

Our results are in agreement with previous studies related to plant acclimation to stress (Loggini et al., 1999; Ruban and Horton, 1999; Chu et al., 2010). An elevation of endogenous carotenoid content was strongly apparent with different light intensities, under prolonged drought and salinity exposure. Carotenoids are considered to be the first line of defense for plants against Singlet oxygen ($^1O_2$) toxicity (Knox and Dodge, 1985). Light stress generates the oxidation of the carotenoid β-carotene, leading to the accumulation of different volatile derivatives. They were found to cause changes in the expression of a large set of genes identified as $^1O_2$ responsive genes. Therefore, next to their antioxidant and light-harvesting functions, carotenoids, through their oxidation by ROS, play a role in the sensing and signaling of oxidative stress conditions as well (Ramel et al., 2012). In addition, carotenoid content induction under salt stress is an attempt to protect the cell against harm and to detoxify plants from the effects of ROS (Verma and Mishra, 2005; Kachout et al., 2013). This is a consequence of H_2O_2 accumulation in spearmint leaves under the same treatment. Results of the present study showed that H_2O_2 accumulation in spearmint leaves was slight in the short term but great under long term drought stress exposure, that sensitivity to the quantity of light was dependent upon the time period of exposure and that salinity was greater after long periods of exposure. Damage from temperature stress was greater from a shock of cold and increased markedly with time. Being subjected to a moderately high temperature caused greater damage than being subjected to a high temperature. Under abiotic stress,

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the production of ROS clearly increased as one of the earliest responses of plant cells to stress (Jajic et al., 2015). H_2O_2 accumulation was found to precede the activation of signaling and to be a consequence of signaling, whereas, H_2O_2 acted as a central player in stress signal transduction pathways, for its features were freely diffusible and relatively long-lived (Hossain et al., 2015). However, H_2O_2 accumulation depends on the balance between the rates of its production and its enzymatic and non-enzymatic utilization.

ROS levels can rise significantly in plants subjected to various types of abiotic stress. Drought induction of H_2O_2 accumulation was in line with the manner of the severity of drought stress since several organelles are the seats, as well as the first target, of reactive oxygen species produced under drought stress (Sharma and Dubey, 2005; Farooq et al., 2009). The accumulation of H_2O_2 is considered to be one of the main features of a plant leaf's response to light stress (Mullineaux et al., 2006; Gondim et al., 2013). In addition, salinity stress has an impact on the H_2O_2 content in leaves, which is in agreement with previous reporting (Gondim et al., 2013). All of these abiotic stress factors rising of H_2O_2 accumulation; result from its role as a signalling molecule for initiating intracellular and systemic signalling or promoting oxidative stress, triggering the signalling process associated with cell death (Mullineaux et al., 2006; Upadhyaya et al., 2007; Oelze et al., 2011). In addition, induction might be associated with the ability of H_2O_2 to generate antioxidant enzymatic defenses in order to reduce abiotic stress deleterious effects, since H_2O_2 is a probable signalling molecule that mediates crosstalk between signalling pathways and contributes to the protection against other types of stress (Neill et al., 2002). However, among the ROS, a variety of plant responses has been found to be triggered by H_2O_2, such as the acclimation to stress and developmental stages (Ślesak et al., 2007; Jajic et al., 2015). Those transcriptome responses include photosynthetic light reaction genes, genes in the phenylpropanoid pathway, ROS scavenging, photorespiration, the reductive pentose phosphate pathway, and hormone biosynthesis and response (Cheeseman, 2007). This was in line with PAL-specific activity changing in response to the same abiotic stress factors (*Fig. 6*).

Non-enzymatically formed RES resulting from ROS accumulation measured by MDA levels tends to correlate with oxidative stress (Pintó-Marijuan and Munné-Bosch, 2014). However, MDA levels do not correlate with the intensity of the stress applied, and supposedly are a latent RES signal synthesized in the plant cells involved in fast-response gene activation (Farmer and Mueller, 2013). Despite its relationship to oxidative conditions, MDA accumulated under abiotic stress and has an activity biased towards abiotic stress gene expression (Weber et al., 2004). For that reason, induced lipid peroxidation is involved in diverse signalling processes in order to protect plants from oxidative stress by scavenging ROS (Mène-Saffrané et al., 2009). This thorough production of lipid fragmentation, including carotenoid fragmentation as well as radical amplification, generates a process by which defense genes protect against oxidative stress (Ramel et al., 2012; Zoeller et al., 2012).

Drought increases the generation of the MDA level particularly after moderate time exposure and exposure to light quantities, enhancing the raising of the MDA level after exposure for a moderate period of time, and at all times, after exposure to low light intensity. Salt stress causes a slight increase in the MDA level after a particular period of exposure. Heat stress raises the MDA level in response to a shock of cold. These changes in the MDA levels in response to different abiotic stress factors are in line with H_2O_2 content changes under the same abiotic stress factors. ROS causes oxidative stress

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in plants (Farooq et al., 2009). ROS can directly attack membrane lipids and increase lipid peroxidation (Mittler et al., 2004). Overproduction of ROS increases the content of MDA and is considered to be an indicator of oxidative damage (Moller et al., 2007).

The production of phenolic compounds in plant tissues rises under abiotic stress conditions (Król et al., 2014). Phenolic compounds include several classes that enhance and prod plant metabolic plasticity, enabling plants to survive under different types of environmental stress. Indeed, plant exposure to environmental constraints results in the harmful impact on plant growth and yield. The impact of severe drought stress on the accumulation of phenolic compounds showed a considerable increase in their protective action against the oxidative stress and dehydration of the leaves (Gregorová et al., 2015). Phenolic accumulation is generated by different light intensities and the UV-B activation of the phenolic biosynthetic pathway. Scavenging role of ROS results in hampering UV-B penetration, thus protecting the photosynthetically active tissues (Mazza et al., 2000; Airaki et al., 2012). Low temperatures alter membrane structure and also affect the activity of membrane-bound enzymes, as the deleterious effects of production of ROS can be associated with chilling. Moreover, low temperatures impair the whole antioxidant plant response. Hence, plants activate ROS scavenging systems (Oh et al., 2009; Airaki et al., 2012).

Secondary metabolites provide resistance that is both passive (continuously available) and active (in response to specific stressors). Phenolic compounds have been shown to be more effective antioxidants *in vitro* and *in vivo* than Vitamin A and Vitamin C, even when synergistically amplifying their biological effects (Blokhina et al., 2003; Kasote et al., 2015). Their antioxidant effectiveness is due to their high reactivity as hydrogen or electron donors, and they stabilize and delocalize unpaired electrons, chelate transition with metal ions, and alter peroxidation kinetics (action of flavonoids) and hydrogen peroxide scavenging (Rice-Evans et al., 1997; Blokhina et al., 2003).

The deleterious effect of high H_2O_2 accumulation was encountered with the rise in anthocyanin and carotenoid content for the same period of drought exposure. At the same time, total phenolic compound accumulation at moderate drought exposure was in line with increased lipid peroxidation levels and with PAL-specific activity changing in response to drought stress at all times of exposure.

The highest total phenolic compound accumulation for abiotic stress factors was in response to the exposure to different light intensities after being transferred from normal light growth intensity. This was done to protect against the highest H_2O_2 accumulation, lipid peroxidation levels and what might be the action of anthocyanin and carotenoid accumulation, including at the highest level and the increase in PAL-specific activity in response to different light intensities, particularly when increasing the time exposure. The change in total phenolic compounds did not reflect being subjected to a high level of salinity, which might be due to the moderate salt concentration (100 mM NaCl). However, prolonged exposure raised both the H_2O_2 accumulation and the MDA levels. This internal imbalance was modulated with a strong accumulation in anthocyanin and carotenoid content by greatly increasing the PAL activity by the same factor. Exposing plants to a sudden temperature shift showed the accumulation of phenolic compounds due to low and moderate temperatures. This results in an elevation in H_2O_2 content, an increase in PAL activity, and affects chlorophyll content. Low temperature impairs the whole antioxidant plant response. Hence, plants activate ROS scavenging systems when

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heat stress has been reported in order to reduce Chl content (Oh et al., 2009; Aien et al., 2011; Airaki et al., 2012).

Phenylalanine ammonia lyase activity was shown to be regulated when exposed to stress (MacDonald and D'Cunha, 2007). Thus, PAL has been recognized as a marker of environmental stress. Long-term drought stress increases PAL activity with consistent effects on lignin content since drought stress causes disruption of lignin deposits in leaves (Terzi et al., 2013). Different light intensities revealed that PAL activity was induced in leaves (Ali et al., 2006). PAL activity increased linearly and positively correlated with increased NaCl concentrations (Gao et al., 2008). High temperatures can mediate changes in secondary cell wall metabolism and possibly regulate the lignin biosynthetic pathway through a differential expression of proteins involved in cell wall biosynthesis (Han et al., 2009). In addition, low temperature had an effect on PAL activity, as well as on the rate of accumulation of different phenolic compounds in leaves (Solecka et al., 1999; Solecka and Kacperska, 2003). Moreover, our results showed that PAL activity increased in response to a prolonged exposure to different abiotic stress factors (*Fig. 6*).

Increased PAL activity could be a response to the cellular damage provoked by either the severity of abiotic stress or the duration of the stress. It seems that in spearmint leaves the enhancement of PAL activity could be related to the involvement of this enzyme as the plant response to abiotic stress factors. Phenylpropanoid compounds act as protectants against adverse environmental stress. The overall pattern of PAL activity was consistent with the change in anthocyanin, carotenoid, H_2O_2, MDA and total phenolic compound levels in response to drought stress (Gholizadeh, 2009).

PAL activity was higher in plants exposed to bright light (1000 μE) than when compared to those in normal light. The enzyme involved in lignin biosynthesis that increased lignification, may be caused by the light intensity level due to the enhancement of phenylpropanoid enzymes as an acclimation response (Ali et al., 2006). Increased PAL activity was linearly and positively correlated with the duration of increased salt stress. This elevation could be a response to the cellular damage caused by the duration of salt stress exposure. Moreover, anthocyanin and carotenoid contents were elevated in line with the change in H_2O_2 content and the increase in PAL activity (Gao et al., 2008)

Concentrations of chlorophyll components are normally used to quantify leaf senescence in stressed plants. Leaf maturation and senescence exhibit chlorophyll degradation and can be induced by stress. However, both are processes involving the regulation of plant hormones (Zhang et al., 2016). Drought can lead to a decrease in chlorophyll content, depending on the duration and severity of drought (Terzi and Kadioglu, 2006). Exposure to a higher light intensity induced a loss of chlorophyll (Jagtap et al., 1998). Salinity adversely affects Chl content (Meloni et al., 2003). Chlorophyll pigments decreased under high temperature treatments (Aien et al., 2011). Our results of Chl content showing a decrease in the early period of drought stress, and with an increase in later periods are in agreement with the findings of Terzi and Kadioglu (2006). Different light intensities exhibited that prolonged exposure to low, moderate and bright light can cause greater degradation, and consequently, a reduction in the levels of total chlorophyll as a result of the limitation of the concentration of carotenoids, which can prevent chlorophyll photo-destruction (Gonçalves et al., 2005; Zervoudakis et al., 2012). Salinity stress (100 mM NaCl) showed slight effects on Chl content, though starting in later periods, it might not be a high concentration.

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Nevertheless, the observed decrease in Chl content may be attributed to both an inhibited synthesis of that pigment and a damaged PS antenna system. Low temperature stress showed a slight gradual decrease in Chl content with the increased time duration of exposure. Low temperature can influence plant photosynthesis and lower the utilization of light Chl biosynthesis (Wu et al., 1997; Tewari and Tripathy, 1998). Moderate and high temperatures also reduced Chl content, which might be an effect of Chl biosynthesis (Tewari and Tripathy, 1998). Temperature stress may have caused a reduction or increase in the synthesis of enzymes or the corresponding mRNA responsible for Chl biosynthesis.

Conclusion

In conclusion, among the several markers used to evaluate the oxidative status of plant tissues in response to abiotic stress are the photosynthetic pigments chlorophyll and carotenoid, Reactive oxygen species, Reactive carbonyl species, Antioxidant systems and Transcriptomics and Proteomics (Pintó-Marijuan and Munné-Bosch, 2014).

The present results lead us to conclude that under different levels of abiotic stress anthocyanin, carotenoid and total phenolic compound content are enhanced in an attempt to protect leaf tissues against any damage. Signals such as ROS, and particularly in H_2O_2, might, due to its features, be the major trigger. The PAL activity and the chlorophyll content changes were used to explore the crosstalk and complexity of the responses.

The present study introduces new data about the *M. spicata* leaves responses to different abiotic stress factors that were focused on some biochemical changing. For that, study data recommend further investigation need to cover other partner and major players in plant responses such as flavonoids, glutathione, ascorbate contents and antioxidant enzymes activities, in particular, total ascorbate peroxidase, glutathione reductase, and polyphenol oxidase.

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BIOCHAR: AN EFFICIENT WAY TO MANAGE LOW WATER AVAILABILITY IN PLANTS

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Abstract. Global food security is continuously being haunted by the rapid increase in population and drastic climate changes. Drought stress (DS) negatively affects the soil fertility and plant growth. Drought often induces cell dehydration and ultimately inhibits cell expansion and division, leaf size, stem elongation, root proliferation, disturbed stomatal oscillations, plant water and nutrient uptake. Biochar (BC), being a carbon-rich material, ameliorates the adverse effects of drought on plants. BC is a carbon-rich (65–90%) solid product of biomass pyrolysis that contains numerous pores and oxygen functional groups and aromatic surfaces. BC application modifies microbial habitats by improving the soil's physical properties. Additionally, BC porosity can decrease soil bulk density and improve soil aeration conditions. BC can increase the available water contents that influence nutrient accessibility to microbial cells. BC application increases the plant growth, biomass and yield of crops under water deficit conditions. It also enhances nutrient uptake, photosynthesis and modifies gas exchange characteristics in plants growing under water paucity. Additionally, it improves the physical, biological properties and water holding capacity of the soil. Keeping in view the tremendous importance of BC, this review highlights its potential in mitigating the DS in plants and sums up by revealing future prospects regarding its role in improvement of soils. Overall, this review can contribute towards the better understanding of the BC-mediated tolerance mechanisms in plants under low water availability.
Keywords: *drought, soil, reclamation, remediation, pyrolysis*

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Introduction

The current world population is approximately 7.3 billion and is predicted to reach 8.5 billion by 2030 (Warner and Jones, 2017). As we know that global food security is continuously being haunted by the rapid increase in population, shrinking resources and drastic climatic changes therefore, to feed the large population, improved cropping systems and strategies are dire need of time (Khalil et al., 2016; Lesk et al., 2016; Rauf et al., 2016). In many regions of the world, biotic and abiotic stresses such as drought, high salinity and extreme temperatures results in the most injurious limitations for the plant's growth and productivity (Khan et al., 2017; Noman et al., 2017; Thalmann and Santelia, 2017; Islam et al., 2018a,b). Among the abiotic stresses, DS is reckoned as the most injurious environmental factor causing significant losses of growth as well as yield of the plants (Ali et al., 2016; Calvo-Polanco et al., 2016; Dubois et al., 2017). The drought has severely affected terrestrial agriculture in the past and recent times. DS along with other abiotic stresses like heavy metal contamination not only limits plant production but also pose more risks to human health (Arshad et al., 2016; Noman and Aqeel, 2017). For example, Liu et al. (2017d) revealed that DS differentially influenced the translocation and redistribution of Cadmium in peanut plants during various developmental stages. It often induces cell dehydration and ultimately result in inhibition of cell expansion and division, leaf size, stem elongation, root proliferation, disturbed stomatal oscillations, plant water and nutrient uptake as well as enhance proline accumulation (Kaushal and Wani, 2016; Khalil et al., 2016; Noman et al., 2015). Moreover, it also curtails photosynthesis (Khalil et al., 2016; Khan et al., 2017). Additionally, it causes oxidative stress through the production of reactive oxygen species (ROS) (Apel and Hirt, 2004; Cruz de Carvalho, 2008). It lowers soil water potential and water use efficiency (WUE) (Kaushal and Wani, 2016). Consequently, combating DS is a challenging task for achieving food security under the drastic climatic changes worldwide.

Historically, several techniques were utilized to improve the soil structural and biochemical properties. For example, thousands year ago, Romans and Greeks incorporated the animal manure and human feces in the soil as an organic amendment to increase the soil quality and fertility. At that time they knew that previously legumes grown field can be beneficial for wheat crop cultivation. Earlier different materials (e.g., plant residues, farm manure sea-shells, and other waste) were adopted as an organic amendment to improve the crop growth. Currently, BC, compost and animal manure as a soil amendment is well-known while crop straw, sewage sludge, peat, wood shavings, and sawdust are also used for soil amendment to improve quality and fertility. BC amendments have large-scale agricultural, economic and environmental benefits (Kumar et al., 2017). BC is a highly porous, fine-grained charcoal that can hold carbon combusted under low oxygen at relatively high temperatures (Fazal and Bano, 2016). Like bioethnol, it can be produced from different organic materials such as plant residues (Llorach-Massana et al., 2017; Tayyab et al., 2018), pecan shells, sawdust (Komnitsas et al., 2016), peanut hulls (Chu et al., 2017), Hardwood (Laird et al., 2017), paper mill sludge (Cho et al., 2017), Palm kernel shell and Palm oil sludge (Lee et al., 2017), pine and jarrah woods (Jiang et al., 2017), rice straw (Liu et al., 2017a), cotton stem, wheat straw, rice husk, corn stem, peach branch, walnut shell, yak manure, eucalyptus wood sawdust (Wang and Liu, 2017), hemlock char and switchblade grass char (Yu et al., 2017) poultry manure (Barati et al., 2018) and pig manure (Liu et al., 2017a). Different types of biomass and the thermochemical conditions are used to

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pyrolyze it significantly to influence the quality of BC and its potential uses (Chen et al., 2011; Yu et al., 2017a). The production of BC is quite similar to charcoal, the production of which is one of the oldest developed technologies (Lehmann and Joseph, 2009). However, it is entirely different from charcoal as it is not primarily used as a fuel, but for atmospheric carbon (C) capture and storage, or bio-sequestration. The porous structure of char particles enhances the water-retaining capacity and nutrient retention of soil, as well microbial accumulation. Due to its perceived benefits of increasing nutrient and water retention, it is used as a soil conditioner that is very promising and useful for soil amendments (Skjemstad et al., 2002; Yu et al., 2017a Sarfraz et al., 2017). Additionally, BC modifies microbial habitats by improving the soil's physical properties. Its porosity can decrease soil bulk density and improve soil aeration conditions. It can also increase the available water contents that influence nutrient accessibility to microbial cells. In addition, it can increase water contents at the permanent wilting point, which indicates the capability of BC, via its high porosity, to store water that is unavailable to plants. Keeping water in this manner is beneficial, especially in sandy and degraded soils (Abel et al., 2013; Skjemstad et al., 2002). Moreover, the improved water retention capacity means that there is a more excellent capability of the soil to hold water against dry-wet cycles in the natural environment, which can favor the maintenance of a stable microbial activity (Liang et al., 2014). A significant body of knowledge reported that BC amendment enhances plant growth, seed production, biomass and nutrient absorption under the drought conditions (Bamminger et al., 2016; Gonzaga et al., 2017; Paneque et al., 2016). However, according to our literature survey, a comprehensive review of the beneficial and effective role of BC upon plants under water deficit conditions is not documented yet. Hence, we reviewed the BC application in agricultural production under DS to give attention to the development of soil health and fertility to feed the increasing population.

Adverse effect of drought stress on soil properties

Drought is one of the most severe abiotic stresses. It strongly affects soil microbial activity, fungal properties, soil respiration and litter decomposition (Geng et al., 2015; Hoogmoed et al., 2016; Mariotte et al., 2015; Schmidt et al., 2017). The significance of the microbial community in soil ecosystem functioning is well known which plays a pivotal role in soil fertility and improvement in soil structure (Wall and Bardgett, 2012; Arafat et al., 2017). For example, microbial communities produce enzymes and are responsible for 90% of soil organic matter (SOM) decomposition (C mineralization), which determines nutrient turnover (Bastida et al., 2013; Swift et al., 1979). Nevertheless, drought can affect soil microbial communities by restricting diffusion of substrate, inhibitory compounds (e.g., plant and antimicrobial secondary metabolites), enzymes and inducing microbial physiological stress (Davidson and Janssens, 2006). As compared to fungal communities, bacterial communities are sensitive to disturbances caused by drought and their response directly affects soil fertility and soil structure (Bardgett and Wardle, 2010).

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Adverse effect of drought stress on plant yield and biomass

Drought negatively influences the plant growth and crop production. It affects the plant development causing severe reductions in crop productivity. Additionally, it can initiate a series of physiological and biochemical changes in plants. It also decreases dry matter production, leaf area index, number of seeds per plant, seed size and finally the grain yield. Moreover, reduction in intercellular carbon dioxide concentration, stomatal conductance and transpiration is also associated with it. Chlorophyll fluorescence is negatively correlated with DS as it has severe effects on leaf gaseous exchange rate and chlorophyll fluorescence depending on various developmental stages of the plant and the duration of DS. Under drought, some processes such as plant hydraulic status, phytohormones, osmotic adjustment, and ROS signaling control the reduction of plant growth.

Production and physicochemical characteristics of biochar amendments

BC is a recalcitrant source of soil organic carbon combusted under low oxygen at relatively high temperatures (Fazal and Bano, 2016). Theoretically, almost any form of organic material can be utilized for its production (Kuppusamy et al., 2016). However, its properties can vary widely depending on feedstock and processing conditions (Brewer et al., 2017). For attaining several benefits of sustainable agriculture, the amendment of BC is being increasingly discussed as a drought mitigation tool. In the last decade, tremendous increase in the number of publications using a variety of BCs addressed as drought mitigation approach shows the high interest of researcher regarding the enhancing scope of BC. Tripathi et al. (2016a) presented the concept of sustainable BC production. Standard techniques used for BC production are rotating kilns, vertical silo-type reactors, hydrothermal carbonization, gasification, and slow and fast pyrolysis. BCs are categorized with a high concentration of total organic carbon (30-70%) depending upon the pyrolysis conditions (aeration, temperature and time), mineral contents (Na, Mg, K, Fe, etc.) EC, pH, volatile matter and ash concentration (Jindo et al., 2014; Qayyum et al., 2015; Yu et al., 2017). However, the BC properties are significantly affected by the feedstock nature (Brewer et al., 2017). It is produced from the thermal conservation of biomass feedstock's (*Fig. 1*). Among various techniques (e.g., pyrolysis, gasification, and hydrothermal carbonization), pyrolysis which heats the biomass at relatively low temperatures (300-900°C) in the absence of oxygen, is the most cost-effective and efficient method for BC production (Cha et al., 2016; Qian et al., 2015). Additionally, pyrolysis can be subdivided into slow and fast pyrolysis, according to the different heating rates. Even though, there exist some disadvantages such as energy inefficiency and demand for long production duration, slow pyrolysis is still widely used for BC production presently due to the comparatively higher BC yield (Liu et al., 2015; Tripathi et al., 2016a). The characterization of sophisticated methods contains the quantification and identification of surface functional groups, polycyclinc aromatic hydrocarbon, aromatic compounds, active surface area and scanning electron microscopy (Hale et al., 2012; Harris et al., 2013). BC has demonstrated its multifunctional roles in carbon sequestration (Crombie et al., 2013). The linkage of BC properties and its application are shown in *Figure 2*. Consequently, before their implementation, BC must be carefully analyzed (Aller et al., 2017; Kuppusamy et al., 2016). BC amendments may have large-scale environmental, economic as well as agricultural benefits. Deep investigation of the BC-mediated plant

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resistance mechanisms against abiotic and biotic stresses will enhance our further understanding of the better agronomic management of economically important crops (Kumar et al., 2017).

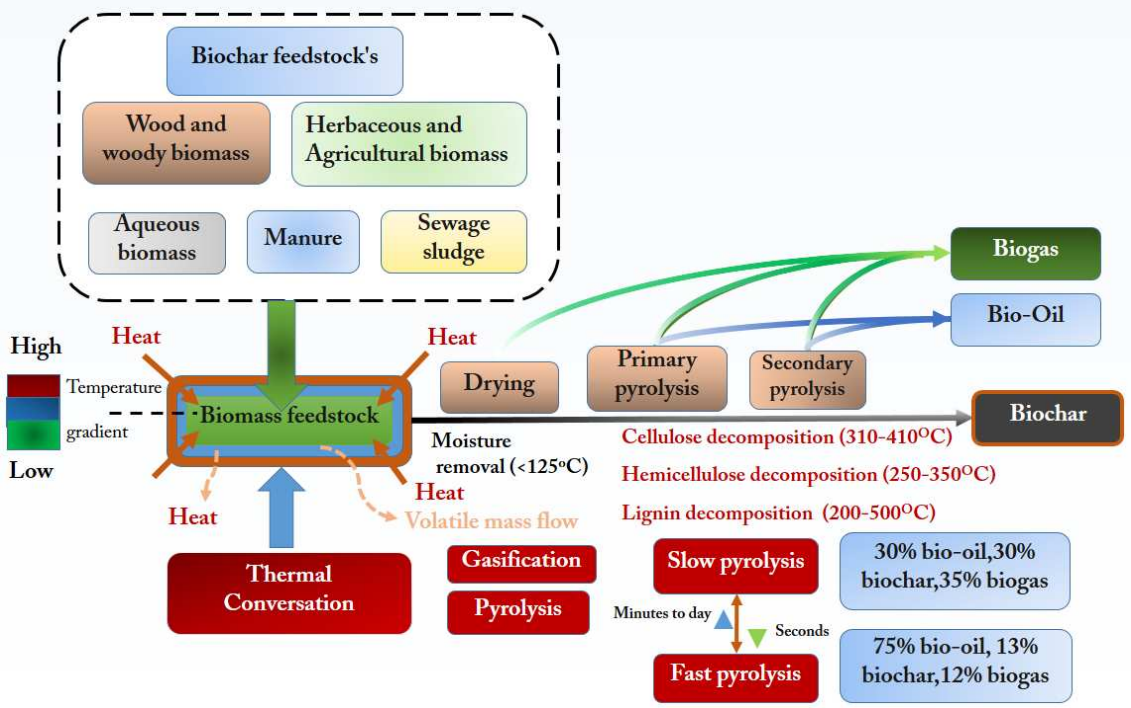


Figure 1. *The brief introduction of biochar feedstock and its production processes (slightly modified from Xiao et al., 2016)*

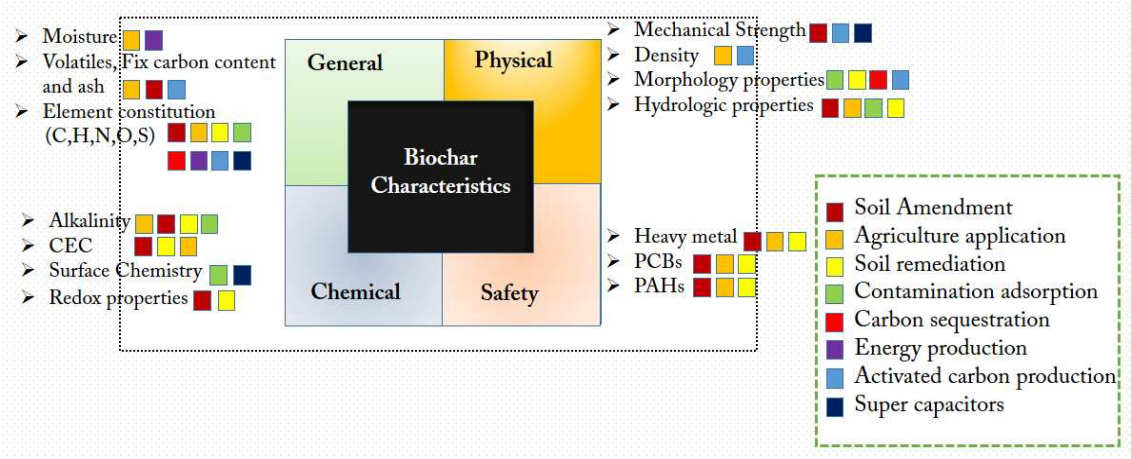


Figure 2. *A brief introduction of the linkage between biochar properties and its application (modified from Xiao et al., 2016). Here, the more number of square boxes designate the improvement in various soil properties via utilization of biochar through various applications which are represented by different color. Moreover, the abbreviations included are C (carbon), H (hydrogen), N (Nitrogen), O (Oxygen), S (Sulpher), CEC (Cation exchange capacity), PCB (Polychlorinated biphenyl) and PAH (Polycyclic aromatic hydrocarbons)*

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Biochar improves plant development and biomass under drought stress

The beneficial effects of BC application under limited water conditions have been widely reported (Akhtar et al., 2015; Paneque et al., 2016; Ramzani et al., 2017; Rogovska et al., 2014). The BC amendment improved growth and biomass of plants under drought-stress (*Table 1; Fig. 3*). Similarly, use of BC exhibited the highest vegetative growth and seed production of field-grown sunflower under non-irrigation conditions (Paneque et al., 2016). Its application enhanced tomato fruit quality, growth and yield under deficit irrigation (Agbna et al., 2017). Similarly, its use supports the growth of winter rapeseed under drought conditions (Bamminger et al., 2016). Likewise, Basso et al. (2013) found a significant increase in soil WHC in a soil column study with hardwood BC and predicted that the increased WHC might enhance available water capacity (AWC = available water between field capacity and permanent wilting point) for crops. Tomato seedlings were protected from wilting due to improved soil moisture content with higher (30% v/v) rates of BC amendments in sandy soils (Mulcahy et al., 2013).

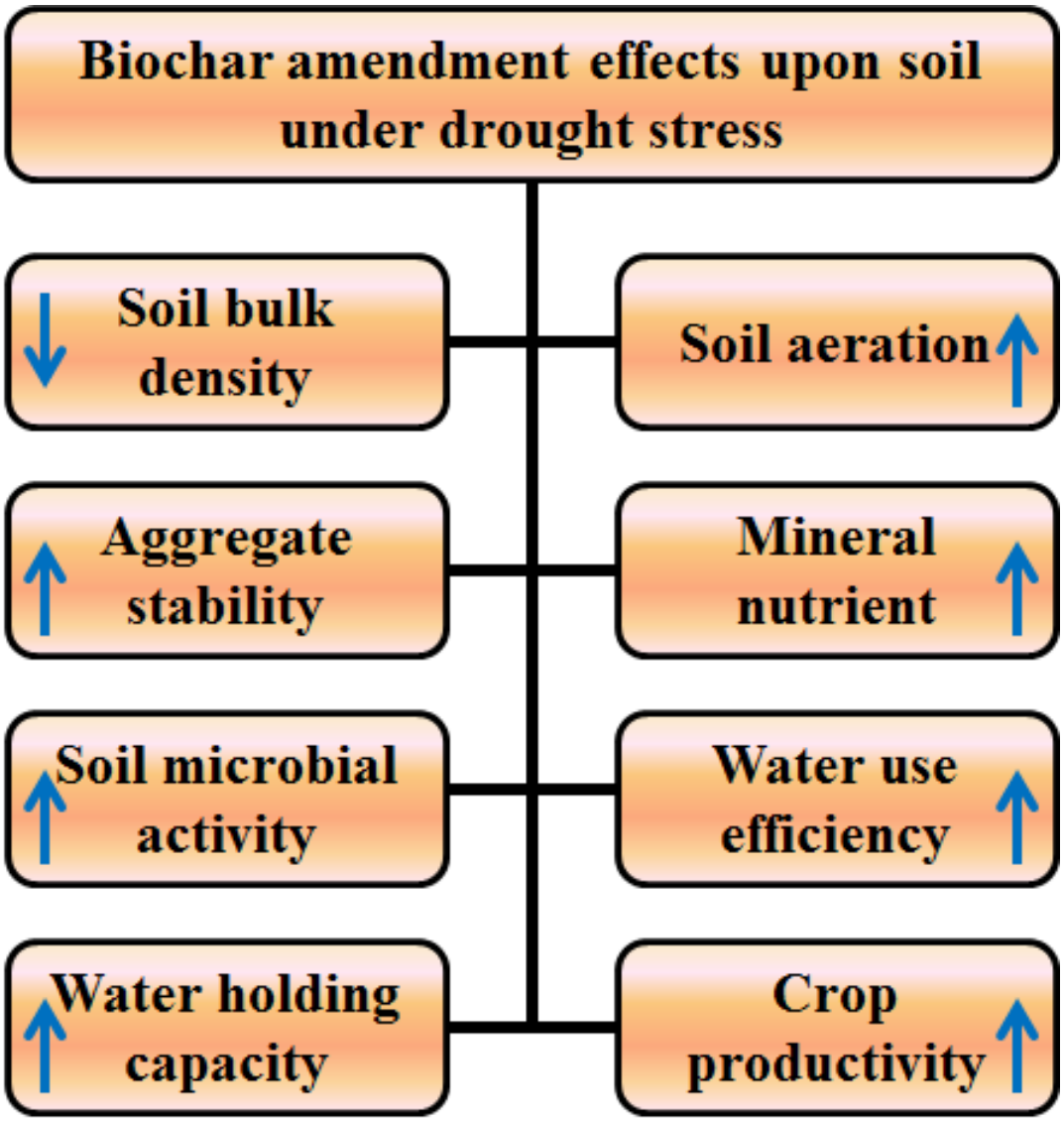


Figure 3. *Biochar possible effects under drought stress at both plant and soil level*

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Table 1. *Impact of biochar on plant growth and soil properties evident by recent research findings*

Feedstock and pyrolysis conditions	Rate of application	Soil type	Experiment condition	Plant species	Effects	References
Corn Cob Biochar, 450 ^{o}C	20 t ha^{-1}	Sandy loam	Field	soybean	Enhanced seedling growth	Hafeez et al., 2017
Wheat straw and sugarcane	5 and 10 t ha^{-1}	Sandy clay loam	Field	-	Increased microbial biomass carbon, phosphatase activity, urease activity and dehydrogenase activity	Mehmood et al., 2017
Rice husk + shell of cotton seed, 400 °C	0 and 5%	Sandy loam	Pot	Tomato	Increased fruit yield, Gs, Pn, RWC and WUE of leaves	Akhtar et al., 2014
Akiko pear trees, 500 °C	9 t hm^{-2}	-	Field	Pyrus ussuriensis Maxim	Improve the defense of plant leaves against drought stress	Lyu et al., 2016
Wheat straw biochar, 500 °C	0, 1, 2, and 3%	Sandy subsoil	Pot	Maize	Maintained leaf water potential, stomatal conductance, photosynthesis	Ahmed et al., 2016
Grapevine wood	15 t ha^{-1}	Sandy loam	Field	Helianthus annuus L.	Improved the plant development and productivity; increased the WHC of the soil	Paneque et al., 2016
Biomass of Lantana camara, 450 °C	0, 1, and 3%	Sandy loam	Pot	Okra	Increased leaf area, plant height, photosynthesis, Gs, and WUE	Batool et al., 2015
Wood-chip sievings at 550–600 °C	0, 1.5, and 3%	Sandy	Pot	Maize	Increased stem and dry leaf mass, RWC, and photosynthesis	Haider et al., 2015
Hardwood (maple), 350 °C	0%, 1%, and 2%	sandy loam	Pot	Milk thistle (*Silybum marianum* L. Gaertn)	Improved soil moisture holding capacity	Keshavarz Afshar et al., 2016
Poultry litter, 400 °C	3%	sandy-clay-loam Soil	Pot	-	Increased the resistance of both the bacterial and fungal networks to drought	Liang et al., 2014

Improving plant WUE has the potential to decrease plant susceptibility to limited water conditions. Amending BC with soil has been proposed as a way to ameliorate the WUE as it has been revealed to improve the WHC of soils (Licht and Smith, 2017). It enhanced hydrology characteristics, soil physical properties, increased soil water content and grain yields and WUE under natural conditions (Xiao et al., 2016). Different BCs have different impacts on soil water relations. For example, amendment of fresh BC with sandy loam soil enhanced the plant available water (PAW) but not aged BC. Addition of fresh and aged BC amendment with silt loam soil did not affect PAW and WUE. It indicates that BC type and BC age have variable impacts on PAW and WUE, meaning that BC amendments can increase plant growth and soil water relations under water-limited conditions for some but not all soils (Aller et al., 2017). Similarly, in another study, Licht and Smith (2017) demonstrated amendment of lignocellulosic BC to forest soils reduced DS in pine-oak systems. Akhtar et al. (2014)

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described that under limited freshwater resources, application of partial rootzone drying irrigation (PRD) in combination with BC might be a promising approach for saving water and enhancing productivity and quality of tomato. Similarly, Agbna et al. (2017) revealed that integration of BC amendment (BA) with deficit irrigation (DI) represents a viable technique that can save water without reducing tomato yields as well as improving the quality of the fruits. This method can mainly be used in areas that regularly struggle with water shortage. More precise optimal levels would likely need to be determined for commercial purposes in future studies as they are likely to be case specific, depending on soil texture, BC type and climate among other factors (Karer et al., 2013). Vaccari et al. (2015) suggests that BC amendment may ensure the same yield while using less external input such as irrigation water. A 10% yield increase of barley (*Hordeum vulgare*) with BC amendment in a chernozem region under prolonged DS was attributed to increased water availability. Githinji (2014) reported that the leaf quality rate of tomato increased with BC application in sandy loam soil as compared to the unamended control. Barley is grown in the coarse sandy subsoil underwater, use of 1% straw gasification BC increased the root and growth shoot. However, gasification BC was not active on barley growth under water stress in sandy loam soils (Hansen et al., 2016). Studies have described that the application of BC improved the photosynthesis of plants under DS (Haider et al., 2015; Lyu et al., 2016; Xiao et al., 2016).

BC significantly improved the relative water contents (RWC), stomatal conductance (Gs), chlorophyll contents, WUE, photosynthetic rate (Pn) and stomatal density of drought-stressed tomato leaves (Akhtar et al., 2014). Its application increased the leaf relative water contents (RWC), transpiration rate and osmotic potential of drought-stressed maize as compared to the control (Haider et al., 2015). Kammann and Graber (2015) study the influence of BC on drought tolerance of Chenopodium quinoa displayed increased drought tolerance, growth and water-use efficiency of quinoa. BC amendment in soil improves the soil water content, photosynthesis, leaf water potential and transpiration under scarce water conditions. However, application of BC resulted in increased soil water content in Danish coarse sandy soil under drought. Leaf water potential, photosynthesis, and transpiration of Maize plants were maintained by 2 and 3% BC addition to the subsoil (Ahmed et al., 2016). Similarly, Haider et al. (2015) reported that BC use in poor sandy soils could improve plant growth by enhancing soil-plant-water relations and photosynthesis under both deficit and excess water. Lyu et al. (2016) revealed the addition of BC could strengthen the defense of plant leaves against DS by influencing electron transfer and regulating the activity of protective enzymes, thereby reducing the effects of drought damage to the photosynthetic apparatus. This showed that BC response under DS might vary with soil, BC type and plant species.

Biochar inoculate with microorganisms mitigate drought stress

Studies have shown that BC may minimize water stress in plants when applied with microorganisms (Liu et al., 2017b; Nadeem et al., 2017). Egamberdieva et al. (2017) reported inoculation of BC with Bradyrhizobium sp. Enhance the growth, biomass, phosphorus (P) uptake and nitrogen (N), and nodulation in lupin (*Lupinus angustifolius L.*) seedlings under DS as compared to the only microbial inoculation. Nadeem et al. (2017) reported inoculation of BC with Pseudomonas fluorescens, reduced the harmful impact of DS on cucumber (*Cucumis sativus L.*) growth, increased the shoot length,

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shoot biomass, root length and root biomass as compared to inoculated control. Besides, significant improvements in chlorophyll and relative water contents, as well as a reduction in leaf electrolyte leakage, demonstrated the effectiveness of this approach. In another study, Liu et al. (2017b) reported that inoculation of BC (birch wood, 500 °C) with Rhizophagus irregularis decreased the WUE, N, leaf area, P in potato and did not adversely impacted the root biomass and soil pH under limited root zone water as compared to control. However, under limited irrigation soil amendment with wood-derived BC (30 $mg.ha^{-1}$) had no significant effect on soil biota groups such as protozoa, bacteria, fungi, nematodes and arthropods (Pressler et al., 2017).

The application of BC with Arbuscular mycorrhizal fungi can enhance drought tolerance of the host plant by physiological mechanisms in nutrient adsorption and biochemical mechanisms, e.g. hormones, osmotic adjustment and antioxidant systems. However, application of BC to the agricultural soil with Arbuscular mycorrhizal fungi stimulated the growth of extra-radical hyphae in soil and increased mycorrhizal colonization of roots. As the water potential of the soil was the same with and without BC amendment, it is unlikely that the observed effects on plant growth were related to possible benefits from the water holding capacity of the BC (Mickan et al., 2016).

Previous studies have revealed that the utilization of BC by microbial inoculation can be helpful in alleviating DS in plants, depending on the type of BC or other factors. Additionally, BC is increasingly discussed as a climate change (drought) mitigation tool, but there is limited knowledge about its interactive effects with future soil DS, possibly influencing its stability as well as its beneficial effects on soil microorganisms and plants.

Biochar improves soil biological and physiochemical properties

Several studies have shown that BC improves the physical properties of soil under DS conditions (Bamminger et al., 2016; Hansen et al., 2016; Zhang et al., 2017). Ye et al. (2015) has shown BC had features that could improve soil properties such as density, acidity, moisture retention, and the stability of heavy metal ions in the soil. Zhang et al. (2017) has shown that soil physicochemical properties improved by the BC and increased abundance and diversity of bacteria have contributed to the ability of BC to protect plants against tobacco bacterial wilt. Total porosity (TP) increased and soil bulk density (BD) decreased, air properties improved (FAC and FAP) and there was an increase in the retention of water available for plants (Pranagal et al., 2017). Liang et al. (2014) also reported that BC application in calcareous soils decreased the significantly bulk density of soil while soil WHC increased. Abel et al. (2013) showed that BC application in sandy soil increased the total pore space, decreased the soil Bulk density (BD) and water retention at permanent wilting point. The equilibration of corn residue BC in a sandy loam soil for 30 days decreased the BD and increased water holding capacity WHC of the soil (Igalavithana et al., 2017). The BC amendment decreases the BD as well as an increase in the soil water contents, have observed in a variety of soils with different soil texture (Herath et al., 2013; Igalavithana et al., 2017). Burrell et al. (2016) showed that straw BC was the most effective at improving plant available water and soil aggregate stability in a coarse-textured soil. Similarly, Baiamonte et al. (2015) reported that application of BC had increased the aggregate stability in sandy-clay soil. However, under crop cultivation, more detailed studies are required to appraise the impact of BC on soil aggregate stability. In general, BC amendment improves the soil

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physical properties under reduced water supply and beneficial for plant growth under semiarid conditions. Though, under drought, additional investigations are required about the BC amendment. In addition, BC aging affected the soil properties, and more studies are needed to evaluate the BC aging effect on soil properties under DS (Arthur et al., 2015). In addition, soil water retention may vary with BC sources and pyrolysis condition. For example, Similarly, Narzari et al. (2017) demonstrated that the BC produced at higher temperature had higher pH and WHC. However, it is suitable for soil having water retention capacity as an amendment. Further investigations, by using different BCs at different rate of application and various pyrolysis conditions might be useful to improve the effectiveness of BC on the soil WHC under DS (Lee et al., 2015). The addition of BC increases the WHC of the soil, which is a critical factor in enhancing plant growth and yield under drought (Akhtar et al., 2015; Basso et al., 2013; Foster et al., 2016; Lehmann et al., 2011). An increase in the WHC of soil under DS might be because of higher CEC and porous structure of BC (Laghari et al., 2016). Studies testified that application of BC improved the soil CEC as compared (Lychuk et al., 2015). Artiola et al. (2012) reported that the application of pine forest waste BC in the soil retained water in the soil, however under laboratory conditions; significant portion of BC pores remained empty. However, there might be the difference in the WHC of BC under field conditions, which need to be investigated in the near future. The application of BC at vineyard improved the soil water content measured on different time (Baronti et al., 2014). BC amendment to sandy soils increased WHC and to retain vast amounts of PAW for crops for more extended periods of times (Basso et al., 2013). Liu et al. (2017c) revealed BC with a high intraporosity and irregular shapes will most efficiently increase water storage in coarse soils. Feedstock, pyrolysis temperature and charring residence time are various factors involved might be useful to produce BC with varying porosity.

Ajayi and Horn (2016) compared the potential of clay and BC in improving mechanical resilience and water retention of sandy soil. Soil water retention capacity increased with Na-bentonite at more negative matric potentials, whereas BC was efficient to increasing soil water retention capacity at saturation which might be caused by the increase in soil porosity. The BC application in the sandy loam soil improved the WHC of the soil used for rice cultivation (De Melo Carvalho et al., 2014). The field application of BC in vertisol increased the wheat growth and developed the ability of soil to retain water under semiarid Mediterranean conditions (Olmo et al., 2014). Githinji (2014) reported that application of BC in the field increased the macroporosity of loamy sand soil.

These studies revealed that BC might be efficient to increase the WHC of soils. Most of the studies were conducted in sandy soils. However, more studies are needed to explore the functions of BC in increasing the WHC of soil with different texture and environmental conditions. Limited research investigated characteristics of BC produced from various feedstocks to determine which are the most effective for improved soil water holding capacity WHC of soil.

How biochar can be applied to improve crop yield

When applying BC to soil for improving its fertility, the BC should be located near the soil's surface in the root zone, where the bulk of nutrient cycling and uptake by plants takes place. Certain systems may benefit from the application of BC in layers

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below the root zone, for example during landscaping for carbon (C) sequestration or moisture management. Similarly, if BC is to be applied to soil solely for C sequestration purposes, placement should be done deeper in the soil. When deciding how to apply BC to soil, the specific cropping system must be taken into consideration. The likelihood of wind and water erosion losses of BC is reduced when it is thoroughly incorporated into soil, however, plowing and soil mixing are not possible or desirable in all cropping systems, at all times. In conventional field cropping systems, BC should ideally be managed using traditional farm machinery and incorporated into routine field operations. This will ensure that the costs of using BC are kept as low as possible. For example, BC can be applied and incorporated together with lime, since lime is often applied as a fine solid which must be well incorporated into soil. The majority of BC field trials reported to date used this method for incorporating BC into soil (Haque et al., 2014; Julie Major et al., 2010; Yamato et al., 2006). Broadcasting can be done by hand on small scales or on larger scales by using lime/solid manure spreaders or broadcast seeders. Moistened BC materials may be better suited to application with manure spreaders than lime spreaders. Incorporation can be achieved using any plowing method at any scale, including hand hoes, animal draft plows, disc harrows, chisels, rotary hoes, etc. As mentioned above, wind losses from applying and incorporating fine BC materials can be significant and precautions must be taken to minimize this. Banding of seeds and fertilizers is a routine operation in mechanized agriculture and involves applying an amendment in a narrow band, usually using equipment that cuts the soil open, without disturbing the entire soil surface. Banding allows BC to be placed inside the soil while minimizing soil disturbance, making it possible to apply BC after crop establishment. However, the amounts of BC that can be applied in this way are lower than those which can be achieved by broadcast applications. Wheat yields in Western Australia were improved by banding BC (Blackwell et al., 2007). When working by hand, BC can be applied in furrows opened using a hoe and closed after applying BC. Mixing it with other soil amendments such as manure, compost or lime before soil application can improve efficiency by reducing the number of field operations required. Since BC has been shown to sorb nutrients and protects them against leaching (Major et al., 2009), mixing with BC may improve the efficiency of manure or other amendment application. BC can also be mixed with liquid manures and applied as a slurry. Fine BCs will likely be best suited to this type of application using existing application equipment, and dust problems associated with these would be addressed. It could also be mixed with manure in holding ponds and could potentially reduce gaseous nitrogen losses as it does when applied to soil (Spokas et al., 2009; Yanai et al., 2007). Where high-resolution data on soil characteristics and farm machinery equipped with geographical positioning systems are available, it would possible to apply BC preferentially to areas of fields where fertility is low. Further studies are needed to address how long BC intraporosity, particle size, and particle shape will last after field application.

Conclusion and future perspectives

DS severely decreases crop yield and growth. Studies reported above showed that BC application increased the plant growth and biomass under DS. BC application in soil increases the nutrient uptake, photosynthesis and modified gas exchange features in plants. More studies are required to evaluate the role of BC under DS conditions. The

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studies mentioned above showed that BC application with microbes might improve the plant tolerance against DS. However, time-course and detailed studies are needed to explore the long-term effectiveness of BC + microbes on the growth of plants and to separate the effects of BC on different stages of microbial life cycles under prevailing conditions of limited water. Amendment of BC with the inoculation of Arbuscular mycorrhizal fungi can enhance drought tolerance mycorrhizal plants through physiological mechanisms in nutrient uptake and biochemical mechanisms regarding adjustment, osmotic, hormones and antioxidant systems. Extensive studies are needed for the development of models for the better recommendation of BC rate based on soil, plant, and environmental conditions.

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ASSESSING SUSTAINABILITY OF THE BAHABAD AQUIFER, CENTRAL IRAN

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Abstract. Bahabad plain in central Iran contains vital groundwater resources, mainly used in agriculture. A well-field at the centre is discharging water for a further located mining and industrial site, results in a closed depression in water-table which divides the aquifer system into southern (S) and northern (N) zones. In this research, the sustainability of groundwater resources was evaluated by groundwater balance studies and safe yield analyses. The annual safe yields are estimated to be 22.3 and 3.7 million cubic meter (MCM) for the zones S and N, respectively. Based on balance studies, total annual abstractions (33.9 and 8.6 MCM, respectively) are greater than the total inputs, exceeding the safe yield, as well. So, the aquifer is over-exploited and groundwater is withdrawn from storage, declining water-table with an average rate of 50 cm annually. In contrast to the public perception of the major impact from well-field abstractions, this research clarifies the role of both industrial and agricultural abstractions in depletion of Bahabad aquifer. Therefore, safe yield must be considered in order to avoid further aquifer mining. It can be attained by decreasing industrial and agricultural abstractions and managing recharge to augment the aquifer storage, assuring the sustainability of the groundwater resources.
Keywords: *aquifer mining, groundwater balance, over-exploitation, safe yield, Yazd*

Introduction

The components of the water cycle are in a balancing state, naturally. However, human interventions, for example over-exploitation of the groundwater resources, disturb the balance leading to unfavorable impacts on the environment. Groundwater resources as a part of the water cycle are important in terms of water supply for different uses in many regions of the world, especially in arid and semi-arid areas where surface-water resources are not generally available. Over-exploitation (abstractions over than the recharge) of these vital resources results in ''groundwater mining'' which is followed by negative effects including depletion of aquifer storage, saline water intrusion, groundwater level decline, land subsidence and other environmental, economic and social problems (Brown et al., 1999). To avoid the problems, a proper management plan is required, assuring the sustainability of the groundwater resources. In this regard, concepts such as safe yield or sustainable yield were applied in groundwater managing strategies, generally restricting the withdrawals at values without negative impacts on the groundwater reservoirs. (Naik and Awasthi, 2003; Alley, 2004; Kalf and Woolley, 2005; Zhou, 2009). Safe yield of the groundwater system is defined as the amount of groundwater that can be exploited continuously without detrimental effects on the groundwater reservoirs (Central Groundwater Board, 1984). It includes all exploitable groundwater originating within the catchment (Naik and Awasthi, 2003).

To ensure sustainability, it is essential that the yield constraints is established based on the hydrological mass balance (Sophocleous, 2000). "The water balance is the

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perfect tool for a better understanding of the valuable groundwater resource which is nothing more than a material balance that accounts for all inputs, outputs and changes in storage within a system defined by prescribed boundaries" (Sophocleous, 2000).

Safe yield, as a feasible managing concept introduced by Naik and Awashti (2003) was implemented by many researchers (Voudouris, 2006; Taheri Tizro et al., 2007; Türker et al., 2013), considering water balance equation to establish the yield restrictions. Voudouris (2006) studied water balance and safe yield in coastal aquifer of Korinthia, an agrotourism center in Greece, where total abstractions are greater than the recharge and the safe yield, resulting in signs of aquifer depletion, seawater intrusion and groundwater quality contamination. The integrated water management through measures that augment the groundwater budget in the coastal aquifer was recommended. Estimating groundwater balance of the alluvial aquifer of Kangavar in west Iran confirmed current abstractions which exceed both recharge and safe yield of the aquifer system are not sustainable and the aquifer system has overexploited. Artificial recharge of the aquifer through river beds, as well as water-saving techniques such as spray and drip irrigation were proposed in order to support sustainable management of groundwater resources in this area (Taheri Tizro et al., 2007). The results of safe yield analysis for the Yeşilköy aquifer in Cyprus (Türker et al., 2013) showed that annual safe yield exceeds the natural aquifer recharge and sustainability of the aquifer is not foreseen. Decreasing groundwater abstraction by lowering the water volume for irrigation use or providing another source like seawater desalination for domestic use were recommended to attain the safe yield.

Bahabad plain is an important agricultural area in Yazd province at central Iran. Excessive groundwater withdrawals for both agricultural and industrial usages impose more stress on the aquifer, results in continuous decline of water table with an average rate of 50 cm annually. Depleting aquifer storage, reduction of groundwater availabilities and increase in pumping costs are the main negative possible impacts which have decreased agricultural activities in the region, enhancing migration to the neighboring cities (personal communication with local governor). Reduction of quantity of the water resources increases the concerns that the safe yield of the aquifer has been exceeded, challenging sustainable development of this arid region. Hence, the aim of this study was to assess the sustainability of groundwater resources, considering estimation of safe yield and water balance. In this regard, the roles of agricultural and industrial abstractions in depleting the aquifer storage were also examined and strategies to achieve the safe yield were discussed.

Study area description

Bahabad plain, 220 km far from Yazd city at central Iran (*Fig. 1*), locates between two parallel NE-SE highlands. The alluvial sediments of the plain are situated principally in a graben structure, where the thickness increases more than 580 m at the center (Yazd Regional Water Authority, 1996). The quaternary alluvial deposits include old terraces, younger terraces, fluvial sediments and salty mud flats. There is a general trend of decreasing grain size of the sediments from south to the north, following the topographic surface slope (*Fig. 2*).

Bahabad unconfined aquifer with an area of about 366 km^2 lies between latitudes of 31° 43' and 32° 12' N and longitudes of 55° 42' and 56° 17' E. Groundwater levels are being monthly monitored in a network of 14 piezometers (*Fig. 2*) since 2002.

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Bahabad aquifer is discharged by 78 pumping wells including 51 agricultural, 22 industrial and 5 municipal wells (*Fig. 1*), extracting the aquifer with an average annual rate of 25.5, 6.2 and 4.8 million cubic meter (MCM), respectively (Shirafkan, 2013). Three qanats in southern part are also discharging the aquifer with an average rate of about 1 MCM yr^{-1}. A qanat is an underground gallery dug out inside the alluvium, in order to transfer groundwater to the ground surface by gravitation force.

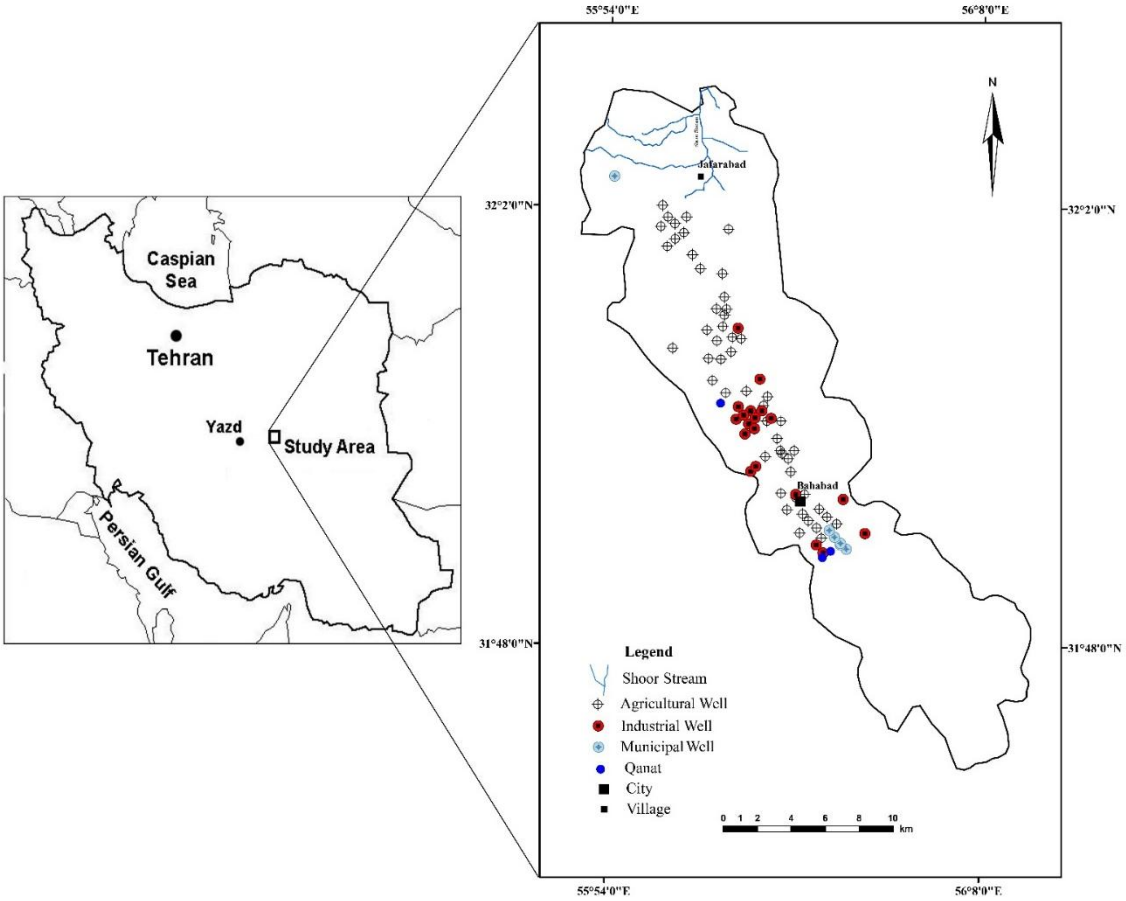


Figure 1. *Map showing location of the study area and spatial distribution of the wells and qanats in the Bahabad aquifer*

Based on iso-potential (contour) map of the aquifer, the groundwater flow is generally directed from south to the north (*Fig. 2a*). A closed depression in water table occurred at the center of the aquifer, where it is locally pumped by a well-field including 10 wells which extract groundwater for industrial uses. It must be mentioned that most of the water pumped by industrial wells is used in the Chadormalu Mining and Industrial Company which is located out of the aquifer basin, 75 km far from the Bahabad city. Due to the well-field abstraction, the flow lines are concentrated toward the center of the well-field and the groundwater divides at the vicinity of the piezometers P7 and P8 (*Fig. 2a*). It results in bisecting the aquifer system into two separated hydrological zones.

Depth to groundwater is highly decreased at the north of the plain, where the aquifer is permanently drained by the Shoor stream (*Fig. 1*).

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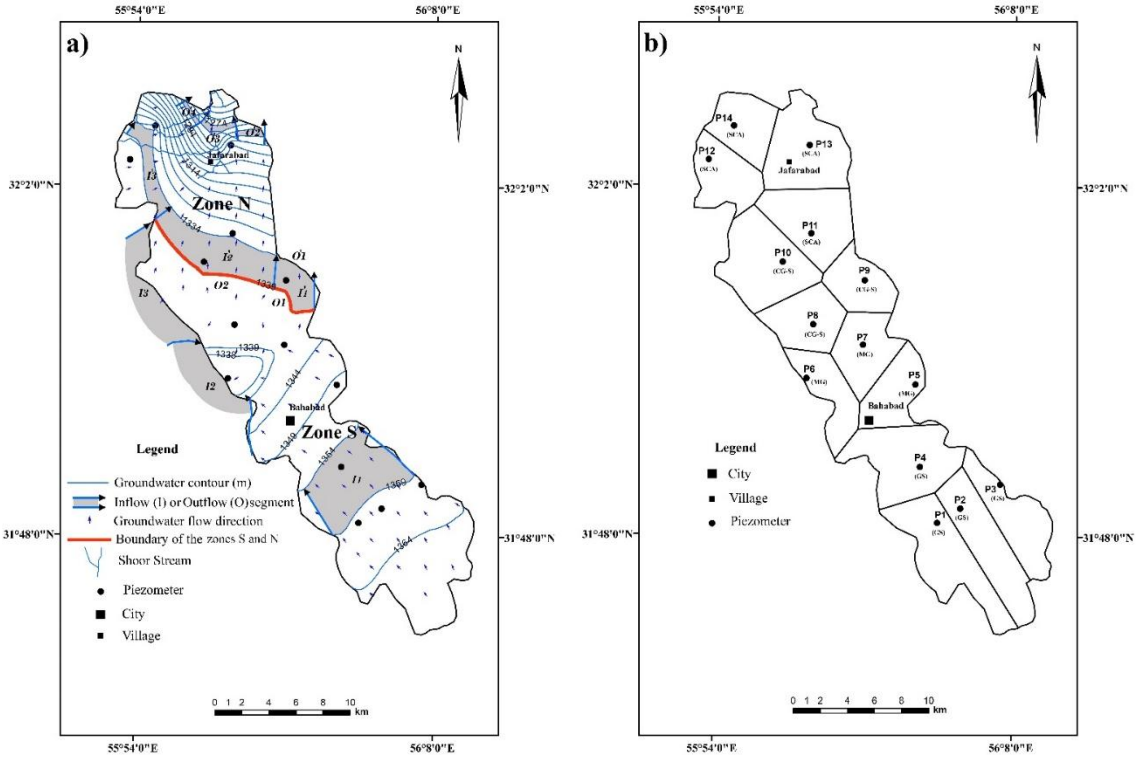


Figure 2. *a) Iso-potential map of the Bahabad aquifer representing groundwater flow direction with inflow and outflow boundaries; b) Thiessen's polygons surrounding piezometers. The dominant lithology (GS: gravelly sand; MG: medium gravel, CG-S: clay-gravel and sand; SCA: silt/clay rich alluvium) is also presented*

Materials and methods

Groundwater Balance

Groundwater balance is based on the comparison of the total inputs and outputs to/from the aquifer during a specified period (balance period), written as:

$$\sum \text{Inputs} - \sum \text{Outputs} = \text{Change in aquifer storage}$$

Considering inputs and outputs to/from the Bahabad aquifer, the following balance equation was applied:

$$\left(G_{in} + R_r + I_{rf} + W_{rf}\right) - \left(G_{out} + G_d + G_w\right) = \pm\Delta V \qquad \text{(Eq.1)}$$

where G_{in} is groundwater inflow through aquifer boundary, R_r rainfall recharge, I_{rf} return flows from irrigation, W_{rf} recharge by municipal wastewater, G_{out} groundwater outflow from boundary of the aquifer, G_d groundwater drainage into Shoor stream, G_w groundwater withdrawal and ΔV change in aquifer storage volume. All terms are expressed as volume of water during the balance period (MCM yr^{-1}).

In addition to water balance formulation (*Eq. 1*), change in aquifer storage volume (ΔV) is also calculated by *Equation 2* to validate the groundwater balance calculations.

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$$\Delta V = A \times Sy \times \Delta H \quad \text{(Eq.2)}$$

where A, Sy and ΔH denote aquifer area (m^2), mean specific yield of the aquifer (dimensionless) and spatially averaged rise or decline in groundwater level during balance period (m), respectively. As the water levels are not uniform in the aquifer, the spatially monthly averaged groundwater level (H) is calculated using *Equation 3*.

$$H = \sum_{i=1}^{n} \frac{(h)_i \times a_i}{A} \quad \text{(Eq.3)}$$

where $(h)_i$ is the height of groundwater table above datum (sea level) in piezometer i (m), a_i the area of the Thiessen's polygon surrounding piezometer i (m^2), A the total aquifer area (m^2) and n the number of piezometers installed in the aquifer.

Specific yield (Sy) is defined as the ratio of the volume of water gravitationally drained, after saturation, to the total volume of the drained soil (Todd and Mayes, 2005). In the present study, Sy was determined using standard values in literatures (Johnson, 1967; Lerner et al., 1990) considering soil texture in aquifer's drilling logs. The method is generally acceptable (Fetter, 2000), employed by many researchers in areas like the study area devoid pumping test data (e.g. Obuobie et al., 2012; Lerner et al., 1990).

Safe yield

In this research the safe yield was estimated by *Equation 4* adapted from Naik and Awasthi (2003). It includes all exploitable/utilizable groundwater originating within the aquifer basin, consisting exploitable dynamic groundwater reserve (Q_{ed}), value of groundwater pumped for irrigation and other uses in wet season (Q_{wet}) and return flows from irrigation and sewage (Q_{rw}). All terms are expressed as volume per time (MCM yr^{-1}).

$$\text{Safe yield} = Q_{ed} + Q_{wet} + Q_{rw} \quad \text{(Eq.4)}$$

The exploitable dynamic groundwater (Q_{ed}) represents the long-term average annual recharge under conditions of maximum groundwater abstraction (dry season). Generally, maximum groundwater extraction occurs in Bahabad region when irrigation starts from beginning of the June until December. Thus, Q_{ed} is estimated using *Equation 5:*

$$Qed = \sum_{i=1}^{n} a_i \times (Sy)_i \times (D_{ld})_i \quad \text{(Eq.5)}$$

where $(Sy)_i$, and $(D_{ld})_i$ denote the specific yield (dimensionless) and the value of water level decline during the dry season (June to December) in piezometer i (m), respectively. Parameters a_i and n were defined before in *Equation 3*.

Value of groundwater pumped in wet season (Q_{wet}) refers to the discharge mainly by agricultural and industrial wells during January to May. In this period the total recharge by the rainfall is occurred and aquifer discharge rates by agricultural wells are mostly

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diminished in response to reduction in evapotranspiration and irrigation demands. This value plus return flows from irrigation and sewage are also considered as utilizable groundwater sources in the region to calculate the safe yield.

Results and discussion

Assessing groundwater balance of the Bahabad aquifer

Regarding the impact of the industrial well-field on the groundwater flow pattern (*Fig. 2a*) the aquifer was divided into two hydrological zones namely as N and S and the groundwater balance in each zone was calculated for water-year 2010-2011 (starting at September 23). Based on *Equation 1* the inputs and outputs to/from Bahabad aquifer were determined as the following:

Inputs

Rainfall recharge, groundwater flow through aquifer boundary and return flows from irrigation and domestic uses are the main inputs into the Bahabad aquifer.

a) Rainfall recharge (R_r)

Rainfall data from Bahabad climatological station at the plain center was used to determine the mean representative rainfall on the aquifer. Regarding the surface area (366 km^2) and relatively flat topography of the aquifer, the rainfall measurements in this station are representative to the whole aquifer, as the rain-gauge density in the plains is proposed be one station per 520 km^2 (Raghunath, 2009). Based on the data measured in this station for a period of 22-year (1988-2010) the depth of annual precipitation varies from 28.5 to 120.5 mm and the average annual rainfall of the plain is 81.5 mm. It mainly occurs during winter (January-March) and spring (April-May) seasons.

The relationship between groundwater recharge and precipitation is governed by different factors such as type of precipitation, climatic conditions, soil moisture prior to storm, storm characteristics (duration, intensity, peak intensity), topography of ground surface, perviousness of ground surface and vegetation cover (Bear, 1979). The rainfall recharge coefficient in adjacent areas having the same climate and lithological formations to the study area was directly measured using the experimental lysimeters (Parab Fars, 2008), which varied from 5 to 10% for the fine and coarse soils, respectively (Parab Fars, 2008). Therefore, the rainfall recharge coefficient was decided to be 10% for the zone N, which is mainly covered by sand and medium gravel sediments and 5% for the zones S with silt/clay rich alluviums (*Fig. 2b*). The annual rainfall during balance period (water-year 2010-2011 starting at September 23) was 30 mm, then annual recharge rate to the aquifer zones of S and N was estimated as the following:

$$R_r = \text{Rainfall depth} * \text{Rainfall recharge coefficient} * \text{Aquifer area}$$

$$R_r = \left(30*10^{-3}\right) * \left(10\%\right) * \left(235*10^{6}\right) = 0.7*10^{6} m^3 yr^{-1} = 0.7 \text{ MCM yr}^{-1} \text{ Zone S}$$

$$Rr = \left(30*10^{-3}\right) * \left(5\%\right) * \left(131*10^{6}\right) = 0.2*10^{6} m^3 yr^{-1} = 0.2 \text{ MCM yr}^{-1} \text{ Zone N}$$

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b) Groundwater inflow through aquifer boundary (G_{in})

The entire inflow boundary of the aquifer was divided into segments named as Ij or I'j where index j denotes the number of the segment. The groundwater inflow through the segment (G_{Ij}) (MCM yr^{-1}) was calculated by Darcy's law (*Eq. 6*) considering average aquifer transmissivity along the segment (T_j) (m^2/day), length of the segment (Wj) (m) and component of the hydraulic gradient normal to it (i_j) (m/m). Flows into the segments were summed to calculate total groundwater inflow through aquifer boundary (G_{in}) (MCM yr^{-1}).

$$G_{in} = \sum_{j=1}^{m} (G_{I_j}) = \sum_{j=1}^{m} (W_j \times T_j \times i_j) \quad \text{(Eq.6)}$$

in which, the index m stands for the maximum number of the segments.

Three inflow segments were identified on the contour map of the groundwater surface in each of the zones S and N of the aquifer (*Fig. 2a*). The average transmissivity of the aquifer along the segments (T_j) has only been measured by pumping test in one exploration well at the south (segment I_1) which is about 3664.5 m^2/day. In other segments the transmissivity values were estimated by multiplying hydraulic conductivity, inferred from soil texture in aquifer drilling logs, and aquifer thickness (Shirafkan, 2013). It varies from 300 to 792.9 m^2/day at the center, decreases to about 5.5 m^2/day at the northern parts of the aquifer (*Table 1*). The decreasing trend in transmissivity values is in consistent with changes in aquifer soil texture from gravelly sand and medium gravel in south and center to silt/clay rich alluvium at the north (*Fig. 2b*).

Table 1. *Values of groundwater inflow and outflow through boundaries of the aquifer balance areas S and N during water-year 2010-2011*

Aquifer zones	Inflow (I) or outflow (O) segment	Segment width (m)	Hydraulic gradient (m/m)	Transmissivity (m^2/day)	Annual inflow or outflow (MCM yr^{-1})
Zone S	I_1	7024.5	0.001	3664.5	9.86
	I_2	5545	0.002	792.9	2.82
	I_3	10000	0.002	300	1.75
	Sum of groundwater inflows				***14.4***
	O_1	3500	0.002	300	0.67
	O_2	9752	0.002	300	2.31
	Sum of groundwater outflows				***3***
Zone N	I'_1	3500	0.002	300	0.67
	I'_2	9752	0.002	300	2.31
	I'_3	7500	0.003	300	2.9
	Sum of groundwater inflows				***5.9***
	O'_1	3500	0.002	300	0.67
	O'_2	2000	0.006	5.5	0.03
	O'_3	4661.5	0.013	5.5	0.12
	O'_4	1900	0.016	5.5	0.06
	Sum of groundwater outflows				***0.9***

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In each segment, hydraulic gradient (i_j) was calculated from iso-potential map of the aquifer (*Fig. 2a*) considering head difference (dh_j) in a selected distance of dl_j ($i_j = dh_j/dl_j$). It varies from 0.001 to 0.003 m/m at inflow boundaries (*Table 1*). The highest value are observed at inflow segment I'_3 in the north of the aquifer where the slit/clay rich alluviums are dominant. Applying *Equation 6,* the total annual volume of groundwater entering the zones S and N of Bahabad aquifer were calculated as 14.4 and 5.9 MCM, respectively (*Table 1*). As a result, the main groundwater flow into the Bahabad aquifer (9.86 MCM yr^{-1}) is taking place through segment I_1 at the south of the plain.

c) Recharge due to water returns (I_{rf} and W_{rf})

Return flow consists of a portion of the water applied for irrigation, industry and domestic uses in the region, which is not used up as "consumptive use" but infiltrates, and eventually reaches the water table. It is estimated that 63.7 and 98.5% of the total extracted groundwaters in the S and N zones of Bahabad aquifer are used for agricultural activities, respectively (Shirakan, 2013). Irrigation return flow coefficients (percent ratio of return flow to the applied irrigation water) in other areas with the same soil texture, climate and irrigation management practices were determined using lysimeters. They varied from 0.4 to 38.7% depending on the total applied water, field length, irrigation management and soil texture (Jafari et al., 2012). In the present study, irrigation return flow coefficients of 15 and 10% were decided for the zones S and N, respectively. The lower value in the zone S is related to the finer texture of the surface soils. The adopted values are nationally acceptable, as they are in the range applied in many groundwater budget studies around the country (Iran Water Resources Management Company, 1991).

Annual volumes of groundwater discharged by agricultural wells and qanats at the zones S and N of Bahabad aquifer are 20 and 6.5 MCM, respectively. Therefore, the annual amounts of recharge from agricultural activities were estimated at 3 and 0.6 MCM, respectively.

$$I_{rf} = \text{Agricultural discharge rate} * \text{Irrigation return flow coefficient}$$

$$I_{rf} = 20 * 15\% = 3 \text{ MCM yr}^{-1} \quad \text{Zone S}$$

$$I_{rf} = 6.5 * 10\% = 0.6 \text{ MCM yr}^{-1} \quad \text{Zone N}$$

Disposal wells in the Bahabad urban and rural areas have the potential to recharge the aquifer, too. Per the norm prescribed by Iran Water Resources Management Company (1991), 50-70% of the water used in urban areas, percolates down in shallow disposal wells and reaches the underlying aquifer. Total annual discharges by municipal wells in the zones S and N of the Bahabad aquifer are 4.7 and 0.1 MCM, respectively. Thus, annual recharge due to the return seepage from water applied for municipal uses was estimated 2.8 and 0.05 MCM, considering return coefficients of 60 and 50% in the zones S and N, respectively. The difference in the coefficients is mostly related to variations in soil texture as it was discussed before.

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$$W_{rf} = \text{Municipal discharge rate} * \text{Municipal return flow coefficient}$$

$$W_{rf} = 4.7 * 60\% = 2.8 \text{ MCM yr}^{-1} \quad \text{Zone S}$$

$$W_{rf} = 0.1 * 50\% = 0.05 \text{ MCM yr}^{-1} \quad \text{Zone N}$$

It must be mentioned that total groundwater discharged by industrial wells in the zone S is transferred and consumed out of the study's aquifer area; therefore, no recharge return flow to the aquifer from industrial uses is expected.

Outputs

a) Groundwater outflow from aquifer boundary (G_{out})

Groundwater flows out of the aquifer balance area through segments symbolized as O and O' in *Figure 2*. Same as the groundwater inflows, the values of groundwater outflows were estimated using Darcy's law (*Eq. 6*). The values of aquifer transmissivity in outflow segments vary from 5.5 to 300 m^2/day and hydraulic gradients change from 0.002 to 0.016 (*Table 1*). Applying *Equation 6,* the total annual volumes of groundwater leaving zones S and N were estimated to be 3 and 0.9 MCM, respectively (*Table 1*).

b) Drainage into the Shoor stream (G_d)

Shoor stream with an average discharge rate of about 40 lit/s (Shirafkan, 2013) is a gaining (effluent) river which drains the Bahabad aquifer only at the northern part (zone N). All the baseflow in the stream is derived from groundwater, as water table elevations in the vicinity of the stream are higher than the stream bed (Shirafkan, 2013). The baseflow was separated from direct runoff using straight-line method (Chow, 1988), considering lowest discharge during the year. It was estimated to be about 0.035 m^3/sec (*Fig. 3*). So, the annual volume of groundwater drained by the Shoor stream was estimated to be about 1.1 MCM.

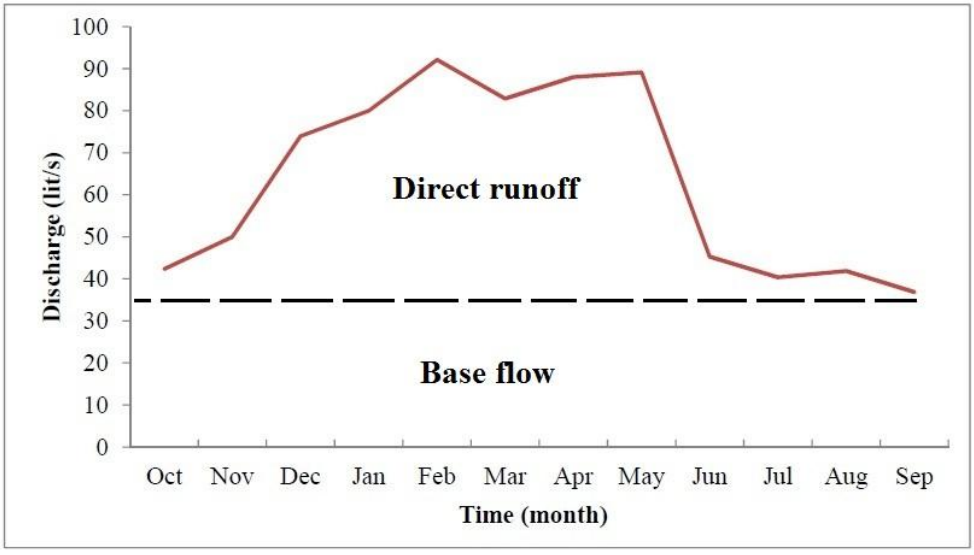


Figure 3. *Mean monthly hydrograph of Shoor stream during 1992 to 2011.*

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$$G_d = 0 \qquad \text{Zone S}$$

$$G_d = 0.035\ m^3/sec = 1.1\ MCM\ yr^{-1} \quad \text{Zone N}$$

c) Groundwater withdrawal (G_w)

Water is withdrawn from Bahabad aquifer by deep and semi-deep wells and qanats. Based on measured data from Yazd regional water authority (Shirafkan, 2013), annual volume of groundwater extracted by agricultural, municipal and industrial wells, as well as, qanats in the zone S were 19, 4.7, 6.2 and 1 MCM, respectively. Discharge by agricultural wells in the zone N was 6.5 and by industrial wells was 0.1 0 MCM yr^{-1}. No qanats and municipal wells are presented in this zone (*Fig. 1*). Total values of groundwater withdrawn from zones S and N of the Bahabad aquifer were calculated as below:

G_w = Discharge by agricultural wells + Discharge by municipal wells + discharge by industrial wells + Discharge by qanats

$$G_w = 19 + 4.7 + 6.2 + 1 = 30.9\ MCM\ yr^{-1} \quad \text{Zone S}$$

$$G_w = 6.5 + 0.1 + 0 + 0 = 6.6\ MCM\ yr^{-1} \quad \text{Zone N}$$

Groundwater balance

The components of groundwater balance in the S and N zones of Bahabad aquifer are presented in *Table 2*. The groundwater balance of the aquifer in the zones S and N was calculated by *Equation 1* as the following:

$$(Gin + Rr + Irf + Wrf) - (Gout + Gd + Gw) = \pm\Delta V$$

$$(14.4 + 0.7 + 3.0 + 2.8) - (3.0 + 0 + 30.9) = 20.9 - 33.9 = -13\ MCM\ yr^{-1} \quad \text{Zone S}$$

$$(5.9 + 0.2 + 0.6 + 0.05) - (0.9 + 1.1 + 6.6) = 6.8 - 8.6 = -1.8\ MCM\ yr^{-1} \quad \text{Zone N}$$

Total annual outputs from S and N zones are calculated 33.9 and 8.6 MCM, exceeding the total inputs of 20.9 and 6.8 MCM, respectively. A negative groundwater balance of about 13 and 1.8 MCM yr^{-1} was estimated for these zones during water-year 2010-2011.

In order to validate the aquifer balance calculations (*Table 2*), change in aquifer storage (ΔV) was also estimated using *Equation 2,* considering measured water levels in piezometers and aquifer specific yield. The values of groundwater height in each month were spatially averaged using *Equation 3* and groundwater hydrographs for the zones S and N were depicted in *Figure 4* for a period of about 9-year from October 2002 to 2011. The basic data used in drawing the hydrographs are presented in the Appendix. The figure shows monthly variations of groundwater level and precipitation in the two zones of Bahabad aquifer, started at October as the first month of the water-year.

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Groundwater level has been declined about 6 m in the zone N and 4.5 m in the zone S during 2002-2011. So, the mean annual declines (ΔH) in the zones S and N are about 65 and 51 cm, respectively.

Table 2. *Groundwater balance at the zones S and N of Bahabad aquifer during water-year 2010-2011*

Aquifer zone	Component of water balance		Annual volume (MCM yr^{-1})
Zone S	**Inputs**	Rainfall recharge	0.7
		Groundwater inflow	14.4
		Irrigation return flow	3.0
		Municipal wastewater seepage	2.8
	Total inputs		**20.9**
	Outputs	Groundwater outflow	3.0
		Discharge by agricultural wells	19.0
		Discharge by Qanats	1.0
		Discharge by municipal wells	4.7
		Discharge by industrial wells	6.2
	Total outputs		**33.9**
	Inputs - outputs		***-13***
Zone N	**Inputs**	Rainfall recharge	0.2
		Groundwater inflow	5.9
		Irrigation return flow	0.6
		Municipal wastewater seepage	0.05
	Total Inputs		**6.8**
	Outputs	Groundwater outflow	0.9
		Discharge by agricultural wells	6.5
		Discharge by municipal wells	0.1
		Groundwater drainage into stream	1.1
	Total outputs		**8.6**
	Inputs - outputs		***-1.8***

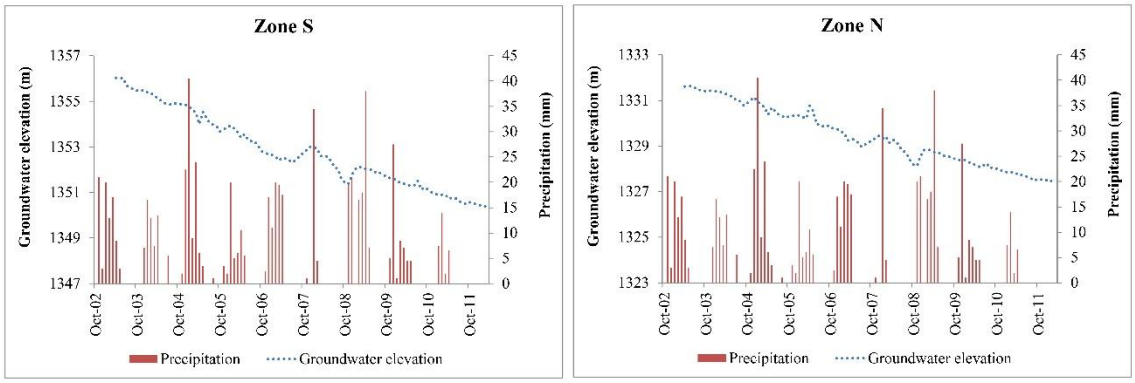


Figure 4. *Groundwater hydrographs for the zones S and N of the Bahabad aquifer during 2002-2011*

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Typical values of Sy in each piezometer were determined based on the referred standard values in literature for dominant lithology of the drilling logs (*Table 3*). One-third of this laboratory measurements were considered as the field values of the Sy, because of the air entrapped near the water table, aquifer stratification and slow drainage of materials above the water table (Mcworther and Sunada, 1977). The spatially averaged values of Sy in the zones S (piezometers P1 to P8) and N (piezometers P9 to P14) were estimated to be 0.07 and 0.03, respectively. The values are in the range accepted for most aquifers in Iran.

Table 3. *Estimated values of specific yield at different locations in the Bahabad aquifer*

Piezometer	Dominant soil texture	Laboratory measured specific yield (dimensionless)	Estimated field specific yield (dimensionless)
P1	Gravelly sand	0.25[1]	**0.083**
P2	Gravelly sand	0.25[1]	**0.083**
P3	Gravelly sand	0.25[1]	**0.083**
P4	Gravelly sand	0.25[1]	**0.083**
P5	Medium gravel	0.23[1]	**0.077**
P6	Medium gravel	0.23[1]	**0.077**
P7	Medium gravel	0.23[1]	**0.077**
P8	Clay-gravel and sand	0.12[1]	**0.04**
P9	Clay-gravel and sand	0.12[1]	**0.04**
P10	Clay-gravel and sand	0.12[1]	**0.04**
P11	Silt/clay rich alluvium	(0.05-0.12)[2] 0.08*	**0.027**
P12	Silt/clay rich alluvium	(0.05-0.12)[2] 0.08*	**0.027**
P13	Silt/clay rich alluvium	(0.05-0.12)[2] 0.08*	**0.027**
P14	Silt/clay rich alluvium	(0.05-0.12)[2] 0.08*	**0.027**

[1]Johnson (1967)
[2]Lerner et al. (1990)
*Mean

Change in groundwater storage volume was calculated as the following:

$$\Delta V = A \times Sy \times \Delta H$$

$$\Delta V = \left(235*10^{6}\right) * \left(0.07\right) * \left(-0.65\right) = -11.2*10^{6} m^{3} yr^{-1} = -11.2 \text{ MCM } yr^{-1} \text{Zone S}$$

$$\Delta V = \left(131*10^{6}\right) * \left(0.03\right) * \left(-0.51\right) = -2.02*10^{6} m^{3} yr^{-1} = -2.02 \text{ MCM } yr^{-1} \text{Zone N}$$

These values are consistent with the negative balances calculated by *Equation 1* (-13 MCM yr^{-1} for the zone S and -1.8 MCM yr^{-1} for the zone N), validating aquifer groundwater balance calculations.

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On the basis of groundwater balance study of the Bahabad aquifer in water-year 2010-2011 (*Table 2*), total outputs are exceeding the total inputs and the balance is negative. It confirms the aquifer system is over-drafted and the groundwater storage has diminished, notifying the current withdrawals mainly by abstraction wells are not sustainable.

Safe yield

The negative balance of the Bahabad aquifer necessitates calculation of safe yield to restrict the withdrawals at rates without detrimental effects. So, the safe yield which includes all exploitable groundwater reserves was calculated using *Equation 4*. In order to calculate dynamic groundwater storage (Q_{ed}), the value of water table decline in each piezometer during dry period (June to December) was determined and presented in *Table 4*. It varies from 22 to 76 cm in the zone S and 10 to 44 cm in the zone N. The values of Sy and area of Theissen's polygon (*Fig. 2b*) surrounding the piezometer (effective area) were also defined and Q_{ed} was calculated using *Equation 5*. It was 6.6 MCM yr^{-1} for the zones S and 1 MCM yr^{-1} for the zone N (*Table 4*).

Table 4. *Dynamic groundwater storage (Qed) at the zones S and N of the Bahabad aquifer*

Aquifer zone	Piezometer	Specific yield (dimensionless)	Effective area (m^2)	Average decline of water table in dry season (m)	Dynamic groundwater storage (MCM yr^{-1})
Zone S	P1	0.08	35649648	0.37	1.09
	P2	0.08	30601717	0.22	0.56
	P3	0.08	25119927	0.13	0.27
	P4	0.08	35677301	0.41	1.2
	P5	0.08	19670291	0.47	0.71
	P6	0.08	19122695	0.57	0.84
	P7	0.08	21564947	0.76	1.3
	P8	0.04	21944326	0.38	0.33
	P9	0.04	4826460	0.44	0.09
	P10	0.04	20996703	0.3	0.25
	Sum				***6.6***
Zone N	P9	0.04	13341523	0.44	0.23
	P10	0.04	14342684	0.3	0.17
	P11	0.03	29003460	0.22	0.17
	P12	0.03	20855296	0.32	0.18
	P13	0.03	33940634	0.1	0.09
	P14	0.03	19401768	0.28	0.15
	Sum				***1***

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Volume of groundwater pumped during wet season (Q_{wet}) was calculated based on data from Yazd regional water authority, multiplying discharge rate and operation time of the wells. It was about 9.9 and 2.0 MCM yr^{-1} for the zones S and N, respectively, generally comprises about 31-32% of the total discharge by wells and qanats in these zones.

Total volume of return flows from irrigation and sewage (Q_{rw}) was deduced from aquifer balance calculations (*Table 2*). Based on the following, it was estimated to be 5.8 and 0.65 MCM yr^{-1} for the zones S and N, respectively.

$$Q_{rw} = Irf + Wrf$$

$$Q_{rw} = 3.0 + 2.8 = 5.8 \text{ MCM yr-1} \quad \text{Zone S}$$

$$Q_{rw} = 0.6 + 0.05 = 0.65 \text{ MCM } yr^{-1} \quad \text{Zone N}$$

Finally, the groundwater safe yields for the S and N zones of the Bahabad aquifer were estimated by *Equation 4* as follows:

$$\text{Safe yield} = Q_{ed} + Q_{wet} + Q_{rw}$$

$$\text{Safe yield} = 6.6 + 9.9 + 5.8 = 22.3 \text{ MCM } yr^{-1} \quad \text{Zone S}$$

$$\text{Safe yield} = 1.0 + 2.0 + 0.65 = 3.7 \text{ MCM } yr^{-1} \quad \text{Zone N}$$

From aforementioned estimates (*Table 2*), it is obvious that the total abstractions from Bahabad aquifer in the zones S (30.9 MCM yr^{-1}) and N (6.6 MCM yr^{-1}) are greater than the safe yield. Therefore, the aquifer system has been over-drafted and the groundwater mining has occurred. It proves that the sustainability of the aquifer system is not foreseen under current circumstances. Depletion of groundwater resources and deterioration of the groundwater quality and subsequent soil salinization of the agricultural lands (Zalidis et al., 2002) are the main environmental problems predicted, as a result of continued groundwater over-exploitation. In this regard, the effect of intensive groundwater abstraction by deep and semi-deep agricultural and industrial wells on storage depletion of the Bahabad aquifer is investigated in more details as follows:

Industrial abstractions: As it was previously mentioned, the pumped water by industrial wells is totally transferred out of the groundwater basin area. Therefore, no recharge by return flows from industrial uses is expected. Assuming this water is consumed on the zone S of Bahabad aquifer, having return flow coefficient of about 60% (Iran Water Resources Management Company, 1991), the negative groundwater balance or aquifer budget deficit will be decreased to 9.2 MCM yr^{-1}. However, change in groundwater flow pattern due to the concentrated pumping would still be occurred.

Agricultural abstractions: The specific role of agricultural discharge on depletion of the aquifer storage was investigated by assuming zero discharge for industrial wells in water balance equation. The results show the budget deficit of about 6 MCM yr^{-1} in the aquifer zone S, drawing attention to the more significant role of agricultural abstractions

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in Bahabad aquifer depletion. This is in contrast to the public perception of considering industrial abstractions as the major cause of depletion.

Conclusions and recommendations

The sustainability of groundwater resources of the Bahabad aquifer in central Iran was evaluated by groundwater balance studies and the safe yield analyses. The aquifer is highly discharged by 78 deep and semi-deep wells for agricultural and industrial uses. A closed depression in water table which formed due to concentrated groundwater pumping by the industrial wells has changed groundwater flow patterns, dividing the aquifer system into southern (S) and northern (N) zones. The main inputs (rainfall recharge, inflow through aquifer boundaries, recharge due to return flow from irrigation and sewage) and outputs (outflow through aquifer boundaries, drainage into stream and groundwater withdraw) to/from the aquifer zones were estimated in water-year 2010-2011 (starting at September 23). The rainfall recharge as well as irrigation and municipal return flow coefficients were decided as 5-10, 10-15 and 50-60%, respectively. The lower values are related to the zone N which mainly covered by silt/clay rich alluvium, as it compared with dominant lithology of gravel and sand in the zone S. As the pumping test results are so occasional (only one in the aquifer south), the values of transmissivity and specific yield of the Bahabad aquifer were determined as 5.5-3664.5 m^2/day and 3-8%, respectively, considering aquifer drilling logs and the values prescribed in literature. The Bahabad aquifer is permanently drained by Shoor stream at the north. Baseflow separation of the stream hydrograph yields drainage volume of about 1.1 MCM yr^{-1}.

On the basis of the water balance study, total inputs to the zone S and N are about 20.9 and 6.8 MCM yr^{-1}, lower than the total outputs of about 33.9 and 8.6 MCM yr^{-1}, respectively. Excess of outputs over inputs which is followed by a water table decline of 65 and 51 cm yr^{-1}, respectively, confirms the aquifer system has overexploited. The safe yield which consists all exploitable groundwater resources was calculated about 22.3 MCM yr^{-1} for the zone S and 3.7 MCM yr^{-1} for the zone N. Obviously, total abstractions from Bahabad aquifer in the zones S (30.8 MCM yr^{-1}) and N (6.5 MCM yr^{-1}) are greater than the safe yield. Therefore, the aquifer system is over-drafted and the groundwater mining has occurred. It proves that the sustainability of the aquifer system is not foreseen under current circumstances. The effects of intensive groundwater abstraction by deep and semi-deep agricultural and industrial wells on storage depletion of the Bahabad aquifer was investigated which highlights, in contrast to the public opinions, the important role of agricultural withdrawals.

This research clarifies the role of both industrial and agricultural abstractions in depletion of Bahabad aquifer. It is evident that the present exploitations are not sustainable and the management of this aquifer is not safe. Therefore, safe yield as a managing tool must be considered avoiding groundwater overexploitation and aquifer mining with negative consequences for the development of the region. For the aquifer system of the study area, the safe yield can be attained by a decrease in groundwater abstractions, increase in recharge or a combination of both. In order to achieve the safe yield, the following recommendations are proposed:

1. Total abstractions for use in both agricultural and industrial sectors must be reduced. In this regard improvement or change of traditional irrigation systems can be effective to reduce groundwater consumption. Industrial abstractions must be

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immediately decreased at least 50%, because their values are currently about two times more than the permissible limits planned by local water authorities. In a similar international problem, management strategies including application of water-saving methods such as spray and drip irrigation were proposed to solve water balance disequilibrium of a coastal aquifer system in Greece (Voudouris, 2006).

2. Aquifer recharge must be managed by reliable methods to increase the aquifer storage. In this regard, artificial recharge projects should be developed in the region to balance the quantity of water exported for industrial uses, having no return flows to the aquifer system. Renovation of the performed projects by removing fine sediments accumulated on the surface of the recharge basins to enhance the infiltration rate is also recommended. Field tests in other places (Hionidi et al., 2002; Voudouris, 2006) confirmed the positive impacts of artificial recharge on augmentation of aquifer storage and improvement of the groundwater quality.

3. Industrial wells should be distributed throughout the aquifer system, avoiding local changes in the flow dynamics. Changes in flow direction due to the closed depression formed by industrial well-field will enhance the intrusion of the more saline groundwater of the northern part and deteriorate quality of the aquifer. An increasing trend of electrical conductivity (EC) from 3000 μmhos/cm to 3800 μmhos/cm is observed in a well located at the north of the well-field during a period of 10-year from 2001 to 2011 (Shirafkan, 2013). Other impacts such as ground settlement (Chen et al., 2003) would also be prevented.

A new research conducted in the study area after completion of the present work (Khatibinia, 2016) revealed continuity in groundwater level decreasing with a measured rate of about 0.5 m yr^{-1}. It forces immediate actions in implication of the appropriate managing strategies like mentioned above, in order to prevent groundwater mining and further depletion of the Bahabad aquifer.

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APPENDIX

Basic data used in drawing the groundwater hydrographs (Fig. 4)

Aquifer Zone	Water-year	Spatially averaged water table height (m) above mean sea level											
		October	November	December	January	February	March	April	May	June	July	August	September
Zone S	2002-2003	-	-	-	-	-	-	1356.0	1356.0	1356.0	1355.7	1355.6	1355.5
	2003-2004	1355.5	1355.5	1355.5	1355.4	1355.4	1355.3	1355.1	1355.0	1354.9	1354.9	1354.8	1354.9
	2004-2005	1354.9	1354.9	1354.9	1354.8	1354.7	1354.5	1354.0	1354.5	1354.3	1354.0	1354.0	1353.9
	2005-2006	1353.7	1353.8	1353.9	1353.9	1353.8	1353.7	1353.4	1353.6	1353.3	1353.2	1353.2	1353.0
	2006-2007	1352.8	1352.7	1352.7	1352.6	1352.6	1352.4	1352.5	1352.5	1352.4	1352.3	1352.5	1352.6
	2007-2008	1352.7	1352.9	1353.0	1353.0	1352.9	1352.6	1352.6	1352.6	1352.4	1352.1	1352.0	1351.6
	2008-2009	1351.5	1351.4	1351.7	1352.0	1352.1	1352.1	1352.0	1352.0	1352.0	1351.9	1351.9	1351.8
	2009-2010	1351.7	1351.6	1351.6	1351.5	1351.4	1351.4	1351.3	1351.3	1351.3	1351.5	1351.1	1351.2
	2010-2011	1351.1	1351.0	1350.9	1350.9	1350.9	1350.9	1350.7	1350.8	1350.7	1350.6	1350.6	1350.5
	2011-2012	1350.6	1350.5	1350.5	1350.4	1350.4	1350.4	-	-	-	-	-	-
Zone N	2002-2003	-	-	-	-	-	-	1331.6	1331.6	1331.6	1331.5	1331.5	1331.4
	2003-2004	1331.4	1331.4	1331.4	1331.4	1331.4	1331.3	1331.3	1331.2	1331.1	1331.0	1331.0	1330.8
	2004-2005	1330.9	1331.0	1331.1	1330.9	1330.8	1330.7	1330.4	1330.7	1330.5	1330.4	1330.3	1330.3
	2005-2006	1330.3	1330.3	1330.4	1330.3	1330.3	1330.2	1330.8	1330.6	1330.0	1329.9	1329.9	1329.9
	2006-2007	1329.8	1329.8	1329.8	1329.6	1329.6	1329.3	1329.3	1329.3	1329.1	1329.0	1329.1	1329.2
	2007-2008	1329.3	1329.4	1329.4	1329.3	1329.4	1329.2	1329.3	1329.2	1329.0	1328.8	1328.7	1328.4
	2008-2009	1328.2	1328.1	1328.6	1328.8	1328.9	1328.8	1328.7	1328.7	1328.6	1328.6	1328.6	1328.5
	2009-2010	1328.4	1328.4	1328.4	1328.4	1328.3	1328.3	1328.2	1328.1	1328.1	1328.3	1328.0	1328.1
	2010-2011	1328.0	1328.0	1327.9	1327.9	1327.9	1327.9	1327.8	1327.7	1327.7	1327.6	1327.6	1327.5
	2011-2012	1327.6	1327.5	1327.5	1327.5	1327.4	1327.4	-	-	-	-	-	-

Water-year	Precipitation depth (mm)											
	October	November	December	January	February	March	April	May	June	July	August	September
2002-2003	0	21	3	20	13	17	8.5	3	0	0	0	0
2003-2004	0	0	7	16.5	13	7.5	13.5	0	0	5.5	0	0
2004-2005	0	2	22.5	40.5	9	24	6	3.5	0	0	1	0
2005-2006	0	3.5	2	20	5	6	10.5	5.5	0	0	0	0
2006-2007	0	2.5	17	11	20	19.5	17.5	0	0	0	0	0
2007-2008	0	1	0	34.5	4.5	0	0	0	0	0	0	0
2008-2009	0	20	21	0	16.5	18	38	7	0	0	0	0
2009-2010	0	5	27.5	1	8.5	7	4.5	4.5	0	0	0	0
2010-2011	0	0	0	7.5	14	2	6.5	0	0	0	0	0
2011-2012	-	-	-	-	-	-	-	-	-	-	-	-

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A TEMPORAL VARIATION OF LEWIS–KOSTIAKOV COEFFICIENTS EQUAL IN DIFFERENT FURROW IRRIGATION MANAGEMENTS IN SUGAR BEET (*BETA VULGARIS* L.) CULTIVATION IN KARAJ, IRAN

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Abstract. Surface irrigation, including border, basin and furrow irrigation is used more than pressurized irrigation due to the low cost and energy requirement. However, more precise selection of design parameters for efficient design of surface irrigation is required for higher irrigation efficiency, and the accurate prediction of the infiltration rate is of prime importance. Field data sets for irrigation events during sugar beet growing season were collected for characterizing infiltration properties in alternate, fixed and conventional furrow irrigation (AFI, FFI and CFI, respectively), carried out on a clay loam soil in Karaj, Iran. The treatment was applied in two consecutive years (2013 and 2014) as randomized complete block design with three replications. Therefore, this research was conducted to determine the parameters of Lewis–Kostiakov infiltration model based on the data obtained in advancing stage and two-pointed method. Less runoff losses and large infiltration rate observed in AFI as compared to CFI due to increased lateral water movement to non-irrigation furrow. Having different infiltration characteristics in AFI, design will be different from CFI in the same field. Results indicated that the final soil infiltration was $1.6*10^{-4}$ $m^3/m/min$ at the beginning of the growing season and decreased in CFI, FFI and AFI management system by 44, 38 and 30% respectively, at the end of growing season. The amount of 'a' coefficient variations in Kostiakov–Lewis model, showed a decreasing trend during the growing season, but the 'k' coefficient had an incremental trend in CFI, FFI and AFI irrigation management system. AFI had greater infiltration rate than FFI, the steady infiltration rate in AFI and FFI was higher than CFI. Consequently, alternate furrow irrigation management with reduced drainage achieved maximum water use efficiency (WUE).

Keywords: *furrow irrigation, final infiltration, drainage, surface irrigation, irrigation management*

Introduction

Water sources suitable for utilization are on the decline in many parts of the world. Therefore, appropriate irrigation management plays a great role in the quality and quantity of these sources, and can help in realizing production potential and in obtaining high yields through reducing runoff losses and by delivering the required water to the root zone (Sepaskhah and Afshar-Chamanabad, 2002). Surface irrigation is used more than pressurized irrigation due to low cost and energy requirements. Innovative surface irrigation techniques are used for higher irrigation efficiency, among which alternate

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furrow irrigation decreases deep percolation, thereby increasing water use efficiency (Sepaskhah and Kamgar-Haghighi, 1997).

The major problem in surface irrigation is its low efficiency resulting from poor irrigation management (Khatri and Smith, 2006). The design, evaluation and simulation of surface irrigation systems dependent on a knowledge of infiltration properties of the soil and the movement of water over the field. Infiltration affects the advance, recession, runoff, and volume of infiltration during irrigation (Fonteh and Podmore, 1993). Spatial variation of infiltration rates makes the management of surface irrigation systems a very complex process (Rasoulzadeh and Sepaskhah, 2003). Infiltration is one of the most important parameters for designing surface irrigation systems, mainly for furrow irrigation (Walker and Skogerboe, 1987). It has a significant effect on water flow and solute transport in the soil surface and subsurface (Ebrahimian, 2014). Quantification of water infiltration into a soil is of great significance in the management of watersheds (Mukheibir, 2008). The performance of the mathematical models to simulate overland flow in fields within surface irrigation systems is dependent on the accuracy of estimation of infiltration parameters. Initial reduction in infiltration is related to the physical process of slaking to the development of an apedal, harvesting surface soil layer (Emdad et al., 2004). There are numerous methods for estimation of infiltration parameters in surface irrigation based on input data requirements, infiltration equations and solution methods (Bautista and Walker, 2010).

Gillies et al. (2007) reported that high irrigation efficiencies in surface irrigation were not out of reach if correct irrigation management was exercised and temporal and spatial changes in soil characteristics were considered. However, precise design for surface irrigation is required for higher irrigation efficiency and accurate prediction of the infiltration rate is of prime importance (Zerhun et al., 1996). There are two types of alternate furrow irrigation: 1) Alternate Furrow Irrigation (AFI) in which irrigated furrow (or non-irrigated furrow) is alternatively changed for every irrigation, and 2) Fixed Furrow Irrigation (FFI) in which irrigated furrow (or non-irrigated furrow) is fixed for every irrigation. This irrigation method has been known to improve water productivity in agricultural lands especially in arid and semi-arid regions (Ebrahimian et al., 2012). Application of alternate furrow irrigation would be expected to alleviate water shortages due to rapid population and decreasing per-capita water. Infiltration rate in alternate (every-other) furrows would be different from conventional furrows in the same field (Gillies and Smith, 2005).

Application of AFI can lead to increased lateral infiltration and decreased deep percolation, which will ultimately result in improved irrigation efficiency (Dialameh et al., 2017).

Slatni et al. (2011) also showed that the CFI treatment had the highest infiltration per unit area as compared to AFI and FFI. Alternate furrow irrigation succeeded in reducing infiltration in the experimental conditions, thus potentially reducing deep percolation losses. Field investigation and modeling on the infiltration process in alternate furrow irrigation would be helpful to reach the precise design of this kind of furrow irrigation system.

Many researchers used the Lewis–Kostiakov equation for infiltration in furrow irrigation and proposed methods for determination of its parameters (Scaloppi et al., 1995). Consequently, several equations have been proposed to describe furrow infiltration as a function of effectual factors on it. The commonly used equations are those proposed by Kostiakov and Kostiakov–Lewis (Nasseri and Neyshabori, 2006).

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Neshat and Parehkar (2007) showed in their research that the Lewis–Kostiakov model was the most suitable model for estimating cumulative infiltration and infiltration rate. Yongyong et al. (2012), determine the effectual infiltration variables, to evalute the performance of four classical infiltration models. The performance of four infiltration models (Philip model, Kostiakov–Lewis model, Kostiakov model, and Horton model) was investigated on the basis of evaluation indices. The Kostiakov–Lewis infiltration model with three parameters provided the best description of the relationship between cumulative infiltration and time.

The objective of this research was to determine the parameters of Lewis–Kostiakov infiltration equation in two types of alternate furrow irrigation (AFI and FFI) and conventional furrow irrigation in several irrigation events during sugar beet growing season. For this purpose, two-pointed method (Elliott and Walker, 1982) used to estimate the infiltration parameters of the Lewis–Kostiakov equation.

Material and methods

Field experiments of furrow irrigated sugar beet were conducted at the research field of the Kamalabad station of the Sugar Beet Research Institute in Karaj, Iran, at 50°55'E longitude, 35°55'N latitude and 1313 m altitude during 2013 and 2014 growing season of sugar beet. Climate in this region is semi-arid with total annual precipitation of 265 mm. The soil in this area no salinity and drainage problems such as water table. Some physical properties of the experimental field soil are presented in *Table 1,* and some chemical irrigated water quality properties are shown in *Table 2.*

Table 1. *Some physical properties of experimental field soil*

Depth cm	Clay %	Silt %	Sand %	Texture	Bulk density gr/cm^3	Field capacity %	Permanent wilting point %
0-30	31.4	42	26.6	Clay loam	1.47	27.4	14
30-60	34.6	27.4	38	Clay loam	1.42	26.6	14.9

Table 2. *Chemical properties of irrigation water at the study area*

HCO_3 (mg/l)	CO_3 (mg/l)	Cl (mg/l)	K (mg/l)	Na (mg/l)	Mg (mg/l)	Ca (mg/l)	EC[1] (dS/m)	SAR[2]	pH
146	0	0.8	0.8	62	16	23	0.5	2.2	8

[1]Electrical conductivity
[2]Specific absorption rate

The soil at the trial site up to 60 cm deep (effective depth for sugar beet root development), a uniform Clay loam soil texture with EC=0.58 dS.m^{-1}, that does not limited growing plants. The soil has a high maintenance capacity that suitable for sugar beet cultivation.

Also water irrigation quality in term of salinity was suitable (0.5 dS.m^{-1}) and not limited for sugar beet cultivation.

The experiments were conducted in the same field for the 2-year period. The experimental design was a randomized block with three replications. Each plot consists

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of 12 row of sugar beet that was 90 m long and 0.5 m wide, the slope of furrow was about 0.00019 $m.m^{-1}$. Sowing density was 3-6 plants per meter.

The design consisted of three irrigation methods. The irrigation methods were alternate furrow irrigation (AFI), fixed furrow irrigation (FFI) and conventional furrow irrigation (CFI).

Outflow rate from the furrows was measured employing a Washington State College (WSC) flume (type II) that was put at the end of the furrow. The inflow rate was measured for each furrow using the volumetric method. The inflow-outflow hydrograph method was employed to calculate the final infiltration rate, which was obtained from the following equation (*Eq. 1*) when a constant outflow rate was reached (Fattah and Upadhyaya, 1996):

$$f_0 = \frac{Q_{in} - Q_{out}}{L} \quad \text{(Eq.1)}$$

Where, Q_{in} is the inflow rate in m^3/min, Q_{out} the outflow rate in m^3/min, L the length of the furrow in m, and f_0 base infiltration rate in m^3/m/min. The volume of infiltrated water was calculated using the inflow and outflow volumes, and the Lewis–Kostiakov equation was employed to obtain the infiltration relation, which was as follows (*Eq. 2*):

$$Z = kt^a + f_0 t \quad \text{(Eq.2)}$$

where, Z is the volume of infiltrated water per unit length of the furrow in m^3/m, f_0 final infiltration rate into the soil in m^3/m/min, t time in minutes, and 'a', 'k' the empirical coefficients of the equation. The two-point method introduced by Elliott and Walker (1982) was employed to calculate the empirical parameters in the Lewis–Kostiakov equation of infiltration. For this purpose, furrows were marked with stakes at 10 m intervals, and the time water reached each station was recorded using a chronometer.

Walker (1989) used the empirical equation describing the advance curve in furrow irrigation to determine the infiltration parameters (k; a) as follows (*Eq. 3*):

$$x = pt^r \quad \text{(Eq.3)}$$

where, x is the distance from the beginning of the furrow in m, t time in minutes, and r, p coefficients of the equation can be evaluated from the two advance points and a simple logarithmic transformation of the power curve equation. The coefficients a, k, in the Lewis–Kostiakov equation can be calculated by using the water balance method in the furrow considering its two middle and end points. The coefficient "a" can be calculated from the following equation (*Eq. 4*):

$$a = \frac{\ln \frac{V_L}{V_{0.5L}}}{\ln \frac{t_L}{t_{0.5L}}} \quad \text{(Eq.4)}$$

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Where t_L and $t_{0.5L}$ are the time water reaches the end and middle of the furrow, respectively, and V_L and $V_{0.5L}$ are determined using the following equations (*Eqs. 5* and *6*):

$$V_L = \frac{Q_0 t_L}{L} - \delta_y A_0 - \frac{f_0 t_L}{1+r} \quad \text{(Eq.5)}$$

$$V_{0.5L} = \frac{2Q_0 t_{0.5L}}{L} - \delta_y A_0 - \frac{f_0 t_{0.5L}}{1+r} \quad \text{(Eq.6)}$$

where the inflow rate into the furrow is in m^3/min, A_0 is the cross section of the inflow (which, in this study, was measured in the field), and δ_y the flow shape factor was considered to be 0.8. The coefficient "k" was determined using *Equation 7:*

$$k = \frac{V_L}{\delta_z t_L^a} \quad \text{(Eq.7)}$$

Where δ_z is the shape factor for infiltration into the furrow that is determined from the following equation (*Eq. 8*):

$$\delta_z = \frac{a + r(1-a) + 1}{(1+a)(1+r)} \quad \text{(Eq.8)}$$

After the parameters in the equation of water infiltration into the furrows were determined, the volume of infiltrated water at various distances from the beginning of the furrow was calculated in irrigations. Infiltration opportunity at any time T_n in minutes was determined using *Equation 9:*

$$T_n = T_r - T_a \quad \text{(Eq.9)}$$

Where, T_a and T_r are the water advancing and retreating time in the furrow, respectively, which were measured in the field in this research. The volumes of water infiltrated at specific points in the furrow were determined by putting the infiltration opportunities in the equation of infiltration into the furrow.

The amount of irrigation water, in the basis of soil moisture in depths of 0-30 and 30-60 cm was measured, and amount of water demand in each layer in the region of plant root relation was obtained by *Equation 10*.

$$dn = \Sigma \left(\theta fci - \theta i\right)\Delta z \quad \text{(Eq.10)}$$

where dn is the net irrigation depth (m), θfci and θi are the volumetric soil water contents in layer i at field capacity and before irrigation, respectively (m^3/m), Δz is the soil layer thickness (m) and n is the number of soil layers. In the first year 13 irrigations were done and second year 11 irrigations were applied.

The objective of this research was to determine the parameters of Lewis–Kostiakov infiltration equation in AFI, FFI and CFI with irrigation events. Various models were

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fitted to the measured values and were studied the various management in order to determine the trend of changes in final infiltration with irrigation events.

Results

ANOVA of final infiltration rate is shown in *Table 3*. ANOVA was significant for irrigation events.

Table 3. *ANOVA of final infiltration rate*

Year	Treatment	df	Sum of squares	MS	F-value	Sig.
2013	CFI	38	1.36×10^{-8}	9.77×10^{-10}	117.87	<0.0001**
	FFI	38	1.64×10^{-8}	1.17×10^{-9}	89.04	<0.0001**
	AFI	21	8.9×10^{-9}	6.2×10^{-10}	97.11	<0.0001**
2014	CFI	32	1.9×10^{-8}	1.59×10^{-9}	81.65	<0.0001**
	FFI	32	2.1×10^{-8}	1.73×10^{-9}	277.3	<0.0001**
	AFI	18	1.99×10^{-8}	1.64×10^{-9}	93.65	<0.0001**

**Significant in 1% level

The effect of irrigation events on final infiltration rate significant, and the result of Duncan's multiple range test showed significant differences ($t < 0.001$) on three managements.

The amount of final infiltration rate in three irrigation managements is shown in *Table 4*.

Table 4. *Total number of final infiltration rate*

Year	Treatment	Irrigation Event												
		1	2	3	4	5	6	7	8	9	10	11	12	13
2013	CFI	0.000161^{a}	0.000115^{b}	0.000109^{c}	0.000106^{cd}	0.000102^{d}	0.000093^{ef}	0.000093^{ef}	0.000092^{ef}	0.000090^{ef}	0.000092^{f}	0.000092^{f}	0.000091^{ef}	0.000091^{f}
	FFI	0.000168^{a}	0.000157^{b}	0.000120^{c}	0.000107^{d}	0.000106^{d}	0.000104^{d}	0.000108^{d}	0.000105^{d}	0.000104^{d}	0.000107^{d}	0.000108^{d}	0.000104^{d}	0.000106^{d}
	AFI	0.000166^{a}	-	0.000135^{b}	-	0.000122^{c}	-	0.000116^{d}	-	0.000118^{e}	-	0.000111^{f}	-	0.000118^{e}
2014	CFI	0.000166^{a}	0.000147^{b}	0.000131^{c}	0.000108^{d}	0.000103^{de}	0.000095^{ef}	0.000097^{ef}	0.000097^{ef}	0.000096^{ef}	0.000092^{f}	0.000093^{f}	-	-
	FFI	0.000176^{a}	0.000175^{a}	0.000144^{b}	0.000128^{c}	0.000114^{d}	0.000112^{de}	0.000111^{de}	0.000110^{de}	0.000110^{de}	0.000110^{de}	0.000108^{e}	-	-
	AFI	0.000161^{a}	-	0.000133^{b}	-	0.000120^{c}	-	0.000114^{d}	-	0.000110^{e}	-	0.000109^{e}	-	-

Mean values with the same superscript letters (a, b, c, d or e) were similar and no statistically significant differences were observed for these samples

Figure 1 shows trends of variation in final infiltration rate into the soil in the various irrigation management during the season with irrigation events in two years.

As shown in *Table 4,* the final infiltration rate decreased at end of the season in all management with logarithmic model. In first year, the final infiltration rate the start of the period $1.6*10^{-4}$ and decreased by 44, 31 and 25% in CFI, FFI and AFI management and in second year, the final infiltration rate the start of the period $1.7*10^{-4}$ and decreased by 45, 37 and 36% in CFI, FFI and AFI management. Finally, *Table 4* shows that final infiltration rate was decreased by 44, 34 and 30% in CFI, FFI and AFI management. Emdad et al. (2004) show that final infiltration rate was decreased 40% at the end of the season in clay loam soil texture.

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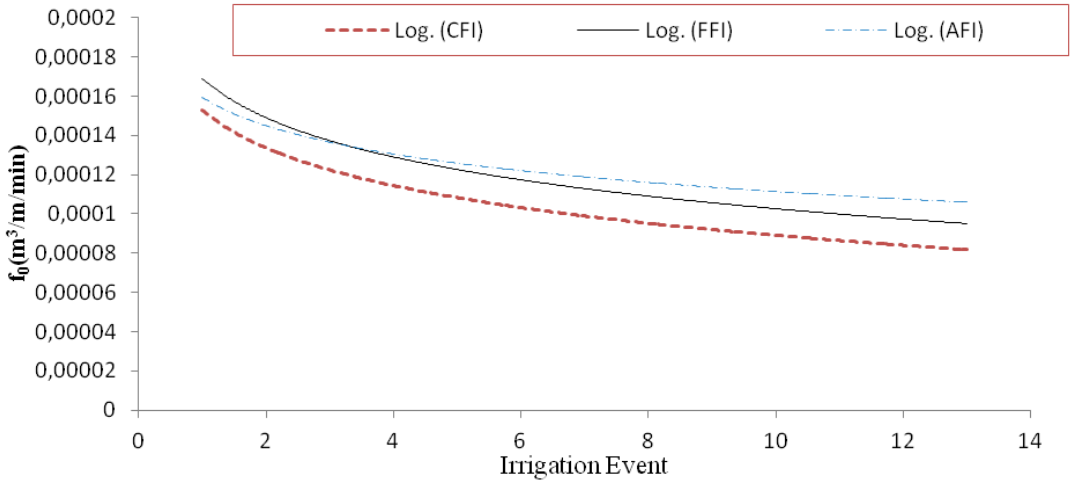


Figure 1. *Final infiltration rate as a function of irrigation event (average of two years)*

Various models were evaluated to determine the best regression fit between values of final infiltration rate and irrigation events. Models were evaluated by SAS software and their significant was investigated. The logarithmic model, presented below, was the best regression model with the highest correlation coefficient (*Eqs. 11-13*):

$$f_{0CFI} = -0.000028ln(\mathrm{IE}) + 0.000153 \qquad R^2 = 0.85 \qquad \text{(Eq.11)}$$

$$f_{0FFI} = -0.000029ln(\mathrm{IE}) + 0.000169 \qquad R^2 = 0.79 \qquad \text{(Eq.12)}$$

$$f_{0AFI} = -0.000021ln(\mathrm{IE}) + 0.000159 \qquad R^2 = 0.91 \qquad \text{(Eq.13)}$$

Where f_{0AFI} is final infiltration rate in AFI management (m^3/m/min), f_{0FFI} is final infiltration rate in FFI management (m^3/m/min), f_{0CFI} is final infiltration rate in CFI management (m^3/m/min) and IE is irrigation event.

Regression analysis showed that there was a logarithmic relationship between final infiltration rate and irrigation event, which is a good function and significant (*Fig. 1*).

ANOVA of 'a' parameter in the Lewis–Kostiakov equation is shown in *Table 5*. ANOVA was significant for irrigation events. The effect of irrigation events on 'a' parameter in the Lewis–Kostiakov equation significant, and the result of Duncan's multiple range tests showed significant differences ($t < 0.001$) on three managements.

ANOVA of 'k' parameter in the Lewis–Kostiakov equation is shown in *Table 6*. ANOVA was significant for irrigation events. The effect of irrigation events on 'k' parameter in the Lewis–Kostiakov equation significant, and the result of Duncan's multiple range test showed significant differences ($t < 0.001$) on three managements.

Table 5. *ANOVA of 'a' parameter in the Lewis–Kostiakov equation*

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Year	Treatment	df	Sum of squares	MS[1]	F-value	Sig.
2013	CFI	38	0.225	0.016	10.53	<0.0001**
	FFI	38	0.324	0.023	9.44	<0.0001**
	AFI	21	0.198	0.014	9.51	<0.0001**
2014	CFI	32	0.601	0.050	28.12	<0.0001**
	FFI	32	0.592	0.049	8.84	<0.0001**
	AFI	18	0.809	0.067	61.4	<0.0001**

[1]Mean square
**Significant in 1% level

Table 6. *ANOVA of 'k' parameter in the Lewis–Kostiakov equation*

Year	Treatment	df	Sum of squares	MS	F-value	Sig.
2013	CFI	38	0.00090	0.000070	12.87	<0.0001**
	FFI	38	0.00089	0.000062	25.06	<0.0001**
	AFI	21	0.00084	0.000095	23.50	<0.0001**
2014	CFI	32	0.00087	0.000072	16.87	<0.0001**
	FFI	32	0.00087	0.000072	23.58	<0.0001**
	AFI	18	0.00107	0.000090	21.75	<0.0001**

**Significant in 1% level

Changes in infiltration parameters in the Lewis–Kostiakov equation were slight so that when the values of 'a', 'k' were studied in one growing season, it was observed that they declined very little. The related results are presented in *Figures 2* and *3,* and the obtained equations are as follows:

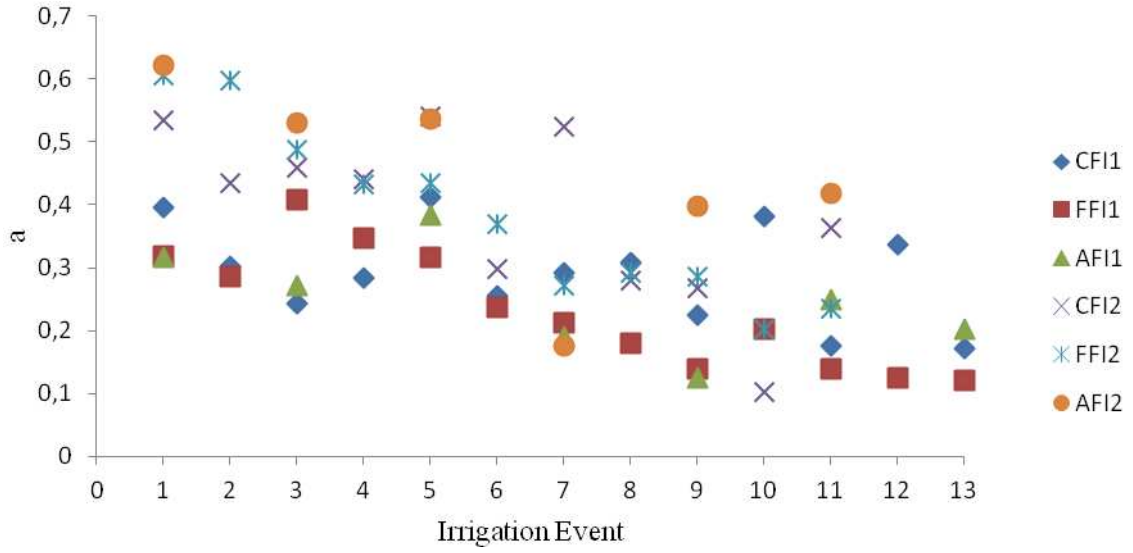


Figure 2. *Lewis–Kostiakov parameters 'a' as a function of irrigation event*

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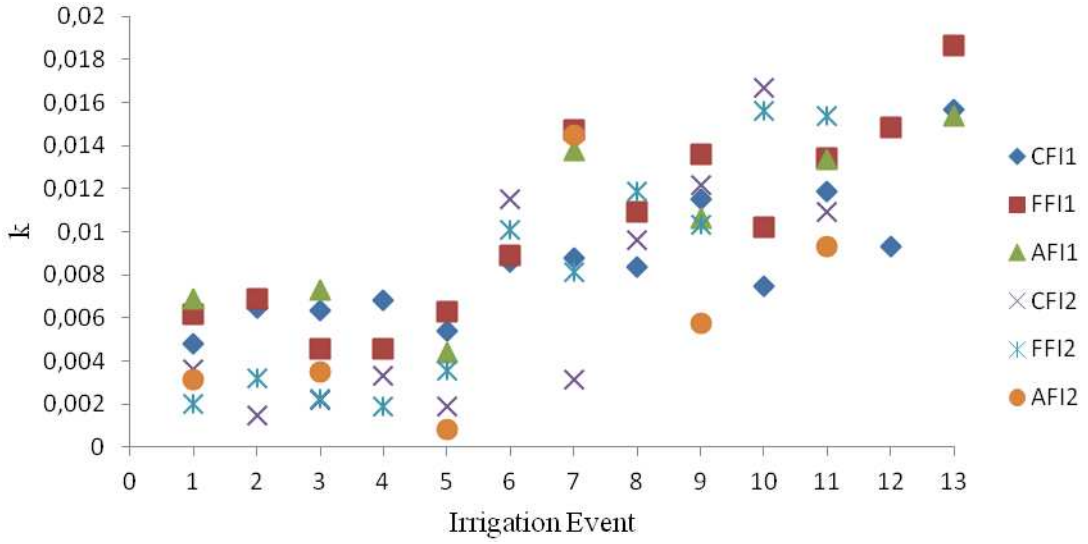


Figure 3. *Lewis–Kostiakov parameters 'k' as a function of irrigation event*

Amount of 'a' coefficient variations in Kostiakov–Lewis model, showed a decreasing trend during the growing season, but the 'k' coefficient had an incremental trend in CFI, FFI and AFI irrigation management system. The average value for 'k' tended to decrease with time during the season for all treatments. Indicated that 'k' and "a" were each significantly different between the early and late season period.

Discussion

The results of this study showed that at the beginning of the cultivating period, the final soil infiltration, due to increasing soil porosity by plowing increases too. Some soil physical properties, such as porosity, decrease due to the surface irrigation and low soil aggregate stability, which directly affects the hydraulic conductivity of saturated soil, in other words, it affects the final soil infiltration, therefore as a result, the final infiltration rate decreases (Slatni et al., 2011). Results show changes in final infiltration rate into the soil followed a descending trend. Soil compaction (subsidence) and destruction of the surface layer (disintegration of aggregates and surface crust formation) were the reasons for the decline in final infiltration rate during the period. Compaction and destruction of soil surface layer, together with decreased aggregate stability and crust formation, reduced final infiltration into the soil.

After the first irrigation, soil aggregate stability decreases due to the surface irrigation by formation of a crack on the surface of the soil. The simultaneous effect of these two factors causes a significant reduction in the final soil infiltration, therefore final soil infiltration according to *Figure 1* is close to a constant limit. final soil infiltration was 1.6×10^{-4} $m^3/m/min$ at the beginning of the growing season in the first year and decreased in CFI, FFI and AFI management system by 44, 37 and 29% respectively, at the end of growing season. Similarly, in the second year, the final soil infiltration at the beginning of the season was 7.4×10^{-4} $m^3/m/min$, which was decreased, by 44, 39 and 32%, at the end of growing season, respectively. Parlange et al. (1982) showed that in furrow irrigation cropping system, the water infiltration rate during the

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first irrigation immediately following cultivation can often be markedly higher than later irrigation.

The AFI management showed the lowest infiltration losses, as compared to FFI and CFI. The infiltration rates in final irrigation were generally smaller in CFI than FFI and AFI. These results are in agreement with those by Sepaskhah and Afshar-Chamanabad (2002), who reported that the infiltration rate in the irrigated furrow of the FFI treatment was higher than in CFI furrows. As a consequence, the advance time would be higher in alternate furrows than in conventional furrows. Slatni et al. (2011), reported that the CFI treatment showed highest infiltration per unit area, compared to AFI and FFI. In this study, the infiltration per unit area was also higher for CFI than for both AFI and FFI management. However, infiltration per irrigated furrow was higher for AFI and FFI than for CFI, due to increased lateral water movement towards non-irrigated furrows.

In this research, indicated that 'k' and 'a' were significantly different between the early and late season period. Results of research conducted by Raine and Mcclymont (1997) and Zapata and Playan (2000) conform to those found in our research.

There was not a particular trend in the amount of coefficient changes during the crop growth period. In other words, the amount of 'a' was not predictable by the model, because of the existence of small seams in heavy soil texture which is an important factor in disturbing of time variation of 'a' coefficient during plant growth periods. According to the results of Hunsaker et al. (1993), Changes in 'a' coefficient have not shown conformity with logarithmic or linear model.

Although the 'k' coefficient is an experimental coefficient which is affected by the initial moisture (before irrigation) (Philip, 1957). Therefore, if in the various irrigation, the initial moisture content is approximately the same, the 'k' coefficient is expected to remain almost constant. The results showed that 'k' coefficient correlates with the failure of specific trend or with the irrigation that has been carried out at different initial moisture levels.

Therefore 'k' and 'a' coefficients of Lewis–Kostiakov equation fixed in estimation equation and amount is 0.1-0.5 for 'a' and 0.001-0.01 for 'k'.

Finally, cumulative infiltration decreased during plant growth season. Therefore to achieve proper management with considerable seasonal decrease of infiltration, time of irrigation must be increased.

AFI was successful in reducing infiltration in the experimental conditions, thus potentially reducing deep percolation losses. Also application efficiency increased in AFI management. Alternate furrow irrigation management in sugar beet may be feasible for water saving and reducing drainage. It can be concluded that using alternate furrow irrigation is a good water management technique to protect the environment by reducing runoff and increase water application efficiency (AE).

Conclusions

The results of this research showed that there were not specific trend changes in the values of the 'k' and 'a' coefficients in Lewis–Kostiakov penetration equation in different irrigation intervals during the growth periods. The initial penetration rate had a decreasing trend, which was predictable from logarithmic models.

Final infiltration rate decreased in CFI, FFI and AFI management system by 44, 38 and 30% respectively, at the end of growing season. High infiltration and steady infiltration rate in alternate furrows were found in this study. More horizontal water

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movement to non-irrigated furrows could establish this large difference between alternate and conventional furrow irrigation managements. Having different infiltration characteristics in alternate furrows, design variables of alternate furrow irrigation will be different from conventional furrow irrigation in the same field. This difference is even existed between AFI and FFI systems because of different infiltration characteristics. Therefore, the design and management of such irrigation methods should differ from conventional furrow irrigation. Field experiments indicated the AFI management could reduce large runoff losses in every irrigation events during sugar beet growing season.

Alternate furrow irrigation management in sugar beet may be feasible for water saving and reducing drainage. It can be concluded that using alternate furrow irrigation is a good water management technique to protect the environment by reducing runoff and increase water application efficiency (AE).

The current study has been done in clay loam texture soil and it might be different in other soil mixtures. Therefore, it is recommended that further experiments can be implemented in various soil textures and furrow lengths to evaluate infiltration parameters on irrigation managements.

Acknowledgments. The research was supported by the Sugar Beet Seed Institute.

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SPATIO-TEMPORAL DISTRIBUTIONS AND TRENDS OF AEROSOL PARAMETERS OVER PAKISTAN USING REMOTE SENSING

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Abstract. Aerosols play a major role in atmospheric solar radiation budget, air quality degradation and climate change. In order to accurately evaluate aerosols effects, we require an in-depth knowledge of spatio-temporal distributions of their parameters. The focus of this paper is to analyze spatio-temporal distributions and trends of aerosol parameters over Pakistan. In present study, Terra-MODIS data have been used during April 2000-March 2014 to analyze AOD (550 nm), Angstrom exponent (470-660) ($AE_{470-660}$), small mode fraction (SMF) and mass concentration (MC). Values of coefficient of determination (R^2) between Terra-AOD and AERONET-AOD are found to be 0.718 and 0.745 for Lahore and Karachi respectively. Terra-AOD has also been compared with other satellite-based observations obtained from Aqua, MISR and SeaWiFS. A notable spatial variability in mean annual Terra-AOD values (0.15-0.87) has been observed with high values (~0.8) over northeastern and southern Pakistan. $AE_{470-660}$ ranges from 0.56 to 1.44 showing high values (greater than 0.8) over northern Pakistan and relatively low values (less than 0.8) over southern Pakistan. MC is found to be in the range from 7.6 μg/cm^2 to 56.2 μg/cm^2 with spatial distribution quite similar to AOD. Overall mean values of AOD, $AE_{470-660}$, SMF and MC are found to be 0.43 ± 0.16, 0.68 ± 0.06, 0.11 ± 0.05 and 28.2 ± 9.71 μg/cm^2 respectively.
Keywords: *AOD, Angstrom exponent, SeaWiFS, small mode fraction*

Introduction

Aerosols contribute to atmospheric climate forcing, air quality degradation, hydrological cycle and precipitation processes (Charlson et al., 1992; Ramanathan et al., 2001; Lohmann and Feichter, 2005; Kosmopoulos et al., 2008). They are also recognized as a major factor that affects the global climate change (IPCC, 2007). It is well established fact that aerosol particles affect the climate directly by scattering and absorption of incoming solar radiation and indirectly by serving as cloud condensation nuclei (Forster et al., 2007; Carslaw et al., 2010; Arneth, A. et al., 2010; Mahowald, 2011). All types of aerosols except black carbon cause cooling at top of the atmosphere and reduce the warming caused by greenhouse gases (Forster et al., 2007). In order to achieve better air quality, reduction in anthropogenic emissions may weaken this useful effect (Arneth, et al., 2009; Mickley et al., 2012; Makkonen et al., 2012).

Aerosols also have harmful effects on human health and ecosystem services (Smart et al., 2011). Long term and detailed studies have shown a significant relationship between aerosol concentrations and human health. The studies have been conducted over various regions of the world and the results have established considerable effects on human health due to aerosols. Due to human health and environmental implications, knowledge of properties and spatio-temporal distribution of aerosols is essential.

Insufficient observations of aerosols have caused dominant uncertainties in the climate modeling (Koukouli et al., 2010; Bennouna et al., 2013). In order to accurately

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evaluate the climate forcing, an in-depth knowledge of optical and physical properties of aerosols at different spatial and temporal scales is required (He et al., 2012; Luo et al., 2014). Different types of aerosols affect the sign and magnitude of climate forcing distinctively (Kaskaoutis et al., 2007). For instance, black carbon aerosols emitted from biomass burning cause positive climate forcing (Jacobson, 2001; Penner et al., 2003), while mineral dust particles show negative forcing at shortwave by scattering the solar radiations (Sokolik and Toon, 1996).

Although ground based observations provide more detailed and authentic information about the atmospheric aerosols, they do not have large-scale spatial coverage (Tariq and Ali, 2015; Tariq, 2017). Aerosol concentrations are extremely variable in both time and space. Satellite remote sensing has the ability to monitor aerosols burden and their properties at large scales (Tariq and Ali, 2015). A number of studies have discussed spatio-temporal variability and trends of aerosols concentrations over the study region and its adjoining areas of Indian subcontinent (Prasad et al., 2006; Alam et al., 2011; Dani et al., 2012; Gupta et al., 2013; Sreekanth, 2013). Prasad et al. (2006) and Dani et al. (2012) found a sharp increase in aerosol optical depth (AOD) over Indian region, due to enhanced urbanization and industrialization. Alam et al. (2011) discussed seasonal variability of MODerate resolution Imaging Spectro-radiometer (MODIS, Chu et al., 2003; Wang and Christopher, 2003) derived AOD for different areas of Pakistan during 2002-2008. They found the highest AOD values during summer season linked to high dust/sand aerosols. Sreekanth (2013) reported an increasing trend in MODIS-AOD during 2002-2011 over Bangalore (India) due to sustained increase in the seasonal averaged AOD during summer. Gupta et al. (2013) analyzed the spatial and temporal patterns of aerosols over two megacities of Pakistan namely Karachi and Lahore by using MODIS data for the period 2001-2010. The major aim of their study was to evaluate the standard of MODIS data for air quality and climate applications. Tariq et al. (2015, 2016) have analyzed the optical properties of aerosols over Lahore using multi-sensor data during intense biomass burning events.

To the best of our knowledge, no detailed study has been conducted so far to analyze the spatio-temporal distributions of aerosol parameters such as mass concentration (MC), small mode fraction (SMF) and Angstrom exponent (AE) over Pakistan. The main objective of this paper is to investigate the spatio-temporal distributions and trends in aerosol parameters over Pakistan using long-term (15 years) data. In the present study, Terra-MODIS data is used for the period April 2000-March 2014 to analyze the AOD (550 nm), Angstrom exponent (470-660) ($AE_{470-660}$), SMF and MC. AOD from different satellite sensors have also been compared with ground-based AOD. Furthermore, Terra-AOD has also been compared with other satellite-based observations obtained from Aqua, MISR and SeaWiFS.

Material and methods

Study area

Pakistan is a South Asian country with estimated population exceeding 191 million and an area of about 796100 km^2. It is bordered by India to the east, China to the northeast, Afghanistan to the west and Iran to the southwest (*Fig. 1a*). It also has a coastline of about 1046 km along the Arabian Sea and Gulf of Oman. Ranging from the coastal areas of the south to the glaciated mountainous areas of the north, Pakistan's land-cover varies from plains to deserts (i.e. Cholistan and Thar), forests, hills and

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plateaus. These different landscapes together with extremely variable climatic conditions make Pakistan a distinctive country for studying spatio-temporal patterns of aerosols (Alam et al., 2011). Major sources of anthropogenic aerosols in Pakistan are petroleum refineries, power plants, fertilizer plants, industries, crop residue burning and fossil fuels combustion in vehicles (Ghauri et al., 1994; Biswas et al., 2008; Tariq and Ali, 2015). High amounts of aerosols are released from industrial areas and biomass burning in northeastern Pakistan and regions across the border in India such as Amritsar, Chandigarh and Bathinda (Raja et al., 2010). *Figure 1b* shows fire aggregates retrieved by MODIS-Terra and MODIS-Aqua during April 2000-March 2014. The figure clearly exhibits high frequency of fire events in Indo-Gangetic plain (IGP). Transboundary aerosols also contribute to the aerosol burden over Pakistan (Tariq et al., 2015, 2016). Winds transport aerosols released from coal based power plants in India to northeastern Pakistan. Dust/sand aerosols are the major source of natural aerosols in Pakistan. High amounts of desert dust dominate the central and southern parts of Pakistan due to comparatively low humidity and high temperature (Tariq and Ali, 2015) (*Fig. 2*). Dust storms in the region are largely associated with the easterly movements of low-pressure systems (Tariq and Ali, 2015).

In order to show prevailing meteorological conditions, specific humidity at 850 mb, wind speeds at 850 mb and surface temperature have been mapped during April 2000-March 2014 using NCEP–Reanalysis-2 data from NOAA/OAR/ESRL PSD, Boulder, Colorado, USA (*Fig. 2*). It can be noted from *Figure 2a* that central and southeastern parts of Pakistan have high temperatures (above 20 °C). *Figure 2b* shows that northeastern parts of Pakistan are characterized by relatively high values of average specific humidity (> 6 g/kg), whereas central and southeastern areas have relatively dry air (specific humidity < 6 g/kg). In *Figure 2c* strong winds (~3 ms^{-1}) can be observed over southern parts of Pakistan.

a



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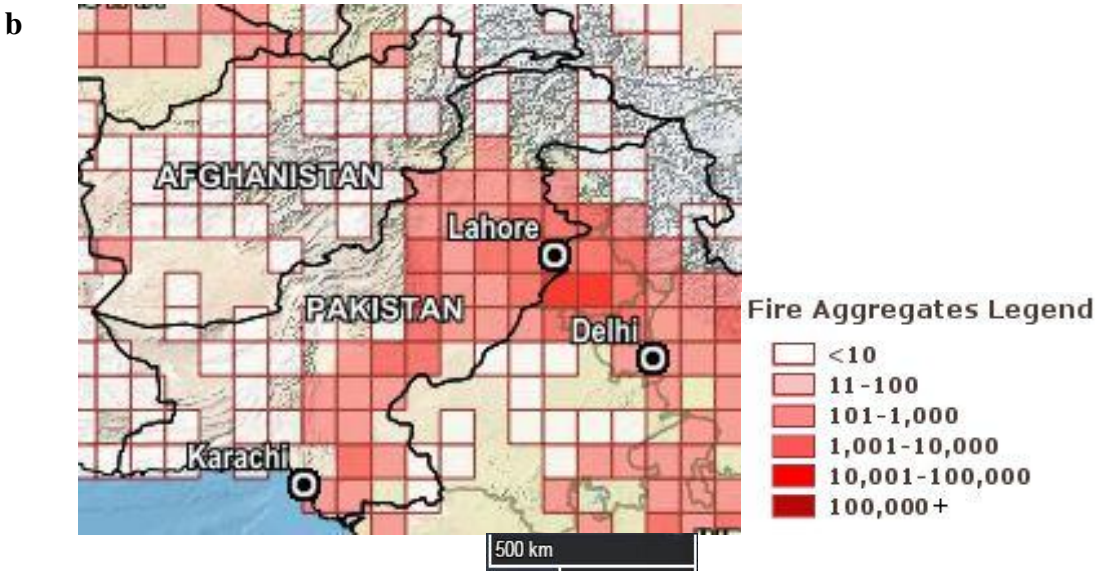


Figure 1. *(a) True color MODIS-image of Pakistan showing major cities, and (b) fire aggregates retrieved from MODIS Terra and Aqua during April 2000-March 2014*

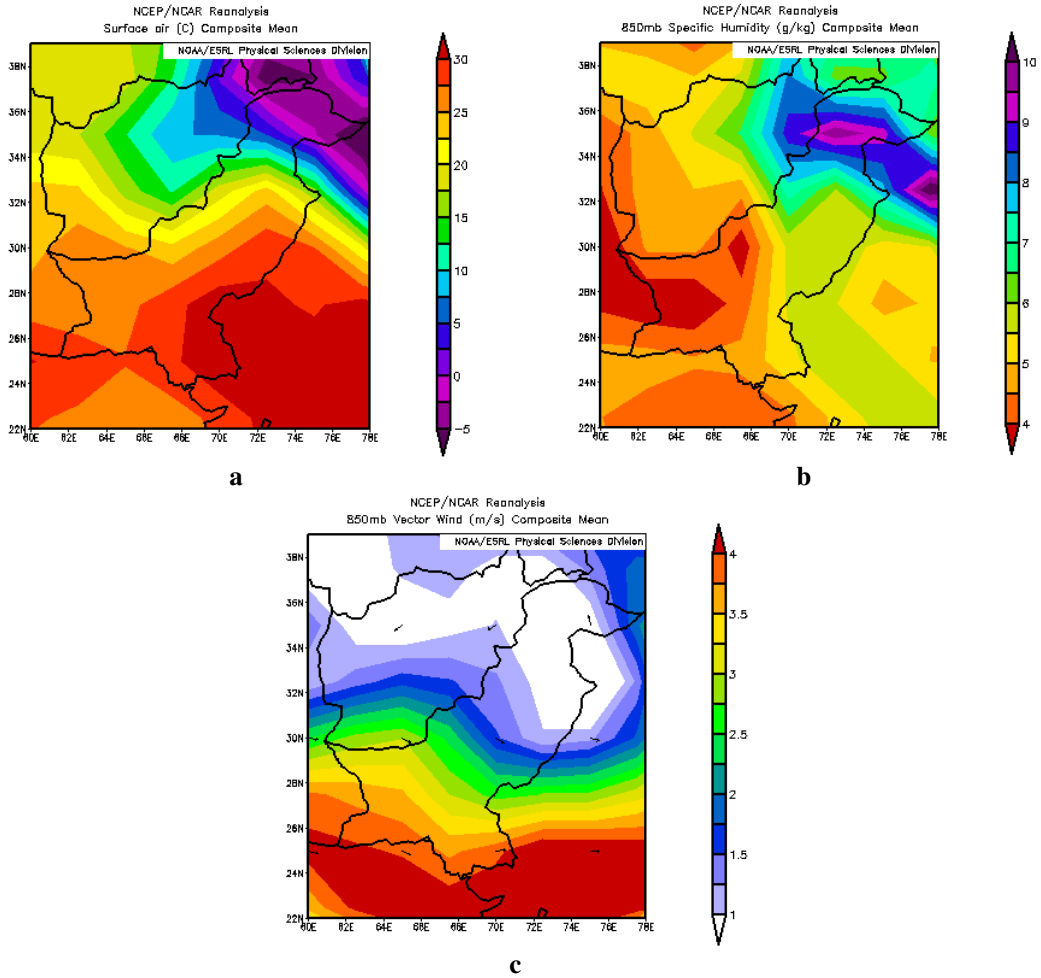


Figure 2. *Maps of (a) surface temperature (°C), (b) specific humidity (g/kg) at 850 mb, and (c) vector wind speeds at 850 mb over Pakistan during April 2000 to March 2014*

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Methodology and datasets

In the present study we have used Terra-MODIS data for the period April 2000-March 2014 to analyze AOD (550 nm), Angstrom exponent ($AE_{470-660}$), small mode fraction (SMF) and mass concentration (MC) of aerosols over Pakistan. AOD from different satellite sensors have also been compared with ground-based AOD. Spatail maps of AOD, $AE_{470-660}$, SMF and MC have been produced uding MATLAB software. The trends in seasonal-mean latitudinal and longitudinal averaged values of Terra-AOD are simulated with the help of linear equations. Linear trend parameters, correlation coefficients and relative changes (%) per year for AOD, AE, SMF and MC on seasonal and annual basis are also calculated.

MODIS

MODIS sensor was launched by NASA onboard Terra satellite in 1999 and Aqua satellite in 2002. MODIS is primarily designed to monitor aerosol fields, cloud cover and radiation budget (King et al., 1992). Terra satellite crosses the equator from north to south in the morning (10:30 local time) while Aqua satellite crosses it from south to north in the afternoon (13:30 local time). The orbits of Terra and Aqua are so designed that they image the whole globe in one to two days. MODIS instrument has a swath width of ~2330 km and acquires data in thirty-six spectral bands. It provides a high radiometric resolution (12 bits) data in all the thirty-six spectral bands from 0.4 μm to 14.4 μm, with spatial resolution of 1 km, 0.5 km and 0.25 km depending on the wavelength band. In the present work Terra-MODIS derived AOD (550 nm), $AE_{470-660}$, SMF and MC have been analyzed for a period from 2000 to 2014. We have used monthly gridded MOD08_M3.051 data product with spatial resolution of 1° × 1° obtained from NASA Goddard Earth Sciences Data and Information Center. These datasets are available via Giovanni website: http://giovanni.gsfc.nasa.gov. Missing values are not considered while calculating annual/monthly means and standard deviations. Due to difference in the surface characteristics, aerosol retrieval algorithms of MODIS are different for oceans (Tanré et al., 1997) and land (Kaufman et al., 1997). The aerosol retrieval algorithms have recently been greatly improved to correct the systematic biases (Remer et al., 2005; Levy et al., 2007). Detailed description of MODIS sensor, data products, retrieval algorithms, retrieval accuracy and validation can be found elsewhere (e.g., Remer et al., 2005 and references therein).

AERONET

The AErosol RObotic NETwork (AERONET) is a network of ground based remote sensing instruments administered by NASA (Holben et al., 1998). It provides detailed data of optical and physical properties of aerosols. AERONET data are processed at three different quality levels: level 1 (unscreened), level 1.5 (cloud screened) and level 2 (quality assured). AERONET instrument measures the direct sun and sky radiances within spectral ranges of 340 nm – 1020 nm and 440 nm – 1020 nm respectively (Holben et al., 1998). AOD and AE are calculated from direct sun radiances while single scattering albedo, asymmetry parameter and refractive index are computed from sky radiances (Dubovik and King, 2000). Currently, there are only two operational AERONET sites in Pakistan: Lahore (a central location in Pakistan) and Karachi (a coastal city of Pakistan). Lahore has a semi arid climate with hot and long summers and cool winters (Ali et al., 2014). From May to September temperatures are high with dust

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storms followed by rainfall. Karachi is the most densely populated city of Pakistan and located in southern region adjacent to Arabian Sea. Being a coastal city, Karachi has relatively mild climate. It has one of the busiest ports of the region and is a hub of industrial and economic activity in Pakistan. In this study Terra-AOD observations have been compared with level-2 AERONET-AOD values over Lahore and Karachi.

MISR

The MISR (Multi-angle Imaging Spectroradiometer) onboard Terra satellite was launched by NASA in 1999 at an altitude of 705 km. It has variable spatial resolutions of 1 km, 275 m and 250 m and temporal resolution of 16 days. It can view the Earth at nine different angles and hence it has the capability to measure the amount of sunlight scattered in different directions (Diner et al., 2001). It is primarily designed to monitor trends and types of aerosol particles produced from natural and anthropogenic activities. In this study we have used MISR AOD at 555 nm and prepared at level 3 version 004 available from NASA Goddard Earth Sciences Data and Information Center. Detailed information of MISR instrumentation, retrieval algorithms and methodology can be found in Diner et al. (2001) and Kahn et al. (2005, 2010).

SeaWiFS

The SeaWiFS instrument onboard SeaStar satellite was launched in 1997. It has a swath width of 1502 km and provides global coverage in approximately 2 days. It was primarily designed to measure ocean color parameters and has a very accurate and stable calibration to measure small contribution of the ocean to the reflected solar radiance at top-of-atmosphere (TOA) (Eplee Jr. et al., 2011). The high quality of calibration and long-term data of SeaWiFS make its AOD more reliable. Its deep blue aerosol retrieval algorithm over land was developed by Hsu et al. (2004, 2006). SeaWiFS SWDB_L3M10_HDF4.004 AOD product at 550 nm is used which can be downloaded from NASA Goddard Earth Sciences Data and Information Center.

Results and discussion

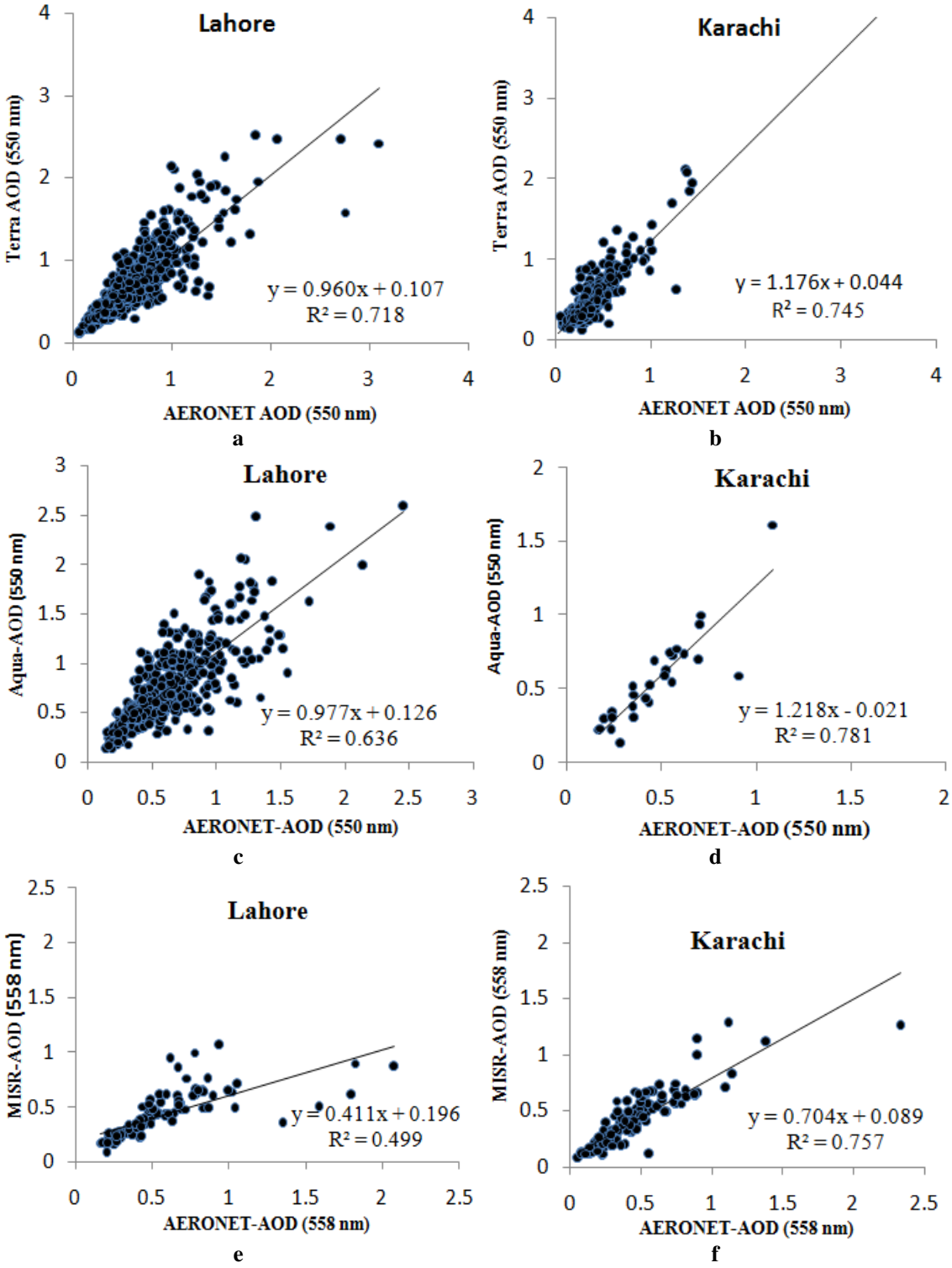
Comparison of satellite sensed AOD with ground based AOD

Ground-based observations are used to validate satellite aerosol products. Daily Terra-AOD, Aqua-AOD, MISR-AOD and SeaWiFs-AOD have been compared with ground based interpolated AERONET-AOD for Lahore and Karachi during 2006-2013 (*Fig. 3*). For the validation of AOD derived from Terra, Aqua, MISR and SeaWiFs, AERONET observations taken at 15-min intervals under cloud free conditions are used (Holben et al., 1998). For the purpose of comparison, only those data pixels have been considered that fall within a radius of about 27.5 km from the selected AERONET site (Petrenko et al., 2012). In order to have observations closest in time, only those observations are taken that fall within ±30 min (30 min before and after) of satellite overpass time (Petrenko et al., 2012).

It can be seen in *Figure 3a-h* that the values of coefficient of determination (R^2) for Lahore are found to be slightly lower than Karachi which is in partial agreement with Bibi et al. (2015). Slope and intercept of linear regression calculated from the comparison of collocated datasets are of great importance (Levy et al., 2010). The slope of linear regression equation demonstrates the closeness of the assumed aerosol type to

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the local aerosol type over a particular region, and the intercept reveals error due to surface reflectance (Tripathi et al., 2005; Hyer et al., 2011). The slope = 1 and intercept = 0 indicate a perfect correlation between satellite observations and ground-based AOD (Tripathi et al., 2005). Slope > 1 indicates over estimation while slope < 1 refers to under estimation. Non-zero intercepts are linked with the errors in calibration or in surface reflectance (Chu et al., 2002; Tripathi et al., 2005).



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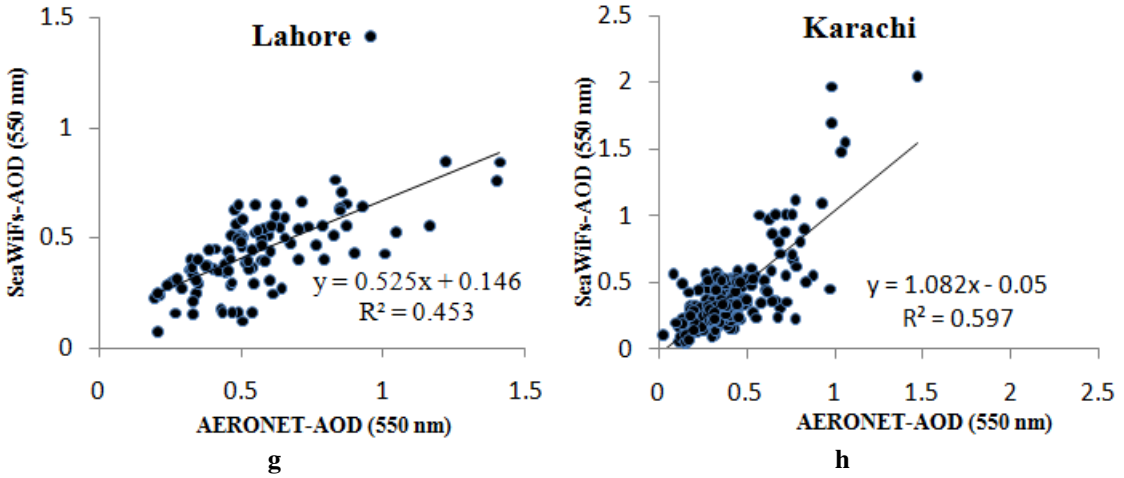


Figure 3. *Validation of daily (a, b) Terra-AOD, (c, d) Aqua-AOD, (e, f) MISR-AOD and (g, h) SeaWiFs-AOD with ground based interpolated AERONET-AOD over Lahore and Karachi for the period 2006-2013. The coefficient of determination (R^2) and equation of linear fit are also given*

All the satellite sensors exhibited under estimation over Lahore while for Karachi all the satellite sensors except MISR showed over estimation. In the present study, values of intercepts for Lahore are found to be 0.107, 0.126, 0.196 and 0.146 for Terra, Aqua, MISR and SeaWiFs respectively. For Karachi, values of intercepts are found to be 0.044, -0.021, 0.089 and -0.05 for Terra, Aqua, MISR and SeaWiFs respectively.

Comparison of Terra-AOD with Aqua, MISR and SeaWiFS observations

To achieve accuracy in measurements and realize the remote sensing capabilities, numerous studies have compared MODIS observations with MISR data (Prasad and Singh, 2007; Xiao et al., 2009; Kumar et al., 2015). Comparison of monthly Terra-AOD with available observations of Aqua-AOD (550 nm), MISR-AOD (555 nm), and SeaWiFS-AOD (550 nm) during the study period has been shown in *Figure 4*. The highest correlation (R^2 = 0.968) observed between Terra-AOD and Aqua-AOD is due to the fact that both Terra and Aqua have the same type of sensor and algorithms for aerosol retrieval (Remer et al., 2005). Also slope of 1.044 and y-intercept of -0.011 indicate very slight difference in the observed AOD from these platforms. This small inconsistency is associated with different overpass times of Terra (10:30 local time) and Aqua (13:30 local time). The coefficient of determination (R^2) between Terra-AOD and MISR-AOD is found to be 0.919. Slope (0.680) and y-intercept (0.018) of linear fit between Terra-AOD and MISR-AOD show that the MISR under-estimates the values of AOD as compared to Terra. These differences are attributed to different algorithms and wavelengths used for the retrieval of aerosols (Martonchik et al., 1998; Diner et al., 1998; Remer et al., 2005). The lowest coefficient of determination (R^2 = 0.750) is found between Terra-AOD and SeaWiFS-AOD. Values of slope (0.359) and y-intercept (0.117) of linear fit for SeaWiFS-AOD also exhibit under estimation. Despite of having same wavelength of operation, the different aerosol model assumptions and instrument calibrations of Terra-MODIS and SeaWiFS cause this under estimation (Hsu et al., 2004, 2006; Remer et al., 2005).

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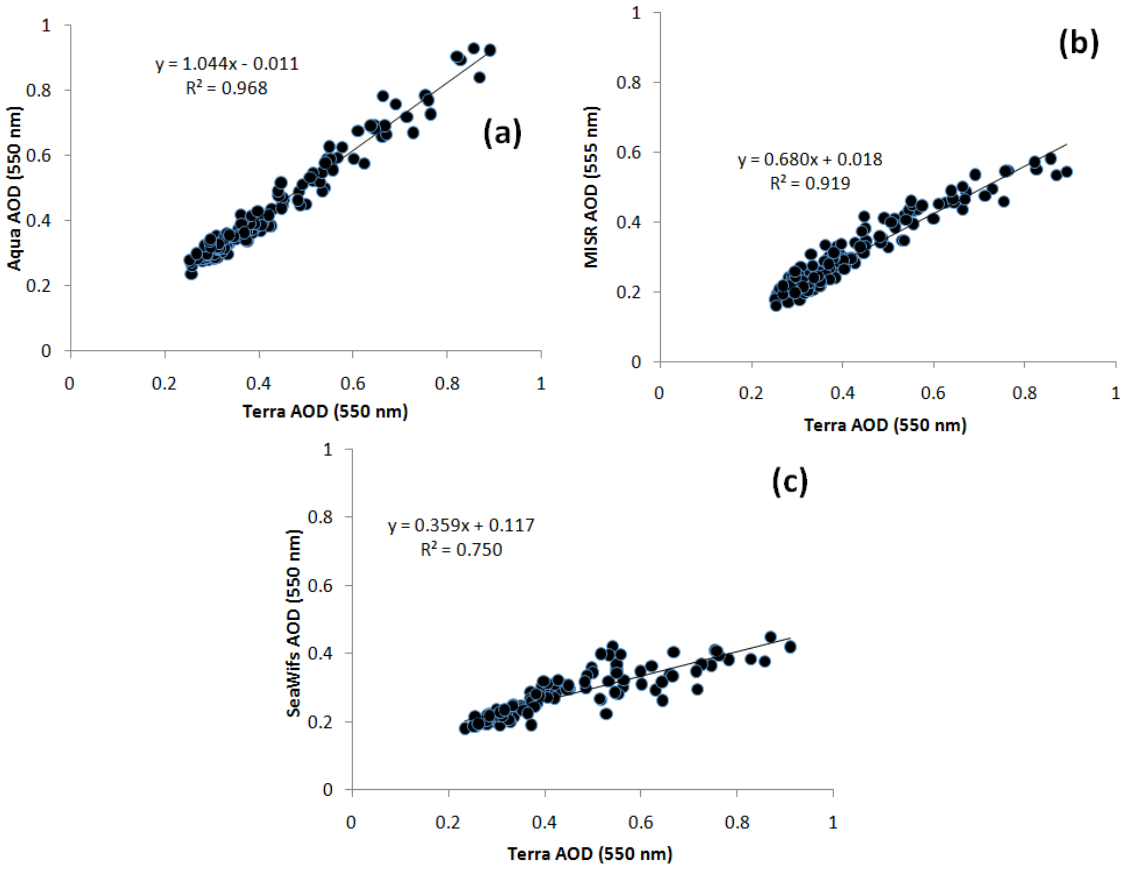


Figure 4. *Comparison between (a) Terra-AOD (550 nm) and Aqua-AOD (550 nm), (b) Terra-AOD (550 nm) and MISR-AOD (555 nm) and (c) Terra-AOD (550 nm) and SeaWiFS-AOD (550 nm) over Pakistan during the study period. The equation of linear fit and coefficient of determination (R^2) are also given*

Spatial distributions of aerosol parameters

Spatial distributions of Terra-AOD, $AE_{470\text{-}660}$, SMF and MC for the period 2000-2014 have been shown in *Figure 5*. White spaces in the figure represent no data/fill values. A significant spatial variability in Terra-AOD values (0.15-0.87) has been observed as shown in *Figure 5a*. It can also be noted from *Figure 5a* that high values (~0.8) of mean annual Terra-AOD exist over northeastern and southern Pakistan. Several studies have reported heavy air pollution due to intense crop residue burning in Pakistan and adjoining regions (Ul-Haq et al., 2015a, b, 2016, 2017). Tariq and Ali (2015) highlighted high aerosol burden over northeastern Pakistan due to open field crop residue burning. Also elevated concentrations of aerosols are associated with the activities at industrial areas in northeastern Pakistan and regions across the border in India such as Amritsar, Chandigarh and Bathinda (Raja et al., 2010). Wind patterns favor transport of aerosols released from anthropogenic activities such as biomass burning and fossil fuel combustion in power plants in India to northeastern Pakistan. On the other hand, high AOD values over the southern Pakistan are mainly contributed by desert/dust aerosols largely transported from local areas including Thar and Cholistan deserts (Tariq and Ali, 2015). Influx of desert/dust aerosols from desert regions of Arabia and Africa (Middleton, 1986; Prospero et al., 2002; Singh et al., 2004; Prasad

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and Singh, 2007; Kumar et al., 2012; Alam et al., 2014; ul-Haq et al., 2016) also contributes to the aerosol burden over this region.

Furthermore, the spatial distribution of Terra-AOD follows the Sulaiman–Kirthar range pattern that acts as a barrier for the desert/dust aerosols coming from the Thar and Cholistan deserts (Tariq and Ali, 2015). Alam et al. (2011) also found high AOD values over southern Pakistan due to high population, increased urbanization, heavy industrialization, and nearness of Thar and Cholistan deserts (Qureshi, 2010). We observe low values of Terra-AOD over northern and northwestern parts of Pakistan due to the fact that most of the areas in northern and northwestern Pakistan are agricultural and less populated in agreement with Alam et al. (2011).

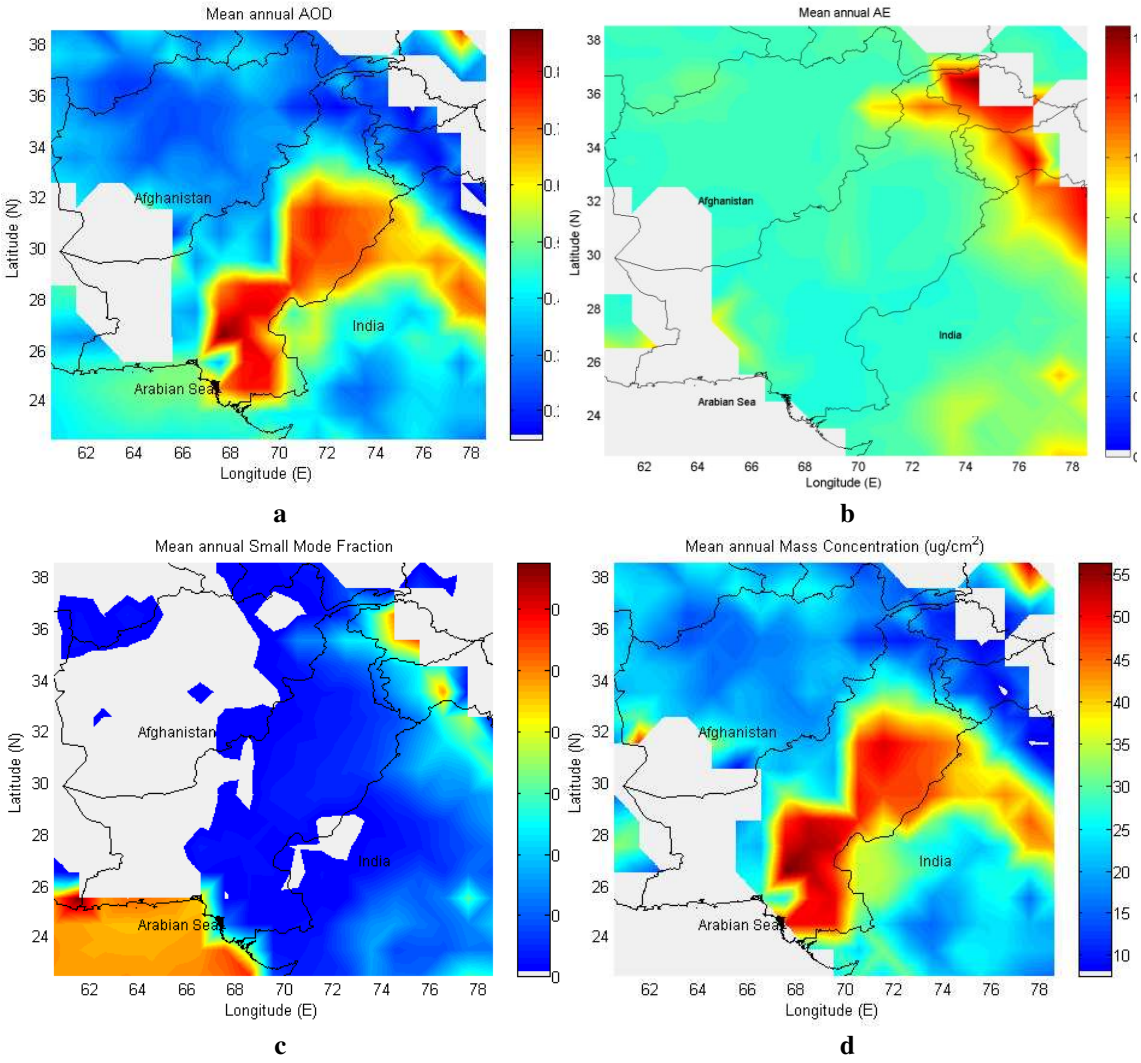


Figure 5. *Mean annual spatial distributions of (a) Terra-AOD, (b) $AE_{470\text{-}660}$, (c) SMF and (d) MC of aerosols over Pakistan from April 2000 to March 2014*

Since AOD only describes the overall aerosol burden without any information of aerosol composition, we have also analyzed other parameters such as Angstrom exponent and small mode fraction to differentiate the fine mode and coarse mode aerosol particles. The Angstrom exponent (AE) is an important qualitative parameter to

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assess the size of aerosol particles (Angstrom, 1929). It is inversely related to the size of aerosol particles; values of AE less than or equal to 1 indicate the dominance of coarse mode aerosol particles (radii ≥ 0.5 μm) such as dust and sea-salt, while AE ≥ 1 indicates the dominance of fine mode aerosol particles (radii ≤ 0.5 μm) such as urban pollution and biomass burning (Westphal and Toon, 1991; Eck et al., 1999). The AE has been used in various studies to assess the size distribution and classify different types of aerosols (Eck et al., 1999, 2001; O'Neill et al., 2001; Schuster et al., 2006; Kalapureddy and Devara, 2008; Kedia and Ramachandran, 2009). *Figure 5b* shows spatial distribution of mean annual $AE_{470\text{-}660}$ over Pakistan during the study period. Wide range of $AE_{470\text{-}660}$ (0.56 to 1.44) indicates considerable variability in the size of aerosol particles. The northern Pakistan is characterized by high values (greater than 0.8) of $AE_{470\text{-}660}$ due to dominance of fine mode aerosols (i.e., anthropogenic aerosols), whereas relatively low values (less than 0.8) over southern Pakistan indicate the dominance of coarse mode aerosols (i.e., sand/dust). Various epidemiological studies have reported a significant correlation between exposure to fine particles in the atmosphere and adverse health effects. Therefore, people residing in the northern parts of Pakistan may experience more harmful health effects due to fine mode aerosol particles.

SMF is the ratio of small mode AOD to the total AOD (Kaufman et al., 2005a, b). High values of SMF are associated with small aerosol particles such as those released from anthropogenic sources (i.e. biomass burning) while low values of SMF indicate naturally occurring large aerosol particles such as sea-salt and dust (Bellouin et al., 2005). The spatial distribution of mean annual SMF (*Fig. 5c*) is found to be almost similar to that of $AE_{470\text{-}660}$ i.e., high values over northern Pakistan and low values over southern Pakistan. It can further be noted from *Figure 5c* that fine mode aerosols are dominating over Arabian Sea. The high values of SMF over Arabian Sea are possibly associated with the presence of sea-salt aerosols. *Figure 5d* shows the spatial distribution of mean annual MC of aerosols over Pakistan. It can be noted that the spatial distribution of MC is quite similar to that of AOD. The MC showed a significant spatial variability (i.e., 7.6 to 56.2 $\mu g/cm^2$). The high values of MC over south and northeastern Pakistan indicate heavy aerosol loading.

Latitudinal and longitudinal gradients in seasonal AOD

Figure 6 shows latitudinal and longitudinal gradients in seasonal Terra-AOD during 2000-2014 over the study region. The latitudinal AODs are averaged over a longitude range of 60.5°E-78.5°E, and longitudinal AODs have been averaged over a latitude range of 22.5°N-38.5°N. *Figure 6a* shows that Terra-AOD remained relatively high on all the latitudes during summer and low during the winter. Unstable atmosphere in summers uplifts the aerosols causing enhanced values of AOD. On the contrary, stable atmosphere during winters entraps the aerosols to lower heights resulting in small values of AOD in agreement with Prasad et al. (2007), Alam et al. (2010) and Ali et al. (2014). Prasad et al. (2007) analyzed the aerosol variations over IGP and they found higher AOD in summer and lower AOD in winter. Alam et al. (2010) found higher values of AOD in summer and lower values in winter over ten different cities of Pakistan. Similarly, Ali et al. (2014) reported high value of AERONET derived AOD over Lahore, a northeastern megacity of Pakistan, due to increased temperatures and frequent dust storms. As a general rule the Terra-AOD in all the four seasons has increased northward from 22.5°N with a maximum value at 28.5°N and then it

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decreases towards 35.5°N followed by a little increase between 35.5°N-38.5°N. The maximum decrease (0.63) in latitudinal Terra-AOD is observed during summer season. It showed averaged peak values of 0.52, 0.92, 0.57 and 0.47 during spring, summer, autumn and winter seasons, respectively, at a latitude of 28.5°N. The peak values at 28.5° N is linked to biomass burning in IGP covering northeastern Pakistan and regions across the border in India (*Fig. 1b*; Tariq et al., 2016; ul-Haq et al., 2016). The lowest AODs are observed to be 0.29, 0.30, 0.20 and 0.19 during spring, summer, autumn and winter seasons, respectively, at a latitude of 36°N.

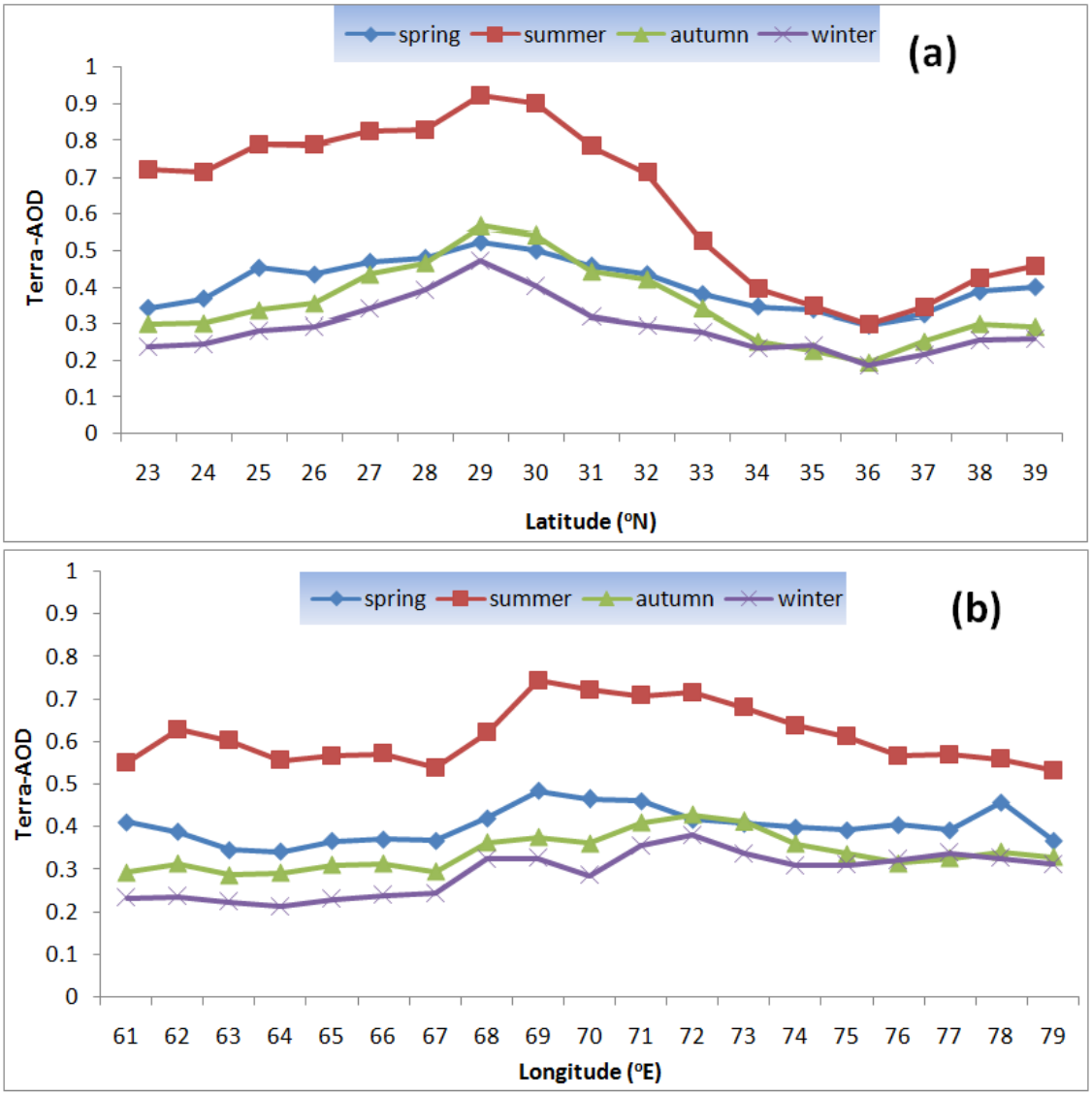


Figure 6. *Seasonal variation in Terra-AOD over different latitudes (a) and longitudes (b) in Pakistan during April 2000-March 2014*

Figure 6b shows seasonal variations in Terra-AOD over different longitudes in the study region. It is observed from the *Figure 6b* that longitudinal Terra-AOD remains higher in summer than all the other seasons. During summer and spring, peak values of longitudinal Terra-AOD have been observed over 69°E, while for winter and autumn

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peak values of Terra-AOD are observed over 72°E. The peak values of longitudinal Terra-AOD are found to be 0.48, 0.74, 0.43 and 0.38 during spring, summer, autumn and winter respectively. The peak values at 69°E and 72°E are associated with biomass burning and dust activity in part of IGP in Pakistan (see *Figs. 1b* and *5a*) (Tariq et al., 2016; ul-Haq et al., 2016). It is noticed that the trends in seasonal-mean latitudinal and longitudinal averaged values of Terra-AOD are better simulated with the help of following linear equations (*Eqs. 1* and *2*):

$$\mathrm{AOD} = a \times \mathrm{Lat} + b \quad \text{(Eq.1)}$$

$$\mathrm{AOD} = a \times \mathrm{Long} + b \quad \text{(Eq.2)}$$

Table 1 shows the values of constants *a* and *b*, and coefficient of determination (R^2) for different seasons over Pakistan during April 2000-March 2014. Some important conclusions drawn from the *Table 1* are as follows:

(i) For latitudinal AOD, the slope *a* is negative during all the seasons showing a decrease in Terra-AOD values towards northern Pakistan. Relatively large values of slope and R^2 in summer indicate less scatter in the linear fit with the largest south to north gradient for latitudinal AOD.

(ii) For longitudinal AOD, positive values of slopes during all the seasons indicate an increasing trend towards eastern Pakistan. Comparatively greater values of slopes and R^2 in winter season indicate less scatter in the linear fit with greatest west to east gradient for longitudinal Terra-AOD.

Table 1. *Values of the a and b coefficients in the equation AOD = a × Lat + b for latitudinal Terra-AOD and AOD = a × Long + b for longitudinal Terra-AOD averaged over the longitudinal region 60.5°E-78.5°E and latitudinal region 22.5°N-38.5°N, respectively, applied to the seasonal-mean values over Pakistan during the study period. The coefficient of determination R^2 represents the scatter of the data values from the fitting linear form*

Season	*a*	*b*	R^2
For latitudinal AOD			
spring	-0.0052	0.4545	0.1564
summer	-0.033	0.9325	0.5977
autumn	-0.0085	0.4321	0.1548
winter	-0.0049	0.3358	0.1141
For longitudinal AOD			
spring	0.0021	0.382	0.085
summer	0.0003	0.6111	0.0007
autumn	0.0032	0.3082	0.174
winter	0.007	0.222	0.5844

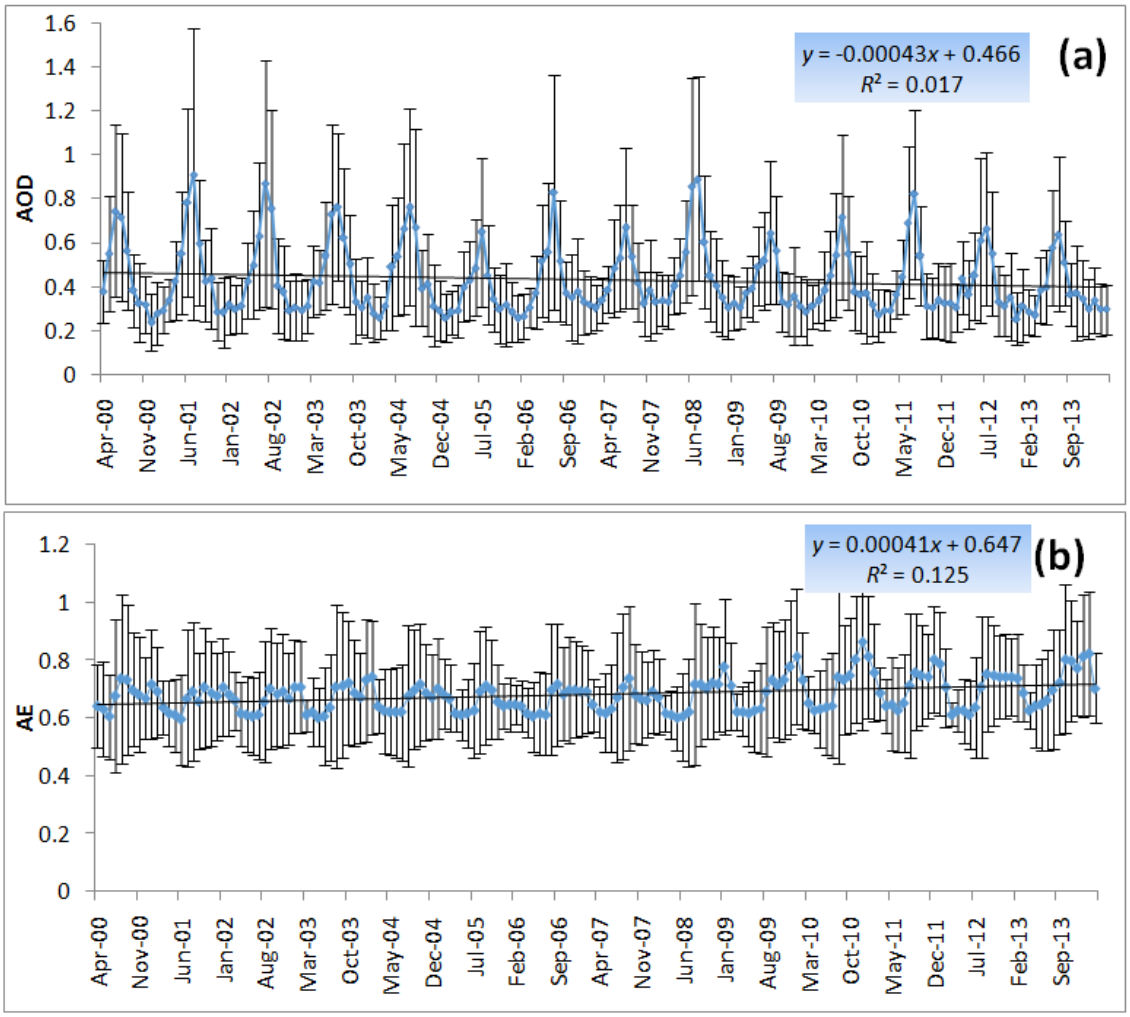
Monthly climatological variations in aerosol parameters

Figure 7a shows a time series of monthly mean Terra-AOD spatially averaged over Pakistan along with standard deviations from April 2000 to March 2014. The Terra-AOD ranges from 0.24 to 0.91 with an overall mean value of 0.43 ± 0.16. The highest value (0.91) of Terra-AOD is observed in July 2001 while the lowest value (0.24) is found in December 2000. The broad range variations in time series of Terra-AOD

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values are attributed mainly to the high values observed over northeastern and southern Pakistan during summer season. The major contributors of high AOD values are dust aerosols originated from the local deserts (i.e., Thar and Cholistan) and anthropogenic activities. Desert/dust aerosols from desert regions of Arabia and Africa (Middleton, 1986; Prospero et al., 2002; Singh et al., 2004; Prasad and Singh, 2007; Kumar et al., 2012; Alam et al., 2014; ul-Haq et al., 2016) also contribute to the aerosol burden over the region. An overall negative change (-15.4%) in the Terra-AOD values is observed indicating that the total aerosol burden is decreasing over Pakistan. However, a positive change (10.6%) has been noticed in $AE_{470\text{-}660}$ values (*Fig. 7b*) indicating that the contribution of fine mode aerosol particles towards total aerosol burden is increasing. The increasing trend in AE with time is linked to anthropogenic activates such as industrial production, residential combustion and biomass burning.

Gupta et al. (2013), while analyzing AOD observations from MODIS during 2001-2010, also showed a decreasing trend in AOD with slope of -0.07 over Lahore (Pakistan). Analysis shows that $AE_{470\text{-}660}$ ranges from 0.60 to 0.86 with a mean of 0.68 ± 0.06. The highest value (0.86) of $AE_{470\text{-}660}$ has been detected in December 2010 and the lowest value (0.60) in June 2001. Similarly, a positive change in SMF is also found (*Fig. 7c*) with an overall mean of 0.11 ± 0.05.



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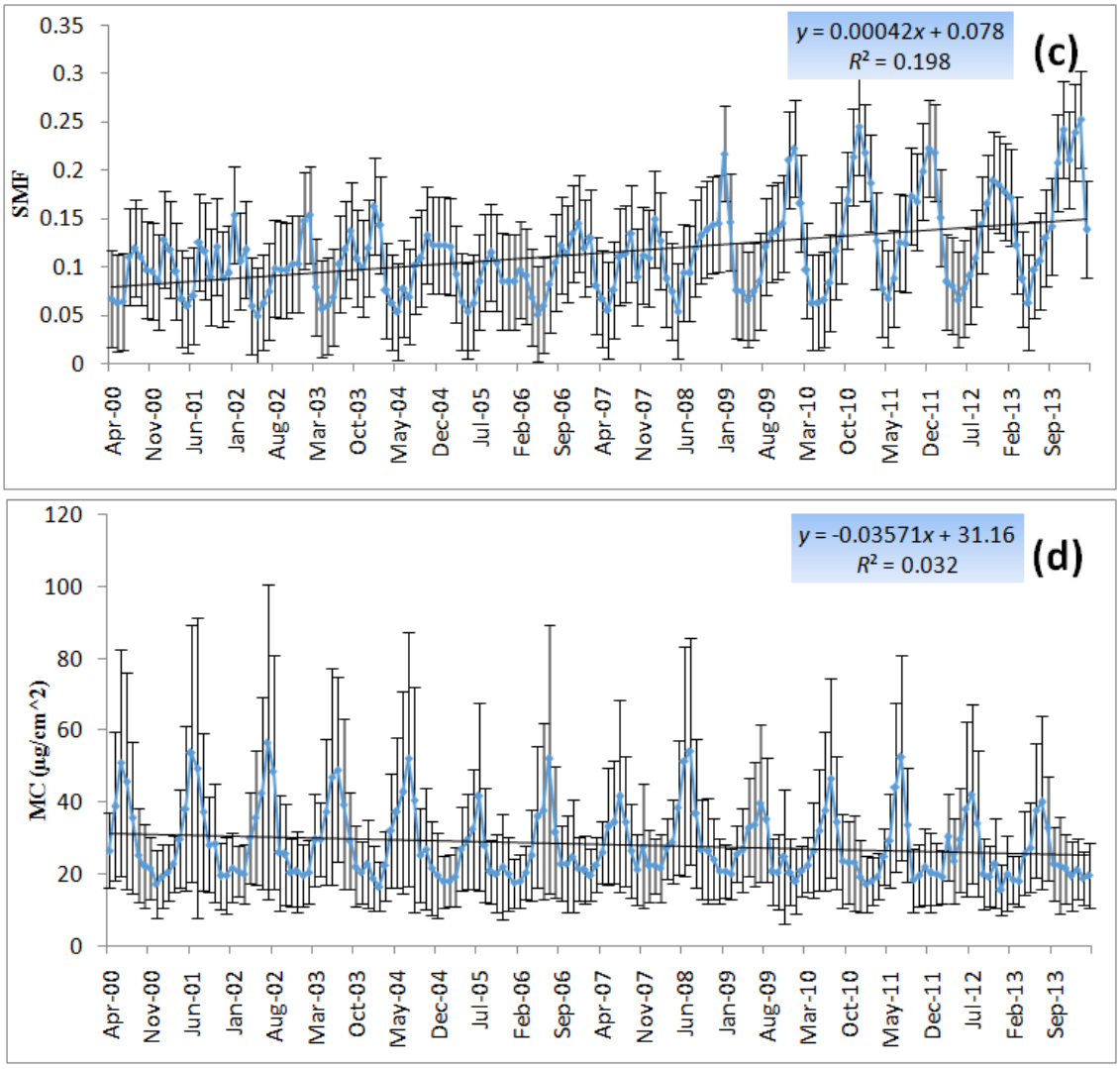


Figure 7. *Time series of (a) AOD, (b) $AE_{470\text{-}660}$, (c) SMF and (d) MC derived from Terra-MODIS over Pakistan during April 2000-March 2014. The error bars show standard deviations from the corresponding mean values*

Extremely low mean value of $AE_{470\text{-}660}$ signifies the dominance of coarse mode aerosols in the atmosphere of Pakistan during the study period. *Figure 7d* shows time series of MC spatially averaged over Pakistan from April 2000 to March 2014. The MC values have been found to be ranging from 15.8 µg/cm^2 to 56.6 µg/cm^2 with an overall mean value of 28.2 ± 9.71 µg/cm^2. Like AOD, MC also shows a negative change (-19.2%) with time. We find the highest value (56.63 µg/cm^2) of MC in July 2002 and the lowest value (15.76 µg/cm^2) in December 2012.

Figure 8a-d shows the variations in monthly average values of AOD, $AE_{470\text{-}660}$, SMF and MC derived from Terra-MODIS over Pakistan during April 2000-March 2014. Error bars represent the standard deviations from the mean values. It can be observed from *Figure 8a* and *d* that AOD and MC derived from Terra satellite have similar behaviors. Both AOD and MC have increased from January to their maximum values in July followed by a decrease to minimum values in December. The peak values of both Terra-AOD (0.71 ± 0.37) and MC (43.36 ± 26.8 µg/cm^2) have been found in July with

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corresponding values of $AE_{470-660}$ and SMF to be 0.64 ± 0.16 and 0.09 ± 0.02 respectively. Elevated values of standard deviation for AOD and MC indicate that aerosols are highly variable in space. The lowest values of standard deviation for Terra-AOD (0.09) and MC (6.7) are found in February indicating almost uniform distribution of aerosols over Pakistan. Our study is in agreement with the results from Alam et al. (2010) who showed that AOD increases from April, reaching its peak value in July and decreases afterwards to attain a minimum value in December. The increase of AOD in summer (June, July and August) is caused by dust transported from the Thar Desert (India) and Dasht Desert (Iran), sea-salt aerosols carried by strong south-westerly winds and increase in humidity within this region (Alam et al., 2010, 2011). Ranjan et al. (2007) analyzed the spectral variation of total column AOD over Rajkot (India) and they showed that water vapor content and AOD are proportional to each other, therefore, enhanced concentration of water vapor in summer results in higher AOD. *Figure 8b* and *c* show the variations of monthly averaged $AE_{470-660}$ and SMF during the study period. It can be noted that both $AE_{470-660}$ and SMF show a quite similar seasonal pattern. Both the parameters are found to be decreasing from January to attain their minimum values during May-June. Peak values of $AE_{470-660}$ (0.73 ± 0.18) and SMF (0.16 ± 0.05) are found in January. Relatively high values of standard deviation are linked to the higher fine mode aerosol concentration over northeastern Pakistan and lower over southern Pakistan. Low values of $AE_{470-660}$ and SMF and their corresponding standard deviations during May-June are associated with dominance of desert dust aerosols over the entire Pakistan.

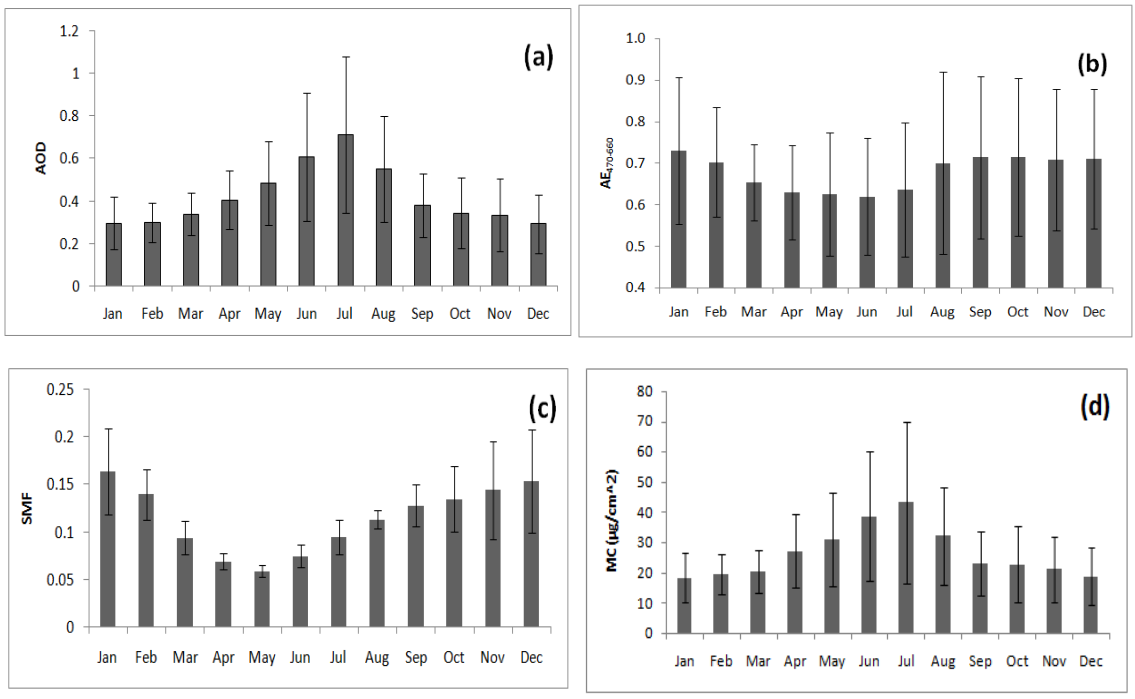


Figure 8. *Temporal variations in monthly average values of (a) AOD, (b) $AE_{470-660}$, (c) SMF and (d) MC derived from Terra-MODIS over Pakistan during April 2000-March 2014. The error bars show standard deviations from the corresponding mean values*

Table 2 shows the linear trend parameters, correlation coefficients and relative changes (%) per year of AOD, AE, SMF and MC on seasonal and annual basis over Pakistan. It can be observed from the table that slopes are negative for Terra-AOD

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during all the seasons except winter. AOD in summer showed the greatest declining trend of -1.170% per year. The highest values of relative change per year of AOD (0.325%) and AE (1.115%) are found during winter while the highest value of percentage change per year of SMF (7.427%) is found during autumn. MC shows decreasing trend both on seasonal and annual basis. Significant (at 0.01 level) positive correlation is found for AE (r = 0.775) and SMF (r = 0.920) on mean annual data. Values of relative change per year are found to be negative for AOD (-0.615%) and MC (-0.986%), and positive for AE (0.570%) and SMF (4.344%).

Table 2. *Linear trend parameters of equation of the form ax + b, where a is slope and b is y-intercept, of AOD, AE, SMF and MC over Pakistan. Correlation coefficient (r) and relative change (%) per year are also given. ** and * represent correlation significance at the 0.01 and 0.05 levels (2-tailed) respectively*

	AOD	**AE**	**SMF**	**MC**
Winter	a=0.001, b=0.285, r=0.366, ΔAOD=0.325%	a=0.008, b=0.658, r=0.741**, ΔAE=1.115%	a=0.007, b=0.096, r=0.789**, ΔSMF=6.273%	a=-0.037, b=19.96, r=-0.135, ΔMC=-0.172%
Spring	a=-0.004, b=0.445, r=-0.619*, ΔAOD=-0.842%	a=0.001, b=0.618, r=0.521, ΔAE=0.150%	a=0.001, b=0.064, r=0.634* ΔSMF=1.420%	a=-0.392, b=31.00, r=-0.681**, ΔMC=-1.189%
Summer	a=-0.009, b=0.723, r=-0.487, ΔAOD=-1.170%	a=0.001, b=0.645, r=0.371, ΔAE=0.144%	a=0.001, b=0.085, r=0.453, ΔSMF=1.073%	a=-0.636, b=46.44, r=-0.551*, ΔMC=-1.289%
Autumn	a=-0.001, b=0.368, r=-0.244, ΔAOD=-0.253%	a=0.006, b=0.673, r=0.850**, ΔAE=0.821%	a=0.007, b=0.080, r=0.920**, ΔSMF=7.427%	a=-0.217, b=24.82, r=-0.681**, ΔMC=-0.819%
Annual	a=-0.003, b=0.456, r=-0.454, ΔAOD=-0.615%	a=0.004, b=0.648, r=0.775**, ΔAE=0.570%	a=0.004, b= 0.081, r=0.873**, ΔSMF=4.344%	a=-0.321, b=30.55, r=-0.623*, ΔMC=-0.986%

Conclusions

In the present study we have used Terra-MODIS data for the period April 2000-March 2014 to analyze AOD (550 nm), Angstrom exponent ($AE_{470-660}$), small mode fraction (SMF) and mass concentration (MC) of aerosols over Pakistan. AOD from different satellite sensors have also been compared with ground-based AOD. Values of coefficient of determination (R^2) between Terra-AOD and AERONET-AOD are found to be 0.718 and 0.745 for Lahore and Karachi respectively. Terra-AOD has also been compared with other satellite-based observations obtained from Aqua, MISR and SeaWiFS. We find significant correlations of Terra-AOD with Aqua-AOD (R^2 = 0.968) and MISR-AOD (R^2 = 0.919). A significant spatial variability in mean annual AOD values (0.15-0.87) has been found with high values (~0.8) observed over northeastern and southern Pakistan. The $AE_{470-660}$ amounts from 0.56 to 1.44 with high values (greater than 0.8) over northern Pakistan and relatively low values (less than 0.8) over

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southern Pakistan. Strong similarity in the spatial distributions of mean annual SMF and $AE_{470\text{-}660}$ has been found. The MC, ranging from 7.6-56.2 μg/cm^2, has spatial distribution quite similar to that of AOD. The latitudinal AOD shows peak values of 0.52, 0.92, 0.57 and 0.47 during spring, summer, autumn and winter seasons, respectively, over latitude of 28.5°N. A negative change (-15.4%) in the AOD values is observed indicating a decrease in the total aerosol burden over Pakistan. However, a positive change (10.6%) is observed in $AE_{470\text{-}660}$ which indicates mounting contribution of fine mode aerosol particles towards total aerosol burden. The peak mean monthly values of both AOD (0.71) and MC (43.36 μg/cm^2) are observed in July with corresponding $AE_{470\text{-}660}$ and SMF values of 0.64 and 0.09, respectively. The overall mean values of the AOD, $AE_{470\text{-}660}$, SMF and MC are found to be 0.43 ± 0.16, 0.68 ± 0.06, 0.11 ± 0.05 and 28.2 ± 9.71 μg/cm^2 respectively. Trends and spatial distributions of different aerosol parameters discussed in this study are important in policy making for human health protection and environmental remediation. Furthermore, the assessment of aerosol concentrations presented in this study can be correlated with harmful effects on human health and drought indices, which can be a possible further research topic.

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COLLABORATIVE PARTNERSHIP PARADIGM IN THE SUSTAINABILITY-ORIENTED SUPPLY CHAIN AS THE WAY TO SUSTAINABILITY

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Abstract. This study extends research into power in the supply chain (SC) on the basis of three "patterns". The first one is the "pattern of sustainability". From the aspect of sustainability, the "golden thread" connecting all actors and influencing relationships between actors in the SC is the sustainability orientation. The second one is the "pattern of sustainability-oriented (SO) actors". Transformation towards sustainability in the SC requires the SO behavior of numerous actors. The third one is the "pattern of SO collaboration". Sustainability results from the complementary effect of numerous actors' behaviors, so this study suggests the SO collaboration paradigm as a way to sustainability. These three patterns of embedding sustainability in the SC can also be observed as the three stages of the transformation of the SC into the sustainability-oriented supply chain (SO-SC). In the first stage of embedding sustainability in the SC, actors voluntarily and/or under pressures accept some sustainability initiatives. In the second stage, the number of SO actors behaving sustainably increases. The SO-SC and the achievement of the goals of the sustainable development (SD) require the sustainable behavior of all (or at least) the majority of actors and SO collaboration.
Keywords: *sustainable development, Agenda for sustainable development, environmental awareness, co-creation of value, consumer*

Introduction

Individuals, companies and countries are faced with an SD challenge. According to "The 2030 Agenda for Sustainable Development" (UN, 2015), all countries and all stakeholders are actors taking part in a collaborative partnership, in which Governments, civil society, the private sector, the United Nations' system and other actors are brought together and all available resources are mobilized. Lozano et al. (2015) proposed the 'Sustainability-Oriented Theory of the Firm', which is based on the holistic perspective of corporate sustainability. SO actors enable transformations in the SC. In compliance with the Agenda for SD (UN, 2015), all actors and all stakeholders are actors taking part in a collaborative partnership in the SC, where consumers, the focal firm, employees, suppliers, regulators and governments, the general public, environmental and social pressure groups, NGOs, neighborhood communities, the media and schools are brought together. Starting from the literature review, the purpose of the study is to present and analyze the collaborative partnership paradigm in the SO-SC as a way to sustainability. "Sustainability-oriented" (SO) means a holistic approach to sustainability by covering all the three dimensions (economic, environmental and social) in the dynamic process (Rakic and Rakic, 2017b). According to the collaborative partnership paradigm in the SO-SC, all actors are engaged in co-creating SO values. Consumers are the most important co-creators of values. Opportunities for firms are the

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speed (of business and change), digitalization and the SO-SC. These are also the sources of companies' competitive advantages.

The structure of the study is as follows. In the next section, the related literature is reviewed. Then, the conceptual framework – the collaborative partnership paradigm is presented and described, as a way to sustainability in the SC. The conclusions and the agenda for further research are presented at the end of the paper.

The engagement of co-creators in sustainable supply-chain management

In firms, there are stakeholders with different priorities because of the protuberance of their values and issues, for which reason the same are often compelled to make trade-offs. For example, owners' and managers' focus is on profitability, whereas concerns of a community's members are probably related to the overall livability of the community and production's environmental impacts. How to balance environmental issues and sound business practices in such a dynamic, complex and uncertain setting is challenging. One research, however, suggests that it is impossible for all stakeholders to be satisfied at all points in time. Ambitiously environmentally targeted strategic decisions can generate real economic costs (Pagell et al., 2010). Firms have increasingly been facing pressure from external stakeholders (e.g. NGOs, customers, regulators) in order for them to maintain sustainable supply chains (Grimm et al., 2014). Searcy (2014) differentiates three groups of stakeholders for the sustainability of an enterprise:

- focal firm stakeholders – employees, unions, managers, owners/investors
- supply chain stakeholders – suppliers, distributors, consumers (individuals, groups or other firms), reclaimers (collectors, re-users, recyclers, remanufacturers or disposers)
- stakeholders beyond the supply chain – government, communities, competitors, NGOs, media.

The engagement of co-creators in the sustainability-oriented internal value chain

Focal firms are those usually (1) ruling or governing the supply chain, (2) providing the customer with a direct contact, and (3) designing the product or service offered (Seuring and Muller, 2008). The focal firm is capable of arranging for the maximum benefit for all the participants of the supply chain through their coordination, the inhibition of an opportunistic behavior, and the maintenance of the sustainability of the supply chain (Li et al., 2014).

The influences of external stakeholders

It is possible to group external drivers at three levels, and by rule they include:

- Regulators and governments at the regulatory level
- Customers, suppliers and competitors at the market level
- Non-governmental organizations (NGOs) and the general public at the societal level (Harms et al., 2013).

From the historical point of view, firms used to accumulate all the resources they were in need of in-house. Today, firms have shifted away from this vertical–integration model and have started programs intended for accessing specialized, global suppliers (Prahalad and Krishnan, 2008). Production processes are frequently dispersed throughout the globe. Supply-chain focal firms might be held responsible for their

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suppliers' environmental and social performance (Seuring and Muller, 2008). Focal firms, buyers, are required to take their suppliers' responsibility, sustainably arranging for their supply chains' actions. It is frequently the case that external stakeholders do not differentiate the focal firm's behavior from the one of its suppliers, simultaneously holding the focal firm responsible for all activities performed within the manufacture of a product (Grimm et al., 2014; Rao, 2002; Koplin et al., 2007). Any party in the supply chain that does not comply with the focal firm's corporate sustainability standards can potentially incur damage to corporate reputation and/or harm customer confidence (Grimm et al., 2014) (Seuring and Muller, 2008). This is particularly the case with brand-owning firms, since they show a tendency of being exposed to pressure from stakeholders, e.g. non-governmental organizations (NGOs). Mattel (Barbie), Nike (Football) and Nestlé (KitKat) are outstanding examples to have demonstrated the way in which firms' brands can suffer from non-compliant sub-suppliers (Barnett and King, 2008; Choi and Linton, 2011; Grimm et al., 2014; Wagner et al., 2009). On the other hand, when the focal firm is exposed to pressure, it usually passes such pressure on to suppliers (Seuring and Muller, 2008). The idea is that the focal firm in the supply chain will compel its upstream suppliers to adopt and adapt the technology and practices resulting in more efficient and environmentally friendly material sources, engage in labor practices deemed to be ethical and result in the lower emissions of the greenhouse gas and a low impact on the environment. As it has been suggested that "a firm is only as sustainable as it suppliers".

Gualandris and Kalchschmidt (2014) recommend that the growing awareness on the stakeholders' part should be acknowledged by managers and that they should be prepared to address strict environmental and social requirements made by the customer. Customer pressure is found to be the essential driver motivating firms to initiate and sustain the process of the SSCM development. Customer pressure refers to end-consumers' and business customers' requests and their requirements, i.e. those made by important primary-stakeholder groups, so that the firm could make its environmental and social performance upgraded (Gualandris and Kalchschmidt, 2014). One of the key criteria for decision making that has so far been missing from green, SSC models is the consumer's role. Many businesses selling environmentally friendly, green or sustainable, low-carbon-emission products show a tendency of costing more. The consumer is usually passed on this cost in the form of higher prices. For the purpose of making green or sustainable products more marketable and sellable, many businesses will not only have to quantify the benefits, but also to justify the value proposition to the customer (Hassini et al., 2012). The factors related to marketing and public relations refer to firms' efforts intended for the creation of a value proposition for the customer, in particular when an "environmentally friendly" product costs more. Also, firms must create the awareness of the practices leading to a more environmentally friendly or sustainable product. Just like focal firms in the supply chain compel their upstream suppliers to be greener and more sustainable, they also try to educate and convince their customers to purchase their green products. As an external pressure, NGO's can turn to boycotts or detrimental publicity campaigns designed to shame the firm into offering more sustainable products (Hassini et al., 2012).

Customer expectations have become the most important external pressure (Doonan et al., 2005). The increasing of his/her environmental, ecological and ethical awareness by the end-customer and demands to save energy, reduce pollution and waste, as well as ensure consumer safety, expose supply management to pressure (Lintukangas et al., 2015).

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The factors related to the policy and regulations originate from governments either through legislation or via a regulator requesting from firms to obey certain environmental standards (Hassini et al., 2012). "If conservationists, environmentalists and animal advocates join forces, perhaps there will be some reason for optimism" (Erdos et al., 2017, p. 188).

From dyadic, via triadic to multiple relationships in supply chain

From a traditional point of view, power relationships are surveyed at the dyadic level (Touboulic et al., 2014), namely most frequently between the local firm (the buyer) and its suppliers (Brammer et al., 2011; Kumar and Rahman, 2015). One side of the relationship dyad has been the subject matter of such studies (Nyaga et al., 2013). Mainly, one actor's responsibilities, namely those on the part of the focal firm (the buying firm) are singled out. Relationships existing between the focal firm other actors, e.g. retailers, are researched into to a lesser extent (Chaurasia, 2014). The end consumer is rarely the focal subject matter of research. When buyers are mentioned and an analysis of them performed, authors actually analyze relationships between the focal firm and suppliers (in which case, the focal firm is considered as the buyer, i.e. the firm as the buyer). This study emphasizes the role of the end consumer.

Apart from dyadic relationships, authors have also done research into triadic relationships (Touboulic et al., 2014), as well as into network relationships (Lacoste and Johnsen, 2015; Tachizawa and Wong, 2015). Touboulic et al. (2014) observe multiple triadic relationships drawing in a large buyer and its small suppliers, in order to check thoroughly into the manner in which relative power may affect the implementation of sustainable supply-management practices. They are analyzed at the triad level of analysis: the buyer–the supplier–the supplier. According to the results of the research, it is possible for a powerful organization to drive sustainability in its SC. When a situation such as the described one occurs, dependent suppliers are not left too much choice – they can but adhere to requirements made by the buyer and it is their obligation to specifically perform a thorough check into the relationship in order to become more sustainable. The findings are conforming to the work previously conducted, in which a suggestion was made that the buyer power stands for a resource to be used for the purpose of compelling the supplier to be compliant with sustainability requirements (Touboulic et al., 2014).

Tachizawa and Wong (2015) propose that a supply network perspective should be used to carry out an analysis of the effectiveness of the mechanisms of governance of green supply chain management (GSCM), not to apply a linear view. There are increasing societal pressures that encircle firms' accountability for the environment, which is accompanied, too, by an ever-larger number of environmental scandals concerning supply networks. It may prove to be insufficient to only apply the traditional frameworks of the governance of the GSCM for the purpose of dealing with this new reality, because the supply network structure/complexity is not taken into consideration, nor is the manner in which these factors interact with different mechanisms of governance is considered, either (Tachizawa and Wong, 2015). It is possible to understand that the supply network is a set of firms taking part, directly or indirectly, in supplying industrial inputs to the focal firm, with or without that company being aware or knowledgeable of it (Choi and Krause, 2006; Choi et al., 2001). Supply networks are viewed by Tachizawa and Wong (2015) as emergent, allowing for different parties

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capable of shaping the supply network and the implementation of the GSCM. For a long time, the importance of power and dependence in relationships has been the subject matter of consideration by IMP. In the IMP literature, power distribution is considered as being dependent on the pattern of the 'interaction' (Hakansson and Waluszewski, 2013; Lacoste and Johnsen, 2015). The IMP paradigm of the interaction approach takes place within the context of relationships between companies embedded in networks (Campbell, 1984; Hakansson, 1986; Lacoste and Johnsen, 2015; Smith and Easton, 1986). Zhao et al. (2008) pointed out the impact of relationship commitment on the integration between actors in the SC. Relationship commitment is one party's willingness to make an investment of its own financial, physical or relationship-based resources in a relationship (Morgan and Hunt, 1994). Quite a large amount of time and resources are invested by firms in the development, maintenance and improvement of the relationships between them in the supply chain since demonstrable benefits in terms of operational and financial performance are offered by such relationships (Nyaga et al., 2013). Firms cooperate with their respective partners in the supply chain so that they could have access to critical resources, mitigate transactional complexity and improve their performance (Fawcett et al., 2011; Nyaga et al., 2013; Zacharia, 2009). It is possible to foster sustainability by making a connection between the concept of co-creation and the concept of relationship management. Co-creation implies an inclusion of well-selected stakeholders, e.g. customers or suppliers, in the innovation process on a targeted basis (Arnold, 2017).

Traditionally in the SC literature, the concept of power in buyer–supplier exchange has been a significant subject matter of researches conducted. When a shift from supply chain management (SCM) to sustainable supply chain management (SSCM) is considered, it is possible for relationship-management strategies to undergo significant changes so as to be able to accommodate sustainability goals with e.g. dominant buyers who do not exploit their power over dependent suppliers but instead treat them as if they were strategic partners (Pagell et al., 2010; Touboulic et al., 2014). In prior researches, a strong accent has been put on cooperation between SC partners for the purpose of facilitating sustainability initiatives (e.g., Gold et al., 2010; Grekova et al., 2016; Lacoste and Johnsen, 2015; Sancha et al., 2016; Seuring and Muller, 2008; Touboulic et al., 2014). While, on the one hand, cooperation has been publically supported as the best manner to manage SC relationships regarding sustainability, that the fact that the major part of the SSCM literature tends to focus on actions performed by large corporations, on the other, is an interesting one to perceive. Cooperation with suppliers has been regarded as a critical component of creating sustainable SCs (Touboulic et al., 2014). Little research has been done that may challenge the collaborative paradigm in SSCM, and power relationships stay underexplored (Hoejmose and Adrien-Kirby, 2012; Walker et al., 2012). A larger number of the case studies appearing in the SSCM literature are a demonstration of some sort of power imbalance, which will not only determine the agenda driver, but also have an impact on the implementation and outcomes of sustainability initiatives (Touboulic et al., 2014).

Conceptual framework – the collaborative partnership paradigm in the sustainability-oriented supply chain as the way to sustainability

This study is focused on the SO collaboration paradigm (*Fig. 1*). According to the SO collaboration paradigm, all actors and all stakeholders act in a collaborative

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partnership, bringing together consumers, focal firm, employees, suppliers, regulators and governments, the general public, environmental and social pressure groups, NGOs, neighborhood communities, media, schools and universities. The full line shows the relations between actors from the aspect of the focal firm. The interrupted (dotted) line shows the relations of other actors which the focal company does not directly participate in.

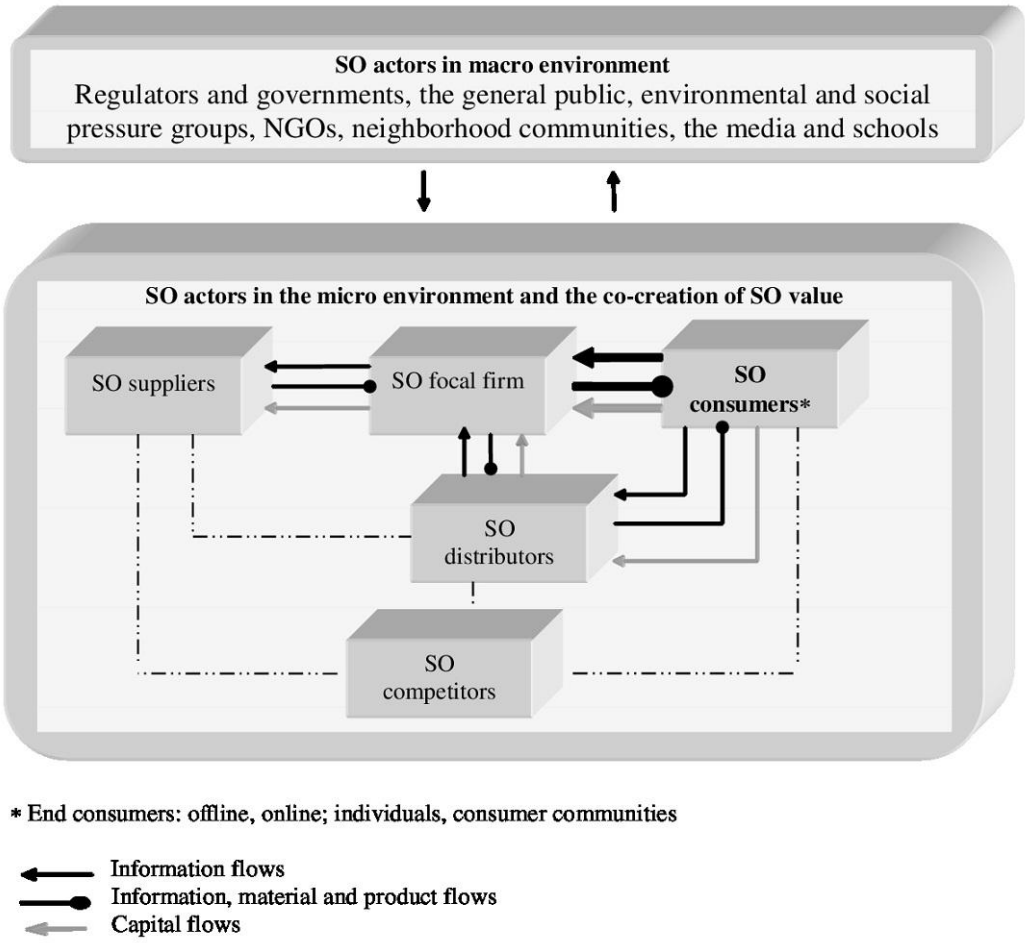


Figure 1. *Conceptual Framework – the Collaborative Partnership Paradigm in the Sustainability-Oriented Supply Chain as the Way to Sustainability (Source: Authors)*

Each of the foregoing actors has its own "patterns of sustainable behavior" and has an influence on other actors and the SC as a whole. SO actors co-create SO value (Rakic and Rakic, 2015a,b, 2017a,b). The focal firm (the brand name) appears in the market in public and is frequently considered to be the main responsible actor. The SO-SC, however, depends on numerous actors' sustainable behavior. The key actors are consumers, focal firms, suppliers, competitors and distributors.

The thickness of the line in Figure 1 between consumers and the focal firm places an accent on the fact that consumers are in focus and that end consumers' needs and wants are (or at least should be) the starting point. Consumer behavior (of end consumers) has an influence on the buying behavior of organizations (B2B). The SO lifestyle of end consumers is "the foundation of sustainability". In the traditional and digital worlds,

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consumers appear as individuals and within consumer communities. The power that consumers have in the digital world is even greater. Consumers in the digital era are in a position to be the most important co-creators of value; yet, they are not aware of their power. The speed of content distribution on the Internet (via social media – especially through social networks and blogs, portals, etc.), the consumers' awareness of a healthy lifestyle and the tendency to preserve the environment will affect the sustainable behavior of an increasing number of consumers. This will further affect the sustainable behavior of all other stakeholders in the supply chain. Consumers can create, comment and share content through social media. Consumers can influence the business and image of firms. On the other hand, the firms based on word-of-mouth marketing can influence the word-of-mouth communication of consumers. Content, like a virus, can be transmitted over the Internet. Responsible actors can use the power of viral marketing to create content about the importance of sustainable behavior for all actors. Companies can encourage influential people (bloggers and various activists in the field of sustainable development) to create, comment and share the content about sustainable behavior.

If end consumers behave sustainably, then firms as buyers also have to behave sustainably. SO consumers have an influence on the "sustainability mindset" and behavior on the B2B market. Then, the focal firm (as the buyer) has to conduct sustainable production, purchase sustainable inputs from SO suppliers and submit sustainable inputs to SO consumers directly and/or through SO distributors. The SO-SC is an opportunity, not a threat, for firms. The speed (of business and change), digitalization and the SO-SC are opportunities for firms.

SO actors in the macro-environment (regulators and governments, the general public, environmental and social pressure groups, NGOs, neighborhood communities, the media and schools) have the roles of setting "the patterns of sustainable behavior", promotions, pressures and controls of actors' sustainable behavior.

This conceptual framework presents:

- SO actors in a macro-environment;
- SO actors in a micro-environment;
- the mutual relations of the macro-environment and the micro-environment;
- the mutual relations of actors in the micro-environment;
- the collaborative partnership of actors in the process of the co-creating of SO value;
- actors in the SC as the co-creators of SO value;
- information, the material, the product and capital flows in the micro-environment;
- end consumers as offline and online consumers; individuals and consumer communities;
- the dominant role of SO consumers in the process of the co-creation of SO value in the SC;
- the power of SO consumers as the co-creators of SO value –the impact of consumer behavior and the consumers' SO lifestyle on the SO behavior of all actors in SC-SO consumers as the initiators of the co-creating of SO value;
- the SO-SC as a result of the complementary action of numerous actors' sustainable behavior; and
- the SO behavior of all actors in the SC for the purpose of achieving SD goals.

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The New Agenda for Sustainable Development emphasizes the need for "transforming our world". Embedding sustainability into the SC is part of the holistic perspective of sustainability. Actors have become involved in embedding the principles of sustainable development in the SC. Adaptations in the SC are based on three patterns. The first one is "the sustainability pattern", i.e. the acceptance and adherence to sustainability initiatives. The second one is the "pattern of SO actors". In order to achieve sustainability in the SC, it is needed that numerous SO actors should be engaged, including consumers, the focal firm, employees, suppliers, regulators and governments, the general public, environmental and social pressure groups, NGOs, neighborhood communities, the media, schools and universities. Many actors' "SO mindsets" have an influence on the achievement of the SD goals. For that reason, SO collaboration and the application of the "pattern of SO collaboration" are needed. These are simultaneously the three stages in embedding sustainability in the SC and the three stages on the pathway from SO actors in the SC to the SSC. In phase one, the introduction of sustainability in the SC is understood and accepted. In the second phase of embedding sustainability in the SC, for a more significant acceptance of sustainability in the SC as many SO actors as possible need to be engaged towards achieving sustainable behavior, amongst them – end consumers in particular. Ultimately, in the third phase, in order to achieve SD goals, the majority of actors need to be SO and behave in a sustainable manner, so that the "pattern of SO collaboration" is in focus.

Conclusion

According to the SO collaboration paradigm, all actors and all stakeholders act in a collaborative partnership for the purpose of achieving SD goals. The SO-SC is a result of a complementary action of numerous actors' sustainable behavior. Embedding sustainability in the lifestyle of end consumers is the basis of end-consumer sustainability. Embedding sustainability in the SC of firms as buyers (B2B) is one part of the holistic perspective of corporate sustainability. Embedding sustainability in actors' behavior is one part of the holistic perspective of the SD. SO actors in the SC influence the sustainability of individuals, companies and countries. Actors' sustainable behavior and collaborative partnership lead "to achieving SD in its three dimensions – economic, social and environmental – in a balanced and integrated manner" (UN, 215, p. 3).

The contributions of this paper are as follows:

- placing an emphasis on the co-creating of SO value in the SC,
- the presentation of the co-creators of SO value in the SC,
- the presentation of the SO behavior of all actors in the SC for the purpose of achieving SD goals,
- the SO-SC as a result of the complementary action of the sustainable behavior of numerous actors,
- placing an emphasis on the power of SO consumers as the co-creators of SO value - the impact of consumer behavior and the consumers' SO lifestyle on the SO behavior of all actors in the SC,
- the presentation of the three patterns of embedding sustainability in the SC,

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- the presentation of the three stages of the transformation of the SC into the SO-SC,
- the presentation and analysis of the conceptual framework: the collaborative partnership paradigm in the SO-SC as a way to sustainability.

Our proposed framework (*Fig. 1*) require empirical validation. Those could be the bases for future research. The research questions could be:

- Are stakeholders in the supply chain aware of the fact that they are the co-authors of values?
- Can stakeholders from the macro-environment influence the SO behaviour of consumers and other actors in the micro-environment?
- Can the focal firm influence the behaviour of other actors in the supply chain?
- Are consumer behaviour and the consumer lifestyle the basis of sustainability?
- Does SO consumer behaviour influence the SO behaviour of other actors in the supply chain?
- Are consumers aware of their having the power and of the fact that, by purchasing products/services, they can affect the survival of companies? Are consumers aware of the fact that their responsible behaviour can affect the responsible behaviour of companies and of all other stakeholders?
- Can sustainability marketing influence the behaviour of actors?
- Is viral marketing an opportunity or threat for actors in the supply chain in today's digital world?
- What and how can the co-creators of value in the supply chain do for sustainable development and sustainability?

The conclusion is that consumers are the most important co-creators of values. The trends in the field, such as digitalization and the orientation towards sustainable development, will affect consumers in such a manner as to make them understand the power they have as the initiators of the co-creation of SO value. Other stakeholders, especially firms, should realize that the sources of a competitive advantage today are: the speed, digitalization and the co-creation of SO value. The conceptual framework – the collaborative partnership paradigm in the SO-SC could be a way to sustainability.

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IMPACT OF SILICON CARBIDE NANOPARTICLES ON HATCHING AND SURVIVAL OF SOIL NEMATODES *CAENORHABDITIS ELEGANS* AND *MELOIDOGYNE INCOGNITA*

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Abstract. Silicon carbide (SiC) nanoparticles are widely used in industrial applications; however, some reports indicate they are safe while others claim the opposite. We aimed to characterize the physicochemical properties of SiC nanoparticles and investigate their impact on the multicellular animal model, *Caenorhabditis elegans* and the plant parasitic nematode *Meloidogyne incognita*. The X-ray diffractogram patterns and chemical analyses confirmed that the content is SiC. Furthermore, N_2 sorption isotherm analyses revealed that Brunauer–Emmett–Teller (BET) surface area was 37.6 m^2/g and single point adsorption total pores volume of pores less than 126.1853 nm in diameter at p/p° = 0.984417366 was 0.124975 cm^3/g. Furthermore, adsorption average pore width was 13.26382 nm. Scanning electron microscopy showed that the SiC nanoparticles have a semi-crystalline shape with uncompleted faces. Some edges are angular whereas others are curved and smooth with an average particle size of 50 nm ± 21.5. The bioassays indicated that SiC nanoparticles did not affect hatching of larvae of both nematodes, whereas they affected dramatically the survival of the first stage larvae (L1) of *C. elegans,* but not the second stage juveniles J_2s of *M. incognita*. There was no effect of SiC on length and width of the dead L1; however, internal organs, particularly the intestine, exhibited black color indicating uptake of the SiC nanoparticles.
Keywords: *physicochemical properties, toxicity, electron microscopy, X-ray*

Introduction

Silicon carbide (SiC), also known as carborundum, is a hard, covalently bonded material produced by the carbothermal reduction of silica using the Acheson process (Acheson, 1895). Approximately 200 SiC poly-types exist due to its periodic stacking in alternate layer arrangements (Sudarshan, 2004). SiC nanoparticles appear in the form of a grayish white powder and cubic morphology with a melting point of 2730 °C with numerous industrial applications. Thus, SiC nanoparticles research is an area of intense scientific interest (Mahawish et al., 2017; Attalla et al., 2018).

SiC nanoparticles hardness, good wear, corrosion resistance, low density and price make them suitable for multiple industries. Initially utilized for grinding and cutting purposes (Cowles and Cowles, 1885; Acheson, 1895). SiC later appeared in ceramic plates, bullet proof vests, glass casting and high performance "ceramic" automobile brake

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discs (Kaufmann et al., 2003; Langhof et al., 2016). Further industrial applications include rubber tire manufacturing, resistance heating element manufacture, and UV protective mirror coatings. SiC nanoparticles are also now used in the copper matrix nanocomposite (Zhang et al., 2004; Ataee-Esfahani et al., 2009; Mula et al., 2011; Zhu et al., 2011).

SiC nanoparticles widespread use including biomedical industry, necessitates an in-depth investigation of their eco-toxicity. To our knowledge, few studies investigated the toxicity of SiC nanoparticles in the environment; however, several studies focused on the potential health impacts. Several studies report that SiC nanoparticles are an inert and do not harm lung tissues (Bruch et al., 1993a, b); however, research also suggests that SiC may have significant cytotoxic and genotoxic effects (Vaughan et al., 1993; Cullen et al., 1997; Akiyama et al., 2007). Vaughan et al. (1993) showed that SiC was toxic for rats and showed chronic inflammation in necropsies during the study. Another study also found that SiC accumulates in rat's lungs over the course of a year (Cullen et al., 1997). These findings necessitate further research to determine the toxicity of SiC to human health, and the potential implications on the environment.

Toxicity depends on the physicochemical characteristics such as particles' size, curvature, shape and surface characteristics charge, functional groups, and free energy (Pourchez et al., 2012). Based on this characterization, some particles generate adverse biological outcomes by causing proteins to unfold, thus resulting in a loss of enzymatic activity. Another pattern is the release of toxic ions in a suspending medium or biological environment (Xia et al., 2008). Thus, several reports have recommended the reevaluation of SiC nanoparticles use (Barillet et al., 2010a, b; Pourchez et al., 2012).

Nematodes as soil organisms were used to evaluate nanoparticles' toxicity (Ma et al., 2009; Cromwell et al., 2014). Nematodes are prevalent and highly distributed in almost every ecological habitat (Hodda, 2011). Some are restricted to a specific geographical environmental condition while others are ubiquitous (Hodda, 2011). The bacterial feeding nematode *Caenorhabditis elegans* is a well-known multicellular model organism and is used extensively in toxicity assessment studies (Ma et al., 2009; Hunt, 2017). *Meloidogyne incognita*, a plant parasitic nematode belonging to the root knot nematodes family (RKN), was also challenged with several nanoparticles, such as silver, titanium, and silicon nanoparticles (Ardakani, 2013; Cromwell et al., 2014). The response of *M. incognita* to the three nanoparticles was recorded in laboratory and pot experiments to assess toxicity (Ardakani, 2013). Results showed that RKN J_2s were highly sensitive to silver nanoparticles while they were less sensitive to titanium and silicon nanoparticles in laboratory. It was also shown that 0.02% (W/W) of silver and titanium nanoparticles totally controlled RKN in pots.

So far, data are missing regarding the effect of SiC on soil nematodes and physicochemical characteristics which are factors determining the nanoparticle´s toxicity. Therefore, the aim of this study is to characterize the physicochemical properties of SiC nanoparticles and investigate their impact on hatching, mortality and morphology of the nematodes *Caenorhabditis elegans* and *Meloidogyne incognita*.

Materials and methods

Characterization of SiC nanoparticles

Synthetic SiC nanoparticles were obtained from Hefei Ev Nano Technology Company (China). Stock concentration of SiC nanoparticles suspensions were prepared (250 mg/l) in sterile, de-ionized water. In order to obtain stable nanoparticles suspension, an

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ultrasonic processor (UP 200S, Germany) was used to disperse the nanoparticles. In addition, carboxyl methyl cellulose (CMC, BDH chemicals Ltd Poole England) was added (1% CMC by weight) for dispersion and stabilization of suspension. The acidity (pH) and salinity (measured as electrical conductivity (EC)) were measured for the two concentrations. A half strength concentration was prepared from the stock solution to obtain a suspension of 125 mg SiC nanoparticles/l with 0.5% CMC. The two concentrations (250 and 125 mg/l) were then used in bioassays.

Chemical and physical properties of SiC nanoparticles were characterized. X-ray powder diffraction (XRD) patterns were recorded using Cu Kα radiation source by Shimadzu X-ray diffractometer (XRD–6000). The chemical composition of the nanoparticles powder was determined by X-ray fluorescence (XRF) analysis measurements using a sequential X-ray spectrometer (Shimadzu XRF-1800). Nanoparticle surface area and pore size distribution were calculated based on nitrogen adsorption method using Micromeritics' Gemini® VII 2390 Series of surface area and porosity analyzers. SiC nanoparticle morphology was investigated using a Scanning Electron Microscope (SEM) from FEI company- Inspect F50/FEG (Schottky Field Gun) high Vacuum (6e-4 Pa Everhart-Thornley SE detector Solid-State BSED, Netherlands).

Nematode cultures

Caenorhabditis elegans

The wild type of *C. elegans*, used in this work, was obtained from the *Caenorhabditis* Genetics Centre (University of Minnesota). The nematodes were cultured on nematode growth medium (NGM) which consists of 20 g/l agar, 2.5 g/l peptone, 5 mg/l cholesterol, 25 mM KPO_4 (KH_2PO_4 and K_2HPO_4) buffer, 51 mM NaCl and 1 mM $MgSO_4$. A loopful of the bacteria *E. coli* strain OP50, obtained from the *Caenorhabditis* Genetics Centre, was added to the medium as a source of food for the nematode. The bacterial isolate was maintained on Luria-Bertani (LB) medium (5 g /l of yeast extract, 10 g /l of tryptone, and 10 g /l NaCl). The *C. elegans* culture was maintained periodically and incubated at 24 °C.

To obtain the synchronized eggs or first stage larvae (L1) for bioassays, the nematodes, including the females and the eggs, were washed off the NGM plates with sterile water and placed in a beaker. The nematode suspension was poured on 2 ml eppendorf tubes with a total volume of 1 ml. Each 1 ml of nematode suspension was lysed by adding 120 µl of 5 N sodium hydroxide (NaOH) and 120 µl of 6.5% sodium hypochlorite (NaOCl). This procedure hydrolyzes the adult and juvenile nematodes, but not the eggs. The tubes were vortexed every 30 s for 5 min. After that the tubes were centrifuged at 6000 rpm for 1 min, supernatant was decanted and the pellets containing the eggs were suspended in 1 ml sterile water. Tubes were vortexed and centrifuged again to clean the eggs from remaining NaOH and NaOCl mixture. The eggs suspension in water was collected and used immediately in the hatching bioassays or eggs were incubated at 24 °C for 8 h until L1 hatched and then were used in the toxicity bioassays.

Meloidogyne incognita

The egg masses and second stage juveniles (J_2s) of the plant parasitic nematode *M. incognita* were used in this assay. The egg masses of *M. incognita* in the hatching assay were handpicked from the roots of infected tomato plants grown in the glass house at the University of Jordan, Amman, Jordan. Egg masses were either directly exposed to the SiC nanoparticles in the hatching bioassay or were incubated in water at 24 °C until the J_2s

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were hatched. The newly hatched J_2s were subjected to the SiC nanoparticles to assess the nematode survival rate.

Nanoparticles suspension

Eggs or larvae of *C. elegans* were exposed to 1 ml of SiC nanoparticle suspensions (688 µl of either SiC nanoparticles concentrations suspended in CMC, 62 µl 0.5 M KCl, 100 µl 0.5 M NaCl, and 150 µl of sterile water contained eggs or larvae). Egg masses and J_2s of *M. incognita* were exposed to 5 ml of either concentrations (3440 µl of either concentration of the nanoparticles suspended in CMC, 310 µl 0.5 M KCl, 500 µl 0.5 M NaCl, and 750 µl of sterile water contained egg masses or J_2s). All bioassays had two final concentrations of SiC nanoparticles, 86 and 172 mg/l. The nanoparticles were vortexed and sonicated for 15 min before the application to prevent the agglomeration of the particles.

Effect of SiC nanoparticles on L1 hatching of C. elegans

Approximately 400 eggs were exposed to the nanoparticles suspensions in a 24-well tissue culture plate. The 0.5% CMC, 1% CMC and water served as controls. Every treatment was replicated three times. Plates were incubated for 24 h at 24 °C. The treated eggs were checked for L1 hatching after 24 h using a dissecting light microscope (Nikon, SMZ645) and the percentages of hatching were calculated and tabulated.

Effect of SiC nanoparticles on L1 survival of C. elegans

In this bioassay, two concentrations of SiC nanoparticles were used: 86 and 172 mg/l. A total of 50 L1 were exposed to 1 ml volume of the nanoparticles in a 24-well tissue culture plate. Treatments contained 0.5% CMC, 1% CMC and water only served as controls. Every treatment was replicated three times. Plates were incubated for 6 h at 24 °C. Numbers of dead and live L1 were counted 24 and 36 h after treatment using inverted binocular compound microscope (MEIJI TECHNO ML VT-T-PC) and the percentages of mortality were calculated and tabulated. The LC50 value was determined by using the polynomial regression curve.

Observations on dead L1 were documented with photos. Lengths and widths of five L1 from each treatment were measured and tabulated.

Effect of SiC nanoparticles on J_2s hatching of M. incognita

In this bioassay, egg masses were exposed to the two concentrations: 86 and 172 mg/l of SiC nanoparticles. Similar size egg masses were used in all replicates of all treatments. Every treatment consisted of three egg masses and was replicated three times. 0.5, 1% CMC and water served as controls. Plates (with a total of 5 ml of SiC nanoparticles) were incubated for seven days at 24 °C. The treated egg masses were checked for J_2s hatching after 2, 4 and 7 days of treatment using a dissecting light microscope (Nikon, SMZ645) and the number of hatched J_2s after 2, 4, and 7 days were calculated and tabulated.

Effect of SiC nanoparticles on J_2s survival of the M. incognita

Approximately 50 J_2s of *M. incognita* were treated with concentrations of 86 and 172 mg/l of SiC nanoparticles. The 0.5% CMC, 1% CMC and water served as controls. Every treatment was replicated three times. Plates (with 5 ml each) were incubated for

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four days at 24 °C. The numbers of dead and live nematodes were counted using a dissecting light microscope (Nikon, SMZ645) for four consecutive days and the mortality rates were tabulated.

Data analyses

Data obtained from all bioassays were subjected to analysis of variance and the means were separated using least significant differences (LSD) (Little and Hills., 1974).

Results

Characterization of SiC nanoparticles

The X-ray diffractogram patterns revealed that the mineral content is Silicon Carbide (SiC) (*Fig. 1*). The major peak was at 2 Θ = 35.68°, 41.42°, 60.01°, and 71.77°. The chemical analyses confirmed that 99.2 wt % of the nano-powder was SiC (*Fig. 2*). The pH and the electrical conductivity (EC) of the stock solution (250 mg/l) was 7.8 and 50 µS/cm respectively. N_2 sorption isotherm analyses showed that BET surface area was 37.6 m²/g and single point adsorption total pore volume of pores less than 126.1853 nm diameter at p/p° = 0.984417366 was 0.124975 cm³/g. Furthermore, adsorption average pore width was 13.26382 nm.

SiC nanoparticle morphology was investigated using scanning electron microscopy (SEM). *Figure 2* shows that the morphology of the SiC nanoparticles has a semi-crystalline shape with incomplete faces. Some edges are angular whereas the others are curved and smooth. The average particle size is 50 nm ± 21.5.

Effect of SCi nanoparticles on the nematode C. elegans L1 hatching and survival

To investigate the effect of SiC on *C. elegans* viability and survival, we exposed the nematode eggs and L1 to 86 and 172 mg/l of the nanoparticles. Results showed that percentage of hatched L1 was significantly higher when eggs were exposed to SiC nanoparticles compared with controls (*Table 1*).

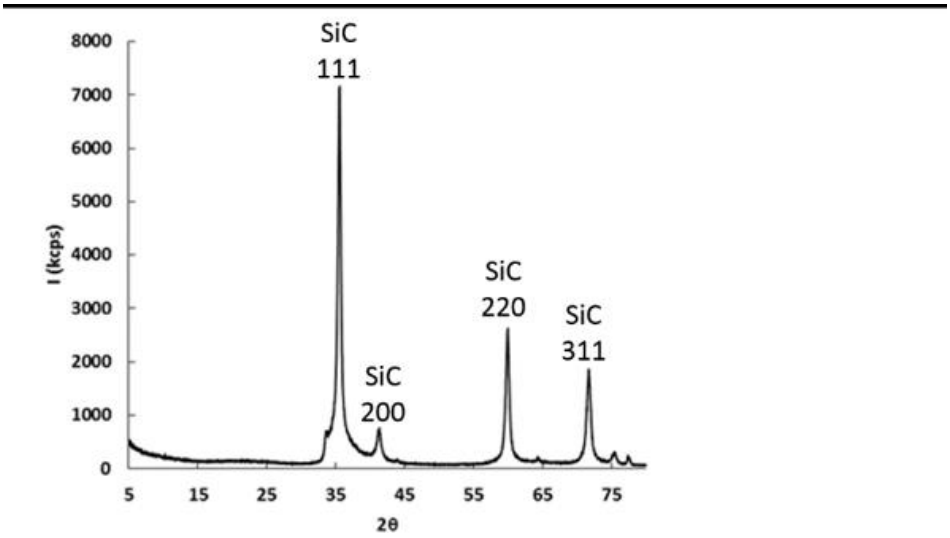


Figure 1. *X-ray diffractometer patterns of the used SiC nanoparticles*

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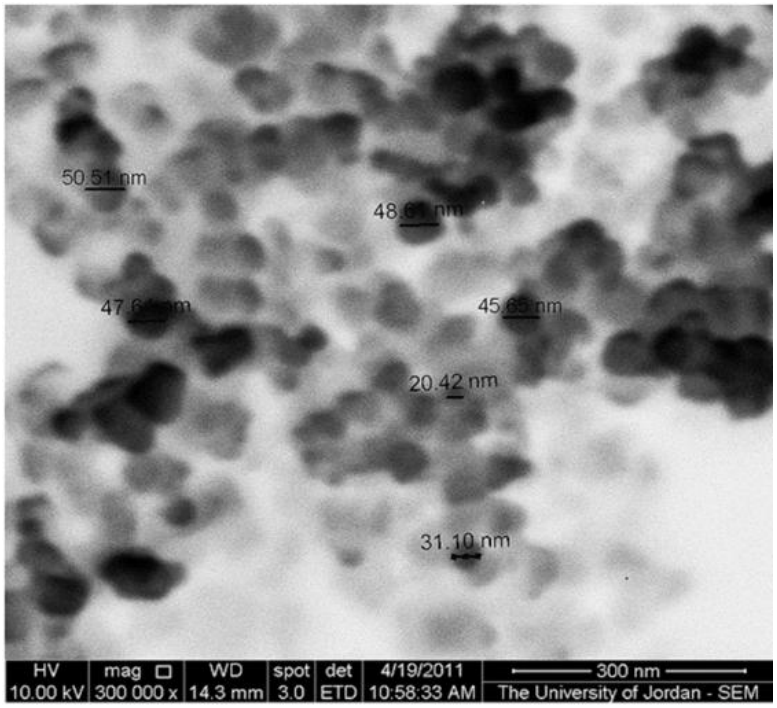


Figure 2. *SEM image of SiC nanoparticles*

Table 1. *Effect of SiC nanoparticles on hatching and mortality of first stage larvae (L1) of a wild type of the nematode Caenorhabditis elegans*

Treatment	Hatching* %	Mortality** %	
		Exposure time	
		24 h	36 h
SiC nanoparticles 172 mg/l	97.5 a ± 0.47	19.3 a ± 1.6	69.6 a ± 5.3
SiC nanoparticles 86 mg /l	98.0 a ± 0.32	17.2 a ± 2.8	66.4 a ± 8.7
CMC 1% and salts	94.0 b ± 1.31	3.5 b ±1.2	17.7 b ± 1.5
CMC 0.5% and salts	94.0 b ± 1.93	2.1 b ± 1.0	15.2 b ± 4.8
Water only	95.0 b ± 1.17	2.6 b ± 2.4	9.6 b ± 3.9

*The data points represent the means of the three replicates followed by ± standard deviation, approximately 400 eggs per replicate. Means with the same letter are not significantly different at 0.05 *P* level

**The data points represent the means of the three replicates, approximately 50 L1 per replicate. Means with the same letter are not significantly different at 0.05 *P* level

Exposure of L1 individuals to SiC nanoparticles for 24 h resulted in a significant ($P < 0.05$) increase in mortality compared to control treatments. Around 17 and 19% of L1 died in SiC nanoparticle treatments compared to up to 3.5% L1 mortality in controls (*Table 1*). After 36 h of exposure to both concentrations of SiC nanoparticle, the survival of L1 was dramatically decreased and reached 30% (70% mortality) and 34% (66% mortality) when treated respectively with 172 mg/l and 86 mg/l; however, the percentage of survived L1 in control treatments were 82, 85 and 90 in 1% CMC, 0.5%

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CMC and water controls, respectively. In general, there was no significant difference of survived L1 exposed to 172 mg/l compared with those exposed to 86 mg/l SiC nanoparticles after 24 and 36 h of exposure (*Table 1*). Using the regression equation ($y = -4E\text{-}05x^2 + 0.0103x - 2E\text{-}15$, $R^2 = 1$), the value of LC50 was 65 mg/l after 36 h of exposure.

The SiC nanoparticles at both concentrations slightly increased the length and width of L1 compared to salt and water only treatments, but the difference was not significant (*Fig.* 3).

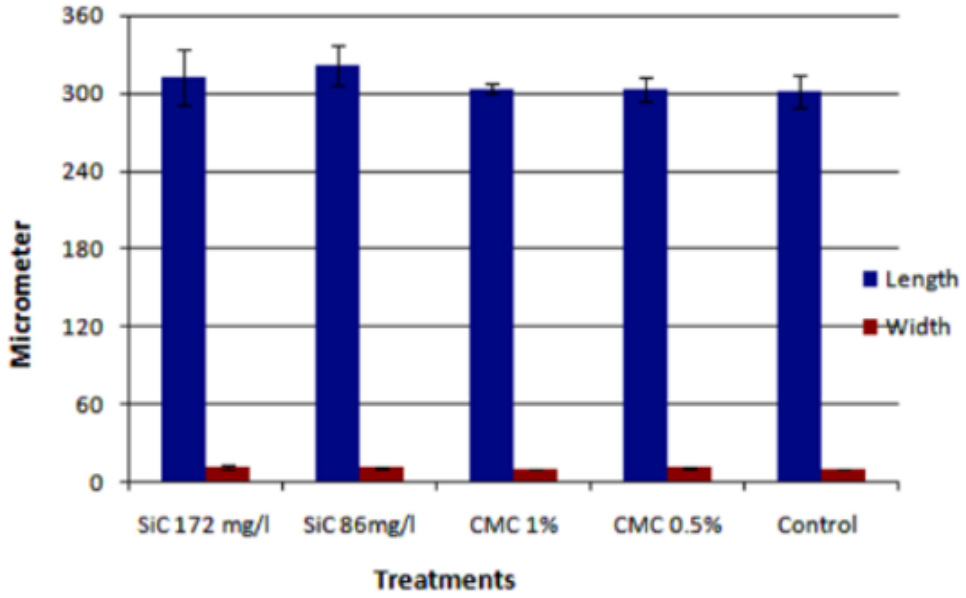


Figure 3. *Effect of SiC on length and width of the soil nematode Caenorhabditis elegans. Length and width of the first stage larvae (L1) of the soil nematode Caenorhabditis elegans after 24 h of exposure to the two concentrations (172 and 86 mg/ l) of silicon carbide nanoparticles and salts (1% and 0.5% CMC) and water controls with E. coli added as a food. The data points and error bars represented the means and standard deviation respectively, of the three replicates, approximately 50 L1 per replicate*

The black color was observed in the internal organs including intestine of the dead L1s that were exposed to the nanoparticles while internal organs were transparent in controls (*Fig. 4*).

Effect of SiC nanoparticles on M. incognita J_2s hatching and survival

To investigate the effect of SiC on J_2s hatching egg masses of *M. incognita* were treated with 86 and 172 mg/l of the nanoparticles. In all treatments hatching of J_2s increased with time. However, treatments with both concentrations of SiC nanoparticles and the two concentrations of CMC salts resulted in higher number of hatched J_2s than those placed in water only at the three monitored times. The use of 86 mg/l SiC nanoparticles caused the highest hatching followed by concentration (172 mg/l) but without significant differences (*Table 2*).

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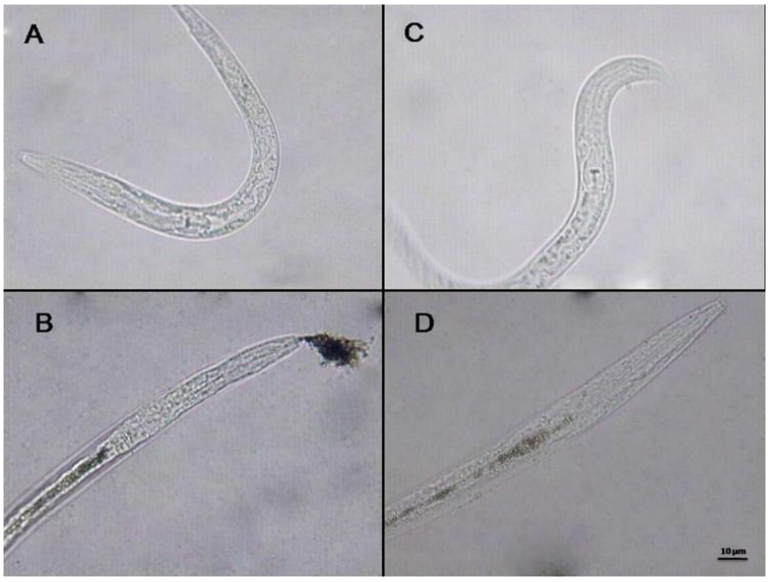


Figure 4. *Aggregation of SiC nanoparticles on First stage larvae (L1) of Caenorhabditis elegans. First stage larvae (L1) of Caenorhabditis elegans exposed to (A) 1% CMC; (B) water only; (C) 86 mg/l SiC nanoparticles showing blackening in intestine and aggregation of SiC nanoparticles at anterior extremity; and (D) 172 mg/l SiC nanoparticles showing blackening in the intestine*

Table 2. *Effect of SiC nanoparticles on hatching of second stage juveniles (J_2s) of the plant parasitic nematode Meloidogyne incognita after the exposure of the eggs for 7 days*

Treatment	No. of hatched J2*		
	Exposure for		
	2 days	4 days	7 days
SiC nanoparticles 172 mg/l	41 ± 23.8	76 ±24	205 ± 82.6
SiC nanoparticles 86 mg /l	41 ± 17.2	90 ±35	254 ± 33.1
CMC 1% and salts	48 ± 30.4	71 ± 35.1	192 ± 93.1
CMC 0.5% and salts	62 ± 35.9	106 ± 83.8	185 ± 133.4
Water only	23 ± 3.51	41 ± 1.5	104 ± 10.5

*The data points represented the means of the three replicates followed by ± standard deviation, approximately 3 egg masses per replicate. No significant differences at 0.05 *P* level

Similar to the *C. elegans* L1, the two concentrations of SiC nanoparticles did not decrease the *M. incognita* J_2s survival. The concentration (172 mg/l) of SiC nanoparticles caused a slight reduction in survival of J_2s where it decreased to 93% (7% mortality) after one day of exposure and continued to decrease to reach 92% (8% mortality) after two days and did not change up to four days of exposure. On the other hand, the low concentration (86 mg/l) of SiC nanoparticles did not cause mortality after two days of exposure and caused only 2% mortality on the third day and stayed steady after the fourth day of exposure (*Table 3*). Few J_2s that were placed in water only died after one day of exposure and no change in the number of live nematodes up to the fourth day of treatment. Similar results were noticed on J_2s placed in 0.5% CMC, while a slight decrease was noted in the number of live J_2s treated with 1% CMC (*Table 3*).

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Table 3. *Effect of SiC nanoparticles on the survival of second stage juveniles (J_2s) of the plant parasitic nematode Meloidogyne incognita after the exposure of the J_2s for 4 days*

Treatment	Mortality* % ± SD of J2			
	Exposure time			
	1 day	2 days	3 days	4 days
SiC nanoparticles 172 mg/l	7.03 ± 6.0	7.89 a ± 5.2	7.89 a ± 5.2	7.89 a ± 5.2
SiC nanoparticles 86 mg/l	0.00	0.00 b	1.45 b ± 1.3	1.45 b ± 1.3
CMC 1% and salts	1.21± 1.1	1.84 b ± 1.9	1.84 b ±1.9	1.84 b ± 1.9
CMC 0.5% and salts	0.78 ± 1.3	0.78 b ± 1.3	0.78 b ± 1.3	0.78 b ± 1.3
Water only	0.83 ± 1.4	0.83 b ± 1.4	0.83 b ± 1.4	0.83 b ± 1.4

*The data points represented the means of the three replicates followed by ± standard deviation, approximately 50 J_2s per replicate. Means with the same letter are not significantly different at 0.05 *P* level

Discussion

In this study, we showed the hatchability and the survival of two soil nematodes under the exposure to the SiC nanoparticles. The nematode *C. elegans* was used to test the toxicity of SiC nanoparticles since basic physiological processes and stress responses are conserved between this nematode and humans (Lee et al., 2007; Hunt, 2017). We used the L1 of *C. elegans* since several reports showed that L1 is more sensitive than both L4 and young adult of *C. elegans* to nanoparticles such as Al nanoparticles and others (Wu et al., 2011).

Hatching of juveniles from eggs of both tested nematodes was not affected and in fact number of hatched juveniles was more in eggs exposed to the SiC nanoparticles suspension than the controls. Similarly, a complete oocyte development was observed and this development ends with the hatching of the L1 within the parent's body of *C. elegans* after the exposure to silica nanoparticles (Pluskota et al., 2009). This suggests that SiC and other nanoparticles did not penetrate the eggshell of the eggs and thus hatching was not affected.

The bioassay showed that SiC nanoparticles were lethal to L1 of *C. elegans* and dead nematodes exhibited black internal organs, indicating that SiC black nanoparticles were taken up during feeding. This supports results from other studies, which showed that nanoparticles taken by the nematode L1 *C. elegans* during feeding were translocated to primary and secondary organs, including epithelia cells, intestines and reproductive organs (Pluskota et al., 2009; Wu et al., 2011). On the contrary, few J_2s of the plant parasitic nematode *M. incognita* died when they were exposed to SiC nanoparticles and lacked the obvious black color in the intestine. This could be attributed to wider mouth of the L1 of *C. elegans,* which allowed for a larger amount of the SiC nanoparticles to be taken up during feeding. Additionally, the root knot nematodes, *Meloidogyne* spp. are obligate sedentary endoparasites and the J_2s do not feed when they are found in the soil or in a solution, thus, the uptake of nanoparticles mechanism by these plant parasites should be investigated. We believe that the lethality of the nanoparticles is not due to pH since nematodes have a wide pH tolerance that ranged from 3 to 12 (Khanna et al., 1997).

This study showed that the SiC nanoparticles of the size of 50 nm with LC50 of 65 mg/l did not affect hatching of either nematode but did affect the survival of *C. elegans*.

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Several reports showed that smaller sizes of nanoparticles are more toxic than larger ones (Khare et al., 2011; Park et al., 2011). It was also shown that silver nanoparticles of a size of 20 nm were more toxic than the larger nanoparticles (80 and 113 nm) (Park et al., 2011). Similarly, TiO_2 and ZnO nanoparticles less than 25 nm were toxic to the nematode *C. elegans* with LC50 of 77 mg/l and 0.32 mg/l, respectively (Khare et al., 2011). Whereas TiO_2 nanoparticles with a size larger than 25 nm were non-toxic and LC50 of 2 mg/l was recorded for < 100 nm ZnO nanoparticles. This might explain the contradictory reports that on SiC nanoparticles' toxicity. However, other characteristics of nanoparticles may affect toxicity and therefore more investigations are needed regarding the comparison of sizes as well as other characteristics.

Conclusion

The physicochemical characterized SiC nanoparticles showed a toxic effect on L1 of the multicellular animal, the soil nematode *C. elegans,* and reduced its survival. The dead L1s that were exposed to either 86 or 172 mg/l of SiC nanoparticles exhibited black internals. In contrast, the two studied concentrations of SiC nanoparticles did not have such an effect on the second stage juveniles J_2s of the plant parasitic nematode *M. incognita*. However, these SiC nanoparticles did not have an effect on the eggs of either nematodes, and instead accelerated hatching. Future work should focus on the physical and chemical characteristics of SiC nanoparticles' toxicity. Additionally, more histological and biochemical studies are needed to assess the mechanism of toxicity.

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THE IMPACT OF CLIMATE CHANGE ON VITICULTURAL POTENTIAL AND WINE GRAPE VARIETIES OF A TEMPERATE WINE GROWING REGION

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Abstract. Climate change causes major shifts in both the spatial distribution of wine-growing areas and their specific types of wine production. With the increase of heliothermal resources, the viticultural potential of temperate wine regions grows, becoming more suitable for quality wine production and for the diversification of the wine types production. The shifts in the viticultural potential of the Averești wine-growing region from Romania during the last decades are evidence of these evolutions. Between 1961-2013, the average annual temperature in the area increased by 0.47 °C, the temperature of July by 1.26 °C, the sunshine duration by 78.6 h and the global radiation by 1.69 kcal/cm^2. Against this background, the area passed from viticultural potential mainly for white table wines to viticultural potential for quality white wines and red table wines. The new viticultural potential is exploited in the Averesti wine-growing region after 2000 by expanding into culture the international winegrape varieties for quality white wines and by introducing the *Cabernet Sauvignon, Merlot* and *Pinot noir* winegrape varieties for red wines into cultivation. The study demonstrates the need to evaluate the new viticultural potential generated by climate change and the need to delineate the viticultural homogeneous zones in the wine-growing regions beneficiary of climate change, in order to correctly zone newly introduced winegrape varieties in such areas.
Keywords: *grapevine, viticulture, Averești, wine, suitability*

Introduction

The specific varieties of wine-growing regions have been established throughout the time by maintaining in their area the winegrape varieties best suited to local relief, climate and soil conditions. These environmental factors have a decisive influence on grapevine growing and fruiting (van Leeuwen and Seguin, 2006; Winkler et al., 1974). In relation to the influence of the climate, winegrape varieties whose grapes reach full ripening each year and which best express their quality potential have been maintained (Seguin, 1986). As a result, today each wine-growing region has its own specific winegrape varieties best adapted to the local environment and which provide the most qualitative wines that can be obtained in the area (Wolkovich et al., 2018).

Due to this close dependence of winegrape varieties on the climate, one of the consequences of climate change (IPCC, 2013) might be their modification and implicitly, the one of the types of wine production specific to affected wine-growing regions. The first predictions in this regard have been made since the 1990s by Kenny and Harrison (1992), and confirmations of this evolution appear more and more

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frequent in recent years (Stock et al., 2005; Bucur and Dejeu, 2016; Irimia et al., 2017b). The modification of wine-growing regions' specific winegrape varieties is, in fact, an adaptation of wine-growing regions to the new climatic context, with particularities from one geographic area to another. For example, in case of the Mediterranean wine-growing regions, where high temperatures already affect the well-established sensory profile of wines (Nemani et al., 2001), it might be necessary to replace local varieties with some more adapted to the warm climate ones (Wolkovich et al., 2018). On the contrary, in cool vineyards where the climate becomes more suitable to viticulture (Gaal et al., 2012; Kryza et al., 2015; Nesbitt et al., 2016; Kovacs et al., 2017; Irimia et al., 2017a) varieties for white table wines, could be replaced by varieties for quality white wines and even by varieties for red wines.

In the context of climate change (Busuioc et al., 2010; Piticar and Ristoiu, 2012; Dumitrescu et al., 2014; Bandoc et al., 2018) recent studies indicate that Romania's viticulture undergoes structural and spatial changes of climatic suitability for viticulture (Bucur and Dejeu, 2016; Irimia et al., 2017a). Climatic suitability for red wines is advancing from S to N, overlapping traditional white wine production areas; climate suitability for white wines is advancing northwards and at higher altitudes, beyond the limits of traditional wine-growing areas. Significant shifts are also recorded in vineyards on a fine scale (Irimia et al., 2017b).

As a result of this evolution of climatic suitability for viticulture, the traditional varieties of vineyards in the northern part of Romania, which is colder, have begun to undergo important changes: In the vineyards of Transylvania, a wine-growing region specialised in white wine production, *Pinot noir, Merlot* and the native *Feteasc*ă *neagr*ă red varieties have recently been introduced (Urcan et al., 2016), while in the vineyards of Moldavia, also producing white wines, *Cabernet Sauvignon, Merlot, Pinot noir* and *Feteasca neagr*ă have been introduced.

The purpose of this study is to reveal the changes in viticultural potential as a result of climate change in the Averești wine-growing region, as well as the changes in its specific winegrape varieties in this context. Previous studies have already revealed the suitability of this area for white wine production (Irimia and Patriche, 2009, 2010). On the background of climatic change, after 2000 winegrape varieties such as *Cabernet Sauvignon, Merlot, Pinot noir* and *Feteasca neagr*ă were introduced in the area. For the accumulation of anthocyanins and the production of quality wines, winegrape varieties for red wine require high heliothermal resources (Dokoozlian and Kliewer, 1996). The location of the new red winegrape varieties in the Averești area was not based on a zoning study, which is why this study aims to evaluate the accuracy of their zoning. Inadequate location of a winegrape variety in a vineyard limits the expression of its qualitative potential and produces ordinary wines (Asselin et al., 2001; Riou et al., 1994).

Assessment of the viticultural potential is based on the analysis of the spatial distribution and suitability for the winegrape varieties of the climate, relief and soil factors (Jones and Duff, 2007; Tomasi et al., 2013; Irimia et al., 2014; Nowlin and Bunch, 2016). Studies based on bioclimatic indices (Blanco-Ward et al., 2017; Quenol et al., 2017), soil and lithological parameters (Morlat and Bodin, 2006) and their combined influence (Jacquet and Morlat, 1997) are also used. This study uses the GIS-based methodology for assessing the viticultural potential in temperate climate conditions of Romania (Irimia et al., 2014), which takes into account the fine-scale

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spatial distribution and the suitability for winegrape growing of 15 parameters representative for vineyards' relief, soil and climate.

The study is designed in four parts, namely: the structure and suitability of the relief, soils and climate in the area; the viticultural potential and the specific winegrape varieties during 1961-1979; the viticultural potential of the 1981-2013 period and the current winegrape varieties; the analysis of the current location of winegrape varieties in the area.

Materials and methods

Study area

The study area is the Avereşti wine-growing region, located in the eastern part of Romania, at 46°46'50" N latitude and 28°0'37" E longitudes (*Fig. 1*).

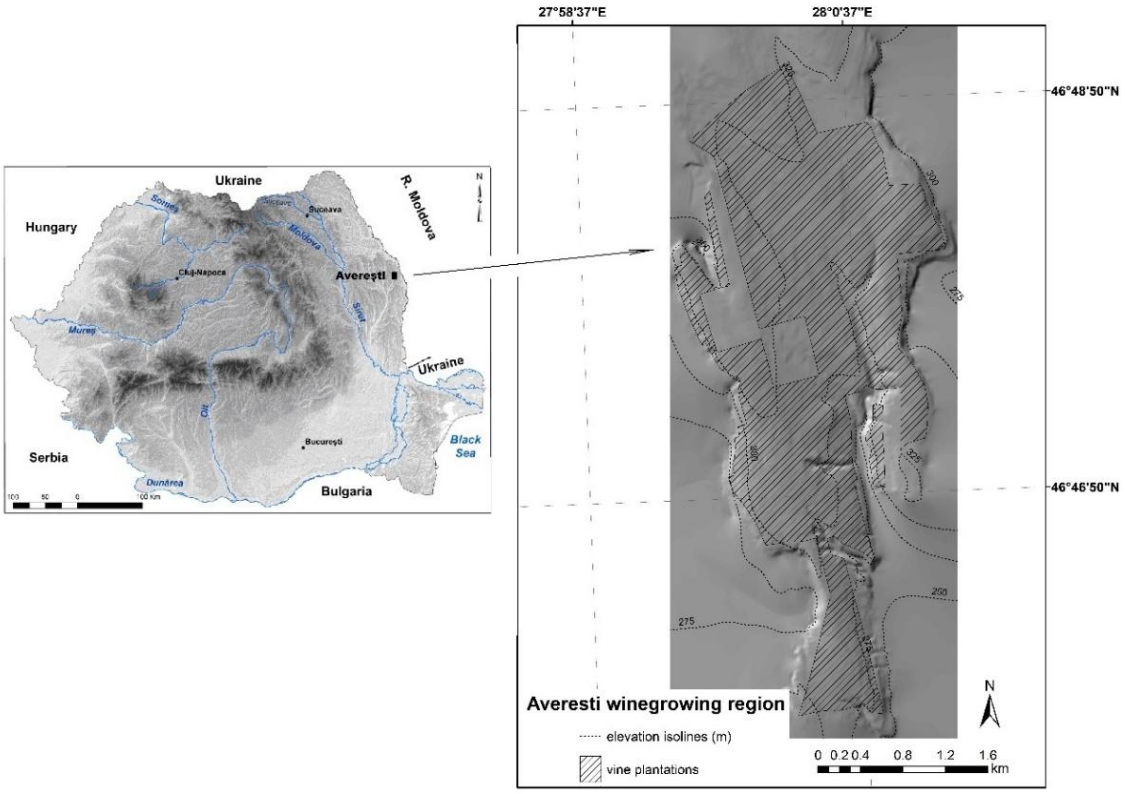


Figure 1. *The map of the Avereşti wine-growing region and its geographical location*

The area has a surface of about 670 ha and it is a compact massive vineyard, situated on a long interfluvial plateau, bordered by river valleys in the east and west and having a slope which causes a difference of about 80 m between the northern (338 m asl elevation) and the southern limit (257 m asl). The area has a temperate continental climate, Dfb in the Koppen-Geiger climate classification (Kottek et al., 2006), with warm and sometimes dry summers, and harsh and sometimes frosty winters. Soils are predominantly represented by Luvisols, with a higher content of clay and humus in the plateau area and thinner, poorly fertile and well drained on east and west slopes.

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Methods

The climate data used for this study were extracted from ROCADA database (Dumitrescu and Bîrsan, 2015) for one point situated in the middle of the vineyard (46°47'94" N latitude, 28°0' 21.6" E longitude, 303.59 m asl). For this location, we extracted daily values of climate parameters, including the mean, maximum and minimum temperatures, precipitations, sunshine duration and relative humidity. The values were exported in Excel for quality check and processing. The spatialization of these punctual data was achieved in GIS environment, starting from a 5 × 5 m digital elevation model (DEM) and vertical temperature and precipitation gradients (Patriche et al., 2011). The DEM was computed with high accuracy, on the basis of the elevation information extracted from 1:5000 topographic maps. The mean annual and monthly vertical gradients for temperature and precipitations were derived from WorldClim database (Hijmans et al., 2005) and SRTM elevation model for the entire Romanian territory.

The potential solar radiation and sunshine duration maps were computed in SAGA-GIS 2.0.8, using the Incoming Solar Radiation module and DEM as input raster. A correction was then applied to the potential global radiation (Entekhabi, 1997), based on the sunshine fraction, in order to achieve the actual global radiation. The actual sunshine duration was derived by multiplying the potential sunshine duration and the sunshine fraction.

The bioclimatic indices were achieved in GIS environment by applying the specific formulas (Irimia et al., 2014).

The terrain slope and aspect were derived based on DEM, and the respective rasters were classified according to the methodology.

For soil cover characterization, we used the data from 11 soil profiles taken from a fine scale agrochemical study of Averești vineyard. The clay content was interpolated by ordinary kriging, while in the case of humus content a spline interpolation was performed. Then, the spatial distributions and clay and humus contents were classified according to the methodology. The analysed soils from Averești vineyard had no skeleton content in the first 50 cm.

The viticultural potential of the area was assessed using Irimia et al. (2014) methodology for viticultural potential assessment in Romanian wine-growing regions conditions. According to this method, the averages of 15 climatic, pedological and topographical parameters for the Averești area for two time periods (1961-1979 and 1980-2013) were mapped and then classified into three suitability classes, ranked from 5 to 10 points (*Table 1*). What is more, the values within the range defined by each suitability class determine and characterize under the temperate continental climate of Romania, a certain type of wine production (Irimia et al., 2014). The choice of the two periods is based on a previous study (Irimia et al., 2017b), which points out that the warming process began consistently in the 1980's for the neighbouring wine-growing region of Cotnari (Romania). Also, the charts made up at Global and European level (EEA, 2017) show that the beginning of abrupt temperature change occurred during the same time period.

The spatial information layers for each ecological category and for each time period were further combined into a GIS database, and the average ranking points (arp) at the pixel level (5 × 5 m) were calculated in order to obtain topographical, pedological and climate suitabilities. The three maps of suitability for each time period were further combined into a GIS database, and the average of ranking points at the pixel level (5 × 5

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m) was calculated in order to obtain the viticultural potential. According to this methodology, the averages of ranking points have the following meanings (*Table 2*): 5 arp = viticultural potential for white table wines, sparkling wines and wines for distillates (WTW, SW, WD); 6 arp = viticultural potential for white table wines, sparkling wines, wines for distillates as well as for white quality wines in warmer growing seasons (WTW, SW, WD + QWW); 7 arp = viticultural potential for quality white wines (QWW); 8 arp = viticultural potential primarily for quality white wines and secondarily for red table wines (QWW + RTW); 9 arp = viticultural potential primarily for quality red wines and secondarily for quality white wines (QRW + QWW); 10 arp = viticultural potential for quality red wines (QRW). The 10 arp and 9 arp were grouped into class I – QRW; 8 arp and 7 arp into class II – QWW; 6 arp and 5 arp into class III - WTW, SW, WD; and the pixels where restrictive values appeared were classified as class IV, as unsuitable for wine production (*Table 2*).

Table 1. *Ecological factors and bioclimatic indices ranked according to their suitability for different types of wine production in temperate continental climatic conditions of Romanian wine-growing regions (according to Irimia et al., 2014)*

Ecological category	Parameters (abbreviation*, units)	Suitability interval within Romanian winegrowing regions	Unsuitable	Suitability classes/ranking points		
			IV/0	III/5	II/8	I/10
			Restrictive for grapevine growing	Type of wine production**		
				WTW, SW, WD	QWW	QRW
Topographical factors	S (%)	0-28	-	< 8	> 15	8-15
	A	-	-	NW, N, NE	E, W	SE, S, SW
Climatic factors and bioclimatic indices	AAT (°C)	8.5-11.2	< 8.5	8.5-9.3	9.4-10.0	10.1-11.2
	TWM (°C)	18.0-22.0	< 18.0	18.1-19.7	19.8-21.0	21.1-22.0
	GR (kcal/cm^2)	80.0-92.0	< 80.0	80-83.9	84.0-86.9	87.0-92.0
	ASD (hours)	1280-1610	< 1280	1280-1450	1451-1550	1551-1610
	PP (mm)	250-390	-	> 390	< 250	251-390
	Σt_u (°C)	1045-1675	< 1045	1045-1200	1201-1400	1401-1675
	LGS (days)	160-210	< 160	160-175	176-190	> 190
	Iha	1.36-2.66	< 1.36	1.36-1.70	1.71-2.20	2.21-2.66
	Ibcv	3.9-13.0	< 3.9	3.9-5.0	5.1-8.0	8.1-13.0
	IAOe	3793-4600	< 3793	3793-4300	4301-4600	> 4600
Pedological factors	Cly (%)	6-40	> 40.0	< 15	25-40	15-25
	Hum (%)	1.5-4.0	-	3.1-4.0	2.1-3.0	1.5-2.0
	Gra (%)	0-40	> 40.0	< 10	30-40	10-30

**S* = slope; *A* = terrain aspect; *AAT* = annual average temperature; *TWM* = warmest month temperature; *GR* = global radiation; *ASD* = actual sunshine duration; *PP* = precipitation during the growing season; Σt_u = sum of effective temperatures; *LGS* = length of the growing season; *Iha* = actual heliothermal index; *Ibcv* = bioclimatic index Constantinescu; *IAOe* = oenoclimate aptitude index; *Cly* = clay; *Hum* = humus; *Gra* = gravel

***WTW, SW, WD* = white table wines, sparkling wines and wines for distillates; *QWW* = quality white wines; *QRW* = quality red wines

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Table 2. *Characterizing viticultural potential according to the average of ranking points (arp) (Irimia et al., 2014)*

Class	Average of ranking points (arp)	Viticultural potential of the area
I	10	Viticultural potential for quality red wines (QRW)
	9	Viticultural potential primarily for quality red wines and secondarily for quality white wines (QRW + QWW)
II	8	Viticultural potential primarily for quality white wines and secondarily for red table wines (QWW + RTW)
	7	Viticultural potential for quality white wines (QWW)
III	6	Viticultural potential for white table wines, sparkling wines, and wines for distillates, as well as for quality white wines in very suitable years, in terms of climate (WTW, SW, WD + QWW)
	5	Viticultural potential for white table wines, sparkling wines, and wines for distillates (WTW, SW, WD)
IV	0	Unsuitable for grape growing (UNS)

The accuracy of the current winegrape varieties zoning was carried out by comparative analysis of the red varieties plots location with the viticultural potential of the area in which they are situated. In the case of red winegrape varieties, we assed the framing of their plots into areas with viticultural potential for RTW; and in the case of white wine varieties, we assed their framing into areas suitable for QWW and RTW.

Results

Structure and suitability of topography, soils and climate of the studied area

The Avereşti winegrowing region is situated in a hilly area with an important variation of the slope and of terrain aspect (*Fig. 2*). The slope varies between 0 and 45%, with a predominance of < 8% on about 80% of the area (*Fig. 2a*), while the terrain aspect is represented by all types, from those unsuitable for grapevine growing such as the northern ones to the very suitable ones like south (*Fig. 2b*). However, the eastern terrain aspect is predominant in the area by 38.6%, followed by northeastern by 11.2% and by southern and southeastern by about 9%.

Spatial variation of the slope and of the terrain aspect make the Avereşti area present all topographical suitability classes for wine production, were classes III and II are predominant (*Fig. 3* and *Table 3*): class III, suitable for WTW, SW and WD, characterizes 50% of the area, while class II, suitable for QWW, characterizes 35.6% of the area. 14% of the surface is characterized by class I of topographic suitability (QRW), and by slopes of 8-15% inclination with southern and predominantly southern terrain aspects.

Regarding the pedological suitability, the whole area is characterized by class II, suitable for QWW, of which 66% has an average of 7 arp (QWW), and 34% has an average of 8 arp (QWW + RTW) (*Fig. 3* and *Table 3*). Pedological suitability is given by the low humus content, ranging from 0.14 to 1.99% (class I) for 100% of the area; lack of limestone fragments from soils (class III) on 100% of the surface; and a clay content of 14-29% (class II) for 100% of the area. The low humus content of the soil is a factor that favours grape quality and is not restrictive when it falls below 1.5% because it is corrected by fertilization.

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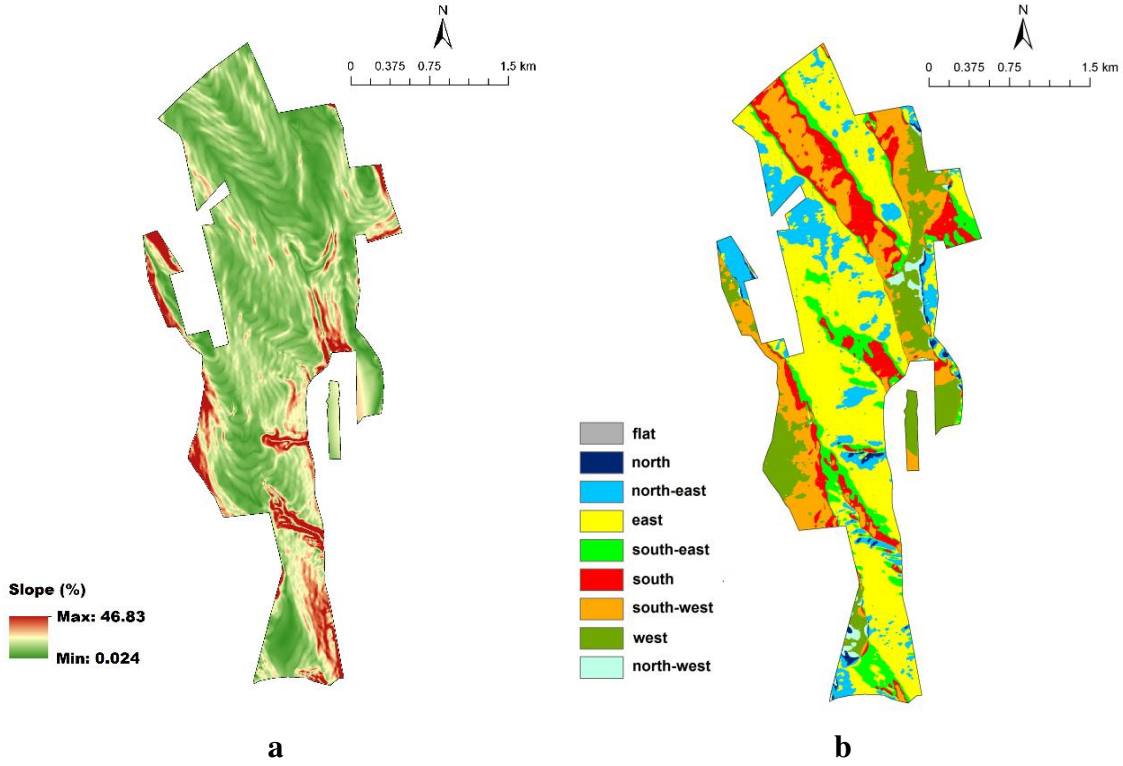


Figure 2. *Slope (a) and terrain aspect (b) in the Avereşti wine-growing region*

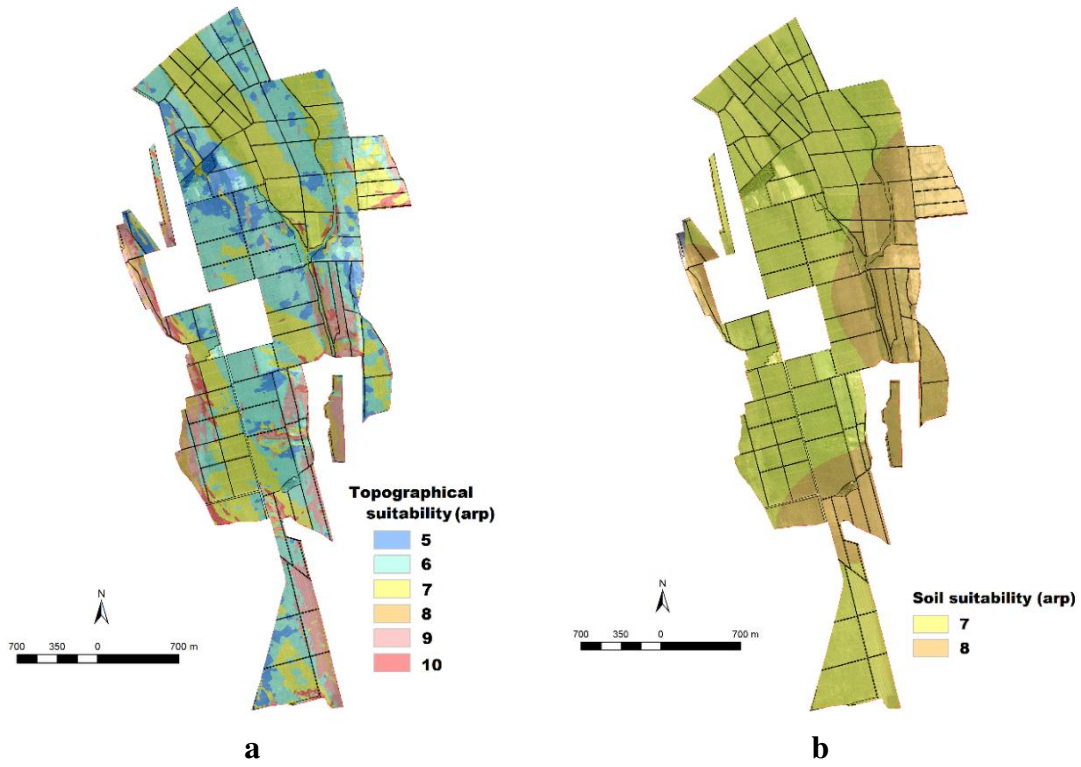


Figure 3. *Topographical (a) and pedological suitability (b) for the Avereşti wine-growing region*

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Table 3. *Structure of the topographical and pedological suitability for wine production in the Avereşti wine-growing region, during the 1961-2013 time period*

Topographical suitability				Pedological suitability			
Class	**arp**	**Share for 1961-2013**		**Class**	**arp**	**Share for 1961-2013**	
		ha	**%**			**ha**	**%**
III	5	71.2	10.6	III	5	0.0	0.0
	6	265.6	39.4		6	0.0	0.0
II	7	220.2	32.7	II	7	443.4	66.0
	8	21.1	3.1		8	228.9	34.0
I	9	76.9	11.4	I	9	0.0	0.0
	10	19.2	2.9		10	0.0	0.0
Total		**674.3**	**100.0**	**Total**		**672.2**	**100.0**

While relief and soils are stable ecological factors that do not cause temporal variations in the quality of wine production, climate is the factor that determines both spatial and temporal variation (Le Roux et al., 2017). In the case of Avereşti wine-growing region, the changes caused by the climate change in climate suitability for wine production between 1961 and 2013 are obvious in the data from *Table 4* and in the maps in *Figure 4*.

Table 4. *Structure of climate suitability for wine production in the Avereşti wine-growing region, for the 1961-1979 and 1980-2013 time periods*

Climate suitability 1961-1979				Climate suitability 1980-2013			
Class	**arp**	**Share**		**Class**	**arp**	**Share**	
		ha	**%**			**ha**	**%**
III	5	8.9	1.3	III	5	0.0	0.0
	6	665.4	98.7		6	1.7	0.2
II	7	0.0	0.0	II	7	434.5	64.4
	8	0.0	0.0		8	238.2	35.3
I	9	0.0	0.0	I	9	0.0	0.0
	10	0.0	0.0		10	0.0	0.0
Total		**674.3**	**100**	**Total**		**672.2**	**100**

Between 1961 and 1979, 100% of the Avereşti wine-growing region was characterized by climate suitability for WTW, SW, WD and for QWW in the warm growing season (*Table 4*). This reduced climatic suitability is due to low values of heliothermic factors (*Table 5*)*:* AAT of 8.47 °C, below the lower limit for the economical cultivation of wine varieties; Σt_u of 1110 °C, value at the lower limit of suitability for wine production; ASD of 1447 h, sufficient only for the production of white wines; LGS of 172 days, sufficient only for early winegrape varieties; IAOe of 4178 units, sufficient only for the production of white wines.

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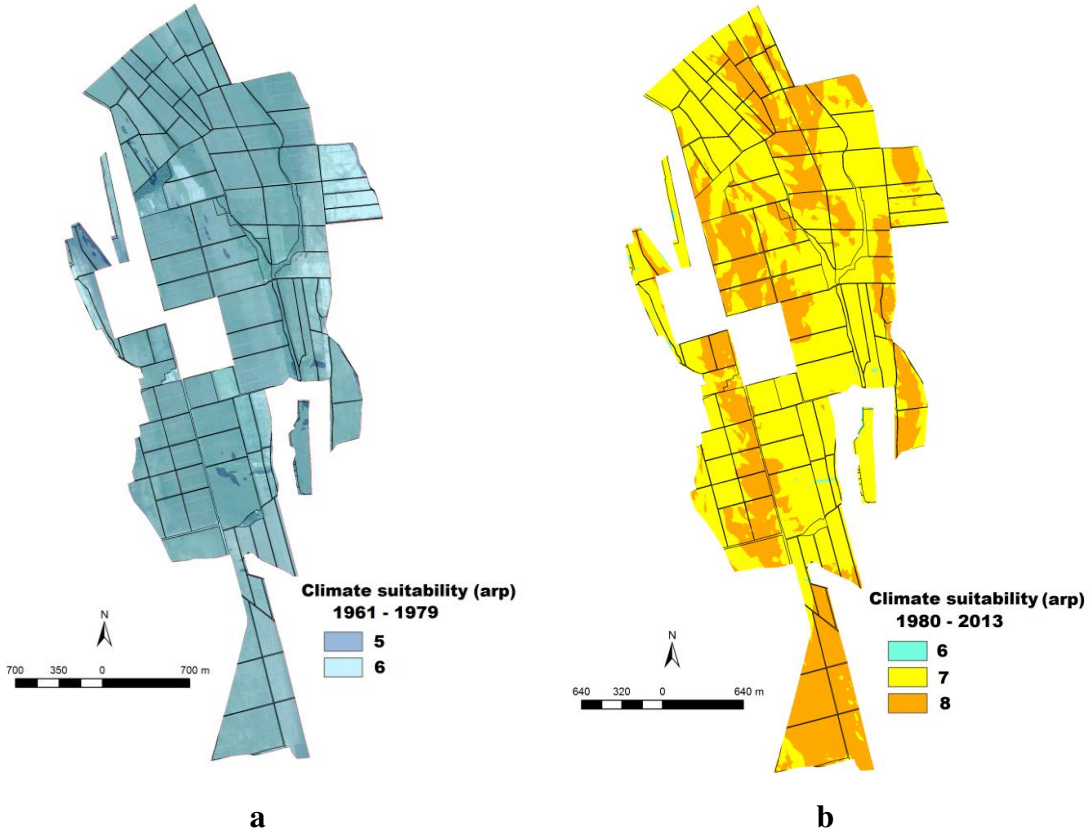


Figure 4. *Climate suitability for the 1961 to 1979 (a) and for the 1980 to 2013 time period (b) for the Avereşti wine-growing region*

Table 5. *Statistics on climate parameters defining climate suitability for the wine production in the Avereşti wine-growing region, for the 1961-1979 and the 1980-2013 time periods*

Parameter	1961-1979				1980-2013			
	Mean	Min	Max	Range	Mean	Min	Max	Range
AAT	8.47	8.31	8.72	0.40	8.94	8.79	9.19	0.40
WMT	19.46	19.27	19.77	0.50	20.72	20.53	21.03	0.50
GR	88.79	72.99	96.10	23.12	90.48	74.37	97.93	23.55
ASD	1447.72	1245.95	1501.97	256.02	1526.30	1313.58	1583.49	269.91
PP	347.63	338.10	353.45	15.34	333.51	323.98	339.32	15.34
Σt_u (°C)	1110.72	1080.04	1161.50	81.46	1225.3	1195.7	1274.1	78.3
LGS	171.78	170.09	174.53	4.43	173.98	172.37	176.62	4.25
IHa	1.61	1.41	1.70	0.29	1.87	1.64	1.97	0.33
Ibcv	6.86	6.00	7.22	1.22	7.55	6.60	7.93	1.33
IAOe	4178.6	3994.5	4266.0	271.4	4311.4	4118.6	4395.7	277.1

Climate suitability during the 1980-2013 period is much higher than the one for the 1961-1979 time period (*Table 4*): class III of climate suitability has practically disappeared, currently characterizing only 0.2% of the area; class II has appeared and currently characterizes 99.8% of the surface, of which 64.4% is suitable for QWW and

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35.3% suitable for QWW and RTW, which is an absolutely new type of suitability for this area. This change in suitability is due to the increase in averages of the heliothermic factors, amid a slight decrease in precipitation (*Table 5*): AAT is of 8.94 °C in the recent period, higher by 0.47 °C than the one between 1961-1979; WMT increased by 1.26 °C reaching the value of 20.72 °C today; GR is higher by 1.69 kcal/cm^2; ASD is also higher by 78.6 h; PP are smaller by 14.1 mm; and IAOe is higher by 133 units, the current value of 4311.4 units placing IAOe in the class of suitability for QWW and RTW.

The viticultural potential and the structure of winegrape varieties during 1961-1979

The results of the research reveal the existence of three viticultural homogeneous zones in the area, characterized by 6, 7 and 8 arp, but whose surface and share in the area radically changed between the 1961 and 2013 as a result of climate change (*Table 6*).

Table 6. *Structure of the viticultural potential for the wine production in the Avereşti wine-growing region, for the 1961-1979 and 1980-2013 time periods*

Viticultural potential 1961-1979				**Viticultural potential 1980-2013**			
Class	**arp**	**Share**		**Class**	**arp**	**Share**	
		ha	**%**			**ha**	**%**
III	5	0.2	0.03	III	5	0.0	0.0
	6	486.2	72.3		6	161.7	24.1
II	7	176.4	26.3	II	7	440.1	65.5
	8	9.4	1.4		8	70.4	10.5
I	9	0.0	0.0	I	9	0.0	0.0
	10	0.0	0.0		10	0.0	0.0
Total		**672.2**	**100**			**672.2**	**100**

Between 1961-1979, Avereşti wine-growing region provided mainly WTW, SW, WD and QWW, its viticultural potential having the following structure (*Table 6*)*:* 0.03% of the area was characterized by a mean of 5 arp, the viticultural potential for WTW, SW, WD; 72.3% of the area was characterized by an average of 6 arp, the viticultural potential for WTW, SW, WD and QWW in warm years; 26.3% of the area was characterized by an average of 7 arp, the viticultural potential for QWW; 1.4% of the area was characterized by an average of 8 arp, the viticutural potential for QWW and RTW. The viticultural potential for QWW and RTW characterized the slopes with western aspects, while the viticultural potential for WTW, SW, WD characterized the plateau area (*Fig. 5a*).

The winegrape varieties for the 1961 to 1979 were the ones selected in time by the cultivation of the best adapted varieties. 100% was represented by varieties for white wines: *Fetească albă* (FA), *Fetească regală* (FR), *Zghihară de Huşi* (Z), *Aligoté* (AL). Due to the viticultural potential for mainly white wines, the winegrape varieties were well located, with ones for quality white wine such as *Feteasca albă* and *Feteasca regală* which were grown on slope areas with QWW potential, and productive ones, such as *Zghihară de Huşi* and *Aligoté* which were grown in areas with viticultural potential for WTW, SW, WD (*Fig. 5b*).

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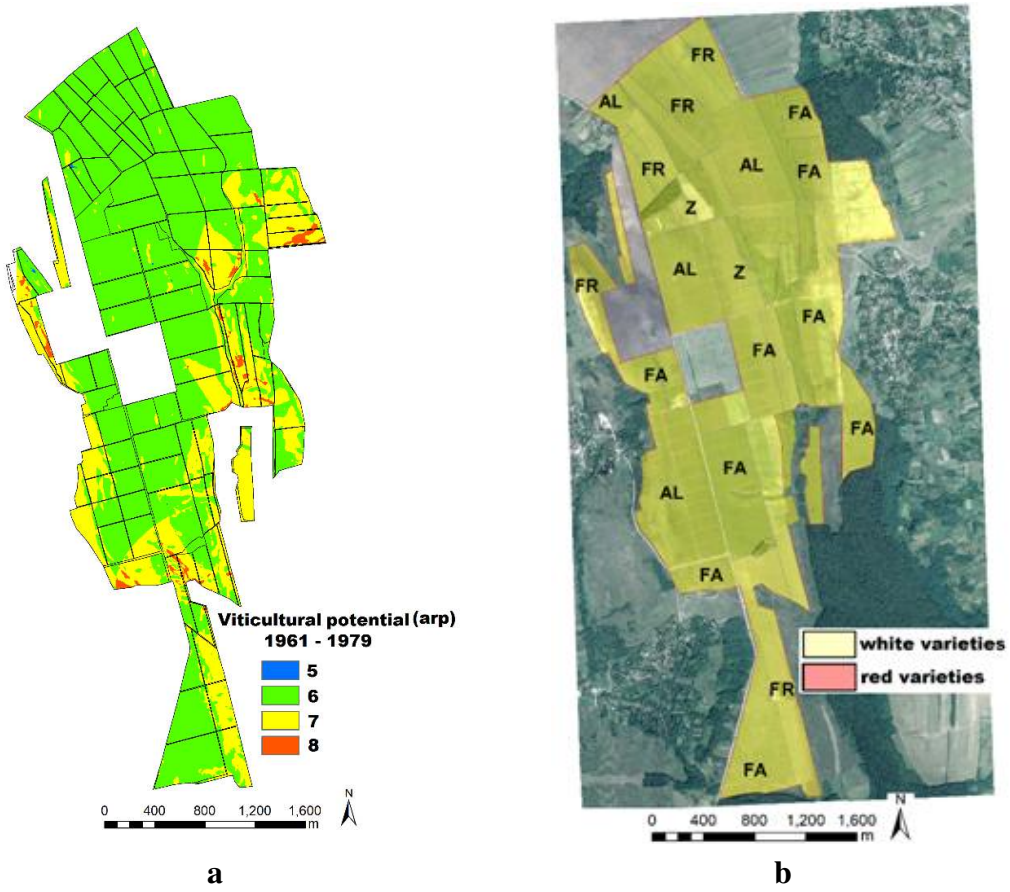


Figure 5. *Viticultural potential (a) and winegrape varieties (b) of the Avereşti wine-growing region between 1961 and 1979*

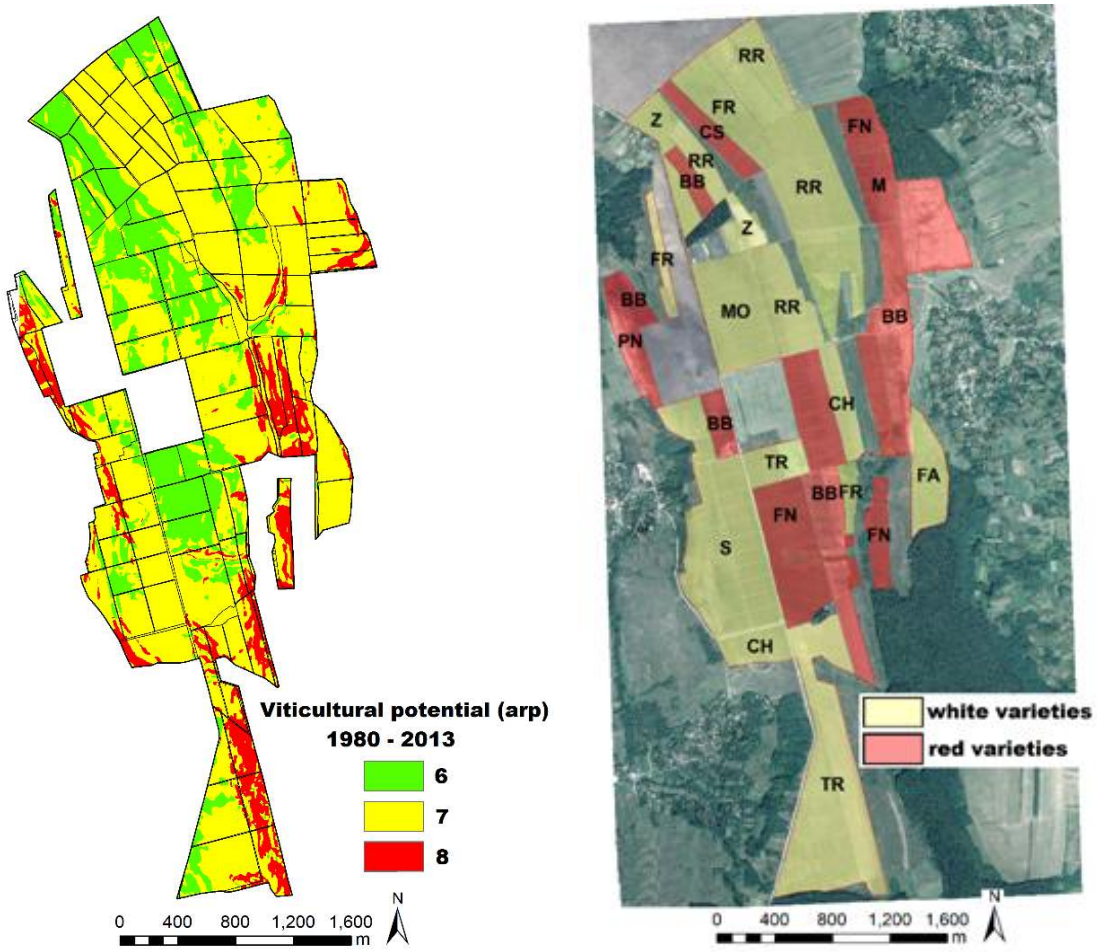
The viticultural potential between the 1980-2013 and the current structure of the winegrape varieties grown within the area

According to the data presented in *Table 6,* the current structure of the viticultural potential of the area is radically different from that of the 1961-1979: the viticultural potential characterized by 5 arp disappeared; the viticultural potential characterized by WTW, SW, WD and QWW in warm years, declined from 72.3% to 24.1%; the viticultural potential with an average of 7 arp increased from 26.3% to 65.5%; and the viticultural potential with an average of 8 arp extended from 1.4% to 10.5%. Basically, after 1980, the wine-growing region became suitable for the QWW and RTW production, unlike the previous period when it was particularly suitable for the WTW, SW and WD production. This new type of viticultural potential, the potential for RTW, characterizes the slopes with western and eastern terrain aspects, where the potential for QWW prevailed in the previous time period, while today's plateau areas, formerly characterized by the WTW, SW and WD potential, have viticultural potential for QWW (*Fig. 6a*).

The current winegrape varieties of the area, grown after the year 2000, are represented mainly by the international varieties for quality white wines such as *Sauvignon* (S), *Chardonnay* (CH), *Muscat Ottonel* (MO), to which Romanian varieties like *Tămâioasa românească* (TR), *Feteasca albă* (FA) and *Fetească regală* (FR) are

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added (*Fig. 6b*). For red wines, *Cabernet Sauvignon* (CS), *Merlot* (M), *Pinot noir* (PN) and Romanian variety *Feteasc*ă *neagr*ă (FN) were introduced in the area for the first time (*Fig. 6b*). In order to capitalize on the new viticultural potential, the *Busuioaca de Bohotin* (BB) variety, of Mediterranean origin (from the *Muscat* group), which requires high heliothermal resources and produces aromatic red wines, was also introduced into area. Still, as an anticipated adaptation to climate change, the *Rhine Riesling*, a late variety that preserve better acidity was introduced in area in the detriment of earlier local variety *Feteasca albă*, whose acidity can be affected by increasing temperatures in the context of climate change. For the area with WTW and SW potential, the *Zghihara de Huşi* (Z) variety, with a high production potential has been maintained in cultivation.



a **b**

Figure 6. *Viticultural potential between for the 1980 to 2013 (a) and spatial distribution of current winegrape varieties (b) in the Averești wine-growing region*

Analysis of winegrape varieties location in the area

The location of winegrape varieties introduced in the area after 2000 was based on empirical observations. As a result, white wine varieties were located mainly on flat terrains, while varieties for red wine on slopes and on some flat terrains, appreciated as suitable.

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However, the analysis of the varieties location reveals 15.6% accuracy for the red wine varieties and 73% for the white wine varieties (*Table 7*). About 66% of the area planted with red wine varieties is located on surfaces with viticultural potential for QWW and 18%, even on surfaces with viticultural potential for WTW, SW and WD. The total area suitable for red wines is of 70.4 ha while the present area growing varieties for red wines is of 220.5 ha, the maximum potential utilization for RTW being of about 32% compared to 15.6% realized. The variability analysis reveals the 100% location of the *Cabernet Sauvignon* variety in the zone with QWW potential (*Fig. 6b*); 88.9% of the *Merlot* surface is located in the area suitable for QWW and only 3.2% in the area suitable for RTW; 59.2% of the *Pinot noir* surface is located in the area suitable for RTW and only 40.6% in the area suitable for QWW (*Table 7*).

Table 7. *Framing of the winegrape varieties for red wines newly planted in the area, within the zones with different types of viticultural potential (arp)*

Viticultural potential (arp)	Framing red and white winegrape varieties within suitability classes (%)		Framing within suitability classes from area (%) of the red winegrape varieties*				
	Red varieties	White varieties	BB	CS	FN	M	PN
6	18.0	27.7	14.1	0.0	36.5	7.9	0.2
7	66.4	64.2	68.8	100.0	53.4	88.9	40.6
8	15.6	8.1	17.1	0.0	10.1	3.2	59.2
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**

*BB = *Busuioaca de Bohotin*; CS = *Cabernet Sauvignon*; FN = *Feteasca neagră*; M = *Merlot*; PN = *Pinot noir*

Growing winegrape varieties for red wine in unsuitable areas limits the expression of their quality potential and the possibility to produce quality wines. In the Avereşti area, the lack of suitability for red wines of the zones suitable for WTW, WD, SW and QWW is given by the small values of IAOe (< 4300 units), ASD (< 1450 h), TWM (< 20 °C), Cly content (< 15%), Gra (< 10%) and northern aspects. Obtaining quality red wines is conditioned by the high exposure of the vine canopy to direct solar radiation, higher temperatures and sunshine duration. In wine-growing areas close to the northern limit of vine growing area, these conditions are more difficult to meet, generally occurring on slopes with predominantly southern terrain aspects, well-drained and less fertile soils, and using training systems that ensure the highest percentage of grapevine leaves exposure to direct solar radiation.

Discussion

The GIS-based multicriteria analysis of the viticultural potential of the Averesti wine-growing region, situated close to the northern limit of grape growing (50° N lat.), reveals its increase during the 1961-2013 time period as a consequence of climate change. With the increase of the viticultural potential, changes in specific winegrape varieties have occurred, new winegrape varieties for quality white wines and even varieties for red wine being planted.

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The originality of this study is that the area's viticultural potential has been assessed by taking into account the values of 15 ecological parameters representative of the vineyards' relief, soils and climate, and determinants of the quality and characteristics of wines. This approach ensures the accurate assessment of the viticultural potential, which must include the influence of relief and soils (Irimia et al., 2014). The accuracy of the study is given by: the use of a digital elevation model with a resolution of 5 × 5 m; the use of daily temperatures, precipitation and sunshine duration for the period 1961-2013 from the ROCADA database (Dumitrescu and Bîrsan, 2015); the use of pedological data representing the whole viticultural area.

Previous research on the ecological characteristics of the Avereşti wine-growing region revealed the significant spatial variation of the terrain aspect, slope, annual average temperature and sunshine duration (Irimia and Patriche 2009, 2010). The complete evaluation of the viticultural potential has revealed the existence of three homogeneous viticultural zones in the area, whose share changed between 1961 and 2013 as a result of climate change. The cause of the change of the viticultural potential is the climate change that in the region manifests by: AAT increase by 0.47 °C between 1961 and 2013; TWM increase by 1.26 °C; LGS extension by 2 days between 1961-2013; PP increase by 14.12 mm; and IAOe increase by 132.8 units. The results are consistent with the outcomes of the research on the evolution of temperatures, insolation and precipitation on Romania territory between 1961 to 2010 (Busuioc et al., 2010; Piticar and Ristoiu, 2012; Dumitrescu et al., 2014) and those communicated for this part of Europe by Karl (1998) and Klein Tank and Köhnnen (2003).

The study reveals that climate change not only modifies the vineyards' specific types of wine production. The Avereşti wine-growing region, characterized in the past by a cool, less suitable climate, passed between 1961 and 2013 from mainly WTW, SW and WD viticultural potential to mainly QWW and even RTW viticultural potential. The results are in line with those recorded for the Cotnari wine-growing region, located 200 km to north, in the same region (Irimia et al., 2017b). So are they in line with the regional developments, the whole of Romania's viticulture being affected by major shifts in climate suitability for the wine production (Bucur and Dejeu, 2016; Irimia et al., 2017a). The results of this study are in accordance with the expected developments for the viticulture of Central and Eastern Europe predicted by Stock et al. (2005), which assessed the evolution of current climate as favoring the expansion of red wine varieties in these areas. In the Avereşti wine-growing region, the *Cabernet Sauvigon, Merlot, Pinot noir, Fetească neagră* and *Busuioacă de Bohotin* red winegrape varieties, not previously existing in the area, were introduced due to the viticultural potential increase determined by climate change.

However, the study demonstrates the need to evaluate the current viticultural potential and to delimit the homogeneous viticultural zones of the wine-growing regions close to the northern limit of vine growing area, that are the beneficiaries of climate change, with a view to the correct zoning of the newly introduced winegrape varieties. In the absence of such an assessment, in the Avereşti wine-growing region only 15.6% of the area planted with red wine varieties is located in zones suitable for RTW; about 66% of the area is located on surfaces suitable for QWW and 18% on area suitable for WTW, SW, WD. It is expected that in the context of climate change, the area's potential for RTW will increase, but currently it is not at the level where high quality red wines can be obtained.

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The results of the study are especially useful for the Avereşti wine-growing region where the management of vine plantations can be optimized and strategies for capitalizing on the wine production can be elaborated. The study also provides information to researchers and to the entire wine sector on the impact of climate change on the vineyards viticultural potential and their specific wine types.

Conclusions

The study has shown that the viticultural potential of the Averesti wine-growing region has radically changed in the context of climate change. Evolutions are similar to those found in previous researches on vineyards climate in this geographical region. They are also consistent with the outcomes of other researches on the evolution of viticulture in this part of Europe, namely the increase of the viticultural potential due to the increase of the heliothermal resources. The Averesti wine-growing region evolved between 1961-2013 from the prevalent white table wine production between 1961-1979, to the prevailing potential for quality white wines and red table wines between 1980 and 2013. The new viticultural potential is being exploited in the Averesti wine-growing area by expanding the international varieties for quality white wines and by introducing red wine varieties in culture, an absolutely new wine type production for the area. These developments are specific to wine-growing regions close to the northern limit of vine culture, but this study demonstrates the need to assess the viticultural potential determined by climate change and to delimit the homogeneous viticultural zones in such areas. Although heliothermic resources have increased, they have not yet reached the level at which to obtain high quality red wines. To this the significant spatial variation of the ecological factors is added, which determines the existence in the vineyard area of the homogeneous viticultural zones with different viticultural potential in relation to winegrape varieties requirements. However, due to the frosty winters in the area that still maintain their harshness, further studies on the matching and behaviour of newly introduced wine-grape varieties into Averești wine-growing region are still needed.

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TECHNICAL STRATEGY FOR REVITALIZING URBAN RIVER WATER QUALITY THROUGH STRUCTURAL APPROACH AT NATIONAL UNIVERSITY OF MALAYSIA (UKM) BANGI CAMPUS, SELANGOR, MALAYSIA

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Abstract. This paper aims to provide technical insights on revitalizing urban river water quality through structural approach in Alur Ilmu urban river, National University of Malaysia @ *Universiti Kebangsaan Malaysia (UKM)* Bangi Campus. The structural approach consists of three main parts: site profiling, water quality assessment to determine spatial distribution of pollutions and on-site water treatment system. The selected water quality parameters were Dissolved Oxygen, Ammoniacal Nitrogen, pH, Total Suspended Solid, Chemical Oxygen Demand and Biological Oxygen Demand. The obtained values were indexed in accordance with Water Quality Index (WQI) of National Water Quality Standard (NWQS) for Malaysia and interpolated to produce thematic maps indicating the point sources of pollution using Kriging model. A series of on-site water treatment system, namely constructed wetland, gross pollutant trap, permeable pavement wall and floating treatment wetland were then strategically constructed using both information obtained from site profiling and spatial distribution. The structural approach has been deployed since 12 June 2015 and has shown significant improvement on average WQI for all thirteen stations from 68.85 to 79.87 in less than 10 months. The most influential point for source of pollution was detected at AL5 (FST Café), it has improved the most, from 51.36 to 73.18. The structural approach has successfully improved Alur Ilmu water quality and should be complemented with non-structural approach to ensure the sustainability of water resource.
Keywords: *site profiling, water quality assessment, spatial distribution, Kriging model, onsite treatment system*

Introduction

Under circumstances of technology advancement, social change and economic development have given impetus to rapid urbanization and land expansion, pristine water quality and natural appealing urban river are apparent if we incorporate environmental consideration into our current practice and future planning. Good governance of urban river through integrated water resource management has tapped valuable potentials for many developed countries towards economic, social and environmental benefits. Cleaner urban river water contributes to alternative water

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resource, i.e. groundwater recharging and stormwater harvesting (Lim and Lu, 2016), fulfilling public demand for better environmental health (Environmental Protection Agency (EPA), 2016), enhancing their quality of life, and providing nature aesthetic appreciation and gentrification for safe, efficient and greener neighbourhood (Reese, 1991; Lim et al., 2013). Besides, it establishes a niche on revitalizing urban rivers through various methodologies and water treatment systems. However, the options available in adopting management and water treatment system have created ambiguity to decision makers and engineers to suit their local context. A flaw adoption of water treatment and management will exacerbate more than only urban river water problems. For example, poor understanding of the urban river water pollution dynamics from introduction of point and non-point sources of pollution (Curriero, 2007), resulting to unnecessary site's geomorphological change and selection of ineffective water treatment system. This creates more loss as more pollutants created from land change, financial strain from high maintenance of unsuitable water treatment system and discontentment from the public.

In revitalizing urban river, structural approach is one of the best management practices (BMP) to remove the pollutants from the water body (Lim and Lu, 2016; Thomas and Reese, 2003). The goal of BMP is to provide sufficient pollution reduction of mass inflow i.e. from point and non-point sources of pollution to meet the targeted mass outflow provided by local standards and guidelines (Thomas and Reese, 2003). Challenges of the structural approach in BMP are the complex urban rainfall-runoff contributing to various types of pollution, myriad of biological, chemical and pollution reactions in the water body. Moreover, despite with careful planning of constructed water treatment systems, the reduction of pollutions was inconsistent (Thomas and Reese, 2003; Brown and Clarke, 2007; Lim and Lu, 2016), therefore it is hard to achieve replicable results from previous case studies. Hence, structural approach is designed in a set of action plan using scientific analyses that optimise both aspects of the site features and the selected water treatment systems by mitigating the pollutions close to its source according to standards stipulated by local authorities or government. Similar to most of the developed countries, Malaysia government has outlined national water quality standard for inland water quality namely Water Quality Index (WQI) to identify and classify the level of acceptance of water quantity and quality for human use and environmental health (Natural Resources and Environment Malaysia (NRE), 2006). The WQI comprises of six water quality parameters, namely dissolve oxygen (DO), Ammoniacal Nitrogen (NH_3-N), total suspended solid (TSS), chemical oxygen demand (COD) and biological oxygen demand (BOD), are calculated into a cumulative value of the 6 parameters in sub-indices with weightage that can be classified into five classes and usages. *Table 1a* is National Water Quality Standard (NWQS) for Malaysia with limits for classes of pollutant concentration acceptance level of the subindices Malaysia's WQI (NRE, 2006). *Table 1b* is DOE WQI Classification for Malaysia with limits for inland water quality (NRE, 2006). *Table 1c* is the description list of the classes and its utilities that have been mentioned in *Tables 1a* and *1b.* Water with Class I in WQI indicates clean and is the most suitable for human consumption and usage. Increasing in WQI indicates the more polluted of urban river water quality is, which is harmful for human use and to the environment.

This paper aims to provide insights on the technical aspects of revitalizing urban river water quality through structural approach using site profiling, spatial distribution analysis of WQI and on-site treatment system. Therefore, the current research enables

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decision makers and engineers to target and apply treatment to remove pollutants in the water body as close as possible to the source of pollution based on the case study in Alur Ilmu, National University of Malaysia @ *Universiti Kebangsaan Malaysia* (UKM) Bangi Campus, Selangor, Malaysia.

Methods

Study area

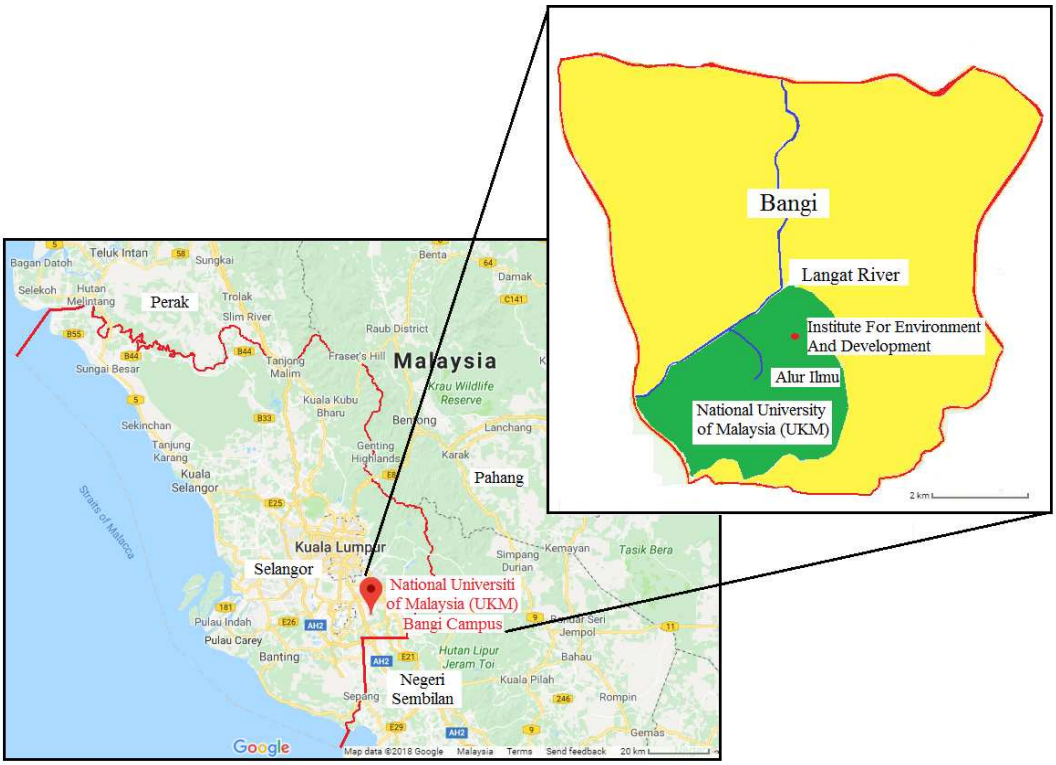
Alur Ilmu is a 1.79 km urban river long stretching across the main campus of the National University of Malaysia (UKM), Bangi, Selangor, Malaysia before flowing into the Langat River. *Figure 1a* is the location of National University of Malaysia (UKM), Bangi Campus, Selangor, Malaysia. *Figure 1b* shows the location of the revitalization of water quality study through structural approach at first 500 m of Alur Ilmu urban river upstream, UKM Bangi, Selangor, Malaysia with sampling sites and the placement of the on-site water treatment system. The first 100 m of Alur Ilmu is located at the lowest elevation point of hilly terrain and flows with normal elevated terrain, closely surrounded by impervious area from building and impervious pavement. It receives water source from the Permanent Reserved Forest and Natural Education Forest (*Hutan Simpan Kekal and Hutan Pendidikan Alam*) UKM, rain water and stormwater runoff. Alur Ilmu has been reinforced from a natural river into a concreted urban river in 1970s concurrent with the construction of the first batch of UKM's faculty buildings without changing its original flow (*Universiti Kebangsaan Malaysia* (UKM), 1978). Alur Ilmu serves as an irrigation system to remove excess water from impervious area nearby the urban river and avoid flooding. Alur Ilmu is a model of an urban river as it receives clean forest water from the upstream and surrounded by the growth of technology, social and economic activities along the urban river which convergences into Langat River at the downstream. In this study, revitalization of Alur Ilmu water quality was conducted within the upstream (500 m length and 5 m width starting from Ghazali Lake to Silt Collection Tank). Average water depth is about 1.8 m with land coverage of 95% impervious area (*Universiti Kebangsaan Malaysia* (UKM), 1978). The average water velocity of the urban river is 0.1 ms^{-1} during dry season and increases to an average of 6 ms^{-1} during rain. In *Figure 1b,* the light blue colour indicates the area selected for spatial distribution analysis. The area was selected to identify point and non-point sources of pollutions in Alur Ilmu and eliminate complication of spatially interpolating within non-convex or irregularly shaped regions (Ghafouri and Swain, 2005). The WQI was reported depreciating from Class II (Chong, 1999) to Class III in 2003 (Mokhtar et al., 2005) to Class IV in 2012 (Din et al., 2012) and Class V in 2017 (Hafiyyan et al., 2017a).

Structural approach

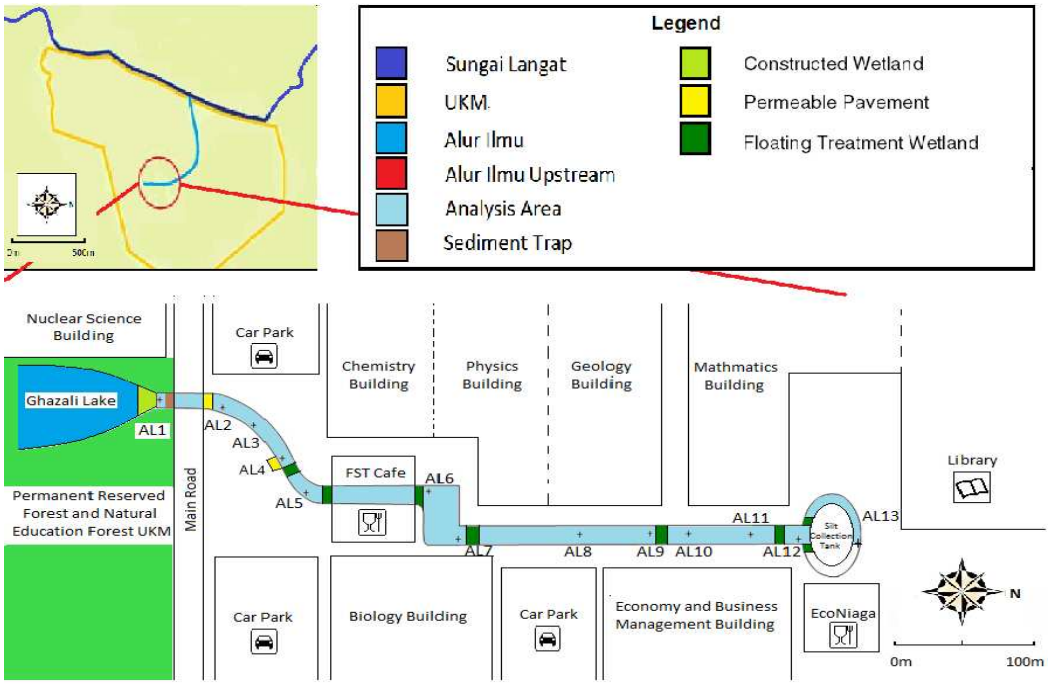
Structural approach is a systematic action plan to understand a complex set of anthropogenic and natural physical, chemical and biological factors which act as pollution to the receiving water and provide effective hardware solution to remove pollutants in the receiving water. The main goal of the structural approach is to obtain optimum pollution reduction perpetuated throughout the years in compliance to management goals and local water quality standards. The approach consists of three components: 1) site profiling, 2) water quality assessment and 3) on-site water treatment

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and should be sequenced as this order. *Figure 2* depicts the research strategy for the structural approach in revitalizing urban river water.



a



b

Figure 1. a *Location of National University of Malaysia (UKM), Bangi Campus, Selangor, Malaysia.* ***b*** *Location of the revitalization of water quality study through structural approach at AlurIlmu urban river (500 m upstream), UKM Bangi, Selangor, Malaysia with sampling sites and the placement of the on-site water treatment system*

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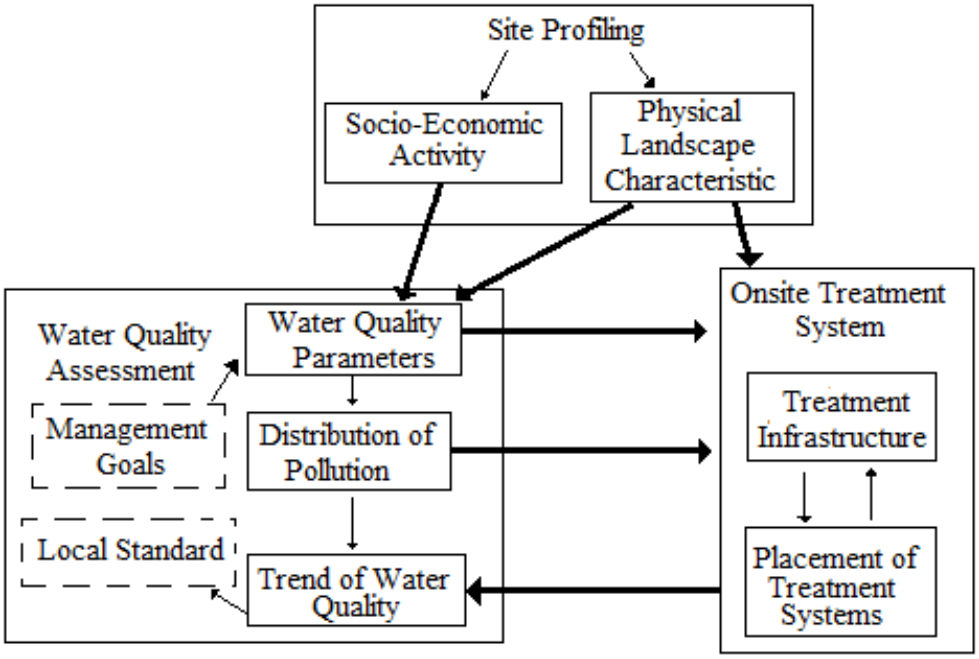


Figure 2. *Research strategy for the structural approach and its components in urban river water revitalization*

The first part of the approach is to develop a site profile of the study location comprises of its socio-economic activities and physical landscape characteristic along Alur Ilmu. Socio-economic activities refer to any human activities that occur nearby the urban river that can influence its water quality through point and non-point sources of pollutions. The physical landscape characteristic encompasses an outline of the urban river and its surrounding location including its geomorphological of the terrain, physical characteristic of the urban river and flow pattern of the urban river. The geomorphology of the terrain encompasses the slopes and nearby land use. Physical characteristic of the urban river is the distinctive feature or quality that describes the urban river and flow pattern is the water depth and water velocity of the urban river. Information of socio-economic activities and physical landscape characteristic are instrumental for identification of the type and cause of pollution.

The second part of the structural approach is water quality assessment of Alur Ilmu. The assessment is conducted by underpinning three aspects which are water quality parameters, distribution of pollution and trend of its water quality. The water quality parameters refer to the physical, chemical and biological characteristic of the water (Water Environment Partnership in Asia (WEPA, 2017) that to be complied with management goals and local standards to protect the end user including human, animals, and ecosystem health or industrial and agriculture activities (Environmental Protection Agency (EPA), Ireland 2001). The water quality assessment for Alur Ilmu was conducted through in-situ monitoring and ex-situ analysis in accordance with National Water Quality Standards for Malaysia, namely Malaysia's WQI (Department of Environment Malaysia (DOE), 2007). Malaysia's WQI is a mathematical value from comprehensive calculation of selected water quality parameters namely dissolved oxygen (DO), pH, Ammoniacal Nitrogen (NH_3-N), total suspended solid (TSS), biological oxygen demand (BOD) and chemical oxygen demand (COD). The purpose of

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the index is to benchmark the water quality of surface water in Malaysia to the public about its uses (American Public Health Association (APHA), 2005). The obtained WQI is used to determine the distribution of pollution through spatial distribution analysis to produce a thematic map determining the distribution of point and non-point sources of pollution entering Alur Ilmu water body.

The selection of on-site water treatment system is based on two aspects, 1) the treatment infrastructure which includes its water treatment application, design criteria, specification, operation and maintenance and 2) the placement of the treatment system. The data obtained from site profiling and water quality assessment are rudimentary for the selection of the on-site treatment system.

Sampling and analytical method

Thirteen stations were selected based on inlets identified along first 500 m of Alur Ilmu and their GPS coordinates were recorded using Garmin Etrex 20 for spatial distribution analysis. Six water parameters were selected for in-situ monitoring and ex-situ analysis for the water quality assessment, based on the National Water Quality Standards for Malaysia (Department of Environment Malaysia (DOE), 2007). The parameter were dissolved oxygen (DO), pH, and Ammoniacal Nitrogen (NH_3-N) for in-situ monitoring and total suspended solid (TSS), biological oxygen demand (BOD) and chemical oxygen demand (COD) for ex-situ analysis. The in-situ monitoring for DO (% saturation), pH (value) and NH_3-N (mg/L) were measured using YSI Multiparameter Sonde during mid-day (11 am – 1 pm) and water samples were collected for analysis. For ex-situ analysis, TSS (mg/L) was sampled using polyvinyl bottle and measured using gravimeter method Method 208D (American Public Health Association (APHA) (2005). BOD (value) was analysed by BOD_5 Method by incubating the water samples for 5 days under temperature of 20 °C in an incubator and analysed using YSI EcoSense Self-stirring BOD Probe. Differences between DO_{first} and DO_{final} were recorded for BOD net value (American Public Health Association (APHA), 2005). COD parameter was carried out using 5220B chemical oxygen demand, closed reflux, titrimetric method and was analysed using HACH Spectrophotometer DR3900 Laboratory VIS Spectrophotometer with RFID* Technology (American Public Health Association (APHA), 2005). The six water quality parameters for each station in Alur Ilmu were used to calculate WQI value using WQI formula and calculation in *Equation 1*. The obtained WQI of each station was compared with DOE WQI Classification in Malaysia and uses (NRE, 2006) in *Tables 1a* and *1b* for water pollution classification and limits. The six parameters: DO, pH, NH_3-N, TSS, BOD and COD are the water quality parameters approved by the Malaysia's DOE for evaluation (*Eq. 1*) of overall status of surface water including urban river water. From *Equation 1*, DO carries the maximum weightage of 22% and pH carries the minimum of 12% in the WQI equation. The WQI equation constitutes of sub-indices of the six water quality parameters calculated according to the best-fit relations given in *Equations 2* to *7*. Variation of the sub-indices with concentrations of the respective pollutants are depicted in *Figure 3*. Better quality of the water represented by high value of the index (Mamun and Zainudin, 2013).

$$\begin{aligned} WQI = &\left(0.22*SIDO\right) + \left(0.19*SIBOD\right) + \left(0.16*SICOD\right) \\ &+ \left(0.15*SIAN\right) + \left(0.16*SISS\right) + \left(0.12*SipH\right) \end{aligned} \quad \text{(Eq.1)}$$

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SIDO = Subindex DO (% saturation)
SIBOD = Subindex BOD
SICOD = Subindex COD
SIAN = Subindex NH3-N
SISS = Subindex SS
SipH = Subindex pH

Subindex for DO (In % saturation)

$$\begin{aligned} SIDO &= 0 && \text{for } x \le 8 \\ SIDO &= 100 && \text{for } x \le 92 \\ SIDO &= -0.395 + 0.030x^2 - 0.00020x^3 && \text{for } 8 < x < 92 \end{aligned} \quad \text{(Eq.2)}$$

Subindex for BOD

$$\begin{aligned} SIBOD &= 100.4 - 4.23\ X && \text{for } x \le 5 \\ SIBOD &= 108*\exp(-0.055x) - 0.1x && \text{for } x > 5 \end{aligned} \quad \text{(Eq.3)}$$

Subindex for COD

$$\begin{aligned} SICOD &= -1.33X + 99.1 && \text{for } x \le 20 \\ SICOD &= 103*\exp(-0.0157x) - 0.04x && \text{for } x > 20 \end{aligned} \quad \text{(Eq.4)}$$

Subindex for NH3-N

$$\begin{aligned} SIAN &= 100.5 - 105x && \text{for } x \le 0.3 \\ SIAN &= 94*\exp(-0.573x) - 5*I\ x\ 2\ I && \text{for } 0.3 < x < 4 \\ SIAN &= 0 \end{aligned} \quad \text{(Eq.5)}$$

Subindex for SS

$$\begin{aligned} SISS &= 97.5*\exp(-0.00676x) + 0.05x && \text{for } x \le 100 \\ SISS &= 71*\exp(-0.0061x) + 0.015x && \text{for } 100 < x < 1000 \\ SISS &= 0 && \text{for } x \ge 1000 \end{aligned} \quad \text{(Eq.6)}$$

Subindex for pH

$$\begin{aligned} SIpH &= 17.02 - 17.2x + 5.02x^2 && \text{for } x < 5.5 \\ SIpH &= -242 + 95.5x - 6.67x^2 && \text{for } 5.5 \le x < 7 \\ SIpH &= -181 + 82.4x - 6.05x^2 && \text{for } 7 \le x < 8.75 \\ SIpH &= 536 - 77.0x + 2.76x && \text{for } x \ge 8.75 \end{aligned} \quad \text{(Eq.7)}$$

* means multiply with

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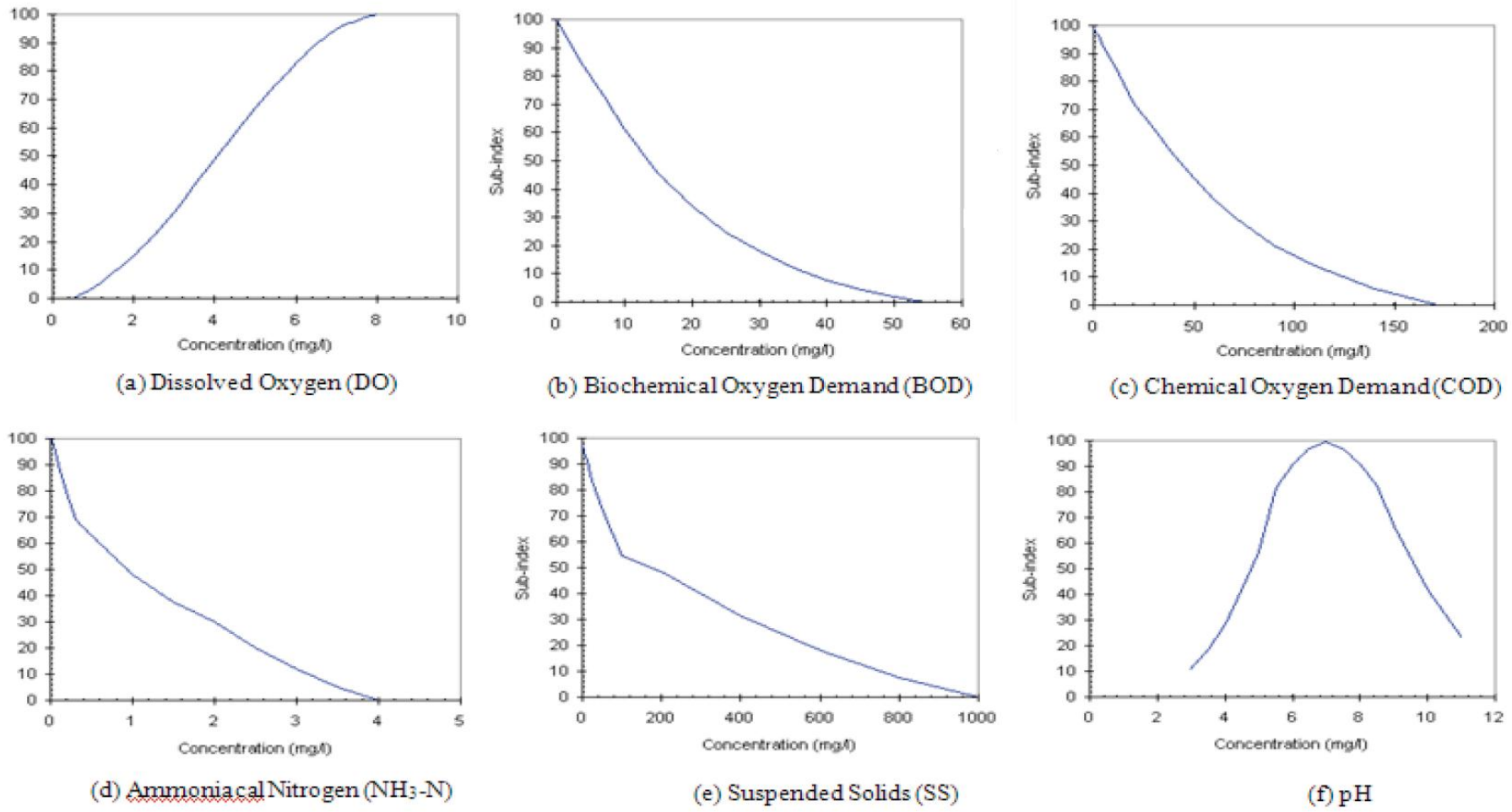


Figure 3. *Variation of sub-indices with pollutant concentrations for the parameters required to determine Malaysia's WQI (Mamun and Zainudin, 2013)*

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Table 1a. *Malaysia's Interim of National Water Quality Standard (NWQS) with limit for classes (NRE, 2006)*

Parameters	Unit	Limits for Classes				
		I	II	III	IV	V
DO	mg/l	> 7.0	5.0 – 7.0	3.0 – 5.0	1.0 – 3.0	< 1.0
pH	-	> 7.0	6.0 – 7.0	5.0 – 6.0	< 5.0	< 5.0
NH_3-N	mg/l	< 0.1	0.1 – 0.3	0.3 – 0.9	0.9 – 2.7	> 2.7
TSS	mg/l	< 2.5	25 - 50	50 - 150	50 - 300	> 300
BOD	mg/l	< 1	1 - 3	3 - 6	6 - 12	> 12
COD	mg/l	< 10	10 - 25	25 - 50	50 - 100	> 100

Table 1b. *Malaysia's WQI classification with limits (NRE, 2006)*

	Limits for Classes				
	I	II	III	IV	V
WQI	< 92.7	76.5 – 92.7	51.9 – 76.5	31.0 – 51.9	> 31.0
Pollution level	Clean 100 - 81		Slightly polluted 80 - 60	Polluted 0 - 59	

Table 1c. *Malaysia's WQI classification (NRE, 2006)*

CLASS	USES
Class I	Conservation of natural environment Water Supply I - Practically no treatment necessary Fishery I - Very sensitive aquatic species
Class IIA	Water Supply II - Conventional treatment Fishery II - Sensitive aquatic species
Class IIB	Recreational use body contact
Class III	Water Supply III - Extensive treatment required Fishery III - Common, of economic value and tolerant species, livestock drinking.
Class IV	Irrigation
Class V	None of the above

Data acquisition and statistical analysis

WQI before treatment was obtained in June 2015 and WQI after treatment was taken in October 2016 to March 2017. The WQI for the thirteen stations were interpolated to produce thematic maps of spatial distribution of pollution using Kriging model via Surfer® 11 for water quality modelling. Kriging model was chosen in this study because it is the most accurate model to present interpolation of water quality data for liquid flowing in a conduit that does not completely enclose the liquid, i.e. concreted stream and river (Ghafouri and Swain, 2005). Kriging is a geostatistical gridding method which expresses trend within the source of data. For example, alternatively of forming isolated peaks at the similar location on the map, it allows the peaks to connect and form as a ridge (Golden Software, 2017). Below is the general linear regression model for Kriging map interpolation (*Eq. 8*), showing how WQI value treated via interpolation (predicted) for notation purposes. From the equation, *s* is denoted as a

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generic spatial location that continuously contrasts over some domain of interest. Y(*s*) is the result of interest from the interpolation value of non-sample WQI data at *s*.

$$\hat{Y}(s) = \beta_0 + \beta_1 X_1 + \varepsilon(s) \quad \text{(Eq.8)}$$

$X_1(s)$ expresses a potential covariate indexed by location *s* which may have more than one covariates. β_1 is the covariate's associated regression effect while β_0 is the constant term and ε(s) is error term. The uncertainty within the universal regression approach is modelled with the random error term ε(s), assumed to have zero means, constant variance and a multivariate correlation structure to accommodate spatial dependence (Murphy et al., 2010). The current study focuses on WQM-generated concentration of the WQI obtained. Using Kriging gridding method, WQI can be generated for all locations within the area, representing as the covariate [$X_1(s)$]. The expected value of a parameter (i.e. $\beta_0 + \beta_1$[Modelled WQI(*s*)]) is different by location and is a function of the WQM-generated concentration. Residual error term is accounted for expected value which deviates in value and varies spatially. Thus, the Kriging gridding method reduces the concern regarding station in a flowing water body by accounting for a spatial varying expected value.

On-site water treatment system

The selection of on-site water treatment system undergoes screening of two general aspects which are treatment infrastructure and placement of treatment system. Both of the aspects should be complemented with the information gathered from site profiling and water quality assessment. Four components were considered in the treatment infrastructure, namely removal efficiency, design criteria, water treatment specification as well as operational and maintenance. Removal efficiency is the ability of water treatment to address targeted water quality parameters in the water body. Design criteria are the installation and development of the water treatment for on-site operation including the size and mechanism involved. Water treatment specification is the requirement for optimal efficiency and better performance and water treatment management suitability (Lim and Lu, 2016; Thomas and Reese, 2003; Din et al., 2012; Murphy et al., 2010). For the placement of the treatment system, two components are deliberated namely space limitation and strategic target close to the source of pollution.

From the aforementioned criteria, the selected on-site water treatment systems were constructed wetland, permeable pavement wall and floating treatment wetland. *Figure 4* depicts the selected on-site water treatment systems used to revitalize Alur Ilmu water quality. All of the water treatment systems were fully deployed to Alur Ilmu on January 2016. The three water treatment systems were opted to complement Alur Ilmu's site profile through which Alur Ilmu is located close to human development and economic activities, i.e. faculty infrastructures, cafe and tarred road, hence the water quality is susceptible to water pollution. Surrounded by impervious hilly terrain, the concreted urban river received large surface runoff during rain. The constructed wetland was placed at the upstream of Alur Ilmu as it is effective to sink suspended solids using Bent Flag Alligator (*Thalia geniculate)*. Permeable pavement wall and floating treatment wetland were selected because of flexible design criteria which can be retrofitted into the minimum available space of Alur Ilmu and effective on domestic water pollution and surface runoff. Four free-floating plants were studied to remove NH_3-N, TSS, and BOD concentration in the water sample.

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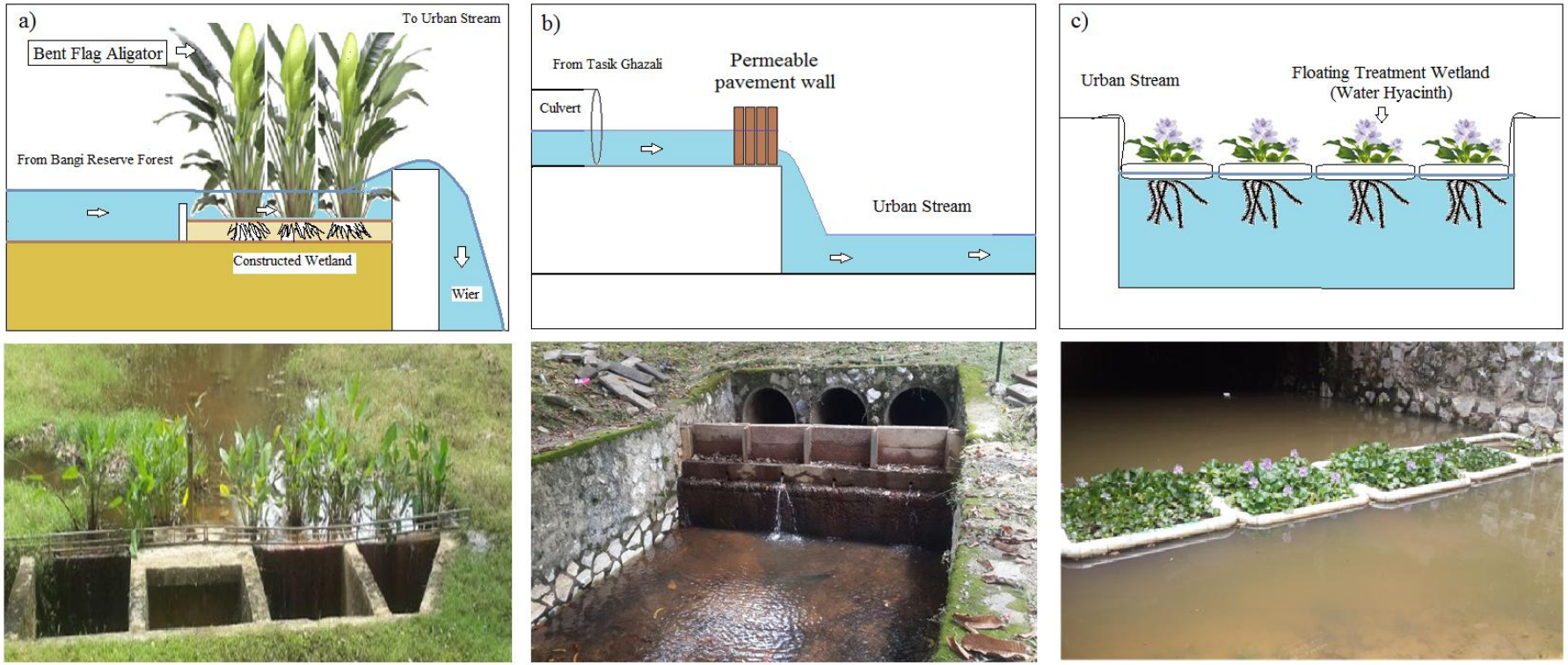


Figure 4. *Schematic diagram of the on-site water treatment systems: CONSTRUCTED wetland (a), permeable pavement wall (b) and floating treatment wetland (c)*

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In order to determine the most effective free-floating plants to remove pollutions from the urban river water, four free-floating plants were selected for feasibility of phytoremediation in pollution removal of Alur Ilmu water sample. The selected free-floating plants are water hyacinth, *Eichhornia crassipes* (Mart.) Solms, water lettuce, *Pistia stratiotes.* L.*,* rose water lettuce, *Pistia sp.* L. and pennywort*, Centella asiatica,* (L.) Urb. Base on the study, Water Hyacinth was chosen to be planted on top of the floating treatment wetland (coverage area ~ 40 m^2) because its effectiveness in removing urban storm water pollution and resilient to urban water dynamic (Hafiyyan et al., 2017b; Wang et al., 2013).

Results

Table 2 shows the inventory of the site profile of Alur Ilmu by which the urban river is bounded with infrastructures and has limited space for large on-site treatment system, namely swale, infiltration basin and wetland.

Table 2. *Site profiling of Alur Ilmu urban river's socio economics activities and physical landscape characteristic*

Site Profile		
Socio-economic activities	● University setting (public) (UKM, 1978; UKM, 2013) ● Two Cafeterias (Economic activities) (UKM, 2013)	
Physical landscape characteristic	Geomorphological of the terrain	● Hilly terrain (UKM, 2013) ● 95% paved area (UKM, 2013)
	Physical characteristic of the urban river	● 500 m study area (from from Ghazali Lake to Silt Collection Tank) ● Concreted urban river (from a natural river into a concreted urban river) (UKM, 1978) ● No change of its original flow ● Bounded with paved area (Building and infrastructures) (UKM, 1978; UKM, 2013) ● First 100 m of Alur Ilmu is located at the lowest elevation point of hilly terrain and flows with normal elevated terrain
	Flow pattern of the urban river	● Receives water source from the Permanent Reserved Forest and Natural Education Forest, rain water and stormwater runoff (Hafiyyan et al., 2017a) ● Average water depth is about 1.8 m ● average water velocity is 0.1 ms^{-1} during dry season ● average water velocity of 6 ms^{-1} during rainy season

The options available are to exercise major remodelling of Alur Ilmu which may harm the integrity of infrastructures in the vicinity or use Low Impact Development (LID) (Thomas and Reese, 2003) by retrofitting on-site water treatment system to the urban river. Based on *Figure 4*, the first part of Alur Ilmu being AL1 is suitable to use constructed wetland as it is the only large area available for effective filtration and detention of soil, sediment and large solids, i.e. plastics, dead branches and debris. The area was planted with Bent Flag Alligator (*Thalia geniculate*) and has the predominant removal mechanism for detention system. It enables to hold water for a period of time,

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allowing for settling of solid pollutants, filtration through vegetation, biological and chemical transformation, i.e. bioabsorptions (Thomas and Reese, 2003).As AL2 to AL13 have limited space for large on-site treatment system, the main culvert was built with permeable pavement wall. In addition, it is a retrofitted stormwater filtration system engineered to physically reduce the concentration of suspended solids, soil and silt by filtering the water vertically past through the porous medium (Thomas and Reese, 2003). Alur Ilmu water body is susceptible to myriad of pollutions from discharged points and surface runoff. Floating treatment wetland (FTW) plots were chosen and deployed into Alur Ilmu. During and after the installation of FTW, no change in any physical landscape characteristic was made because the plots were retrofitted into the urban river hence it is ideal to limited space urban setting. Previous study in 2017 has revealed the feasibility of phytoremediation in pollution removal of Alur Ilmu water sample (Hafiyyan et al., 2017b). Four free-floating plants were studied to remove NH_3-N, TSS, and BOD concentration in the water sample. The free-floating plants were water hyacinth, *Eichhornia crassipes* (Mart.) Solms, water lettuce, *Pistia stratiotes.* L.*,* rose water lettuce, *Pistia sp.* L. and pennywort*, Centella asiatica,* (L.) Urb. The result showed rose water lettuce has the highest concentration reduction followed by water lettuce, water hyacinth and pennywort. However, among all four plants, water hyacinth was the most adaptable plants thus make it a better option for on-site phytoremediation of Alur Ilmu water. Water hyacinth (*Eichhornia crassipes*) was used for floating treatment wetland as regional stowmwater control (Hafiyyan et al., 2017b) The regional stormwater control is defined as facilities designed to manage stormwater runoff from multiple inlets for on-site control (Thomas and Reese, 2003; Martin et al., 2007). The floating treatment wetland designed for the regional stormwater control can maximize the utilization of developable land, to reduce operation and maintenance cost, retrofit potential and highly visible for enhancing natural aesthetical value. Hence, Water hyacinth was selected because of its effectiveness in large application of urban water body (Wang et al., 2013; Hafiyyan et al., 2017b). The thematic map in *Figure 5* depicts the strategic design of the on-site treatment system layout at Alur Ilmu urban river and in thematic map of WQI improvement.

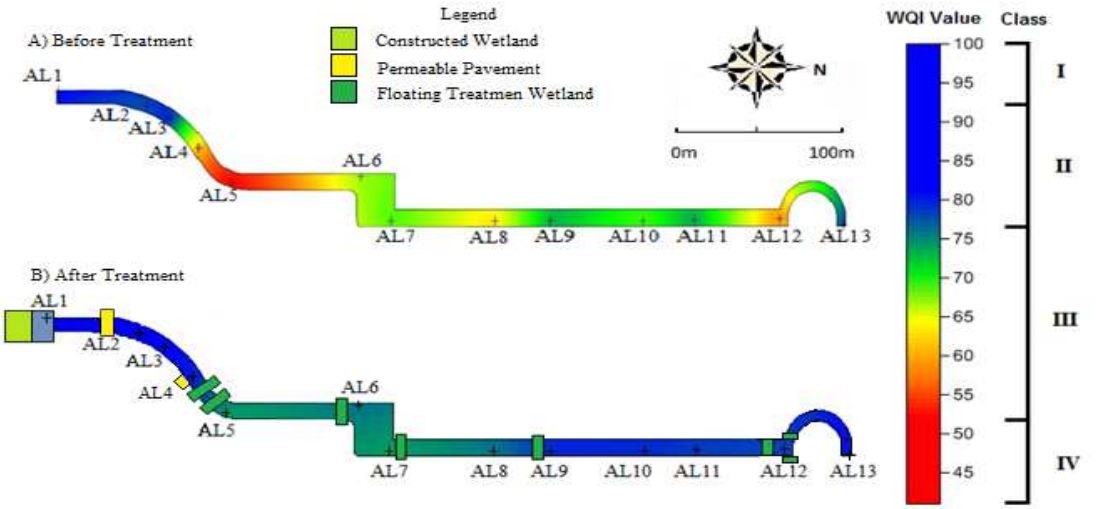


Figure 5. *Comparison of spatial distribution of WQI in Alur Ilmu before and after the structural approach*

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As shown in *Table 3,* the average WQI was 68.85 (Class III) in June 2015 (before treatment) and it has been improved to 79.87 (Class II) in October 2016 (after treatment) within 10 months with 16.00% of improvement in WQI. The highest average WQI after implemented structural approach was reported in Feb 2017 being 81.98 (Class II) with 19.07% of improvement in WQI. After implementing treatment, average WQI stabled at Class II ranging from 78.60 to 81.98 in WQI. The thematic maps show a general and clear pattern difference of WQI distribution before and after implementing the structural approach in Alur Ilmu. All the thirteen stations along 500 m of Alur Ilmu have generally improved spatially in WQI. The highest improvement of WQI was recorded at AL5 with 46.47% of improvement from 51.36 (Class IV, June 2015) to 75.23 (Class III, Feb 2017).

Discussion

Figure 5 shows that structural approach has a positive effect in revitalizing the urban river water quality. *Figure 6* is a visual comparison of Alur Ilmu urban river before (top) and after (bottom) implementation of the structural approach. Based on *Figure 5* and *Figure 6*, the structural approach has successfully improved both aesthetic and water quality of Alur Ilmu. The overall design of the structural approach is depending on the understanding of the complex pollution dynamic from natural phenomenon and anthropogenic factors of physical, chemical and biological forces emanating into the urban river (Thomas and Reese, 2003). Based on the aforementioned methodology, the water quality assessment and spatial distribution analysis are the tools used to understand pollution accumulation and urban hotspot (Thomas and Reese, 2003) of the land use prior to improve water quality of the urban river. Alur Ilmu is located at the lowest elevation level of hilly terrain, surrounded by imperious surface area and economic activities. Therefore, it is vulnerable to point and non-point sources of pollution.

The methodology of the current study followed the urban stromwater management manual for Malaysia (*Manual Saliran Mesra Alam, MSMA)* (Department of Irrigation and Drainage – DID, 2012), a guideline for non-point source of pollution control. The on-site treatment system used in the current study complied the key aspect of the manual which is to control pollution source using methods that produce the least impact towards the environment.

The thematic map before treatment is the superlative example to represent the pollution dynamic in the water body caused by point and non-point sources of pollution. The map indicates that pollutions accumulate at station AL5, AL8 and AL12. In relation to urban hotspot, AL5 and AL12 are both nearby to Cafeteria and AL8 is nearby to car park and faculty buildings. The introduction of pollution into the water body may come from the leakage of pipes and poor maintenance of pipes to channel waste produced into designated water treatment system and discarded properly through schedule waste disposal (Chong, 1999).

Wang et al. (2013) has shown positive results on revitalizing Lake Caohai (10.5 km^2), China water quality which is bounded by urban infrastructure using onsite treatment system by deploying a large scale confined growth of Water hyacinth (coverage area ~ 4.3 km^2) on N removal (761 t) (64% removal) within 6 months. The current study and Wang et al. (2013) were differ in water parameter tested because of different type of water pollutants were introduced into the water body respectively. Both of the studies used similar methodology to revitalize the water quality by profiling the study site and defining water quality parameters.

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Table 3. *WQI of Alur Ilmu before and after treatment*

Location of stations			Before treatment			After treatment																	
			Jun 2015 (hafiyyan et.al 2017)			Oct 2016			Nov 2016			Dec 2016			Jan 2017			Feb 2017			Mar 2017		
Station	**Long**	**Lat**	**WQI**	**Class**	**Status**	**WQI**	**Class**	**Status**	**WQI**	**Class**	**Status**	**WQI**	**Class**	**Status**	**WQI**	**Class**	**Status**	**WQI**	**Class**	**Status**	**WQI**	**Class**	**Status**
AL1	2.9219861	101.7817889	79.12	II	S.P	99.15	I	C	99.03	I	C	98.21	I	C	99.00	I	C	99.05	I	C	92.35	I	C
AL2	2.9227833	101.781675	76.34	III	S.P	90.40	II	C	90.61	II	C	92.11	II	C	90.11	II	C	92.90	I	C	90.43	II	C
AL3	2.9226806	101.7817306	78.12	II	S.P	80.81	II	C	81.43	II	C	82.51	II	C	80.68	II	C	81.20	II	C	81.01	II	C
AL4	2.92285	101.7817944	61.26	III	S.P	79.11	II	S.P	79.02	II	S.P	79.87	II	S.P	79.81	II	S.P	81.01	II	C	78.56	II	S.P
AL5	2.9230278	101.7818667	51.36	IV	P	73.18	III	S.P	71.24	III	S.P	71.53	III	S.P	72.91	III	S.P	75.23	III	S.P	70.11	III	S.P
AL6	2.9234722	101.7818139	66.23	III	S.P	75.13	III	S.P	75.36	III	S.P	75.65	III	S.P	75.05	III	S.P	78.23	II	S.P	73.02	III	S.P
AL7	2.923625	101.7819861	68.46	III	S.P	72.94	III	S.P	73.39	III	S.P	72.31	III	S.P	73.47	III	S.P	75.15	II	S.P	71.34	III	S.P
AL8	2.9239780	101.7819970	62.42	III	S.P	74.90	III	S.P	74.22	III	S.P	73.78	III	S.P	75.23	III	S.P	76.24	III	S.P	74.18	III	S.P
AL9	2.9242810	101.7820720	73.13	III	S.P	78.89	II	S.P	79.07	II	S.P	79.54	II	S.P	80.23	II	C	81.67	II	C	78.82	II	S.P
AL10	2.9245940	101.7821030	68.65	III	S.P	78.45	II	S.P	77.36	II	S.P	78.01	II	S.P	79.21	II	S.P	80.48	II	C	78.11	II	S.P
AL11	2.9248440	101.7820580	73.49	III	S.P	77.58	II	S.P	74.66	III	S.P	74.58	III	S.P	77.81	II	S.P	78.86	II	S.P	75.76	II	S.P
AL12	2.9250780	101.7820280	58.88	III	P	77.36	II	S.P	78.28	II	S.P	78.53	II	S.P	79.32	II	S.P	82.56	II	C	79.59	II	S.P
AL13	2.9251780	101.7819190	77.53	II	S.P	80.37	II	C	78.61	II	S.P	78.51	II	S.P	79.57	II	S.P	83.17	II	C	78.53	II	S.P
Average	-	-	68.85	III	S.P	79.87	II	S.P	79.40	II	S.P	79.63	II	S.P	80.18	II	C	81.98	II	C	78.60	II	S.P

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Constructed wetland + gross pollutant trap **Permeable pavement wall** **Floating treatment wetland**

Figure 6. *Pictures of Alur Ilmu urban river before (top) and after (bottom) implementation of the structural approach*

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However, using spatial distribution analysis of water quality, the current study is able to determine optimal area for strategic placement of on-site treatment system needed that close to pollution source and using only approximate 1.6% of the study area to improve 16% of average WQI compared to Wang et al. (2013) that used 40.95% of the study area for 64% average N removal.

Figure 7 depicts WQI temporal trend of Alur Ilmu. The WQI trend projected that Alur Ilmu WQI will be more polluted if conventional management and practices are still continued. The intervention of structural approach has been proven able to bring back the water quality to Class II (Mokhtar et al 2005) after 10 months. Further improvement of the urban river WQI can be obtained if both structural and non-structural approaches are used through integrated management.

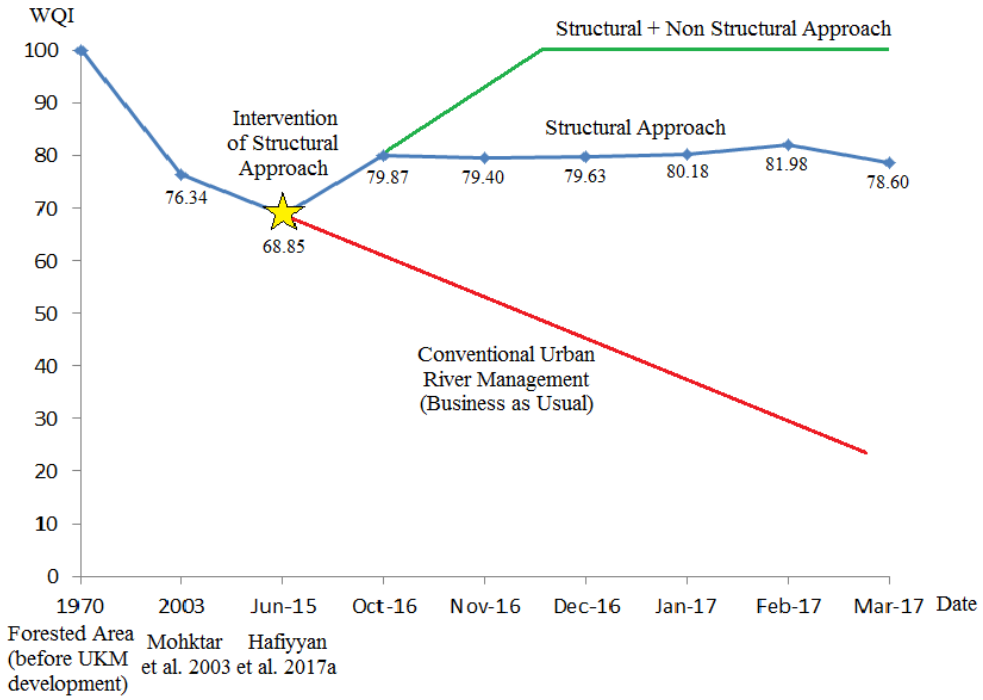


Figure 7. *WQI temporal trend of Alur Ilmu*

Conclusion

The result shows that the structural approach using constructed wetland, gross pollutant trap, permeable pavement wall and floating treatment wetland in revitalizing urban river's water quality have a positive effect to revitalize the water quality. The average WQI was 68.85 (Class III) in June 2015 (before treatment) and it has been improved to 79.87 (Class II) in October 2016 (after treatment) within 10 months with 16.00% of improvement in WQI. However, the structural approach should be complemented with a non-structural approach to control pollution before and after entering the urban river's water body as conventional infrastructure developments and economic activities with less consideration of environmental protection will cause various pollutions that can impair the capacity of the on-site treatment system. Realizing sustainable water resource management through revitalization urban river water quality, it is recommended to incorporate the urban river uses and functions into the urban masterplan that include environmental protection and gentrification as well as values for money.

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APPLICATION OF ECOTOURISM OPPORTUNITIES SPECTRUM METHOD IN ECOTOURISM RESOURCES: A CASE STUDY OF SAMANDAĞ COASTAL AREAS IN SOUTHERN TURKEY

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Abstract. The aim of the study is to identify the areas that might serve as ecotourism focuses in the Samandağ coastal zone and its surroundings, located on the southern border of Turkey, and to develop an optimum ecotourism route. The methodology used is Ecotourism Opportunity Spectrum (ECOS). The methodology is comprised of three stages. In the first stage areas that can serve as ecotourism focuses have been identified. In the second stage ECOS method has been applied to the identified focuses. In this regard, 10 criteria and 40 sub-criteria that determine the level of suitability for identifying an ecotourism route have been identified. Both these criteria and the sub-criteria have been scaled and ecotourism opportunity spectrum value has been determined for each criterion. According to these calculations, the highest ecotourism opportunity spectrum value is 400. In the study, the focus that is closest to this value is considered as the most suitable for identifying an ecotourism route according to the planning criteria. As a result of the study, Samandağ coastal zone and its surroundings has very high importance with respect to preserving, planning and development of the resources with a sustainable approach. In this area, 20 focuses have been identified for being used for ecotourism. The highest spectrum value calculated by applying this method is 271.86 and it belong to Vakıflı focus, while the lowest spectrum value is 136.92 for the Milleyha focus.
Keywords: *ecotourism focuses, ecotourism route, natural resources, ecotourism activities, criteria and values*

Introduction

It is known that the idea of ecotourism, its use as a technical term and its applications had existed before it was termed conceptually. The concept of ecotourism had begun in the 1980s, with alternative tourism debates, as a search for a different kind of tourism that serves as a new alternative to mass tourism and does not cause negative social and ecological effects like mass tourism (Long et al., 2013). In the following years the concept of ecotourism developed with an increasing awareness towards environmental protection and the general acceptance of sustainability principles. Some researchers such as Fennell (2001), Blamey (2001), Goodwin (1996), who study ecotourism, define it basically as a sustainable kind of tourism that is based on learning and experiencing the nature, has lower ethical impacts, focuses on the local rather than consumption.

In this regard, ecotourism has strong ties with sustainable tourism due to its characteristics like preserving the natural habitats or taking the requirements of local people into consideration.

Suitable management for ecotourism development is essential in order to conserve and maintain the biological richness of the area. In addition, ecotourism can be defined as an opportunity to promote the values in the protected areas. In this respect, ecotourism evaluation should be regarded as an important tool for sustainable development of tourism in a protected area. This can be judged with the help of criteria and indicators approach, which is basically a concept of sustainable ecotourism

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planning developed in a set of principles, criteria and indicators (Bunruamkaew and Murayam, 2011).

The selected area of study is the Samandağ coastal zone and its surroundings located in the Hatay province of Turkey and it has numerous characteristics such being the habitat of Mediterranean seals, nesting area of sea turtles, buxus gene preservation forest, wild life development area, and involving endemic plants, monumental trees, and archaeological protected areas. The region has a high potential for ecotourism with its resources. Therefore, the purpose of the study has been to identify the areas that could serve as ecotourism focuses within the region of Samandağ coastal zone in the Hatay province and its surroundings, and identify an optimum ecotourism route. In this regard, Ecotourism Opportunity Spectrum Method has been used.

Materials and methods

Study area

The study area is the Samandağ coastal zone and its surroundings, located in the Hatay Province in the Eastern Mediterranean region of Turkey (*Fig. 1*). The area is approximately 14 km long. It is surrounded by Musa Mountain in the northwest, Keldağ in the southeast, and the Mediterranean in the south. The Orontes River that flows through the area is born in Syria and meets the Mediterranean in Samandağ. The delta formed by the river is the breeding ground for "Green Sea Turtles" (*Chelonia mydas*), which is an endangered species according to the International Union for Conservation of Nature (IUCN) criteria (IUCN, 2006). The study area is therefore highly rich in terms of biodiversity.

Since the study area contains protected areas such as breeding sites for sea turtles and features venues that shall support religious tourism, it has been declared as "Hatay Samandağ Tourism Zone" in the official journal no. 21731 dated 17.10.1993 based on the decree of the Cabinet of Ministers no. 93/4833 within the framework of the Tourism Strategy Action Plan of Turkey.

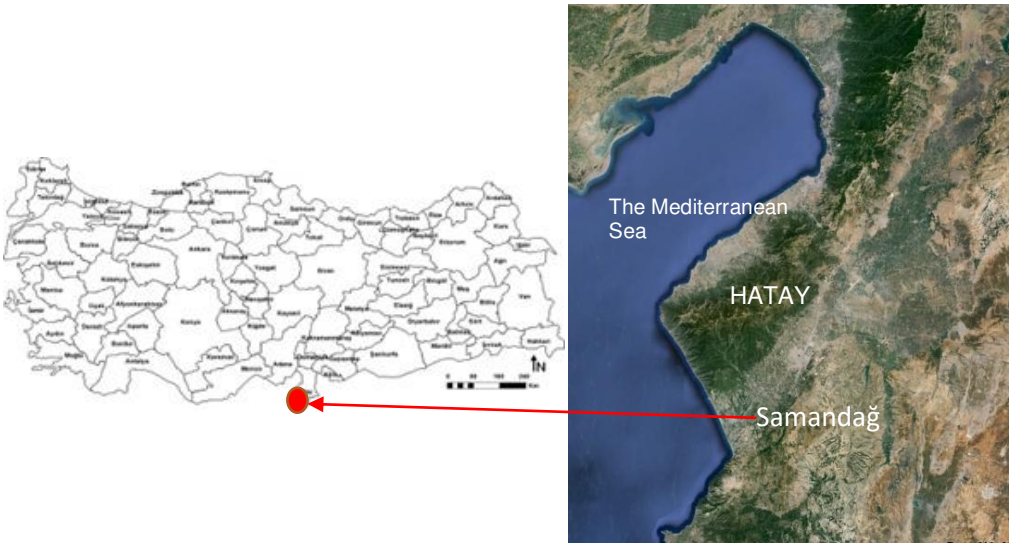


Figure 1. *Site location of study area*

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Methodology

The methodology of the study, which was formed to identify an ecotourism route involving quality ecotourism resources, is as follows:

1. Identifying the areas that can serve as ecotourism focuses,
2. Applying the ECOS method in order to determine the source values of the identified spots,
3. Identifying the optimum ecotourism route in line with the data obtained through applying ECOS.

Identifying the focuses

The areas that are mentioned as ecotourism areas and fall within the study area according to the Hatay Province 2016 Environmental Status Report, UCTEA Samandağ Study Group Report, Hatay Province Tourism Strategy and 2018-2023 Action Plan Report and maps obtained from the local administrations related with the study area, have been accepted as ecotourism focuses.

Applying the ECOS method on the identified spots

When the studies on the planning of ecotourism areas are analyzed, it is seen that methods like Limits of Acceptable Change-LAC, Environmentally Based Tourism Planning Model-EBT, Recreation Opportunities Spectrum (ROS), and Tourism Opportunities Spectrum (TOS) have been used. Drawn up on the basis of all these methods, the Ecotourism opportunity spectrum (ECOS) attracts attention as both an opportunity and a new method put forward depending on the available approaches for the development of ecotourism in rural areas.

Even though the ECOS method was developed to provide a conceptual management approach to ecotourism, it depends on the already available models in the literature. The method particularly combines the methods of ROS and TOS and this is called the Ecotourism Opportunity Spectrum (ECOS) with several fundamental alterations and pluses. The method comprises eight sub-components in total; (1) accessibility, (2) relationship between ecotourism and other resource uses, (3) attractions in a region, (4) presence of existing tourism infrastructure, (5) level of user skill and knowledge required, (6) level of social interaction, (7) degree of acceptance of impacts and control over level of use, (8) type of management needed to ensure the viability of areas on a long-term basis. Such 8 criteria are the determinative amongst decision-makers and users in long-term use of the area as an ecotourism area (Body and Butler, 1996).

The Ecotourism Opportunity Spectrum (ECOS) method has been applied in order to evaluate the availability of the focuses identified in the study. Since the study area involves critical regions, the criteria used in the ECOS method have been enhanced and applied accordingly.

In this regard, 10 criteria have been determined relying on the studies of Hvenegaard (1994), Boyd and Butler (1996), Fennell and Weaver (2005), Tsaur et al. (2006), Bender (2008), Barzekar et al. (2011), Yılmaz et al. (2013), Ashok et al. (2017), Dhami et al. (2017), Nino et al. (2017). These 10 criteria have been presented in *Table 1*. These criteria have been divided into sub-criteria in order to offer relative values that define the level of availability for determining an ecotourism route (*Table 1*).

It has been observed that researchers who study ecotourism assign relative values that vary between 1-3, 1-5, -3-+3, 1-10, 1-100 to the sub-criteria. Gold (1980) and

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Giles-Corti et al. (2005) emphasize that the range of weight values may be small if the elements under evaluation are similar in terms of concept. Additionally, the literature used as references of the study and the opinions of experts and scholars of the subject have been taken into consideration. As a result, each sub-criteria has been given relative values between 1-4 in accordance with their suitability for determining an ecotourism route. High values between 1-4 represent the availability for being an ecotourism focus and high sustainability of naturalness. In low values this situation is the contrary.(4. Extremely Important, 3. Important, 2. Unimportant, 1. Extremely Unimportant).

Table 1. *The criteria, sub-criteria and relative values for ECOS method*

Criteria	Sub-criteria	Relative values
1. Accessibility	Presence of road easily accessible during all seasons of the year	4
	Presence of road accessible during certain seasons of the year	3
	Presence of road where transportation is frequently interrupted	2
	No means of transportation	1
2. Level of responding to distinct ecotourism activities	High	4
	Medium	3
	Low	2
	Not usable	1
3. Source diversity of the focus	Rich and preserved flora and fauna presence	4
	Partially damaged flora and fauna presence	3
	Extremely damaged flora and fauna presence	2
	Insignificant flora and fauna presence	1
4. Protected ecosystem elements	High level of specific habitat presence	4
	Medium level of specific habitat presence	3
	Low level of specific habitat presence	2
	No specific habitat present	1
5. Visual attractiveness of the focus	High	4
	Medium	3
	Low	2
	Conditions with negative effect on attractiveness	1
6. Available tourism infrastructure	Adequate recreational infrastructure	4
	Inadequate recreational infrastructure, but suitable for improvement	3
	Inadequate recreational infrastructure	2
	No recreational infrastructure	1
7. Available tourism superstructure	Adequate recreational superstructure	4

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	Inadequate recreational superstructure, but suitable for improvement	3
	Inadequate recreational superstructure	2
	No recreational superstructure	1
8. Social interaction level	Intense interaction level between both the local populace and the eco-tourists	4
	Intense interaction level between eco-tourists and the local populace	3
	Intense interaction level only between eco-tourists	2
	Insignificant interaction level between all groups	1
9. Knowledge level of the eco-tourists on both the area and the source, and their attitude and behavior concerning the core of the ecotourism activities	High	4
	Medium	3
	Low	2
	Very inadequate knowledge and behaviors	1
10. Effects of visitor use on the ecotourism source	High level of positive impact due to use	4
	Positive impact due to use	3
	Negative impact due to use	2
	High level of negative impact due to use	1

While finalizing the planning decisions the comparative weighted scores of criteria should be determined. This stems from the fact that the level of importance of each criterion in terms of identifying an ecotourism route is not the same. Thus, the criteria have been weighted in the second stage. In this regard, a total of 30 experts with at least 5 years of professional experience from Mustafa Kemal University Faculty of Architecture Department of Landscaping Architecture (4 individuals), Hatay Metropolitan Municipality (10 individuals), Hatay Provincial Directorate of Culture and Tourism (5 individuals), Hatay Directorate of Environment and Urbanization (6 individuals), Branch Offices of Forestry and Rural Affairs (5 individuals) were given a questionnaire. During the execution of the study, 34 experts working in these institutions were identified and 30 of them could be given the questionnaire. A score ranging from 1-10 was assigned for each criterion at the questionnaire. All the points obtained for each criterion have been added and their weighted arithmetic mean scores were calculated. The weighted arithmetic mean scores have determined the weighted score of each criterion that ranges from 1 to 10. The ecotourism opportunity spectrum value of each criterion was calculated by multiplying the weighted scores and the score each focus has according to *Table 1*. According to these calculations, the highest total value is 400. In the study, the focus that is closest to this value is considered as the most suitable for identifying an ecotourism route according to the planning criteria.

Identifying the optimum ecotourism route

In order to take correct and rational planning decisions, the obtained ecotourism opportunity spectrum values have been categorized, taking the level of availability to be used as ecotourism areas into consideration and the areas that would form the optimum ecotourism routes have been determined.

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Results

The results of the study have been gathered under three titles as 1. Findings about ecotourism focuses, 2. Findings obtained by applying ecotourism opportunity spectrum, 3- optimum ecotourism route.

Findings about ecotourism focuses

In accordance with the reports and maps obtained from the local administrations 20 focuses suitable for ecotourism purposes were identified in the study area (*Fig. 2*). Six of these focuses are located in Samandağ coastal zone and the remaining 14 are located in the surroundings of Samandağ coastal zone.

The identified focuses incorporate many features in terms of both nature and culture. The availability of such focuses for ecotourism activities has been evaluated relying on the basic approach of the institutions and organizations conducting research on ecotourism, such as "World Tourism Organization-UNWTO" (UNWTO, 2002), "The International Ecotourism Society-TIES" (TIES, 2005), and the works of researchers such as Butler and Waldbrook (2003), Turoğlu and Özdemir (2005), Stepanova (2008), Şenkaya et al. (2012), Shieh et al. (2014). The types of activities applicable on the focuses within this context are given in *Table 2*.

Findings Obtained From "Ecotourism Opportunity Spectrum"

First, the condition of the focuses in accordance with the specified criteria was propounded in order to implement the ecotourism opportunity spectrum method. The results obtained are presented in *Table 3*.

According to the accessibility criteria presented in *Table 1,* the focuses that are accessible throughout the year are Hıdırbey, Çubukçu, Tomb of St. Hızır and surroundings, Çevlik port and surroundings, Eriklikuyu, Yoğunoluk and Sutaşı. Therefore, the ECOS method relative value of these focuses is set as 4 (*Table 3*).

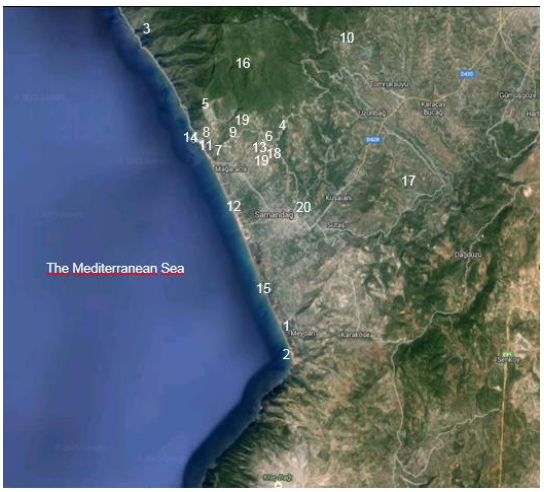


Figure 2. *Ecotourism focuses in the study area. (1. Meydan village, 2. Keldağ, 3. İskenderun-Arsuz wildlife improvement site, 4. Yaylıca, 5. Amanos mountains Musa mountain zone, 6. Batıayaz, 7. Mağaracık, 8. Kapısuyu village, 9. Hıdırbey village, 10. Çubukçu, 11. Titus tunnel and surrounding, 12. Tomb of St. Hızır and surrounding, 13. Vakıflı village, 14. Çevlik port and surrounding, 15. Milleyha wetland, 16. Çamlıyayla, 17. Aknehir, 18. Eriklikuyu, 19. Yoğunoluk, 20. Sutaşı)*

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Table 2. *Ecotourism activities applicable on the ecotourism focuses*

EF*	EA**																			
	P	**B**	**CUT**	**AGT**	**MAT**						**CAT**	**HİT**	**CAM**	**PA**	**HRT**	**TRK**	**BN**	**MC**	**N**	**W**	**OT**
					S	Y	C	F	SD	A											
1					**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**				**x**			**x**	**x**	**x**
2					**x**	**x**	**x**	**x**	**x**	x	**x**	**x**	**x**			**x**			**x**		
3		**x**												**x**	**x**	**x**	**x**		**x**	**x**	
4	**x**	**x**		x						x						**x**			**x**	**x**	**x**
5		**x**										**x**		**x**		**x**		**x**	**x**		
6	**x**	**x**		x									**x**		**x**	**x**			**x**		
7											**x**	**x**									
8				x							**x**	**x**				**x**			**x**		
9	**x**			**x**								**x**	**x**								
10																					
11		**x**									**x**	**x**				**x**			**x**		**x**
12			**x**		**x**	**x**	**x**	**x**		x		**x**	**x**		**x**	**x**	**x**			**x**	
13	**x**	**x**	**x**	**x**								**x**	**x**		**x**	**x**	**x**	**x**	**x**		
14					**x**	**x**	**x**	**x**	**x**	x			**x**		**x**	**x**	**x**		**x**		
15		**x**			**x**										**x**				**x**		**x**
16	**x**			x									**x**	**x**	**x**	**x**					
17			**x**																		
18				x											**x**	**x**					
19				**x**								**x**			**x**	**x**					
20				x							x	x	x		x		x				x

*EF (Ecotourism focuses): 1. Meydan village, 2. Keldağ, 3. İskenderun-Arsuz wildlife improvement site, 4. Yaylıca, 5. Amanos mountains Musa mountain zone, 6. Batıayaz, 7. Mağaracık, 8. Kapısuyu village, 9. Hıdırbey village, 10. Çubukçu11. Titus tunnel and surrounding, 12. Tomb of St. Hızır and surrounding, 13. Vakıflı village, 14. Çevlik port and surrounding, 15. Milleyha wetland, 16. Çamlıyayla, 17. Aknehir, 18. Eriklikuyu, 19. Yoğunoluk, 20. Sutaşı

**EA (Ecotourism activities): P: Plateau tourism, B: Botanical tourism, CUT: Cultural tourism, AGT: Agricultural tourism, MAT: Marine tourism (S: Swimming, Y. Yatching, C: Canoe, F: Fishing, SD: Scuba-diving, A: Amateur hand-linefishing), CAT: Cave tourism, HİT: Historical tourism, CAM: Camper Tourism, PA: Paraliding, HRT: Horse trekking, TRK: Trekking, BN: Bike ride in nature, MC: Motorcross, N: Nature photography, W: Wildlife watching, OT: Ornithological tourism

Keldağ, İskenderun-Arsuz wildlife improvement site, Amanos mountains Musa mountain zone, Titus tunnel and its surrounding areas are very close to the beach at Samandağ coastal zone on the Mediterranean. Accessibility of these focuses is frequently interrupted due to flood tides during winter. Therefore, the ECOS method relative value is set as 2 in terms of accessibility (*Table 3*).

İskenderun-Arsuz wildlife improvement site is one of the 79 wildlife improvement sites in Turkey. Covering an area of 27.063 ha, this site was taken under protection in 2006 according to Law on Land Hunting of the Ministry of Forestry and Water Affairs of Turkey in order to protect Chevrotain (*Capra aegagrus*) and Roe-deer (*Capreolus capreolus*) species which have limited habitats in Turkey. 7000 ha of the improvement

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site intersects with the study area. This site is one of the 20 focuses identified and it is important for ecotourism activities such as Nature photography and Wildlife watching. Therefore, ECOS method relative value of the site is set as 4 (*Table 3*).

Table 3. *Relative values for the focuses according to the specified criteria*

Ecotourism focuses	Criteria***									
	AC	**LEA**	**SD**	**PEE**	**VAF**	**ATİ**	**ATS**	**SİL**	**KEC**	**EVE**
1	3	4	4	3	4	2	3	4	3	2
2	2	3	4	3	4	1	1	2	2	2
3	2	2	4	4	4	1	1	2	2	3
4	3	3	3	4	1	2	2	3	2	1
5	2	3	3	4	4	1	1	3	2	2
6	3	3	3	4	4	1	2	4	3	1
7	3	3	3	4	3	3	2	4	3	1
8	3	4	4	3	4	2	2	2	3	2
9	4	4	3	3	4	2	1	4	4	2
10	4	2	3	2	3	2	1	3	4	3
11	2	2	3	3	4	1	2	2	4	3
12	4	3	2	1	2	1	3	3	4	2
13	3	4	3	2	4	2	3	4	4	3
14	4	3	2	3	3	2	3	3	2	3
15	3	2	2	4	3	1	1	2	2	2
16	3	3	2	2	4	2	2	2	3	3
17	3	3	2	2	3	2	2	4	3	3
18	4	4	2	2	4	1	1	4	4	3
19	4	4	2	2	4	2	1	4	3	3
20	4	3	4	2	3	2	1	2	2	3

***Criteria: AC: Accesibility, LEA: Level of responding to distinct ecotourism activities, SD: Source diversity of the focus, PEE: Protected ecosystem elements, VAF: Visual attractiveness of the focus, ATİ: Available tourism infrastructure, ATS: Available tourism superstructure, SİL: Social interaction level, KEC: Knowledge level of the eco-tourists on both the area and the source, and their attıtude and behavior concerning the core of the ecotourism activities, EVE: Effects of visitor use on the ecotourism source

In Batıayaz there is the buxus (*Buxus sempervirens*) gene forest. This forest has been announced as a Gene Preservation Forest in order to be used as a gene source for improvement studies today and in the future. The ECOS relative value is set as 4 for Batıayaz due to this situation and its incorporating many habitats in accordance with the protected ecosystem elements criterion listed in *Table 1*.

All the other focuses have been evaluated in accordance with the criteria listed in *Table 1* and the results obtained are given in *Table 3*.

The criteria were weighted in the second phase of the ECOS method. For this purpose, a questionnaire was conducted among a group of 30 experts directly involved in the subject matter and work at Mustafa Kemal University Faculty of Architecture Department of Landscaping Architecture (4 individuals), Hatay Metropolitan Municipality (10 individuals), Hatay Provincial Directorate of Culture and Tourism (5

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individuals), Hatay Directorate of Environment and Urbanization (6 individuals), Branch Offices of Forestry and Rural Affairs (5 individuals). All the scores obtained for each criterion through the questionnaire have been added and the weighted scores given in *Table 4* have been calculated.

Table 4. *Weighted scores for the focuses depending on the criteria*

Ecotourism focuses	Criteria***									
	AC	**LEA**	**SD**	**PEE**	**VAF**	**ATİ**	**ATS**	**SİL**	**KEC**	**EVE**
1	8.54	8.26	8.37	8.83	8.74	4.9	5.83	8.03	6.13	4.83
2	8.03	8.76	8.03	7.8	9.76	4.73	1.4	7.7	5.43	5.86
3	9.68	8.7	8.9	9.5	9.63	4.2	1.5	5.74	4.33	7.03
4	9.97	9.37	7.03	8.03	6.47	4.36	6.43	9.62	8.8	4
5	8.37	8.13	7.3	9.04	9.8	3.36	1.4	7.03	5.03	5.5
6	8.27	9.03	7	8.7	9.1	4.2	3.27	9.47	9	8.2
7	8.9	8.3	7.7	8.97	8.8	5.73	5.6	8.7	8.5	5.24
8	8.43	8.9	7.5	9.23	7.03	5.6	2.6	6.6	6.47	7.87
9	9.2	9.63	8.8	8.84	7.7	2.96	3.36	9.57	8.9	7.12
10	9.2	8.27	9.2	9.13	9.17	3.13	3.1	5.5	9	4.98
11	7.8	9.56	9.46	9.54	9.56	3	5.6	6.94	7.7	7.7
12	9.85	8.9	9.2	8.97	5	5.34	5.7	8.83	9.5	4.9
13	9.67	9.8	9.16	8.7	9.2	5.5	6.3	9.27	9.85	5.2
14	9.93	8.8	8.13	9.03	8.73	2.7	5.58	4.83	9.03	7.03
15	9.34	7	7	8.76	4.14	2.167	5.45	3.4	5	4.5
16	5.23	8.03	8.7	8.3	7.36	3.14	2.15	4.2	4.2	5.8
17	8.53	8.9	8.63	8.6	7.17	2.1	1.73	3.54	4.47	5.44
18	7.8	8.3	8.56	8.9	7.47	4.5	1.5	3.8	8.27	5.15
19	8.17	8.9	8.8	9.2	7.3	5.12	1.38	3.5	5.6	5.52
20	9.4	8.83	8.3	9.6	6.6	5.57	2.06	3.07	5.74	4.83

In the last phase of the ECOS method, the ecotourism opportunity spectrum values have been determined. The values are calculated by multiplying the relative values and weighted scores are of each focus. The determined ecotourism opportunity spectrum values have been given in *Table 5*.

As seen in *Table 5,* a multitude of ecotourism opportunity spectrum values that range from 1.37 to 39.73 are available for the study area. Achieving practicable results for assessments in such a broad range is only possible by categorizing the ecotourism opportunity spectrum values. In this case, calculating the weighted mean value of the ecotourism opportunity spectrum values can prove to be indicative in determination of the availability of the focuses.

The resulting weighted mean value of the figures given in *Table 5* is calculated to be around 20.20. This value means that the focuses with a spectrum value of 20.20 on average, or higher values, can be considered to have low improvement priority in terms of being used as an ecotourism site. It might be deduced that when the spectrum value wanes from the mean value, the possibility of that site being used as an ecotourism site

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shall decrease and the improvement priority shall increase. In this context, ecotourism opportunity spectrum values are categorized in 8 classes as indicated in *Table 6*.

Table 5. *Ecotourism opportunity spectrum value of the focuses*

Ecotourism focuses	Criteria***										
	AC	**LEA**	**SD**	**PEE**	**VAF**	**ATİ**	**ATS**	**SİL**	**KEC**	**EVE**	**Total EOSV**
1	25.6	33.06	33.47	26.5	34.96	9.8	17.5	32.13	18.39	9.67	241.08
2	16.07	26.3	32.13	23.4	39.04	4.73	1.4	15.4	10.86	11.73	181.06
3	19.33	17.4	35.6	38	38.52	4.2	1.5	11.46	8.66	21.1	195.77
4	29.9	28.1	21.1	32.13	6.47	8.73	12.86	28.9	17.6	4	189.79
5	16.73	24.4	21.9	36.14	39.2	3.37	1.4	21.1	10.06	11	185.3
6	24.8	27.1	21	34.8	36.4	4.2	6.53	37.87	27	8.2	227.9
7	26.7	24.9	23.1	35.86	26.4	17.2	11.2	34.8	25.5	5.24	230.9
8	25.3	35.6	30	27.7	28.12	11.2	5.2	13.2	19.41	15.73	211.46
9	36.8	38.54	26.4	26.5	30.8	5.93	3.38	38.27	35.6	14.27	256.49
10	36.8	16.53	27.6	18.27	27.51	6.26	3.1	16.5	36	14.9	203.47
11	15.6	19.13	28.4	28.6	38.24	3	11.2	13.87	30.8	23.1	211.94
12	39.3	26.7	18.4	8.96	10	5.33	17.1	26.5	38	9.8	200.09
13	29	39.2	27.5	17.4	36.8	11	18.9	37.06	39.4	15.6	**271.86**
14	**39.73**	26.4	16.27	27.1	26.19	5.4	16.7	14.5	18.06	21.1	211.45
15	28	14	14	35.06	12.42	2.17	5.47	6.8	10	9	136.92
16	15.7	24.1	17.4	16.6	29.44	6.26	4.35	8.4	12.6	17.4	152.25
17	25.6	26.7	17.27	17.2	21.51	4.2	3.48	14.13	13.41	16.3	159.8
18	31.2	33.2	17.14	17.8	29.88	4.5	1.5	15.2	33.08	15.4	198.9
19	32.67	35.6	17.6	18.4	29.2	10.27	**1.37**	14	16.8	16.6	192.51
20	37.6	26.5	33.2	19.2	19.8	11.14	2.06	6.13	11.48	14.5	181.61

Table 6. *Ecotourism opportunity spectrum value classes*

Class number	Value Classes	
1	0-5	High improvement priority
2	5.1-10	
3	10.1-15	
4	15.1-20	
5	20.1-25	Low improvement priority
6	25.1-30	
7	30.1-35	
8	35.1-40	

The following findings could be obtained when the data in *Tables 5* and *6* are analyzed:

- In terms of accessibility, Keldağ, İskenderun-Arsuz wildlife improvement site, Amanos mountains Musa mountain zone, Titus tunnel and surroundings, and Çamlıyayla are the 5 focuses with high improvement priority. The other 15 focuses

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have low improvement priority. The improvement priority of 5 focuses is high since their availability for being used as ecotourism sites is insufficient. The other 15 focuses are available as ecotourism sites.

- In terms of hosting different ecotourism activities, 4 focuses including İskenderun-Arsuz wildlife improvement site, Çubukçu, Titus tunnel and surroundings, and Milleyha wetland have high improvement priority. The other 16 focuses have low improvement priority. These 4 focuses mentioned are categorized among areas with low availability for hosting different ecotourism activities and high improvement priority due to their incorporating protected areas.

- When the focuses are analyzed in terms of resource variety, 7 focuses including Tomb of St. Hızır and surroundings, Çevlik port and surroundings, Milleyha wetland, Çamlıyayla, Aknehir, Eriklikuyu, and Yoğunoluk have high improvement priority. This stems from partial damage done to the flora and fauna of the focuses.

- The focus with the highest value (36.14) in terms of protected ecosystem elements is Amanos mountains Musa mountain zone. This is because the focus has a variety of habitat. The focuses with low values in terms of protected ecosystem elements are Çubukçu, Tomb of St. Hızır and surroundings, Vakıflı village, Çamlıyayla, Aknehir, Eriklikuyu, Yoğunoluk, and Sutaşı.

- The focuses with the highest value in terms of visual attractiveness are Amanos mountains Musa mountain zone (39.20), Keldağ (39.04), İskenderun-Arsuz wildlife improvement site (38.52), Titus tunnel and surroundings (38.24), Vakıflı village (36.8), and Batıayaz (36.4), respectively. These sites have visually rich landscape. The focus with the lowest score in terms of visual attractiveness is Yaylıca'dır (6.47).

- All focuses are inadequate with respect to available tourism infrastructure. Therefore, they should be improved with priority.

- All focuses have high improvement priority in terms of available tourism superstructure.

- In terms of social interaction level criterion, the focuses with the highest values are Hıdırbey village (38.27), Batıayaz (37.87), Vakıflı village (37.06), Mağaracık (34.8), Meydan village (32.13), respectively. At these focuses the interaction level is quite high both among local people and eco-tourists.

- The focuses with the highest value in terms of the knowledge level of eco-tourists about both the site and the resource, and their attitudes and behaviours in relation to the core of ecotourism activities, are Vakıflı village (39.4), Tomb of St. Hızır and surrounding (38.0), Çubukçu (36.0), Hıdırbey village (35.6), Eriklikuyu (33.08), Titus tunnel and surrounding (30.8), respectively.

- In terms of usage effect of visitors on the ecotourism resource, Çevlik port and surrounding (21.1), Titus tunnel and surrounding (23.1), İskenderun-Arsuz wildlife improvement site (21.1) have been affected positively from the visitors. Among the other 17 focuses, the most negatively affected one was Yaylıca (4.0).

- The highest ECOS value of Meydan village is visual attractiveness of the focus (34.96), and the lowest is effects of visitor use on the ecotourism source (9.67). The focus needs prior development in terms of available tourism infrastructure (9.8), available tourism superstructure (17.5), knowledge level of the eco-tourists on both the area and the source, and their attitude and behavior concerning the core of the ecotourism activities (18.39), effects of visitor use on the ecotourism source (9.67).

- Keldağ focus needs prior development in terms of accessibility (16.07), available tourism infrastructure (4.73), available tourism superstructure (1.4), social interaction

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level (15.4), knowledge level of the eco-tourists on both the area and the source, and their attitude and behavior concerning the core of the ecotourism activities (10.86), and effects of visitor use on the ecotourism source (11.73).

- İskenderun-Arsuz wildlife improvement site has high scores as an ecotourism focus in source diversity of the focus (35.6), protected ecosystem elements (38.0), visual attractiveness of the focus (38.52), effects of visitor use on the ecotourism source (21.1). But this focus should be developed priorly in other criteria. The lowest value of the focus is available tourism superstructure (1.5).

- Yaylıca got the highest ECOS value in protected ecosystem elements (32.13) and the lowest in effects of visitor use on the ecotourism source (4.0).

- Amanos mountains Musa mountain zone has the highest ECOS value in visual attractiveness of the focus (39.2) and the lowest ECOS value in available tourism superstructure (1.4). Its use as en ecotourism focus is low in terms of accessibility, available tourism infrastructure, available tourism superstructure, knowledge level of the eco-tourists on both the area and the source, and their attitude and behavior concerning the core of the ecotourism activities, effects of visitor use on the ecotourism.

- Batıayaz has the lowest ECOS value in terms of available tourism infrastructure (4.2), available tourism superstructure (6.53), effects of visitor use on the ecotourism source (8.2) criteria. The social interaction level (37.87) value of the focus is the highest among other criteria.

- Mağaracık has the lowest values in terms of available tourism infrastructure (17.2), available tourism superstructure (11.2), effects of visitor use on the ecotourism source (5.24). On the other hand, it has the highest value in protected ecosystem elements with 35.86.

- Kapısuyu village is inadequate as an ecotourism focus in terms of available tourism infrastructure (11.2), available tourism superstructure (5.2), social interaction level (13.2), knowledge level of the eco-tourists on both the area and the source (19.41), and their attitude and behavior concerning the core of the ecotourism activities, and effects of visitor use on the ecotourism source (15.73).

- Hıdırbey village is inadequate as an ecotourism focus in terms of available tourism infrastructure (5.93), available tourism superstructure (3.38), and effects of visitor use on the ecotourism source (14.27). This focus got the highest value in level of responding to distinct ecotourism activities (38.54).

- Çubukçu has the highest values in accessibility (36.8), source diversity of the focus (27.6), and visual attractiveness of the focus (27.51). In terms of other 7 criteria, the focus is inadequate as an ecotourism focus.

- While Titus tunnel and surroundings is available for ecotourism in terms of source diversity of the focus (28.4), protected ecosystem elements (28.6), visual attractiveness of the focus (38.24), knowledge level of the eco-tourists on both the area and the source, and their attitude and behavior concerning the core of the ecotourism activities (30.8), and effects of visitor use on the ecotourism source (23.1), it should be developed in other 5 criteria.

- Tomb of St. Hızır and surroundings is available for ecotourism in terms of accessibility (39.3), level of responding to distinct ecotourism activities (26.7), social interaction level (26.5), knowledge level of the eco-tourists on both the area and the source, and their attitude and behavior concerning the core of the ecotourism activities (38.0).

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- Vakıflı village is inadequate as an ecotourism focus in terms of protected ecosystem elements (17.4), available tourism infrastructure (11), available tourism superstructure (18.9), and effects of visitor use on the ecotourism source (15.6). However, this focus got the highest ECOS value calculated for optimum ecotourism route.

- Çevlik port and surroundings got the highest value compared to other focuses in accessibility (39.73). In addition, it is available as an ecotourism focus in terms of level of responding to distinct ecotourism activities (26.4), protected ecosystem elements (27.1), visual attractiveness of the focus (26.19), and effects of visitor use on the ecotourism source (21.1).

- Milleyha wetland is among low priority focuses only in accessibility (28.0) and protected ecosystem elements (35.06). The other 8 criteria should be developed in priority.

- Çamlıyayla is among the low priority group only in terms of level of responding to distinct ecotourism activities (24.1) and visual attractiveness of the focus (29.44). The other 8 criteria should be developed in priority.

- Aknehir is among the focuses with low priority in terms of accessibility (25.6), level of responding to distinct ecotourism activities (26.7), and visual attractiveness of the focus (21.51).

- Eriklikuyu needs development in terms of source diversity of the focus (17.14), protected ecosystem elements (17.8), available tourism infrastructure (4.5), available tourism superstructure (1.5), social interaction level (15.2), and effects of visitor use on the ecotourism source (15.4).

- Yoğunoluk has the lowest value compared to other focuses in terms of available tourism superstructure (1.37). The focus has low priority in accessibility (32.67), level of responding to distinct ecotourism activities (35.6), and visual attractiveness of the focus (29.2).

- Sutaşı has low priority in accessibility (37.6), level of responding to distinct ecotourism activities (26.5), and available tourism infrastructure (33.2).

Optimum ecotourism route

The total ecotourism opportunity spectrum values of the focuses have been calculated in order to determine the ecotourism route of the study area (*Table 5*). The highest spectrum value for the ecotourism route is 400. When total spectrum values of the focuses in *Table 5* are analyzed, the highest value among the focuses that comprise the ecotourism route belongs to Vakıflı village (271.86), and the lowest value belongs to Milleyha wetland (136.92).

The other focuses have been categorized in terms of spectrum values that make up the ecotourism route and in this respect

- the focuses with the highest values for identifying the ecotourism route are Vakıflı village (271.86) and Hıdırbey (256.49), respectively;

- the focuses with the medium values for identifying the ecotourism route are Meydan village (241.08), Mağaracık (230.9), Batıayaz village (227.9), Titus tunnel and surroundings (211.94), Kapısuyu village (211.46), Çevlik port and surroundings (211.45), Çubukçu (203.47) and Tomb of St. Hızır and surroundings (200.09), respectively;

- the focuses with the lowest values for identifying the ecotourism route are Eriklikuyu (198.9), İskenderun-Arsuz wildlife improvement site (195.77), Yoğunoluk

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(192.51), Yaylıca (189.79), Amanos mountains Musa mountain zone (185.3), Sutaşı (181.61), Keldağ (181.06), Aknehir (159.8), and Çamlıyayla (152.25), respectively.

Conclusion

In the light of the obtained findings, the results that can contribute to identifying the optimum ecotourism route among the studied focuses are as follows:

- The Samandağ coastal zone and its surroundings, selected as the study area, involves many ecotourism areas rich in natural, cultural, religious, and historical heritage. Considering these areas/focuses, which are evaluated individually, collectively in such a way as to form an ecotourism route demonstrates the significance of the study.
- The Samandağ coastal zone and the surrounding ecotourism sites close by have been accepted as ecotourism focuses within the scope of this study and 20 focuses have been identified. The "ecotourism opportunity spectrum" method has been applied in order to analyze these focuses in such a way to determine an optimum ecotourism route.
- By determining the optimum ecotourism route, the purpose was to evaluate the availability of the options that would comprise the route. The highest spectrum value calculated by applying the method was 400. The aim was forming an ecotourism route with focuses at this value.
- However, when the spectrum values of the focuses were analyzed, it was seen that they could not reach the 400 target. While the highest spectrum value belonged to Vakıflı village with 271.86, the lowest spectrum value was 136.92 for Milleyha wetland.
- The spectrum values between 271.86 and 136.92 have been divided into three categories according to their weighted scores. In this categorization the focuses with the highest value were considered to be the most suitable ones for the route. The other two categories involve medium and low value focuses.
- The suitable medium and low value focuses have been analyzed at the results section in terms of criteria determined with the Ecotourism opportunity spectrum method. The analysis is both important for determining the availability of focuses for the ecotourism route according to criteria and the improvement priority of focuses for being used as ecotourism sites. The spectrum value of focuses improved in terms of their lower criteria will rise and thus they will reach the optimum level for ecotourism route.
- The ECOS method used in the study is advantageous in evaluating the availability of the focuses compared to other methods. The results obtained through this method can serve as a basis for studies like ecotourism management plan or site use plan.

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A NOVEL ACTIVATED CARBON INJECTION METHOD TO ENHANCE PCDD/FS REMOVAL EFFICIENCY IN FLUE GAS FROM A MEDICAL WASTE INCINERATOR IN SHANGHAI, CHINA

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Abstract. To reach the emission standard of PCDD/Fs, different influencing factors of removal efficiency of traditional activated carbon injection (ACI) process were considered in this research. Besides, a modified activated carbon injection (ACI) process was developed to improve the removal efficiency of PCDD/Fs. Results showed that removal efficiency of PCDD/Fs using activated carbon A (fitted the dioxin molecular size) was higher than using B (commercially available powder activated carbon) with the same dosage of 1000 mg/m^3. And contact time extended to 8 s was a better choice. Vapor pressures of PCDD/Fs congeners influenced the gas/particle partitioning of PCDD/Fs removal efficiencies. The modified ACI - pulse ACI process demonstrated higher dioxins removal efficiency (~99.6%) than the traditional method due to the increased thickness of carbon layer and total residence time of carbon.
Keywords: *pulse activated carbon injection, dioxins, carbon species, dosage, contact time, vapor pressure*

Introduction

The incineration of medical waste discharges large quantity of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), widely known as dioxins. Dioxins are potentially toxic chemicals with serious health hazard even at extremely low doses. The majority of dioxins tend to travel long distances before falling to earth with rainfall or settling as particle (Singh and Prakash, 2007). Additionally, dioxins are extremely persistent with an estimated half-life of 7-11 years in humans (Schecter et al., 2006). Currently, activated carbon injection (ACI) in combination with bag filter has been found to be an effective alternative to the conventional process for the removal of vapor-phase dioxins at low concentrations in the flue gas from waste incinerator (Abad et al., 2003; Gunes et al., 2015; Chi et al., 2008). However, the low attainment rate of the PCDD/Fs discharge from medical waste incineration flue gas in the whole country indicates that the traditional ACI is far less efficient to meet the national requirement 0.5 TEQ ng/m^3 (MEP, 2001). Especially in Shanghai, the new emission standard of 0.1 TEQ ng/m^3 is much stricter. Thus, there is an urgent need for a more efficient PCDD/Fs removal method for old projects transformation and new projects construction. Besides, the use of the adsorbents is less efficient and may lead to an increase in the total amount of emissions from the incineration plant (flue gas + cyclone ash + reacted ash) (Chang and Lin, 2001). Another issue is the reliability of results on dioxins formation based on laboratory studies, which depends on the use of model compounds (Debecker et al., 2011). Several factors influence the removal efficiency of ACI and bag filter system, including gas/particle partitioning of PCDD/Fs

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(Chang et al., 2002), species and dosage of activated carbon (AC) (Abad et al., 2003; Kim et al., 2007; Tejima et al., 1996), injection rate (Gunes et al., 2015), contact time of activated carbon and PCDD/Fs (Karademir et al., 2004), gas/particle partitioning (Karademir et al., 2004), and injection method among others. Tejima et al. (1996) reported that the removal efficiency of PCDD/Fs could reach over 99% with activated carbon dosage of 50-100 mg/m^3. Similar results were reported by Abad et al. (2003) and Everaert et al. (2003). Gunes et al. (2015) reported that the optimum ACI rate for PCDD/Fs was 66 mg/m^3 with removal efficiency was 97% and no significant difference in efficiency was observed at ACI rate of 197 mg/m^3. Some studies have reported the use of a double-bag activated carbon adsorption process on the basis of the traditional ACI process with a single bag for the removal of PCDD/Fs in a medical waste incinerator system (Kim et al., 2007; Mckay, 2002; Chen et al., 2014; Lin et al., 2008). Though higher removal efficiency was achieved using less amount of activated carbon, the capital and operating costs increased significantly. Li et al. (2008) added a bio-solution (NOE-7F) to the raw materials, which inhibited PCDD/F formation and combining both ACI and NOE-7F had a high potential for practical application. By the addition of NOE-7F in the stack flue gas, the fraction of total PCDD/F was reduced by 92.4%, which was not high enough to meet the EU emission standard. Ooi et al. (2008) reported the removal of vapor phase PCDD/Fs by adsorbing into plastics instead of activated carbon and the removal efficiency was over 99%. The experiments, however, were conducted in an electric arc furnace steel plant. There have been no reports so far about modification of ACI method as applied to medical waste incinerators.

Therefore, this paper introduces the very first modified injection method for PCDD/Fs removal examined in a commercial-scale medical waste incineration facility in Shanghai and evaluates its efficiency. Influences of traditional injection method on PCDD/Fs removal are also examined for comparison, including species and dosage of activated carbon, contact time, and gas/particle partitioning.

Materials and methods

Full-scale medical waste incinerator

The medical waste incinerator researched is located in Shanghai, China. The dealing capacity of the incinerator plant is 2400~2500 metric ton/day and operates 300 days per year. The incinerator has a two-stage combustion system consisting of a rotary kiln of 4.3 m diameter. The temperatures of the rotary kiln head and kiln are 870~890 °C and 1020~1050 °C separately. The original air pollution control system included quench tower, dry-type reactor, traditional ACI, bag filter, and wet scrubbing tower, and the efficiency was 97.5%, which met the Chinese emission standard of dioxins (0.5 TEQ ng/m^3). However, to reach the Shanghai limit (0.1 TEQ ng/m^3), the ACI process needs to be retrofitted. Besides, SO_2 and HCl are captured by powdered $Ca(OH)_2$ injection.

Activated carbon A used in the test fitted the dioxin molecular size (the average relative molecular size of dioxins and furans is 10 angstroms (1 nm)). Activated carbon B is commercially available powder activated carbon. Their characteristics are presented in *Table 1*.

The International Theoretical and Applied Chemistry Federation has defined the pore size as follows: small pores: < 2 nm in diameter, middle pores: 2-5 nm in diameter, large pores: > 50 nm in diameter. Iodine value is an index reflecting the level of iodine adsorption in water, which shows the micro pore volume of activated carbon. Molasses

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(mg) is the number of key indicators to measure the sugar decolorization, and shows the number of middle pores and large pores.

Table 1. *Characteristics of the tested AC*

Species	Activated carbon A (GL50)	Activated carbon B
Iodine value	700	1100
Molasses (mg)	525	200
Surface area (m/g)	800	1225
Bulk density (kg/m)	490	250
Particle size D50 (%)	20	10
Acid-base properties	Alkaline	Alkaline

Description of the Pulse ACI Unit

Activated carbon injection is an important approach to control dioxins in flue gas. Traditional ACI process injects activated carbon continuously for cleaning the flue gas (Abad et al., 2003; Gunes et al., 2015). Since the activated carbon adsorption process is carried out at high temperature and humidity, and at a wide concentration range of dioxins in the flue gas, the equilibrium adsorption capacity and contact time is limited by the thermodynamics. Therefore, to achieve a dioxins removal efficiency of over 99%, a modified ACI technology is needed in addition to using the increased amount of activated carbon and contact time.

The AC injection method was modified in this study and is known as pulse activated carbon injection method. During the bag cleaning cycle, first dose of large amount of activated carbon was injected in a short time and then a small amount of AC was added steadily. The whole process was carried out periodically. Since the diagram of the activated carbon dosage and time represents like a pulse signal, the method is termed as pulse ACI. The improved method does not increase the amount of activated carbon dosage needed. The bag cleaning cycle is one hour. During the bag cleaning cycle, there were two dosage stages. In the first stage, it took 30 s to dose 50% of the total activated carbon dosage. In the next stage, it took the one hour to dose the remaining 50% of the total activated carbon continuously and uniformly. The pulse method is shown in *Figure 1*. The dotted line represents the traditional method while the solid line represents the pulse method. Keeping the total activated carbon dosage same in both methods, the areas of the shadow in the *Figure 1* are equal in one bag cleaning cycle.

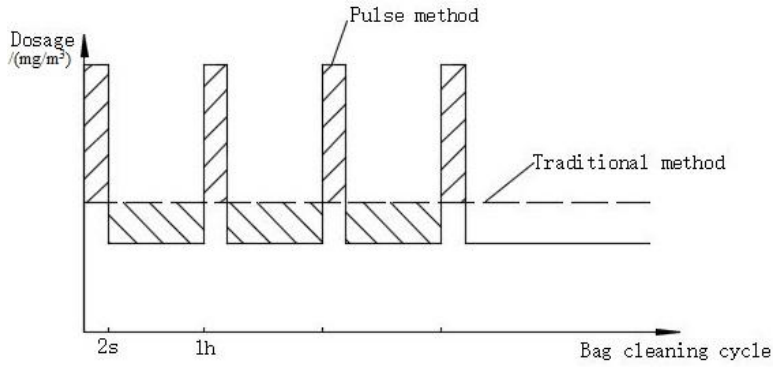


Figure 1. *Pulse injection method of AC in medical waste incinerator, Shanghai, China*

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Sampling and analyses

Flue gas samples were taken from four sampling points and the locations of flue gas sampling points are shown in *Figure 2*. The emission of PCDD/Fs in flue gas was measured simultaneously at four sampling points.

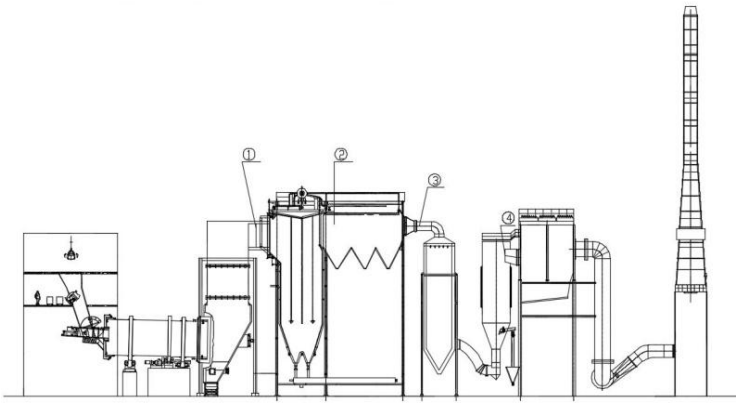


Figure 2. *Sampling points: (1) the post-combustion chamber outlet; (2) the high temperature point in the waste heat boiler (entrance point); (3) the waste heat boiler outlet; (4) the bag filter inlet*

Sampling procedures are as follows. First, the sampling device was installed according to European Standard Protocols EN1948-1 and the air tightness of the sampling system was checked. Then the sampling tube was inserted into the flue sampling point, and the sampling hole was closed. And then the sampling nozzle was aligned with the gas flow direction, and the sampling pump was started to adjust the sampling rate. During the sampling period, when the pressure and temperature changed greatly, it was necessary to adjust the flow rate in time. If the resistance of the filter cylinder increased too large to keep a constant sampling speed, the filter cartridge should be replaced and then continue to sample. In the process of sampling, special attention was paid to keeping the gas phase adsorption column out of light and below 30 °C. After sampling, the sampling pump was stopped and the sampling tube was drawn out at the same time. Besides, time, cumulative flow and other parameters were recorded. At last, the sampling device was removed without light and the cartridge was also removed and wrapped with aluminum foil. The sampling tubes and connecting pipes were washed with toluene. Washing liquid and condensed water were saved in brown reagent bottle.

Main apparatus and equipment used in field sampling (shown in *Fig. 3*) include four sets of quartz cooling, buffer bottle, adsorption bottle, and so on (self-designed); four ice baths with circulating pump sampling box; three sampling device lifting frames; one aneroid barometer; digital temperature and humidity meter (Finland HM34); digital pressure gauges (German MUR DM9100); S-type Pibtot's tubes and hot bulb anemometers.

Analysis was conducted by the Belgian SGS company. The analytical method was US EPA Method 23 (US EPA1995). Identification and quantification of PCDD/Fs were performed by HRGC/HRMS using a 7890 Series gas chromatograph (Agilent, USA) and AutoSpec Premier (Waters).

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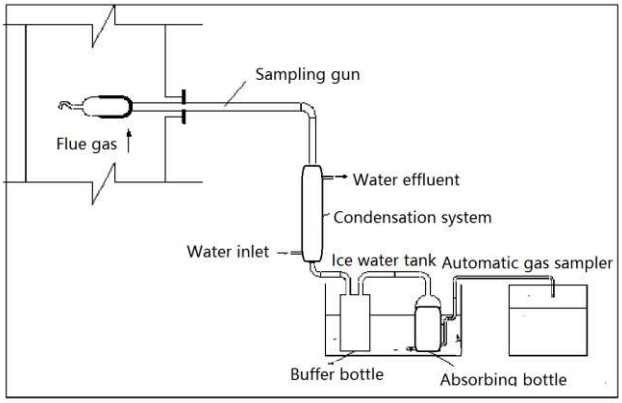


Figure 3. *Flow chart of sampling system*

The results of each selected influencing factors of PCDD/Fs removal efficiency were statistically analyzed. If the factor had significant influence on PCDD/Fs removal efficiency, the probability in the result of variance analysis must be less than 0.05 or at least equal to 0.05 ($P \leq 0.05$).

Results and discussion

PCDD/Fs removal efficiencies of conventional ACI process

Effect of activated carbon types and dosage on PCDD/Fs removal efficiencies

ACI method involves injecting a certain amount of solid adsorbent into the flowing gas before bag filter and then separating the adsorbent before discharging the gas (Karademir et al., 2004). ACI technique transfers dioxins from gas phase to solid phase and further treatment (such as bag filter) is usually required. As discussed above, activated carbon type and dosage are two important factors that affect the removal efficiency of dioxins. These two factors were examined in this study. Both activated carbon A and activated carbon B were added at the entrance of the bag to study the effect of activated carbon types on dioxins removal. The dosages of activated carbon A and B were 100 mg/m^3 and 1000 mg/m^3. The results are summarized in *Table 2*.

It can be seen from *Table 2* that ACI technology showed high removal efficiency for PCDD/Fs. ACI system and bag filter are used to remove the gas phase and particle phase PCDD/Fs, respectively (Gunes et al., 2015). Since PCDD/Fs compounds in gas phase comprised of the majority of the pollutants tested, adsorption of gas phase compounds on the AC and then removal of AC and compounds in particle phase on filter was proved to be an effective way of removal of total PCDD/Fs in flue gas instead of using bag filter alone.

The addition of same dosage (i.e., 100 mg/m^3) of activated carbon, the average removal efficiency of activated carbon A was much higher (92%) than that of activated carbon B (82%). Abad et al. (2003) reported 92-96% PCDD/Fs removal efficiency at activated carbon dosage of 100 mg/m^3. Everaert et al. (2003) also found the removal efficiency of 93-94% at activated carbon dosage of 50-100 mg/m^3 in ACI. However, the results showed that the PCDD/Fs removal efficiency at this dosage is still very low to meet the strict emission requirements of 0.1 TEQ ng/m^3.

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Table 2. *Removal efficiencies of PCDD/F congeners by different AC species and dosage*

Congener	Removal efficiency (%)			
	A 100 mg/m^3	**A 1000 mg/m^3**	**B 100 mg/m^3**	**B 1000 mg/m^3**
2378-TCDF	93.7	97.5	88.8	96.6
12378-PeCDF	95.2	96.3	84.8	94.9
23478-PeCDF	92.5	96.0	86.4	92.5
123478-HxCDF	95.5	96.0	87.1	94.2
123678-HxCDF	95.9	96.5	87.7	91.6
234678-HxCDF	93.2	95.8	82.4	92.7
123789-HxCDF	93.4	93.8	87.5	89.1
1234678-HpCDF	91.6	96.3	88.1	90.7
1234789-HpCDF	90.6	95.8	81.7	90.1
OCDF	85.5	96.1	82.6	87.1
2378-TCDD	85.1	94.2	81.2	79.2
12378-PeCDD	93.0	94.6	89.0	91.8
123478-HxCDD	94.1	95.7	86.3	90.8
123678-HxCDD	94.1	96.3	89.4	90.5
123789-HxCDD	92.6	95.3	87.1	89.1
1234678-HpCDD	90.1	97.1	87.8	89.7
OCDD	84.2	97.9	85.6	88.2
PCDD/Fs	89.5	96.0	86.6	92.1

Single factor analysis of the data of these two types of AC had P-value of 1.22×10^{-5} (< 0.05), indicating that AC types has a significant effect on the PCDD/Fs removal efficiency. Since activated carbon A has higher specific surface area and pore size, which is ideal for the removal of dioxins, it has higher adsorption capacity and removal efficiency.

Higher dosage of activated carbon was needed to achieve higher removal efficiency. The dosages of the activated carbon A and B were both increased from 100 to 1000 mg/m^3. The single factor analysis of the data for activated carbons A and B had P-values of 2.15×10^{-5} and 0.000347, respectively, which are far below 0.05, indicating that the dosage also has a significant effect on the removal efficiency. For both types of activated carbon, removal efficiency improved with increasing the dosage. However, the increase in efficiency was not obvious. For activated carbon A, removal efficiency of low and high chlorine content congeners increased more compared to medium chlorine content congeners. For activated carbon B, the efficiency showed variability with the increase of the chlorine atom number. For the congeners with same number of chlorine atoms, the efficiency differed with the position of chlorine atom substituted and differed from dioxins to furans, especially 2,3,7,8-TCDD and 2,3,7,8-TCDF, which showed significant difference. Although activated carbon A at 1000 mg/m^3 had the highest removal efficiency for all congeners, the advantage was not significant; likely due to saturation of its adsorption capacity. Since the injection point of activated carbon at the batch experiment was at the inlet of the bag filter, the contact time of the flue gas and activated carbon was limited, which could have resulted overall low removal efficiency (~96%).

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Since the flue gas generation rate from medical waste incinerator is significantly less than that from large domestic waste incinerator, the dosage of the activated carbon A was increased to 1000 mg/m^3 which resulted in increase in the removal efficiency of PCDD/Fs from 90 to 96%. However, the increase of PCDD/Fs removal efficiency was not always consistent with the increased dosage of activated carbon. Everaert et al. (2003) reported no significant change in removal efficiency at injection rates over 200 mg/m^3. Chang et al. (2001) expressed that the higher AC injection would not significantly increase the dioxin removal efficiency when the injected AC concentration was over 50 mg/Nm^3 and a large amount of activated carbon would lead to an increase in total dioxin emissions (flue gas + cyclone ash + reacted ash). Gunes et al. (2015) also reported no significant difference in removal efficiency at 197 mg/m^3 and 66 mg/m^3 AC injection rate, and the efficiency decreased at 332 mg/m^3 of AC injection rate. Although the efficiency greatly improved at 1000 mg/m^3 of AC dosage, it still failed to consistently meet the EU 2000 guidelines for dioxin emission standards. Considering the dosage of 1000 mg/m^3 was extremely high, no further test was conducted at higher dosage of activated carbon from the thermodynamics stand point. Similarly, increasing the dosage of activated carbon B by 10-folds could only reduce the dioxin emissions by about 50% (from 0.15 to 0.08 TEQ ng/m^3). No further significant improvement was achieved at higher dosage of activated carbon injection. Thus, there is a need to explore advanced technology to improve the removal efficiency of the flue gas contaminants.

Effect of contact time on PCDD/Fs removal efficiencies

Contact time or residence time, is very important factor affecting adsorption of organic compounds on activated carbon (Karademir et al., 2004). However, only very limited studies has explored the effect of contact time on adsorption of organic compounds on activated carbon at full-scale medical waste incinerator. Since the concentration of dioxins in flue gas is at TEQ ng/m^3 level, the driving force for the mass transfer from flue gas to activated carbon is relatively small compared to emissions of other pollutants at significantly high concentrations. In order to improve the mass transfer rate, a reasonable increase in contact time of activated carbon and flue gas becomes an important factor to increase the adsorption rate and efficiency. The previous injection point was at the entrance of the bag filtration and the average contact time was less than 2 s. After modification of the injection point to the dry fluidized bed, the average contact time was more than 8 s. The removal efficiency was compared under the same injection and burning conditions as shown in *Figure 4*. The injected activated carbon types and dosage were specialized ones with 1000 mg/m^3.

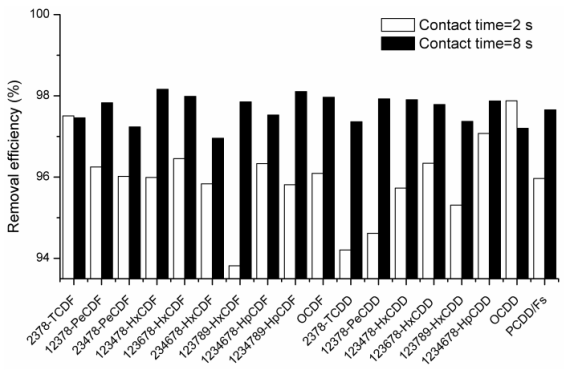


Figure 4. *Removal efficiencies of PCDD/F congeners through different contact time*

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Figure 4 shows that removal efficiency of PCDD/Fs improved from 96 to 98%. The removal efficiency of 17 congeners except 2,3,7,8-TCDF and OCDD increased. The highest increase in removal efficiency was from 93.8 to 97.9% for 1,2,3,7,8,9-HxCDF and the lowest increase was from 97.1 to 97.9% for 1,2,3,4,6,7,8-HpCDD. Although the increase in contact time improved the removal efficiency, the removal efficiency was still below the emission standard. Thus, modified ACI process was examined to improve the efficiency as discussed in the following section.

Effect of activated carbon on the removal of dioxins in the gas and solid phases

The distribution of dioxins in gas and solid phases is a very important factor for dioxins removal. The effect of activated carbon on the removal of dioxins in gas phase and solid phase was studied in order to further study the improvement of the active carbon injection method. The injection condition was activated carbon A dosage of 1000 mg/m^3 at the dry fluidized bed and the burning condition was discussed in section 2.1. The results are presented in *Figure 5*.

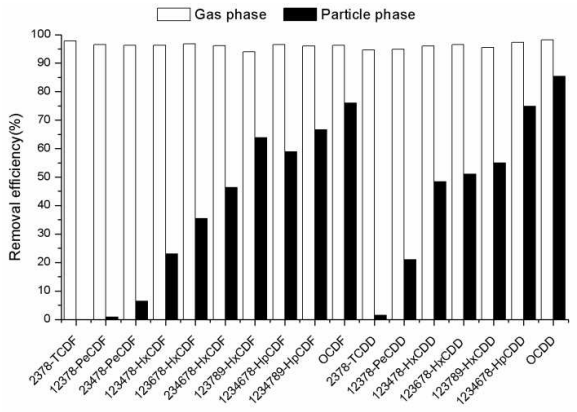


Figure 5. *Removal efficiencies of PCDD/F congeners for gas and particle phase*

Activated carbon has high removal efficiency of dioxins in the flue gas. The removal efficiency of PCDD/Fs in flue gas was 97.1%. But the removal efficiency was much lower for the particle state (~75.5%), indicating that ACI was more effective to treat dioxins in gas phase. The removal efficiencies of particulate dioxins and PCDD/Fs with higher molecular weight were higher than that with lower molecular weight. The increase in removal efficiency showed a linear trend with increase in molecular weight, which indicates that part of PCDD/Fs with low molecular weight was in the nanometer aerosol state leading to low efficiency of traditional ACI process. However, considering that the proportion of dioxins in the gas phase was more than 98%, gaseous dioxins were the main target of the dioxin emission control.

From other aspect, the removal efficiencies of both low chlorinated and high chlorinated congeners in gas phase were high in PCDD and PCDF, while the efficiencies of congeners in particle phase increased with the increase of chlorine content of both PCDD and PCDF. This is mainly attributed to different vapor pressures of congeners and adsorption capacity of activated carbon for dioxins in gas and solid phases (Chang et al., 2002). Less chlorinated congeners have higher vapor pressure and tend to exit in gas phase, which are more likely to be adsorbed by activated carbon

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(Gunes et al., 2015). On the other hand, higher chlorinated congeners tend to exist in solid phase due to their lower vapor pressures. Hence, the removal efficiency of less chlorinated congeners in gas phase is higher than that in the solid phase, while the removal efficiencies of higher chlorinated congeners show the opposite trend. With the increase in chlorine content, the removal efficiencies of congeners in particle phase increased because more congeners tend to exist in particle phase. *Figure 6* shows the average efficiencies of the three tests for PCDD/F homologues and their relationship with the vapor pressure (log P) at 25 °C (Li et al., 2008).

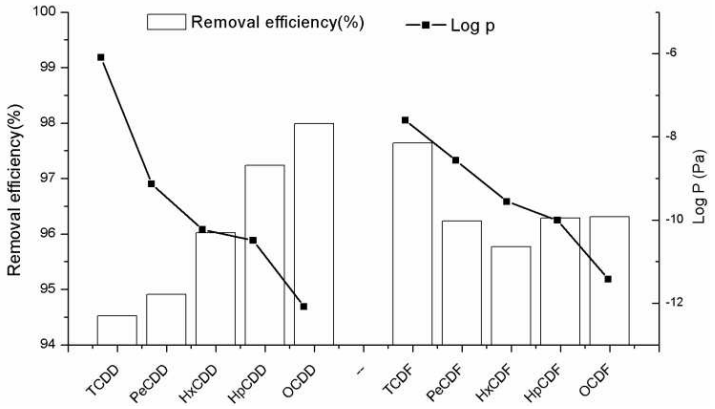


Figure 6. *Average removal efficiencies of PCDD/F homologues*

Figure 6 shows that as chlorination level of PCDD homologue increased, removal efficiencies increased while vapor pressure declined. For PCDF, both removal efficiencies and vapor pressure declined with increasing chlorination. The results of PCDF removal were in consistent with other studies (Chang et al., 2002; Karademir et al., 2004). The PCDD removals, however, showed the opposite trend with that of the above literature. Further studies are needed to identify the discrepancy.

The traditional ACI tests showed that the highest removal efficiency was 97.7% with 1000 mg/m^3 of activated carbon A injected in the dry fluidized bed (contact time is 2 s). In practical projects, activated carbon dosage is far less than 1000 mg/m^3 because of the reasons mentioned above, so the highest removal efficiency was 96%. When the TEQ concentration of PCDD/Fs in the flue gas was higher than 5 TEQ ng/m^3 (the lowest emission concentration), the emission standard of 0.1 TEQ ng/m^3 could not be achieved through the traditional ACI process. Therefore, to achieve higher removal efficiency, activated carbon injection method needs to be modified besides increasing the amount of activated carbon, selecting an appropriate activated carbon, and increasing contact time.

PCDD/Fs removal efficiencies of pulse ACI process

The two methods, namely pulse ACI and continuous ACI, were tested under the same conditions with the same activated carbon dosage to evaluate their efficacies in PCDD/Fs removal. The results are shown in *Figure 7*.

The removal efficiency of total dioxins increased from 96.9 to 99.7%, and the emissions were reduced by more than 90% (from 49.62 TEQ ng/m^3 to 1.56 TEQ ng/m^3).

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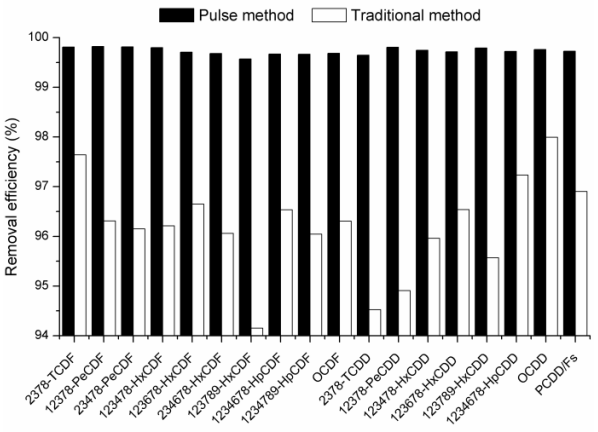


Figure 7. *Removal efficiencies of PCDD/F congeners by traditional and pulse method*

From *Figure 7* it can be seen that for the 17 PCDD/F congeners, PCDD/Fs removal efficiency of pulse ACI method was obviously higher than that of continuous ACI method. The results clearly show that the removal efficiency of dioxins by pulse method was higher than that by continuous method, especially for middle chlorine (4-6) compounds.

The average removal efficiencies of PCDF and PCDD with continuous stable method were nearly same (e.g., 96.2% and 96.1%, respectively). The removal efficiencies of both PCDF and PCDD with pulse method were much higher at 99.7%, indicating that modified method was effective for both PCDD and PCDF removal. Removal efficiencies of 17 congeners with pulse method were very consistent while the efficiencies of continuous stable method varied greatly from PCDF to PCDD and with the chlorine content. Since congener profiles and formation mechanism of PCDD/Fs in flue gas are influenced significantly by chlorine content (Wang et al., 2003) and temperature (Mckay, 2002), and high emission of MWIs (medical waste incinerators) may result from non-continuous operations, irregular waste feeding (Choi et al., 2008), and poor operating conditions (WHO, 2011), it is often difficult to maintain stable emission. Hence, the pulse method, which provides relatively stable removal efficiency, has broad application prospects.

As discussed earlier, activated carbon mainly adsorbs dioxins in the gas phase. Taking into account that dioxins and activated carbon are basically maintained at the same flow rate for contact during the continuing activated carbon adsorption process and since the movement between gas and the solid is relatively small and the gas film on the surface of activated carbon is thick, the mass transfer process mainly relies on molecular diffusion. Under such circumstance, the adsorption rate in gas phase is low and thus the utilization of activated carbon. Another drawback is that the activated carbon contained pollutants is enriched on the surface of the bag, increasing the possibility of partial overheating and further release of the pollutants to the flue gas.

Large amount of activated carbon are injected in pulse ACI process in a short time in order to form activated carbon layers on the surface of the bag quickly and then reduce the injection rate, while in continuous ACI process, activated carbon injection rate is constant. The surface of the bag type dust collector also acts as a fixed bed when in contact with flue gas. These two types of activated carbon processes were combined in one system thereby saving the space. Due to different gas and solid velocities, turbulent

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flow occurs in the gas phase thereby reducing the thickness of gas film around the surface of the activated carbon. This results in an improvement in mass transfer efficiency. Moreover, the length of activated carbon layers exceeds the length of the mass transfer zone that prevents penetration at the beginning and the reduction in efficiency. Thus, the inner layer of the activated carbon layer remains relatively cleaned for further purification of the gas through the bag to avoid gaseous phase PCDD/Fs release from the contaminated filters of the aged bag filters because of memory effect (Li et al., 2011).

Studies are being currently conducted focusing on reducing the time of second period which is continuing the PAC adsorption process, in order to reduce the total amount of injected carbon.

Conclusions

In this study, different influencing factors of traditional ACI method removal efficiency and a developed ACI technology in a medical waste incineration plant were investigated.

Vapor pressures of PCDD/Fs congeners influenced the gas/particle partitioning of PCDD/Fs and dioxin removal efficiencies. The vapor pressure of PCDD/Fs congeners decreases with increasing chlorination and ACI was more effective to remove dioxins in gas phase than in the solid phase.

The removal efficiency could be improved by selecting appropriate adsorption activated carbon species, increasing the dosage of activated carbon, and increasing the gas solid contact time. Activated carbon A was better than B with dosage of 1000 mg/m^3 at a contact time exceeding 8 s.

At the current optimal conditions using the traditional continuously stable activated carbon injection method, average removal efficiency was not high enough to meet the flue gas dioxin emission standards of 0.5 TEQ ng/m^3. Thus, the EU dioxin emission limits in waste incineration of 0.1 TEQ ng/m^3 is very unlikely to be achieved. The pulse activated carbon injection (Pulse ACI) method developed in this study has shown that higher dioxin removal efficiency of 99.7% than the current traditional method could be obtained, making it possible to achieve the dioxin emission limit of 0.1 TEQ ng/m^3.

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THE EFFECTS OF SOIL CONDITIONERS ON TOTAL PROTEIN AND CRUDE FIBER CONCENTRATION IN SELECTED GRASS SPECIES

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Abstract. The aim of this study was to determine the influence of biological preparations and mineral fertilizers on total protein and crude fiber in *Dactylis glomerata* and *Lolium perenne*. The study was carried out in years 2011-2014 in three repetitions, on 3 m^2 experimental plots. There were mineral, biological or both mineral and biological fertilization applied on the grass. Mineral fertilization included nitrogen, phosphorus, potassium. There were used three biological preparations named UGmax, Humus Active Papka and Eko-Użyźniacz in the studies. For both grass species Eko-Użyźniacz applied together with mineral fertilizers increased protein content in fodder. Application of Humus Active Papka with mineral fertilizers lowered crude fiber concentration in fodder. The highest concentration of total protein was in *Dactylis glomerata* and *Lolium perenne* from the plots where soil conditioners together with mineral fertilizers were applied. The most advantageous concentration of crude fiber was in *Dactylis glomerata* growing on the plot with Humus Active Papka together with mineral fertilizers applied, while in *Lolium perenne* on the plot with Eko-Użyźniacz applied, without mineral fertilizers.
Keywords: *Dactylis glomerata, Lolium perenne, biopreparate, soil amendment, soil fertilizer, grass*

Introduction

The vegetation of pastures and meadows consists mainly of grass, so important in ruminant nutrition, with *Dactylis glomerata* being one of the most nutritional species there (Stewart and Ellison, 2011; Billman et al., 2017; Jones et al., 2017). It contains a high amount of total protein, but a lot of substances, like crude fiber, lower its digestibility (Ellis et al., 2011; Tilvikiene et al., 2014; Cullen, 2017). Another popular grass species in permanent grasslands is *Lolium perenne* but, contrary to *Dactylis glomerata*, its total protein content is lower even if it is more digestible because of low fiber content (Turner, 2006a; Xiong et al., 2006; Robins and Lovatt, 2016,). According to many publications total protein content in fodder from permanent grasslands in Poland is diversified (Heinsoo et al., 2010; Jankowska-Huflejt and Wróbel, 2010; French, 2017). This content varies dependent on the species, its variety, the amount of nutrients in the soil (mainly nitrogen), the growing stage of the plant, the weather, and the grass cutting (Walter et al., 2012; Grant et al., 2014; Zielińska et al., 2014; Loaiza, 2017). Minimum content of total protein should range from 150 to 170 g kg^{-1}. This amount of protein is indispensable for proper absorption of nutrients in the digestive system of dairy cows (Peeters, 2008; Grygierzec, 2012). Crude fiber has a big impact on nutritional value of the feed and animal health, and its concentration is dependent on the species, the variety of a plant, its age, the cutting, the fertilizers used, and precipitation

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(Mertens, 2002; Pozdisek et al., 2003; Arzani et al., 2004; Jankowska-Huflejt and Wróbel, 2010). For a balanced level of nutrient digestibility and energy value of feeds, the concentration of fiber should not exceed 28 - 30% DM (Bumane, 2010).

Now there is a growing interest in organic farming and biological products such as fertilizers, soil conditioners, and similar substances approved by the Institute of Soil Science and Plant Cultivation (IUNG) in Puławy. This growing interest in biological products enhancing quality and quantity of crops is reflected in many publications (Sosnowski, 2012; Sosnowski et al., 2014; Sosnowski and Jankowski, 2015; Truba et al., 2017). However, not many studies are concerned with the effects of those products on the content of total protein and crude fiber in forage grass.

Considering the above, the aim of this paper is to study the effects of some soil conditioners on the content of total protein and crude fiber in *Dactylis glomerata* and *Lolium perenne*.

Materials and methods

Characteristic of field site

A randomised experiment in blocks replicated three times was set up in the experimental station of the Department of Grassland and Green Areas Creation of the University of Natural Sciences and Humanities in Siedlce in Poland (52.169°N, 22.280°E). According to the Polish soil classification system (Systematics of Polish Soils, 2011), the soil used in the experiment was the culture soils with the hortisole type, developed from loamy sand (*Table 1*).

Table 1. *Soil granulometric composition*

Percentage share of soil fractions (diameter in mm)			
2.0-0.05	0.05-0.002	>0.002	Granulometric group
85	13	2	ps

Based on the analysis performed at the Regional Chemical Station in Wesoła, it was found that the soil was of neutral pH (pH in 1n KCL = 6.8), with a high content of humus (3.78%), available phosphorus ($H_2PO_4^-$ - 170 mg kg^{-1}), magnesium (Mg^{2+} - 84 mg kg^{-1}), and moderate content of total nitrogen (N - 1.3 g kg^{-1}) and available potassium (K^+ - 114 mg kg^{-1}).

Characteristic of preparations used in experiment

The aim of the experiment was to compare soil conditioners of the following trade names: UGmax, Eko-Użyźniacz, and Humus Active Papka, used separately and supplemented with NPK fertilizers. In the experiment, there were the following experimental units:

1. control (with no fertilizers),
2. UG (UGmax soil conditioner),
3. EU (Eko-Użyźniacz soil conditioner),
4. HA (Humus Active Papka soil conditioner),
5. NPK,
6. UG + NPK,

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7. EU + NPK,
8. HA + NPK.

The UGmax soil conditioner is an extract from compost, containing macronutrients (N, P, K, Mg, Na) and micronutrients (Mn). It also contains lactic acid bacteria, photosynthetic bacteria, *Azotobacter*, *Pseudomonas*, yeast, and *Actinomycetes* (*Table 2*). The microorganisms in the conditioner have a capacity of processing organic and natural fertilizers into compost and humus. These processes are conducive not only to the production of humus but also to improving soil structure, which in turn has a positive effect on water balance in the soil. In addition, the UGmax soil conditioner increases disease resistance, keeping plants healthy, but it also stimulates the development of the root system and supports the biological reduction of molecular nitrogen.

Humus Active Papka contains macronutrients (N, P, K, Ca, Mg), trace elements (Mn, Fe, Zn, Cu), and active humus with useful microorganisms (*Table 2*). According to the manufacturer Humus Active Papka, among other beneficial effects, positively affects plant health as well as soil structure, and releases nutrients not readily available to plants.

Eko-Użyźniacz is extracted from bovine vermicompost and contains the main macronutrients (N, P, K), microorganisms, and enzymes related to metabolism of earthworms (*Table 2*). This soil conditioner stimulates biological life in soil degraded chemically, mechanically, or biologically, and increases plant resistance to different stress factors.

Table 2. *Ingredients of the soil conditioners*

Soil conditioner	Macronutrients (g kg^{-1})						Micronutrients (mg kg^{-1})					Microorganism and others
	N	P	K	Ca	Mg	Na	Mn	Fe	Zn	Cu	Mo	
Humus Astive Papka (HA)	0.2	1.3	4.6	3.0	0.5	-	15	500	3	1	-	Active humus with useful microorganisms
Eko-Użyźniacz (EU)	0.6	0.3	0.7	-	-	-	-	-	-	-	-	Endo micorhizza, fungi, bacteria, enzymes of earthworms
UGmax (UG)	1.2	0.2	2.9	-	0.1	0.2	0.3	-	-	-	-	Lactic acid bacteria, photosynthetic bacteria, *Azotobacter, Pseudomonas*, yeast, *Actinomycetes*

The above biological soil conditioning products were applied in the experiment every year in spring at the following doses: UGmax - 0.6 L ha^{-1}, Eko-Użyźniacz - 15 L ha^{-1}, and Humus Active Papka - 50 L ha^{-1}.

Mineral fertilizers with nitrogen, phosphorus, and potassium (NPK) were applied at the following doses: N – 150, P (P_2O_5) – 80, and K (K_2O) – 120 kg ha^{-1}. Mineral nitrogen was supplied in the form of ammonium nitrate (NH_4NO_3), phosphorus in the form of granulated triple superphosphate ($Ca(H_2PO_4)_2$), and potassium as high

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percentage potassium salt (KCl). Phosphorus was applied once a year in spring, while potassium and nitrogen were used in three doses, before the growing season and before the second and third cuttings.

Plant material and analysis

The above products were tested on two grass species: *Dactylis glomerata* of the Bora variety and *Lolium perenne* of the Info variety, sown in the autumn of 2011 according to the standards of 18 and 23 kg ha^{-1}, respectively. The area of the experimental plot was 3 m^2.

During the three-year experiment in each growing season the grass was harvested three times with dry matter yield determined and chemical analyses carried out. Chemical tests for all the cuttings of the last three years of the experiment were done at the Institute of Technology and Life Sciences in Falenty using near infrared spectroscopy (NIRS) with the Infra Analyzer 450. First of all, the content of total protein and crude fiber was determined.

Weather conditions

Meteorological data for the years of research were obtained from the Hydrological and Meteorological Station in Siedlce. Sielianinov's hydrometric coefficient (K) was worked out to determine variability of weather conditions and their impact on plant vegetation (Skowera and Puła, 2004).

In the first year (2012) optimal weather conditions, with regard to temperature and rainfall, were only in June and October (*Table 3, Fig. 1*). In the rest of the growing season, there was not enough rain, with periods of drought and severe drought. In the next years, dry periods were followed by wet periods, with optimal conditions only in April of 2013 and 2014 but also in July of 2014. In each year of the experiment, the best weather conditions were in the first part of the growing season.

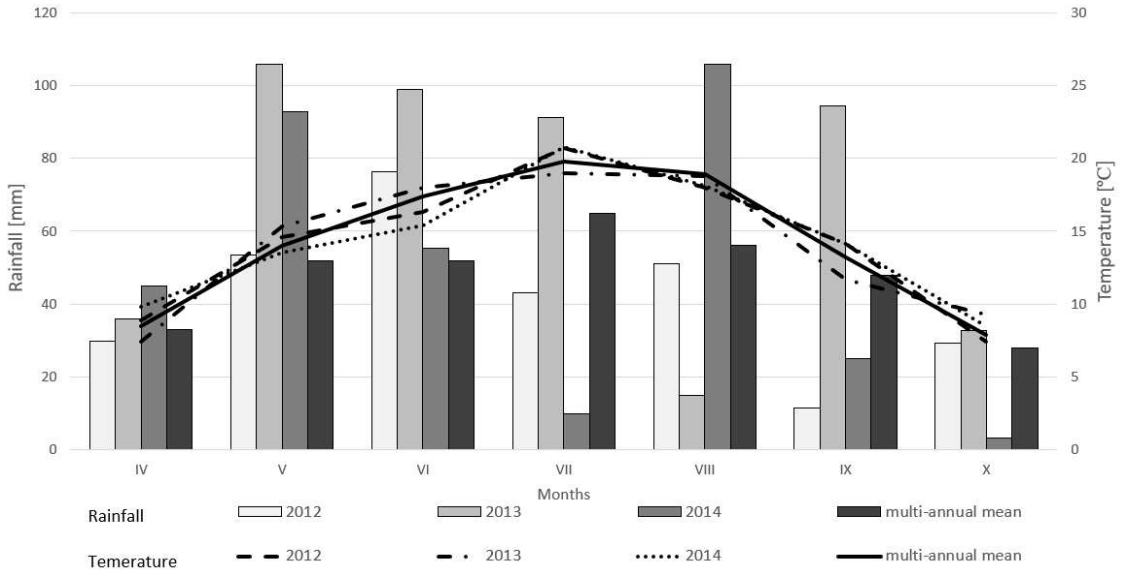


Figure 1. *Average of air temperature and sum of atmospheric precipitation in the months of the growing seasons during the research*

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Table 3. *Sielianinov's hydrometric coefficient (K) values during the growing season*

Years	Months						
	IV	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
2012	1.12 (md)	1.22 (md)	1.56 (o)	0.69 (sd)	0.94 (d)	0.27 (ed)	1.32 (o)
2013	1.60 (o)	2.20 (w)	1.80 (mw)	1.50 (o)	0.25 (ed)	2.70 (sw)	1.22 (md)
2014	1.53 (o)	2.29 (w)	1.20 (md)	0.16 (ed)	1.95 (mw)	0.59 (sd)	0.13 (ed)

$K \leq 0.4$ extreme drought (ed); $0.4 < K \leq 0.7$ severe drought (sd); $0.7 < K \leq 1.0$ drought (d); $1.0 < K \leq 1.3$ moderate drought (md); $1.3 < K \leq 1.6$ optimal (o); $1.6 < K \leq 2.0$ moderately wet (mw); $2.0 < K \leq 2.5$ wet (w); $2.5 < K \leq 3.0$ severely wet (sw); $K > 3.0$ extremely wet (ew)

Statistics analysis

The collected data were analysed statistically using the Statistica 12.0. The mean arithmetic were calculated. Effects of tested factors (grass species, preparations used in experiment, year or cutting) on the content of crude fiber and total protein in plant were estimated using three-way analysis of variance. Detailed comparisons of the mean values were based on Tuckey's test at $p \leq 0.05$.

Results and discussion

Total protein content

Total protein content in the grass species tested in the experiment (*Tables 4* and *5*) varied depending on the fertilizers used, the year of the experiment and the cutting. Generally, no matter what grass species it was, the highest total protein content (*Table 4*) was in experimental variants where bio products together with mineral fertilizers were applied. The highest content of total protein (158.6 g kg^{-1}) was found in the dry matter with Eko-Użyźniacz together with NPK applied. The lowest content of total protein was in grass where the bio product UGmax (127.6 g kg^{-1}) was applied. Low content of total protein could be caused by a lack of nitrogen fertilization or by a late harvest. A lot of publications (Valk et al., 2000; Johnson et al., 2001; Tas et al., 2006; Grant et al., 2014) hold that total protein concentration does not always rise with fertilizer doses. Walter (2012) points out that it may be caused by dilution of total protein concentration in the high amount of yield.

Comparing all years of the experiment (*Table 4*) it was found that no matter what the combination of fertilizers and conditioners was, the highest amount of total protein in dry matter (151.0 g kg^{-1}) was found in the second year (2013) of the experiment, with the lowest (141.9 g kg^{-1}) in the first year (2012) of the experiment, but these differences were not statistically significant. Differences in total protein concentration were caused through the various weather conditions. In the first year of the research objects contained less total protein, in second year compound content increased when time of the regrowth was moderated by the weather. In the last year in growing season occurred a lot of dry periods, therefore protein content decreased.

In the study, there were no significant differences in total protein content between *Dactylis glomerata* and *Lolium perenne* (*Table 5*). According to Turner et al. (2006b) nitrophilous grass species like *Dactylis glomerata*, which contains 212 g of total protein in 1 kg DM, accumulate the highest amounts of this nutrient. A little less protein is produced by *Lolium perenne,* with 129 g in 1 kg DM.

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Table 4. *The effect of fertilizer and the year of the experiment on total protein concentration (g kg^{-1}) in dry matter of the grass species*

Species	Year	Fertilizer								Mean
		0	NPK	UG	EU	HA	UG+NPK	EU+NPK	HA+NPK	
Dactylis glomerata	2012	121.0	140.6	122.9	137.5	147.3	157.9	147.9	156.3	141.4
	2013	143.8	141.3	134.2	133.8	137.8	148.6	153.7	159.7	144.1
	2014	149.1	141.9	144.1	155.9	159.4	159.1	161.6	150.9	152.8
Lolium perenne	2012	126.0	142.3	109.2	137.8	132.7	153.7	174.8	161.4	142.2
	2013	141.0	159.5	140.0	161.6	165.8	151.7	178.1	165.9	158.0
	2014	155.0	127.6	114.8	138.9	145.5	168.7	135.2	144.5	141.3
Mean effect of fertilizer		139.4	142.2	127.6	144.3	148.1	156.6	158.6	156.5	
Mean effect of year										
2012		123.5	141.4	116.1	137.7	140.0	155.8	161.4	158.9	141.9
2013		142.4	150.4	137.1	147.7	151.8	150.1	165.9	162.8	151.0
2014		152.1	134.8	129.5	147.4	152.5	163.9	148.4	147.7	147.0

$HSD_{0.05}$ for: fertilizer = 21.9; year = insignificant; fertilizer/year = insignificant; species/year = 11.0; species/year/fertilizer = 44.3

Table 5. *The effect of the fertilizer and the cutting on total protein concentration in the dry matter (g kg^{-1}) of grass species (average of the years)*

Species	Cutting	Fertilizer								Mean
		0	NPK	UG	EU	HA	UG+NPK	EU+NPK	HA+NPK	
Dactylis glomerata	I	127.7	124.0	114.2	141.0	130.6	143.9	153.9	132.6	133.5
	II	134.0	142.0	142.7	142.3	156.0	163.7	153.3	167.9	150.2
	III	152.3	157.7	144.4	144.0	157.9	157.9	155.9	166.5	154.6
Lolium perenne	I	129.6	125.8	108.3	124.3	143.3	148.7	143.3	160.5	135.5
	II	125.1	128.1	115.6	142.5	140.2	151.6	169.7	140.5	139.2
	III	167.3	175.5	140.2	171.5	160.4	173.7	175.1	170.8	166.8
Mean effect of species										
Dactylis glomerata		138.0	141.2	133.8	142.4	148.2	155.2	154.4	155.7	146.1
Lolium perenne		140.7	143.1	121.3	146.1	148.0	158.0	162.7	157.3	147.2
Mean effect of cutting										
I		128.6	124.9	111.2	132.6	137.0	146.3	148.6	146.6	134.5
II		129.5	135.0	129.1	142.4	148.1	157.7	161.5	154.2	144.7
III		159.8	166.6	142.3	157.7	159.2	165.8	165.5	168.6	160.7

$HSD_{0.05}$ for: species = insignificant; cutting = 8.7; fertilizer/species = insignificant; fertilizer/cutting = insignificant; species/cutting = 10.8; species/cutting/fertilizer = 39.8

As a result of application of mineral fertilization and type of soil conditioner together, was observed increase in total protein content in the both species of grass (*Table 5*).

Concerning total protein content in individual cuttings (*Table 5*), was significantly differentiated. The grass of the third cutting contained the highest concentration of

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protein (160.7 g kg^{-1}), while the lowest was in the grass of the first cutting (134.5 g kg^{-1}). The difference between following cutting was approximately 7 to 11%. Despite of dry period before third cutting, content of total protein in grass was high. It could be caused through the concentration of total protein in mass of grass. Pontes et al. (2007) come up with different results when protein concentration in the biomass of *Dactylis glomerata* and *Lolium perenne* was the highest in the second and third cutting and was the lowest in the first cutting (*Table 5*).

Crude fiber content

The concentration of crude fiber was not significantly varied depending on the dose of fertilizers (*Table 6*). However, in data was observed decrease of crude fiber content in the Humus Active Papka plot and Humus Active with mineral fertilization plot approximately 4 to 6% relative to the control object. On the others experimental objects crude fiber content was similar to the control plot.

Comparing experimental years (*Table 6*) it was found that no matter what fertilizers were used and which species of grass was tested, the highest concentration of crude fiber (230.9 g kg^{-1}) was in the grass of the last year (2014) and the lowest (212.1 g kg^{-1}) in the second year (2013) of the experiment. There was a statistically significant difference between crude fiber concentrations in each year. Variable content of crude fiber in the years 2012-2014 was caused through the difference in weather conditions, where in the last one year dry period could caused significant increase of this factor.

Table 6. *The effect of the fertilizer and the experimental year on crude fiber concentration (g kg^{-1}) of grass species*

Species	Year	Fertilizer								Mean
		0	NPK	UG	EU	HA	UG+NPK	EU+NPK	HA+NPK	
Dactylis glomerata	2012	261.4	250.7	265.4	250.6	227.5	231.1	257.7	227.5	246.5
	2013	223.6	226.5	251.8	233.4	245.1	248.6	221.9	207.3	232.3
	2014	249.3	243.1	243.1	238.4	222.2	231.7	236.7	244.4	238.6
Lolium perenne	2012	208.3	217.3	189.6	152.8	192.6	175.3	189.0	184.8	188.7
	2013	171.5	201.2	211.6	183.9	192.0	202.0	194.8	177.2	191.8
	2014	213.8	260.1	269.5	195.7	195.8	210.1	233.3	207.3	223.2
Mean effect of fertilizer		221.4	233.2	238.5	209.2	212.6	216.5	222.3	208.1	
Mean effect of year										
2012		234.9	234.0	227.5	201.7	210.1	203.2	223.4	206.1	217.6
2013		197.6	213.9	231.7	208.7	218.6	225.3	208.4	192.3	212.1
2014		231.6	251.6	256.3	217.0	209.0	220.9	235.0	225.9	230.9

$HSD_{0.05}$ for: fertilizer = insignificant; year = 15.5; fertilizer/year = insignificant; species/year = 14.1; species/year/fertilizer = 57.9

The study showed significant interaction between such variants as fertilizers, grass species and experimental years. Taking into account both different fertilizers and experimental years (*Table 6*) it was found that the highest concentration of crude fiber was in *Dactylis glomerata* (265.4 g kg^{-1}) in the first year (2012) with the UGmax soil

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conditioner applied, and in *Lolium perenne* the highest concentration (269.5 g kg^{-1}) was also in the plot with the UGmax but in the last year (2014) of the experiment.

Comparing both grass species (*Table 7*) it was found that no matter what fertilizers were applied *Dactylis glomerata* had a higher concentration of fiber than *Lolium perenne* (239.1 g kg^{-1} and 201.3 g kg^{-1}, respectively). The difference between crude fiber concentrations was statistically significant. Tendency to accumulate of crude fiber in *Dactylis glomerata* species is widely documented in the literature (Ates and Tekeli, 2005; Skládanka et al., 2008; Tilvikiene et al., 2014).

***Table 7**. The effect of the fertilizer and the cutting on crude fiber concentration (g kg^{-1}) of grass species*

Species	Cutting	Fertilizer								Mean
		0	NPK	UG	EU	HA	UG+NPK	EU+NPK	HA+NPK	
Dactylis glomerata	I	279.0	275.8	279.4	249.9	244.9	251.4	260.4	249.0	261.2
	II	240.6	233.4	249.0	233.8	222.2	229.7	225.6	217.5	231.5
	III	214.8	211.2	231.9	238.7	227.7	230.2	230.4	212.7	224.7
Lolium perenne	I	186.6	258.4	253.3	215.8	196.5	215.5	226.0	195.9	218.5
	II	224.3	236.7	229.0	160.3	216.7	193.3	198.0	200.5	207.4
	III	182.8	183.5	188.4	156.3	167.3	178.6	193.1	172.9	177.9
Mean effect of species										
Dactylis glomerata		244.8	240.1	253.4	240.8	231.6	237.1	238.8	226.4	239.1
Lolium perenne		197.9	226.2	223.6	177.5	193.5	195.8	205.7	189.8	201.3
Mean effect of cutting										
I		232.8	267.1	266.4	232.9	220.7	233.4	243.2	222.5	239.9
II		232.4	235.1	239.0	197.1	219.4	211.5	211.8	209.0	219.4
III		198.8	197.3	210.2	197.5	197.5	204.4	211.8	192.9	201.3

$HSD_{0.05}$ for: species = insignificant; cutting = insignificant; fertilizer/species = insignificant; fertilizer /cutting = insignificant; species/cutting = insignificant; species/cutting/fertilizer = 53.4

Content of crude fiber in the both species of grass and the all combinations of fertilizer were not statistically significant. The lowest content of crude fiber in *Dactylis glometara* was on the plot with the Humus Active Papka with mineral fertilization 226.4 g kg^{-1} DM (the value 8% lower than on the control object), while in *Lolium perenne* biomass from the plot with Eko-Użyźniacz fertilizers 177.5 g kg^{-1} DM (11% lower than on the control object).

Differences between crude fiber content in the cutting was not statistically significant. However, there was seen decreasing trend of crude fiber content from first cutting (239.9 g kg^{-1} DM) to third cutting (201.3 g kg^{-1} DM). Similarly, Jankowska-Huflejt and Wróbel (2010) found the lowest concentration of crude fiber in the grass of the third cutting (261.8 g kg^{-1} DM), and the highest in the grass from the second cutting (313.7 g kg^{-1} DM). Contrary to that Skládanka et al. (2008) found that crude fiber concentration in the biomass of *Dactylis glomerata* was the highest in the third cutting and successively increased in the following cuttings. Large variety of crude fiber content in the cuttings between literature and this publication was probably caused by differences in hydro-thermal conditions of the weather.

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Conclusions

1. For both grass species Eko-Użyźniacz applied together with mineral fertilizers increased protein content in fodder.
2. Application of Humus Active Papka with mineral fertilizers lowered crude fiber concentration in fodder.
3. The highest concentration of total protein was in Dactylis glomerata and Lolium perenne from the plots where soil conditioners together with mineral fertilizers were applied.
4. The highest concentration of crude fiber was in Dactylis glomerata growing on the plot with Humus Active Papka together with mineral fertilizers applied, while in Lolium perenne on the plot with Eko-Użyźniacz applied, without mineral fertilizers.
5. Taking into account the studied grass species and fertilizer combinations, Lolium perenne had better quality parameters than Dactylis glomerata, especially fertilized with EU+NPK or HA+NPK. Therefore, the species of this grass fertilized with these fertilizers should be recommended in agricultural practice.

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RELATIONSHIP BETWEEN PHYSIOLOGICAL INDICATORS OF OAT AND METEOROLOGICAL FACTORS IN TIBET'S ALPINE PASTORAL AREAS

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Abstract. According to large daily temperature difference, strong solar radiation and significant change of air humidity in the alpine area of Tibet, a field experiment was conducted to analyze the oat's diurnal variation of the leaf water potential (ψ_L), transpiration rate (Tr) and net photosynthetic rate (Pn). Meanwhile, the relationship between oat's physiological indicators and typical meteorological factors (atmospheric temperature (T), solar radiation (R_s), relative air humidity (RH)) were investigated with regression models. Results showed that the oat's daily change curve of ψ_L showed 'V-type' in the seeding and heading stages and 'W-type' in the jointing and tillering stages. As shown in the results, oat's Pn presented a decreasing tendency in a day and the peak appearing between 9:00 and 10:00 at one day in each growing stage. The Tr presented double-peak curves and midday depression in the seeding and heading stages. The variation tendency about oat's Tr in the tillering stage and jointing stage was "Multi-peak" type without distinct midday depression and parabolic tendency. By multiple regression analysis between the data of oat's physiological indexes and comprehensive factors, the ψ_L, Tr and Pn of oat in alpine region can be predicted under condition of adequate water supply.
Keywords: *oat, leaf water potential, transpiration rate, net photosynthetic rate, meteorological factor, high-altitude area*

Introduction

In recent years, with the development of pasture land water conservancy, the study of artificial grass water requirement and irrigation program is a public concern. Then the moisture physiological characteristics of pasture are of special note. For example, Liu et al. (2009) studied the effect of soil water stress on osmoregulation substance content of oat leaf. However, the relationship between leaf water potential (Ψ_L), transpiration rate (Tr), photosynthetic rate (Pn), which are the important parameters in the water cycle processes and meteorological factors are rarely investigated.

The leaf water potential is a basic indicator to reveal the status of crop water. The change of leaf water potential depends on many requirements of soil-plant-atmosphere continuum and complex interaction among the regions of plant. The daily variation rule of leaf water potential about winter wheat in the filling stage under the condition of

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sprinkling irrigation and surface irrigation were investigated by Yao et al. (2009). It found that the leaf water potential of winter wheat irrigated by sprinkling irrigation was higher than that by surface irrigation. Due to the individual and environmental variations, the leaf water potential of different plants will be different. The value of leaf water potential is associated closely with its yield, which is also varied with environmental factors. Kang et al. (1994) found that the Ψ_L of winter wheat will decrease with crop growing. Fu et al (2012) showed that the soil moisture content and temperature were the main factors for the leaf water potential change of *Phragmites* in arid areas. However, the relationship between leaf water potential of oat and meteorological indexes is remains unclear.

Transpiration regulates the water balance in plants and is influenced indirectly by environmental factors through effects on stomatal opening (Tamang and Sadok, 2018). Liu et al. (2003) studied the transpiration diurnal characteristics of spring wheat and found that the solar radiation and temperature will affect transpiration obviously. Peng et al. (2005) considered that the reduction of soil water content would put off the diurnal maximum of transpiration of late rice. His study also showed that there were different effects degree of vapor pressure deficit and stomatal conductance on transpiration of late rice in the different growth stages. Evapotranspiration and stomatal conductance are known to decrease as leaves age increasing in many species (Sobrado et al., 1994). Meanwhile, stomatal conductance for transfer of water vapor from the interior of the leaf to the atmosphere is affected by irradiance, leaf age, temperature, leaf water status, and the difference in absolute humidity between ambient air and the interior of the leaf (Wardlaw et al., 1967; Johnsonet al., 1983; Ku et al., 1977; Li and Zhou, 2004; Zhao et al., 2011). Some studies found that there was significantly correlational relationship between stomatal conductance and temperature, humidity and leaf water potential. At present, the model about relationship between stomatal conductance and environmental factors is composed of two classes. One is empirical model, such as Jarvis, Ball and so on (Ball et al., 1987; Jarvis et al., 1976; Leuning, 1995). Others are the optimization theory of stomatal regulation proposed by Cowan and Farquhar (1977) and optimization model of stomatal conductance established by Medlyn et al. (2011).

The diurnal variation of plant photosynthesis is not only the basic unit of material accumulation and physiological metabolism in the plant's production process but also an important method to analyze the impact of environment on plant growth and metabolism. Under normal circumstances, the daily change curve of plant photosynthesis is a bimodal type or unimodal type. Yu et al. (2015) reported that the diurnal variation of net photosynthesis of *maize* presenting the bimodal curve with apparent photosynthetic "noon break" phenomenon. The diurnal variation of plant photosynthesis is different from the separate plant species and the surrounding environment change (Farquhar and Sharkey, 1982). Many environmental factors will affect the plant photosynthesis, such as Photon flux density, temperature, relative humidity and so on. Under natural conditions, the influence of environmental factors on plant photosynthesis is the result of combination. However, there is hardly any research on the impact of meteorological indexes on oat photosynthesis is rarely.

The objectives of this research are:

(1)To investigate the daily variation of oat's Ψ_L, *Pn* and *Tr* in different growth stages on the condition of full irrigation in plateau area.

(2)To determine the relationships between oat's physiological indexes and meteorological factors in plateau area.

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Materials and methods

Experimental site

Tibet, which is mainly located in Qinghai-Xizang Plateau, is in the average elevation of 4000 m. There are 820 million ha natural grasslands in the alpine area of Tibet and the meteorological characteristics are large daily temperature difference, strong solar radiation and significant change of air humidity in this area. The problems of uneven distribution of water-heat resource in space-time and soil depletion are severe in the northern of Tibet plateau region especially. The special physiographic factors and production custom make the grass irrigation technology lagged far behind.

Field experiments were conducted in the central Tibet with longitude 90°45'-91°31' and north latitude 29°31'-31°04' in the Damxung County in Lasa (*Fig. 1*). The region with an average altitude of 4200 m is plateau-continental climate and the rainy season and dry season are clear. The annual mean precipitation is 480 mm and the rain mainly fall between June and September. In the region, with multi-year average evaporation being 1966 mm, the frost-free period is only 60 days and percentage of sunshine is above 60% in a year. The study area has adequate solar resource but is lack of hot resource. *Table 1* presents information on the physical properties of the soil in the field area. Surface soil above 40 cm mainly consists of loam, while the lower layer mainly consists of silver sand and rubble.

Table 1. *Physical properties of the soil in the field area*

Soil horizon (cm)	Field capacity (%)	Soil bulk density (g/cm³)	Percent clay (%)	Percent loam (%)	Percent sand (%)	Soil texture
0-10	17.96	1.48	7.8	44.07	48.13	Loam
10-20	16.14	1.59	7.66	43.48	48.85	Loam
20-30	18.03	1.55	7.51	42.8	49.69	Loam
30-40	18.28	1.56	8.08	47.11	44.81	Loam

Note: Soil texture classification according to the quality standard of America



Figure 1. *The location of study site*

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Agronomy

The experimental design was a randomized complete block with 3 replications. Each experimental plot was 12 m long by 5 m wide (60 m^2) with 30 cm height ridge. A buffer zone spacing of 2.00 m was provided between the plots. The oat (Danmai 444) was used for the experiment and planted on June 15th in 2013. The sowing rate was 187.5 kg/ha and the planting method was strip seeding with line spacing of 25 cm. Before planting, 4.5 t/ha organic fertilizer and 224.9 kg/ha N (as ammonium nitrate) were fertilized. One and half months after planting, a further 74.96 kg/ha of N (as ammonium nitrate) was applied. Crops were manually harvested when ripe rate reached about 95% (early September). Soil moisture was measured once every 7 days using TDR and validated by soil samples using soil drilling. The experiment was carried out without water stress. When the soil moisture was lower than the lower irrigation limit (W_{cg}), the irrigation would be carried out with ground irrigation. The detailed growing period information of oat and lower irrigation limit are shown in *Table 2*.

Table 2. *Growing period of oat and lower irrigation limit*

Growth period	Seeding stage		Tillering stage	Jointing stage	Heading stage
	Pre-emergence	After emergence			
P_g	Early June-late June	Late June-early July	Early July-middle of July	Late July- middle of August	Late August-harvest
W_{cg}	70(%) FC	70(%) FC	70(%) FC	75(%) FC	70(%) FC

Notes: (a) Each stage water condition refers to the percentage of maximum field capacity (FC); W_{cg}: Lower irrigation limit in different growth period with full irrigation (b) P_g: The beginning and ending of growth period

Meteorological factors

Due to the large daily temperature difference, strong solar radiation and significant change of air humidity, in the research district, we selected the *T*, R_s and *RH* as the typical meteorological factors. The data of *RH* and *T* were measured by the meteorological station in farmland of Damxung and R_s was calculated from the *Equations 1* and *2* (Allen et al., 1998).

$$R_a = \frac{12\times60\times G_{sc}\times dr[(\omega2-\omega1)\sin(\Phi)\sin(\delta)+\cos(\Phi)\cos(\delta)\sin((\omega2)-\sin(\omega1))]}{\pi} \quad \text{(Eq.1)}$$

$$R_s = \left(0.25 + 0.5\times\frac{n}{N}\right)\times R_a \quad \text{(Eq.2)}$$

where R_a is extraterrestrial radiation, MJm^{-2} h^{-1}; R_s is solar radiation, MJm^{-2} h^{-1}; G_{sc} is solar constant, 0.082; *dr* is the reciprocal of relative distance between the sun and earth; δ is the solar declination; *Φ* is the geographical latitude; *ω1*(*ω2*) is the solar hour angle in the early (late) period and n/N is the relative sunshine duration.

Measurements

Oat blade with well growth, normal color and size (second leave from the top of plant in seedling stage and third leave from the top of plant in tillering stage, jointing

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stage and heading stage) were selected to monitor the leaf water potential (ψ_L, MPa) with 3 plants replication using the water dew point meter (Psypro, Wescor Inc in USA) every one hour. Oat blades with well growth and normal color and size were selected to monitor the transpiration rate (*Tr*, millimol•m^{-2} s^{-1}) and net photosynthetic rate (*Pn*, μmol•m^{-2} s^{-1}) using automatic portable photosynthesis system (LCPro-SD, ADC in British) every 1 h with 3 plants replication. The meteorological characteristics of measurement's days are better sunshine, gentle breeze and no rainfall. The ψ_L was measured for one day in seeding, tillering and heading stage, and measured for 6 days at jointing stage. The *Tr* and net *Pn* were measured for one day in seeding, tillering, jointing and heading stage, respectively.

Data analysis

The data were subjected to the analysis of regression for meteorological factors and crop physiological indexes using SPSS software. The significance of regression model was determined with an F test. The F-protected least significance difference (LSD) was calculated at the 0.05 and 0.01 probability level, respectively.

Results and discussion

Daily variation of oat's leaf water potential, net photosynthetic rate and transpiration rate at different growth stages

Daily variation of oat's leaf water potential in different growth stages

Under conditions of full irrigation, ample sunshine and suitable temperature, diurnal variation of ψ_L in different growth stages were selected to contrast (*Fig. 2*). According to the data, the diurnal ψ_L in growth periods ranged largely with maximum of 6 MPa under the special environment of high altitude, strong radiation and large diurnal temperature range in Tibet. However, the ψ_L of winter wheat's variation value was only 3 Mpa and the ψ_L of Mongolian blue-beard grew in the alpine region is only 1.24 Mpa (Han et al., 2008; Tong et al., 2005).

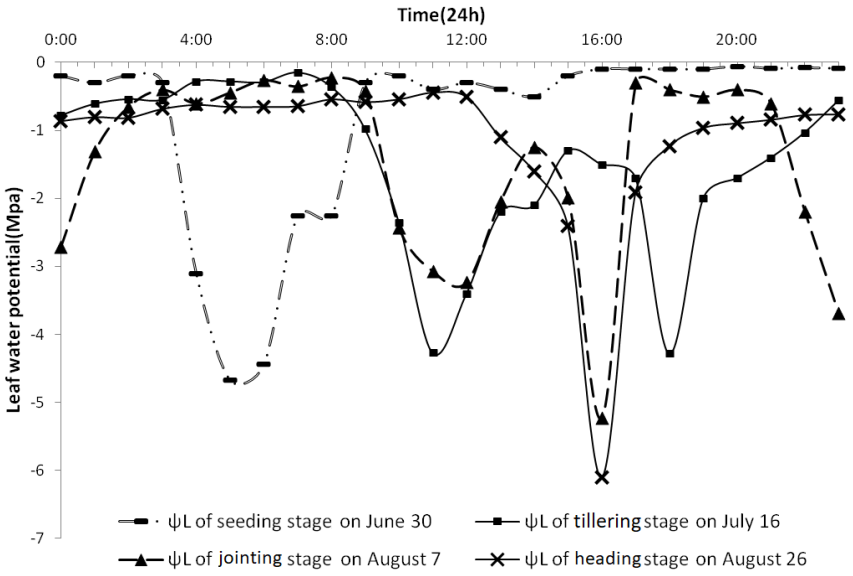


Figure 2. *Daily variation of ψ_L under different growing periods*

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In the seedling stage, the ψ_L usually changed at night and the minimum of -4.67 MPa appeared at day break, and the change trend showed "V-type". The trough of ψ_L happened five hours before sunrise, when T and R_s were lower with the RH being larger. The ψ_L would reach the larger value in the day time. This is due to cells of seedling leaf avoiding to be damaged by radiation or avoiding the effect of water stress on crops by excessive transpiration. The frequent fluctuations of ψ_L at dawn reflected the frequent water exchange with external and stronger life activity in the seedling stage of oat. This indicates that the oat could adapt to the low temperature at night in the seedling stage. In contrast, high temperature and radiation will inhibit the growth of oat in the seedling stage, which is related to the undeveloped oat root and immature mechanism of stomata cell for opening and closing.

(2) In the tillering stage, the oat grew quickly and exchange moisture with external environment frequently. The ψ_L fluctuated significantly at daytime and the trend showed "W-type". The lowest trough of ψ_L appeared at the 10:00 to 11:00 with a minimum value of -4.27 MPa and 18:00 with a minimum value of -4.28 MPa (*Fig. 1*). With the rise of R_s and T, the oat reduced their own tissue water potential to absorb more moisture for the life activity. The ψ_L decreased after sunrise. The temperature reached the maximum at noon. Through regulating the stomata opening and closing, the oat reduced water loss. Then as the sun set, the T fell, ψ_L reduced again to absorb more moisture. The ψ_L returned to a higher value of -1.7 MPa at 21:00. The results support the conclusions of Guo, et al. (2006) and Tong et al. (2005) that the trend of ψ_L showed "W-type" on the condition of full-irrigation. The oat had already certain adaptability to the change on meteorological factors in the tillering stage. The life activity at daytime was going to be vigorous than it at night.

(3) The jointing stage is the critical period for the organic synthesis and accumulation of plant (Tian et al., 2012). The diurnal variation of ψ_L tended to be stable and the trends were similar to that in the tillering stage with two troughs. The time when the first trough appeared at 11:00 was consistent with the moment of tillering stage's first trough; however, the second trough was about two hours upfront than that in tillering stage with a minimum value of -5.22 Mpa. This phenomenon can be attributed to the special meteorological conditions. The general trend of ψ_L was higher in the morning and evening and the ψ_L was significantly reduced with great fluctuations. At 14:00, the ψ_L was at a high value of -1.24 MPa, when the oat's stomatal conductance decreased rapidly, guard cell shrinkage and partial stomata closure in response to intense light and drying air. Therefore through short "nap", the oat could adapte the change of weather factor and control water balance of the body (Kang et al., 1991).

(4) In heading stage, the reproductive growth replaced the vegetative growth gradually. The ψ_L maintaining a relatively high value tended to "V-model" with a minimum of -6.1 MPa at 16:00 (*Fig. 1*). The occurrence time of trough agree with the moment of the second trough appeared in tillering and jointing stages. The results are keeping with the conclusions of Guo et al. (2006) that the trend of ψ_L showed "V-type". This proved that water currently had strong driving force to promote water from soil to root at 16:00-18:00 under non-water stress condition. To some extent, it indicates that the heading stage is the critical period to translate the energy to organics and so the lack of water will affect the quality of artificial grassland. However, further study is needed to confirm why the ψ_L with a minimum of -6.1 Mpa at high altitudes lower than other areas obviously.

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Daily variation of net photosynthetic rate of oat in different growth stages

In low elevation regions, previous studies suggested that the curves of diurnal variation on *Pn* of gramineous plants presented double-peak, and an obvious midday depression at 12:00 o'clock (Li et al., 2013; Liu et al., 2006). This study showed that *Pn* has its unique change rule at high altitudes. The daily variation patterns of *Pn* of oat are presented in *Figure 2*. As the trend lines implied in *Figure 3,* it presented a decreasing tendency in a day. The variation tendency of oat's *Pn* were basic consistent without fixed peaks and troughs model in different growth stages. The *Pn* on the morning was higher than that on the afternoon and the peak appeared between 9:00 and 10:00 at one day. After the peak value appeared, there would be a reduction and the downturn would last a long time in the tillering stage and heading stage. The maximum of *Pn* in the seeding stage, tillering stage, jointing stage and heading stage were 10.56 μmol m^{-2} s^{-1}, 9.91 μmol m^{-2} s^{-1}, 10.01 μmol m^{-2} s^{-1} and 9.01 μmol m^{-2} s^{-1}, respectively.

At 12:00 am and 4:00 pm, an obvious valley appeared in the seeding stage; likewise the valley appeared on the morning and afternoon in the jointing stage, while the minimum values of P_n were 5.1 μmol m^{-2} s^{-1} and 5.4 μmol m^{-2} s^{-1}, respectively. The *Pn* in tillering stage witnessed significant downturn at 12:00-14:00, which was similar to the *Pn* in heading stage at 11:00-14:00, troughs appeared with the minimum value of 5.2 μmol m^{-2} s^{-1} and 4.4 μmol m^{-2} s^{-1}, respectively.

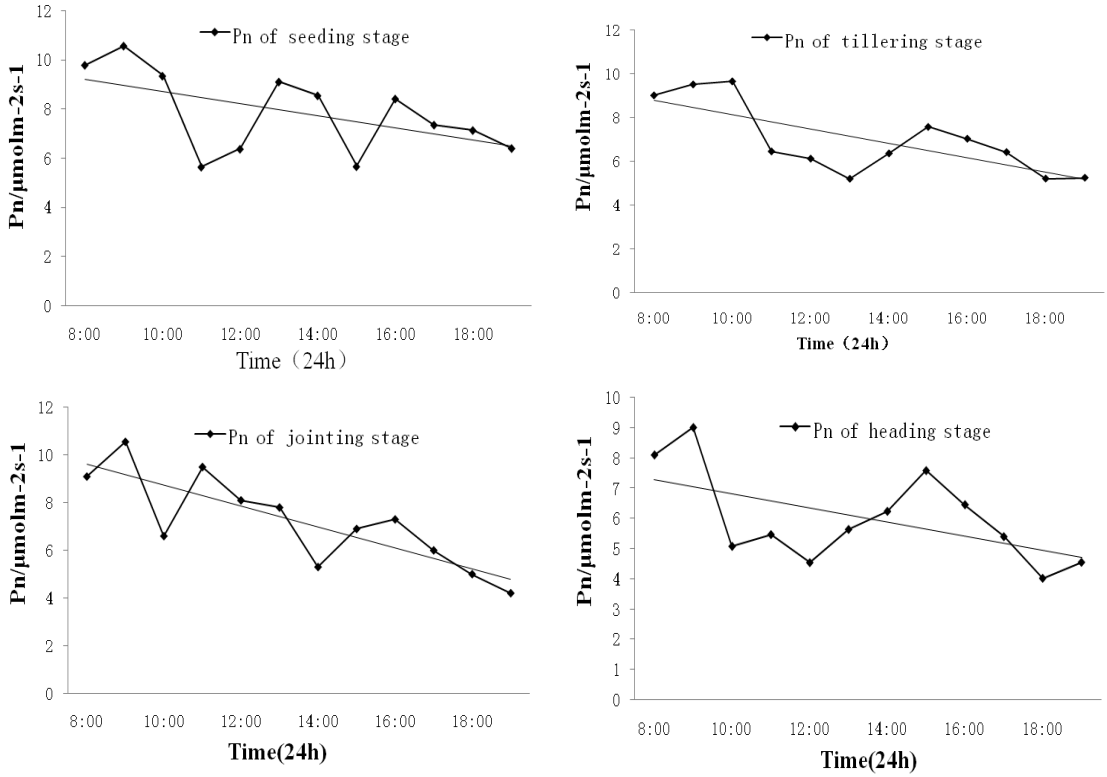


Figure 3. *Daily variation of Pn under different growing periods*

Daily variation of oat's transpiration rate at different growth stages

The *Tr* of oat in the seeding stage is obviously less than that in the tillering stage, jointing stage and heading stage (*Fig. 4*).

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(1) The *Tr* presents double-peak curves and midday depression in the seeding and heading stage

The obvious tendency of bimodal patterns about *Tr* are appeared in the seeding stage and heading stage of oat. This changing trend is similar to the result reported by Luo et al. (2011), the curves of diurnal variation in *Tr* of Medicago sativa had two peaks and an obvious midday depression with or without water stress at lower altitude. At 12:00, the *Tr* in the seeding stage peaks at the maximum of 45.3 millimol•m^{-2} s^{-1}, while the midday depression appearing from 13:00 to 16:00. The *Tr* value is the minimum of one day with being 15.2 millimol•m^{-2} s^{-1} at 8:00 in the morning. In heading stage, the maximum value (64.5 millimol•m^{-2} s^{-1}) of *Tr* appeared at 11:00 and the minimum value (13.7 millimol•m^{-2} s^{-1}) of Tr appeared at 20:00. The curves of diurnal variation in *Tr* had an obvious midday depression between 12:00 and 14:00.

(2) The *Tr* presents "Multi-peak" type in the tillering stage and jointing stage

Different from the current research about grass, the variation tendency on *Tr* of oat in the tillering stage and jointing stage was "Multi-peak" type without distinct midday depression and parabolic tendency. In tillering stage, the *Tr* would rise rapidly after sunrise and reach to the first summit (66.1 millimol•m^{-2} s^{-1}) at 11:00, and there were more peak values which appeared at 14:00 and 17:00 respectively in one day. In jointing stage, the *Tr* reached to the first peak (73.8 millimol•m^{-2} s^{-1}) at 11:00 similarly, and there were more peak values which appeared at 13:00, 15:00 and 17:00 respectively in one day. In this research, the variation tendency of in the tillering stage and jointing stage was different from most graminaceous plants with a single peak (Philip et al., 1966; Yuan et al., 2009).

This study investigated its emergence reason and regulation. On one hand, the result is due to the strong nutritional growth of oat in the tillering stage and jointing stage. There are frequent matter and energy exchange with the environment in these two phases. On the other hand, it is related to the specific weather conditions (such as amounts of moisture in atmosphere vary greatly as a direct result of stronger solar radiation and frequent rainfall in a day) in Tibet alpine pasture.

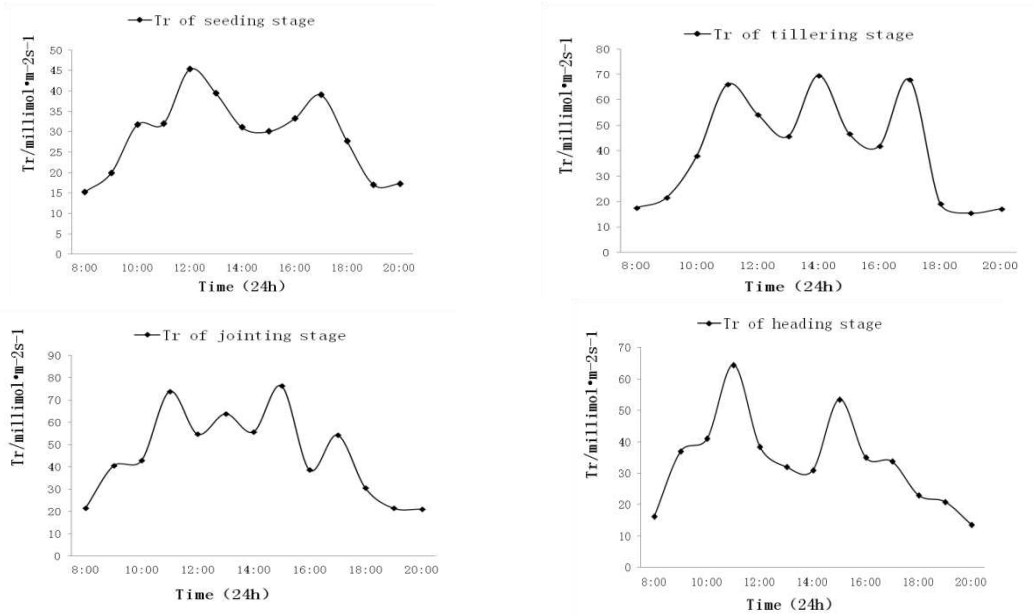


Figure 4. *Daily variation of Tr under different growing periods*

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Relationships between physiological indexes of oat and single meteorological factor

The daily variation relationships between ψ_L, *Tr*, *Pn* and *T*, R_s, *RH* were analyzed with SPSS and presented in *Table 3*. Results showed that the change trend of ψ_L could be predicted simply by typical meteorological factors, as many studied reported that there were obvious relationships between the *T*, R_s, *RH* and ψ_L (Zhang et al., 2008). The correlation order of *T*, R_s, *RH* with ψ_L was: $T > RH > R_s$with correlation coefficients of 0.728, 0.676 and 0.353, respectively. From the models, the ψ_L decreased by 1.96 Mpa with the temperature increasing by 10°C. Due to the R_s was always positive number, the relationship between ψ_L and R_s was inversely proportional quadratic parabola trend. Different from the other two meteorological factors, the logarithmic relationship appeared between ψ_L and *RH*.

Table 3. *Regression model of single meteorological factors and crop physiological indexes*

Crop physiology index	Meteorological factor	Regression model	Determinate coefficient R^2	F test value
ψ_L (Mpa)	*RH*	ψ_L=-30.46+6.65*ln(RH)	0.676**	45.413
	R_s	ψ_L=-2.066-0.647R_s-0.077R_s^2	0.353*	4.523
	T	ψ_L=-0.101-0.196T	0.728**	58.578
Tr (millimol•m^{-2} s^{-1})	*RH*	*Tr*=-43.544+2.481RH-0.021RH^2	0.504*	5.584
	R_s	*Tr*=12.121*exp(0.2880*R_s)	0.360*	6.180
	T	*Tr*=-19.369+13.897*ln(T)	0.328*	5.867
*P*n (μmol•m^{-2} s^{-1})	*RH*	*P*n=-43.465+2.198*RH-0.0002*RH^3	0.529*	6.173
	R_s	*P*n=28.165+8.008R_s	0.354*	3.041
	T	*P*n=12.947*$T^{0.392}$	0.538*	8.166

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level
Number of samples for ψ_L was 216 (24 × 9 = 216), number of samples for *Tr* was 48 (12 × 4 = 48) and number of samples for *P*n was 48 (12 × 4 = 48)

The R_s guarantees the energy for crop transpiration. The crop transpiration rate (*Tr*) increases with the increase in water potential gradient, caused by stronger R_s, higher temperature and reduced atmospheric water. The correlation order between *Tr* and *T*, R_s, *RH* was: $RH > R_s > T$ with correlation coefficient of 0.504, 0.36 and 0.328, respectively. The relationship trend between *Tr* and *RH* was the quadratic downward. The relationship between *Tr* and *RH* was different from ψ_L with the trend of inversely proportional quadratic parabola. The maximum *Tr* appeared when the *RH* equaled to 59.07% and the *Tr* would decreased with *RH* increasing when the *RH* was greater than 59.07%. Differently from others, the relationship trend between *Tr* and R_s was exponential. The minimum *Tr* was 12.121 millimol•m^{-2} s^{-1} while R_s was 0. Logarithmic relationship was shown between *Tr* and *T* and there was not transpiration when the temperature was less than 4.03 °C. Then the transpiration rate will increase with the increase in temperature.

The impact factors on *Pn* of oat are not only the temperature but also the crop stomatal opening, transpiration changes and intercellular carbon dioxide concentration.

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Nevertheless, the *Pn* will affect the crop development. The correlation order between *Pn* and *T, R_s, RH* was: $T > RH > R_s$ with correlation coefficient of 0.538, 0.529 and 0.354, respectively. Different from the *Tr*, the relationship trend between *Pn* and *RH* was the cubic polynomial. The linear relationship was presented between *Pn* and R_s. An 80.08 μmol•m^{-2} s^{-1} *Pn* increment appeared when the R_s increased by 10 MJ m^{-2} h^{-1}. The relationship trend between *Pn* and *T* was different from others. Power function was showed between *Pn* and temperature with the power exponent of 0.392.

Then the relationship between ψ_L and physical signs will be introduced in great detail. If irrigation is sufficient, the change trend of ψ_L is affected not only the different growth stages but also the meteorological factors. The change of ψ_L can reflect the crop ability adapting to the environment.

With the uncertain meteorological condition, the data of ψ_L and meteorological factors collected from August 7th to August 12th when the weather conditions and humidity were suitable for analysis, in order to eliminate the particular of observation data as much as possible in one day. The metrological and ψ_L data observed over six days were averaged, which is an effective measure to reduce the error caused by the particular data.

(1) The diurnal variation relationship between ψ_L and R_s

From the change curve (*Fig. 5*), the change trend about the ψ_L and R_s are negative correlation ($R^2 = 0.353$). The R_s started to change from negative value to positive value at 5:00. It reached maximum (3.45 MJ m^{-2} h^{-1}) at 12:00. However, the ψ_L decreased rapidly from -0.32 MPa at 7:00, reached the first minimum at 13:00 and started to increase after two slight rebounds between 13:00-15:00. The fluctuation of ψ_L may be impacted by other meteorological factors (Yang et al., 2012). From the above analysis, the changes of ψ_L lagged behind the changes of R_s.

(2) The diurnal variation relationship between ψ_L and *RH*

The change trend of ψ_L and *RH* are a positive correlation ($R^2 = 0.676$; *Fig. 5*). After the sunrise, *RH* tended to drop off over time with the maximum 87% at 8:00. The minimum appeared at 18:00 with 42.67%. Both ψ_L and *RH* started to rise after the sunset and the ψ_L reached to -1.8 Mpa in the end.

(3) The diurnal variation relationship between ψ_L and *T*

The change trend of ψ_L and *T* are a negative correlation obviously ($R^2 = 0.728$) (*Fig. 5*). At daylight, the ψ_L decreased while *T* was rising, and *T* reached to minimum of 3.15 °C at 7:00, however ψ_L was at a higher value with -0.32 Mpa. *T* tended to drop off after it reached the maximum value of 27.5 °C at 15:00. In addition, the continuous high temperature would lead to ψ_L picks up again with "nap".

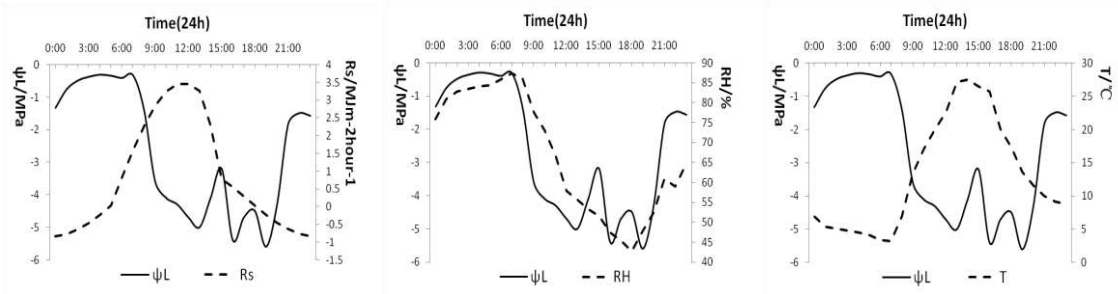


Figure 5. *The relationship between daily variation of leaf water potential and meteorological factors*

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Relationships between physiological indexes of oat and comprehensive factors

The crop physiological indicators are usually influenced by multiple meteorological factors comprehensively, such as temperature, humidity and so on. For example, the daily variation of ψ_L is little on cloudy days with large humidity and wind speed, while the daily variation of ψ_L is larger on sunny day with small wind speed and moderate humidity. By multiple regression analysis between the data of oat's physiological indexes and comprehensive factors, the ψ_L, *Tr* and P_n of oat in alpine region can be predicted under condition of adequate water supply. The regression models between ψ_L, *Tr, Pn* and meteorological factors are presented in *Table 4*. The correlation coefficients are 0.86, 0.78 and 0.66, respectively.

Table 4. *Regression model of multi-meteorological factors and crop physiological indexes*

Crop physiology index	Regression model	Determinate coefficient R^2	F test value
ψ_L (Mpa)	ψ_L=-11.271-0.882R_s+0.076T+0.123RH	0.86**	40.95
Tr (millimol•m^{-2} s^{-1})	*Tr*=-39.375+1.606T+0.453RH+0.056R_s	0.78*	11.90
*P*n (μmol m^{-2} s^{-1})	*P*n=-7.603+0.919T+0.319RH+5.848R_s	0.66*	10.40

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level
Number of samples for ψ_L was 216 (24 × 9 = 216), number of samples for *Tr* was 48 (12 × 4 = 48) and number of samples for *P*n was 48 (12 × 4 = 48)

Conclusions

With the water resources becoming scarcer, the water-saving agriculture in Tibet plateau has drawn much attention. At the same time, the oat physiological indexes research is indispensable for water-saving agriculture. The results show that the daily oat's ψ_L changed obviously during the different growth stages. The daily change curve of oat's ψ_L showed 'V-type' in the seeding and heading stages, and the lowest value appeared at 5:00 and 16:00 respectively. However, oat's ψ_L showed 'W-type' in the jointing and tillering stages in which the lowest value both appeared in the morning and afternoon. The maximum change range of ψ_L can reach to 6 MPa in a day.

The variation tendency of oat's *Pn* was basically consistent without fixed peaks and troughs model in different growth stages. As the facts mentioned above, *Pn* presented a decreasing tendency in a day. The *Pn* on the morning was higher than that on the afternoon and the peak appeared between 9:00 and 10:00 at one day. The maximum of *Pn* in the seeding stage, tillering stage, jointing stage and heading stage were 10.56, 9.91, 10.01 and 9.01 μmol m^{-2} s^{-1}, respectively.

The *Tr* of oat at the seeding stage was obviously less than that in the tillering stage, jointing stage and heading stage. The *Tr* presented double-peak curves and midday depression in the seeding and heading stage. Different from the current research about grass, the variation tendency about *Tr* of oat in the tillering stage and jointing stage was "Multi-peak" type without distinct midday depression and parabolic tendency.

Based on the regression analysis, the correlation coefficients between meteorological factors and oat's four physiological indicators were: $T > RH > R_s$; $RH > R_s > T$; $T > RH > R_s$, respectively for ψ_L, *Tr* and *Pn.* By multiple regression analysis between the data of oat's physiological indexes and comprehensive factors, the ψ_L, *Tr* and *Pn* of

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oat in alpine region can be predicted under condition of adequate water supply. These results are useful to understand the hydrologic cycle of the irrigated grass and determine the scientific irrigation schedule to improve water use efficiency of grass in the alpine area.

The studies about ψ_L, *Tr* and *Pn* in this paper are under the condition of full irrigation. Therefore, in order to determine the scientific irrigation schedule, the influence of water stress on ψ_L, *Tr* and *Pn* will be the focus of the further study.

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HOUSEHOLD CHARACTERISTICS AND FOREST RESOURCES DEPENDENCE IN THE RUMPI HILLS OF CAMEROON

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Abstract. Rural households have over decades depended on forests and forest products for sustenance and livelihood values. This dependence is usually driven by the individual household's dynamics, forests availability and accessibility. However, with overexploitation and agricultural expansion, these forests are rapidly declining putting in peril household livelihoods. This study aims at examining and analyzing household dynamics and how these dynamics combine with other factors to influence household dependence on forest resources in the Rumpi hills of Cameroon. Data were collected from 484 households, across 20 villages and at four selected sites through household surveys and 20 focus group discussions. Household characteristics such as uneducated heads (p-value 0.000381) significantly influenced the use of forest resources than male heads (P-value 0.000642) and years of residence (11–15) (p-value 0.00624) in the village at the 95% level of significance. Forest dependence was mostly conditioned by the household's ability to farm, forest abundance, and unemployment. Additionally, income from agriculture and forest resources respectively constituted 36.7% and 34.1% of the average total rural household income. Investment in climate-smart agriculture, non-wood domestic cooking energy, agroforestry systems and reliable rural transport systems is necessary in order to curb forest dependence and improve on livelihoods and sustainable forest management initiatives.
Keywords: *rural livelihoods, household income, forest dependency, sustainable forest management*

Introduction

With a national forest cover estimated at 42%, Cameroon harbours enormous forests and marine resources embedded in a mosaic of landscape structures along basins, plains and mountains. These forests are mostly situated in the south where they make-up the

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western margins of the large tract of the Congo Basin Forests (Robiglio et al., 2010; Ekoungoulou et al., 2018a). However, rural communities live in and around these forests where they depend on several forest resources and products for sustenance and livelihood. Besides serving as sources of wood, farmlands, medicinal plants and hunting grounds, these forests also serve sociocultural and recreational purposes (Etongo and Glover, 2012; Tieguhong et al., 2012; Mukete et al., 2016a).

This dependence which is likely to increase in the coming decades challenges the very existential purposes of these forests often resulting in associated forest degradation, deforestation and constituent biodiversity loss. This stems from the observable depletion of forests due to the combined effects of agricultural expansion, wood harvesting for cooking, heating and wood works (Kimengsi and Lambi, 2015; Mukete et al., 2018b).

Various studies have examined household dependence and the contributions of forest resources to household income and rural livelihoods. For instance, Makoudjou et al. (2017) found forests to contribute on average 38% of total annual household income. Here, 19, 13 and 6% of such contributions according to their study came from illegal logging, hunting and harvesting of plant-related non-timber forest products (NTFPs). NTFPs include goods of biological origin other than wood and timber, derived from forests and other land uses such as fallows, woodlots, agroforestry systems, and plantations (Dembner and Perlis, 1999). In the Rumpi hills forest, NTFPs primarily gathered from forests and other land uses include land snails (*Archachatina marginata*), bush mango (*Irvingia gabonensis*), bitter cola (*Garcinia cola*), njansang (*Ricinodendron heudelotii*), country onion (*Afrostyrax lepidophyllus*), bush pepper (*Piper guineense*), and several edible species of mushrooms (Ofundem et al., 2017; Mukete et al., 2018b).

In another study, rural households with better access to forested land such as national parks and forest reserves in Malawi were found to have higher total income, forest income, and relative forest income (Kamanga et al., 2009). Meanwhile, the amount of forest income that households earned was found to vary significantly across study sites in Zambia. This variation was influenced by market and household level factors as well as the occurrence and abundance of forest resources and products (Bwalya, 2013). Accordingly, Belcher et al. (2015) affirmed that, households whose members were relatively more educated are likely to obtain more income from forest related activities than less educated households. Similarly, households made-up mostly of younger members were more dependent on collecting forest products. This is attributed to their starting families and possessing lower agricultural household assets than older and better-established households (McElwee, 2008). On the other hand, households with relatively larger family sizes were found to be more forest resource dependent than those with smaller family sizes (Mamo et al., 2007). Other factors such as geographical location and topographical conditions have also been observed to influence forest resource dependence and use (Zenteno et al., 2013).

Many other studies have linked household forest resource dependence vis-a-vis sustainable forest management initiatives. For instance, Lambi et al. (2012) observed that management and development initiatives that influence households' use of forest resources and forest dependence will invariably affect household employment status and skills through capacity building. Beside household's characteristics, limited opportunities for non-forests livelihood diversification options still prevails, thereby creating a situation of more reliance on forest resources. Such overdependence on forest resource use does not only induce more deforestation and forest degradation, but also

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questions the effectiveness and sustainability of protected area (PAs) initiatives (Ewane et al., 2015).

Sustainable forest management initiatives especially PAs initiatives, can provide significant socioeconomic benefits especially to forest dependent rural communities. Jindal et al. (2012) are of the opinion that, forest management and development initiatives are likely to be compromised should they not tally with household's dynamics and their livelihood activities. Accordingly, Arnold and Perez (2001), Ambrose-Oji (2003), Lambi et al. (2012) and Hajost and Zerbock (2013) observed that, the unabated active participation and interest of local forest dependent rural communities in forest management and development initiatives is closely related to their individual household's characteristics and requirements. Lambi et al. (2012) and Ofoegbu et al. (2017) also found direct links between household characteristics and their decision to carry-out forest related activities. In related studies, factors linked to household conditions were found to influence their motivation and willingness of host communities to participate in forest carbon conservation activities in Mozambique (Jindal et al., 2012), Cameroon (Chia et al., 2013) and Bolivia (Zenteno et al., 2013).

Some studies on sustainable forest management activities have also found that, the long-term sustainability of such initiatives are likely to be efficient if and only if the socioeconomic needs and characteristics of local communities are taken into cognizance. For example, Ambrose-Oji (2003) in her study on the contributions of NTFPs to local livelihoods observed that, forest value could be increased by valorizing the use and importance of NTFPs to local forest dependent rural communities in the Southwest Region of Cameroon. Accordingly, this valorization could then translate into incentives and encourage local villagers to embrace sustainable forest management strategies for the production of livelihood benefits (Ambrose-Oji, 2003). Similarly, Levang et al. (2015) studied the importance of forests concessions to the lives of local forest dependent communities of the east and south regions of Cameroon. Their study found agricultural expansion, unregulated hunting and logging inside the concessions to be setbacks to forest resources utilization for the local communities. They concluded that curtailing these setbacks would necessitate the inclusion of these local communities into stakeholder decision making discussions. Also, Ewane et al. (2015) reported on the importance of a general national reform policy in forest governance and management such as to ensure future sustainable forest management in Cameroon. The study proposed a participatory approach involving both local forest-dependent communities and government institutions which will foster collaboration and adaptive management in forest management. However, tackling the multiple challenges related to forest-dependent rural communities and the implications for strategic and practical forest planning has not been carefully done in Cameroon (Lang, 2015; Mukete et al., 2018a). Therefore, additional studies that identify drivers of household forest dependence and the determinants of livelihood patterns for forest-dependent communities are necessary. These would aid in reducing negative impacts on forest resources while ensuring forests are sustainably utilized.

Within the past three decades, the absence of reliable overall quantitative data on household forest resources dependence has preoccupied researchers in Cameroon. In this regard, several studies started addressing different aspects of household forest resources dependence, contributions to household income and rural livelihoods such as: economic contribution to livelihoods (Levang et al., 2015; Makoudjou et al., 2017), poverty alleviation (Ambrose-Oji, 2003; Tieguhong et al., 2012), role of NTFPs (Timko

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et al., 2010; Ofundem et al., 2017), drivers of dependence (Etongo and Glover, 2012; Ewane et al., 2015) and Reducing Emissions from Deforestation and Forest Degradation (REDD+) initiatives (Chia et al., 2013; Awono et al., 2014). These REDD+ initiatives are mechanisms via which developing countries are financially rewarded for achieving carbon emissions reductions and associated with a decrease in the conversion of forests to alternate land uses (Parker et al., 2009; Chia et al., 2013; Awono et al., 2014). Aside financial rewards, reduced deforestation and forest degradation, it facilitates the conservation and enhancement of forest carbon stocks as well as promotes sustainable forest management (Peskett et al., 2008).

Studies that directly address the topic of household characteristics and forest resource dependence in Cameroon are lacking and this is the very first study that addresses the topic in the Rumpi hills of the South West Region of Cameroon. This study bridges that knowledge-gap as it examines household characteristics and forest resource dependence. It also makes an analysis of how household characteristics may influence a household's dependence on forest resource use. This baseline information may help improve macro-level poverty estimates and serve as an input into conservation policy. It may also serve as a knowledge platform for effective collaboration between forest dependent communities, forestry stakeholders and policy makers to design effective community-based forest management and rural development programs aimed at reducing deforestation and forest degradation.

Materials and methods

Study area

The Rumpi hills forests (4°49' N 9°10' E) and their characteristic horse-shoe shaped Rumpi Hills Forest Reserve, (RHFR) (Latitude: 4°51′26″ Longitude: 9°07′15″) are located in Ndian division (Latitude: 4° 50' 00Longitude: 8° 50' 00"). Ndian division is an administrative unit in the South West Region of Cameroon covering an estimated area of 6626 km^2. Its mountainous areas are characterized by undulating Rumpi hills ranging from 400-1800 m with the highest peak at Mount Rata located in the eastern borders of the study area (*Fig. 1;* SWPA, 2016; Mukete et al., 2018b).

The RHFR is one of the oldest protected areas in Cameroon. Previously known as the Rumpi Hills Native Administration Forest Reserve, it was created in 1941 and occupies an area equivalent to 455 km^2 spread across four local council areas in Ndian division. The eastern borders are in Dikome Balue, southern borders in Ekondo Titi, western borders in Mundemba and northern borders in Toko local councils, respectively (Mukete et al., 2018b).

Mangroves and creeks dominated estuary forms a very low and indented point of contact with the sea in the Rio del Rey Estuary along the Gulf of Guinea making-up over 40% of Ndian. About 25% of the remaining land area is flat while the other 35% is mountainous and dominated by the Rumpi hills – our study area. Based on 2001 population survey in addition to future projections, the population of Ndian division is estimated at 362201 of which 17% are semi-urban and 83% rural. The average population density is 54.7 inhabitants per km^2. This density drops to an estimated 20 inhabitants per km^2 in the Rumpi hills and maritime areas. Meanwhile, around the semi-urban centers in Ekondo Titi and Mundemba, the population density increases to about 220 inhabitants per km^2 (BUCREP, 2015; Mukete et al., 2018b).

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Covering over 2300 km^2, the Rumpi hills forests constitute a combination of montane, coastal evergreen, drier northern semi-evergreen forests, and other vegetation types (Mukete et al., 2017). These forests are very rich in terrestrial and marine ecological diversity with plant and animal species that range from simple to complex organisms. About 198 bird species including the endangered white-throated mountain-babbler (*Kupeornis gilberti*) and white-necked pigeon (*Columba albinucha*) have been identified. Also, many tropical montane mammal, reptile and amphibian species such as the endangered drill (*Mandrillus leucophaeus*) and forest shrew (*Myosorex rumpii*) have also been identified (Linder, 2013; Birdlife International, 2014).

The soils are composed of Precambrian clays and sandstones which may have emanated from various volcanic eruptions (Yerima and van Ranst, 2005). The area exhibits two seasons, a dry season from December to February and a rainy season from May to October with annual rainfall ranging from 4027 to 6368 mm. Temperature varies little throughout the year with mean monthly maximum temperatures in the dry season being 31.8 °C and in the rainy season 18.2 °C. The relative humidity is high during most of the year with minimum monthly values ranging between 78% and 90% (Etongo and Glover, 2012; Mukete et al., 2017).

Approximately 90% of the local population directly or indirectly relies on forests for their livelihood where food and cash crops including yams, spices, vegetables, cocoyams, plantains, cocoa, and oil palm are widely cultivated. The local population especially in the rural areas also depends on the harvesting of NTFPs primarily as an offseason job when pressure on crop cultivation is reduced. In the high altitude forest areas of Dikome Balue and Toko local councils, hunting and NTFPs gathering are important activities for the local communities. The rural population also benefits from employment as labourers provided by the agroindustrial giant Pamol Plantations Plc, smallholder plantations and in small private businesses. The national road N16 from Kumba in Meme division passes through the major semi-urban areas of Ekondo Titi and Mundemba in the southern and western zones (*Fig. 1*). Moreso, the different maritime ports of the Rio del Rey estuary also provide excellent access points by water to neighbouring Nigeria, Equatorial Guinea and Gabon which facilitates the evacuation of forest resources (Malleson, 2000; Mukete et al., 2018b).

Sampling of rural communities

Data on the drivers of household characteristics and forest resources dependence were obtained through different methods. They include household surveys – semi-structured, close and open-ended recall questions, and 20 focus group discussions (FGDs) with household heads and forestry stakeholders (Mukete et al., 2017). Majority of the studied villages are adjacent to the RHFR and are among those that had existed and had been using the forest even before the horse shoe-shaped RHFR was created. These villages thus represent an important source for historical data. The number of households in each village was obtained from the local village chiefs because official census data are lacking. In villages with less than 25 households, only semi-structured and open ended questionnaires through a door to door survey were used. Meanwhile, villages with more than 25 households were divided into groups for easy implementation of FGDs. The number of participants in a FGD was limited to 10 and included volunteers and or randomly selected farmers of at least 18 years old (as required by Cameroon Law). Their National Identity Cards were verified and in cases where this was unavailable, recommendation was sought from the local village chief or

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his representative. In instances where potential participants were unable to effectively communicate in English language, "Pidgin English" or Oroko dialect was used by the first author to ensure proper understanding. The interview captured at least 70% of the households per village giving a total of 484 interviews of households across the 20 adjacent villages.

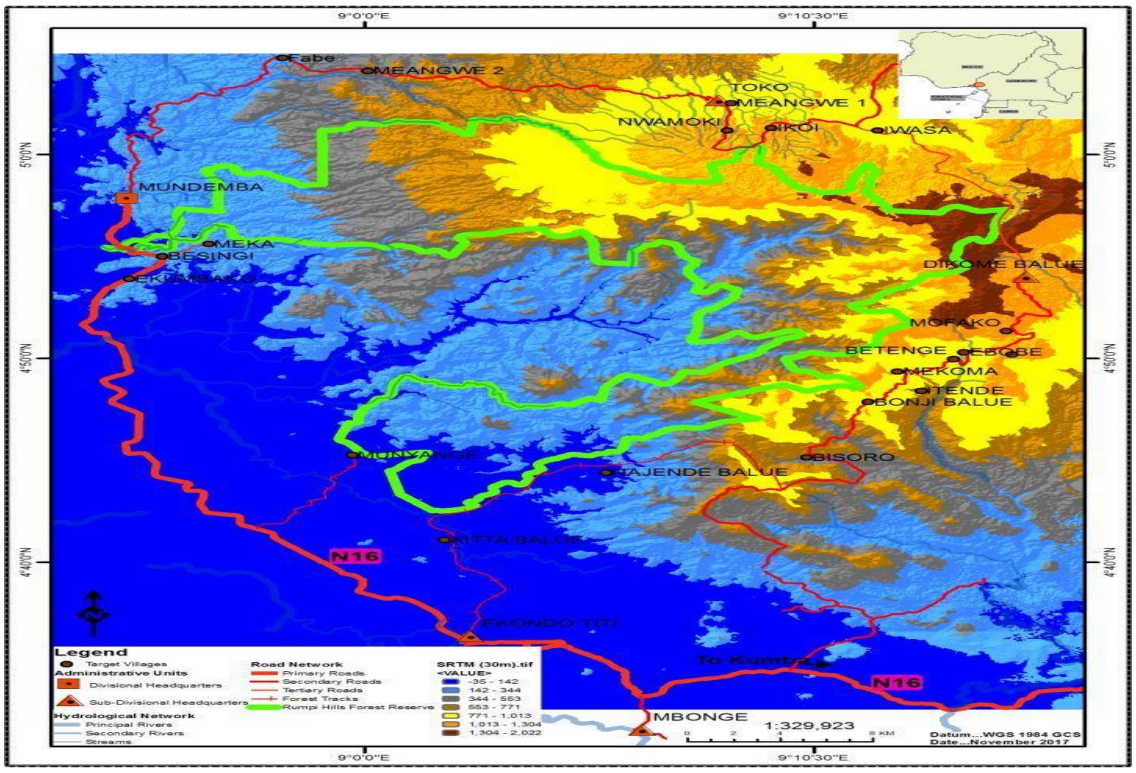


Figure 1. *Location map of the study area*

The household which was the basic unit for this research was defined *as a group of people living together in the same house who regularly cook and eat from the same pot*. Here, questions were centered on the gender, age, annual income, income sources, educational level, marital status, ethnicity; farming practices and off-farm activities of the household head. Information was also collected from local forestry stakeholders on the drivers of deforestation at the micro level, their engagement of rural households in mitigating the effects of deforestation in addition to the implementation of forest management policies. The questionnaires were designed so as to gain an understanding of household characteristics, the driving causes of their dependence on forests and its implications for forest sustainability. A two-point type scale (Yes = important, No = not important) was used to examine the importance of forests to household income, sustenance, livelihood and access into protected areas (Ofoegbu et al., 2017).

Statistical analyses

Data collected through the questionnaire-based survey were analyzed using Stata 13.0 for Mac. A regression analysis was also conducted to determine the relationship between the dependent and independent variables. Hence, the dependent variables were regressed on independent variables in order to determine if there was a statistically

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significant relationship and how much variation of the dependent variables was explained by the independent variables. The p-value ≤ 0.05 represented statistical significance at the 95% confidence interval and the data analysis procedure used in this paper was adopted from Kleinbaum et al. (1998) and Mbatu (2006).

The econometric model is expressed as follows (*Eq.1*):

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + ? + \beta_n X_n + E \quad \text{(Eq.1)}$$

where Y is the dependent variable and represents household forest dependence. The eight variables examined in this study and used as dependent variables are included in *Table 1*.

Meanwhile, X_n are the independent variables which represent the potential causes or human forces that influence the variability of dependent variables. These are the household characteristics that affect forest dependence or 20 explanatory variables that have been classified in *Table 1*.

β_o is a constant or intercept and it represents the change in the dependent variable when there is no change in the independent variables.

β_n are the regression coefficients and represent the percentage change in the dependent variable when the independent variables increase by 1%.

E is the error term or error component. It represents the variations of the dependent variable that are not explained by the independent variables included in the model.

Table 1. *Variables used in the econometric model*

Dependent variables (Y)	Independent variables (X_n)
Little or no financial cost to use forests	Gender of household heads: male and female
Forest resource abundance	Age of household heads: 0-21 years, 22-60 years, over 60 years old
Easy access to forest	Residence period in village: 0-5 years, 6-10 years, 11-15 years, 16-20 years, and over 20 years
Absence of alternative energy	Educational level of household heads: university, secondary, primary, and uneducated
Unemployment	Residential status of household heads: indigenous and migrant
Shock avoidance	Marital status of household heads: married, single, divorced, and widowed
Farming ability	
Animal rearing ability	

Results

Age and gender characteristics of the household

Out of the 484 households (164 in Dikome Balue, 96 in Ekondo Titi, 119 in Mundemba and 105 in Toko, respectively), 57% were male-headed while 43% were female-headed, most of whom were either widows or divorced and were in the 21-40 age group. Besides Ekondo Titi — a hotspot for immigrants from other parts of the country, the respondents especially in the villages have lived there for more than 20 years. Therefore, the period of residence in the villages was greater than one year and majority of the respondents were indigenous and also married. For household age

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composition, 18.8% belonged to the 0-21 years age group, 39.6% in the 21-40 years (very active age group), 25.6% in the 41-60 years (active group) and 16% in the 61+ years age (retirement or inactive) age group. Additionally, a minuscule 2.3% respondents had acquired university education, 20.4% had secondary education while 37.3% had completed primary education. Therefore, an average of 40% respondents across the case study villages had no formal education and could neither read nor write. This lack of formal education does not qualify them for formal jobs either with the government or private sector but were actively involved in agriculture and other extractive activities (*Table 2*).

Table 2. *Demographic and socioeconomic characteristics of the household heads across the four study sites. Here, 57% were male-headed while 43% were female-headed, and where 39.6% were in the 21-40 years (very active age group)*

Demographic characteristics	Dikome Balue %	Ekondo Titi %	Mundemba %	Toko %	Total (%) of respondents
Household head males	54.2	67.7	53.8	52.4	57
Household head females	45.8	32.3	46.2	47.6	43
Age of household head: 0-21	20.1	13.5	15.9	25.7	18.8
21-40	41.6	40.6	39.3	37.1	39.6
41-60	24.3	27.1	26.2	24.8	25.6
61+	14	18.8	18.6	12.4	16
Period of residence: 0-5	17.1	17.7	18.5	14.2	16.9
6-10	12.2	12.5	6.7	12.4	11
11-15	15.2	26	9.2	10.5	15.2
16-20	12.2	23	13.4	10.5	14.7
>20	37.2	20.8	52.2	52.4	42.2
Educational level: university	3.1	6.2	0	0	2.3
Secondary school	20.7	16.7	24.3	20	20.4
Primary	31.1	30.2	53.7	34.3	37.3
No education	45.1	46.9	21.8	45.7	40
Indigenous	75.6	77.1	82.4	73.3	77.1
Migrant	24.4	22.9	17.6	26.7	22.9
Marital status: single	24.4	21.9	28.6	15.2	22.5
Married	56.7	66.7	59.6	62	61.2
Divorced	12.2	8.3	10.1	9.5	10
Widowed	6.7	3.1	4.2	13.3	6.3
Cash crop farming	90	93	91	66	85
Food crop farming	97	94	100	100	98
Animal rearing	44	86	55	71	64
NTFPs harvesting	32	88	35	63	54.5
Hunting	14	35	12	34	23.7
Petty business	20	39.5	51	14	31.1
Fishing	7.5	5	7	6	6.4
Public transport	7	14	9	12	10.5
Wood extraction	100	100	100	100	100
Others	5	4	2	1	3

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Occupation, income sources and levels

Households are mainly involved in cash and food crop cultivation with the latter mainly practiced through shifting cultivation and agroforestry systems. Farm sizes were instrumental in the choice of practice engaged by farm households because those having 0.2 to 2.5 hectares (ha) of land mostly cultivated both food and limited amount of cash crops in the so-called agroforestry systems. Meanwhile, cash crop cultivation primarily occurred on farmlands ranging between 2.5 and 10 ha. In agroforestry systems, cash crops (cocoa, oil palm), fruits trees (mango, African plum and oranges) and food crops (cassava, maize, and yams) are intercropped or farmed together.

Aside farming, domestic animals have been part of the culture, tradition and lifestyle of the local populations for as long as they have existed. Many of the households (64%) domesticate animals like fowls, goats, pigs and dogs. This culture, tradition and lifestyle is also embedded in household environmental resources consumption and marketing of forest products including wood, rattan and bamboo products, fruits and nuts; hunting and fishing products such as rodents, birds, snails, tilapia, mudfish and shrimps.

Off-farm income mainly from petty businesses was reported by 31.1% of the surveyed households. In addition, fewer numbers of households corresponding to 3% also earned income from rendering services such as transportation. Other additional income sources particularly during the farming season include shared labour in the form of hire, meeting groups and two-party. *Two party is a practice whereby at harvest time, the crops are shared with the land owner on the basis of usually a verbal agreement* (Mukete et al., 2017). Similarly, households derived 65.9% of their average total household income from farming and animal rearing. Meanwhile, forest products including fuelwood collection and wood harvesting were also important livelihood and household income sources contributing over 34.1% of household income.

From *Table 3,* fruits and nuts include banana, mango, oil palm, plantain, cashew, plum; spices are mostly bush pepper, grains of paradise, ginger; vegetables are pepper, okra, tomato, egusi, garden eggs; root crops refer to tubers such as yams, cocoyams, cassava, and potatoes; cereals comprise simply of corn while others include cocoa, tea, coffee, rubber and sugar cane.

Table 3. *Percentage contribution of farming, animal rearing and forest resources harvesting to household income*

Farming income source	Percentage (%) contribution	Animal income source	Percentage (%) contribution	Forest income source	Percentage (%) contribution
Fruits/nuts	10.6	Fowls	22.5	Medicine	13.2
Spices	11.2	Goats	13.4	Wood harvesting	23.9
Vegetables	19.4	Dogs	8.2	Bamboo products	13.8
Root crops	25.8	Sheep	5.3	Fuelwood	28.3
Cereals	22.4	Ducks	2.1	Rattan products	11.1
Others	10.6	Cats	7.1	Others	9.7
		Snails	20.7		
		Pigs	5.6		
		Others	15.1		
Total percentage (%) contribution	36.7		29.2		34.1

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Aside domestic animals, others include hunted animals such as rodents, birds, monkeys, antelopes, and deer. Meanwhile, forest products include wood harvested for construction, chewing stick, woodwork and charcoal production; bamboo products include mats, bags, thatches, and fishing tools; rattan products include baskets, chairs. Others include ropes, gum, fish, njangsang, bush onion, bush mango.

Drivers of household forest resources dependence

Results indicated that agricultural skill is the most important driver of household forest dependence as reported by 91% of the surveyed households (*Table 4*). Other drivers that influenced forest dependence as mentioned by respondents included easy access to forests in a village, little or no financial cost to use forests, forest abundance, absence of alternative energy sources and unemployment. Statistical analysis also found these drivers to be statistically significant (P-value = 0.000) at the 95% confidence interval although differently across the study area.

Table 4. *Reasons for household forest resources dependence in the study area. Here, agricultural skills or ability to farm was the most important factor responsible for household forest dependence*

Socioeconomic drivers	***Response***	***Number of household heads in subdivision (%)***				***Total response %***
		Dikome Balue	***Ekondo Titi***	***Mundemba***	***Toko***	
Little or no financial cost to use forests	Yes	86	77.6	67	82.6	78.3
	No	14	22.4	33	14.4	21.7
Forest abundance	Yes	78.2	69.3	45.9	78.8	68
	No	21.8	30.7	54.1	21.2	32
Easy access to forest	Yes	92.1	68.5	61.2	96.4	79.5
	No	7.9	31.5	38.8	3.6	20.5
Absence of alternative energy	Yes	81.7	57.8	49.3	88.9	69.4
	No	18.3	42.2	51.7	11.1	30.6
Unemployment	Yes	73.7	48.1	78.6	84.8	71.3
	No	26.3	51.9	21.4	15.2	28.7
Shock avoidance	Yes	82.4	44.3	49	88.3	66
	No	15.6	55.7	51	11.7	34
Agricultural skills	Yes	93.9	95.5	91.2	83	90.9
	No	6.1	4.5	8.8	17	9.1
Animal rearing skills	Yes	44.7	86	55.6	71.2	64.3
	No	55.3	14	45.4	28.8	35.7

Results of the statistical analysis are presented in *Table 5* while causal relationships between drivers of household forest resources dependence and household characteristics are presented in *Table 6*. Each model represents the relationship between a dependent variable and the two independent variables with most significant affects. For instance, easy access to forest resources correlated significantly ($P < 0.01$) with male household heads and also with uneducated household heads (*Table 6*). Furthermore, forest abundance was statistically significant ($P < 0.05$) and correlated significantly with household heads that were over 60 years of age and have acquired just primary education. Similarly, the desire to avoid shocks as a result of poor harvest and job loss

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was significant ($P < 0.01$) and related positively with household heads whose status was single and divorced (*Table 6*).

The people that have lived in study areas for 6 to 10 years and who were also married were the two variables that significantly affected the dependent variable, little or no financial cost to use forests (*Eq. 2*). Here, a 1% increase for households that have lived for 6 to 10 years in the communities, the lower their cost of using forest resources by 3.02%. Therefore, forest dependence is expected to increase especially if majority of the surveyed households have lived in the area for 6 to 10 years. Meanwhile, a 1% increase for household heads that were married would decrease forest dependence by 0.92% although the impact is not statistically significant.

$$Cost = 102 + 3 * R_610 - 0.9 * Married \quad \text{(Eq.2)}$$

Similarly, the two variables – household heads that were over 60 years old and with just primary education have significant impacts on forest resources abundance as expressed in *Equation 3*. Hence, a 1% increase in this age group and education level decreases household dependence on forests by 2.11% and 1% respectively. Therefore, as the number of elders and children increases, forest dependence decreases.

$$Abundance = 139.4 - 2.1 * Age_60 - 1 * Primary \quad \text{(Eq.3)}$$

Easy access to forests is significantly affected by the number of male household heads and people with no education. Contrary to conventional thinking that male-headed households are likely to have more access to forest resources, results indicated that a 1% increase in male headed households also decreases their easy access to forests by 1.93% and by implication their dependence (*Eq. 4a* and *Table 6*). On the other hand, a 1% increase among uneducated household heads resulted to a 1.36% increase in their easy access to forest resources. It shows therefore that, local communities are likely to become more forest-dependent as illiteracy rate increases given that all other factors are held constant (*Eq. 4b* and *Table 6*).

$$Access = 135.3 - 1.9 * M_heads + 1.3 * Uneducated \quad \text{(Eq.4a)}$$

$$Access = -57.5 + 1.9 * F_heads + 1.3 * Uneducated \quad \text{(Eq.4b)}$$

Furthermore, a 1% increase for household heads that were over 60 years of age resulted to a 6.27% decrease on the lack of alternative sources of energy. For household heads that have lived in their respective communities for 11 to 16 years, an increase of 1% also increases their lack of alternative energy sources by 0.58%. This implies that, as the number of household's increases especially for those that have resided in the area for 11 to 16 years and those who also lack alternative energy sources beside the use of wood energy, their dependence on forests will also increase (*Eq. 5*).

$$Alternative\ energy = 160.6 - 6.2 * Age_60 + 0.5 * R_115 \quad \text{(Eq.5)}$$

Based on *Equation 6* and *Table 6*, a 1% increase for households who have resided in the respective communities for a period of 6 to 10 years increases forest dependence

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especially for those that were unemployed by 199.3%. It is observed here that, a 1% increase among uneducated household heads also decreases unemployment by 47.26%.

$$Unemployment = -226.2 + 199.2 * R_610 - 47.2 * Uneducated \quad \text{(Eq.6)}$$

Accordingly, *Equation 7* represents the relationship between the number of people that depended on forests in order to avoid shocks based on two marital status — single and divorced. Results showed that a 1% increase for those that were single decreases their shock avoidance by 4.3% or people who are single are less forest-dependent. Additionally, a 1% increase for divorced households would cause shock avoidance to increase by 12.52%, hence forest dependence is likely to increase as the number of divorced households increases.

$$Shock\ avoidance = 34.7 - 4.3 * Single + 12.5 * Divorced \quad \text{(Eq.7)}$$

In *Equation 8,* the number of people with farming ability was regressed at two levels: households who lived in the study area for 16 to 20 years and who were also married. It was observed that an increase by 1% for households, who have lived in the villages for 16 to 20 years, also increased their farming activities by 1.58%. Therefore, as the number of households who have lived in these communities for 16 to 20 years increases, forest dependence will also increase as a result of farming activities. This is because households search for new farmlands in the forests when the productivity of their current fields shows signs of decrease. On the other hand, a 1% increase for household heads that were married also decreases farming activities by 1.54%.

$$Farming\ ability = 162.1 + 1.5 * R_1620 - 1.5 * Married \quad \text{(Eq.8)}$$

The number of people with animal rearing ability was modeled against the number of people aged 21 years at most and who were also married. Regression results showed that a 1% increase in household heads that are married would cause animal rearing to increase by 4.46%. Therefore, a 1% increase for household heads that were at most 21 years of age increases animal rearing by 0.5%. Thus, forest dependence also increases as the number of young people increases and as more people get married (*Eq. 9*).

$$Animal\ rearing = -218.2 + 0.5 * Age_021 + 4.46 * Married \quad \text{(Eq.9)}$$

Table 5. *Regression analysis on the drivers of household forest resources dependence and where farming ability was the most important driver of household forest dependence*

Variable	*Mean*	*Standard deviation*	*Minimum*	*Maximum*
Little or no financial cost to use forests	78.3	8.285731	67	86
Forest abundance	68.05	15.39231	45.9	78.8
Easy access to forest	79.55	17.32291	61.2	96.4
Absence of alternative energy	69.425	18.88657	49.3	88.9
Unemployment	71.3	16.11976	48.1	84.8
Shock avoidance	66	22.55467	44.3	88.3
Farming	90.9	5.557577	83	95.5
Animal rearing	64.375	18.05849	44.7	86

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Table 6. *Regression results on socioeconomic variables that explained drivers of household forest dependence. Results of the statistical analysis are presented in Tables 5 and 6 with causal relationships between drivers of household forest resources dependence and household characteristics*

Variables	**(1)** ***Little or no financial cost to use forests***	**(2)** ***Abundance***	**(3)** ***Easy access***	**(4)** ***No alternatives***
Res6to10 (Years of residence, 6-10 years)	3.020**	-	-	-
-	(0.162)	-	-	-
Married (Married household heads)	-0.927*	-	-	-
-	(0.108)	-	-	-
AgeOver60 (Household heads over 60 years)	-	-2.114**	-	-6.273***
-	-	(0.0651)	-	(0.0147)
Primary (Household heads with primary education)	-	-1.009**	-	-
-	-	(0.0191)	-	-
MHeads (Male household heads)	-	-	-1.930***	-
-	-	-	(0.000642)	-
Uneducated (Household heads with no education)	-	-	1.360***	-
-	-	-	(0.000381)	-
Res11to15 (Years of residence, 11-15years)	-	-	-	0.582***
-	-	-	-	(0.00624)
Constant	102.0**	139.4***	135.4***	160.6***
-	(6.347)	(0.991)	(0.0345)	(0.215)
Observations	4	4	4	4
R-squared	0.997	1.000	1.000	1.000

Variables	**(5)** ***Unemployment***	**(6)** ***Shock avoidance***	**(7)** ***Farming***	**(8)** ***Animal rearing***
Res6to10 (Years of residence, 6-10years)	199.3***	-	-	-
-	(2.265)	-	-	-
Uneducated (Household heads with no education)	-47.26***	-	-	-
-	(0.532)	-	-	-

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Single (Single household heads)	-	-4.302***	-	-
-	-	(0.0248)	-	-
Divorced (Divorced household heads)	-	12.52***	-	-
-	-	(0.0727)	-	-
Res16to20 (Years of residence, 6-10years)	-	-	1.581***	-
-	-	-	(0.0147)	-
Married (Married household heads)	-	-	-1.545***	4.460***
-	-	-	(0.0195)	(0.00862)
Age0to21 (Household heads aged 0-21 years)	-	-	-	0.503***
-	-	-	-	(0.00682)
Constant	-226.3**	34.70**	162.1***	-218.3***
-	(3.641)	(0.722)	(1.032)	(0.588)
Observations	4	4	4	4
R-squared	1.000	1.000	1.000	1.000

Standard errors in parentheses ***p < 0.01, **p < 0.05, *p < 0.1

Discussion

Household characteristics and forest resource dependence

Households' forest dependence was found to be significantly influenced by characteristics such as gender and the level of education of household heads, age, marital status, and years of residence in the village. As the number of uneducated household heads increases, forest dependence due to easy access to forest resources also increases. This implies that, rural households become more forest-dependent as the illiteracy rate increases. Farm households primarily lack the basic education that could enable them gain any type of formal employment. As such, these households largely depend on agriculture (food and case crop cultivation) and other extractive activities such as the harvesting of NTFPs from the forest and other land uses. Even when farmers are aware of other options for livelihood diversification, translating such knowledge into practice is confronted with socio-cultural, economic, institutional and even environmental constraints. Therefore, with constraints on rural households that depend primarily on land and forests, their dependence on forests resources is a more feasible option to augment household income and improve on livelihoods (Etongo et al., 2015; Mukete et al., 2018b).

Additionally, households' forest dependence due to minimal or no financial costs associated to the use of forest resources was mostly determined by the number of household heads who had lived in the villages for 6 to 10 years and also among those that were married. Therefore, as the number of households who have lived in these villages for 6 to 10 years increased, forest dependence also increased. This is due to the

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absence of other options for livelihood such as in the industrial sector unavailable in most towns of Cameroon. Similarly, household forest dependence as a result of forest resources abundance was determined by the quota of household heads that were over 60 years old with primary education as their highest qualification. Hence, an increase in the number of elderly people and children decreased forest dependence. This may be related to the fact that both groups of people do not have the physical capabilities required for exploiting forest resources especially across a roughed terrain such as the Rumpi hills.

Also, household forest dependence as a result of no alternative source of household domestic energy beside wood energy was observed to be driven by the number of respondents above 60 years and those who had lived in these villages for 11 to 16 years. Just as in the little or no financial cost in the use of forest resources, forest dependence decreased as the people get older. Given the youthful population in the study area, dependence on forest resources is a common process because harvesting of forest products requires not only lots of energy but in most case longer distances have to be cover in the forests — conditions that are likely to be fulfilled by the younger and energetic youthful male population. Therefore, as the male population grow older; their ability to harvest forest resources decreases often due to lack of physical strength and in some cases poor health conditions.

In addition, forest dependence in order to avoid shocks correlated with the number of people who were single and also those that were divorced. This infers that singles are less likely to use forest resources because they have less responsibility in the form of the number of mouths to be fed. On the other hand, forest dependence increased as the number of divorced cases increased. In this case, divorced people may still have mouths to feed and were more likely to avoid shocks (poor crop harvests and job loss) through the use of forest resources for household subsistence.

From another perspective, forest dependence due to subsistent activities such as animal rearing increased as more people got married. Most of the household heads that were married with relatively younger families had low capital and labour inputs. For majority of such households, forest dependence is greatest as nearby bushes and forests are cleared for cropping and harvesting of fodder for livestock. The relatively younger families also bear the brunt of early child-bearing years and face the greatest pressures to increase forest dependence in order to augment their limited labour force which likely insufficient to allow the household to self-sufficient on food crop cultivation (Murphy et al., 1999; Mukete et al., 2018b).

According to Malleson (2000), households that are involved in animal rearing and who have also lived long in the villages are likely to be more forest depended. This is due to the relatively low cost associated with supporting their livelihood strategies. Therefore, forest resources, their availability, access and abundance around rural communities generally favour animal rearing as a livelihood strategy. Related studies Bwalya (2013), Tieguhong et al. (2012) and Angelsen et al. (2014) observed that income and the rate of consumption of forest products by a household were more likely to increase its demand for forest-related livelihood activities.

Economic drivers and household forest resources dependence

Generally, there is a high dependency on forest and farm related income sources which are a reflection of low household annual income, an overview of the differences in income levels and a lack of access to credit schemes as well as government subsidies

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(Mukete et al., 2018a). Estimates on poverty based on the United Nations global poverty index correspond to people living on less than 1.25 USD (United State Dollars) or 625 FCFA (Central African Franc) a day and where majority are located in developing countries particularly in rural areas (Phimmachanh et al., 2015). From these various estimates, 49.2% of the households were found to be poor and belong to the lowest income group of less than 460 USD per annum. This explains the high dependency on forest and agriculture related activities in order for these households to meet their daily livelihood requirements. Also, rural households have not fully engaged off-farm sources of income and a minuscule or in most cases, a complete absence of the industrial sector additionally explain their high dependency on forest and agriculture related activities (Mukete et al., 2016b). Meanwhile, household daily income was estimated in the local currency, the Franc CFA at 1 USD = 500 FCFA (Etongo and Glover, 2012).

From another perspective, the high dependency on the harvesting of forest resources, food and cash crop cultivation, and hunting portrays a higher price advantage with respect to the other income sources at both village and distant markets. These have provided strong incentives for agricultural and forest resource extraction. Additionally, majority of households lack access to credit schemes and government subsidies. As such, they primarily depend on family circles, friends and farmers' groups for loans. This lack of access to finance has also been a driving force for agricultural and forest resource dependence in the Rumpi hills (Malleson, 2000; Mukete et al., 2017). This is also because poverty limits the household's ability to invest in agriculture at a larger scale, thus local household agricultural investments continue to remain at the subsistence level.

Similar studies by Babulo et al. (2009) and Angelsen et al. (2014) found a relationship between household income and resource dependency. These studies observed that low income households were more dependent on natural resources leading to ecological degradation. Meanwhile Kamanga (2009) and Nguyen et al. (2015) observed a high variability in the relationship between poverty and ecological degradation. Narain et al. (2008) and Bwalya (2013) on the other hand found middle income households to be less dependent on forest resource as compared to the poorest and relatively richer households. Also Zenteno et al. (2013) used social ecological systems theory (SES) to analyze change in forest communities in the northern Bolivian Amazon. Their study observed that despite the various changes in both internal and external policies, population growth, competition among households and more profitable economic opportunities still threaten forest resources dependence. Garekae et al. (2017) studied the level of household forest dependency using a range of socio-economic factors around the Chobe Forest Reserve of Botswana. Their study found age and education to significantly influence household forest resource dependence. Their study also showed forest dependence to decrease with increasing respondent age and educational level. Therefore, the youth were likely to be more forest dependent when compared to the elderly age group with higher levels of educational attainment who are likely to be less forest dependent.

Conclusion

It is necessary to integrate household dynamics and surrounding forest conditions in forest related studies especially in remote forest depended communities. This is because

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household reliance on forest resources is conditioned by various household characteristics and forest outcomes — structure, composition and abundance. As such forest dependence by households can be comprehensively evaluated through the lens of household and farm characteristics, access to forests and forest incomes.

Household characteristics such as gender and education of household heads, age, marital status, and years of residence in the village influence household's dependence on forest resources. Aside from household characteristics, other factors particularly related to surrounding forest conditions such as easy access to forests in a village, minimal or no financial costs on the use of forests and forest abundance are likely to influence a household's dependence on forest resources. These household characteristics when considered in isolation did not usually influence forest resources dependence except when considered in combination with surrounding forest conditions. For instance, as the number of uneducated household heads increases, forest dependence due to easy access to forest resources increases. This implies that, rural households become more forest-dependent as the illiteracy rate increases ceteris paribus. Formal education and the level of attainment especially higher education are important not just for formal employment but can position farm households to effectively engage in agriculture in a sustainable way with little reliance on NTFPs collection. But this is not the case in the rural areas in Cameroon and the Rumpi hills in particular as majority of the farm households are uneducated. The capacity building that is to be provided by the agricultural extension workers also run short of staff and contacts with farmers are minimal and not available in most cases.

Meanwhile, household characteristics such as the number of people above 60 years and those who have lived for 11 to 16 years in their villages influenced household forest dependence as a result of the lack of alternative source of energy beside fuelwood. Therefore, forest dependence decreased as the people get older and given the youthful population in the study area, dependence on forest resources is forecasted to increase in the coming decade except a transition from wood to more renewable energy sources becomes evident. Meanwhile, a household's forest dependence is also influenced by its desire to avoid shocks and this is conditioned by household characteristics such as the number of single and divorced households in the village. This implies that singles are less likely to use forest resources because they have less responsibility in the form of the number of mouths to feed.

This study also observed that a household's dependence on forest resources portrays a higher price advantage with respect to the other income sources at both village and distant markets. These have provided strong incentives for agricultural and forest resource extraction because majority of households lack access to credit schemes and government subsidies, but depend primarily on family circles, friends and farmers groups for loans. This lack of access to finance which in turn limits a households' ability for livelihood diversification has also been a driving force for agricultural and forest resource dependence in the Rumpi hills.

The study also corroborates existing scientific literatures that forest resources dependence at the household level is usually conditioned by household characteristics, surrounding forest conditions and prevailing household economic dynamics. It has also brought forward an understanding of the major factors that influence household forest resources dependence especially in remote communities. Some of these factors include household characteristics (gender, educational level, marital status, and age of household heads); surrounding forest conditions (forest resource abundance and easy

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access to forest) and economic dynamics (absence of alternative energy, shock avoidance, unemployment, income sources and levels as well as the ability to farm and rear animals). Using these factors, effective policies can be developed that take into cognizance socioeconomic needs and characteristics of forest dependent communities. These policies will also include a broad-based assessment of local community socioeconomic conditions before the development, implementation and monitoring of forest management and exploitation initiatives.

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APPENDIX

Drivers of household forest resources dependence

The econometric model is expressed as follows:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + ? + \beta_n X_n + E \quad \text{(Eq.1)}$$

where Y is the dependent variable and represents household forest dependence. The eight variables examined in this study and used as dependent variables are included in *Table 1*.
Xn are the independent variables and represent the potential causes or human forces that influence the variability of dependent variables. These are the household characteristics that affect forest dependence or 20 explanatory variables.
βo is a constant or intercept and it represents the change in the dependent variable when there is no change in the independent variables.
βn are the regression coefficients and represent the percentage change in the dependent variable when the independent variables increase by 1 percent.
E is the error term or error component and represents the variations of the dependent variable that are not explained by the independent variables included in the model.

1. For ***Little or no financial cost to use forests:*** cost variable

```
. regress cost R_610 Married

Source | SS df MS  Number of obs = 4
-------------+------------------------------  F (2, 1) = 179.00
Model | 205.386304 2 102.693152  Prob > F = 0.0528
Residual | .573695658 1 .573695658  R-squared = 0.9972
-------------+------------------------------  Adj R-squared = 0.9916
Total | 205.96 3 68.6533333  Root MSE = .75743

------------------------------------------------------------------------------
cost | Coef. Std. Err. t P>|t| [95% Conf. Interval]
-------------+----------------------------------------------------------------
R_610 | 3.019815 .1617292 18.67 0.034 .9648504 5.07478
Married | -.927085 .1084228 -8.55 0.074 -2.304727 .450557
_cons | 102.017 6.346992 16.07 0.040 21.3708 182.6632
------------------------------------------------------------------------------
```

$$Cost = 102 + 3 * R_610 - 0.9 * Married \quad \text{(Eq.2)}$$

2. For ***Forest resource abundance:*** abundance variable

```
. regress abundance Age_60 Primary

Source | SS df MS  Number of obs = 4
-------------+------------------------------  F (2, 1) = 3246.42
Model | 710.660547 2 355.330274  Prob > F = 0.0124
Residual | .109452874 1 .109452874  R-squared = 0.9998
-------------+------------------------------  Adj R-squared = 0.9995
```


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Total | 710.77 3 236.923333 Root MSE = .33084

---
abundance | Coef. Std. Err. t P>|t| [95% Conf. Interval]
---+---
Age_60 | -2.114391 .0651459 -32.46 0.020 -2.942148 -1.286633
Primary | -1.00905 .0191059 -52.81 0.012 -1.251813 -.7662861
_cons | 139.4373 .9911498 140.68 0.005 126.8436 152.0311

$$Forest\ abundance = 139.4 - 2.1 * Age_{60} - 1 * Primary \quad \text{(Eq.3)}$$

3. For ***Easy access to forest:*** access variable (Note: Need to create a categorical variable named Gender to represents male and female household heads)

. regress access M_heads Uneducated

Source | SS df MS Number of obs = 4
---+--- F (2, 1) = .
Model | 900.249944 2 450.124972 Prob > F = 0.0002
Residual | .000055528 1 .000055528 R-squared = 1.0000
---+--- Adj R-squared = 1.0000
Total | 900.25 3 300.083333 Root MSE = .00745

---
access | Coef. Std. Err. t P>|t| [95% Conf. Interval]
---+---
M_heads | -1.929889 .0006424 -3004.08 0.000 -1.938052 -1.921726
Uneducated | 1.359561 .0003809 3569.32 0.000 1.354721 1.364401
_cons | 135.3894 .0345478 3918.90 0.000 134.9504 135.8284

$$access = 135.3 - 1.9 * M_heads + 1.3 * Uneducated \quad \text{(Eq.4a)}$$

. regress access F_heads Uneducated

Source | SS df MS Number of obs = 4
---+--- F (2, 1) = .
Model | 900.249944 2 450.124972 Prob > F = 0.0002
Residual | .000055528 1 .000055528 R-squared = 1.0000
---+--- Adj R-squared = 1.0000
Total | 900.25 3 300.083333 Root MSE = .00745

---
access | Coef. Std. Err. t P>|t| [95% Conf. Interval]
---+---
F_heads | 1.929889 .0006424 3004.08 0.000 1.921726 1.938052
Uneducated | 1.359561 .0003809 3569.32 0.000 1.354721 1.364401
_cons | -57.59947 .0360972 -1595.68 0.000 -58.05813 -57.14081
---

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$$Access = -57.5 + 1.9 * F_heads + 1.3 * Uneducated \quad \text{(Eq.4b)}$$

4. ***For Absence of alternative energy:*** alternative variable

. regress alternative Age_60 R_1115

Source | SS df MS Number of obs = 4
---+--- F (2, 1) =96080.75
Model | 1070.10193 2 535.050966 Prob > F = 0.0023
Residual | .005568763 1 .005568763 R-squared = 1.0000
---+--- Adj R-squared = 1.0000
Total | 1070.1075 3 356.7025 Root MSE = .07462

---
alternative | Coef. Std. Err. t P>|t| [95% Conf. Interval]
---+---
Age_60 | -6.2726 .0146781 -427.35 0.001 -6.459103 -6.086098
R_1115 | .5818325 .0062372 93.28 0.007 .5025817 .6610832
_cons | 160.6146 .215213 746.31 0.001 157.88 163.3491

$$Alternative = 160.6 - 6.2 * Age_60 + 0.5 * R_115 \quad \text{(Eq.5)}$$

5. For ***Unemployment:*** unemployment variable

. regress unemployment R_610 Uneducated

Source | SS df MS Number of obs = 4
---+--- F (2, 1) = 4406.68
Model | 779.45156 2 389.72578 Prob > F = 0.0107
Residual | .08843977 1 .08843977 R-squared = 0.9999
---+--- Adj R-squared = 0.9997
Total | 779.54 3 259.846667 Root MSE = .29739

---
unemployment | Coef. Std. Err. t P>|t| [95% Conf. Interval]
---+---
R_610 | 199.2705 2.265035 87.98 0.007 170.4905 228.0505
Uneducated | -47.25916 .5320716 -88.82 0.007 -54.01977 -40.49855
_cons | -226.2528 3.640868 -62.14 0.010 -272.5144 -179.9912

$$Unemployment = -226.2 + 199.2 * R_610 - 47.2 * Uneducated \quad \text{(Eq.6)}$$

6. For ***Shock avoidance:*** shock variable

. regress shock Single Divorced

Source | SS df MS Number of obs = 4
---+--- F (2, 1) =21448.44

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Model | 1526.10442 2 763.052212 Prob > F = 0.0048
Residual | .035576124 1 .035576124 R-squared = 1.0000
----------+---------- Adj R-squared = 0.9999
Total | 1526.14 3 508.713333 Root MSE = .18862

---
shock | Coef. Std. Err. t P>|t| [95% Conf. Interval]
----------+---
Single | -4.301556 .0247619 -173.72 0.004 -4.616186 -3.986926
Divorced | 12.51899 .0726625 172.29 0.004 11.59572 13.44225
_cons | 34.70121 .721896 48.07 0.013 25.52866 43.87377

$$Shock = 34.7 - 4.3 * Single + 12.5 * Divorced \quad \text{(Eq.7)}$$

7. For ***Farming ability:*** farming variable
. regress farming R_1620 Married

Source | SS df MS Number of obs = 4
----------+---------- F (2, 1) = 5840.30
Model | 92.6520679 2 46.3260339 Prob > F = 0.0093
Residual | .007932127 1 .007932127 R-squared = 0.9999
----------+---------- Adj R-squared = 0.9997
Total | 92.66 3 30.8866667 Root MSE = .08906

---
farming | Coef. Std. Err. t P>|t| [95% Conf. Interval]
----------+---
R_1620 | 1.581469 .0146697 107.80 0.006 1.395072 1.767865
Married | -1.544749 .0194568 -79.39 0.008 -1.79197 -1.297527
_cons | 162.1497 1.032367 157.07 0.004 149.0322 175.2671

$$Farming = 162.1 + 1.5 * R_1620 - 1.5 * Married \quad \text{(Eq.8)}$$

8. For ***Rearing ability:*** rearing variable
. regress rearing Age_021 Married

Source | SS df MS Number of obs = 4
----------+---------- F (2, 1) = .
Model | 978.324048 2 489.162024 Prob > F = 0.0019
Residual | .003451875 1 .003451875 R-squared = 1.0000
----------+---------- Adj R-squared = 1.0000
Total | 978.3275 3 326.109167 Root MSE = .05875

---
rearing | Coef. Std. Err. t P>|t| [95% Conf. Interval]
----------+---

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Age_021 | .5027929 .0068157 73.77 0.009 .4161911 .5893946
Married | 4.46013 .008616 517.65 0.001 4.350653 4.569607
_cons | -218.2605 .5876051 -371.44 0.002 -225.7267 -210.7942

$$Animal\ rearing = -218.2 + 0.5 * Age_021 + 4.46 * Married \quad \text{(Eq.9)}$$

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DIFFERENCES IN SELENIUM UPTAKE AND TRANSPORT, AND RELATED GENE EXPRESSION IN THREE *BRASSICA* VEGETABLES

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Abstract. Selenium (Se) is an essential micronutrient for humans, animals and plant growth. The consumption of plant products containing a high Se concentration, is the most efficient and safe way of Se supplementation. Selenium concentrations in plants vary depending on exogenous selenium levels, plant species and variety. In the recent pot experiment, the effects of exogenous selenium (0, 0.5, 1.0, 2.5 and 5 $mg \cdot kg^{-1}$) on the selenium uptake and transport of three *Brassica* vegetables (*Brassica rapa ssp. chinensis* L., *Brassica rapa ssp. pekinensis* L. and *Brassica oleracea* var. *capitata* L.) were studied. The expression levels of selenium metabolism related genes families, such as APS, APR, SAT, SMT, CysD and MMT, were also detected using qRT-PCR assays. The results showed that selenium concentration in leaves, stems and roots of the three *Brassica* vegetables increased with the increase of soil selenium level. The selenium concentration in the leaves of the three *Brassica* vegetables was the highest at selenium level of 5 $mg \cdot kg^{-1}$. Leaf selenium concentration of *Brassica rapa ssp. chinensis* L. was significantly 1.39-3.02 times higher than that of the other two vegetables. Under the same selenium treatment, selenium concentration, selenium accumulation coefficient and transport coefficient of edible parts of *Brassica* vegetables were the highest in *Brassica rapa ssp. chinensis* L. and the lowest in *Brassica oleracea* var. *capitata* L. At soil selenium level of 5 $mg \cdot kg^{-1}$, the expression levels of SAT, SMT and MMT in leaves of *Brassica rapa ssp. chinensis* L. and the expression levels of SAT, APS, SMT, APR and MMT in leaves of *Brassica pekinensis* L. significantly increased. *Brassica rapa ssp. chinensis* L. had the best selenium enrichment effect among the three *Brassica* vegetables.
Keywords: *selenium, Brassica, bioconcentration factor, qRT-PCR, selenium metabolism related genes*

Abbreviations: Se – selenium, APS – ATP sulfurylase, APR – APS reductase, SAT – Serine acetyltransferase, CysD – Cysteine desulfurase, MMT – S-adenosyl-L-Met:L-Met S-methyltransferase, SMT – selenocysteine methyltransferase, MeSeCys – methylselenocysteine, SeMet – Selenomethionine, OAS – O-acetylserine, BCF – bioconcentration factor, TF – translocation factor, SeMM – methyl-Semethionine, SMM – thiomethyl methionine

Introduction

Selenium (Se), a vital trace element for humans, is important for preventing disease, improving health, and preventing aging and has been considered a life-protecting agent (Gupta et al., 2016). Most soils of the world has a selenium content of 0.01-2.0 $mg \cdot kg^{-1}$, with an average content of 0.4 $mg \cdot kg^{-1}$. In selenium-deficient soil, Se content is less than 0.15 $mg \cdot kg^{-1}$. The world's selenium-deficient population reaches 1.5 billion (Zhu et al., 2009). About 72% of China's land area has varying degrees of selenium deficiency (Zhou et al., 2016). Selenium content of less than 0.05 $mg \cdot kg^{-1}$ in human

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foods results in selenium deficiency, but greater than 5 $mg \cdot kg^{-1}$ can be toxic (Wen, 2006). Inorganic selenium supplementation not only has the problem of low uptake and conversion rate, but also will adversely affect human health because of the narrow safe dose range of selenium (Keskinen et al., 2010; Zhao et al., 2017). Bio-selenium is the safest and most effective way to supplement human selenium (Ellis and Salt, 2003; Song et al., 2017). By absorbing and enriching exogenous selenium and converting it to safe and effective bioorganic selenium, crops improve human or animal selenium nutrition in low and selenium-deficient areas (Zhang et al., 2014; Li et al., 2015; Liu et al., 2017).

Selenium levels in plants vary depending on exogenous selenium levels, plant species and variety (Longchamp et al., 2015; Bañuelos et al., 2016). Under the same soil selenium level, the selenium concentrations in fruits of different tomato varieties were ranked as Luobeiqi > KT30 > Defumm-8 (Zhao et al., 2017). The selenium concentration in shoot, stem and root of *Brassica rapa* Linn L. was higher than that of *Brassica tumida* Tsenet Lee L. at the same soil selenium level (Xie, 2016). However, there are also reports to the contrary. Yawata et al. (2010) conducted a study on three cruciferous crops [Indian mustard (*Brassica juncea*), nozawana (*Brassica rapa* var. *hakabura*) and komatsuna (*Brassica rapa* var. *peruviridis*)] and found that there was no significant difference in selenium concentration among the three crops. However, due to its rapid growth and larger biomass, komatsuna has higher selenium accumulation.

ATP sulfurylase (APS) exerts a rate limiting effect on selenate reduction and selenium assimilation (Matich, et al., 2012; Zhang, 2014). Overexpression of APS in transgenic plants promoted APSe separation into AMP and selenite and increased selenium accumulation (Xie, 2016; Zhao et al., 2017). APR (APS reductase) is the APS reductase gene. Methylation is the major metabolic pathway for selenium in plants. After selenomethionine (SeMet) production, selenium is converted to methylselenocysteine (MeSeCys) by the methylation pathway under the action of selenocysteine methyltransferase (SMT) (Song et al., 2017). SMT activity is closely linked to the high accumulation of selenium in eight species of Astragalus with different selenium enrichment ability (Fang et al., 2017; Schiavon and Pilon-Smits, 2017). McKenzie et al. (2009) reported that 200 μM selenium application resulted in an over-expression of SMT transgene in crops, which increased selenium accumulation in crops by 2-4 times, made MeSeCys content reach 20% of the total selenium, with MeSeCys directly converted to methyl second-generation selenium. In addition, CysD (Cysteine desulfurase), MMT (S-adenosyl-L-Met:L-Met S-methyltransferase) and SAT (Serine acetyltransferase) were also found to be the main genes for plant metabolism and selenium accumulation. Co-expression of SAT and SMT promoted the increase of OAS (O-acetylserine), which in turn promoted the expression of sulfur transporter as well as plant uptake and accumulation of selenium (Çakır et al., 2012; Song et al., 2017).

Brassica has the strongest selenium enrichment ability (Wiesner-Reinhold et al., 2017). At present, there are many reports on selenium-enriched crops at home and abroad (Matich, et al., 2012; Zhao et al., 2017). However, there are few reports of Se enrichment difference and Se related gene expression in *Brassica* vegetables (Xie, 2016). In this experiment, *Brassica rapa ssp. chinensis* L. (pakchoi), *Brassica rapa ssp. pekinensis* L. (Chinese cabbage) and *Brassica oleracea* var. *capitata* L. (cabbage) were used as materials to compare the differences in selenium uptake, selenium transformation and transport among the three *Brassica* vegetables. Also, selenium metabolism related gene expression of the three *Brassica* vegetable s was tested.

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Materials and methods

Plant material, soil and Se treatments

The seeds of *Brassica rapa ssp. chinensis* L., *Brassica rapa ssp. pekinensis* L. and *Brassica oleracea* var. *capitata* L. were purchased from Xiema farmer's market in Beibei district, Chongqing, China. The experimental soil was taken from Jiulongpo District of Chongqing, China. The soil pH was 6.70, soil organic matter was 9.08 $g \cdot kg^{-1}$, soil total nitrogen was 0.64 $g \cdot kg^{-1}$, soil available nitrogen was 72.77 $mg \cdot kg^{-1}$, soil available phosphorus was 54.81 $mg \cdot kg^{-1}$, soil available potassium was 216.7 $mg \cdot kg^{-1}$, soil cation exchange capacity was 30.3 $cmol \cdot kg^{-1}$, soil total selenium was 0.42 $mg \cdot kg^{-1}$ and soil available selenium was 0.029 $mg \cdot kg^{-1}$.

Pot experiment was conducted at No. 1 glass greenhouse of College of Resources and Environment of Southwest University, Beibei District, Chongqing, China (106.4N, 29.8E), from November 4, 2014 to February 25, 2015. Five selenium levels (0, 0.5, 1.0, 2.5 and 5 $mg \cdot kg^{-1}$) prepared from Na_2SeO_3 were set up. Considering the larger biomass of *Brassica oleracea* var. *capitata* L. than *Brassica rapa ssp. chinensis* L. and *Brassica rapa ssp. pekinensis* L., two $plant \cdot pot^{-1}$ of *Brassica oleracea* var. *capitata* L., three $plant \cdot pot^{-1}$ of *Brassica rapa ssp. chinensis* L. and *Brassica rapa ssp. pekinensis* L. respectively were planted in black plastic basin (25 cm in diameter and 17 cm in height), each basin was loaded with 5 kg of air-dried soil screened with 5 mm sieve. The total dosage of nitrogen fertilizer, phosphate fertilizer and potassium fertilizer was 180 $mg \cdot kg^{-1}$, 100 $mg \cdot kg^{-1}$ and 150 $mg \cdot kg^{-1}$, respectively. Ammonium nitrate, monopotassium phosphate and potassium sulphate were used as nitrogen, phosphate and potassium fertilizers, respectively. Each treatment was repeated 3 times and arranged randomly. During the cultivation period, pure water was used to maintain soil water content at 60% -80% of the maximum water holding capacity of the field. Considering the longer growth period of *Brassica oleracea* var. *capitata* L., *Brassica oleracea* var. *capitata* L. was harvested after 85 days, and *Brassica rapa ssp. chinensis* L. and *Brassica rapa ssp. pekinensis* L. were harvested after 65 days. Edible parts of vegetables were taken, quick-frozen by liquid nitrogen and placed in -80℃ refrigerator for later RNA extraction.

Analysis of soil physicochemical properties

The soil pH was ascertained in 1:5 (soil: water), and available phosphorus, available potassium and total nitrogen in soil was determined in term of a previously report (Rayment and Higginson, 1992). The soil cation exchange capacity and soil organic matter content was determined on the basis of a previously published method (McCleod, 1975).

Analysis of Se concentrations in soil and plants

Soil and plant samples were ground and digested at 170°C using 10 mL of acid mixture (8 mL of ultrapure nitric acid and 2 mL of perchloric acid) (Kyodan et al., 1988). The acid mixture was heated until white smoke appeared; further, 10 mL of 6 $mol \cdot L^{-1}$ hydrochloric acid was added, and the mixture was heated until the white smoke reappeared (Kyodan et al., 1988). The residual sample was then diluted in 25 mL of ultrapure water (Kyodan et al., 1988). The concentration of Se in the solution was

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analyzed using hydride generation atomic fluorescence spectrometry (PF6.3, Beijing Purkinje General Instrument Co. Ltd., China).

Detection of gene expression

RNA was extracted from edible part of the sample stored in the refrigerator at -80 ℃. The specific procedure followed the operation manual of the RNA extraction kit purchased from Tiangen Biotechnology Company (Beijing, China). Purification of total RNA, RNA detection, synthesis of reverse transcription cDNA, primer design and synthesis, PCR amplification of cDNA and real-time quantitative PCR were done according to Zhao et al. (2017) method.

The statistical analysis

Data were analyzed by one-way analysis of univariate ANOVA and followed by Duncan's multiple comparison tests using SPSS version 21.0 (IBM Corp., Armonk, NY, USA). The variables analyzed separately were Se concentration, bioconcentration factor (BCF) and translocation factor (TF) of Se in three *Brassica* vegetables. The BCF refers to the ratio of the Se concentration in tissues to the exogenous Se. TF is the ratio of Se concentration in shoots to that in roots (Renkema et al., 2011). The level of significance was 0.05.

Results

Selenium concentration

As can be seen from *Figure 1,* the selenium concentration in leaves, stems and roots of the three *Brassica* vegetables increased with the increase of soil selenium level. The selenium concentration in leaves, stems and roots of the three *Brassica* vegetables was the highest at the maximum level of soil selenium (5 $mg \cdot kg^{-1}$). At this time, leaf selenium concentration of *Brassica rapa ssp. chinensis* L. was significantly higher than that of the other *Brassica* vegetables by 1.39-3.02 times. Under the same selenium level, selenium concentration in stem and root of *Brassica oleracea* var. *capitata* L. was the lowest among the three *Brassica* vegetables.

Selenium transport ability

In order to better compare the differences in selenium uptake, transport and accumulation among the main *Brassica* vegetables, bioconcentration factor (BCF) and transport factor (TF) were introduced in this study to characterize the enrichment and transport of selenium in different *Brassica* vegetables. A higher BCF value indicates stronger selenium enrichment ability. A higher TF value suggests stronger ability of plants to transport selenium absorbed by the roots to shoot.

As can be seen from *Table 1,* $BCF_{leaves/soil}$ of *Brassica rapa ssp. chinensis* L. reached the maximum at 0.5 $mg \cdot kg^{-1}$ selenium, and decreased with the increase of soil selenium level. Selenium treatment had no significant effect on the $BCF_{leaves/soil}$ of *Brassica rapa ssp. pekinensis* L. and *Brassica oleracea* var. *capitata* L. With the increase of soil selenium levels, $BCF_{stem/soil}$ of three *Brassica* vegetables firstly decreased and then increased, reaching the highest at 0.5 $mg \cdot kg^{-1}$ selenium.

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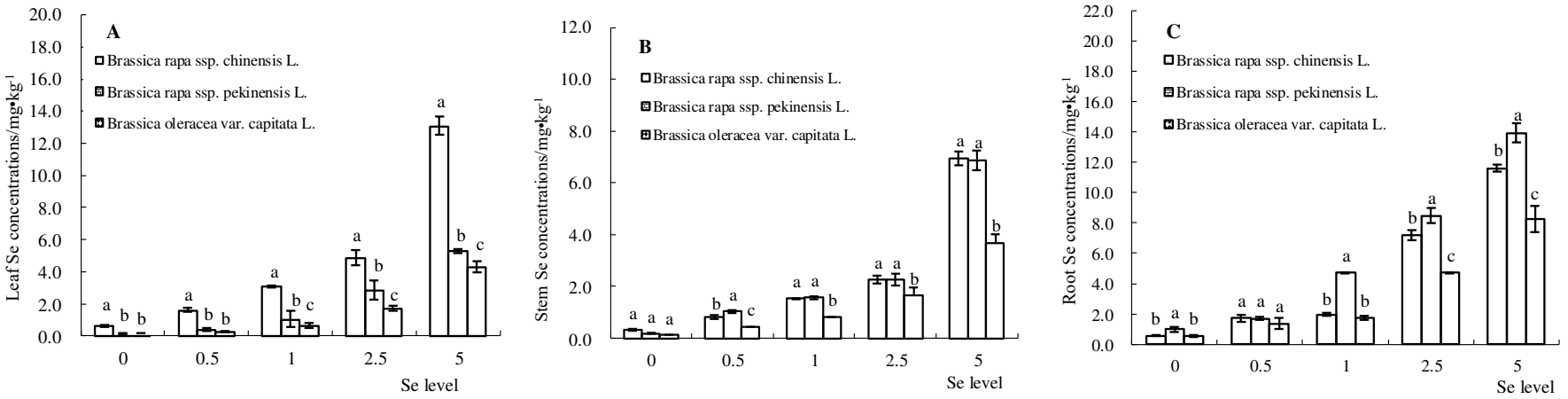


Figure 1. *Effects of different concentrations of selenium on the selenium concentrations of leaf, stem and root in different Brassica vegetables. Different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different species.*

Table 1. *Bioconcentration factor (BCF) and translocation factor (TF) of Se in different Brassica vegetables*

Se level /mg·kg^{-1}	$BCF_{leaevs/soil}$			$BCF_{stem/soil}$			$BCF_{root/soil}$			TF		
	Brassica rapa ssp. chinensis L.	*Brassica rapa ssp. pekinensis* L.	*Brassica oleracea* var. *capitata* L.	*Brassica rapa ssp. chinensis* L.	*Brassica rapa ssp. pekinensis* L.	*Brassica oleracea* var. *capitata* L.	*Brassica rapa ssp. chinensis* L.	*Brassica rapa ssp. pekinensis* L.	*Brassica oleracea* var. *capitata* L.	*Brassica rapa ssp. chinensis* L.	*Brassica rapa ssp. pekinensis* L.	*Brassica oleracea* var. *capitata* L.
0	-	-	-	-	-	-	-	-	-	-	-	-
0.5	3.24±0.24a	0.80±0.95b	0.50±0.67c	1.66±0.16b	2.06±0.14a	0.89±0.03c	3.46±0.46a	3.39±1.0a	2.71±0.70b	1.43±0.30a	0.93±0.52b	0.56±0.38c
1.0	3.09±0.06a	1.05±0.50b	0.69±0.14c	1.52±0.02a	1.56±0.06a	0.66±0.12b	1.98±0.08b	4.72±0.03a	1.89±0.01b	2.34±0.10a	0.55±0.09c	0.72±0.01b
2.5	1.95±0.18a	1.15±0.24b	0.65±0.16c	0.91±0.07a	0.90±0.09a	0.84±0.05a	2.88±0.33b	3.40±0.19a	1.73±0.65c	0.99±0.02a	0.60±0.06b	0.94±0.42a
5.0	2.62±0.11a	1.06±0.03b	0.86±0.01c	1.39±0.01a	1.37±0.07a	0.74±0.07b	2.32±0.31b	2.79±0.61a	1.65±0.25c	1.74±0.27a	0.89±0.16b	0.99±0.20b

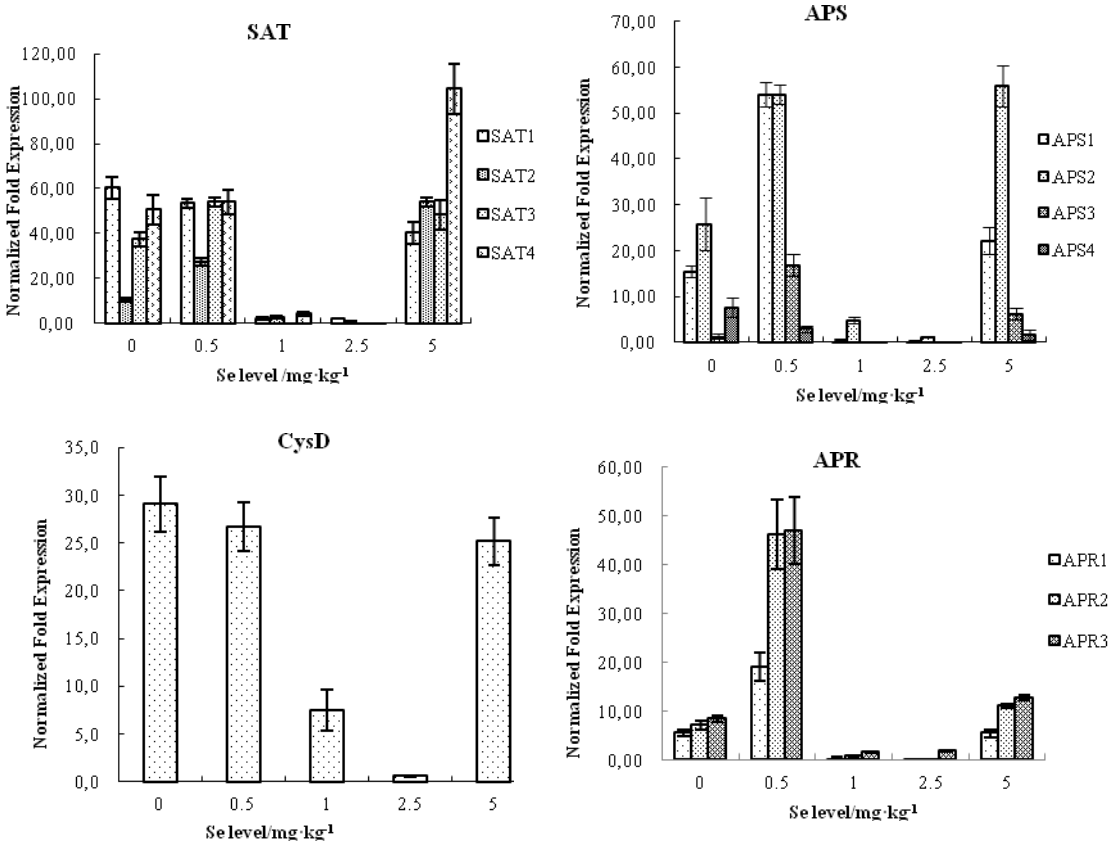
Different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different species. "-" indicates selenium concentrations below the detection limit (0.005 mg·kg^{-1}). The results listed are mean ± standard deviation.

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With the increase of soil selenium level, $BCF_{root/soil}$ of the three vegetables decreased first, reaching the lowest value at 1 $mg \cdot kg^{-1}$ soil selenium level, and then increased again, but still lower than that of non-selenium treatment. $BCF_{root/soil}$ of *Brassica oleracea* var. *capitata* L. was significantly reduced at high levels of selenium (5 $mg \cdot kg^{-1}$), which was 39.01% lower than in the case of 0.5 $mg \cdot kg^{-1}$ selenium level. Selenium transport coefficient of *Brassica rapa ssp. chinensis* L. and *Brassica rapa ssp. pekinensis* L. increased with the increase in exogenous selenium, indicating that greater soil selenium levels enhanced the ability of the two *Brassica* vegetables to transport selenium from the root to the shoot. On the contrary, *Brassica oleracea* var. *capitata* L. showed the opposite trend. For selenium transport coefficient (TF), that of *Brassica rapa ssp. chinensis* L. > *Brassica rapa ssp. pekinensis* L.≈*Brassica oleracea* var. *capitata* L., indicating that among the three tested *Brassica* vegetables, *Brassica rapa ssp. chinensis* L. has the strongest ability to transport selenium to shoot. In comparison of selenium enrichment factor of edible parts among the three *Brassica* vegetables, *Brassica rapa ssp. chinensis* L. ranked the top, while *Brassica oleracea* var. *capitata* L. was at the bottom.

Expression of selenium metabolism related gene

As can be seen from *Figure 2,* at soil selenium level ≤ 0.5 $mg \cdot kg^{-1}$, the SAT expression level was not significant in the leaves of *Brassica rapa ssp. chinensis* L. When the soil selenium level increased to 1 $mg \cdot kg^{-1}$, SAT expression level decreased drastically, reaching the lowest at 2.5 $mg \cdot kg^{-1}$ selenium, but significantly increased at 5 $mg \cdot kg^{-1}$ soil selenium level.



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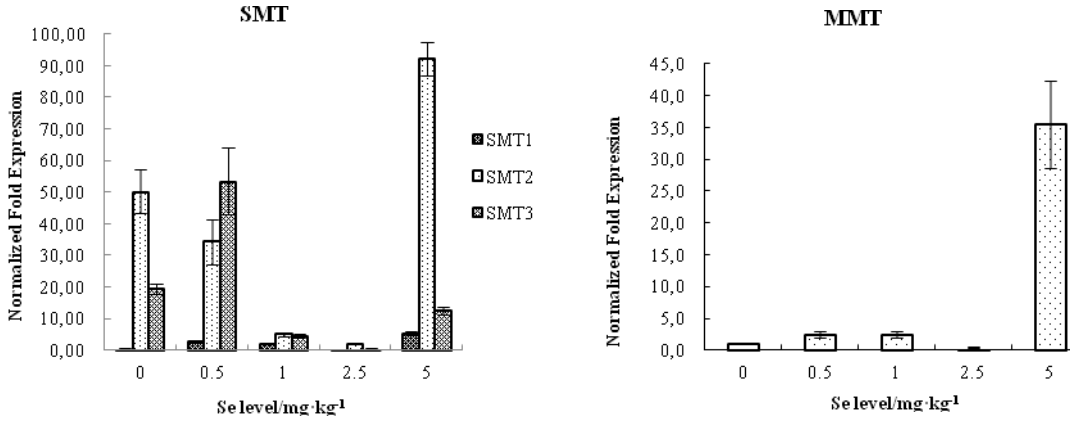


Figure 2. *Real-time PCR analysis of Se metabolism associated genes in leaf of Brassica rapa ssp. chinensis L.*

The expression level of SAT4 gene was the highest in SAT genes family of *Brassica rapa ssp. chinensis* L. at the same selenium level. Exogenous selenium inhibited the expression of APS4. At 0.5 mg·kg^{-1} soil selenium level, the expression levels of APS1, APS2 and APS3 increased by 1.10-12.20 times compared with those of non-selenium treatment. At soil selenium level ≥ 1 mg·kg^{-1}, expression level of APS was in order of APS2 > APS1 > APS3 > APS4 under the same selenium treatment. CysD gene expression level decreased first with the increase of soil selenium level, reaching the lowest level at 2.5 mg·kg^{-1} selenium, and then increased. However, CysD expression level in all selenium treatments was lower than that of non-selenium treatment. With the increase of soil selenium level, APR gene expression level first increased, reaching the highest level at 0.5 mg·kg^{-1} selenium, and then decreased. Under the same selenium treatment, APR expression level was ranked as APR3 > APR2 > APR1. With the change of soil selenium level, SMT2 expression level was the lowest at 2.5 mg·kg^{-1}, reaching the peak at 5 mg·kg^{-1}. SMT2 expression level was 1.84 times that of non-selenium treatment. Variation trend of SMT1 and SMT3 gene expression was consistent with that of APR. At soil selenium level ≤ 2.5 mg·kg^{-1}, there was no significant difference in MMT gene expression level. When selenium level increased to 5 mg·kg^{-1}, MMT expression level significantly increased, which was 33.42 times that of non-selenium treatment. Except MMT, selenium metabolism-related gene expression in leaves of *Brassica rapa ssp. chinensis* L. was inhibited at 1 mg·kg^{-1} and 2.5 mg·kg^{-1} selenium.

As can be seen from *Figure 3,* at soil selenium level ≤ 2.5 mg·kg^{-1}, expression levels of SAT, APS, SMT and APR in leaves of *Brassica rapa ssp. pekinensis* L. were low, but increased significantly at 5 mg·kg^{-1} selenium level. The expression level of SAT2, SAT4, APS2, SMT2 and APR2 was found the highest in the SAT, APS, SMT and APR genes family respectively. Exogenous selenium inhibited CysD expression level in leaves of *Brassica rapa ssp. pekinensis* L. MMT expression level first decreased with the increase of soil selenium level, reaching the lowest at 1 mg·kg^{-1} selenium level, and then increased, reaching the highest level at 5 mg·kg^{-1} soil selenium level.

As can be seen from *Figure 4,* except that APR1 expression in leaves of *Brassica oleracea* var. *capitata* L. was inhibited by exogenous selenium, the expression of other genes in leaves of *Brassica oleracea* var. *capitata* L. increased and then decreased with

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the increase of soil selenium levels, reaching the highest at 0.5 mg·kg^{-1} selenium level. The APS expression in wild cabbage leaves was the highest among all the six genes, and APS4 expression level in leaves of *Brassica oleracea* var. *capitata* L. was up to 994.62 at 0.5 mg·kg^{-1} selenium.

The qRT-PCR assays of all Se metabolism related genes showed that the expression levels of detected genes in *Brassica oleracea* var. *capitata* L. were more severely regulated than that in other two vegetables at the same soil Se level. Moreover, the expression levels of all detected genes in *Brassica oleracea* var. *capitata* L. was much higher than that in other two vegetables even at soil selenium level ≤ 2.5 mg·kg^{-1} (*Figs. 2, 3* and *4*). It indicated that *Brassica oleracea* var. *capitata* L. might be more tolerant to exogenous Se and may has more complicate Se metabolism processes than *Brassica rapa* ssp. *chinensis* L. and *Brassica rapa* ssp. *pekinensis* L.

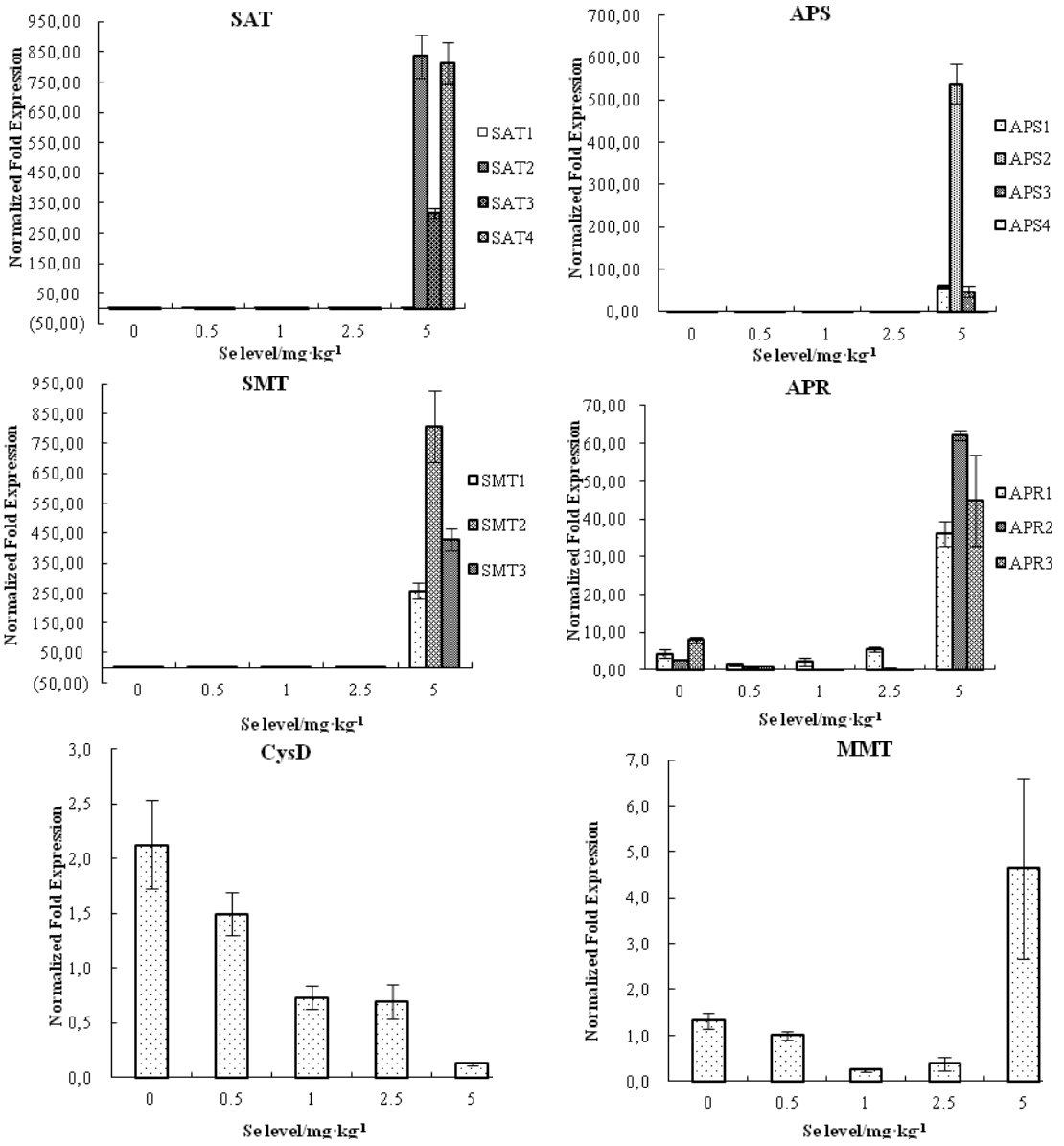


Figure 3. *Real-time PCR analysis of Se metabolism associated genes in leaf of Brassica rapa ssp. pekinensis L.*

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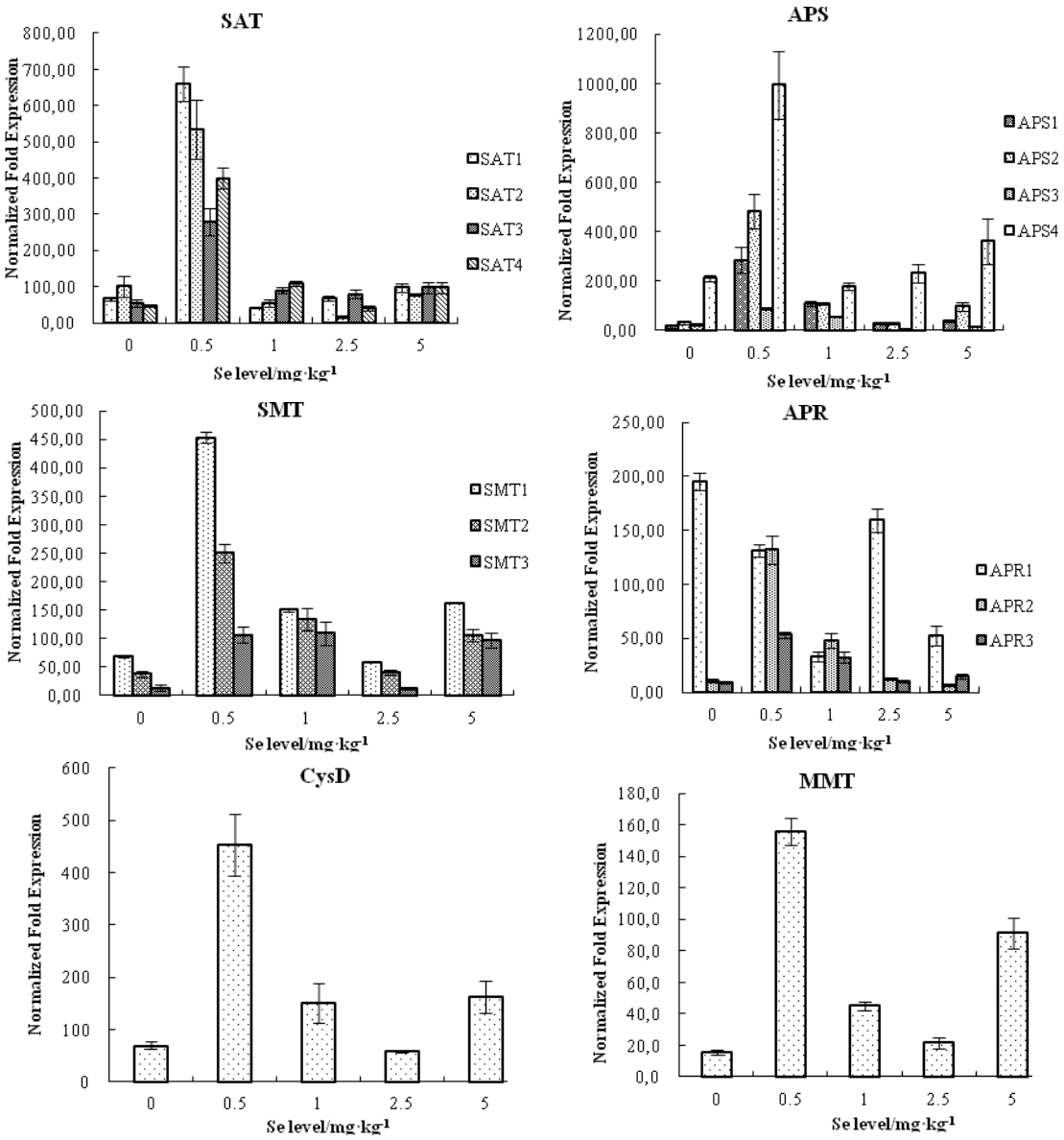


Figure 4. *Real-time PCR analysis of Se metabolism associated genes in leaf of Brassica oleracea var. capitata L.*

Discussion

In pot experiment, the accumulation of selenium in *Brassica* vegetables increased with the increase of soil selenium level, but selenium was extravagantly absorbed in *Brassica* vegetables at 5 mg·kg^{-1} selenium. This result was similar to that reported by Zhao et al. (2017). The uptake of selenium by plants varies according to the plant species (Šindelářová et al., 2015). For the three *Brassica* vegetables, *Brassica rapa ssp. chinensis* L. had the highest selenium uptake, while *Brassica oleracea* var. *capitata* L. had the lowest, probably because selenium action sites differ for different vegetables. To discover the specific reasons, in depth study is needed of the expression levels of selenium metabolism related genes.

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Much of the selenite absorbed remains in the roots, with only a small fraction transported to the stems and leaves (Wiesner-Reinhold et al., 2017). In this experiment, $BCF_{root/soil} > BCF_{leaf/soil}$ of *Brassica rapa ssp. pekinensis* L. and *Brassica oleracea* var. *capitata* L. suggested that the selenium absorbed by the two *Brassica* vegetables mainly accumulated in the roots. $BCF_{stem/soil} < BCF_{root/soil} < BCF_{leaf/soil}$ of *Brassica rapa ssp. chinensis* L. indicated that it was more able to transport selenium to shoots. Moreover, during selenium transportation in the body of *Brassica rapa ssp. chinensis* L., there was no hindrance in selenium transport from root to leaf. In comparison among the three *Brassica v*egetables, $BCF_{leaevs/soil}$, $BCF_{stem/soil}$ and transport coefficient (TF) were the lowest in *Brassica oleracea* var. *capitata* L., indicating that it had relatively weak capacity of selenium uptake and selenium transport. In this experiment, selenium content, selenium enrichment coefficient and transport coefficient was the highest in *Brassica rapa ssp. chinensis* L. among the three *Brassica* vegetables, so *Brassica rapa ssp. chinensis* L. had the best selenium enrichment among the three *Brassica* vegetables.

Selenite has been found to be bioabsorbable by high-affinity proactive transport mechanism of ATP. Overexpression of ATP sulfurylase in transgenic plants accelerates this process and increases selenium tolerance and accumulation (Araie et al., 2008). Leduc et al. (2006) team over-expressed mustard ATP sulfurylase and SMT genes in Indian mustard at the same time, finding that mustard selenium content increased by 4-9 times. Terry et al. (2000) obtained transgenic plants that had increased selenium tolerance and enrichment by overexpression of seleno methyltransferase (SMT) in Arabidopsis and Indian mustard. In this experiment, ATP and APS expression levels of *Brassica rapa ssp. chinensis* L. and *Brassica oleracea* var. *capitata* L. increased significantly under selenium treatment. Meanwhile, exogenous selenium also increased SMT gene expression level, but high selenium level ($\leq$2.5 $mg \cdot kg^{-1}$) would down-regulate transcription level of SMT gene mRNA in *Brassica oleracea* var. *capitata* L., showing that SMT could enhance the tolerance of vegetables to selenium under high-selenium treatment, thus increasing the accumulation of selenium. SAT and CysD are two important enzymes involved in the synthesis and regulation of cysteine, and the two combine to form cysteine synthase (Çakır et al., 2012). In this experiment, SAT expression level in leaf of *Brassica rapa ssp. pekinensis* L. firstly increased and then decreased with the increase of soil selenium level. Exogenous selenium inhibited CysD expression in leaf of *Brassica rapa ssp. pekinensis* L., which may be due to the competitive effect of selenium and sulfur that caused inorganic sulfur hare to mix with cysteine, thus the transcription level of CysD gene mRNA was down-regulated. The expression levels of leaf genes SAT and CysD in the other two *Brassica* vegetables showed the same trend under the same treatment, probably due to the synergistic effect of the complexes formed by the two in plant cells (Liu et al., 2017). Methionine methyltransferase plays a key role in the production of volatile compounds in plants. SeMet and Met produce methyl-Semethionine (SeMM) and thiomethyl methionine (SMM) by homocysteine methyltransferase and methionine methyltransferase (MMT), and these compounds have toxic effects on non-selenium plants (Araie et al., 2008). In this experiment, the expression of MMT in leaves of *Brassica rapa ssp. chinensis* L. and *Brassica rapa ssp. pekinensis* L. was not significant at soil selenium level $\leq$ 2.5 $mg \cdot kg^{-1}$, but significantly increased at soil selenium level of 5 $mg \cdot kg^{-1}$. Under the same selenium level, the expression level of the same gene in different vegetables was significantly different, which may be related to the different transcriptional levels of mRNA.

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Conclusion

The selenium concentrations in leaves, stems and roots of the three *Brassica* vegetables increased with the increase of soil selenium level, and selenium concentration in leaves was the highest at soil selenium level of 5 $mg \cdot kg^{-1}$. At 5 $mg \cdot kg^{-1}$ selenium level, the expression levels of SAT, SMT and MMT in leaves of *Brassica rapa ssp. chinensis* L. and the expression levels of SAT, APS, SMT, APR and MMT in leaves of *Brassica rapa ssp. pekinensis* L. were significantly increased, while expression levels of SAT, APS, SMT, APR, MMT and CysD in leaves of *Brassica oleracea* var. *capitata* L. were significantly decreased. Under the same exogenous selenium level, the selenium concentration, selenium enrichment coefficient and transport coefficient of edible parts were highest in *Brassica rapa ssp. chinensis* L. and the lowest in *Brassica oleracea* var. *capitata* L. among the three *Brassica* vegetables. *Brassica rapa ssp. chinensis* L. had the best selenium enrichment effect among the three *Brassica* vegetables. However, the field application of selenium-enriched vegetables should be studied in the future.

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DIFFERENCE OF CD ENRICHMENT AND TRANSPORT IN ALFALFA (*MEDICAGO SATIVA* L.) AND INDIAN MUSTARD (*BRASSICA JUNCEA* L.) AND CD CHEMICAL FORMS IN SOIL

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Abstract. In order to compare the application potential of alfalfa (*Medicago sativa* L.) and Indian mustard (*Brassica juncea* L.) on cadmium contaminated soil, difference in dry weight, Cd content and accumulation, transport and enrichment coefficient between alfalfa and Indian mustard were studied under different Cd levels (0, 75, 150, 300 and 600 $mg \cdot kg^{-1}$) using soil culture test. Also, Cd forms in soil were analyzed. The results showed that the dry weight of shoots and roots of alfalfa and Indian mustard decreased significantly ($P < 0.05$) with the increase of soil Cd levels, decreasing by 50.00-71.90% and 29.60-59.30% (alfalfa), 59.60-89.00% and 64.30-74.80% (Indian mustard) compared with the control. With the increase of soil Cd level, Cd concentration and Cd accumulation in shoots and roots of alfalfa and Indian mustard increased significantly ($P < 0.05$). At same soil Cd level, Cd concentration in shoots and roots was in order of alfalfa > Indian mustard, while Cd accumulation was in order of Indian mustard > alfalfa. At the soil Cd level of 75 $mg \cdot kg^{-1}$, Cd concentration in shoots of alfalfa and Indian mustard exceeded the critical value of Cd hyperaccumulator (100 $mg \cdot kg^{-1}$), 356.463 $mg \cdot kg^{-1}$ and 308.735 $mg \cdot kg^{-1}$ respectively. The enrichment coefficient (BCF) of alfalfa and Indian mustard was above 1 under all Cd levels, and the transport coefficient (TF) and BCF was in order of Indian mustard > alfalfa. Based on comprehensive consideration of biomass, Cd accumulation, transport coefficient and enrichment coefficient, Indian mustard is more suitable as repair material for soil Cd pollution.
Keywords: *soil Cd pollution, Cd hyperaccumulator, transfer coefficient, enrichment coefficient, soil Cd forms*

Introduction

Cadmium (Cd) is the top soil heavy metal pollutant. According to statistics, about 30,000 tons of Cd is released into the environment each year in the world, and of which Cd released into the soils reaches about 82-94% (Xiong et al., 2015). The total amount of Cd emitted into the environment from industrial waste is 680 tons per year in China (Ning et al., 2015). Cadmium pollution in farmland is 280,000 hm^2, and annual output of agricultural products with excessive Cd is nearly 1.5 million tons in China (Li et al., 2017b; Liu et al., 2017). Cadmium can be absorbed by plants and can be enriched in the human body through the food chain, endangering human health. Therefore, soil Cd pollution control and remediation has drawn wide attention from researchers (Li et al., 2017b; Marzban et al., 2017).

Indian mustard (*Brassica juncea* L.) is cruciferous *Brassica* with short growth cycle and great aboveground biomass. Capable of enriching a variety of heavy metals. When Cd was added at 0-200 $mg \cdot kg^{-1}$, the biomass of Indian mustard was unaffected, and

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shoot Cd concentration was up to 7.824-102.672 mg·kg^{-1}, and root Cd concentration was up to 0.374-191.910 mg·kg^{-1} (Yang et al., 2011). Alfalfa (*Medicago sativa* L.) a leguminous perennial herb, is a high-yielding cash crop. Sun and Song (2015) reported that Cd concentration below 25 mg·kg^{-1} can promote the growth of alfalfa shoot and root. With the increase of Cd level in soil, Cd concentrations in alfalfa shoot and root increase, and also has a higher enrichment effect on Cd ions. At present, there is little research on comparison of Cd uptake by alfalfa and Indian mustard. Therefore, in this study, the differences in biomass, Cd concentration and accumulation in alfalfa and Indian mustard, as well as Cd forms in soil were studied using soil culture test with different Cd concentrations, and plant suitable for soil Cd pollution remediation was screened out.

Materials and methods

Plant material, soil and Cd treatments

The seeds of alfalfa (*Medicago sativa* L.) were purchased from Jiangxi Scarecrow Agricultural Park, and the seeds of Indian mustard (*Brassica juncea* L.) were purchased from Angoo Plant Science and Technology, China. The soil was collected from the purple soil base of Southwest University in Beibei District of Chongqing, China. The soil pH was 5.23, the content of organic matter was 8.87 g·kg^{-1}, that of available phosphorus was 38.59 mg·kg^{-1}, that of available potassium was 65.07 mg·kg^{-1}, that of total nitrogen was 1.54 g·kg^{-1}, that of available nitrogen was 74.60 mg·kg^{-1}, that of total Cd was 0.110 mg·kg^{-1}, and that of available Cd < 0.005 mg·kg^{-1}.

The soil culture experiment was conducted in the glass greenhouse of College of Resources and Environment of Southwest University, China from March to May in 2015. An opaque black plastic basin (25 cm in diameter and 17 cm in height) was used in the experiment. The 5 kg of air-dried soil screened by 5 mm sieve was loaded in each basin. The $CdCl_2 \cdot 2.5\ H_2O$ solution was added to the soil and mixed well. The soil Cd^{2+} pollution levels were simulated at 0, 75, 150, 300 and 600 mg·kg^{-1}, and kept balance for two weeks. The seedlings of Indian mustard and alfalfa with 5 cm high were then transplanted 6 and 10 plants for each pot. The moisture content in soil was kept 70-75% of the maximum moisture in the fields with deionized water. Fast- measurement of Soil Moisture (TZS-IW, Zhejiang Tuopu Instrument Co., Ltd., China) was used to determine the moisture content in soil. In the basic fertilizer the used amount of P ($NH_4H_2PO_4$) and K (KCl) were 100 and 150 mg·L^{-1} respectively and Nitrogen content ($NH_4\ H_2\ PO_4$ and urea) was 180 mg·L^{-1}. N fertilizer was applied in three installments: 40% for basal and 60% each for seedling stages which applied with 15 days' interval after transplanting, each time 30%. All experiments were performed in triplicate and arranged at random. After 60 days, 6 (Indian mustard) and 10 (alfalfa) plants for each pot were harvested, and were separated into shoots and roots, washed free of soil with tap water and then rinsed with 0.1 M HCl followed by several rinses with deionized water (Kachenko and Singh, 2006). The harvested plants were oven-dried at 105 °C for 15 min, and oven drying to constant weight in 60 °C.

Analysis of soil physicochemical properties

The soil pH was ascertained in 1:5 (soil:water), and available phosphorus, available potassium and total nitrogen in soil was determined in term of a previously report

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(Rayment and Higginson, 1992). The soil organic matter content was determined on the basis of a previously published method (McCleod, 1975).

Analysis of Cd concentration in soil and plants

Soil containing Cd was first boiled with HNO_3–$HClO_4$ (*v*:*v* = 4:1), and its concentration was determined using an atomic absorption spectrophotometer (SIMMA 6000; PerkinElmer, Norwalk, CT, USA). The plant samples were first air-dried and ground and the Cd concentration in the plants was determined using a similar method to that used to quantify the levels of Cd in the soil. The results were monitored for quality control in following with plant standard reference material (GBW08513) and soil (GBW08303) obtained from the National Institute of Standards and Technology, China. The recovery rates of all the plants and soils were higher than 95%, and the relative standard deviation (RSD) for the precision of the tests was less than 10%.

1. Transport coefficient (TF) = Cd concentration in shoot ($mg \cdot kg^{-1}$) / Cd concentration in root ($mg \cdot kg^{-1}$) (Sun et al., 2016).
2. Enrichment coefficient (BCF) = Cd concentration in shoot (root) ($mg \cdot kg^{-1}$) / soil Cd ($mg \cdot kg^{-1}$) (Feng et al., 2016).

Soil Cd form determination

Method proposed by Tessier et al. (1979) was used to extract Cd from soil. The Cd concentration of each form was determined by atomic absorption spectrophotometry (Perkin Elmer SIMMA 6000, Norwalk, USA). The detection limit of atomic absorption spectrophotometer was 0.005 $mg \cdot kg^{-1}$. Soil standard substance (GBW # 08303) from the National Institute of Standards and Technology was used to monitor the quality of the results. The Cd recovery of all soil samples was higher than 95%, and the accuracy of relative standard deviation (RSD) was within 10%.

Statistical analysis

Three-way analysis of univariate ANOVA and correlation analysis were performed using SPSS version 21.0 package (SPSS, 2009). The variables analyzed separately were Cd concentration and Cd accumulation in alfalfa and Indian mustard, and Cd chemical forms in soil. The level of significance was 0.05.

Results

Biomass

As shown in *Table 1,* with the increase of soil Cd level, shoot and root dry weight of alfalfa and Indian mustard decreased significantly ($P < 0.05$), decreasing by 50.00-71.90% and 29.60-59.30% (alfalfa), 59.60-89.00% and 64.30-74.80% (Indian mustard) respectively. At same soil Cd levels, biomass of Indian mustard shoot and root was significantly higher than that of alfalfa. The tolerance coefficients of alfalfa shoot and root were 28.21-50.00% and 40.74-70.37% when exposed to Cd, respectively. When the Cd pollution level was above 150 $mg \cdot kg^{-1}$, the tolerance coefficients of each part were lower than 50%. The tolerance coefficients of Indian mustard shoot and root were 10.97-40.37% and 25.22-35.65% when exposed to Cd, respectively, both lower than 50%.

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Table 1. *Dry weights and resistance coefficient of two plants in different soil Cd levels*

Soil Cd levels ($mg \cdot kg^{-1}$)	Dry weight ($g \cdot plant^{-1}$)				Resistance coefficient (%)			
	Shoot		Root		Shoot		Root	
	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard
0	0.078±0.007a	0.857±0.033a	0.027±0.002	0.115±0.013a	—	—	—	—
75	0.039±0.008a	0.346±0.041a	0.019±0.001b	0.041±0.001a	50.00	40.37	70.37	35.65
150	0.023±0.003b	0.157±0.020a	0.013±0.002b	0.032±0.003a	29.49	18.32	48.15	27.83
300	0.022±0.001b	0.140±0.013a	0.013±0.002b	0.030±0.002a	28.21	16.34	48.15	26.09
600	0.022±0.001b	0.094±0.017a	0.011±0.001b	0.029±0.001a	28.21	10.97	40.74	25.22

Note: tolerance coefficient = Cd treatment plant biomass / control plant biomass × 100. The results listed are mean ± standard deviation. Different lowercase letters indicate the difference between different plants under the same Cd level ($P < 0.05$). The same below

Cd concentration in shoot and root

As shown in *Figure 1,* with the increase of soil Cd level, the Cd concentrations in shoots and roots of the two plants increased significantly ($P < 0.05$).

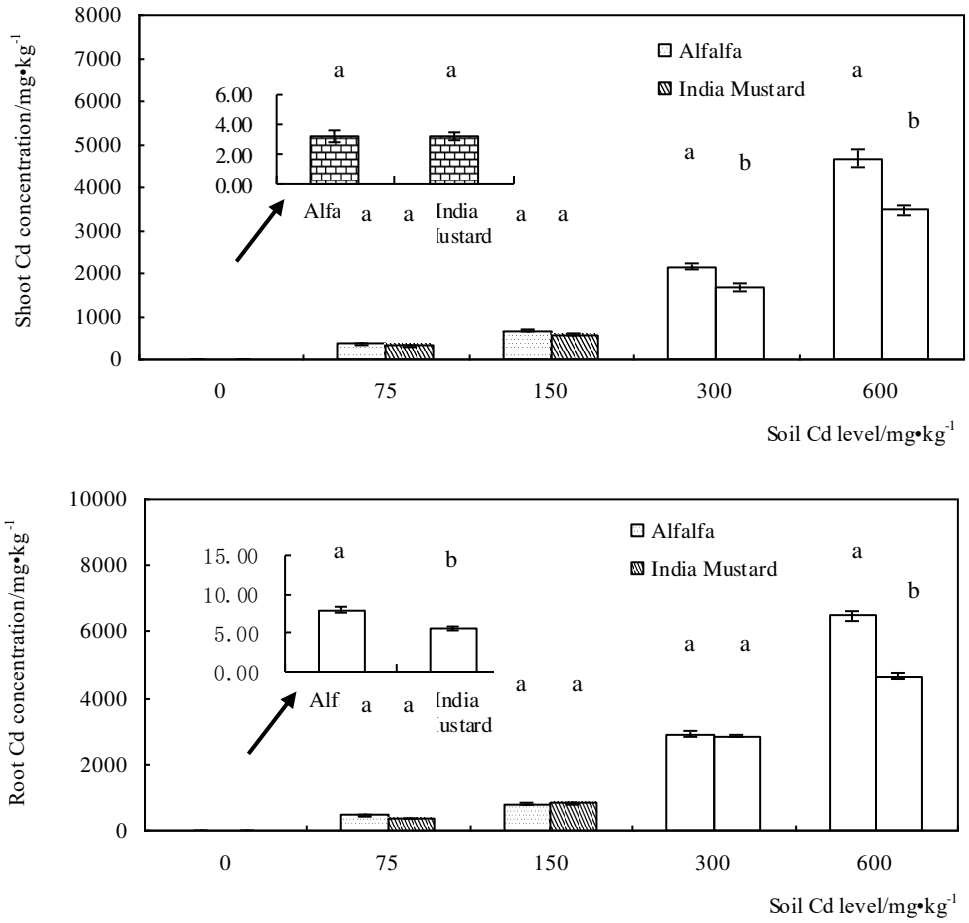


Figure 1. *Effect of different soil Cd levels on Cd concentration of shoots and roots in two plants. Note: Different lowercase letters indicate the difference between different plants under the same Cd pollution level ($P < 0.05$). The same below*

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Under the same Cd level, the Cd concentration in roots of the two plants was higher than that in shoots, and Cd concentrations in shoot and root were in order of alfalfa > Indian mustard. At 75 mg·kg^{-1} soil Cd level, shoot Cd concentrations in alfalfa and Indian mustard passed the critical level of Cd hyperaccumulator (100 mg·kg^{-1}) which were 356.463 mg·kg^{-1} and 308.735 mg·kg^{-1} respectively.

Cd accumulation in shoot and root

As can be seen from *Figure 2,* with the increase of soil Cd levels, Cd accumulation in shoots and roots of the two plants increased with the increase of soil Cd level. At the 600 mg·kg^{-1} soil Cd level, Cd accumulations of shoot and root in alfalfa and Indian mustard reach the peak by 175.540, 101.632 and 324.70 μg·plant^{-1} respectively. Under the same Cd level in soil, Cd accumulations of shoot and root was in order of alfalfa < Indian mustard.

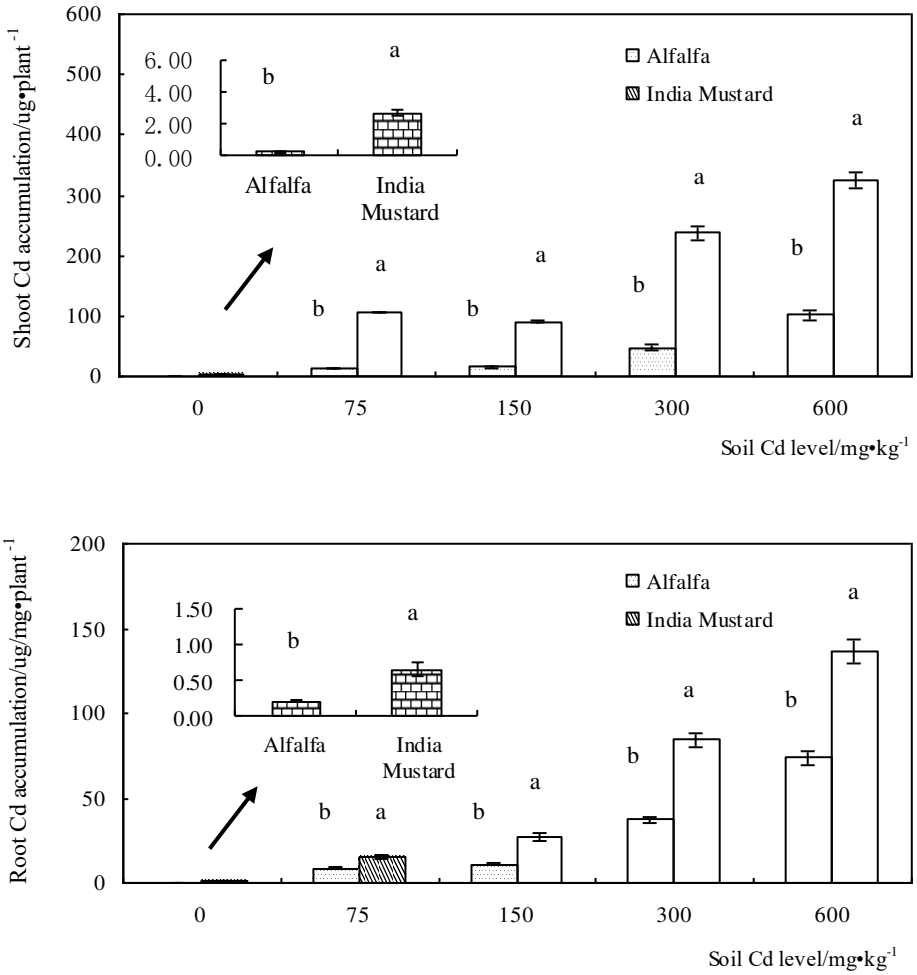


Figure 2. *Effect of different soil Cd levels on Cd accumulation in two plants*

Comparison of enrichment and transport capacity

As shown in *Table 2,* BCF of shoots of alfalfa and Indian mustard decreased firstly, reaching the lowest level at 150 mg·kg^{-1} Cd level, and then increased. Compared to that

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at 150 mg·kg^{-1} Cd level, the BCF of shoots of alfalfa and Indian mustard increases by 62.94% and 76.16% (alfalfa), 47.30% and 50.85% (Indian mustard) at the 300 and 600 mg·kg^{-1} Cd levels. With the increase of soil Cd level, the $BCF_{root/soil}$ of alfalfa decreased first, reaching the lowest at 150 mg·kg^{-1} Cd level, down by 10.48% compared with that at 75 mg·kg^{-1} Cd level, and then increased. For Indian mustard, the $BCF_{root/soil}$ first increased and then decreased, reaching the peak at 300 mg·kg^{-1} Cd. The BCF of the two plants was above 1, with that of Indian mustard > alfalfa. The TF of alfalfa and Indian mustard decreased with the increase of soil Cd levels, and the TF value was below 1. Under the same Cd levels, the TF was in order of Indian alfalfa > alfalfa.

Table 2. *The values of BCF and TF in two plants*

Soil Cd levels mg·kg^{-1}	$BCF_{Shoot/Soil}$		$BCF_{Root/Soil}$		$BCF_{Plant/Soil}$		TF	
	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard
0	-	-	-	-	-	-	-	-
75	4.753±0.274a	4.116±0.023b	6.042±0.278a	4.917±0.163b	10.795±0.005a	9.034±0.186b	0.789±0.082b	0.838±0.023a
150	4.421±0.159a	3.837±0.068b	5.409±0.006a	5.541±0.026a	9.830±0.165a	9.379±0.041a	0.817±0.028a	0.693±0.0150b
300	7.204±0.052a	5.652±0.305b	9.714±0.237a	9.521±0.116a	16.918±0.185a	15.173±0.421b	0.742±0.023a	0.593±0.025b
600	7.788±0.200a	5.788±0.219b	10.803±0.243a	7.780±0.105b	18.591±0.443a	13.568±0.324b	0.721±0.002a	0.744±0.018a

Note: BCF (Enrichment coefficient) = Cd concentration in shoot (root) (mg·kg^{-1}) / soil Cd (mg·kg^{-1}) (Feng et al., 2016); TF (Transport coefficient) = Cd concentration in shoot (mg·kg^{-1}) / Cd concentration in root (mg·kg^{-1}) (Sun et al., 2016)

Soil Cd chemical forms

As shown in *Table 3* and *Figure 3,* the Cd content in different forms of soil (EXC, CAB, FeMn, OM and RES) increased significantly with the increase of soil Cd level ($P < 0.05$). Cadmium fraction distribution coefficient (FDC) also demonstrated a corresponding trend. At the same Cd level, soil Cd of the two plants mainly exists as EXC-Cd. Cadmium fraction distribution coefficient (FDC) of EXC-Cd in soil of alfalfa and Indian mustard were 63.92-81.87% and 57.97-80.71% respectively. The OM-Cd content had the lowest distribution proportion under the same soil Cd, with FDC at only 0.87-13.33%. At the 300 mg·kg^{-1} and 600 mg·kg^{-1} Cd levels, exchangeable Cd content in the soil planted with Indian mustard was significantly lower than that planted with alfalfa.

Discussion

Earlier reports suggested that Cd had no significant effect on plant growth at a low Cd level (Liu et al., 2013; Yu et al., 2017) and even stimulated and promoted plant growth (Wei et al., 2004). In this experiment, the biomass of alfalfa was significantly lower than that of the control at the 75 mg·kg^{-1} Cd level, indicating that 75 mg·kg^{-1} Cd had a serious injury to alfalfa growth. The results are consistent with those of Lou et al. (2015). In this study, with the increase of soil Cd level. It may be probable that growth and development of plants were directly related to Cd level, soil physicochemical property, fertility and soil type, but the specific reason remains to be further studied.

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Table 3. *Effect of different Cd levels on Cd chemical forms in soil*

Soil Cd levels ($mg \cdot kg^{-1}$)	EXC-Cd		CAB-Cd		FeMn-Cd		OM-Cd		RES-Cd	
	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard	Alfalfa	Indian mustard
0	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
75	46.093±0.521a	40.141±0.551b	9.093±0.834a	10.437±2.011a	8.694±0.328b	10.088±2.509a	2.522±0.499b	5.540±1.491a	5.708±0.667a	3.033±0.308b
150	95.442±0.090a	66.357±1.262b	14.273±1.675a	11.933±1.001b	10.879±0.840a	10.586±1.999a	3.525±1.494b	8.288±1.247ba	6.221±0.161a	5.385±0.339b
300	142.382±22.228a	117.148±7.278b	20.886±0.359a	19.150±0.242a	14.508±1.807a	8.578±0.003b	3.528±0.170b	12.283±0.758a	7.702±0.654a	7.890±0.167a
600	330.700±3.299a	291.113±18.349b	37.603±0.311a	36.098±0.705a	19.884±0.488a	15.802±3.222b	4.863±0.164	7.776±2.234a	10.892±0.160a	9.888±0.178a

Note: EXC-Cd, CAB-Cd, FeMn-Cd, OM-Cd and RES-Cd indicate exchangeable Cd, carbonate-bound Cd, Fe-Mn oxides-bound Cd, Organic Cd and residuals Cd, respectively

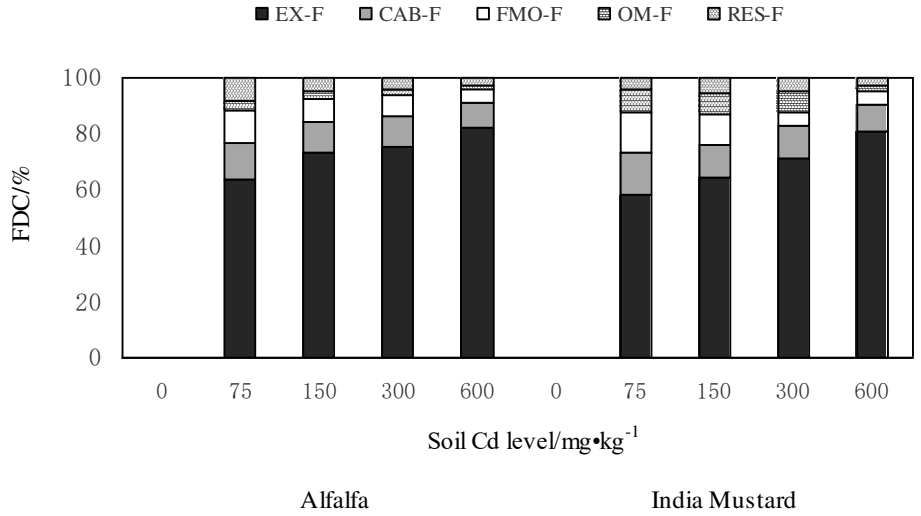


Figure 3. *Effect of different soil Cd levels on Cd fraction distribution coefficient (FDC) in soils. Note: EX-F, CAB-F, FMO-F, OM-F and RES-F indicate Cd distribution coefficient (FDC) of exchangeable Cd, carbonate-bound Cd, Fe-Mn oxides-bound Cd, Organic Cd and residuals Cd in soil, respectively*

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For Cd hyperaccumulator plants, Cd concentration in shoot should be 100 times higher than that of common plants (the critical value of Cd is 100 $mg \cdot kg^{-1}$); Cd concentration of plant root should be lower that of shoot; the plant growth does not appear obvious toxicity symptoms and has strong resistance to Cd (Li et al., 2017a; Lin and Feng, 2017; Sun and Song, 2015;). The results of this study showed that, Cd concentration in shoots of alfalfa and Indian mustard exceeded the 100 $mg \cdot kg^{-1}$ threshold of hyperaccumulator plant at the 75 $mg \cdot kg^{-1}$ Cd level. This result was similar to that reported by Yang et al. (2011). In comparison of Cd concentration in shoots and roots of the two plants under the same soil Cd level, that of alfalfa > Indian mustard. However, in the analysis of biomass and Cd accumulation, the biomass of Indian mustard was much higher than that of alfalfa, therefore, its Cd accumulation was also significantly higher than that of alfalfa. Different plant organs have different levels of heavy metals (Li et al., 2017b). In this experiment, it was found that, for both alfalfa and Indian mustard, that in root > shoot at different soil Cd levels, and their roots had strong Cd enrichment ability, but their ability to transport Cd to shoots was weak. Similar results were reported by Chen et al. (2014), Jiao et al. (2014), Hattab et al. (2013), and Sun et al. (2016), but different from the report by Yang et al. (2011). In this experiment, with the increase of Cd concentration, Cd transport index of Indian mustard first increased and then decreased, while alfalfa Cd transport index also increased. This result was different from earlier reports (Li and Song, 2016). In addition, BCF of alfalfa and Indian mustard were above 1 when exposed to Cd, while TF was lower than 1. The higher root BCF value of Indian mustard suggests that Indian mustard has the strongest Cd enrichment ability.

It has been reported that there is a significant difference in soil Cd form among different crops (Zhang et al., 2017). In this experiment, EXC-Cd, CAB-Cd and FeMn-Cd of alfalfa were significantly higher than that of Indian mustard under high concentration of Cd, while RES-Cd and OM-Cd were lower than that of Indian mustard, which was similar to the results of previous studies (Huang et al., 2000). It may be because different root exudates produced by different crops change Cd forms in the soil and mitigate Cd damage to the crop. In this experiment, the Cd in the two plants at different Cd levels both existed in the form of highly active EXC-Cd, and Cd concentration in different forms increased with the increase of Cd added concentration, which may be because Cd entered soil in the form of Cd solution with the highest activity (Xiong et al., 2015).

Conclusion

With the increase of soil Cd level, the biomass of shoots and roots of alfalfa and Indian mustard decreased significantly. The EXC-Cd was the dominating form of soil Cd. With the increase of Cd level in soil, the percentage of EXC-Cd in total Cd also increased continuously. At 75 and 150 $mg \cdot kg^{-1}$ Cd levels, Cd concentrations in shoots of alfalfa and Indian mustard exceeded the critical value of hyperaccumulators, The BCFs of both alfalfa and Indian mustard were above 1. At the same soil Cd level, for BCF and TF values, those of Indian mustard > alfalfa. Based on comprehensive consideration of biomass, Cd accumulation, transport coefficient and enrichment coefficient, Indian mustard has stronger Cd accumulation ability, thus more suitable as repair material for soil Cd pollution.

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APPLICATION OF SILVER NANOPARTICLE PRODUCTION FOR LIPASE MODIFICATION USING PUER TEA

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Abstract. Application of cystine capped silver nanoparticles synthesized using puer tea as the reducing agent to decorate lipase has been reported in the present work. Biodiesel production technology is competitive in terms of low cost and alternative source of energy which should be not only sustainable but also environmentally friendly. Designing of the lipase immobilization for biodiesel production has a remarkable impact and is still challenging. In this work, biodiesel production from soybean oil was enhanced and facilitated by using a novel biocatalyst consisting of commercial lipase (EC 3.1.1.3), silver nanoparticles, and polydopamine. Various parameters such as cystine concentration, pH, temperature and amount of reducing agent were standardized and their effect on the synthesis process has been initially evaluated by surface plasmon resonance peak analysis. Further the synthesized nanoparticles were also characterized using X-ray diffraction (XRD) and scanning electron microscope (SEM). The transesterification reaction conditions were as follows: oil/alcohol molar ratio 1:4; temperature 40 °C and total reaction time 6 h. Lipozyme TL-100L lipase provided the highest yield of fatty acid methyl esters as 92%. Operational stability was determined with immobilized lipase and it indicated that a small enzyme deactivation occurred after used repeatedly for 10 consecutive batches with each of 24 h. Since the process is yet effective and enzyme does not leak out from the polymer, the method can be proposed for industrial applications. Research highlights Lipozyme TL-100L and Novozym 388 were immobilized onto micro porous polymeric matrix by both physical adsorption and covalent linking. Immobilized enzymes were used for synthesis of fatty acid methyl esters by transesterification of canola oil and methanol using semi-continuous operation system. According to chromatographic analysis, Lipase Lipozyme TL-100L resulted in the highest yield of methyl ester as 92%. The bioconjugates saw 15% loss in its initial activity at the end of five reusability cycles. This decorated reusable system has the potential to be utilized for various applications pertaining to the exploitation of lipase in various industries.
Keywords: *silver nanoparticles, cystine, application, puer tea, reducing agent*

Introduction

Biodiesel production from vegetable oil has been increasing in the last decade in ASEAN countries, for example, Thailand, Malaysia, Indonesia, Vietnam, and The Philippines, since many of them have diverse and high yield crops of oleaginous plants, for example, palm, soybean, and sunflower (Mukta et al., 2010; Murphy et al., 2012). This is relevant to the world's rapid industrialization and increasing population (Preechajarn et al., 2012). Since the use of enzyme in its free form makes the process very expensive, efforts are being made worldwide to develop a stable decorated enzyme system so that the enzyme can be reused and the processing can be made cost

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effective. Transesterification of vegetable oils by short chain alcohols (methanol or ethanol) catalyzed by lipase (EC 3.1.1.3) is a worldwide biochemical mechanism to produce biodiesel (Fjerbaek et al., 2009; Fan et al., 2012) and there are two modes of lipase usage: free or soluble lipase and immobilized lipase. In early 2001, using free lipases from Candida rugosa and Pseudomonas cepacia along with methanol as acyl acceptor, a method was reported to yield biodiesel from soybean oil up to 80 and 90%, respectively (Kaieda et al., 2001). Various matrixes has been successfully used for enzyme immobilization application (to name some alginate, gelatin, polyacrylamide, cellulose, polyvinyl fibers, silica and nanoparticles etc.), among them nanoparticles can be a promising choice (Tan et al., 2010; Salis et al., 2008). Immobilized lipase methods have been introduced to improve lipase stability and reusability. Herein immobilized enzymes are defined as "enzyme physically confined or localized in a certain defined region of space with retention of their catalytic activities, which can be used repeatedly and continuously" (Lee et al., 2007).

Employed lipases for immobilization have been derived from microorganisms to animals, for example, from pancreatic porcine, Burkholderia cepacia, Pseudomonas spp., and Candida spp. (Bu et al., 2012; Peng et al., 2013). Immobilization methods are also various, for example, adsorption on acrylic resin, celite, and anion resin, covalent bonding using silica-PVA and styrene-divinylbenzene, entrapment using hydrophobic sol-gel support, and cross-linking using glutaraldehyde (Ren et al., 2011). These methods can yield biodiesel from soybean oil using adsorbed lipase (Lee et al., 2009) and from mahua oil using cross-linked lipase (Veerapandian et al., 2011). However, these techniques are still sophisticated and require multiple-steps material consumption in case of Pseudomonas cepacia cross-linked lipase (Jierwiriyapant et al., 1992), as well as time consumption for oil transesterification (49 h) in case of absorbed Pseudomonas fluorescens lipase on macroporous polypropylene matrix (Sangla et al., 2006).

In current study silver nanoparticles were synthesized using puer tea extract as the reducing agent. The formation and morphology of each composition were characterized by UV-Vis spectroscopy and scanning electron microscope (SEM). Polydopamine has also been studied for enzyme immobilization, for example, trypsin (Kanmani et al., 2016), glucose oxidase, and lipase (Rivera et al., 2012). Enzymes can be covalently immobilized on a polydopamine surface via nucleophiles (Walt and Agayn, 1994). The effects of various parameters like temperature, pH and reducing agent concentration were standardized during the course of nanoparticle synthesis. Therefore, nanoparticles were characterized for their surface morphology using SEM. Initial screening of the nanoparticle synthesis was done by monitoring the surface plasmon resonance with help of UV-Vis spectroscopy for each parameter under consideration. This study brings us one step closer to the continuous use of lipase for various biotechnological processes.

Materials and methods

Chemicals

Silver nitrate, glutaraldehyde, Triton X-100 and sodium dodecyl sulfate (SDS) were obtained from Aladdin Chemistry (Shanghai, China). P-nitrophenol palmitate (pNP) was obtained from sigma; L-Cystine was obtained from Aladdin Chemistry (Shanghai,

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China). Deionized water was used throughout the process from Millipore and puer tea was procured from Puer, Yunnan province, China.

p-nitrophenol palmitate substrate preparation

420 μM of substrate solution was prepared in Tris-Cl buffer (0.1 M, 8.2 pH) by weighing 0.004 g of pNP and adding it to 25 mL of buffer along with 125 μL of Triton X-100 and 0.0045 g of SDS. The mixture was heated at 65 °C for 20 min and then stored at 4 °C to be used within a week.

Puer tea extract preparation

3.0 g of puer tea leaves were boiled with 50 mL of deionized water at 65 °C for 10 min in a closed container. The extract was first filtered through whatman filter paper No.1 and then centrifuged at 10000 rpm for 10 min at room temperature. The supernatant was collected in a clean falcon tube and stored at 4 °C for further use. The extract was used within a week.

Enzyme production and enzyme activity assay

Lipase was isolated from *B. subtilis* cultured in nutrient broth supplemented with 1% olive oil for 24 h. The broth was then centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant was collected after centrifugation was stored at 4 °C as enzyme source for further use. The crude enzyme was tested for activity using assay mixture comprising of p-nitrophenol palmitate as a the substrate (Gupta et al., 2003). For the activity assay 2.5 mL of substrate was added to 1.5 mL of Tris-Cl buffer and 1 mL of crude enzyme preparation. The assay mixture was incubated at 37 °C for 30 min. The absorbance was recorded at 410 nm. The enzyme activity was calculated as U/mL based on the concentration of p-nitrophenol obtained from the standard curve. The total protein content of the crude enzyme was calculated using the method of (Lowry et al.).

Nanoparticle synthesis and characterization

The nanoparticles synthesis was initiated by mixing 100 mL of 1 mM silver nitrate solution and 1 mL of 1 mM L-cystine. This solution was slowly stirred at room temperature for 10 min. 5 mL of puer tea extract was then added slowly with continuous stirring at room temperature. pH of the reaction mixture was adjusted to 11 by dropwise addition of 1 M sodium hydroxide. The change in the colour of the reaction mixture was recorded at 65 °C for 30 min. The colour of reaction mixture changes from transparent (before addition of tea extract) to light brown (after addition of tea extract) to dark brown (after adjusting pH and heating at 65 °C for 30 min). This change in the colour of the reaction mixture corresponds to synthesis of silver nanoparticles. Thus obtained nanoparticles were then centrifuged at 20000 rpm for 40 min to pelletize the nanoparticles which were dried at 37 °C overnight. The formation of nanoparticles was also confirmed using nanodrop spectrophotometric method in terms of surface plasmon resonance (SPR) at 405 nm. The particles were analyzed by particle size analyzer to determine average size of the particles synthesized. Transmission electron microscopy was performed to observe the surface characteristics of the synthesized silver nanoparticles, while XRD analysis was done to confirm the crystalline structure of the synthesized nanoparticles.

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Results and discussion

Optimization of nanoparticle synthesis parameters

Different parameters affecting the synthesis of silver nanoparticles were optimized so that mono- dispersed nanoparticles of uniform size and shape can be obtained. The parameters optimized were analyzed in terms of SPR peaks and the effect of each parameter was recorded on the basis of the SPR peaks achieved for each parameter. To check the consistency of data the experiments were carried out in triplicates.

Effect of varying concentrations of cystine

The effect of cystine concentration on nanoparticle synthesis was analyzed by changing the concentrations of cystine in the reaction mixture. Cystine concentration was varied from 0.5 mM, 1 mM, 2 mM, 5 mM and 10 mM. The effect was observed by monitoring SPR peaks in the complete UV-Vis range for each test concentration of cystine.

Effect of varying concentrations of reducing agent (puer tea)

Various concentrations of silver nitrate solutions: puer tea extract was used to carry out the synthesis of nanoparticles i.e. 100:1, 100:2, 100:3, 100:4 and 100:5. The effect of varying the concentration of reducing agent on the synthesis of nanoparticles was observed by viewing the SPR peaks. *Figure 1a* shows the effect of various concentrations of cystine on the synthesis of nanoparticles. Higher concentration of cystine resulted in slight shift of the peak towards higher wavelength with a broader peak size. This could be because of the aggregation of nanoparticles to form super clusters leading to redshift. Redshift is a phenomenon which is represented by increase in the emitted wavelength by the nanoparticles because of increase in the crystalline structure (size) of the particles. As the size of the particles increases, the distance between the valance band also increases resulting in the increase of the dielectric constant (Yon-Rui et al., 2013).

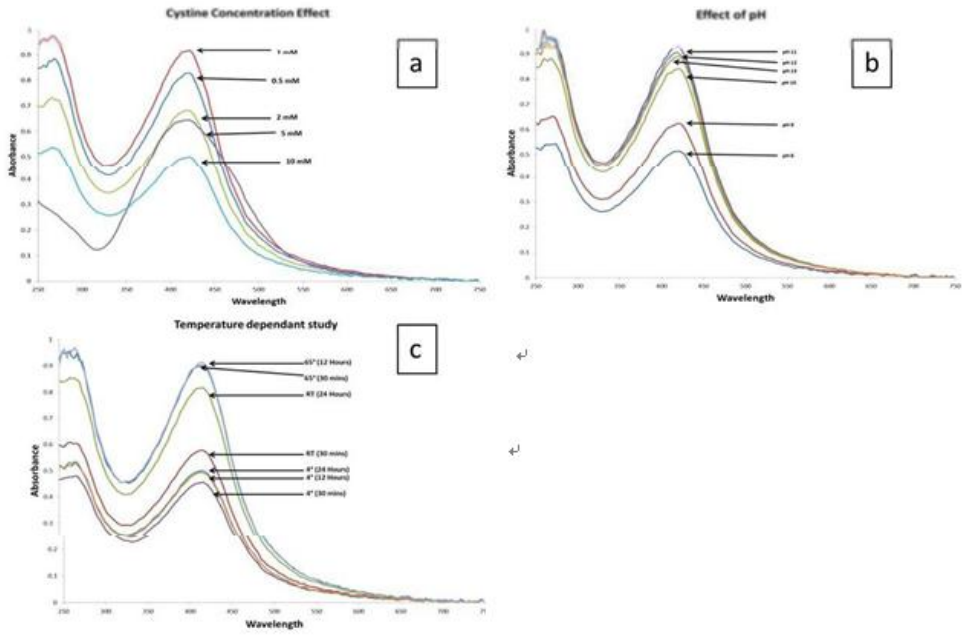


Figure 1. *Process optimization for synthesis of cystine capped silver nanoparticles*

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Effect of pH

pH plays an important role in the formation of nanoparticles since acidic conditions interfere and hinder the synthesis of nanoparticles in most of the cases studied and reported. Under our standard condition, also reduction of silver nitrate was not observed even with prolonged incubation under acidic conditions. Therefore, pH of the reaction mixture was set towards alkaline side by addition of 1 M sodium hydroxide. pH 8, 9 and 10 exhibited slight change in reaction mixture colour and it gave very low intensity peaks indicating low or negligible reduction rate. At pH 11 and 12, a very rapid change in the colour was observed with an intense peak within 30 min of incubation (*Fig. 1b*). Result shown in *Figure 1c* suggests that the synthesis was achieved significantly at high temperature.

Effect of reaction temperature

The study of temperature effect was performed at 3 different temperatures, such as 4 °C, room temperature (25 °C) and 65 °C. The effect of temperature on nanoparticle synthesis was observed by viewing the SPR peaks. Effect of temperature on the synthesis of nanoparticles was studied at three different temperatures i.e. 4 °C, RT and 65 °C. The temperatures selected were from low to medium to high range. Initiation of nanoparticle synthesis took place at every temperature tested which is evident from the colour development at all three temperatures. Result shown in *Figure 1c* suggests that the synthesis was achieved significantly at high temperature. Low temperature resulted into low intensity peaks after 12 h of incubation and did not enhance further even after prolonged incubation, while room temperature lead to medium intensity peaks in about 4 h of incubation and took 24 h to match the intensity observed at high temperature incubation. At 65 °C, a high intensity peak was obtained within 30 min of incubation.

Nanoparticle synthesis and characterization

The puer tea used as reducing agent for the synthesis of cystine capped silver nanoparticles in present work exhibits reducing properties mainly due to presence of anti-oxidant compounds such as catachins in it. The mechanism for the formation of cystine capped silver nanoparticles involves formation of silver cystine complex followed with reduction in presence of anti-oxidant components of puer tea at pH 11 at 65 °C. Cystine binds to silver through thiolate bonding (Mandal et al., 2001). Initially formation of nanoparticle was confirmed by checking SPR peaks spectrophotometrically. A sharp peak at 405 nm (*Fig. 2a*) indicates the formation of silver nanoparticles. The peak arises due to the absorption of electromagnetic waves in the visible region by the nanoparticles that provides them the ability to give a peak at a specific wavelength. The reaction colour changes from light brown to dark brown within 10 min of incubation at 65 °C under alkaline conditions (*Fig. 2b*). The time-dependent study was carried out for formation of silver nanoparticle for 2 h. It can be concluded from the SPR peaks obtained that the maximum nanoparticles synthesis is achieved within 30 min of incubation as there is no significant change in the peak value after that. A higher temperature mediated synthesis was preferred over synthesis at RT to avoid super-clustering of the nanoparticles due to carboxyl or amino group present on the cystine molecules. The peaks obtained from the spectrophotometric analysis even gave a rough idea about the size of nanoparticles since the SPR peaks obtained were broad; it indicates that the particles formed might be of larger size.

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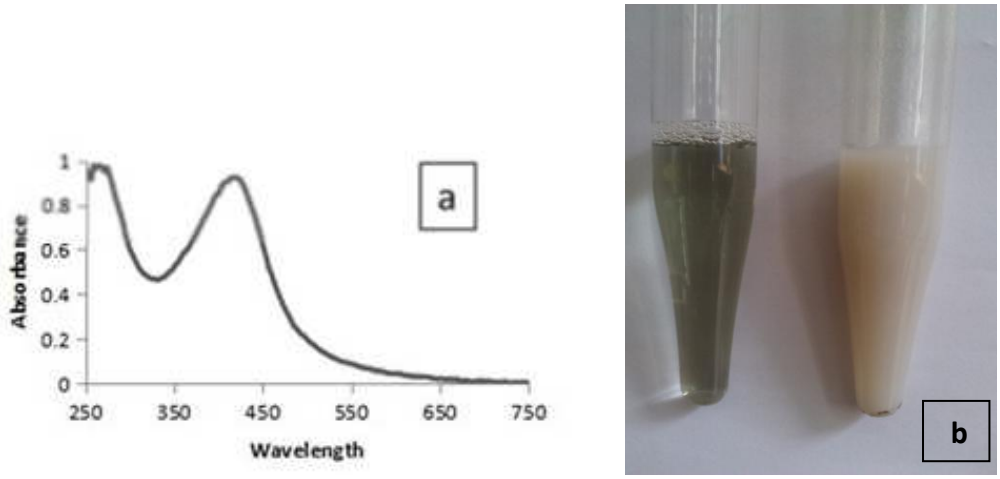
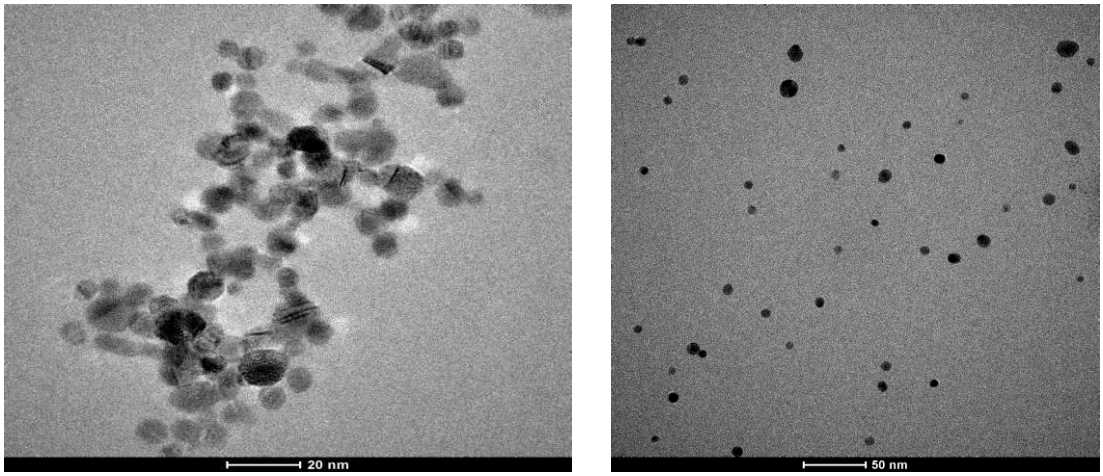


Figure 2. *(a) SPR analysis of synthesized cystine capped silver nanoparticles. (b) Confirmatory colour change during the synthesis of nanoparticles*

SEM analysis of nanoparticles

The particle size analysis of the nanoparticles revealed the average size of the particles to be around 20 nm (*Fig. 3a*). The same was also confirmed in the SEM analysis of the samples. Spherical nanoparticles which were around 20 nm in diameter can be clearly seen in the SEM analysis result represented in *Figure 3b*. Nanoparticles of different sizes have been reported using green synthesis like Prakasha et al. (2013) synthesized silver nanoparticles of 55-83 nm in size using leaf extract of *Mimusops elengi*. Another research by Jyoti et al. (2016) demonstrated the synthesis of silver nanoparticles of 36 nm in diameter using the leaf extract of *Urtica dioica*. Thus, it can be concluded that reducing agents and reaction conditions play very critical role in deciding the shape and size of the nanoparticles synthesized. These reflections correspond to the facets of face centered cubic crystal of silver nanoparticles (Jyoti et al., 2016).



a **b**

Figure 3. *(a) Particle Size analysis of the synthesized silver nanoparticles. (b) SEM images of the synthesized nanoparticles*

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Lipase isolation and activity assay

The crude lipase was isolated from strain of *Bacillus subtilis* (Acc. No. KT591343). The crude lipase was quantified for its activity using p-nitrophenol palmitate as the substrate. The lipase acts on the substrate and hydrolyzes the palmitate group resulting in release of p-nitrophenol. The release of p-nitrophenol brings a change in colour of the assay mixture from colorless to light yellow. The change in the colour intensity is spectrophotometrically recorded at 410 nm. The standard plot for pnitrophenol is represented in *Figure 4*. The activity of the enzyme was calculated as the amount of pnitrophenol released from 1 mole of p-nitrophenol palmitate per minute per mL of reaction mixture. The activity of lipase from an unidentified strain isolated from dairy waste (Sahu et al., 2011) and from a *Staphylococcus sp.* isolated from oil contaminated area has been reported by (Sirisha et al., 2010) to be 38-46 and 25 U/mL respectively.

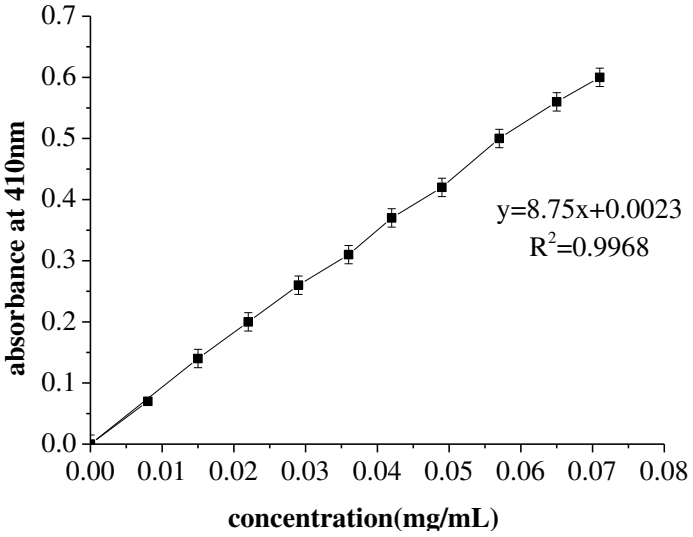


Figure 4. *Standard curve for p-nitrophenol*

Stability and reusability

The enzyme system so formed was stable for a period of 2 months with a loss of 15% of its original activity which is marginally better than the soluble enzyme which showed the loss of 25% from its original activity. Even though the activity lost comparison does not reflect a significant change, the reusability of the system gives it a significant advantage over the soluble enzyme. The use of enzyme system repeatedly showed the average loss of activity by 3-4% per cycle over the course of 5 cycles as shown in *Table 1.* This loss in the activity can also be attributed to the loss of nanoparticles after every assay cycle which could not be recovered during centrifugation step. The loss can also be possible because of leeching out of the enzyme from the decorated nanoparticles. Even though the covalent bond binds the nanoparticles with the enzyme the possibility of the nanoparticles releasing the enzyme into the system cannot be ruled out either during the activity assay or during the centrifugation step to pelletize the system.

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Table 1. *Reusability test for lipase-silver nanoparticles bioconjugates*

Cycle number	Absorbance	Activity U/mL
Cycle 1	0.17	24.9
Cycle 2	0.16	23.1
Cycle 3	0.16	23.1
Cycle 4	0.15	21.2
Cycle 5	0.15	21.2

Conclusion

Original research article represents the immobilization of lipase on the surface of economically synthesized capped silver nanoparticles. The synthesis involved the production of cystine capped silver nanoparticles using puer tea as the reducing agent. The covalent coupling of the enzyme to the amino group present on the cystine capping acted as a bridge to decorate the enzyme to the nanoparticles. This improved the shelf life of expensive soluble enzyme with a reasonable specific activity in the decorated state. The development of such decorated system can be taken to the next level as to work on specific industrial applications with minor modifications as per application demand. This decorated reusable system has the potential to be utilized for various applications pertaining to the exploitation of lipase in various industries. Further experiments will include studies regarding the recovery of AgNPs from other media and the implementation of other metal nanoparticles (Au, Pt and Pd).

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COMPARING PERFORMANCE OF FIXED AND VARIABLE AREA SAMPLING PLOTS IN IRANIAN SUB-HUMID FORESTS

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Abstract. In this study, two inventory methods, including use of plots with 300 m^2 fixed-area and Relascope with variable-area plots were compared to evaluate biodiversity, quantitative and qualitative characteristics of Arasbaran forest stands in the North West of Iran. For this purpose, 58 sample plots were specified using 100 m×100 m regular randomized grid and tree characteristics were measured by two inventory methods. In each plot, the quantitative characteristics including diameter at breast height (DBH), basal area at breast height, tree number (density) and volume per hectare, sampling time and the qualitative characteristics such as percentage of species composition, health and origin of trees were recorded. Species richness, Shannon Weiner diversity and Simpson evenness indices were calculated for each sample plot. Independent t-test was applied for comparing the means of quantitative characteristics and biodiversity indices between the two methods. The results indicated that the quantitative characteristics are significantly different between two methods, whereas, no significant differences were observed in richness, biodiversity indices and qualitative characteristics. Average inventory duration in the fixed-area plot method (706.10 s) was considerably higher than that of the variable-area plots method with Relascope (485.91 s). The square of sampling error by inventory time ($T \times E\%^2$) index was used to evaluate the two methods precisely. This coefficient was less for volume per hectare and basal area at breast height per hectare in the variable-area plot method.
Keywords: *Arasbaran forests, species diversity, fixed area plot, relascope, quantitative characteristics*

Introduction

Arasbaran area is a crossing zone among multi-habitats due to its vegetative elements related to numerous climates. Existence of specific climatic conditions, high biodiversity, presence of rare plant and animal species as well as existence of vegetative elements related to various climates are factors distinguishing these forests from other regions (Sagheb Talebi et al., 2005). Stand structure and species diversity are two important ecological and functional features of forest ecosystems (Pretzch, 1997), affecting the landscape beauty and recreational value of the forest (Su et al., 2012). According to the sustainable management criteria, maintaining and enhancing diversity are important factors in forest conservation (Nath et al., 2009). Protecting the natural ecosystems and biodiversity of Arasbaran forests is necessary due to its importance and status in a variety of aspects. They include species diversity, vital effects on water balance of the Aras River, preservation of biological reserves, impact on climate purification, preventing soil erosion, providing ecotourism values and achieving sustainable development (Mohammad Zadeh et al., 2014). Monitoring this valuable resource is the most important way to reach a management model and maintain ecosystem biodiversity, which can be achieved by quantitative and qualitative forest inventory and understanding the forest community dynamics, making possible subsequent planning (Eshaghi Rad et al., 2003; Islam et al., 2015; Motz et al., 2010; Hassanzad Navroodi et al., 2015). However, foresters often face problems with

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choosing the right method to measure and obtain accurate scientific information on biodiversity and forest structure (Pique et al., 2011).

An appropriate measurement method is one that offers reasonable information about the inventory area with suitable accuracy and cost (Parker, 1979; Husch et al., 1982; Avery and Burkhart, 2015). Accordingly, decision making on the measured variable and the appropriate measurement method is necessary (Pique et al., 2011). In forest inventory, various methods are used to design the sampling plots that are generally divided into two groups: sample plots with fixed area and sample plots with variable area (Zobeyri, 2007). Relascope is suggested for variable-area plot sampling method which is also known as n-tree sampling methodology (Bitterlich, 1984). In the fixed-area plot sampling method, the probability of selecting each one of the trees is the same within the sample unit, but in contrast with the variable-area plots method, the trees have a selection chance depending on their dimensions (Bitterlich, 1984; Ducey, 1999). In sampling with variable-area plot, it is attempted to increase sampling efficiency by overcoming the drawbacks associated with the fixed-area plot sampling method (Sheil et al., 2003). Zare et al (2016) selected a wild Pistachio stand in Fars Province of Iran where in a 100×100 m^2 grid, 46 sample points and six single-distance sampling methods were applied. In general, nearest single-distance methods were efficient enough to estimate the biometric characteristic of wild Pistachio trees precisely and accurately.

Relascope is a multipurpose device for measuring samples with variable area that is used by researchers for estimating biodiversity and stand structure (Barabesi and Fattoriu, 1999; Motz et al., 2010). In the Relascope method, the probability of choosing trees is proportional to their dimension and size (Piqueet al., 2011). In order to determine the best sampling method, several studies have been conducted. Some of these studies have introduced fixed-area plot sampling methods as the suitable ones for estimating quantitative, qualitative information and biodiversity (Motz et al., 2010). Whereas in the others, the sampling method with variable area or sample line has been introduced as the superior method (Eshaghi Rad et al., 2003; Alijanpour et al., 2003; Henttonen and Kangas, 2015).

Fallah et al. (2000) compared two sampling methods with fixed-area plots (10 acre) and variable-area plots (Relascope) in Loveh - Gorgan oak forests using characteristics including number of trees per hectare, basal area per hectare and time required for data recording. Results showed that the sampling method with 10 acre area was more suitable for the forest inventory. In another study, two methods of measuring with fixed-area plot and Relascope were used to study quantitative variables in northern Spain. It was found that the use of Relascope has a faster estimation of variables compared to the fixed-area sampling method. This means that although the Relascope is a costly sampling instrument, it significantly reduces the cost of inventory and, if needed, facilitates increased sampling density for a specific budget (Pique et al., 2011). Despite numerous studies in the Arasbaran region using sampling with fixed-area plots and sample line (Alijanpour et al., 2009; Moradi et al., 2015), the use of Relascope has not yet been used to measure quantitative and qualitative characteristics and species diversity.

This study intends to use two methods, namely circular sample plots with an area of three acre and Relascope (Band number of 2) to estimate the diversity of species, quantitative and qualitative characteristics (by the criterion of integrating inventory error and plot sampling time in the forest stands of the Kaleibar Chay watershed (Armeni Olan) of the Arasbaran region.

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Material and methods

Study area

The Armeni Olan forests unit, with an area of 60 hectares, located at longitude of 46° 45' 7" to 46° 45' 52" East, and latitude of 38° 55' 43" to 38° 56' 22" North was selected for this research (*Fig. 1*).

Annual rainfall is about 500-600 mm. Relative humidity in June reaches its highest level, about 85%. Arasbaran dense forests are located at altitudes of 1000 to 1600 m above sea level (Alijanpour, 2011). The climate condition of the region is moderate semi-humid with cool and dry summers based on the De Martonne formula. The soil in the forest areas is mostly of brown and calcareous brown types. These soils are located on top of hard rock limestone, marl and sandstone (Alijanpour, 2011).

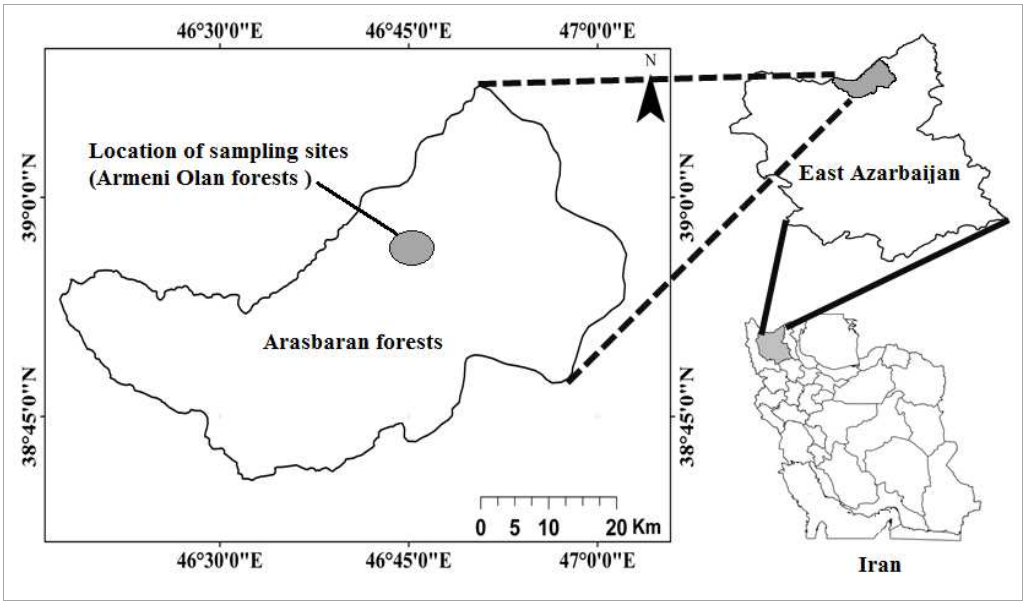


Figure 1. *Location of sampling site in Arasbaran forests*

Research methodology

Initially, a 60-hectare area was selected in the forest area. For the sampling of plots, a regular randomized grid system with 100 × 100 m dimensions was established.

Then, 58 points were specified on the map (1:10,000 scale) at the intersections of the network sides as plot centers. According to the conducted field survey and the previous studies carried out in the forest stands of the Arasbaran region and the placement of 15-10 trees in the sample, the area and shape of sample plots were decided to be circular fixed-area of three acre (Alijanpour et al., 2009; Alijanpour et al., 2011). At first, the general characteristics of each sampling plot location were recorded. Then, diameter at breast height (DBH) (cm) was measured using Calliper (Ushikata, Japan). Finally, the type of species, origin, and quality of trees were recorded.

The trees were studied in terms of the origin (seed regeneration stems and coppiced stems) as well as health status of the stem (healthy and unhealthy trees). Individuals infested by fungi or insect and also decayed trees were considered unhealthy. Then, the heights (m) of five trees, comprising of four trees close to the center of the sample plot

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in the four geographic directions, and one tree as the thickest one were measured (Suunto clinometer, Finland) in the sampling plot. Sampling time was also recorded by stopwatch. In order to record data with Relascope, based on the initial controlling of Band No. 2 and considering the placement of 10-15 trees in the sample plot, the quantitative and qualitative characteristics of the trees were measured (Spiegel Relascope, Austria) in accordance with the instructions for using the Relascope device (Avery and Burkhart, 2015). In this method, the sampling time of the plots was also recorded.

The measured DBH in each variable area-plot was entered into Microsoft Excel 2013 to calculate the basal area per hectare of fixed-area plot samples by using *Equation 1*.

The coefficient of basal area for Band 2 of Relascope was used to calculate basal area per hectare (Avery and Burkhart, 2015).

$$g = \frac{\pi}{4} \times d^2 \quad \text{(Eq.1)}$$

Where g is the basal area at breast height (m^2) and d is DBH (m).

In order to calculate the volume in a sample plot with fixed-area, it is necessary to use a local volume table, which unfortunately has not been yet provided for Arasbaran forest stands.

Therefore, the relationship between DBH and the measured heights of the trees in the fixed area plot (five stems per plot) was used to calculate such a table. Based on the calculated height graph and the estimation of the height of each diameter class, its volume was estimated using *Equation 2*.

Next, the volume in the sample plot and the volume per hectare of trees in the sample plot were calculated (Zobeyri, 2007).

$$V = 0.4 \times d^2 \times h \quad \text{(Eq.2)}$$

Where V is tree volume (m^3), d is DBH and h is tree height (m).

To calculate the volume per hectare for each sample plot in the Relascope method, the trees were arranged in diameter classes of 1 cm and the coefficient per hectare was calculated for each of the diameter classes.

Taking into account the selected strip coefficient for sampling and diameter class, the coefficient in hectare for each diameter class was calculated (Avery and Burkhart, 2015). *Equation 3* was used to calculate the coefficient per hectare.

$$F = 10000 \div (d^2 \times f^2 \times \pi) \quad \text{(Eq.3)}$$

Where F is the coefficient per hectare, d is the diameter of each class (m), f is the coefficient of each strip for the tree (in this study, tape number 2 was used for the sampling and the coefficient of the strip was 35.35).

Then, considering the number of trees in each diameter class, the number per hectare was calculated and, taking into account the volume of each class (by the volume table prepared in this study), the volume per hectare was calculated for each sample plot.

For statistical analysis of the species type data, DBH, quality, origin, slope and sampling time for each sample were entered in the SPSS software version 22 and, the

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normal distribution of the quantitative data was evaluated using the Kolmogorov-Smirnov test.

The calculated mean DBH, basal area at breast height, volume and time for each of sample plot in two methods of sampling with fixed-area and variable-area plots were compared by using the independent t-test. Origin, species composition and quality of species were compared by the Chi-Square test.

In addition, the species frequency data were used in each sample plot to calculate the species evenness and diversity indices.

Species richness, Simpson diversity index, Shannon Wiener diversity and evenness indices for each plot were calculated using the PC-ORD software version 5. Then, the obtained indices were compared with the independent t-test using SPSS version 22.

Finally, in order to evaluate the two methods of inventory, *Equation 4* was used, where E% is the standard error of estimation at the specified confidence level and (T) is the sampling time (Husch et al., 1982).

$$A = (E\%)^2 \times T \quad \text{(Eq.4)}$$

Results

Quantitative characteristics of the studied stand

Obtained mean, standard error and percentage of standard error for DBH, number per hectare, basal area at breast height per hectare, volume per hectare and height of trees from 58 sampling plots with fixed and variable-areas (Relascope) are provided in *Table 1*. Based on this table, mean DBH, basal area at breast height, number per hectares and volume per hectare in the variable-area plots sampling method is higher than fixed-area plots.

Qualitative characteristics of the study stand

Percentage of mixture of species

As shown in *Table 2,* in the method of using plots with fixed-area, *Carpinus betulus* had the highest frequency (76.6%) and in the sampling method with variable-area plots, this species, with 74.4%, was the most frequent species in the stand.

The origin of the trees

The results of sampling with fixed-area plots showed that 86.3% of the trees in this stand were coppiced stems and 13.7% were seed regeneration stems. On the other hand, the results of sampling plots with variable-area showed that 84.1% of the trees in this stand were coppiced stems and 15.9% were seed regeneration stems (*Fig. 2*).

Tree health

Another characteristic studied for the trees was the health of the trees whose DBH values were measured.

From the total number of trees measured by the fixed-area plots sampling method, about 91.2% were healthy and 8.8% were unhealthy. While, Sampling by variable-area plots yielded 92.7% healthy and 7.3% unhealthy trees (*Fig. 3*).

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Comparison of quantitative and qualitative characteristics of the studied stand in the two methods

The t-test results between the two inventory methods for parameters including mean diameter at breast height, mean basal area per hectare and average volume per hectare showed that there were significant differences at the 95% probability level (*Table 1*).

In addition, according to the Chi-Square test, it was found that the parameters including percentage of species composition, origin and health of the stems had no significant difference between the two inventory methods at 95% probability (*Table 3*).

Table 1. *Quantitative characteristics of the studied stand and t-test results*

Variables	Method of sampling	Mean	Standard error	% Standard error	t	Significance
DBH (cm)	Fixed area plot	12.34	0.19	3.08	-5.027	0.000*
	Relascope	13.83	0.24	3.47		
Basal area (m^2/ha)	Fixed area plot	12.41	0.69	11.12	-2.805	0.006*
	Relascope	14.96	0.6	8.02		
Volume (m^3/ha)	Fixed area plot	64.77	4.2	12.97	-1.93	0.057*
	Relascope	74.96	3.26	8.70		
Number of trees (N/ha)	Fixed area plot	939.65	44.37	9.44	-	-
	Relascope	1195.27	54.62	9.14		
	Fixed area plot	10.19	0.22	4.32		

Table 2. *The percentage of species composition in the fixed-area plot and variable-area plot*

Type of plot area	*Carpinus betulus*	*Quercus petraea*	*Acer copmestre*	*Acer monspessulanum*	*Quercus macranthera*	*Cornus mas*	*Prunus avium*	*Taxus baccata*	*Euonymus sp*
Variable area plot	74.4	12.9	5.1	0.9	4	0.1	2.2	0.2	0.1
Fixed area plot	76.6	11.3	5.3	1.3	3.1	0.3	1.3	0.6	0.3

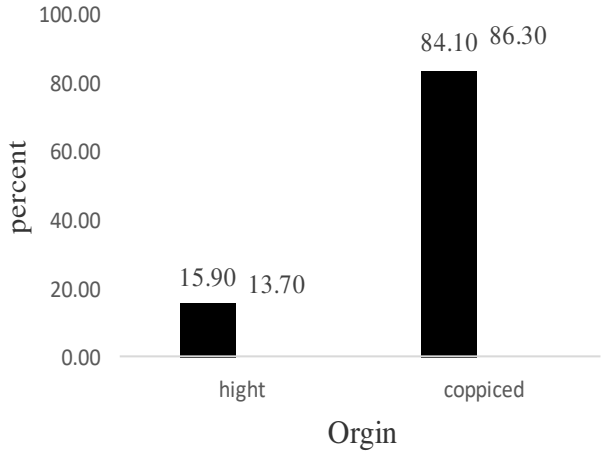


Figure 2. *The origin of the stands in the fixed-area plot and the Variable-area plot*

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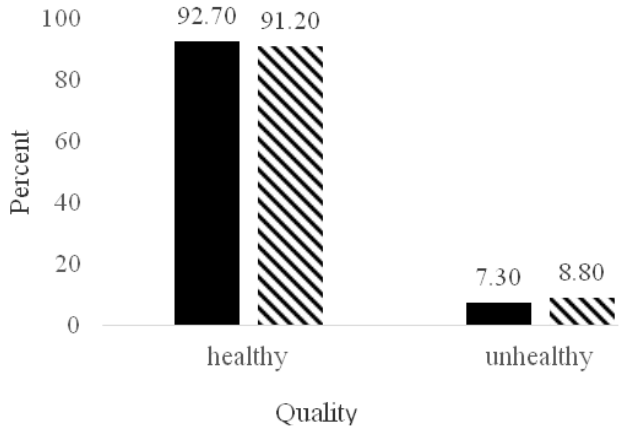


Figure 3. *Health of the stems in the fixed-area and variable-area sample plots*

Table 3. *Comparison of qualitative characteristics of the stand in the two methods using Chi-square test*

Variables	Chi-square	Degree of freedom	Significance
Percentages of mixture	9.8	8	0.28^{ns}
Origin	2.197	1	0.12^{ns}
Quality of species	1.805	1	0.179^{ns}

Sampling time

Comparison of average sampling time in the two methods showed that there was a significant difference between the two inventory methods at the 5% level (*Table 4*) and the recording time of the fixed-area plot was significantly higher than the recording time associated with the variable-area plot.

Table 4. *Comparison of plot sampling time for two methods*

Variables	Fixed area plot	Variable area plot	t	Significance
Mean sampling time for each plot (second)	706.10(20.24)	485.91(14.56)	8.83	0.000*

The significance is at 5% and the numbers in brackets indicate the standard error

Evaluation of the two inventory methods using the $(E\%)^2 \times T$ index

The evaluation of the two inventory methods, taking into account the time and accuracy of the inventory (*Equation 4*), showed that the calculated values of this index for the variables of volume and basal area per hectare were less in the sampling method with variable area (*Table 5*).

Table 5. *Comparison of two methods by using the $(E\ \%)^2 \times T$ index*

Variables	Fixed area plot	Variable area plot
Volume (m3/ha)	118780.77	36778.50
Basal area (m2/ha)	88573.18	31253.92

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Species diversity

Mean and standard error of species richness, evenness and Shannon Wiener diversity and Simpson species diversity index of forest stands are shown in *Table 6*.

Although the evenness index and Simpson diversity derived from variable-area plots were higher than fixed-area plots and the species richness index and Shannon-Weiner species diversity index were higher in fixed-area plots, the t-test did not show a significant difference in the mean of different indices between the two methods ($\alpha = 5\%$).

Table 6. *Comparison of species diversity of the studied stand with t test results*

Variable	Fixed area plot	Variable area plot	t	Significance
Richness	2.79(0.16)	2.40(0.11)	1.960	0.52^{ns}
Simpson diversity indices	0.32(0.03)	0.33(0.03)	-0.125	0.9^{ns}
Shannon diversity indices	0.58(0.052)	0.55(0.045)	0.396	0.69^{ns}
Shannon evenness index	0.51(0.043)	0.58(0.043)	-1.108	0.27^{ns}

n.s: The difference is not significant

Discussion

In this research, the fixed-area and Relascope sampling methods were used (for the first time in Arasbaran forests) to obtain quantitative and qualitative characteristics and species diversity indices. Different stand characteristics can be used as indicators of management practices in forests. Meantime, some stand characteristics are sensitive to the measurement method (e.g., volume, quadratic mean diameter and small stem density and basal area); while others are less sensitive (Misik et al., 2013: Rice et al., 2014). The results showed that the estimated quantitative parameters of the forests in this research were similar to the other studies conducted in the same forest stand (Alijanpour et al., 2003, 2009, 2011; Moradi et al., 2015). However, there were significant differences between the two methods regarding DBH, basal area at breast height per hectare and volume per hectare values. These results were consistent with existing literature (Fallah et al., 2000; Sadiqi et al., 2014; Lowell, 1997; Rios et al., 2000; Motz et al., 2010; Pique et al., 2011).

In terms of accuracy of estimating quantitative characteristics, the results showed that the standard error of estimation for average volume per hectare, basal area per hectare and number per hectare when using Relascope were less than those of sampling method with a circular fixed-area plot. More accurate estimations were made regarding basal area at the breast height and volume per hectare by variable-area plot (Relascope) method in comparison to fixed-area sampling method. In addition, in a study the methods were used to estimate variables including basal area at breast height, density, and diameter distribution of trees. It has been shown that in multi-layered habitats and semi high-density forests, the Relascope had more precision in estimating basal area than density (Pique et al., 2011). It has been suggested that the precision of measurements made by Relascope in surveying the basal area at breast height per hectare, volume per hectare and DBH was generally higher than that of circular sampling method (Druszcz et al., 1995).

In the investigation of qualitative characteristics such as species composition percentage, species origin and stem health, the results of the present study were

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consistent with the findings of the other researchers conducted in the same region (Amir ghasemi, 2001; Alijanpour et al., 2007; Moradi et al., 2015). Regarding the less measurement time using Relascope method, sampling method with variable-area plots (Relascope) for examining the species composition percentage and the origin and quality of the stems is suggested. Average time of sampling in fixed-area method was significantly higher than that of variable-area sampling method which was in good accordance with more recent report by Portela et al. (2016). Fallah et al. (2000) and Sadiqi et al. (2014) also reported the similar results. In determining the optimum method of sampling in Arasbaran forests stands, Alijanpour et al. (2003) showed that the average recording time of circular plots, rectangular and line samples were significantly different from each other and they reported that the time taken for line sampling was less than others.

In calculating the Shannon species diversity, Simpson species diversity and evenness, there was no significant difference between the two sampling methods. In this regard, other studies have shown that the accuracy of basal area and diversity indices were also similar (Motz et al., 2010). Moreover, by comparing the circle sample and distance methods in estimating diversity indices, the distance approach has been proposed for forest sampling strategies (Kint et al., 2004). Some researchers have introduced variable-area sampling for surveying species richness due to its speed and accuracy (Potela et al., 2016).

Conclusion

According to $(E\%)^2 \times$ index (Hush et al., 1982), It is concluded that for determining the characteristics of the basal area at breast height and volume per hectare, the sampling method with variable area was more appropriate regarding the standard error of estimation percentage and cost. In addition, due to the fact that there was no significant difference between the two methods for determining species diversity, using the Relascope could be suggested to estimate some quantitative characteristics as well as richness and diversity indices of the Arasbaran forests.

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ABIOTIC AND BIOTIC FACTORS AFFECTING THE OCCURRENCE OF THRIPS ON LOTUS FLOWERS

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Abstract. *Frankliniella schultzei* Trybom and *Scirtothrips dorsalis* Hood are two important insect pests of lotus flowers (*Nulumbo nucifera* Gearn). Both are active throughout the year and are difficult to control during pre- and post- harvest management. This study is focused on factors that influence the relationship among the thrips, their host plants and abiotic factors. Therefore, the distribution of these thrips species within lotus flowers of different flower ages was assessed using randomly sampling from commercially growing areas in Bangkok, Thailand and investigated thrips abundance on these flowers every week from April to September, 2016. The results revealed that there was a higher thrips population on older lotus flowers. *S. dorsalis* was much more abundant in summer than in the rainy season. It was found mainly on the pedicel and the outer corolla of the flowers. In the summer and rainy season, *F. schultzei* occurred most often and were found on the distal parts of lotus petals and pollen areas. Hindu lotus and East Indian lotus could support more of *F. schultzei* and *S. dorsalis* than Album Plenum and Roseum Plenum. The correlation among abiotic factors (temperature, amount of rainfall and relative humidity) and population fluctuations of *S. dorsalis* and *F. schultzei* was assessed. The studies showed a positive correlation of temperature (r = 0.7918) and a negative correction of relative humidity (r = -0.7588) to population of *S. dorsalis* but no relation with amount of rainfall (r = 0.0260). Whereas, there were non-significant correlations among *F. schultzei* and temperature (r = 0.43650, relative humidity (r = -0.2113) and rainfall (r = -0.4164). Interactions between different temperatures, relative humidity and rainfall effects on the abundance of the thrips were seen here. Both fundamental and applied perspectives will provide better understanding the thrips population abundance, and help to develop management plans for thrips control.
Keywords: *F. schultzei, population fluctuation, S. dorsalis, relative humidity, rainfall*

Introduction

Currently, insect pests are an important factor in lotus farming that affect the quantity and quality of the agricultural product. Farmers use chemicals as the primary measure to prevent damage caused by insects. Thrips are major enemies of both vegetable crops and ornamental plants. They use their stylets to obtain water and liquid nutrients from various plant cells including shoots, flowers and leaves, which results in a pale color or dry edges of affected plant tissue. The insects are highly polyphagous. Both *Scirtothrips dorsalis* Hood (chilli thrips) and *Frankliniella schultzei* Trybom (common blossom thrips) are two important insect pests of lotus flowers (*Nulumbo nucifera* Gearn) grown for export (Mungnimitr and Bumroongsook, 2007). The chilli thrips is a serious insect pest of lotus, agricultural crops and various floral plants. It is most often found feeding on young shoots and leaves and causes scars on fruit surface (Seal and Klassen, 2008).

The common blossom thrips are abundant on sacred lotus in Thailand (Mound and Palmer, 1981). It is an anthophilous insect and often feed on the floral parts of its host plant (Kakkar et al., 2012b), and is capable of destroying parts of the lotus flower. Both larvae and adults usually stay at the base of the petals and feed on plant cells (Mungnimitr and Bumroongsook, 2007), but can feed on other plant parts such as petals and leaves (Milne et al., 1996). It is a highly polyphagous insect, infesting a wide range of vegetable

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crops and ornamental plants (Moritz et al., 2013). The thrips aggregate and colonize on plant petals (Milne et al., 2002) and can also pollinate various host plants (Kirk, 1997), although they can disfigure ornamental flowers in the process. Heavy infestation can discolor of flowers and distort the growth of young shoots (Amin and Palmer, 1985). Both forms of *F. schultzei* (dark and pale forms) can also be insect vectors for virus transmission (Nagata and De Avila, 2000; Cho et al., 1988).

Abiotic and biotic factors work in combination to shape species distributions and abundance (Lewis et al., 2017). Temperature is one of the important abiotic factors that affects abundance, distribution and population dynamics of insects (Kang et al., 2009; Yadav and Chang, 2014). Thrips population had a positive correlation with temperature and showed a negative correlation with relative humidity (Shuaib et al., 2008). Climatic factors are important factors that significantly influence thrips density as well (Kirk, 1997; Waiganjo et al., 2008). High temperatures with no rainfall increased thrips populations, while high relative humidity and rainfall had a negative relationship (Hamdy and Salem, 1994).

Biotic factors associated with host plants can affect species distributions and abundance. Plant aging increased mortality and influenced larval development of thrips (Campos et al., 2003). A nutrient supplement (nectar/pollen) is essential for insect survival in the absence of prey, or a modified microhabitat which can protect it against severe abiotic conditions (Xiao et al., 2011). Asian lotus flower is a concave shape and without nectar, having a cavity surrounded by petals and stamens and releasing scent. The flower has a thermoregulation process that can control temperature during anthesis and an optimum temperature between 30 and 36 °C when the environmental temperature is between 10 and 45 °C (Seymour et al., 1998; Seymour and Schultze-Motel, 1996; Xiao et al., 2011). Thrips are a lotus flower pollinator. The level of plant damage is dependent on *S. dorsalis* density (Mannion et al., 2014).

The objectives of this research are to determine seasonal population fluctuation and within lotus flower distribution of *F. schultzei* and *S. dorsalis*. The correlation of abiotic factors: temperature, relative humidity and amount of rainfall and population abundance of thrips on lotus flowers were evaluated. Understanding the influence of these abiotic and biotic factors on thrips abundance can help to further develop more efficient management programs for thrips control.

Materials and methods

Location of the sample sites

The experimental sites were located in Ladkrabang district of Bangkok province. Eleven sites of sample collection were chosen based on lotus varieties and non pesticide-treated areas (*Fig. 1*). GPS coordinates for location and the distance from sample sites to the data logger including the lotus plant variety were illustrated in *Table 1*. S1 and S2 are the experimental sites for collection of Hindu lotus (a single white flower); S3 and S4 for Album Plenum (a double white flower); S5-S6 for East Indian lotus (a single pink flower); and S7-S11 for Roseum Plenum (a double pink flower, *Table 1*).

Population density of S. dorsalis and F. schultzei on different stages of lotus flowers

Differently aged lotus flowers (*N. nucifera* var. Roseum Plenum), i.e., small bud, standard bud and full bloom (5, 9 and 13 days above water level, respectively; n = 30),

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were collected weekly from non-chemical lotus farms in Ladkrabang area, Bangkok, Thailand. Direct thrips count was performed using a stereomicroscope and total number of thrips found on each type of flower was recorded.

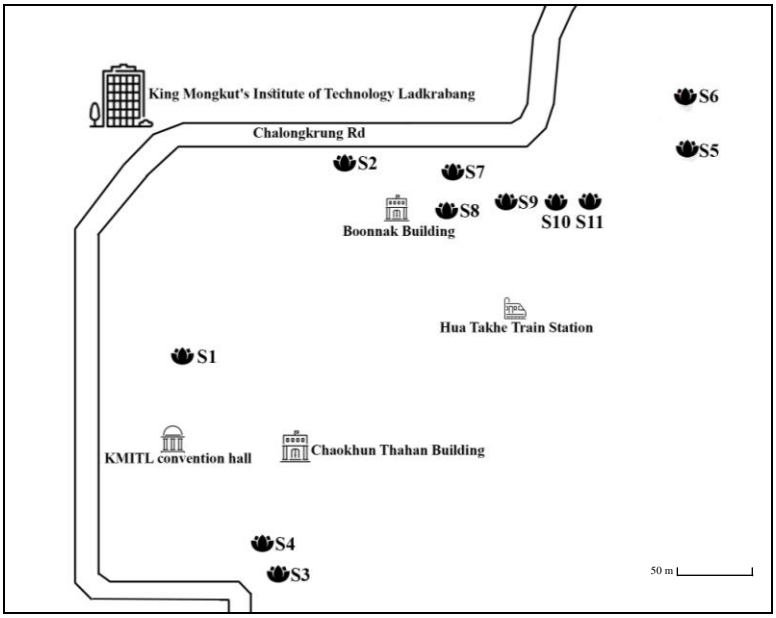


Figure 1. *Location of the sample sites in Ladkrabang district*

Table 1. *Description of eleven sample sites located in Ladkrabang district, Bangkok, Thailand*

Sample sites	Location	Distance to the data logger (km)	Lotus varieties
S1	13°43'38.0"N 100°46'46.9"E	0.200	Hindu lotus
S2	13°43'48.6"N 100°46'53.4"E	0.126	Hindu lotus
S3	13°43'30.1"N 100°46'49.7"E	0.286	Album Plenum
S4	13°43'30.8"N 100°46'49.3"E	0.578	Album Plenum
S5	13°43'49.2"N 100°47'09.3"E	0.318	East Indian lotus
S6	13°43'51.7"N 100°47'09.2"E	0.340	East Indian lotus
S7	13°43'48.3"N 100°46'58.2"E	0.020	Roseum Plenum
S8	13°43'46.9"N 100°46'57.7"E	0.047	Roseum Plenum
S9	13°43'47.6"N 100°46'59.5"E	0.043	Roseum Plenum
S10	13°43'47.6"N 100°47'00.7"E	0.074	Roseum Plenum
S11	13°43'47.6"N 100°47'01.5"E	0.101	Roseum Plenum

Comparison of the abundance of S. dorsalis and F. schultzei on different parts of lotus flower

Standard buds of Roseum Plenum lotus flowers (n = 30) were collected and thrips count on its pedicel, petals and pollen areas of lotus cut flower were performed. Thrips density of *S. dorsalis* and *F. schultzei* from these flower parts was identified and recorded**.**

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Number of thrips on the different corolla of lotus flowers

Roseum Plenum lotus flowers are composed of six corolla: the first corolla are the outermost petas, and the innermost petals are the sixth corolla. Standard lotus flowers var. Roseum Plenum (n = 30) were collected weekly. Thrips count on each corolla were investigated.

Thrips abundance on different varieties of lotus flowers

A total of 30 lotus flowers were randomly selected from each of four varieties of *N. nucifera*: Hindu lotus (a single white flower), Album Plenum (a double white flower), East Indian lotus (a single pink flower) and Roseum Plenum (a double pink flower). Number of thrips found on lotus was investigated and recorded.

Correlation among thrips population abundance and temperature, relative humidity and rainfall

A total of 30 Roseum Plenum lotus flowers were randomly selected. Thrips counts of *S. dorsalis* and *F. schultzei* on each flower and its pedicel was performed. For the temperature and relative humidity dataset, a data logger (DT172-CEM G M Electronics) was used to record air temperature and relative humidity every hour The weather data (daily average observation for air temperature and relative humidity) was computed. The data logger was placed on the wall of the storage house (5 m from S7) at 1.5 m above the ground with protective roof. The amount of rainfall at Ladkrabang area obtained from the Thai Meteorological Department.

Data collection and statistical analysis

The experimental period was spread out across two seasons: summer (April-May, 2016) and rainy season (June-September, 2016). Samples were collected once a month for six months and processed independently for each experiment. To reduce light intensity variation effect on thrips population, all samples were collected between 7 and 9 am. Thrips were collected from plant parts, then transferred in 70% ethanol for later analysis. They were identified to species using a stereomicroscope. The SPSS statistical software was used to perform data analysis. Population density of thrips were analyzed separately for each experiment using the paired sample t-test to determine the mean difference between density of *S. dorsalis* and *F. schultzei*. ANOVA was applied to assess the mean difference of thrips occurrence among lotus varieties. Linear regression analysis was used to assess correlation among thrips occurrence and abiotic factors**.**

Results

Population density of S. dorsalis and F. schultzei on different stages of Roseum Plenum lotus flowers

Thrips counts on the same type of flower buds were compared and the results showed that the older the flower buds were, the more thrips were found. In the small flower buds, no difference was found in the density of *F. schultzei* detected than *S. dorsalis* (t-value = 1.29 and p = 0.1125). *F. schultzei* were found more than *S. dorsalis* in the standard-sized buds (t = 2.19 and p = 0.0264) and the full bloom lotus flowers

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(t = 3.11 and p = 0.0054; *Table 2*). *F. schultzei* was found the most on full bloom lotus flowers.

Comparison of the abundance of S. dorsalis and F. schultzei on diffirent parts of Roseum Plenum lotus flowers

S. dorsalis was found on flower pedicel and petals and none were detected in the middle area of flowers (containing pollen and stamen). Comparing *S. dorsalis* and *F. schultzei* occurrence on flower pedicels, the result showed no significant difference (t = 1.46 and p = 0.0869). *F. schultzei* were detected more than *S. dorsalis* on lotus flower petals (t = 2.82 and p = 0.0091), as well as stamen and pollen areas (t = 2.56 and p = 0.0141; *Table 3*).

Table 2. *Population density of S. dorsalis (S) and F. schultzei (F) on different stages of Roseum Plenum lotus flower*

Months (2016)	Thrips density[1] on different stages of flower					
	Small bud		Standard bud		Full bloom	
	S	F	S	F	S	F
April	0.16	3.42	1.20	3.04	7.09	14.31
May	0.68	2.03	2.55	16.03	2.75	48.70
June	0.44	0.49	0.96	7.73	0.68	20.69
July	1.18	0.90	0.63	1.78	0.35	16.58
August	1.08	0.91	0.08	9.01	0.01	30.51
September	0.00	0.00	0.00	0.00	0.73	2.867

Table 3. *Number of S. dorsalis (S) and F. schultzei (F) found on different parts of Roseum Plenum lotus flowers*

Months (2016)	Thrips density					
	Flower pedicel		Petal		Pollen and stamen	
	S	F	S	F	S	F
April	1.87	0.00	1.29	0.86	0.00	4.99
May	0.38	0.42	0.52	7.24	0.00	17.43
June	0.37	0.05	0.87	0.78	0.00	3.02
July	0.09	0.00	0.96	2.94	0.00	3.48
August	0.30	0.00	0.08	7.01	0.00	6.46
September	0.00	0.00	0.22	6.69	0.00	1.21

Number of thrips on the different corolla of Roseum Plenum lotus flowers

S. dorsalis were found from the first to the third corolla of Roseum Plenum flowers (*Table 4*). *F. schultzei* could be located from the outermost to the innermost corollas (*Table 5*). Density of *F. schultzei* appeared higher than *S. dorsalis* on different corolla.

Thrips abundance on different varieties of lotus flowers

S. dorsalis occurrence peaked in April and decreased gradually from May to September. They preferred Hindu lotus and East Indian lotus followed by Album Plenum and Roseum Plenum (*Table 6*). *F. schultzei* was found most in East Indian lotus during April and May, 2016 and its population was highest in Hindu lotus from June to

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September, 2016 (*Table 7*). *F. schultzei* was detected more in Roseum Plenum in May and June than Album Plenum.

Table 4. *Population density of S. dorsalis on different corolla of Roseum Plenum lotus flowers*

Months (2016)	Population density of *S. dorsalis* on the corolla no.					
	1	2	3	4	5	6
April	5.98	1.93	1.35	0.00	0.00	0.00
May	1.40	0.62	0.73	0.00	0.20	0.00
June	0.22	0.11	0.04	0.00	0.00	0.00
July	0.62	0.15	0.33	0.00	0.00	0.00
August	0.04	0.00	0.00	0.00	0.00	0.00
September	0.11	0.02	0.02	0.00	0.00	0.00

Table 5. *Population density of F. schultzei on different corollas of Roseum Plenum lotus flowers*

Months (2016)	Population density of *F. schultzei* on the corolla no.					
	1	2	3	4	5	6
April	0.02	.0.31	0.00	0.00	0.00	0.00
May	0.31	2.24	0.47	1.80	0.82	0.91
June	0.18	9.40	0.04	5.44	0.71	0.91
July	0.15	22.58	0.31	8.56	2.27	2.24
August	0.20	19.27	0.13	7.56	1.91	0.78
September	0.00	8.04	0.00	3.31	1.49	0.02

Table 6. *Population density of S. dorsalis on different varieties of lotus flower*

Months (2016)	Number of *S. dorsalis*[1]			
	Hindu lotus	Album Plenum	East Indian lotus	Roseum Plenum
April	21.80b	16.04b	36.04a	3.60c
May	21.75a	3.91bc	9.04b	2.07c
June	6.51a	1.02b	1.42b	3.27ab
July	6.62a	1.02b	1.42b	3.27ab
August	0.47a	0.09a	2.71a	0.44a
September	0.00b	0.18b	1.69a	0.35b

[1]Within rows, means followed by the same letters are not significantly different (P = 0.05, DMRT)

Table 7. *Population density of F. schultzei on different varieties of lotus flower*

Months (2016)	Number of *F. schultzei*[1]			
	Hindu lotus	Album Plenum	East Indian lotus	Roseum Plenum
April	14.05b	1.07b	57.27a	2.58b
May	12.09c	3.33c	73.62a	28.95b
June	34.49a	1.36c	11.53bc	14.60b
July	143.67a	20.07c	54.00b	11.78c
August	185.15a	10.24c	141.95b	35.07c
September	20.37a	0.00c	7.33b	0.27c

[1]Within rows, means followed by the same letters are not significantly different (P = 0.05, DMRT)

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Correlation among thrips population abundance and temperature, relative humidity and rainfall

The climatic conditions from April to September, 2016 in the study area ranged from 27.20–32.57 °C, RH 72–83% and rainfall 0–19 mm (*Table 8*). *S. dorsalis* density on lotus flower was highest in April and deceased gradually to 10.85, 11.68 and 7.85 in May, June and July, respectively. In August and September, there were 1.17 and 1.45 thrips/flower, respectively (*Fig. 2*). *F. schultzei* density was highest in August and lowest in June. The correlation between the amount of thrips on lotus flower over the five months of sampling and the abiotic factors temperature, relative humidity and rainfall was statistically analyzed. The results showed that temperature and relative humidity were significantly correlated with the change in *S. dorsalis* (r = 0.7918 and -0.7588, respectively). *F. schultzei* density on lotus flowers was not significantly correlated to temperature, relative humidity and rainfall (*Table 9*).

Table 8. *Abiotic condition of temperature, relative humidity and rainfall during the sampling periods*

Months (2016)	Temp (^{o}C)	RH (%)	Rainfall (mm)
April	32.57	72	0.00
May	28.20	80	8.95
June	30.36	75	19.00
July	28.60	79	1.50
August	29.50	75	0.68
September	27.20	83	0.00

Discussion

Both abiotic and biotic factors affect insect population abundance (Lewis et al., 2017). *S. dorsalis* is a polyphagous insect pest with a global distribution (Dickey et al., 2015). It feeds on meristem and young plant parts. *S. dorsalis* prefers the outer corolla of lotus flower and its pedicel. In addition, it feeds on lotus leaves (Mungnimitr and Bumroongsook, 2007). *F. schultzei* primarily attacked lotus flowers and caused discoloration. The female deposits egg on flower tissue and it takes about 13 days to complete life cycle (Kakkar et al., 2012a). *F. schultzei* was observed on full bloom lotus flowers. Aggregation density of insects on host plant corollas induce mating success (Kang et al., 2009). High density of *F. schultzei* on lotus flowers was similar to the finding of Kakkar et al. (2012b), who reported that adult are the most numerous in tomato flower.

As noted above, Hindu lotus and East Indian lotus are categorized as a single flower and Album Plenum and Roseum Plenum are a double flower (Mandal and Bar, 2013). The structure of single lotus flowers could allow *F. schultzei* to build up populations more easily than double lotus flowers. Further studies should be investigated if various colors of lotus flowers attract thrips to different degrees.

The lower the relative humidity value and the higher the temperature, the higher the number of *S. dorsalis* observed. Previous research reported similar results on relationship among temperature, relative humidity and thrips population (Hamdy and Salem, 1994). *S. dorsalis* was found on leaves and the outer parts of lotus flowers, therefore it is more exposed to the environmental condition than *F. schultzei*. On the contrary, *F. schultzei* density on lotus flowers might not depend on the abiotic factors

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like temperature, relative humidity and rainfall due to its cryptic habitat. The microhabitat within flowers could play important roles on observed population of thrips (Salguero_Navas et al., 1991).

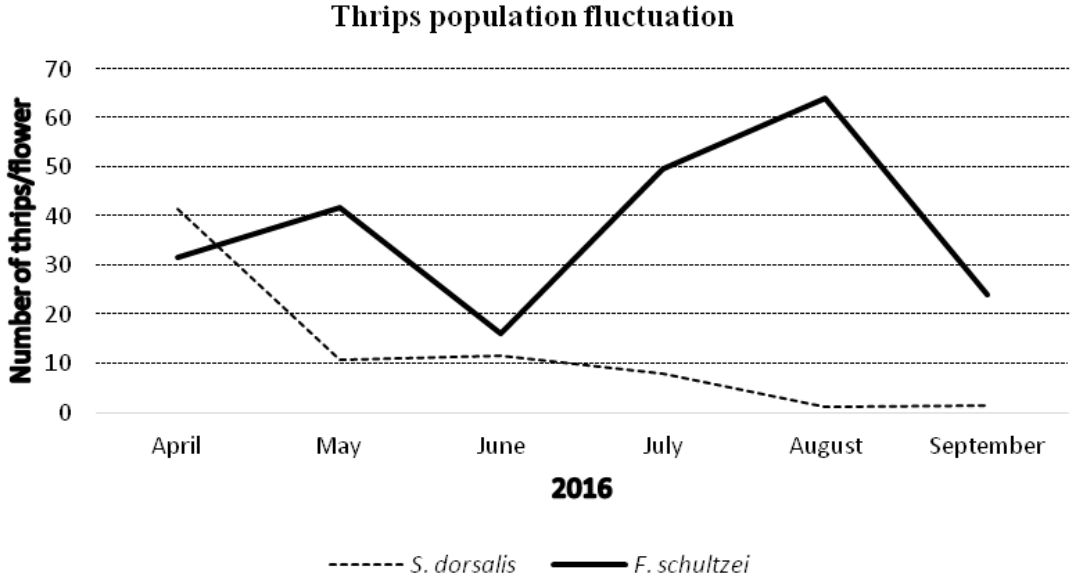


Figure 2. *Population density of thrips on lotus flowers*

Table 9. *Correlation of population density of S. dorsalis and F. schultzei and temperature, relative humidity and rainfall*

Thrips species	Correlation coefficient		
	Temperature	**Relative humidity**	**Rainfall**
S. dorsalis	0.7918*	-0.7588*	-0.0260
	(0.8899)	(0.8711)	(0.1613)
F. schultzei	0.4365	0.2113	0.4164
	(0.6607)	(0.4597)	(0.6453)

(R) = values in parentheses are R adjusted
*Statistically difference at p = 0.05

Conclusions

S. dorsalis attacked flower pedicels and the outer part of lotus petals and population peak during high temperature and dry weather. Abiotic factors such as the temperature and relative humidity played a remarkable role on *S. dorsalis* abundance. Regarding *F. schultzei*, this is the first report that they attack four varieties of lotus flowers. The *F. schultzei* mainly inhabit and feed on the petals and the pollen areas of lotus flower. Biotic factors (age of host plant and food selection) shaped the distribution and abundance of *F. schulzei*. In addition, lotus flower structures as protective shelter and microclimate within the flowers are factors that influenced the population abundance of *F. schulzei*. Size and cryptic behavior of these thrips make them difficult to control. Therefore, phytosanitary treatment of these thrips for lotus cut flowers export is probably the most challenging task.

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EFFECT OF MICROWAVE RADIATION ON GROWTH AND GERMINATION OF STONE PINE (*PINUS PINEA* L.) SEEDLINGS

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Abstract. Highly successful afforestation applications and good performance development is linked to adoption of correct afforestation techniques, quality of seedling material, selection of appropriate origin, seed material, and treatment of seedlings. One of these treatment processes is subjecting seeds to a magnetic field. The aim of this study is to determine the effects of magnetic field on the germination of seeds and growth of seedlings of stone pine (*Pinus pinea* L.). For this purpose, seeds (Aydın-Koçarlı origin) were used in nursery garden beds (Isparta, Turkey) with three replications based on completely randomized design. A magnetron tube setup was used to generate a magnetic field. A 220V AC magnetron tube was positioned 15 cm above the seed container, and 280, 336, 462, and 595 W output for 15, 30, and 60 s was applied, respectively. As a result of being subjected to the magnetic field, stone pine seeds experienced changes in seedling morphological characteristics and positive effects on seedling growth and development. It is believed that application of 280 W microwave of 160 micro Tesla intensity for 60 s could suppress live plants by encouraging quick seedling development, help seedlings benefit from water and nutrient elements in the earth, and contribute to the economy of the country by reducing development time.
Keywords: *magnetic field, seed, morphological characteristics, seedling quality, afforestration*

Introduction

Forests in Turkey cover 21.7 ha, or 27.6% of total area. The country has a tree value of total 1.49 billion m^3 and rich bio-diversity. Half of the forest resources of the country consist of pervasive and infertile needle-leaved and broad-leaved forests. The fertiliy of our forests is low, at approximately 0.750 m^3/ha per year (Urgenc, 1998). Therefore, it is necessary to fertilise pervasive forest areas with artificial rejuvenation techniques in the near future and create new forest areas with afforestation applications.

Today, in developed countries where intensive forestry techniques have been applied, determining afforestation areas only to obtain wood raw materials has been abandoned. These applications were replaced with multi-functional afforestation techniques that aim to produce raw wood material and by-products (Ilter and Ok, 2004). Through such multi-functional afforestation as used in Mediterranean and south European countries, it is obvious that such countries commonly prefer stone pine (*Pinus pinea* L.) because it is more durable than other pine types, adapts to high temperatures and drought, tolerates water stress with its deep roots, provides effective protection against erosion, and has economic benefits (Ansin, 2001).

In terms of worldwide distribution of stone pine, large forests are observed in Spain, Portugal, Turkey, and Italy, as well as Greece, Syria, Lebanon, France, Yugoslavia, Montenegro, and other North Africa countries (Firat, 1943).

The stone pine is a typical Mediterranean tree, and it is one of five pine species that naturally grow in our country. Stone pine generally has a natural distribution in regions with a Mediterranean climate, which have an annual temperature average of 11.4-18.7ºC, relatively high annual precipitation average of 635.7-1288.1 mm, and relative humidity of 58-80.8% (Sayman et al., 2006).

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Highly successful afforestation applications and good performance development is linked with adoption of correct afforestation techniques, quality of seedling material, selection of appropriate origin, seed material, and treatment of seedlings (Urgenc, 1998). One of these treatment processes involves subjecting seeds to a magnetic field. The effects of magnetic fields on growth and development of plants and practical applications have long been discussed. Recently, some studies have indicated positive effects of magnetic fields on plant growth. Magnetic fields have been found to have positive effects on fertility and number of flowers (Matsuda et al., 1992), seed germination ratio and speed (Souza Torres et al., 1999), and earliness of flowering (Samy, 1998) of various plant types and species.

Accordingly, in afforestation applications with stone pine, which has economic and forestry importance for manufacturing of by-products, it is possible to discuss the significant effects that magnetic fields have on product yield and growth performance (Kirdar et al., 2016). Based on these facts, this study was conducted to evaluate the effects of magnetic fields on stone pine (*Pinus pinea* L.) seed germination and seedling growth.

Materials and methods

The material of this study consisted of seeds collected in December 2015 from naturally grown stone pine stands in the Aydın-Koçarlı region (Turkey) (37.690503 north latitude, 27.688276 east longitude, 796 m altitude) that showed superiority to other individuals in the stand in terms of height, diameter, and body smoothness. Before harvested seeds were planted, they were divided into 6 groups (including a control group) and subjected to magnetic fields of varying microwave power and duration via magnetron tube (*Table 1*).

Table 1. *Properties of microwave application by the groups*

Group	Microwave intensity (μT)	Microwave output power (W)	Duration of exposure (s)	Post-Exposure seed temperature (ºC)
1	160	280	60	40
2	192	336	30	60
3	192	336	60	78
4	264	462	15	94
5	340	595	15	108
Control Group				

A magnetron tube setup was used for generating the magnetic field. A 220V AC magnetron tube was positioned 15 cm above the seed container, and 280, 336, 462, and 595 W output power was applied for 15, 30, and 60 s. Seeds were not applied at high output powers (462 and 595 W) for 30 and 60 s due to overheating. The frequency of the microwave was 2450 MHz.

Seeds were planted in 2016 in the Suleyman Demirel University-Yenisarbademli Vocational School application nursery garden beds with three replications based on completely randomized design. The nursery garden field has a semi-continental climate in a Mediterranean climate transition area and an altitude of 1178 m. Annual average temperature was 11.2ºC, and annual average precipitation was 727.2 mm. The 0-30 cm

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deep planting bed on the trial's planting parcel was 57.9% sand, 23.3% silt, and 18.8% clay with a silty loam texture (*Figure 1*).

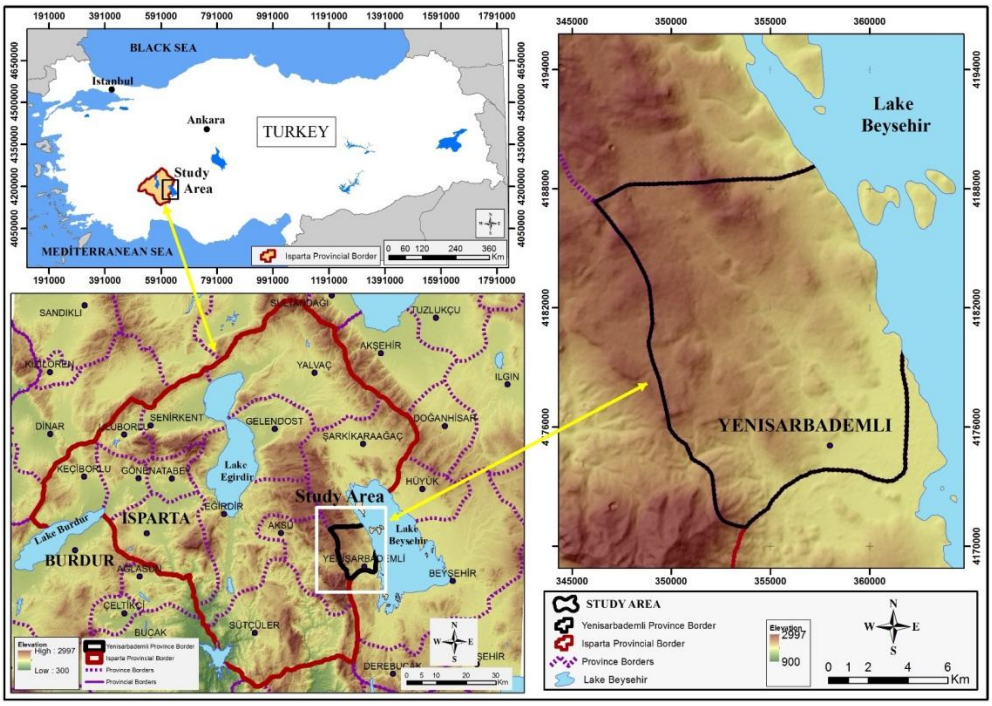


Figure 1. *Study area*

Germinated seeds were counted once every 3-4 days starting from the first germination. Germination percentage (GP) was calculated for each treatment. The formula used for calculating the germination percentage is as follows (Eq. 1):

$$GP = \frac{\sum ni}{N} x100 \qquad \text{(Eq.1)}$$

with ni as the number of seeds germinated on the i^{th} day, and N indicating the total number of seeds in the germination experiment.

During the 1-year growth period, routine seedling growth applications of weeding, hoeing, and irrigation were applied. Seedling removal was conducted without harming the roots of seedlings in the fall season when growth and development was completed. After removing the seedlings, they were placed in a bucket with water without harming roots or offshoots and were monitored carefully. Later, the shoot height (cm), shoot fresh weight (g), root collar diameter (mm), tap root length (cm), and root fresh weight (g) of seedlings were measured. For this purpose, a total of 540 seedlings, or 90 seedlings in each group, were measured. Heights were measured with a ruler with 1 mm sensitivity, weights were measured with a sensitive scale, and root collar diameters were measured with an electronic calliper with 0.01 mm sensitivity.

SPSS 20.0 Windows package program was used for comparing the measured morphological and physiological characteristics, and variance analysis and the Duncan test were applied.

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Results

Germinations were recorded to measure the germination percentage of planted seeds. Germinations were completed at the end of 1 month. First germination was observed in seeds in the control group, which had the highest germination percentage (61%). Based on this information, after the 1-month period, the number of germinated seeds was divided by the total number of planted seeds and germination percentages were calculated (*Figure 2*).

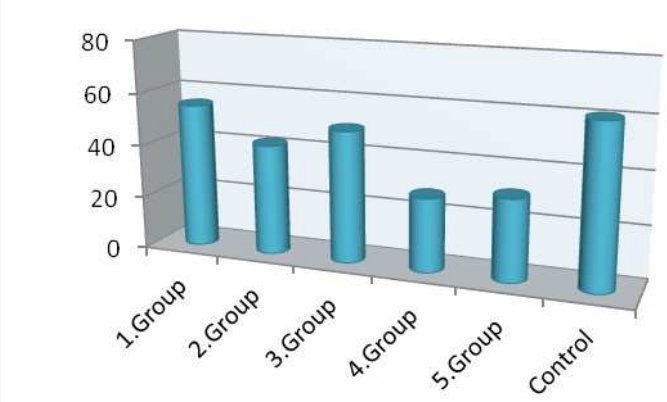


Figure 2. *Germination percentage (%) of groups*

Based on variance analysis of seedling morphological characteristic and following Duncan test results, it was shown that magnetic fields have statistically significant ($p < 0.001$) effects on shoot height (SH), shoot fresh weight (SFW), root collar diameter (RCD), tap root length (TRL), and root fresh weight (RFW) of stone pine. Average values, standard deviation, F value, importance level, and classification of groups are given in *Table 2*. When groups were analyzed in terms of seedling morphological characteristics, seedlings in group 1 had higher average values compared to the other groups, whereas most of groups 3, 4, and 5 had the lowest values.

According to variance analysis results, it was determined that in terms of morphological characteristics, the highest F value was observed in tap root length and the lowest F value was observed in root fresh weight, where all groups were differentiated with the same significance level ($p < 0.001$) (*Table 2*).

Table 2. *Variance analysis results of groups by morphological characteristics (mean and standard error)*

Group	RCD(mm)	SH(cm)	TRL(cm)	SFW(gr)	RFW(gr)
Control	2.39+0.1b	12.2+0.5b	21.6+1.1b	21.4+2.3b	4.5+0.3b
1	2.84+0.2a	14.9+0.6a	24.9+0.6a	28.9+2.9a	5.7+0.5a
2	2.04+0.1c	13+0.8ab	19.8+1.3b	15.7+1.5c	3.8+0.5cd
3	2.44+0.1b	11.7+0.8b	24.3+0.4a	21.3+1.9b	4.3+0.4bcd
4	2+0.1c	13.7+0.5ab	16.7+1.2c	15.4+0.9c	2.9+0.3d
5	2.24+0.1bc	8.3+0.5c	16+0.6c	19.9+0.6bc	4+0.3bc
Significance level	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
F	8.47	11.1	16.9	7.01	6.16

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Stone pine seedlings were classified according to shoot height and root collar diameter as specified in the Turkish Standards Institute's Seedling Standards (TSE, 1988). Based on this classification, group 1 had the highest percentage (92%) and group 5 had the lowest percentage (4%) among 1st-grade seedlings in terms of shoot height. For root collar diameter, group 1 and group 3 had the highest values (both 100%) and group 4 had the lowest value (52%) among 1st-grade seedlings (*Table 3*).

Table 3. *Distribution to quality classes of shoot height and root collar diameter based on TSI Seedling Standards*

	TSI Seedling Standards (1988)		**Distribution to Quality Classes (%) of Group**					
	Class	Range	1	2	3	4	5	Control
Shoot Height (SH)	I	SH>12cm	92	64	48	68	4	70
	II	10cm<SH<12cm	8	20	22	22	32	22
	Cull	SH<12cm	0	16	30	10	64	8
Root Collar Diameter (RCD)	I	RCD>2mm	100	60	100	52	98	93
	Cull	RCD<2mm	0	40	0	48	2	7

Discussion and conclusions

In this study, the effects of magnetic fields on certain morphological characteristics of germinating seedling stone pine trees were investigated. Stone pine seeds were subjected to microwaves of different power settings (280, 336, 462, and 595 W) and durations (15, 30, or 60 s) and were planted along with control group seeds. At the end of one vegetation period, the germination status of seeds and shoot height, shoot fresh weight, root collar diameter, tap root length, and root fresh weight of 1+0 seedlings were investigated.

Based on the findings of this study, it was determined that magnetic field application influenced the development of seedlings with 99.9% statistical confidence. It was determined that seeds subjected to a magnetic field had a lower germination percentage compared to the control group. A similar study that investigated the effects of magnetic fields on germination of mung bean plants indicated that certain magnetic field groups (30, 40, and F50 Hz) had a lower germination percentage compared to the control group (Huang and Wang, 2008).

When seeds are subjected to microwave radiation, the microwaves first pass through the cell wall and later are absorbed by the water molecules in the seed. As a result, tissues containing water heat significantly, and cytoplasm collapses the cell wall and leaks out. Furthermore, due to excessive heating, proteins in the seed may denature and lose functionality. Therefore, it is believed that certain seeds subjected to microwave energy were deformed and had a lower germination percentage compared to the control group (Brodie et al., 2012).

It is also believed that this negative effect is due to increased gibberellin and cytokinin synthesis or activity. In particular, gibberellin has a high ratio in developing seeds. It plays a significant role in controlling and stimulating seed germination. Gibberellin stimulates enzymes for germination of seeds and the α-amylase enzyme that carries those enzymes from embryo to endosperm to convert starch to sugar for energy supply. Cytokinin has a role in cell multiplication and stimulation of cell expansion

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(Hartmann et al., 1997; Hilhorst and Karssen, 1992). For these reasons, gibberellin and cytokinin may cause lower a germination percentage in test groups compared to the control group.

One-way variance analysis (ANOVA) was conducted to test whether there was significant difference between groups' averages in seedling morphological characteristics. Test results indicated that there was a statistically significant difference between groups ($p < 0.001$). The Duncan test was applied to determine which group(s) caused such difference. Test results indicated that the magnetic field had a statistically significant effect ($p < 0.001$) on shoot height, shoot fresh weight, root collar diameter, tap root length, and root fresh weight of stone pine. When groups were analysed in terms of all morphological characteristics, the highest average of germinated seeds was identified in the group that was subjected to 280 W microwave energy with 160 micro Tesla intensity for 60 seconds.

Another study that investigated the relationship between certain magnetic field applications and development of stone pine indicated that a magnetic field with 9.42 mT intensity had positive effects on development of seedlings (Kirdar et al., 2016). Similar results were obtained for different plants, such as by Matsuda et al. (1992) for strawberry (Fragaria sp.); Vashisth and Nagarajan (2008) for chickpea (Cicer arietinum L.); Souza Torres et al. (1999) and Martinez et al. (2009) for tomato (Lycopersicon esculentum Mill.); Cakmak et al. (2010) for wheat (Triticumaestivum L. cv. Bezostaya) and bean (Phaseolus vulgaris L. cv. Gina); Tanvir et al. (2012) for white siris (Albizia procera Benth.); Racuciu (2012) for corn (Zea mays L.); and Cramariuc et al. (2005) for potato.

Magnetic fields may increase seedling development in particular due to effects on auxin, cytosine, and gibberellin metabolism. The most important effect of cytokinin in plants is in cell multiplication, and the most important effect of auxin and gibberellin is in increasing cell elongation (Jansen, 1982; Arteca, 1996). Increased synthesis or activity of auxin, cytokinin, and gibberellin may cause an increase in shoot height, shoot fresh weight, root collar diameter, tap root length, and root fresh weight of seedlings.

When groups were analysed, the highest average in terms of morphological characteristics was found in seedlings in group 1. It is believed that the lower seedling morphological characteristics of other groups was caused by an increase in seed temperature in line with increased magnetic field intensity and duration (*Table 1*). Similarly, Kazanci (2014) investigated the effects of high temperature on seed germination and found that among 37 of 71 taxa, high temperature (100-140ºC) had negative effects on seed germination.

As a result, the magnetic field applied to stone pine seeds had effects on seedling morphological characteristics and positive effects on seedling growth and development. In this sense, in afforestation applications with stone pine, which has economic and forestry importance for manufacturing of by-products, it is believed that application of 280 W microwaves with an 160 μT intensity for 60 s could suppress live plants by developing seedlings quickly, help seedlings to benefit from water and nutrient elements in the earth, and contribute to the economy of the country by reducing development time. Seeds should not applied at high output power (minimum 462 W) for more than 15 s due to overheating. It is recommended to applied at low output powers for more than 60 s for further studies. A study that investigated the relationship between certain magnetic field applications and development of stone pine indicated that a magnetic field (9.42 mT intensity) with 45 m had positive effects on development of

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seedlings (Kirdar et al., 2016). Similarly, Vashisth and Nagarajan (2010) investigated the effects of magnetic field applications on sunflower (*Helianthus annuus* L.) seeds germination and found that 50 and 200 mT for 2 h exposure yielded superior results.

Studies of the effect of the microwave on the seeds of the forest trees are very limited. So it is thought that it will lead similar studies to be done in this field.

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OVERVIEW OF IMPROVING PATROLLING EFFORTS: A CASE STUDY OF FOREST STATION IN PU HU NATURE RESERVE, VIETNAM

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Abstract. Effective management of the special-use forests in Vietnam, e.g. nature reserves and national parks, depends on information about disturbances of illegal activities due to the effective protection as patrol ranger efforts (PREs). Patrol rangers are indispensable for protecting forest in conservation areas. Moreover, to enhance PREs efficiency is regularly to reject illegal activities and support conservation. This paper explores the quantification of PREs in terms of illegal activity encounters collected by rangers in Nam Tien Forest Station (FS). Based on a monitoring system of ranger's efforts, the study conducted surveys for monthly activity reports at the central office. The results revealed that the patrol efforts such as patrol-day and distance were inversely proportional to the number of illegal activity encounters and fluctuated in accordance with the time when they were conducted each year. This study will be useful for forest management at the local level in relation to the planning and monitoring of patrol activities. Usefully, this study's finding are critical for application to the various protected areas with respect to curbing illegal activity and enhancing forest resources-based forest management practices.
Keywords: *patrol ranger effort, patrol, conservation, illegal activity, forest*

Introduction

Globally, continued biodiversity loss is undoubted (Squires, 2014; Adhikari et al., 2017) and even forest managers of Protected Areas (PAs) have variable success in protecting the biodiversity of their areas (Bradshaw et al., 2015). In particular, tropical forest areas all over the world have been experiencing widespread and rapid deforestation and forest degradation, because of pressures of logging, fire, cultivated encroachments, and poaching in their immediate surroundings, and inadequate protection inside the reserves (Baillie et al., 2004; Koh et al., 2013; Bradshaw et al., 2015) such as biodiversity declines and this cause a massive loss of species (Brooks et al., 1997; Harrison et al., 2016). Protected areas such as nature reserves and national

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parks, which often present viable biodiversity, are extremely important in conservation (Gray and Kalpers, 2005; Kimdung et al., 2013). The striking economic growth in the regional buffer zone of PAs shows several profits, but also brings impacts to its ecosystems and biodiversity. On the other side, it has advanced conservation opportunities (Squires, 2014).

The decline in natural resources in the tropical forest requires much attention and the efforts to arrest these declines have become a part of the Ghana government policies and law enforcement efforts (Wiafe, 2016). Much of the flora and fauna of the region in Southeast Asia like Vietnam is not found elsewhere in the world (endemic) (Koh et al., 2013). It has the highest recorded relative rate of deforestation, compared to other tropical forest areas and it could lose three-quarters of its original forests and up to 42% of its biodiversity by 2100 (Sodhi et al., 2004; Miettinen et al., 2011). The impact of human activities on natural resources might have a cascading effect on the whole biodiversity (Wright, 2003). The exploitation of any species of fauna and flora is commonly prohibited and controlled in PAs (Mubalama, 2010). Yet knowledge of protection effort is often limited because the true extent of natural resource exploitation is hard to recognize, especially if it is illegal (Nuno et al., 2013).

As a result of conservation targets, a number of the PAs have been established over the world over the last few decades. It aims is to reduce the first humanity's pressures on the natural environment (Steffen et al., 2015; Venter et al., 2016) and secondly, the source of the primary threats to a country's natural resources and its long-term persistence regarding its biological diversity (Lan et al., 2002). The arious activities are essential for human subsistence but lead to biodiversity loss, and this trend is likely to continue in the future (Baillie et al., 2004). Recent policy commitments aim to reduce biodiversity loss (Dulvy et al., 2006) because a consequence of illegal activities is their increasingly serious effect on natural resources (Gray and Kalpers, 2005) which cause biodiversity loss (Critchlow et al., 2015). Previous researchers have noted that illegal activities seem to vary in accordance with law enforcement efforts (Gandiwa et al., 2013). For example, poor law enforcement performances have been significantly linked to decreases in conservation as a result of increase illegal activities (Bassett, 2005; Ogutu et al., 2011). Additionally, effective law enforcement mechanisms are only weakly implemented by voluntary compliance in the PAs (Rowcliffe et al., 2004). Enhancing law enforcement performance like PEFs, by contrast, has been linked to declines in illegal activities such as poaching (Jachmann, 2008a, b; Martin, 2010; Wiafe, 2016).

The uninterrupted struggle between law enforcement practice and illegal activities suggests some difficulties in enforcing conservation (Lamarque et al., 2009; Wiafe, 2016). Key to biodiversity conservation and the target of forest management is to retain the representative biodiversity of all ecological regions and to conserve the integrity of ecosystems along with their biodiversity from illegal activities (Wiafe, 2016). The stakeholders as rangers in forest stations respond to the threats to conservation in several ways (Albers and Grinspoon, 1997). Previous studies have provided proof which suggests that illegal activities such as illegal logging and agricultural expansion (Khoi and Murayama, 2010) and poaching (Mubalama, 2010) have emerged as serious threats to conservation.

In Vietnam, PEFs in PAs have been applied as conservation tools of forest management. There is little doubt that planning, monitoring, and evaluation by patrol ranger efforts (PREs) are essential tools for enhancing law enforcement practices and

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compliance in such areas (Monteiro et al., 2010). Furthermore, there is proof that that investment in conservation law enforcement is effective (St. John et al., 2014). For example, anti-poaching patrols increased the effectiveness of patrol efforts to deter hunting (Jachmann, 2008a). However, there is a gap in the research considering the occurrence of illegal activity and law enforcement efforts in PAs. In Vietnam, all illegal activities have been politically observed and controlled by forest officers and a management board (Vietnam Government, 2010). Additionally, law enforcement efforts have continually been identified as one of the most important factors in making parks work (Mubalama, 2010). This research focused on contributing to the understanding of patrol efforts and illegal activity in natural resources management, specifically Nam Tien FS in Pu Hu Nature Reserve (NR). Understanding the patterns and extent of illegal activities is vital for effective patrol efforts, which constitute essential elements of illegal activities reduction (Critchlow et al., 2016) and of biodiversity loss prevention (Critchlow et al., 2015).

The aim of this study was to focus on patrol ranger efforts (PREs) carried out at the largest forest station in Pu Hu NR, namely the Nam Tien Forest Station (FS) when the short-term law enforcement dataset was available. Therefore, from January to December 2017, the objectives of this study were to: (i) evaluate the quantified patrol ranger efforts against illegal activities; (ii) find out the relationship among relevant factors that might influence patrol effort and illegal activity encounters; and, (iii) recognize fluctuations in the illegal activities encountered during the study months. This exploratory research provided insight into patrol performance related to law enforcement ranger's efforts in patrol planning, which could resulting in PAs in Vietnam and a simple analysis of them that could be applied in enforcement patrolling, which could result in illegal activities reduction aimed at halting decreases in species of conservation concern (Plumptre et al., 2014).

Material and methods

Location and general description

Pu Hu NR lies in two districts (including Quan Hoa and Muong Lat) in Thanh Hoa province. The area covers over 28.000 ha, is rugged and is located at latitude 20°30' to 20°40'N and longitude 104°40' to 105°05'E in northeast Vietnam. Amongst the six forest stations and one sub-forest station in Pu Hu, Nam Tien FS is one of the extreme pressure from illegal activities occurrence because of its dense population and the people's demands, compared to other forest stations. As explained above, this research focuses on Nam Tien FS, which is supposed to control an area of around 5,700 ha divided into nine forest sub-area plots of the Pu Hu NR. The three permanent forest officers are responsible for protecting the forest, and there are 423 households and 1,512 inhabitants in 12 villages of 2 communities in the buffer zone, namely the settlements Nam Tien and Thien Phu communities. These are the villages with the highest population density and they have the most crowded immediate inhabitants in the buffer zone areas under Nam Tien FS, compared to other buffer zones in Pu Hu. The protection efforts needed to meet conservation objectives are often different from the requirements of the fringe residents (Ormsby and Kaplin, 2005). As in the NR areas, there have been hunting, logging, cutting, and grazing illegal activity encounters. Once a nature reserve area has been established, all extraction of activities by the immediate population area is illegal (Dong et al., 2017). Rangers in Nam Tien FS have protected

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inside the NR through the forbidding of logging, poaching, and grazing by the local population, and other confrontations with forest-edge communities (Plumptre et al., 2014; Risdianto et al., 2016).

Data collection

The data on illegal activities during patrols were collected from January to December 2017. Feasible patrols recorded any observations indicating non-permitted activities by humans or livestock. Basically, rangers had been properly trained in how to record GPS-tracks through an area that had illegal activities or drastic negative effects on biodiversity conservation.

In Nam Tien FS and other stations in Pu Hu, administrative management still use conventional law enforcement in the form of foot patrols that frequently start from each of the sub-stations or from the central stations (Albers and Grinspoon, 1997; Jachmann, 1998, 2008a, b; Burton, 2010; Gandiwa et al., 2014; Wiafe, 2016). Simple patrol forms were applied for record: the numbers of rangers on patrol, and the types, quantities, and places of illegal activity encountered. They presented the exact duration of using the Global Positioning System (GPS) (Mubalama, 2010). After each patrol, field data were collected on data sheets or in patrol staff logbooks (Burton, 2010; Gandiwa et al., 2014), which were summarily tabulated in Excel sheets, then submitted to the head office at the end of each month.

For each sighting, GPS waypoints and tracks, types of human signs were legally identified and the management area where the species or signs had been detected was noted accordingly (Mubalama and Mushenzi, 2004). The typical patrol was conducted by one or two rangers patrolling and recording the patrol route, using a GPS unit in liaison with a topographic map of the particular area (Burton, 2010; Gandiwa et al., 2014). Any apparent changes or unusual situations along the patrol track were recorded (Burton, 2010). After recording for different points, it was properly compared with information from the ranger's patrolling, and added into map software for accounting the new appearances of illegal activity found (Gandiwa et al., 2014). Regularly, rangers were conducted at the Nam Tien FS, although sometimes mobile teams from central office were also deployed and re-evaluated the previous information about the site. Location points (waypoints) of illegal activities were recorded and written down, including the current name and number of officers on patrol, duration of patrol, name of the forest sub-area plot, and numbers as well as longitude and latitude of the illegal activities discovered (Jachmann, 1998, 2008a, b; Burton, 2010; Gandiwa et al., 2014; Wiafe, 2016).

Informal patrol efforts planning

Officially, regular monthly meetings and discussions were held at the Pu Hu office by The Management Board to analyse each station's patrol efforts. Due to the number of patrol tracks and the collected information from each patrol track, a short patrol report was presented in detail about the sub-area forest plot. According to the current information and planning from the monthly meeting, Nam Tien FS prepared its own plans for patrolling during the following month (Gandiwa et al., 2014; Wiafe, 2016). Additionally, patrol movements and starting times from the forest station had to be unpredictable (Jachmann, 2008b). However, the patrol teams could not always follow the given patrol plan, often made a change of strategy necessary.

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Plans for patrol routes were produced by the head of the forest station and patrollers assembled the team for each patrol of the sub-area forest (Jachmann, 1998, 2008a, b; Gandiwa et al., 2014; Wiafe, 2016). During the patrolling, the leader of a team had a decision to make about whether to use an 'arrow head' or a 'single file' formation for his officers, depending on previous information obtained from the earlier patrols (Wiafe, 2016). Based on specific planning, each ranger had to patrol at least five-time patrols per month in their protected areas.

Data analysis

The Finding per Effort index (F/E) was applied which gives an overview of indicators of illegal activities per unit (Jachmann, 1998b; Wiafe, 2016). Monthly, finding is the total number of illegal activity encounters, and effort refers to the total number of effective patrol-days (patrol-time) (Jachmann, 2008b, c). Kilometric Index of Abundance (KIA) is the proportion of illegitimate activities that were encountered to distance per kilometers (km) walked per month (Groupe, 1991). Kilometers (km) per Effort index (P/E) is the rate of the distance in kilometer (km) walked on the effective patrol-day or patrol-time per unit. Data were analyzed using Microsoft Excel software for descriptive statistics using the Statistical Package Social Sciences (SPSS) version 20.0 to determine any significant difference among various variables by testing function ($p = 0.05$); and mean values were presented as mean ± SD (standard deviation). In particular, descriptive statistics were used to summarize the illegal activity found in the data set. Data on illegal activity by different tracks were grouped together and totals, averages and percentages for patrolling routes were calculated. Burton (2010) showed that given trait data were not the normal distribution, therefore, non-parametric statistics were used to analyze correlation and compare means (Spearman rank correlation and Kruskal-Wallis test if more than two events and Man-Whitney test with two variables). So, data were analyzed by using both tests in SPSS to determine if there is any significant ($a = 0.05$) in the variables of two, or more than two events.

Results and discussion

The brief description of possible patrol routes through each sub-area forest plot presented (*Table 1*; *Fig. 1*). Establishment of all patrol routes covered sensitive biodiversity loss and illegal activity occurrence. Rangers (FS) reached to the sub-area forests from Nam Tien forest station by motorbike.

Patrol ranger efforts

The average patrol-days (patrol-hours) in the period of the first quarter to the fourth quarter in 2017 were as follows: 0.82 (SD = 0.49), 0.68 (SD = 0.51), 0.57 (SD = 0.43), and 0.69 (SD = 0.56) respectively. The average quarterly patrol-days of patrol effort fluctuated from the first quarter to the fourth quarter in 2017, i.e., from 0.82 (SD = 0.49) to 0.69 (SD = 0.56) respectively. In particular, the mean for the monthly patrol-days was not significantly different among these months ($H = 18.14$, $p > 0.05$; *Fig. 2*). Despite potentially large punishment costs (Jachmann, 2008b, c), the enforcement efforts might be sufficient, possibly due to fitting staff to salary, to create a low expected cost of the punishment relative to the gains from illegal activities (Albers and Grinspoon, 1997; Wiafe, 2016).

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Table 1. *Summary of technical tracks on the protected area*

Name of sub-areas	Name of communities	Size of sub-areas (ha)	Patrol distance (km)	Absolute height (m)
102	Nam Tien	1240.21	9 - 10	1,200 - 1,320
120	Nam Tien	1081.03	7 - 9	926 - 1,025
121	Nam Tien	754.46	7 - 8	650 - 730
124	Nam Tien	574.09	5 - 6	541 - 635
132	Nam Tien	658.82	4 - 5	230 - 335
142	Nam Tien	100.35	2 - 4	353 - 430
146	Nam Tien	678.08	1 - 3	454 - 540
147B	Nam Tien	36.06	5 - 6	600 - 752
142	Thien Phu	545.2	7 - 8	720 - 820

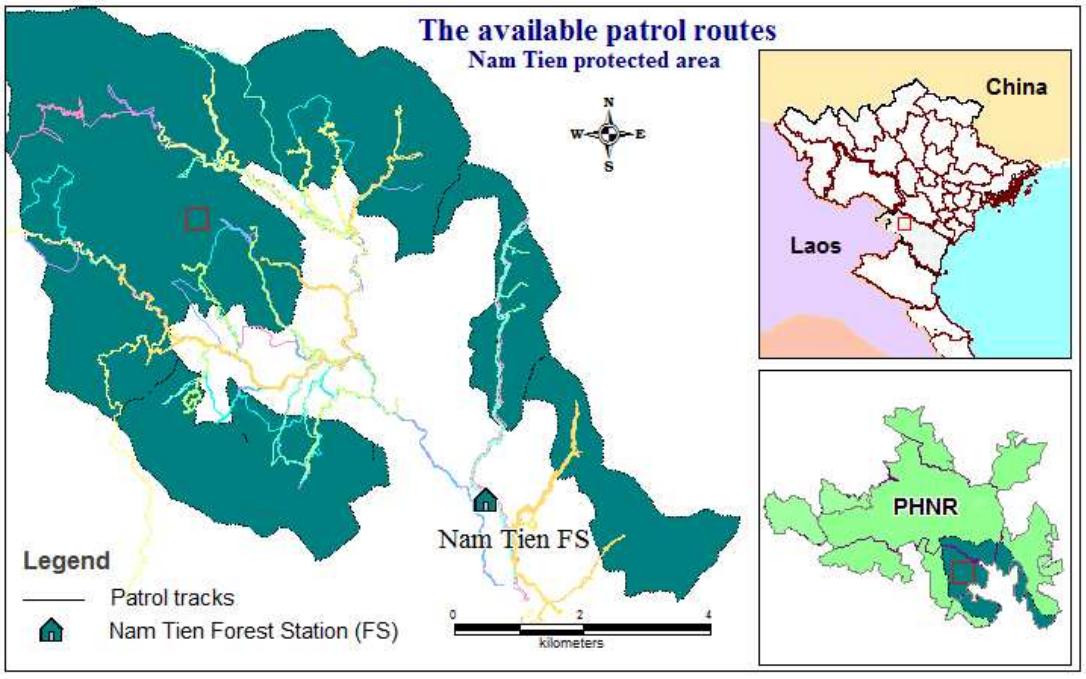


Figure 1. *The location of study area*

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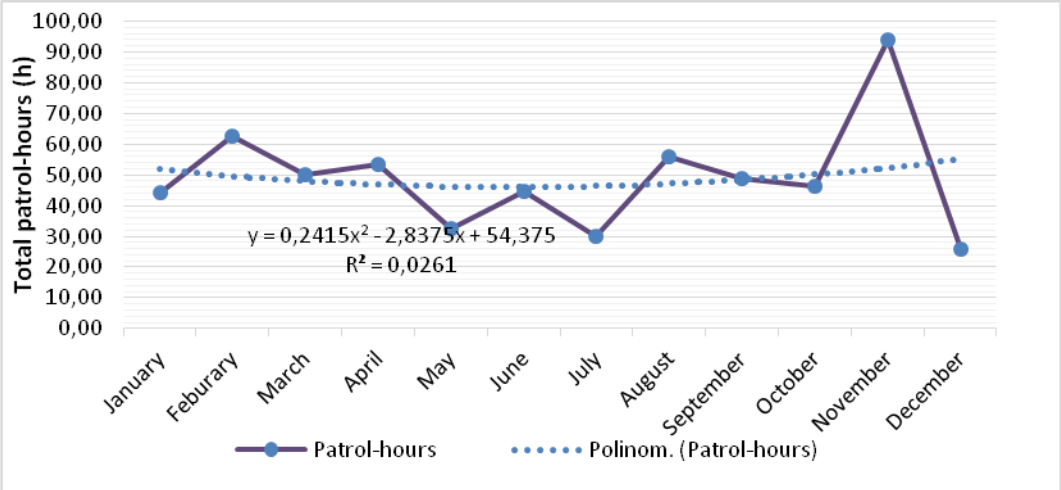


Figure 2. *The total of average patrol-day*

Nonetheless, the distance of patrol rangers differently walked was a quarterly average of 9.25 (SD = 6.18) km in the first quarter; 8.98 (SD = 6.19) in the second one; 8.10 (SD = 6.40) and 8.61 (SD = 5.93) in the last two quarters of 2017 respectively. However, quarterly distances walked during this year did not differ among months ($H = 1.81$, $p > 0.05$). Monthly distances covered that significantly varied months ($H = 20.39$, $p < 0.05$) and the trend of the distance merely increased, after being constant in August (*Fig. 3*).

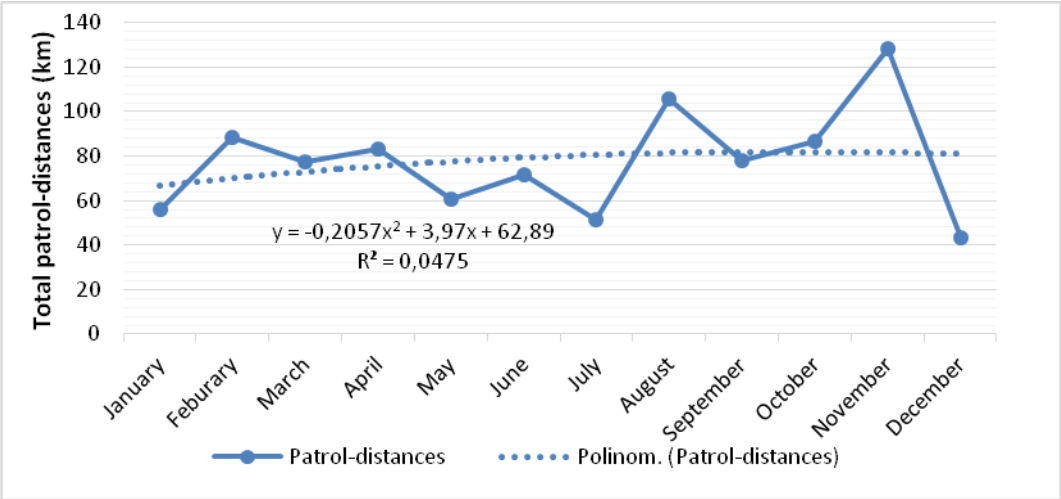


Figure 3. *The total of average patrol distances walked out by rangers*

Generally, during 1-year of patrolling, the number of patrol-days per month was positively correlated with the number of kilometers walked in those months ($rs = 0.88$, $p < 0.01$) similar to Wiafe's (2016) findings in the research on Ghana. The greater number of staff on patrol the larger the area covered and the longer the distance because of increasing patrol-days and so patrol distances ($rs = 0.61$, $p < 0.01$; $rs = 0.65$, $p < 0.01$ respectively) (Jachmann, 1998, 2008a, b; Gandiwa et al., 2014; Risdianto et al., 2016; Wiafe, 2016). The influence of patrol-day on the distance covered could be accounted

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for with the increase in the number of staff patrolling in the field, who were able to patrol for a long period and covered longer distances (Jachmann, 2008a, b; Dhanjal-Adams et al., 2016; Wiafe, 2016).

Crucially, the factors of elevation area, and dissatisfied rangers, correlated with patrol-day and walked distance ($rs = 0.47$, $p < 0.01$; $rs = 0.38$, $p < 0.01$ respectively). Nonetheless, the trend of average elevation area was significantly stable within 12 months (*Fig. 4*). Actually, the patrol routes, based on previous small paths, were identical and rangers did not attempt to open new routes since they had used the same way of coming to NRs. Similarly, the speed of patrolling was negatively correlated with the patrol-day and patrol-elevation ($rs = -0.36$, $p < 0.05$; $rs = -0.25$, $p < 0.01$ respectively). By contrast, it was not correlated with distance ($rs = 0.72$, $p > 0.05$). The monthly speed of patrolling during this year did not vary significantly ($H = 23.62$, $p > 0.05$), even when the trend of patrol-speed was marginally increased (*Fig. 5*).

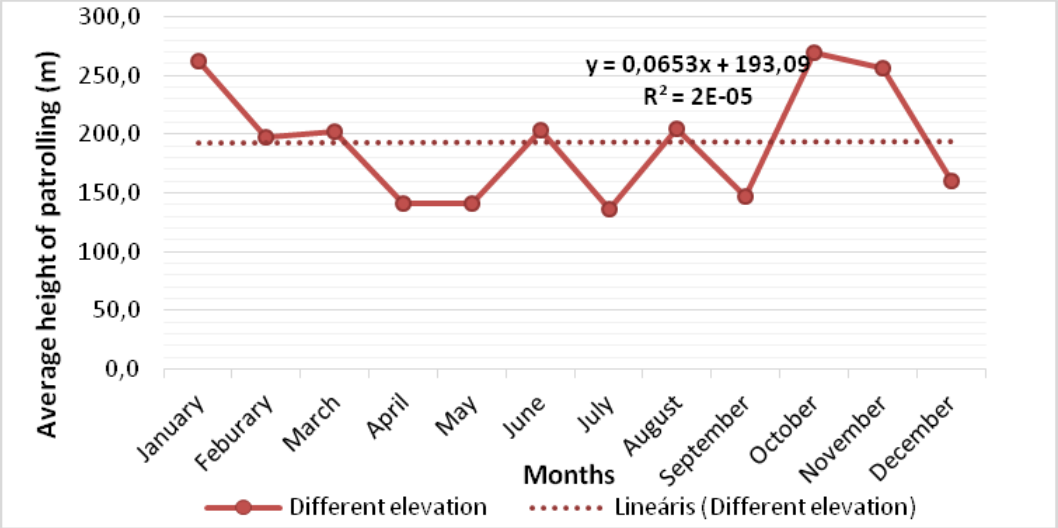


Figure 4. *The average patrol-elevation by ranger's performance*

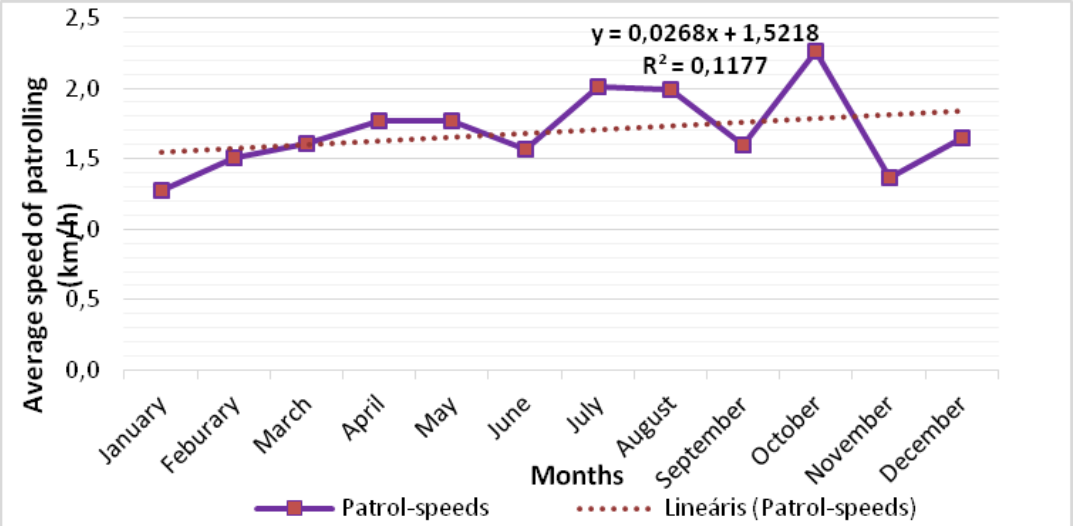


Figure 5. *The average patrol-speeds by ranger's performance*

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Illegal activities encountered from patrol efforts

Considering on the number of illegal activities found was fluctuated during the period of time (the specific quarters), the average number of illegal activities was recorded for the four quarters in 2017 as follows: 6.83 (SD = 3.69), 3.00 (SD = 1.29), 2.41 (SD = 1.45), and 4.3 (SD = 1.58) respectively. The average quarterly number of illegal activity encounters from patrol efforts declined from the first quarter to the fourth quarter in 2017, from 6.83 (SD = 3.69) to 4.3 (SD = 1.58) respectively. When rangers pushed their efforts at patrolling on the field related to the increasing hour-patrol and distance (*Figs. 2* and *3*), the illegal activity seemed to reduce. Further, there were significant differences in illegal activities found during the months ($H = 57.27$, $p < 0.001$). The increasing number of illegal activity encounters in terms of patrol-days and distance significantly increased after sharply declining in August (*Figs. 6* and *7*).

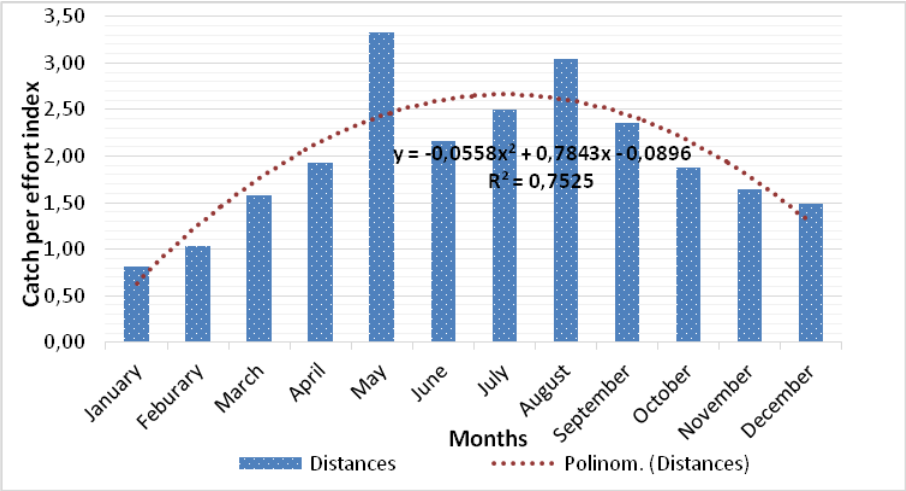


Figure 6. *The distance of indicators of illegal activities found*

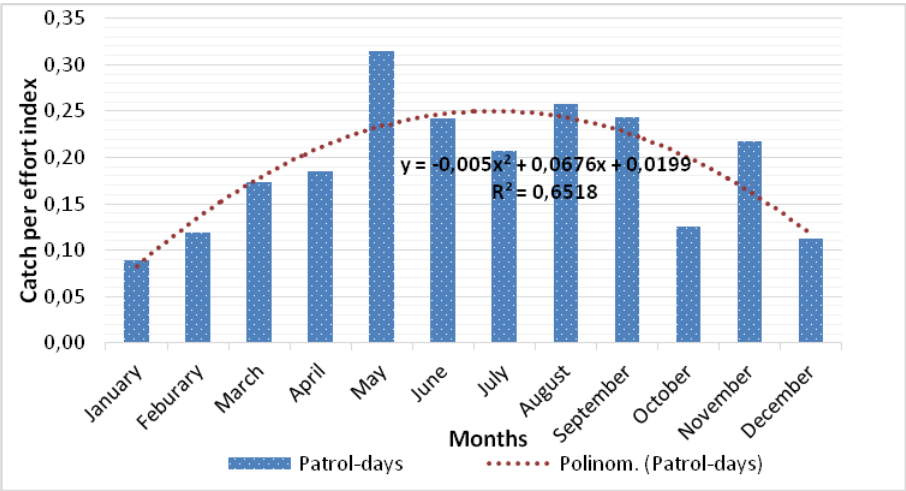


Figure 7. *The patrol-days (hours) for indicators of illegal activities found*

However, the number of illegal activities encountered was significantly correlated with patrol-day and walked distance ($rs = 0.55$, $p < 0.01$ and $rs = 0.46$, $p < 0.01$

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respectively) similar to the finding by Gandiwa et al. (2013) and Wiafe (2016). In contrast to Jachmann (2008), Risdianto et al. (2016), and Wiafe (2016), this study found that there was not a significant relationship between illegal activities found and the number of patrol rangers ($rs = 0.14$, $p > 0.05$). When illegal activity significantly declined at the current place, increasing the number of patrol staff was not a great conservation choice.

Patrol rangers in each sub-forest area plot were at liberty to alter their patrol planning and style, depending on whether or not they had attained the information on patrolling practises and in doing so they also relied on the experience (Wiafe, 2016), education and motivation of rangers (Jachmann, 2008a, b). In addition to this, patrol efforts in Nam Tien FS were also strengthened by the involvement of the Pu Hu NR which had more resources available for law enforcement practices (Gandiwa et al., 2013). Interestingly, the fluctuation of encounter rates in terms of patrol-days and distance was merely different during months (*Fig. 8*). Thus, there was no doubt that Pu Hu managers could consider pushing their staff to prevent illegal activity efficiently with their indicators of patrol-day or distance in the field.

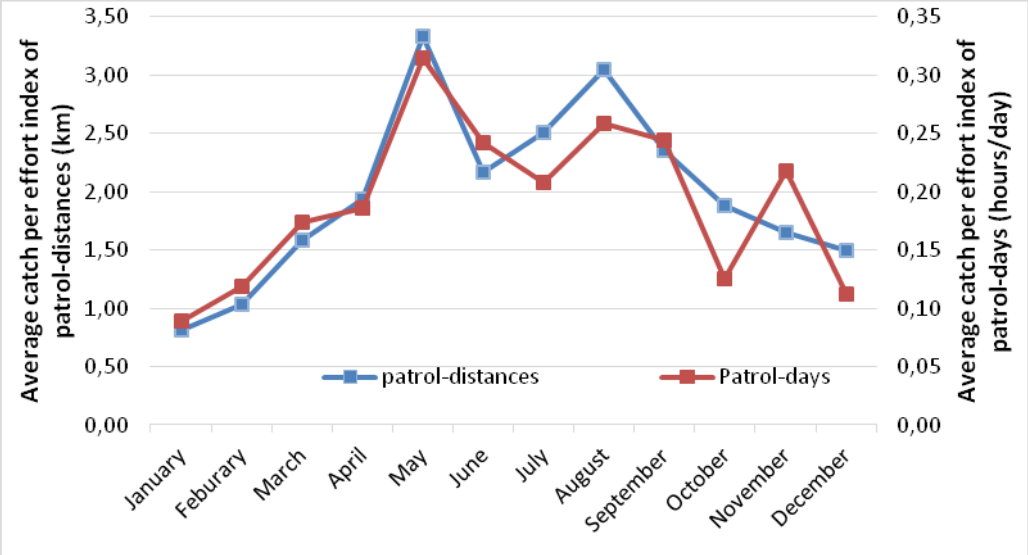


Figure 8. *Trend of illegal activities encounters in terms of patrol-day and distance*

Fluctuation in patrol ranger efforts

Almost all rangers in forest stations have regularly declined to patrol during the Lunar New Year (holiday) and rainy season (limited patrol efforts) because of difficult situation on the fields. It has therefore been speculated that these periods of time disrupt patrol efforts indicators such as days, rangers, distance, and time (Wiafe, 2016). Lunar New Year is in February-March, it confronted with inadequate staff (Moreto, 2016), because a half of staff in Nam Tien FS haves vocation with several days off. Local people attempted to enjoy the NRs by buying something for welcoming the New Year. Interestingly, the number of patrollers still correlated with patrol-distance in 2016 ($rs = 0.62$, $p < 0.05$). The monthly average number of patrol-days and illegal activity encounters were significantly different between the period of February-March and other months ($U = 411$; $p < 0.05$) and ($U = 95$; $p < 0.001$), and opposite to total of patrol-

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distances, there was no difference in two period of time ($U = 537$; $p > 0.05$). Rangers strived for covering forest areas in terms of distances; however, the insufficient number of rangers affected the number of patrol-days. Especially, rangers had issues living at forest station locations, as they were homesick because a few rangers lived in such areas (Moreto, 2016). There was no doubt that shifting patrollers might be necessary for patrolling during these months. Furthermore, as mentioned above, the potential for illegal activity significantly increased because of greater demand from society. The mean number of patrol-days and patrol-distance was higher for the day-off months 0.89 (SD = 0.43) and 9.63 (SD = 5.61) compared to other periods: 0.65 (SD = 0.51), and 8.55 (SD = 6.19) respectively.

Commonly, there are two months August and September in each year, when the number of illegal activities was significant between the precipitation season and other seasons ($U = 450$; $P < 0.05$). Locally, influences on illegal activity occurrence could be considered in terms of different seasons. By contrast, the number of patrol-days and distances in the rainy season was not different in other seasons ($U = 868$; $P > 0.05$) and ($U = 862.05$; $P > 0.05$) respectively. Interestingly, the number of illegal activities in the rainy season was also found to be significant when correlated with number of patrol-days ($rs = 0.70$, $p < 0.01$) and distance ($rs = 0.53$, $p < 0.05$), but it was not correlated with staff number ($rs = 0.22$, $p > 0.05$). This is different from Wiafe (2016), who reported that precipitation disturbed patrol efforts when the number of patrol staff was significantly correlated with patrol-days ($rs = 0.65$, $p > 0.01$) and distances ($rs = 0.69$, $p < 0.01$) that was also found to be not significantly correlated with illegal activity encounters. Almost all patrollers as rangers still prevented to the illegal hunting activities even under uncomfortable weather conditions when the criminals might have taken the opportunity to carry out their activities (Wiafe, 2016).

Conclusion

This study may be applied for evaluation of the protection efforts made by management in PAs. Locally, the leader of each forest station should pay attention to whether or not each ranger could carry out the job of protecting its natural resources effectively. From the results, it could be seen that there was a trend of illegal activity encounters that proportionately fluctuated with patrol-distances and patrol-days or patrol-times. There is an urgent need for management boards of nature reserves to increase law enforcement, based on the recording of information (Damnyag et al., 2013) and on current staff levels. In addition to this, this study focused on all the illegal activities that could similarly impacted conservation aspects. There is a need for a specific research on different activities. Moreover, the PEFs were analyzed only in a short-term period of patrolling - around one year - and extension needs to be considered in the future.

The advantage of this paper was that it presented a strategy for activity that provides planning that can be enhanced for sustainable forest protection at the local level. Improvement of law enforcement performance by inspection activity is extremely important for increasing biodiversity conservation. In some areas, the main driver of the chronic factors is the large in-migration of people to the local communities, particularly into the short and medium paths of the conservation protection areas. The energetic patrolling efforts also plays a warning role and helps prevent illicit activity in several situations in the forestry fields.

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As Wiafe (2016) reported there were various external factors (such as local policy, illegal activity occurrence, number of rangers, and financial support) that needed the increasing contribution of illegal activity and effort to bring it down. Additionally, factors like work experience, leadership style, qualification, and logistics provided could influence the walking field. However, this research did not consider it. Further research should explore the essential relationship between ranger's social aspects and patrol efforts. A step change involving increased funding support and education is urgently needed for fulfilling the improvement of motivated rangers (Watson et al., 2014). Overall, this study highlights the fact that management boards should evaluate locations of forest stations based on the final results of threat reduction and management effort (Gong et al., 2017).

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Conflict of interest. The authors definitely confirm that this article content has no conflict of interest.

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RESEARCH ON CULTIVATION POTENTIALITIES OF 'LOCAL KARACADAG' AND 'OSMANCIK-97' RICE VARIETIES IN ORGANIC AGRICULTURAL CONDITIONS

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Abstract. This research was conducted to determine the potentialities of organic rice cultivation in Karacadag region and to set an example for farmers in transition to organic farming. The Local Karacadag paddy the variety grown in the Karacadag region and the Osmancık-97 breeding variety, the most cultivated variety in Turkey, were used as material. The research was carried out in three replications according to the split parcel trial design in random blocks in the 2011-2012-14 rice growing periods on the farmer field in Karahan village of Karacadag region, in Turkey. As a result of the agricultural researches made, organic values showed superior rates compared to conventional applications in terms of the number of days of flowering (92.3 days) and non-fractured rice yield (65.6%). It was observed that regarding the kernel yield in unit area the Karacadag variety was highest at 4 816 kg ha^{-1} in the conventional application while the organic application of the Karacadag variety was in second place with 4 179 kg ha^{-1}. It is concluded that the organic rice farming is thought to be economical when the organic rice in the market is sold with about 30% more the price compared to the conventional rice.
Keywords: *Karacadag region, organic rice, rice cultivation, yield and yield components*

Introduction

Rice is an important cereal type that is used as a food source for the human nutrition in the world's cereal production with 672 021 180 tons and it ranks second after the corn production. Rice obtained after processing is an important staple food in the far eastern countries especially where it is consumed intensively, it is rich in amino acids even has small amounts of protein in its composition. According to the data in 2010, the countries where rice cultivation is intensively carried out were: 197 million t with China, 120.6 million t with India, 66.4 million t with Indonesia, 49.4 million t with Bangladesh, 40 million t with Vietnam, 70% of total rice production in the world is carried out in these countries (Anonymous, 2013).

According to the agricultural statistics, there are approximately 37.2 million ha of organic farming area in 160 countries in the world in 2009. These areas constitute only 0.9% of the total agricultural land in the world (Willer and Klicher, 2011).

Although the rice cultivation area in our country shows fluctuations from year to year, the rice cultivation area was 115 856 ha, production was 920 000 t and the yield was 7 940 kg ha^{-1} in 2015. Rapid population growth and the difficulty of sowing in certain areas limit paddy production by making imports inevitable. There are about 40 provinces in Turkey which conduct rice cultivation. Some of them are 386 907 t with Edirne, 122 600 t with Samsun, 113 994 t with Balıkesir, 91 880 t with Çanakkale and

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52 298 t with Çorum. The rice production in the country cannot meet the needs (Anonymous, 2015).

In the Southeast Anatolia Region, rice cultivation is 2 356 ha, production is 11 250 t and the yield is 4 770 kg ha^{-1}. All of the rice production in the region takes place in Diyarbakır and Sanlıurfa provinces. The total area of rice cultivation in Diyarbakır is 1 747 ha with 8 514 t of production and in Sanlıurfa the cultivated area is 608 ha with 2 736 t of production (Anonymous, 2015).

According to the organic agricultural statistics, there are approximately 558 837 ha, where production was 922 682 t in Turkey in 2013. Organic paddy production are 49 t with Samsun, 42.5 t with Diyarbakır and 4.38 t with Artvin (Anonymous, 2013).

In Karacadag region, the main sources of livelihood in the villages where rice is grown, are rice production and small ruminant livestock. The local rice is produced based on traditional techniques inherited through hundreds of years. As the fields are stony, no machine tillage is possible and all practices are done by hand. The water for irrigation is used from an artifical lake with gravity, so no energy is used. This variety is only produced and consumed in this area.

Karacadag is in Southeastern Anatolia Region. Its soil is very rich in minerals. But all the surface is full of rocks and stones. The local community has been producing 'Karacadag rice' since hundreds of years with the local knowledge heritage from their fathers. They sow rice once in two or seven years in the stony and sloping field, without tillage.

Organic agriculture, a form of agricultural production that protects human and environmental health, including the use of natural substances and methods instead of synthetic chemical pesticides, hormones and mineral fertilizers, has been gradually increasing in recent years in the world and in Turkey as a result of conventional agricultural practices (Inci and Sonmez, 2006).

The Southeast Anatolia Region has a considerable potential for organic agriculture production when taking into consideration the surplus of agricultural population, climate and soil structure of the area (Gursoy et al., 2009). With the Southeast Anatolia Project (GAP), an irrigation water deficiency which is the biggest bottleneck in the rice farming will be eliminated largely. The soil of the region has not been contaminated with excessive fertilizers and chemicals. As the soil is fertile, productivity can be achieved at low costs in agricultural production. The Karacadag rice variety is the reason for the success of the critical years with bad environmental conditions as well as its quality characteristics that meet the local consumers demands.

Clearing the stony areas which covers large areas and opening these areas to the organic farming will raise the life standards of the local farmers in the Karacadag region. Thus, the non-contaminated areas outside the farming land will be cultivated and high value added products will be manufactured. In addition, appropriate breeding techniques in terms of water, soil and environmental protection will bring display to the local farmers. However, the ecology of the Southeast Anatolia Region is extremely suitable for rice farming.

The Karacadag rice is a high quality local variety that adapts to the ecological conditions of the region. The fact that the region has adapted to the special soil structure and irrigation water distinguishes paddy product from the other varieties. The most important feature of the Karacadag rice variety is that it is the kind that the people in the region look for the most with its colour, flavour and taste. The ability of its kernels to draw water during cooking is high. It does not have lapping and sticky properties. Its

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shape and size are also important distinguishing features. The Karacadag rice variety has medium kernel size. The high amount of protein and starch in the rice makes pilaf (traditional food) delicious. It can be said that it has adapted to the local ecology and it is resistant to some stressful conditions, especially diseases and pests. Because of these properties, it has value as breeding material. This variety is highly resistant to the cold snow waters of Karacadag; it has a stable yield potential. This variety is cultivated for centuries and it has been genetically clarified (Alp, 2011).

Primitive forms and local varieties are important genetic repositories of cultural herbs in resolving future problems or transferring new traits to the culture plants. Because local varieties have been selected for many years in a certain region, they are well adapted to the environment and succeed in extreme climatic conditions of some years. It should be kept in mind that genetically clarified local varieties that have completed their ecological adaptation are genitors that can be used more easily than the wild plant in modern breed development studies.

Recently, with the introduction of the advanced technology-based farming and entrance of highly productive varieties into the production areas, many of the local varieties have disappeared and many of them are still disappearing. Continuity in crop production will only be possible with the protection of the wild and local varieties.

The Karacadag is one of the important planting areas of the endemic and rare crops as well as wild relatives of many Gramineae and leguminous plants. The Karacadag is an extinct volcano in the Southeast Anatolia Region and it is located on the southwest side of the Diyarbakır province. The volcano mass of the Karacadag has a very small slope towards the peak of the surrounding areas and extends in three directions: Diyarbakır, Viransehir and Hilvan. The part of the Karacadag in the direction of the Diyarbakır which is spread over an area of approximately 7.200 km^2 is suitable for plant production (Alp, 2011).

General position, when considering its unpolluted nature and climate, is that Karacadag region has a great potential in organic production. The promotion of organic farming, rice flour, baby food, healthy food will present significant contribution in terms of accessibility and sustainability.

With the help of this work, in the first stage it is aimed to reveal the differences between the conventional and organic farming and to show the potentialities of utilizing local rice varieties in organic production conditions.

Materials and methods

This research was conducted to determine the potentialities of organic rice cultivation in Karacadag region and to set an example for farmers in transition to organic farming. The Local Karacadag paddy the variety grown in the Karacadag region and the Osmancık-97 breeding variety, the most cultivated variety in Turkey, were used as material. The research was carried out in three replications according to the split parcel trial design in random blocks in the 2011-2012-14 rice growing periods on the farmer field in Karahan village of Karacadag region, in Turkey.

Karacadag Local rice variety is a late bloomer and its vegetation duration is over about 130-140 days, it is also resistant to cold and drought. It has poor durability to inclination, it is tall and stringy. The Osmancık-97 rice variety has an Italian origin. Its structure is stemmed, and it is resistant to inclination. The structure of the variety is without strings and it does not spill its kernels.

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The soil samples taken from soil at 0-20 cm depth were analysed and the results were: total salt concentration was 0.011%, soil pH was 7.68, the ratio of phosphorus from the useful nutrients from the plants was 1.75, the organic matter was 1.42%.

The location is approximately 900-1000 m above the sea level. In the province of Diyarbakır where the field experiments were conducted the annual rainfall is almost completely happens between September and June in the summer months, rainfall is almost never seen and the proportional humidity rate is quite low. The annual average rainfall of the zone is 488.1 mm, the relative humidity is 53% and the average temperature is around 14.8 °C (*Table 1*).

In the experiment made, pre-germinated seeds were planted as 400 units per square meter for conventional parcels and 440 units for organic parcels with the spreading sowing method on 23.05.2011, 30.04.2012 and 12.05.2014. The size of the parcel was set on 15 m^2 (3 x 5 m) and 10.0 m^2 (2.5 x 4.0 m) at harvest. Soil cultivation was not applied before the sowing in the stony terrain. Less seeds were used in the local experiment was due to the fact that about 20-30% of the land is stony. That 10% more seeds were used in the organic plot is for fighting weeds.

In the conventional rice parcels, 120 kg/ha pure nitrogen and 60 kg/ha pure phosphorus were dropped. 1/3 of the nitrogen and all of the phosphorus were used at the same time with planting. The second 1/3 of the nitrogen was provided during the tillering and the last 1/3 was given as sprinkles before the earing period.

In the organic rice parcels, the solid fertilizer was planted at 1 400 kg/ha and half the B5A liquid organic fertilizer (4 000 g/ha) was sown at the planting site. The other half of the liquid organic fertilizer was given one month before harvesting through the leaves.

Table 1. *Sum of precipitation, humidity and temperatures in each year and long-term average*

Meteorological data	Years	Months						
		April	May	June	July	August	September	October
Average temperature (°C)	2011	13.0	17.7	25.5	31.4	30.7	25.0	18.1
	2012	15.2	19.6	27.7	31.3	31.1	26.1	16.4
	2014	14.7	19.8	26.6	31.6	31.1	24.7	17.5
	Long T.	13.8	19.3	26.3	31.2	30.3	24.8	17.2
Average max. temperature (°C)	2011	18.4	24.5	33.5	39.5	38.5	32.9	31.0
	2012	22.6	27.1	35.7	38.6	38.6	34.4	24.0
	2014	22.0	28.1	34.1	39.3	39.6	32.2	24.2
	Long T.	20.2	26.5	33.7	38.4	38.1	33.2	25.2
Precipitation	2011	209.0	80.1	13.6	0.6	0	9.2	63.0
	2012	26.2	41.0	7.0	1.6	0	1.8	11.8
	2014	39.9	48.8	21.4	0.6	0	27.4	34.2
	Long T.	68.7	41.3	7.9	0.5	0.4	4.1	34.7
Average humidity (%)	2011	75.7	67.6	38.0	22.5	21.7	30.2	41.6
	2012	58.5	58.0	27.8	20.2	20.8	23.1	55.2
	2014	63.1	53.5	29.2	22.2	21.3	35.5	61.5
	Long T.	63.0	56.0	31.0	27.0	28.0	32.0	48.0

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Chemical herbicide was applied against wild weeds in conventional rice parcels while weeds in organic parcels were picked by hand. Chemical pesticide was applied against grasshoppers in conventional rice parcels while against grasshoppers in organic parcels was applied by NeemAzal organic certified pesticide (active substance 'Azadirachtin A').

Irrigation water was provided from the pond in the countryside. Irrigation was done in the form of surface irrigation. Irrigation was interrupted 10 days before full ripening to ensure the dryness of the harvest fields. The harvesting took place on 10.10.2011, 24.09.2012 and 02.10.2014 when the rice plants turned yellow and bunches dangled from trunks.

Observations and the measurements of the following features were made in 10 plants: number of days of flowering (day), number of maturation days (day), plant height (cm), number of tillering in plants (number), kernel number of a bunch (gram), kernel weight of a bunch (gram), thousand kernel weight (gram), length of the bunch (cm), non-fractured rice yield (%), and the measurements were made on 10 m^2: biological yield (kg/ha), unit area kernel weight (kg/ha).

The data obtained from the study were analysed in the JUMP statistical package program. The LSD test was applied and the coefficient of variation (CV) was calculated with the differences between the averages (Yurtsever, 1984; Duzgunes et al., 1987).

Results

In this research, when the average values of varieties were examined in terms of number of days of flowering; 96.7 days in conventional conditions, 97.6 days in organic condition for Karacadag variety; 86.7 days in conventional conditions and 87.1 days in organic conditions for Osmancık-97 variety. The average values of the varieties with regard to the growing conditions were 92.3 days in organic farming and 91.7 days in conventional farming. The average values of the varieties were found to be 97.1 days for Karacadag variety and 86.9 days for Osmancık-97 variety (*Table 2*).

Table 2. *Average values of number of days of flowering (day), number of ripening days (day) and the formed groups according to the LSD (least significant difference) test*

Applications	Number of days of flowering		Average	Number of ripening days		Average
	Karacadag	Osmancık-97		Karacadag	Osmancık-97	
Conventional	96.7	86.7	91.7	141.6	127.2	134.4 a
Organic	97.6	87.1	92.3	140.9	126.6	133.8 b
Average	97.1 a	86.9 b		141.2 a	126.9 b	
CV (%)	0.44			0.35		
LSD variety	0.30**			0.34**		
LSD application	N.S.			0.38**		
LSD interaction	N.S.			N.S.		

*The difference between the averages shown with the same letter is not important (N.S.) at 0.05

When the average values of the varieties were examined in terms of number of maturity days; Karacadag variety was 141.6 days in conventional condition, 140.9 days

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in organic conditions; Osmancık-97 variety was 127.2 days in conventional conditions and 126.6 days in organic conditions. The average values of the varieties with regard to the growing conditions were 133.8 days in organic farming and 134.4 days in conventional farming. The average values of the varieties were found to be 141.2 days for Karacadag variety and 126.9 days for Osmancık-97 variety (*Table 2*).

When the average values of the varieties were examined with regard to the plant height; Karacadag variety was 95.4 cm in conventional conditions and 92.1 cm in organic conditions; Osmancık-97 was 77.1 cm in conventional conditions and 75.1 cm in organic conditions. It is concluded that the average values of the varieties were 83.6 cm in organic application and 86.2 cm in conventional application. The average values of the varieties were found to be 93.7 cm for Karacadag variety and 76.1 cm for Osmancık-97 variety (*Table 3*). Karacadag Local variety showed longer plant height values in both conventional and organic conditions than Osmancık-97 variety.

When the average values of the varieties were examined with regard to the number of tillering in plants, the Local Karacadag variety was 2.61 in conventional conditions and 2.59 in organic conditions; the Osmancık-97 was 1.66 number both in conventional and organic conditions. In terms of growing conditions, the average values of the varieties were 2.12 in organic application and 2.13 in conventional application. The average values of the varieties were found to be 2.60 for Karacadag variety and 1.66 for Osmancık-97 variety (*Table 3*). It has been observed that the Local Karacadag rice variety is more abundant in terms of tillering in both farming conditions than the Osmancık-97 variety.

Table 3. *Average values of plant height (cm), number of tillering (number) and the formed groups according to the LSD (least significant difference) test*

Applications	Plant height		Average	Number of tillering		Average
	Karacadag	Osmancık-97		Karacadag	Osmancık-97	
Conventional	95.4	77.1	86.2	2.61	1.66	2.13
Organic	92.1	75.1	83.6	2.59	1.66	2.12
Average	93.7 a	76.1 b		2.60 a	1.66 b	
CV (%)	3.59			6.55		
LSD Variety	2.21**			0.10**		
LSD Application	N.S.			N.S.		
LSD Interaction	N.S.			N.S.		

*The difference between the averages shown with the same letter is not important (N.S.) at 0.05

In this research, when the number of bunches in per m^2 was examined, the Local Karacadag variety was 393.9 in conventional conditions and 376.0 in the organic conditions; Osmancık-97 variety was 313.7 in conventional conditions and 307.6 in organic conditions. In terms of growing conditions, the average number of bunches in per m^2 was found to be 341.8 in organic application and 353.8 in conventional application. The average values of the varieties were found to be 384.9 for Karacadag variety and 310.6 for Osmancık-97 variety (*Table 4*). It is observed that the Local Karacadag rice variety yields a larger number of bunches than the Osmancık-97 variety in both conventional and organic conditions.

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Table 4. *The average values of bunch number in square meter (number), kernel number of bunch (number) and the formed groups according to the LSD (least significant difference) test*

Applications	Bunch number in m^2		Average	Kernel number of bunch		Average
	Karacadag	Osmancık-97		Karacadag	Osmancık-97	
Conventional	393.9	313.7	353.8	60.1	63.9	62.0 a
Organic	376.0	307.6	341.8	53.4	60.1	56.8 b
Average	384.9 a	310.6 b		56.8 b	62.0 a	
CV (%)	2.92			8.07		
LSD Variety	7.39**			3.48**		
LSD Application	N.S.			4.02*		
LSD Interaction	N.S.			N.S.		

*The difference between the averages shown with the same letter is not important (N.S.) at 0.05

When the average values of varieties were examined with regard to the kernel number of bunch, the Karacadag variety was 60.1 in conventional conditions and 53.4 in organic conditions; the Osmancık-97 was 63.9 in conventional conditions and 60.1 in organic conditions. In terms of growing conditions, the average number of kernel in bunch was found to be 56.8 in organic conditions and 62.0 in conventional conditions. The average values of the varieties were found to be 56.8 for Karacadag variety and 62.0 for Osmancık-97 variety (*Table 4*).

In this research, when the average values of the varieties were examined related to the kernel weight of bunch; the Local Karacadag variety was 1.79 g in conventional conditions, 1.57 g in organic conditions; Osmancık-97 variety was 2.08 g in conventional conditions and 1.97 g in organic conditions. In terms of growing conditions, the average kernel weight of bunch was 1.77 g in organic conditions and 1.93 g in conventional conditions. The average values of the varieties were found to be 1.68 g for Karacadag variety and 2.02 g for Osmancık-97 variety (*Table 5*).

Table 5. *The average values of kernel weight of bunch (g), thousand kernel weight (g) and the formed groups according to the LSD (least significant difference) test*

Applications	Kernel weight of bunch		Average	Thousand kernel weight		Average
	Karacadag	Osmancık-97		Karacadag	Osmancık-97	
Conventional	1.79	2.08	1.93 a	29.65	32.68	31.16
Organic	1.57	1.97	1.77 b	29.43	32.45	30.94
Average	1.68 b	2.02 a		29.54 b	32.56 a	
CV (%)	9.87			2.14		
LSD Variety	0.13**			0.48**		
LSD Application	0.12*			N.S.		
LSD Interaction	N.S.			N.S.		

*The difference between the averages shown with the same letter is not important (N.S.) at 0.05

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In this research, when the average values of the varieties were examined related to the thousand kernel weight it was found that the Local Karacadag variety was 29.65 g in conventional conditions and 29.43 g in organic conditions; the Osmancık-97 variety was 32.68 g in conventional conditions and 32.45 g in organic conditions. In terms of growing conditions, the average thousand kernel weight of varieties was determined as 30.94 g in organic application and 31.16 g in conventional application. The average values of the varieties were found to be 29.54 g for Karacadag variety and 32.56 g for Osmancık-97 variety (*Table 5*).

When the average values of the varieties were examined with regard to bunch length, it was determined that the Local Karacadag variety was 16.3 cm in conventional conditions and 16.1 cm in organic conditions; the Osmancık-97 was 12.7 cm in conventional conditions and 12.4 cm in organic conditions. In terms of growing conditions, the average values of bunch lengths of the varieties were 14.2 cm in organic applications and 14.5 cm in conventional application. The average values of the varieties were found found to be 16.2 cm for Karacadag variety and 12.5 cm for Osmancık-97 variety (*Table 6*).

When the average values of varieties were examined with regard to the biological yield, it was determined that the Local Karacadag variety was 13 514 kg ha^{-1} in conventional conditions and 11 663 kg ha^{-1} in organic conditions; the Osmancık-97 variety was 10 332 kg ha^{-1} in conventional conditions and 9 057 kg ha^{-1} in organic conditions. In terms of growing conditions, the average values of the biological yields of the varieties were 10 360 kg ha^{-1} in organic application and 11 923 kg ha^{-1} in conventional application. The average values of the varieties were found to be 12 589 kg ha^{-1} for Karacadag variety and 9 694 kg ha^{-1} for Osmancık-97 variety (*Table 6*).

Table 6. *The average values of bunch length (cm), biological yield (kg/ha) and the formed groups according to the LSD (least significant difference) test*

Applications	Bunch length		Average	Biological yield		Average
	Karacadag	Osmancık-97		Karacadag	Osmancık-97	
Conventional	16.3	12.7	14.5	13 514	10 332	11 923 a
Organic	16.1	12.4	14.2	11 663	9 057	10 360 b
Average	16.2 a	12.5 b		12 589 a	9 694 b	
CV (%)	6.37			4.64		
LSD Variety	0.66**			37.52**		
LSD Application	N.S.			94.97**		
LSD Interaction	N.S.			N.S.		

*The difference between the averages shown with the same letter is not important (N.S.) at 0.05

When the average values of the varieties were examined with regard to the kernel yield in unit area, it was determined that the Local Karacadag variety was 4 816 kg ha^{-1} in conventional conditions and 4 179 kg ha^{-1} in organic conditions; the Osmancık-97 variety was 3 784 kg ha^{-1} in conventional conditions and 3 234 kg ha^{-1} in organic conditions. In terms of growing conditions, the average kernel yield in unit area was 3 706 kg ha^{-1} in organic application and 4 300 kg ha^{-1} in conventional application. The average values of the varieties were found to be 4 498 kg ha^{-1} for Karacadag variety and 3 509 kg ha^{-1} for Osmancık-97 variety (*Table 7*).

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In this research when the average values of varieties were examined with regard to the non-fractured rice yield it was found that the Local Karacadag variety was 64.9% in conventional conditions and 67.5% in organic conditions; the Osmancık-97 variety was 62.9% in conventional conditions and 63.7% in organic conditions. The average non-fractured rice yield of the varieties in terms of growing conditions was found 65.6% in organic application and 63.9% in conventional application. The average values of the varieties were found to be 66.2% for Karacadag variety and 63.3% for Osmancık-97 variety (*Table 7*). It has been observed that the Local Karacadag variety has a significant superiority over the Osmancık-97 variety both in conventional and organic conditions.

Table 7. *The average values kernel yield in unit area (kg/ha), non-fractured rice yield and the formed groups according to the LSD (least significant difference) test*

Applications	Kernel yield in unit area		Average	Non-fractured rice yield		Average
	Karacadag	Osmancık-97		Karacadag	Osmancık-97	
Conventional	4 816	3 784	4 300 a	64.9	62.9	63.9
Organic	4 179	3 234	3 706 b	67.5	63.7	65.6
Average	4 498 a	3 509 b		66.2 a	63.3 b	
CV (%)	2.63			3.64		
LSD Variety	7.65**			1.71**		
LSD Application	39.92*			N.S.		
LSD Interaction	N.S.			N.S.		

*The difference between the averages shown with the same letter is not important (N.S.) at 0.05

Discussion

Kıran (1992) found that the number of days of flowering in Diyarbakır was between 64-118 days. Savsatlı and Gulumser (2006) stated that in the ecological conditions of Samsun, the number of days of flowering rice changes between 79-89 days with the broadcast seeding method according to the variety used. Savsatlı et al. (2008) determined the number of days of flowering in rice between 82-110 days.

Savsatlı and Gulumser (2006) stated that the number of ripening days in rice is between 120-136 days with the help of broadcast seeding method among the varieties used. Kıran (1992) found that the number of ripening days in Diyarbakır conditions ranged between 96-143 days. Genctan et al. (1994) reported that the number of ripening days varies between 119-143 days in a research conducted on a total of 10 different rice varieties.

The plant height affecting rice farming indirectly depends on the level of fertility of the soil, the method of sowing, the amount of applied fertilizer, environmental conditions and the genetic structure of the variety. Alp et al. (2010) stated that plant height of rice farming in the conditions of Diyarbakır changes between 81.82-99.50 cm. Taşer (2011) stated that the average plant height varied between 67.5-110.3 cm in the Southeast Anatolia Region and Karacadag Local rice variety is longer than any other variety.

The number of tillering in plants depends on the genetic characteristics of the varieties and it is greatly influenced by the environmental conditions (Kun, 1988). Lin (1974) stated that the number of tillering per plant decreased when the rice was planted frequently in the field, and the companions that formed after the maximum period of

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tillering decreased because of their inability to complete their development. Gevrek (2000) pointed out that the average number of tillering between applications in the rice plants varied between 1.3-2.9.

In a research Choi et al. (2002) conducted in South Korea, they stated that the number of bunches per m^2 was found to be 364 in conventional application and in organic application it was 420-444. Kıran and Oktar (1994) stated that the number of bunches in m^2 varies between 372-639 in Diyarbakır conditions and the number of bunches in m^2 of Karacadag variety is higher than other varieties and lines.

In a research conducted in South Korea, Choi et al. (2002) found that the kernel number of bunches was 79.6-88.0. Kıran and Oktar (1994) found that the kernel number of bunches varies 52-147 with Diyarbakır conditions. Alp et al. (2010) stated that the kernel number of bunches in rice farming varies 42.08-99.50 with Diyarbakır conditions. Sahin et al. (2012) determined the kernel number of bunches to be 43.6 to 113.1. Taser (2011) stated that the kernel number of bunches changes between 89.33-164.0 and the values of local rice samples were higher than those of many breeding varieties with regard to the kernel number of bunches.

Alp et al. (2010) stated that the kernel weight of bunch varies between 1.22-2.69 g in Diyarbakır conditions. Taser (2011) stated that the kernel weight of bunch varies between 1.463-1.942 g under the conditions of the Southeast Anatolian Region.

Taser (2011) determined that the bunch length changes between 12.40-23.20 cm, the bunch length of Osmancık-97 variety was 14.17 cm and the average of Karacadag population lines was around 21.06 cm. Sahin et al. (2012) determined that the bunch length varies between 11.7-18.5 cm.

It has been observed that the Local Karacadag variety has a significant superiority compared to the Osmancık-97 variety with regard to the biological yield in both conventional and organic conditions. It can be said by looking at the results of the research that biological yield decreases significantly under organic conditions.

It has been observed that the Local Karacadag variety has a distinctive superiority with regard to the kernel yield in unit area compared to the Osmancık-97 variety in both conventional and organic conditions. It can be said that the Osmancık-97 variety showed a lower kernel yield in unit area than the Local Karacadag variety due to the fact that the ripening period corresponds to high temperatures and it does not adjust to the Diyarbakır's ecological environment. In a research conducted in South Korea, Choi et al. (2002) found the kernel yield of the hairy vetch as 5 680-5 870 kg ha^{-1} in organic conditions and 5 760 kg ha^{-1} in conventional conditions. They also stated that in farmyard manure application, the kernel yield was 5 340-6 660 kg ha^{-1} and 6 210 kg ha^{-1} in conventional application. They pointed out that farmyard manure can be used in the production of hairy vetch. In an organic research conducted in South Korea, Lee et al. (2003) stated that the application of 100 kg nitrogen and 30 kg phosphorus per hectare yielded the highest number of yield with 5 690 kg ha^{-1}, 20 t of hairy vetch application for hectare yielded 5 290 kg ha^{-1}, 12 t compost application for hectare yielded 4 380 kg ha^{-1}. In a research conducted in Malaysia, Abu Bakar et al. (2003) reported that they achieved a yield of 1 760-4 100 kg ha^{-1} with organic applications, the average yield value was obtained as 3 200 kg ha^{-1}, and the rice yield in the region was around 5 000 kg ha^{-1}. Beser and Genctan (1999) stated that the yields of rice vary according to the ecological conditions, variety and technology used.

Savsatlı and Gulumser (2006) stated that the factors affecting the rice yield are kernel length, kernel shape and thousand kernel weight; for instance K-424 rice variety is

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shorter and fuller than the other varieties which leads the variety to have high rates of fractured and non-fractured rice yield. Therefore, they expressed that the genetic properties of the varieties affect rice yield to a great extent. Taser (2011) stated that the yield values of rice samples varied between 51.67-68.67% in the conditions of the Southeast Anatolian Region, and the highest non-fractured rice yield was found in the samples of the Local Karacadag rice variety.

Conclusions

As a result of agricultural research, organic and conventional applications; organic application showed higher values compared to the conventional application with regard to the number of days of flowering (92.3 days), and non-fractured rice yield (65.6%). However, it was observed that following values fell behind in organic conditions compared to the conventional conditions: the number of ripening days, plant height, number of tillering, bunch number in m^2, kernel number of bunch, thousand kernel weight, kernel weight of bunch, bunch length, biological yield and kernel yield in unit area.

Local Karacadag rice paddy variety which is cultivated under the organic conditions performed superior values compared to the Osmancık-97 breeding variety in terms of number of days of flowering (97.6 days), number of ripening days (140.9), plant height (92.1 cm), number of tillering in plants (2.59), number of bunches in m^2 (376.0 piece), bunch length (16.1 cm), biological yield (11 663 kg ha^{-1}), yield for unit area (4 179 kg ha^{-1}) and non-fractured rice yield (67.5%).

The Local Karacadag rice variety cultivated in conventional and organic conditions showed superior values compared to the Osmancık-97 variety in terms of number of days of flowering, number of ripening days, plant height, number of tillering in plants, bunch number in m^2, bunch length, biological yield, kernel yield in unit area, and non-fractured rice yield. However, it was observed that the Local Karacadag variety fell behind the Osmancık-97 variety with regard to the kernel weight of bunch, kernel number in bunch and thousand kernel weight.

When all the features examined in the research were taken into consideration, in addition to many features of the Karacadag variety, it was observed that the kernel yield in unit area was highest at 4 816 kg ha^{-1} in the conventional application while the organic application of the Karacadag variety was in second place with 4 179 kg ha^{-1}. It is concluded that the organic rice farming is thought to be economical when the organic rice in the market can be sold with about 30% higher price compared to the conventional rice. It is concluded that the use of Karacadag variety in organic agriculture may extend the organic agriculture in the region.

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FOLIAR APPLICATIONS OF $FeSO_4$ ALONE AND IN COMBINATION WITH CITRIC ACID CAN REDUCE IRON DEFICIENCY INDUCED CHLOROSIS IN TWO PAKISTANI PEANUT (*ARACHIS HYPOGAEA* L.) VARIETIES

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Abstract. Present study was conducted to check the ameliorative effect of foliar applications on iron (Fe) deficiency in peanut. The hydroponics experiments were performed at National Agriculture Research Centre, Islamabad, Pakistan. Various foliar treatments were used to check $FeSO_4$ alone and its combined effect with citric acid and surfactants. $FeSO_4$ alone and in combination with citric acid showed ameliorative effect on Fe deficiency in peanut. Active Fe concentration of BARI-2000 and BARD-699 increased up to 52% and 25% as compared to control, when foliar treatment of $FeSO_4$ was applied. Combined effect of $FeSO_4$ and citric acid resulted 55% and 56% increase of active Fe concentrations compared to control. However, foliar application of surfactant (sodium dodecyl sulphate) suppressed the growth of peanut. Various morpho physiological parameters showed that BARD-699 was more responsive to foliar applications, while citric acid alone and in combination can be used in correcting Fe deficiency in both genotypes.
Keywords: *BARI-2000, BARD-699, Calcareous soils, Pothwar region, hydroponics experiments*

Introduction

Iron (Fe) deficiency chlorosis is a common problem in calcareous soils reducing yield significantly (Inal et al., 2007; Boodi et al., 2016). Peanut is susceptible to Fe deficiency while grown on calcareous soils with high pH and high bicarbonate levels.

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The problem is common in Pothwar tract of Pakistan (Rashid et al., 1997). Various strategies are in practice to combat Fe deficiency. These strategies include either improving the mechanism of Fe uptake or enhancing the Fe in soil solution. Among various fertilizers in practice, EDDHA and analogues (the most stable chelates) proved to be effective in maintaining Fe in soil and transporting it to the roots of plants (Lucena, 2003).

Breeding (Cianzio, 1995) and genetic modifications (Robinson et al., 1999) are commonly used strategies for uptake of Fe from soil. Due to high cost of these strategies, other solutions should also be focused. Other methods to provide Fe to the plant are foliar applications and trunk injection. Foliar applications has been advocated by various researchers (Tagliavini and Rombola, 2001; Reed et al., 1988). Foliar applications produced good results and can be practically used (Song et al., 2017a,b). Being an expensive strategy, trunk injection is used for garden trees only (Fernández-Escobar et al., 1993).

Foliar applications are useful in mitigation of Fe deficiency. Foliar applications of $FeSO_4$ increased leaf Fe concentration, however, basal treatments are more effective for long results (Mann et al., 2017). Foliar applications of salicylic acid and sodium nitroprusside in combination can increase chlorophyll, active Fe and increased Fe accumulation in cell organelles by decreasing interveinal Chlorosis (Kong et al., 2014). In *Prunus persica* L. foliar applications of Fe(III)-EDTA, Fe(III)-citrate, $FeSO_4.7H_2O$ and Fe(III)-DTPA, Fe(III)-IDHA always resulted in leaf chlorophyll increase. Re-greening varies with application of different compounds (Fernández et al., 2006).

Fe-sulphate, Fe-citrate and Fe-EDTA were very effective in re-greening of effected leaves of peach (*Prunus persica* L.). After one week of spraying, Fe sources induced physiological and biochemical responses in Fe deficient low-chill peach plants in the following order Fe-sulphate>Fe-citrate>Fe-EDTA. Different peach cultivars showed different responses towards different Fe sources (Chakraborty et al., 2012). Foliar applications are cheap source to recover Fe deficiency symptoms (Song et al., 2017a). Present study was aimed to explore the effect of different foliar application alone and in combination in correcting Fe deficiency in BARI-2000 and BARD-699.

Materials and Methods

Purpose of Experiment

The purpose of this experiment was to check the effect of $FeSO_4$ alone and with different treatments on the morpho-physiological parameters of peanut. As $FeSO_4$ is cheaper than other chemicals so it can be used alone or in combination with other chemicals for correction of Fe deficiency induced chlorosis.

Selection of Genotypes

The experiments were performed at National Agriculture Research Centre, Islamabad, Pakistan. Two genotypes were selected as Fe deficiency tolerant (BARI-2000) and Fe deficiency sensitive (BARD-699) genotypes based on previous hydroponics and pot experiments (Akhtar et al., 2014; Akhtar et al., 2013).

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Seed Germination

After surface sterilization with H_2O_2, seeds of two genotypes were washed with distilled water and germinated on wet sterile sand at 25°C in the darkness.

Hydroponics Setup

After germination uniform seedlings were transferred to aerated Hoagland's nutrient solution (Epstein, 1972). The seedlings were aerated for 24 h. The setup was kept in controlled conditions of 14/10 light /dark period with 30/20°C ± 2°C temperature and 800 cd of light. The pH of nutrient solution was maintained at 6.2. The experiment was performed in four replications.

Foliar Treatments

Following treatments were made to two weeks old seedlings;(a) Fe limited (without any treatment) (b) 0.1% citric acid (c) 0.5%$FeSO_4$ (d) 0.5%$FeSO_4$+0.1%citric acid (e) 0.5% $FeSO_4$+0.1%citric acid+1%surfactants and (f) 1%surfactant. SDS (sodium dodecyl sulphate) was used as surfactant.

SPAD Values

Young fully expanded leaves from three plants were used to record chlorophyll content by SPAD-502 (Minolta, Japan). The average of three leaves of one plant was considered as one replication and data was recorded for four replications. During the experiment, after every week data was recorded for SPAD values and average values were calculated.

Active Fe Content

Active Fe content was measured from young fully expanded leaves (Gao and Shi, 2007). Each plant was considered a single replication.

Photosynthetic and Transpiration Rates

At first detection of chlorotic symptoms, Photosynthetic rate (A) and transpiration rate (E) were recorded using Infra-Red Gas Analyzer (LCA4 Bioscientific Ltd.).

Morphological Parameters

Seedlings were harvested before the anthesis stage (Three weeks after emergence) and number of leaves was counted. Root lengths, plant height and root and shoot fresh weights were recorded. The samples were dried at 60°C for three days to record dry weights of root and shoots.

Total Fe Content

Total Fe content was measured by dry ash method by considering single plant a replication and data was recorded for four replications (Rashid et al., 2001).

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Data Analysis

Pearson's correlation coefficients were recorded by Minitab 13 at 5% confidence interval. Mean ± S.E was calculated by MS Excel. Percentage increase was calculated by formula:

$$\frac{(100 - \text{Values in control(-Fe)} \times 100)}{(\text{Values in treatment})} \quad \text{(Eq.1)}$$

Replications were compared by using SPSS. Pairwise comparison was done by applying t-test using SPSS.

Results

Photosynthetic Rate

Fe deficiency is a widespread problem in calcareous soils. Significantly higher photosynthetic rate was recorded for BARD-699 with foliar application of $FeSO_4$, 53% increase was recorded as compared to Fe deficient treatment. With same treatment, BARI-2000 showed 44% increase in photosynthetic rate as compared to its Fe limited condition. Surfactant significantly lowered the photosynthetic rate of BARI-2000 (56%) as well as BARD-699 (20%). With foliar application of citric acid, $FeSO_4$+citric acid and combination of $FeSO_4$, citric acid and surfactant resulted increased photosynthetic rate of both genotypes. In case of BARI-2000, 3, 27 and 23% increase in photosynthetic rate was recorded as compared to Fe deficient treatment. With same treatments BARD-699 showed 35, 39 and 30% increase in photosynthetic rate as compared to Fe deficient treatment (*Figure 1*).

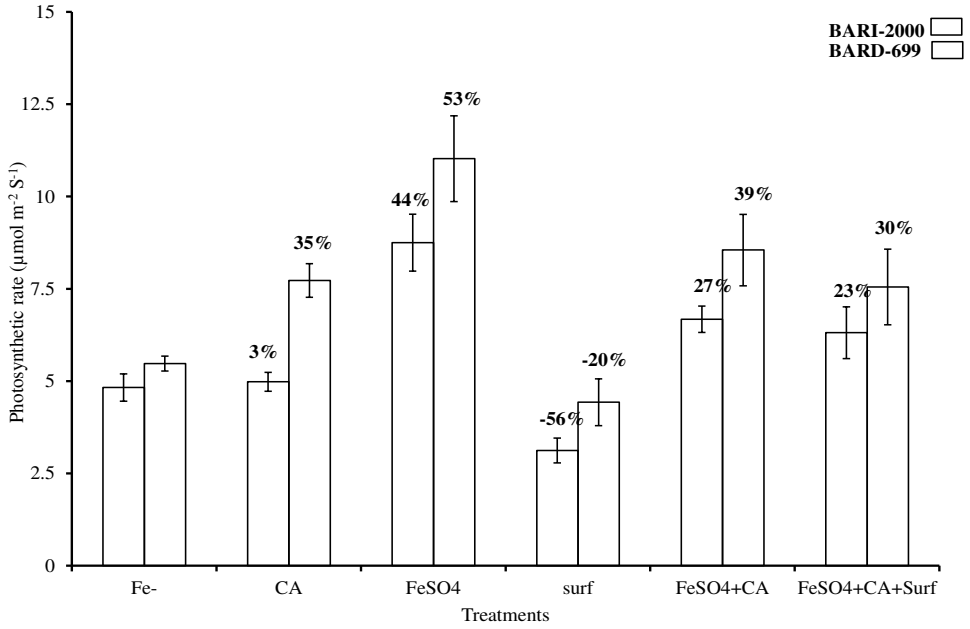


Figure 1. *Photosynthetic rates of Peanut genotypes in response to different foliar applications of equal concentration of Fe (CA: Citric acid; Surf: Surfactant)*

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Transpiration Rate

Foliar application of $FeSO_4$ along with citric acid significantly increased the transpiration rate of BARI-2000 (28%) and BARD-699 (35%) as compared to the respective Fe limited conditions. Transpiration rate with foliar application of citric acid, $FeSO_4$ and citric acid+$FeSO_4$+surfactant resulted increase of transpiration rate for both genotypes as compared to their respective Fe limited treatments. BARI-2000 resulted 20, 21 and 1% increase in transpiration rate, whereas BARD-699 showed 11,13 and 9% increase as compared to their respective Fe limited treatments. Surfactants resulted decreased transpiration rate of both genotypes *(Figure 2*).

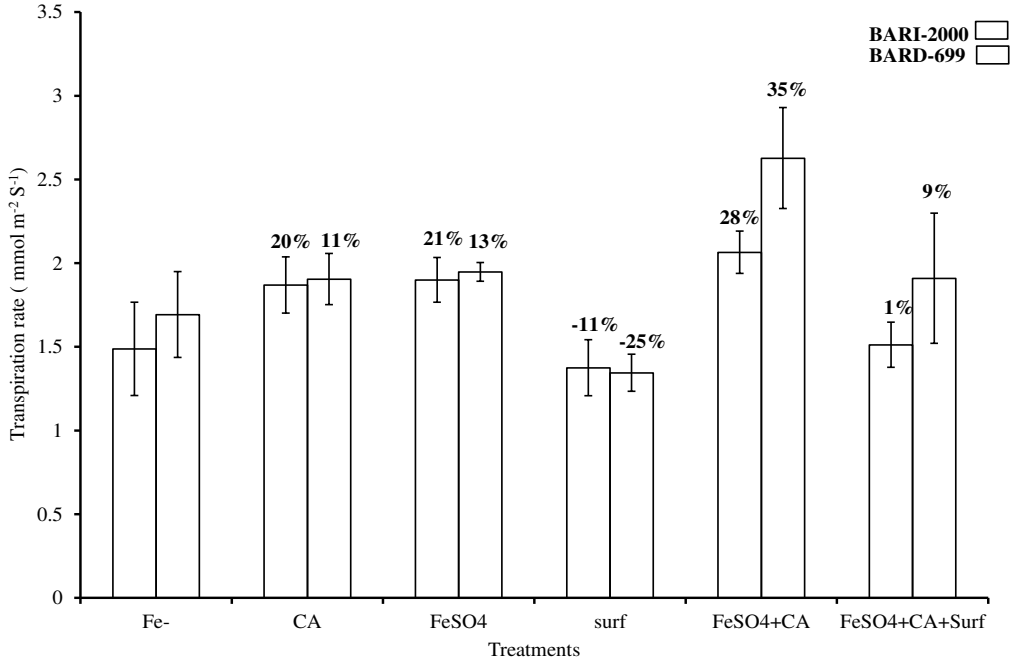


Figure 2. *Transpiration rate of Peanut genotypes in response to different foliar applications of equal concentration of Fe (CA: Citric acid; Surf: Surfactant)*

SPAD Values

More chlorotic symptoms will result in lower SPAD values. SPAD values decreased significantly with foliar application of surfactant. The decrease was 61% in BARI-2000 and 15% in case of BARD-699 as compared to their respective Fe limited conditions. This showed that surfactant disturbed plant functions resulting in decreased SPAD values. Other treatments including citric acid, $FeSO_4$, combination of citric acid and $FeSO_4$ and citric acid+$FeSO_4$+surfactant resulted increase in SPAD values of both genotypes. With these treatments BARI-2000 showed 24, 24, 25 and 12% increase in SPAD values as compared to Fe limited treatments. BARD-699 showed 28, 24, 28 and 17% increase in SPAD values in respective treatments as compared to Fe limited treatments (*Figure 3*).

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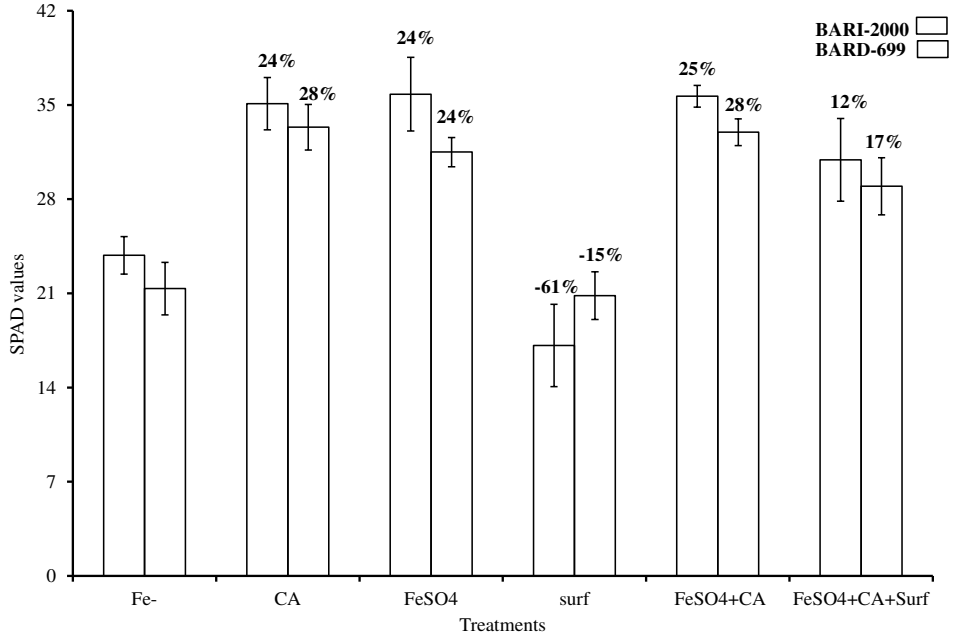


Figure 3. *SPAD values of Peanut genotypes in response to different foliar applications of equal concentration of Fe (CA: Citric acid; Surf: Surfactant)*

Correlation

SPAD values were positively correlated with active (R^2=0.697) and total Fe concentrations (R^2=0.587). Photosynthetic (R^2=0.625) and transpiration rate (R^2=0.620) was also positively correlated with SPAD values (*Table 1*).

Table 1. *Pearson correlation coefficients along with probability of significance between different parameters recorded under Fe deficient conditions and different foliar applications (Citric acid, $FeSO_4$, citric acid+$FeSO_4$, surfactant, citric acid+$FeSO_4$+surfactant)*

	NL	RL	PH	RFW	RDW	SFW	SDW	A	E	SPAD	Actv.Fe
RL	0.613**										
PH	0.454**	0.514**									
RFW	0.497**	0.432**	0.589**								
RDW	0.400**	0.512**	0.518**	0.420**							
SFW	0.583**	0.491**	0.712**	0.583**	0.448**						
SDW	0.585**	0.493**	0.570**	0.588**	0.499**	0.772**					
A	0.558**	0.798**	0.552**	0.451**	0.687**	0.529**	0.535**				
E	0.371**	0.510**	0.585**	0.434**	0.561**	0.475**	0.557**	0.552**			
SPAD	0.484**	0.495**	0.643**	0.599**	0.719**	0.512**	0.697**	0.625**	0.620**		
Actv.Fe	0.520**	0.724**	0.592**	0.467**	0.819**	0.551**	0.627**	0.850**	0.697**	0.740**	
Total Fe	0.459**	0.760**	0.527**	0.397**	0.657**	0.562**	0.658**	0.836**	0.738**	0.587**	0.853**

NL; number of leaves, RL; root length, PH; plant height, RFW; root fresh weight, RDW; root dry weight, SFW; shoot fresh weight, SDW; shoot dry weight, A; photosynthetic rate, E; transpiration rate, SPAD, active Fe and total Fe content

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Active Fe concentration was significantly higher with foliar application of $FeSO_4$ alone and in combination with citric acid.

Active Fe Content

In case of $FeSO_4$ increase in active Fe for BARI-2000 was 53% and BARD-699 gave value 58%. Similarly with combination of citric acid and $FeSO_4$, BARI-2000 showed 56% while BARD-699 showed 55% increase as compared to their respective Fe limited treatments. Citric acid showed 25% increase of active Fe of BARI-2000 and 52% increase in case of BARD-699 as compared to Fe limited treatment. $FeSO_4$ in combination with citric acid and surfactant showed 25% increase in BARI-2000 while 34% increase in BARD-699 as compared to Fe limited treatments. Surfactant resulted decrease in active Fe concentration of both genotypes as compared to Fe limited treatments (*Figure 4*).

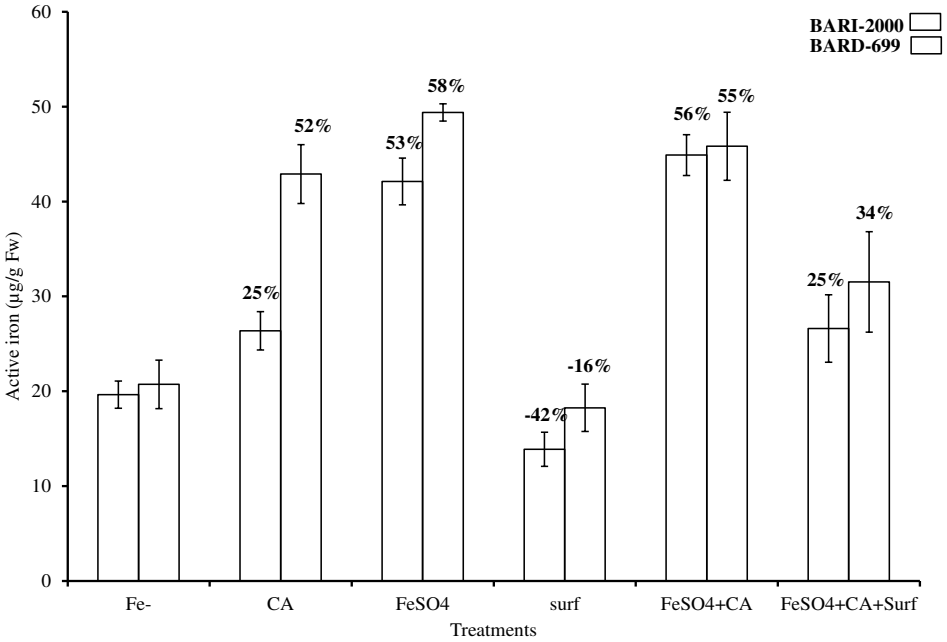


Figure 4. *Active Fe content of Peanut genotypes in response to different foliar applications of equal concentration of Fe (CA: Citric acid; Surf: Surfactant)*

Total Fe Content

Total Fe content increased significantly in BARD-699 with foliar application of $FeSO_4$ (66%) and citric acid +$FeSO_4$ (68%) as compared to Fe limited treatments. With same treatments, BARI-2000 showed 43% and 35% increase in total Fe respectively as compared to Fe deficient conditions. Citric acid resulted 6% increase in BARI-2000 while 45% increase in total Fe of BARD-699 as compared to their respective Fe deficient treatments. $FeSO_4$ in combination with citric acid and surfactant increased the total Fe concentration of BARI-2000 up to 11% while that of BARD-699 up to 44% as compared to Fe limited conditions. Foliar application of surfactant decreased the concentration of total Fe of both genotypes when compared to total Fe in Fe limited conditions (*Figure 5*). Total Fe concentration was positively correlated with active Fe concentrations (R^2=0.853) (*Table 1*).

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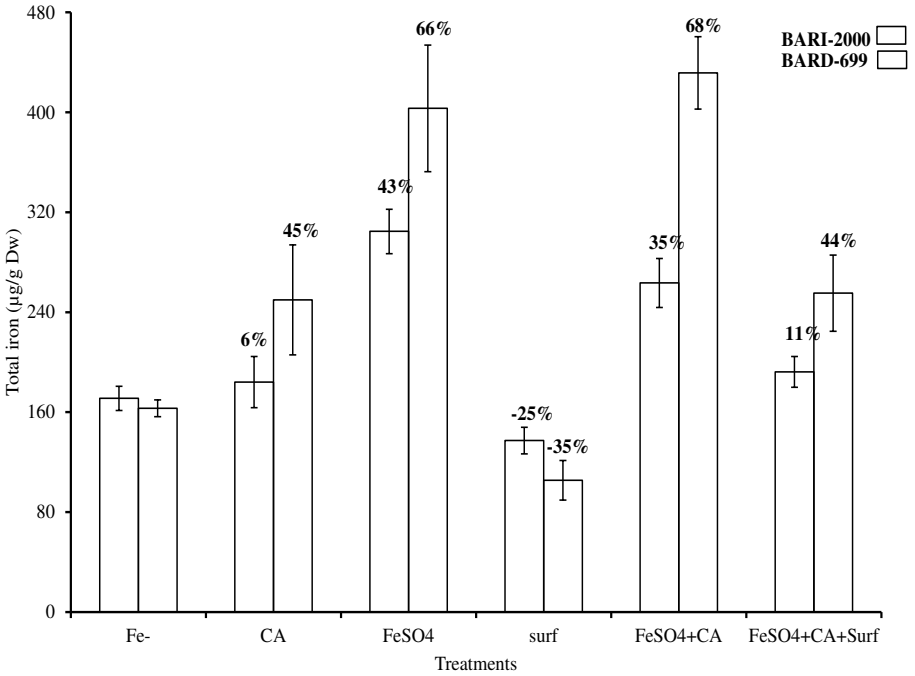


Figure 5. *Total Fe content of Peanut genotypes in response to different foliar applications of equal concentration of iron (CA: Citric acid; Surf: Surfactant)*

Comparison of Replications

The replications in both genotypes were non-significant (*Table 2*).

Table 2. *Comparison of replications in two genotypes*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Genotypes	7237.538	1	7237.538	9.169	0.003
Parameter	2448495.072	11	222590.461	281.993	0
Treatment	59821.011	5	11964.202	15.157	0
Rep	324.295	3	108.098	0.137	0.938

Pairwise Comparison of Genotypes

Pairwise comparison showed that both the genotypes were highly significant in few parameters when different foliar applications were made. The genotypes were non-significant in –Fe conditions except for shoot dry weight and transpiration rate. No. of leaves and root lengths were significantly different, when foliar applications of $FeSO_4$ were made. Shoot fresh weights and SPAD values were significantly different with foliar applications $FeSO_4$ were made in combination with citric acid. Foliar applications of Citric acid and $FeSO_4$ showed significant differences for Active Fe and total Fe content in both genotypes. SPAD values and total Fe content were significantly different with foliar applications of $FeSO_4$ and Citric acid when applied in combination. Genotypes were significantly different with respect to total Fe when $FeSO_4$+Citric acid +Surfactants were applied in combination (*Table 3*).

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Table 3. *Pairwise comparison of BARI-2000 and BARD-699*

	-Fe	Citric Acid	$FeSO_4$	Surfactant	$FeSO_4$+Citic acid	$FeSO_4$+CA+Surfactant
NL	-1.273	-0.217	-4.264**	-1.112	1.754	-0.891
RL	-1.163	-1.973	-4.013**	-2.898*	-2.472*	-0.107
PH	1.362	-0.993	0.116	-2.774*	-1.022	-3.516*
RFW	0.42	-0.194	2.316	-1.001	-2.112	-0.41
RDW	0.745	-2.165	2.959*	-0.299	1.855	-1.378
SFW	3.386*	-3.374*	-1.192	-1.828	-3.769**	-2.335
SDW	-25.927***	-24.026***	-16.172***	-10.235***	-14.161***	-10.235***
A	-3.081*	-10.473***	-3.262*	-3.634*	-3.632*	-1.995
E	-1.083	-0.308	-0.656	0.299	-3.438*	-1.931
SPAD	2.067	1.355	2.919	-2.094*	4.179**	1.056
Active Fe	-0.738	-8.926***	-5.551 ***	-2.842*	-0.445	-1.539
Total Fe	1.352	-2.709*	-3.668**	3.334*	-9.609***	-3.831**

CA; Citric acid, NL; number of leaves, RL; root length, PH; plant height, RFW; root fresh weight, RDW; root dry weight, SFW; shoot fresh weight, SDW; shoot dry weight, A; photosynthetic rate, E; transpiration rate, SPAD, active Fe and total Fe content

Discussion

Being an important oil seed crop, peanut ranks fifth in vegetable oil seed production throughout the world (Song et al., 2017a). The Fe deficiency is the yield limiting factor in dicot plants with main symptoms of interveinal chlorosis due to inhibition in chlorophyll synthesis (Song et al., 2017b). The problem is more pronounced in the Pothwar tract of Pakistan with calcareous soils resulting in reduced peanut yield. The study was planned to check the effect of foliar applications on the Fe deficiency induced chlorosis in peanut. When nutrients are applied in the form of foliar application, leaf cuticle is the first obstacle to absorb nutrients (Kannan, 1990). In our results foliar applications increased the SPAD values, Photosynthetic rate, transpiration rate, active and total Fe content of peanut. Similarly foliar applications of Salicyclic acid and Sodium Nitroprusside alleviated the Fe deficiency of peanut by increasing the photosynthetic rate and carbohydrate increase (Kong et al., 2014). Similar results were also reported by Song et al. (2017b), where lower SPAD values were recorded in Fe deficient plants as compared to the plants treated with foliar applications.

Active Fe content is an important parameter that shows the concentration of physiologically active Fe in plant tissue (Rashid et al., 1997). Our results showed an increase in active and total Fe content when foliar applications were applied. Similar results were reported by Kong et al. (2014). In peanut plants, foliar applications of SNP and NA enhanced the active Fe content. As compared to total Fe, physiologically active Fe is considered a better nutritional Fe indicator (Hakan and Vahap, 2007). Active Fe content was increased after foliar application of SNP and SA (Song et al., 2017b). In another study, exogenous application of Nitric oxide improved Fe availability in plants (Graziano et al., 2002), that could improve Fe storage by ferritin protein (Briat et al., 2010). Active Fe content was significantly different by foliar applications as described

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by Pairwise comparison of BARD-699 and BARI-2000. This suggests the different responses of genotypes towards foliar applications. BARD-699 showed earlier response towards foliar applications, that may be related to its sensitive nature. In order to cope with Fe deficiency, the genotypes increased the uptake of Fe.

SPAD values were positively correlated with active Fe and total Fe content. Photosynthetic and transpiration rates were also positively correlated. This shows that by applying Fe exogenously, Fe deficiency induced chlorosis can be recovered and hence yield can be improved. SPAD values were significantly different in both genotypes when citric acid was applied in the form of foliar application. Foliar application of Fe is a common mean to correct Fe deficiency in agricultural crops (Salahi et al., 2017; Álvarez-Fernández et al., 2005). These applications included the foliar sprays of sequestrene (FeEDDHA, $FeSO_4$, FeEDTA) and other treatments (Lucena, 2003; Rashid et al., 1997). The treatments are used either alone or in combination with acids to lower down the pH of leaf tissues (Kumawat et al., 2006). With all foliar treatments, total Fe was significantly different among two genotypes. Citric acid is a good source to recover Fe deficiency induced chlorosis by reducing the pH as indicated by our study. FeEDDHA is the most common in Fe deficiency areas for correction of Fe chlorosis in fruit trees (Lucena, 2003). After foliar Fe treatment to chlorotic plants, various effects have been reported due to many factors involved in the process of leaf penetration, plant translocation and cell Fe uptake (Curie and Briat, 2003). When subjected to Fe deficiency, plants responded in different ways (Rombolà et al., 2002), such as chlorophyll synthesis inhibition and disturbed enzyme activities, which requires Fe as cofactor (Bisht et al., 2002). Surfactants are frequently added to the different foliar sprays, as they can increase the penetration of these substances to the leaf cuticle (Stock and Holloway, 2006). However, few surfactants are considered less toxic or less effective, as Agarol-600 resulted negative effect on wheat shoot growth when combined with $ZnSO_4$ (Haslett et al., 2001), while other surfactants are not effective at all (Kannan, 1984). Our results suggested that Sodium Dodicyl Sulphate cannot be used as surfactant as SDS alone resulted decreased growth of both genotypes. It may be concluded that citric acid and $FeSO_4$ alone and in combination can increase the growth of both genotypes particularly of BARD-699.

Conclusion

It may be concluded that citric acid and $FeSO_4$ alone or in combination can increase the growth of both genotypes particularly of BARD-699. Citric acid and $FeSO_4$ together was most effective among all treatments tested. Both the treatments can be successfully used to ameliorate Fe deficiency induced chlorosis. SDS can not be recommended as a surfactant.

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FRACTURE CHARACTERIZATION FOR CARBONATE RESERVOIRS: A CASE STUDY ON NT OILFIELD IN THE EASTERN MARGIN OF THE PRE-CASPIAN BASIN

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Abstract. In order to make full use of well logging information to accurately predict the fracture distribution of carbonate reservoir, we use several log curves to comprehensively identify and delineate the structural fractures, dissolved fractures and sutured fractures of different occurrences, scales and geneses in the North Truwa oilfield of the Pre-Caspian Basin in Kazakhstan. The resistivity difference-ratio method may be used to identify high-angle fractures. The 3-porosity ratio method, which tends to be affected by dissolved pores and vugs, is applicable to low-angle fractures and microfractures. By using each single method separately, only 54.8% of fractures on the average may be identified. The comprehensive probability density method which integrates the above two methods improves the accuracy of fracture identification to 73.6% as per the validation of core and imaging logging data. Fracture distribution in the study area is dominated by sedimentation, structure and diagenesis. In the lateral direction, the structural highs and areas with intense karstification may demonstrate high fracture probability density and thus areinterpreted to be rich in structural and dissolved fractures. In the vertical direction, more fractures may occur in the brittle intervals with small shale and gypsum contents. The results of fracture prediction are consistent with the sedimentary diagenesis of carbonate reservoirs, indicating that the method is feasible to predict the fracture.
Keywords: *fracture classification, fracture signatures, fracture prediction, conventional logging, comprehensive probability density, distribution*

Introduction

It is challenging to characterize the fractures in carbonate formations. Carbonate fractures may be delineated in the perspective of genesis and geometry by using seismic, logging and stress data (Lai et al., 2017; Ameen et al., 2012; Kazatchenko et al., 2007). In a field with a number of wells drilled, fracture characterization tends to be accomplished by using logging methods, such as the method of Wavelet Transform (Dong et al., 2013) which obtain the high-frequency weak signal of fracture in logs by means of wavelet decomposition and reconstruction, Matrix index method (Sun et al., 1999) which identify the fracture by calculating the acoustic skeleton index and the density skeleton index and constructing the rendezvous skeleton index, 3-porosity ratio (Gong et al., 2007; Luo et al., 2001) which characterize fracture development by density, neutron and acoustic plot, fractal dimension method (Tang et al., 2012) which determine the fractal dimension that reflects the complexity of fracture reservoir by multiple mesh encryption, and resistivity difference-ratio (Yan et al., 2009; Cai et al., 2003) which characterize fracture development by the difference ratio of deep and shallow resistivities. But the accuracy of the methods is related to fracture geometry,

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occurrence and scale, especially when only a single method is used to identify multi-geometry multi-occurrence fractures of complex origins. A solution is comprehensive identification by using a combination of various log curves. We make a case study on the North Truwa oilfield at the east margin of the Pre-Caspian Basin in Kazakhstan. The objective is to improve fracture characterization by analyzing fracture signatures and the capability of existing methods and finding improved methods with conventional log curves.

Materials

Geological setting

The North Truwa oilfield tectonically lies in the eastern slope of the Zharkamys-Enbek palaeohigh at the east margin of the Pre-Caspian Basin in Kazakhstan. It is a faulted anticlinal structure whichextends in the NE direction and is bounded by a NE-strike boundary fault on the east (*Fig. 1*). A package of thick carbonate rocks were deposited in the Paleozoic Carboniferous Period (He et al., 2014; Zhao et al., 2013; Zhao et al., 2010; Bian et al., 2011; Zheng et al., 2005). KT-I in the Middle and Upper Carboniferous Series is the major hydrocarbon bearing formation and is vertically subdivided into three oil layers, i.e. A, Б and B. During KT-I deposition, the open platform in southeastern deep water changed into restricted platform and evaporate platform in shallow water toward the northwest.

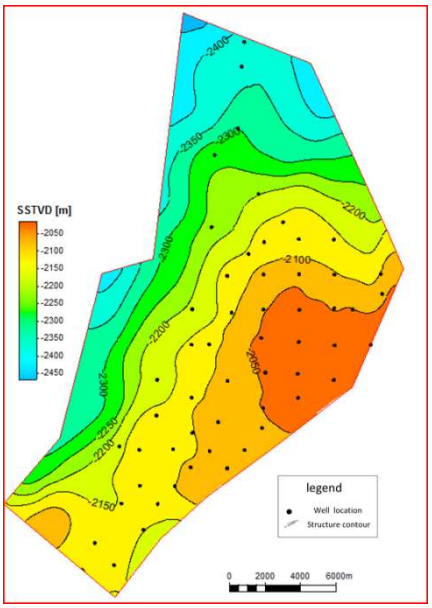


Figure 1. *Location and structure map of the study area. (SSTVD: subsea true vertical depth)*

Due to the elevation of the whole area before Permian deposition, the layer A at the top experienced differential denudation and early deposited carbonate rocks were exposed to the leaching process of atmospheric water (Wang et al., 2012). Lithologies vary with sedimentary environment and change from grainstone in the open platform to dolomitic limestone and limy dolostone to dolostone, gypseous dolostone and gypsum rocks in the evaporate platform (*Fig. 2*). Vertically B3-B5 in the lower section deposited

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in deep water at the early stage of KT-I deposition has a high shale content of 19.9% on the average, dolomite content of 10.7% and average calcite content of 69.4%. Б1-B2 is composed of relatively clean limestone and dolomitic limestone with small shale content of 7.0% on the average and increased dolomite content of 10.6% and calcite content of 82.3%. A2-A3 in the upper section has an increased average shale content of 15.7% as well as increased average dolomite content of 24.0%, average anhydrite content of 5%, and average calcite content of 55.4% in the evaporate platform.

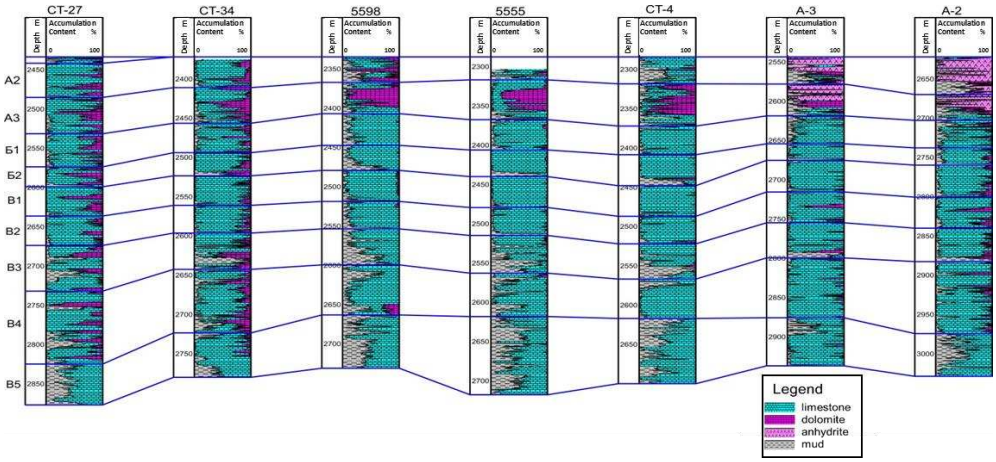


Figure 2. *Lithologic section of the study area*

Fractures

As per fracture geneses, three types of fractures, i.e. structural fracture, dissolved fracture and sutured fracture, could be observed on cores and image logs. Large fractures could be visually inspected and microfractures of which the apertures are generally less than 0.1 mm (Anders et al., 2014; Lai et al., 2017) may only be examined by using a microscope. Fracture dip ranges from 10° to 90° and there are also irregular fractures. Most fractures in the oil field are open seams, and only few are filled with calcite.

Structural fractures

Structural fractures are common in the study area due to the effect of tectonic stress. Fracture surface is flat or distributes in en-echelon (*Fig. 3a*). Two groups of fractures with different occurrences may co-exist (*Fig. 3b*). These fractures vary greatly from large fractures which could be identified on seismic sections to small fractures which could be identified on cores and imaging log curves to microfractures which may only be observed by using a microscope (*Fig. 3c*). Fracture dip Angle is widely distributed from 10° to 90° (*Fig. 3d*).

Dissolved fractures

Dissolved fractures surface is irregular and fracture width is in the range of 0.1-5 mm. Both large fractures and microfractures could be inspected (*Fig. 3e, f*). Generally there are no oriented fractures. Some oriented fractures are early structural fractures

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corroded and enlarged by acidic fluids at the late stage (*Fig. 3g*). Fluids usually flowed through fractures; thus dissolved fractures are often associated with structural fractures.

Sutured fractures

According to the core observation, most sutured fractures are opened and filled with crude oil; this means the existence of sutured fractures is important to oil accumulation and flow. Sutured fractures exhibit various occurrences, including intra-stratal low-angle sutured fractures, vertical sutured fractures and high-angle oblique sutured fractures. A single or a group of sutured fractures may be examined (*Fig. 3h*). Dissolved fractures may also occur together with sutured fractures (*Fig. 3i*) because of fluid flow through sutured fractures.

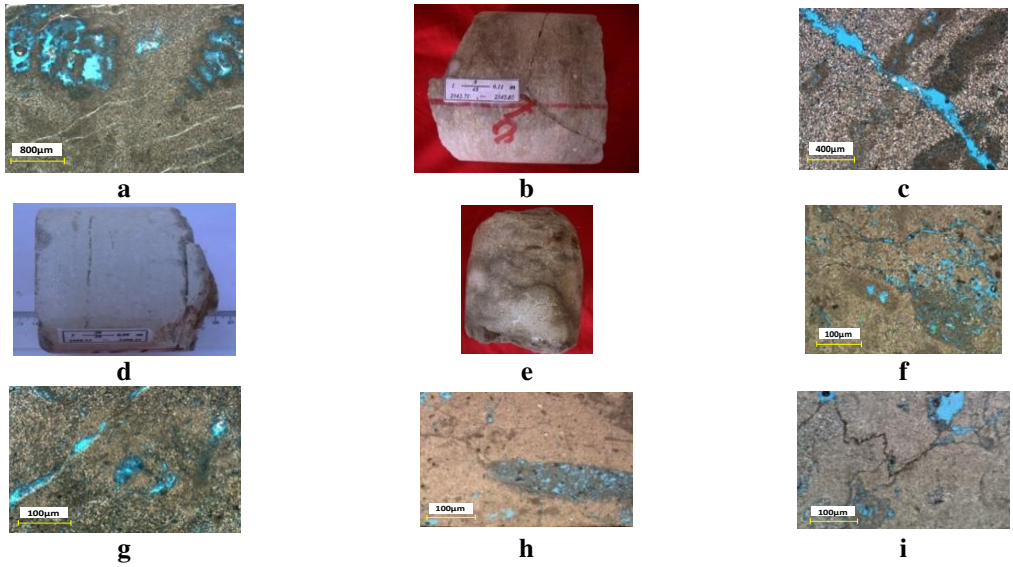


Figure 3. *Fractures in the study area.* ***a.*** *En-echelon structural fractures.* ***b.*** *Structural fractures with two occurrences.* ***c.*** *Microstructural fractures observed under a microscope.* ***d.*** *Low-angle structural fractures.* ***e.*** *Macro dissolved fractures.* ***f.*** *Micro dissolved fractures.* ***g.*** *Early structural fractures corroded and enlarged.* ***h.*** *Net-shaped sutured fractures.* ***i.*** *Corroded sutured fractures*

There are seldom fractures with single genesis, single occurrence or single scale separately occurring in the area. Fractures with various geneses and occurrences usually exist together with varying degrees of development. In this circumstance, it is hard to identify single-genesis or single-scale fractures by using conventional log curves. But it is possible and significant to quantitatively predict fractures in an interval.

Methods

We used some published techniques, i.e. resistivity difference-ratio and 3-porosity ratio, based on conventional logs to identify fractures in the North Truwa oilfield and validated the results with core and imaging log data. Both techniques were demonstrated to be of low credibility. In view of the complexity of fracture geneses and

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occurrences, two techniques were combined to identify multi-genesis multi-occurrence fractures in carbonate rocks.

Resistivity difference-ratio

The resistivity close to and far away from the wellbore will be measured by deep and shallow resistivity logging. The resistivity around the wellbore will decrease if the mud in the wellbore flows into the formations through fractures (Babadagli and Al-Salmi, 2004). For high-angle fractures (above 75°), both deep and shallow resistivity curves exhibit remarkable decrease, especially the shallow resistivity due to more impact of mud invasion on near-hole area. Consequently there is a positive difference between deep and shallow resistivity (Sibbit and Faivre, 1985) (*Fig. 4a*). The magnitude is much higher than that caused by the existence of pores. For low-angle fractures (below 30°) or horizontal fractures, the mud may flowperpendicularly to the wellbore into the fractures far away from the wellbore; thus deep resistivity exhibits larger decrease than shallow resistivity because various fluids (oil, gas, water or mud) in the fractures have much lower resistivity than tight rocks. This negative difference reaches a maximum at the interval with horizontal fractures (Sima, 2009) (*Fig. 4b*). Owing to the impact of fluids and filling materials in fractures, positive difference or zero difference may also occur in addition to negative difference at the interval with low-angle fractures.

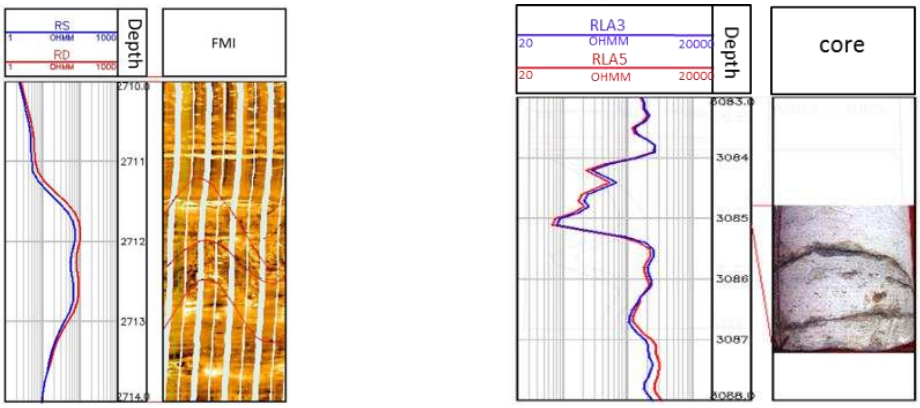


Figure 4. *Fractures and corresponding resistivity responses*

Inaccordance with resistivity responses to fractures, the method of resistivity difference-ratio was widely applied to fracture identification and prediction (Sun et al., 1999; Dond et al., 2013) by using the equation $RTC = (R_t - R_{lls}) / R_{lls}$, where RTC is deep-shallow resistivity difference ratio, R_{lls} is the shallow resistivity, R_t is the true resistivity after invasion correction by $R_t = 2.589\ R_{lld} - 1.589\ R_{lls}$, and R_{lld} is the deep resistivity.

In view of the uncertainty of resistivity difference for low-angle fractures, we substitute the deep resistivity with true resistivity to calculate the difference for fracture and pore discrimination. Generally at the interval with fractured hydrocarbon reservoirs, $R_t > R_{lls}$ and $RTC > 0$; RTC shows positive anomalies higher than the base line. At the fractured interval with water or tight interval, $R_t \approx R_{lls}$ and $RTC \approx 0$.

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Three-porosity ratio

Acoustic log curve also demonstrates some responses to the existence of fractures because of acoustic attenuation by fractures. At the fractured interval, later arrivals instead of the head wave will be recorded; thus the log curve exhibits increased acoustic time or cycle skip. This leads to large acoustic porosity. At the interval with high-angle fractures, there are no distinct responses on the curve because acoustic waves bypass the fractures as per the principle of least distance. At the interval with oblique, horizontal or net-shaped fractures, the log curve exhibits slightly jagged profile due to increased acoustic time or cycle skip caused by severe acoustic attenuation (He et al., 2014; Bian et al., 2011) (*Fig. 5*). Therefore the variations in acoustic porosity somewhat indicate the existence of fractures, especially horizontal, net-shaped and micro fractures (Sima et al., 2009; Wang et al., 2006). Acoustic wave is compressional wave and thus more sensitive to matrix pores uniformly distributing in the rock and less sensitive to secondary dissolved pores irregularly appearing in the rock. In such a circumstance, the increment of acoustic porosity is small (Li et al., 2013).

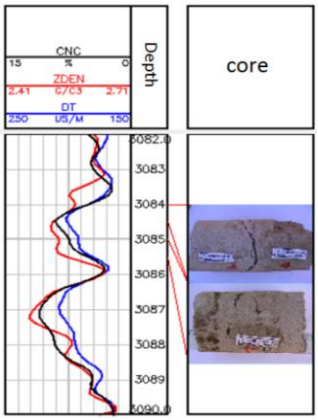


Figure 5. *Responses of porosity logs to fractures*

Neutron porosity and density porosity indicate the total porosity of matrix pores, secondary dissolved pores and fractures. The existence of fractures and secondary dissolved pores may lead to larger increments of neutron porosity and density porosity than that of acoustic porosity. In other words, the gap between total porosity (neutron porosity and density porosity) and acoustic porosity will increase at the interval with secondary dissolved pores and fractures.

The porosity difference-ratio, RP, is used for fracture characterization and defined as the function of neutron porosity Φ_N, density porosity Φ_D, acoustic porosity Φ_S and total porosity Φ_T, as shown by (*Eqs. 1* and *2*).

$$\Phi_T = \sqrt{\frac{\Phi_D^{\,2} + \Phi_N^{\,2}}{2}} \qquad \text{(Eq.1)}$$

$$R_p = \frac{|\Phi_T - \Phi_S|}{\Phi_T} \qquad \text{(Eq.2)}$$

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When RP > 0, the development of secondary reservoir space such as fracture is indicated. A large RP indicates more secondary pore space including fractures, dissolved pores and microfractures.

Comprehensive probability density

The area has more low-angle fractures than high-angle fractures, so the resistivity difference-ratio method is inferior to 3-porosity ratio method in fracture identification. For both methods, only 55% of fractures could be identified on the average. It is hard to identify the fractures with multi-genesis, multi-scale and multi-occurrence by using a single method. Thus two methods were combined to determine the comprehensive probability density of fracture. The procedure is described as follows.

Step 1 (*Eqs. 3* and *4*): calculate the normal or nearly normal distribution of resistivity (Yan et al., 2009) because of the original skewed distribution (non-uniform numerical distribution).

$$RTC' = \ln(RTC+1) \quad \text{(Eq.3)}$$

$$RP'=\ln(RP+1) \quad \text{(Eq.4)}$$

Step 2 (*Eqs. 5, 6, 7* and *8*): normalize RTC derived from the resistivity difference-ratio method and RP derived from the 3-porosity ratio method, and then calculate corresponding probability density for fracture characterization.

$$RTC' \geq 0: P_{RTC}=1-\exp(-RTC') \quad \text{(Eq.5)}$$

$$RTC' \leq 0: P_{RTC}=0 \quad \text{(Eq.6)}$$

$$RP' \geq 0: P_{RP}=1-\exp(-RP') \quad \text{(Eq.7)}$$

$$RP' \leq 0: P_{RP}=0 \quad \text{(Eq.8)}$$

Step 3 (*Eqs. 9* and *10*): validate the results and feasibility for reservoir characterization by using core and imaging log data. The thickness of the fractured interval was calculated based on above two methods and core and imaging log data. The percentage of accuracy was then used as the weighting factor for each method.

$$X_{RTC} = H_{RTC} / H \quad \text{(Eq.9)}$$

$$X_{RP} = H_{RP} / H \quad \text{(Eq.10)}$$

Here, H_{RTC}, H_{RP} and H represent the thickness of the fractured interval calculated by above two methods and core or imaging log data, respectively (*Eqs. 11* and *12*).

$$W_{RTC}=X_{RTC}/(X_{RTC}+X_{RP}) \quad \text{(Eq.11)}$$

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$$W_{RP}=X_{RP}/(X_{RTC}+X_{RP}) \quad \text{(Eq.12)}$$

Here, W_{RTC} and W_{RP} represent the weighting factor for each method, respectively.

Step 4 (*Eqs. 13*): calculate the comprehensive probability density.

$$P= W_{RTC}\times P_{RTC}+W_{RP}\times P_{RP} \quad \text{(Eq.13)}$$

Results

As per the validation by core and image logs data from 5 wells, RTC results are more sensitive to high-angle fractures and less sensitive to low-angle and horizontal fractures; 82% of high-angle fractures on the average were identified and only 26% of low-angle fractures were identified. Due to the complexity of fracture occurrence, on the whole, 31.9-74.1% of fractures, with an average of 49.5%, were identified in the study area. As shown in *Figure 6a,* Well UT-1 was examined to have high-angle fractures at 2707-2714 m and the average dip angle is 70.6°. The fractured interval was interpreted to be 1.8 m thick on the imaging log curve, which is reconciled with the thickness of 2 m in terms of high positive RTCs. As shown in *Figure 6b,* Well CT-20 was observed to have nearly horizontal fractures at 3164.4-3165.1 m and 3167.5-3168.1 m. The fractured intervals were interpreted to be 0.7 and 0.6 m thick, respectively. But on the RTC profile, the upper interval with apparent positive anomalies was interpreted to be a fractured interval of 0.6 m thick, whereas the lower interval with no distinct anomalies was interpreted to have 0 m fractured interval. This means only 46.2% of fractured intervals were identified.



a **b**

Figure 6. *RTC-based fracture identification*

The Three-porosity ratio result was validated by core and imaging log data to be of limited accuracy. For example, the existence of low-angle fractures at 3101-3104 m in Well CT-20 is in agreement with high RP (*Fig. 7a*) and microfractures at 3213.47-3213.53 m in Well CT-4 is in agreement with the average RP of 5.7 (*Fig. 7*b). The interval at 2600-2601 m in Well CT-10 with no fractures but with secondary dissolved pores also exhibits high RP (*Fig. 7c*), whereas the interval at 2711-2712 m in Well UT-I with abundant high-angle fractures does not illustrate significant RP variations (*Fig. 7d*). This means that the method is applicable to the identification of low-angle fractures and microfractures. The results are not affected by the existence of water layers but tend to be affected by secondary dissolved pores. In addition, high-angle

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fractures cannot be identified accurately. As per the statistics of 5 wells, 48-72.5%, with an average of 61.5%, of fractures could be identified.

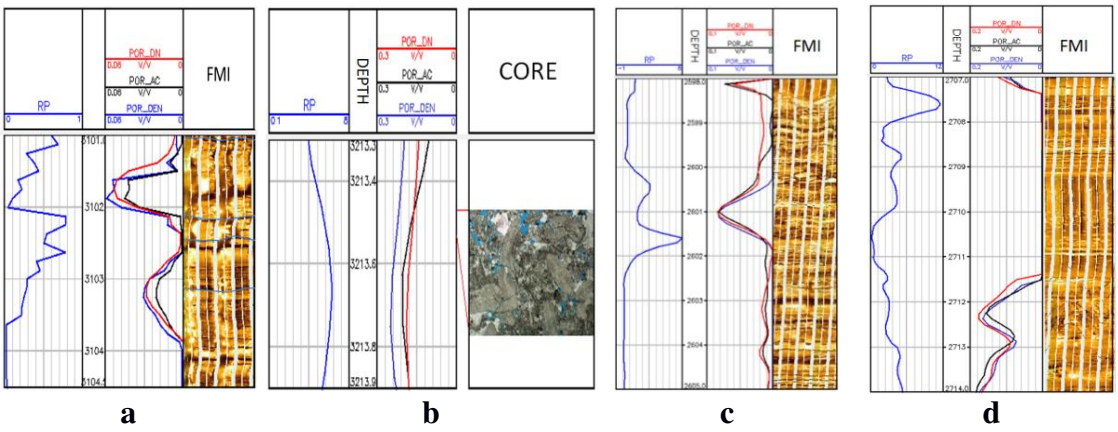


Figure 7. *Fracture identification by 3-porosity ratio method*

According to core and image logs, the P value of fracture development period is generally greater than 20%. Therefore, the position of 20% above the baseline of P value is used as the lower limit of fracture development, and the further the deviation from the baseline, the higher the degree of fracture development. Five cored wells were included in the calculation. The weighting factor for the resistivity difference-ratio method was estimated to be 0.395-0.531 with an average of 0.44; the factor for the 3-porosity ratio method was estimated to be 0.469-0.635 with an average of 0.56 (*Table 1*).

Table 1. *Parameters for the comprehensiveprobability density method*

Type	Well no.	Thickness of cored interval by core observation (m)	Thickness of cored interval by RTC method (m)	Thickness of cored interval by RP method (m)	X_{RTC}	X_{RP}	W_{RTC}	W_{RP}	Thickness of cored interval by P interpretation (m)	Percentage of P accuracy
Parametric calculation well	CT4	63.5	34.5	30.5	0.544	0.48	0.531	0.469	47.685	75.09%
	CT10	32.9	10.5	19.3	0.319	0.555	0.365	0.635	22.376	68.01%
	CT22	70	51.9	50.8	0.741	0.725	0.506	0.494	50.943	72.78%
	CT41	34.2	14.2	20.9	0.415	0.612	0.404	0.596	28.1288	77.40%
	5555	40.5	18.5	28.4	0.457	0.701	0.395	0.605	29.646	73.20%
	Avg.	48.2	25.9	30.0	0.495	0.615	0.4402	0.5598	35.75576	73.30%
Verified well	5598	12.6	5.3	7.2	0.424	0.568			9.475	75.20%

The average weighting factors were then used to calculate the comprehensive probability density, P. For these 5 cored wells, the percentage of accuracy (X_{RTC}) for the resistivity difference-ratio method was 31.9-74.1% with an average of 49.5% and the accuracy (X_{RP}) for the 3-porosity ratio method was 48-72.5% with an average of 61.5%. The accuracy for the comprehensive method was 68.01-77.4% with the increase by 23.8% and 11.8%, respectively, to 73.3% on the average. According to the weight

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parameters Calculated using 5 coring Wells, with another 1 new coring well 5598 as testing well, P is calculated. The thickness of fracture development of this well is 12.6 m. The RTC recognition rate is 42.4%, and the RP recognition rate is 7.2%. The fracture development thickness predicted using comprehensive probability density P is 9.5 m, and the recognition rate is 75.2 (*Table 1* and *Fig. 8*). For example, there are 4 fractured intervals at 3084.5-3085.5 m, 3086.2-3087.2 m, 3090-3091 m and 3092-3093.5 m, respectively, in the cored interval from 3084.5-3098 m in Well CT-4. The fracture density is 38, 12, 13 and 28/m, respectively. These observations basically answer to the P-based results.

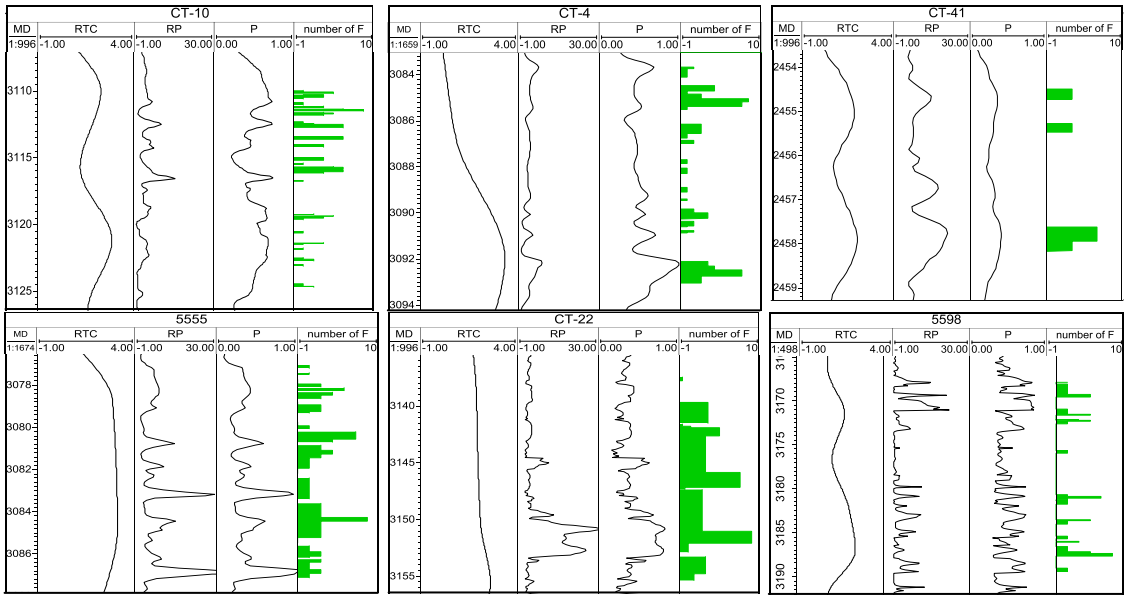


Figure 8. *Fracture predictions and core observations*

Discussion

Lateral and vertical distributions of fractures (*Figs. 9* and *11*) were delineated by the probability density calculated above. These maps and sections show that fracture distribution is related to sedimentation, karstification and tectonic movements (Braissant, 2003; Zhang et al., 2004).

Laterally more fractures occur at the structural high of the anticline, where there are 3-4 zones rich in fractures. In lower Б and B layers, the appearance of more fractures at the structural high is attributed to large tectonic stress, where the probability density of fracture is high. The appearance of more fractures at the structural high in the upper layer A is related to two factors. One is large tectonic stress which gave rise to structural fractures. The other is the leaching process which was caused by the uplifting before the Permian Period and resulted in dissolved fractures at the structural high. Early fractures were also enlarged by the chemical erosion of atmospheric water. Thus both structural fractures and dissolved fractures occur at the structural high of the anticline in layer A.

In the intervals with shale content of 4%-6% and gypsum content of 0 in B2, the probability density of fracture is related to structural location. For the same lithofacies, the structural high tends to exhibit more fractures than the structural low (Andrea et al., 2016) (*Fig. 10*).

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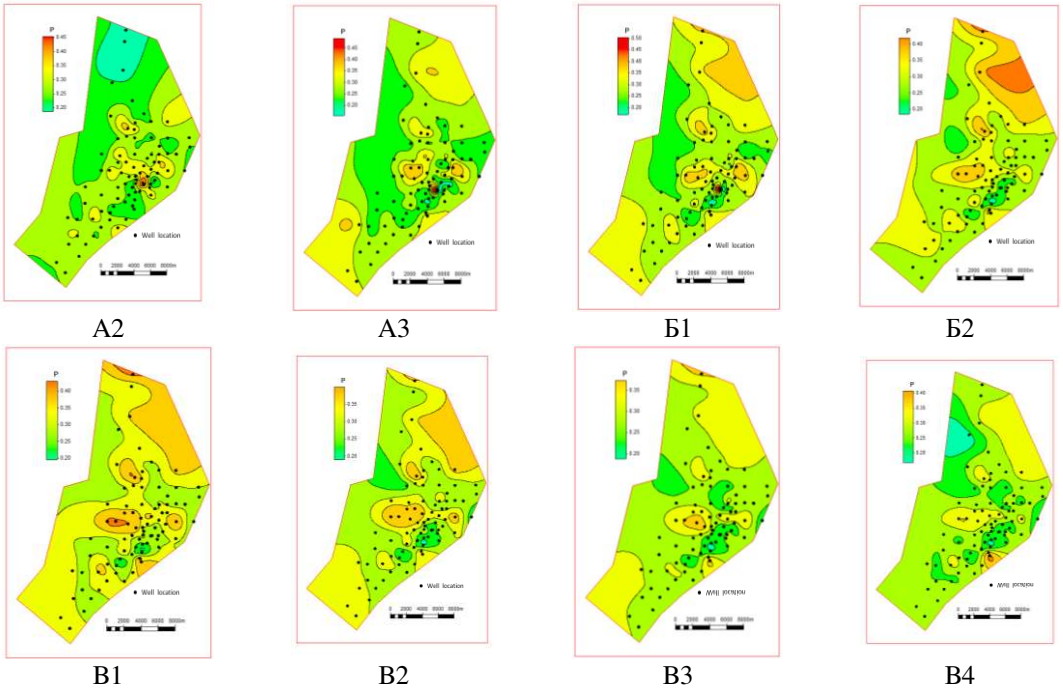


Figure 9. *P contour maps for layers*

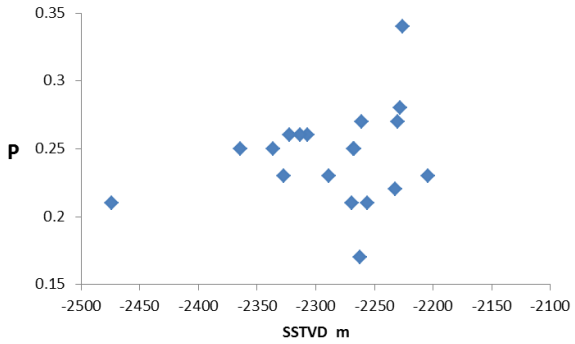


Figure 10. *Fracture probability density vs. structural location. (P: the comprehensive probability density; SSTVD: subsea true vertical depth)*

Vertically the appearance of fractures is dominated by sedimentary environment and varies with lithofacies. In lower B3-B5 with high shale content, fractures tend to occur in the intervals with high calcareous content instead of the ductile intervals with plastic deformation under tectonic stress (Lander and Laubach, 2015; Olson et al., 2009; Mauro et al., 2013). The probability density of fracture is only 0.21 on the average in this layer. In middle Б1-B2 with minute shale content, limestone and dolomitic limestone tend to break under tectonic stress to form structural fractures; thus the probability density of fracture is 0.069-1 with an average of 0.32. In upper A with shallow water and high shale content, the evaporate platform with gypsum rocks has strong plasticity. Except local fractures at the structural high, other areas are less fractured. The probability density of fracture is 0.28 on the average. Layers A and B3-B5 at the bottom have similar shale content. But due to the effect of the leaching process, layer A has more

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dissolved fractures than B3-B5 (*Table 2* and *Fig. 11*). Generally the probability density of fracture is negatively correlated with shale and gypsum contents and positively correlated with calcareous (calcite) content. The dolomite in the study area is mainly powder crystalline dolomite. A reservoir rock with high dolomite content is usually less fractured due to its high porosity and consequent small brittleness. Thus the appearance of fractures is negatively correlated with dolomite content (*Fig. 12*).

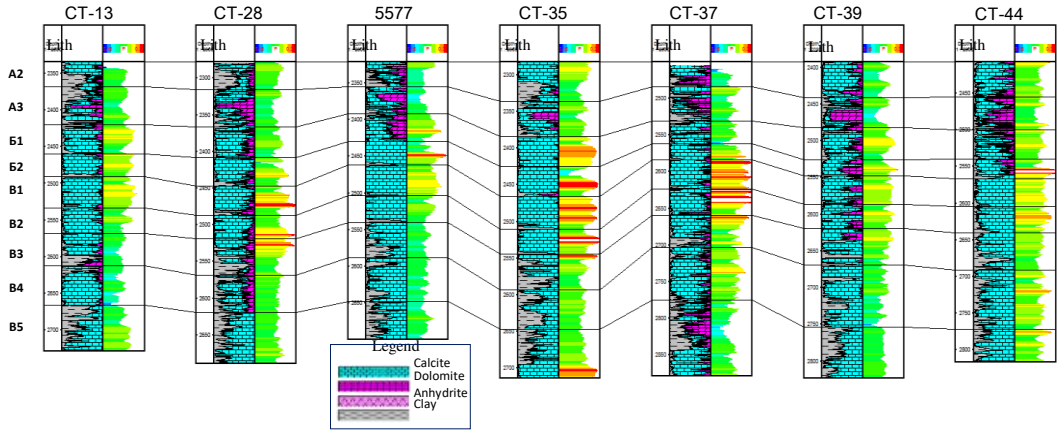


Figure 11. *Vertical fracture probability density and lithologies*

Table 2. *Lithologies and fracture signatures in layers*

Layer	Sedimentary environment	Lithology	Shale content (%)	Dolomite content (%)	Calcite content (%)	Anhydrite content (%)	P
A	Restricted platform – evaporate platform, shallow water	Dolostone, limy dolostone, gypseousdolostone, gypsum rocks, dolomitic limestone and limestone	15.7	24.0	55.4	5.0	0.28
Б1-B2	Open platform - restricted platform, shallow water	Limestone, dolomite-bearing limestone, dolomitic limestone and limy dolostone	7.0	10.6	82.3	0.0	0.32
B3-B5	Open platform, deep water	Argillaceous limestone, shale-bearing limestone and limy mudstone	19.9	10.7	69.4	0.0	0.21

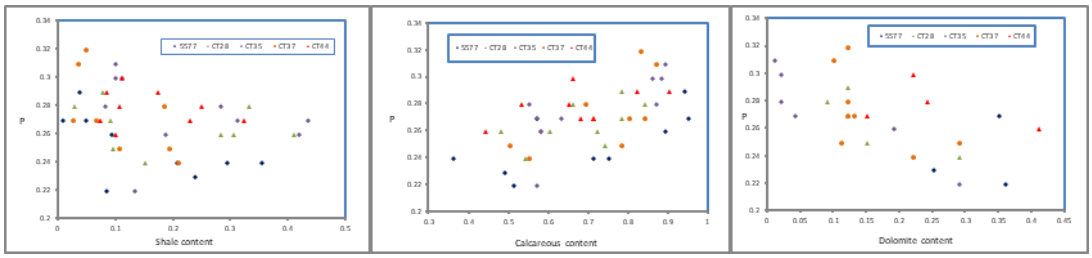


Figure 12. *Fracture probability density vs. rock composition*

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Conclusions

Due to the impacts of sedimentary environment, karstification and tectonic movements, the North Truwa oilfield at the east margin of the Pre-Caspian Basin has various low-angle, high-angle and net-shaped structural fractures, dissolved fractures and sutured fractures. By using a single method, i.e. resistivity difference-ratio or 3-porosity ratio, only 55% of fractures on the average were identified. The fracture probability density method which integrates above two methods improves the accuracy to 73.6%. The results show fracture distribution in this area is related to sedimentation, structure and diagenesis. Laterally dominated by structures and karstification, more fractures may occur at the structural high. Vertically dominated by lithofacies, more fractures occur in the brittle intervals with small shale and gypsum contents.

The purpose of carbonate reservoir fractures studing is to improve the of oil fields, so how and how much the fracture effect the production is strongly recommended to study in the future.

Acknowledgements. This study was supported by the National Major Science and Technology Project (2017ZX05030-002).

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LITTERFALL PRODUCTION OF OAK FORESTS IN NORTHWEST TUNISIA

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Abstract. Litterfall is considered a key process in determining the carbon and nutrient cycle of the forest ecosystems, and in controlling the main respiration substrates on the forest floor. Litterfall and its seasonal changes were measured monthly for one year, in two sites and three stands of oak forests in Northwest Tunisia. The results show that the total annual litterfall varied significantly according to forest types ($P<0.01$) in the range of 7.71–9.89 t ha^{-1} yr^{-1}. Litterfall of *Quercus suber*, *Quercus canariensis* and mixed stand occur mostly in autumn, winter, spring and summer seasons, respectively. An annual decay coefficient is calculated from litter accumulation data, which varies according to the site and the stand. The decomposition rate is more pronounced in the *Quercus canariensis* stand, followed by the mixed stand and a slow decomposition in *Quercus suber* stand.
Keywords: *forest ecosystem, Quercus canariensis, Quercus suber, decomposition, annual decay coefficient*

Introduction

In the biogeochemical cycle of organic matter and mineral elements, litterfall plays an important part in the relations of soil, vegetation and surrounding environment, constituting one of the essential ecological phenomena in the wooded ecosystems (Vitousek et al., 1995).

The environmental signals activate a series of events leading to the fall of the leaves. The fallen leaves on the soil degraded by the microorganisms allow the recycling of the constituents of the plant material, which are uptaken again by the tree.

The fall of the leaves is not considered as a great loss for the tree because their metabolism is slowed down due to ceased photosynthesis and their constituents are recycled and reused.

Litter dynamics play an important part in the return of nutrients to the forest soil. It is considered an important component in the accumulation of soil organic matter (SOM). It is also one of the major inputs of C to the soil and an important C source for SOM (Sayer, 2006). Therefore, litterfall is a key parameter in measuring and predicting soil C sequestration (Liski et al., 2005) and one of the most important variables in the global C cycle (Janssens et al., 2003). The litterfall is characterized by a regular rate from one year to another.

The estimation of the foliar proportion of litter is, however, directly tributary of the other components of the litter whose production is rather variable from one year to

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another because it depends on events which occur with irregular time intervals. Such factors are likely to vary the annual litterfall (Ben Yahia et al., 2016).

The fall of the leaves is considered a very important periodic event in these forests. It represents approximately 65% of the total litterfall (Vogt et al., 1986) and it contributes to the mobilization of the organic matter. The litterfall is influenced by climate variability and latitude. Berg and Meentmeyer (2001) observed that litterfall is positively correlated with annual evapotranspiration or other climatic indices including average air temperature. Litterfall decreases with the increase in latitude (Bray and Gorham, 1964).

In Tunisia, few studies have been made on the leaf fall and decomposition of organic matter in forests. In this context, the aim of our study is to analyse the seasonal variation of litterfall in *Quercus canariensis* and *Quercus suber* forest in Northwest Tunisia and to estimate the decay coefficient from the litter stock.

Materials and methods

Study area

The study was carried out in a natural oak forest site in Northwest Tunisia, the site of Khroufa (36° 55.5' N; 08° 56' E) and the site of Ain Zena (36° 46.98' N; 8° 43.79 E). These sites belong to the forest subdivision of Tabarka and Ain Draham, respectively (*Fig. 1*).

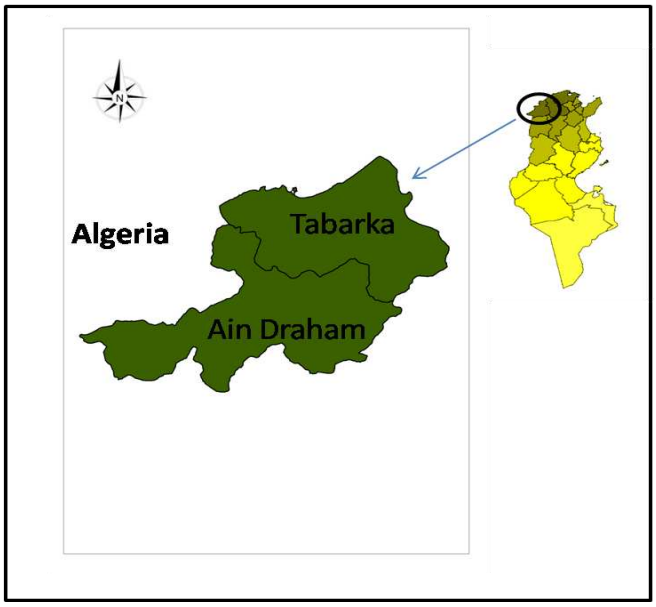


Figure 1. *Study area in Northwest Tunisia*

The climate of Khroufa forest is characterized by mild winters while the climate of Ain Zena forest is described as having temperate winter with snowy precipitations (*Fig. 2*).

Each of the two study sites consists of three stands. The dominant species in stand 1, 2 and 3 are *Quercus canariensis* (*QC*), *Quercus suber* (*QS*) and *Quercus canariensis* and *Quercus suber* (mixed), respectively (*Fig. 3*). A detailed description of the sites is indicated in *Table 1*.

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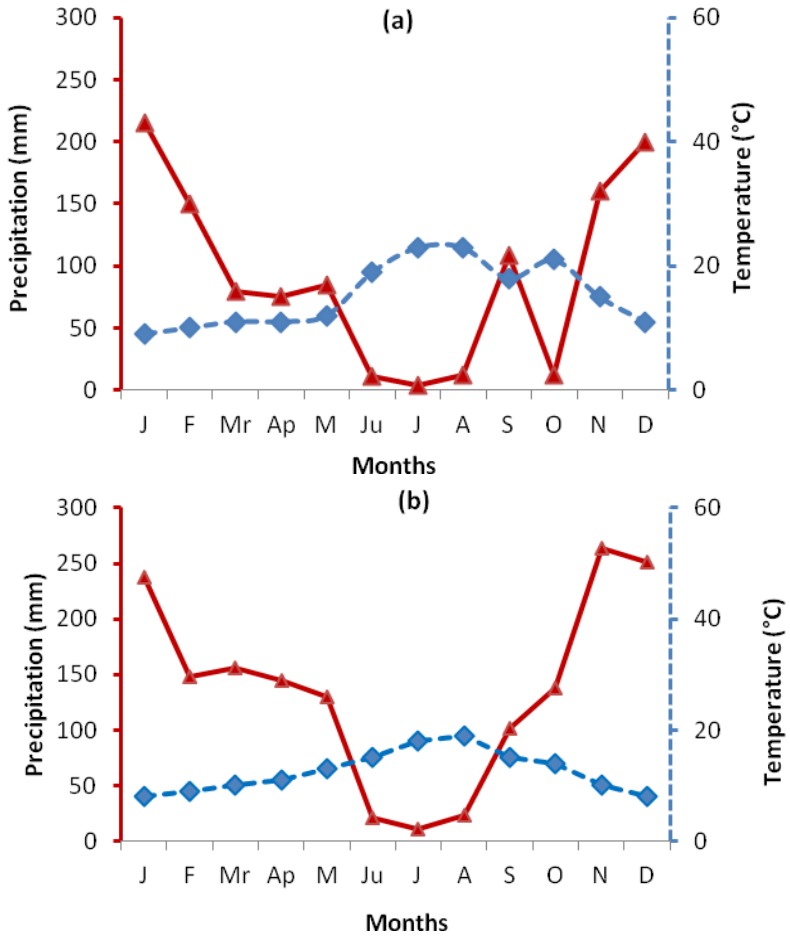


Figure 2. *Ombrothermic diagram of Bagnouls and Gaussen (P = 2 T) for the year 2011 for the two sites (a) Khroufa and (b) Ain Zena*

Table 1. *Characteristics of the study sites*

Sites	**Khroufa**			**Ain Zena**		
Stands	**1**	**2**	**3**	**1**	**2**	**3**
Coordinates	36°55'27.0''N 08°57'03.7''E	36°55'53.8''N 08°56'37.7''E	36°55'35.4''N 08°56'26.6''E	36°44'09.4''N 08°51'38.1''E	36°44'59''N 08°52'11.8''E	36°44'23.4''N 08°52'05.7''E
Altitude (m)	530 - 590	430 - 465	500 - 530	850 - 870	800 - 820	820 - 845
Soil type	Cambisols humic	Cambisols humic	Cambisols humic	Luvisols	Luvisols	Luvisols
Natural vegetation	*Q. canariensis*	*Q. suber*	*Q. canariensis* + *Q. suber*	*Q. canariensis*	*Q. suber*	*Q. canariensis* + *Q. suber*
Plant associations	*Erica arborea, Arbutus unedo*	*Erica arborea, Myrtus communis, Pistacia lentiscus, Phillyrea angustifolia, Smilax aspera*	*Erica arborea, Myrtus communis, Pistacia lentiscus*	*Erica arborea, Arbutus unedo*	*Erica arborea, Myrtus communis, Pistacia lentiscus, Phillyrea angustifolia, Smilax aspera*	*Erica arborea, Myrtus communis, Pistacia lentiscus*

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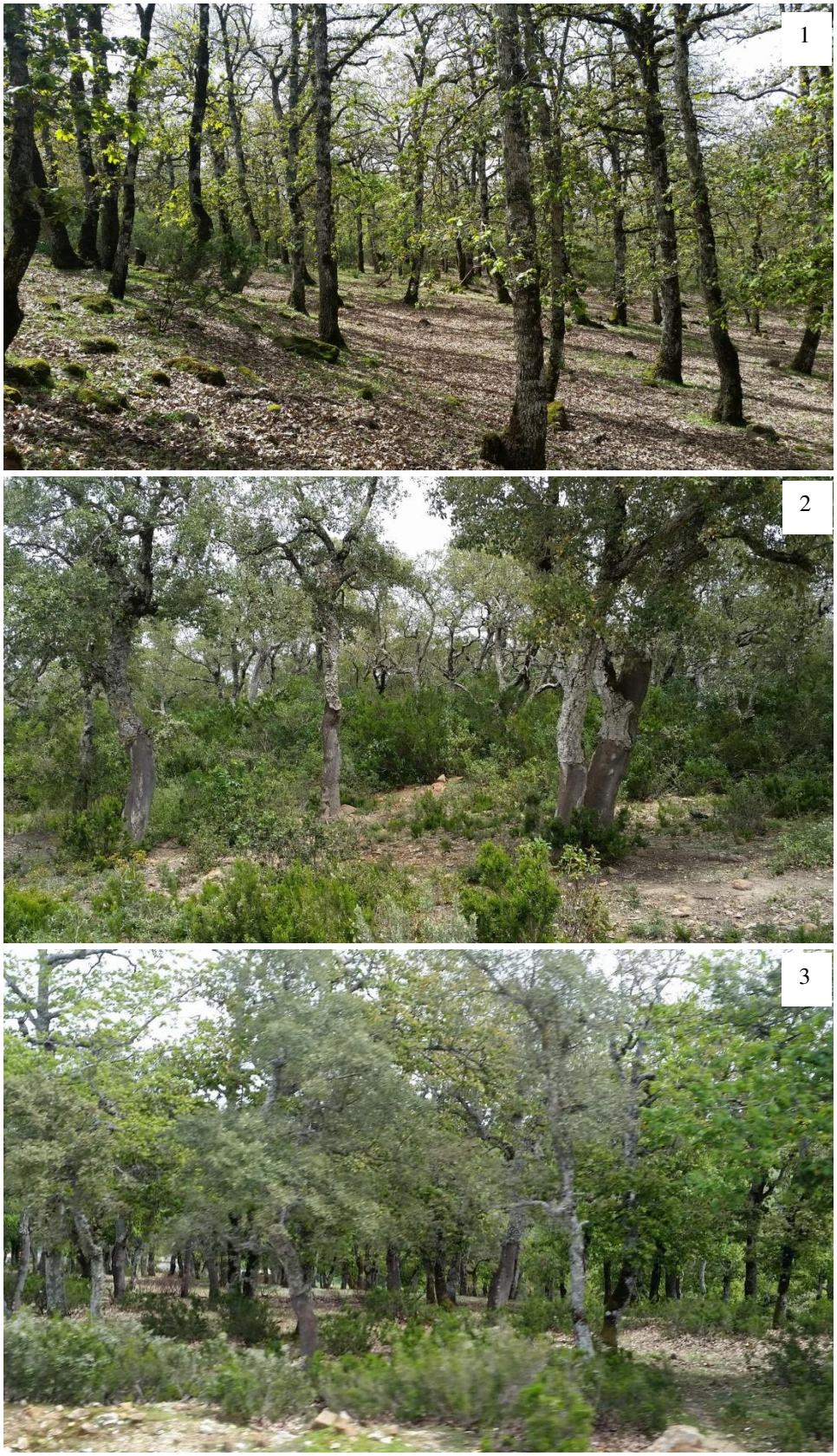


Figure 3. *Photos of the different stands studied (1, 2 and 3)*

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Quercus suber and *Quercus canariensis* are important forest species in the Mediterranean region, specifically in Tunisia. They cover 69.870 ha and 6614 ha, respectively

Litterfall collection

Litterfall was collected monthly throughout a year (from June 2011 to May 2012) for two species (*Quercus Canariensis* and *Quercus Suber*) at all sites and stands.

Five plots are established at each stand to measure the litterfall. Five litter traps in each plot (1 m^2 collecting surface) were installed and located randomly at each plot.

The samples were collected from the five traps and transported to the laboratory, where they were dried at 75 °C for 48 h, and weighed each month throughout a whole year. The samples were sorted into four components: leaves, twigs, fruits and flowers. Twigs with a diameter of more than 2 cm were discarded.

After calculating for each plot the litter quantity per litter trap and per year for each fraction, an average value per plot were used to extrapolate litterfall per hectare and per year.

Data analysis was performed using SAS software: Statistical significance of differences between means of different variables were determined by the Student Newman Keuls (SNK) test using a significance threshold of 5%.

Results

The litterfall of the two species in the two study sites was estimated. The results show that the dynamics of the litterfall differ significantly with time, sites and stand (*Table 2*). In the pure *Quercus canariensis* stand, we noted that the litterfall is highly important during the months of January, February and March.

We noticed that the fall of the leaves and twigs in the pure *Quercus canariensis* stands in the Khroufa and Ain zena sites are important in winter season (*Fig. 4*).

Table 2. *Variance analysis of the different fractions of the litterfall. Correlation is significant at the 0.05, 0.01 and 0.001 level. (*P < 0.05, **P < 0.01, ***P < 0.001)*

Variables	Leaves	Twigs	Flowers	Fruits	Total litterfall
Sites	0.43	22.09***	0.71	54.97***	2.31
Stands	17.20***	3.41*	33.94***	16.51***	8.86**
Times	27.67***	8.42***	10.94***	9.03***	22.87***

We observed in the two sites that in the pure *Quercus suber* stand, the highest number of falling leaves was observed during May and June.

At Khroufa's site, the highest number of falling twigs is in June, November and January, whereas it is higher in the summer at Ain Zena's site. The maximum number of leaves dropped in the mixed *Quercus canariensis* and *Quercus suber* stands, is in May, January and March in Khroufa, while in Ain zena stands, the highest number of leaves fallen occur from May to July.

The fall of the twigs is intense during winter and summer season in Khroufa and Ain Zena, respectively (*Fig. 4*).

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Leaf litter contributes to total litterfall, substantially. The percents of leaf litter vary slightly among forest types in a range of 77.3–85%, followed by twigs.

Regarding fruits and flowers (mentioned as other in *Fig. 4*), there is a significant difference among stands and times ($P < 0.001$), it is observed that the rates are very low in the two sites and stands. The fall of flowers generally occurs during summer months.

For fruits, the values found are low, especially, in Ain Zena stand; this is probably due to the cattle action in this station.

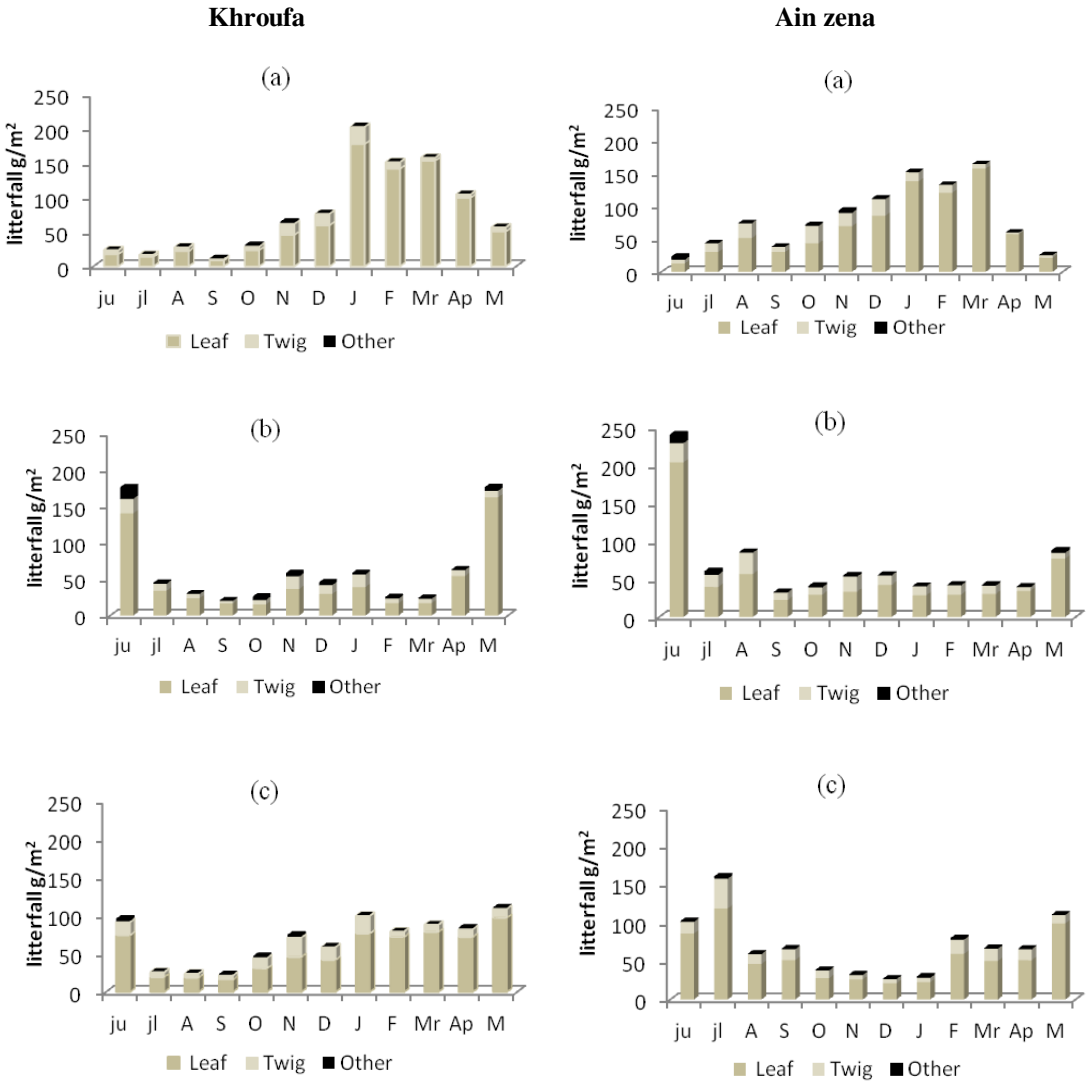


Figure 4. *Monthly variation of litterfall in (a) stand 1, (b) stand 2 and (c) stand 3 in Khroufa and Ain Zena sites in northwestern Tunisia*

The total litterfall is the most pronounced in the pure *Quercus canariensis* stand, it is on the order of 9.33 t ha^{-1} yr^{-1} and 9.89 t ha^{-1} yr^{-1} respectively at Khroufa and Ain Zena sites, moderate in the mixed stand (8.18 and 8.45 t ha^{-1} yr^{-1}) and low in *Quercus suber* stand (7.71 and 8.26 t ha^{-1} yr^{-1}) (*Table 3*).

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Litter decomposition rate

Mass balance techniques are used to estimate litter decomposition for the whole ecosystem. The mass balance for the aboveground litter decomposition, suggests that annual litter decomposition should equal the annual input of fresh litter as long as the mass of stock litter stored in the ecosystem remains constant (Olson, 1963; Schlesinger, 1997).

The coefficient $k = \frac{A}{L}$ (for which A = annual litterfall and L = litter stock) can be used to estimate the percentage of litter decomposed by year. Thus, it is possible to obtain the necessary time for the litter to disappear to 100% (in the absence of input).

The results obtained (*Table 3*) highlight that the decomposition rate is higher in Khroufa site than Ain Zena. Similarly, the decomposition rate is more pronounced in *Quercus canariensis* stand; this rate reached 99% yr^{-1} at Khroufa and 81% yr^{-1} at Ain Zena sites, followed by the mixed stand (90.1% yr^{-1} and 75% yr^{-1}) and, finally, a slow decomposition in *Quercus suber* stand 75% yr^{-1} and 67% yr^{-1}).

Table 3. *Annual litterfall, litter decomposition rate (k) and time of 100% litter decomposition for the different sites and stands*

Sites	Stands	Annual litterfall (t ha^{-1} yr^{-1})	k (% yr^{-1})	Time of 100% litter decomposition (days)
Khroufa	QC	9.33	99	369
	Mixed	6.45	90.1	405
	QS	6.06	75	487
Ain Zena	QC	9.89	81	451
	Mixed	8.45	75	487
	QS	8.26	67	545

Discussion

The litter input of the present study (7.71 to 9.89 t ha^{-1} yr^{-1}) in the forest stands is higher than the values of 5 t ha^{-1} yr^{-1} recorded by Selmi (1985); and other studies carried out on the oak forests of the Mediterranean Basin.

The litterfall of the *Quercus suber* is about 5.6 t ha^{-1} yr^{-1} in Portugal and Spain (Sa et al., 2001; Caritat et al., 2006). In May and June, the peak of the leaf fall changes depending on the year (Bellot et al., 1992; Caritat et al., 1996; Martin et al., 1996). The fall of leaves at the end of spring may be due to an adaptation to the hydric deficit that can occur during the dry season (July and August), in the Mediterranean areas (Escudero et al., 1987).

However, the estimation of the proportion of leaf litter is directly dependent on other components of litter (Ben Yahia et al., 2016). The input is variable from one year to another because it depends on events that occur at irregular intervals. Therefore, some factors are likely to vary the annual litter production. The high values found in our study are probably due to higher temperatures and lower precipitation.

Dendroclimatological studies in Tunisia and Morocco (Aloui, 1982; Raouane, 1985) have shown that *Quercus canariensis* has a greater sensitivity to precipitation than to

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temperatures. But on the other hand, it is indifferent to the physico-chemical nature of the soil.

Our study shows that the fall of the twigs takes place in winter for *Quercus canariensis* stand and at the beginning of summer and winter for the two others stands. This may vary from one study to another since the twig fall depends significantly on wind and storms. According to Caritat et al. (2006), the fall period takes place mostly at the beginning of springtime and at the end of autumn, and it varies a lot depending on the year as observed for the same species in Sicily (Leonardi et al., 1992).

According to Pérez-Suarez (2009), the strong seasonality in leaf-litterfall recorded in other ecosystems with a variety of deciduous and evergreen species (West, 1985; Berg and Meentemeyer, 2001; Liu et al., 2001) has been attributed to interactive effects of weather patterns (e.g. drought), species phenology (Binkley et al., 1992; Liu et al., 2001; Berg and Meentemeyer, 2001) and the temporal distribution of precipitation events (Liu et al., 2004).

For the decomposition rate, the values observed for *Quercus canariensis* stand at Khroufa indicate that the decomposition of litter is relatively fast (369 days), which can be explained by the climatic conditions favorable to the decomposers and the biological activity in this stand. On the other hand, it has been found that there is a significant difference with the coefficients found following the decomposition experiment using the litterbag method.

Van Wesemael (1992) studied decomposition in the Mediterranean forests of Italy (Tuscany) by the litterbag method, and found a rate of decomposition respectively equal to 50% yr^{-1} and 47% yr^{-1} in *Quercus cerris* and *Quercus suber* after one year of decomposition.

This difference highlights the critical importance of mesofauna, prevented from acting by using litterbag method (Bernhard, 1970).

The decomposition rate of *Quercus canariensis* and *Quercus suber* reached 1.69 yr^{-1} and 1.1 yr^{-1} in Southern Spain (Aponte et al., 2012). Moreover, the decomposition of *Quercus serrata* is rapid over time. For instance, according to Pandey et al. (2007) the decomposition rate is 2.37 yr^{-1} and the half life time is 107 days.

These high values are due to the climate characterized by an alternation of dry and wet periods which play an essential part in the regulation of decomposition rates (Tripathi and Singh, 1992) by changing microbial communities in the process of organic matter decomposition (Arunachalam et al., 1997).

Conclusion

The examination of the seasonal variation of litterfall at different oak forests in Tunisia shows that there are no differences between the two study sites of Khroufa and Ain Zena; the only difference is between stands. This could be related to the differences phenological alterations of the species studied. The results suggested that total annual litterfall vary significantly according to forests, with a range of 6.06–9.89 t ha^{-1} yr^{-1}, and that leaf litter is the main component.

In *Quercus suber* the observed links between leaf fall and climatic variables are as they were be expected in typical Mediterranean forests, in which low rainfall and high temperatures favor leaf fall in spring. As for deciduous species, the opposite is true.

Our results will help develop litterfall models in response to climate change in the future studies.

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In addition, the litterfall is an important phase in the biogeochemical cycle of nutrients that ensure the productivity of ecosystems. The knowledge of the quantities of biomass returning to the soil and the released minerals allow, with other criteria such as decomposition rate, to give an idea about their use by plants and to determine the excesses or deficiencies in a given ecosystem.

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STUDY ON THE EFFECTS OF IRRIGATION INTERVALS AND DROUGHT STRESS ON YIELD AND YIELD COMPONENTS OF FOUR MAIZE CULTIVARS IN IRAN

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Abstract. This study was conducted in order to examine the effects of irrigation intervals and drought stress on crop yield and its components for four maize cultivars. The split-plot in the (RCBD) design was employed to conduct this study with three replications over a period of two years. After the establishment of all plants the irrigation was controlled in the following intervals at seven treatments: 7 days, 10 days, 12 days, 14 days until the end of the growing period and from the 3-leaf to 7-leaf stages withholding the irrigation. After that, irrigation was performed every 10 days, 12 days and 14 days respectively as the main factor. The cultivars included KSC 704, 703, 705, and KSC706 as a sub-factor. As expected, there were significant differences between the cultivars: KSC705 and KSC704 showed the highest mean yield (199.9 g/plant) and the lowest mean yield (184.4 g/plant), respectively. Under conditions of severe stress, the best cultivar was KSC705 with a yield of 189.9 g/plant. In addition, under normal conditions, KSC705 turned out to be the best cultivar with a yield of 594.3 g/plant with a 10-day irrigation interval. Therefore, this cultivar has been introduced as the most stable cultivar under the conditions of drought stress.
Keywords: *corn, cultivars, drought stress, irrigation interval, yield*

Introduction

Maize (*Zea maize L.*) is one of the most important cereal crops, grown principally during the summer season, in Iran and other Middle East countries. Corn is the staple food for many human populations in the world. It is a tropical plant, but, at present, it is intensively cultivated worldwide in subtropical and temperate regions. It can be successfully cultivated twice in a year. In developing countries, maize is a major source of income for many farmers, and is widely used in the food industry (Tagne et al., 2008).

Drought is one of the main environmental constraints that affects crop growth and production (Ashraf and Harris, 2004; Farooq et al., 2009). It has been estimated that up to 45% of the world's agricultural lands are subjected to drought (Bot et al., 2000). Moisture stress in crops depends on the different developmental stages and it could significantly affect the yield and other physiological traits (Chaves et al., 2009). An adequate supply of water for irrigation is one of the main factors that directly affects the growth and productivity of corn plants. Frequent water deficit is the primary limiting factor for crop production under arid and semi-arid conditions (Hussain et al., 2004). It

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affects nearly all the growth processes of the plant. The stress response depends upon the intensity, rate and duration of exposure, and the stage of crop growth (Wajid et al., 2004). Sun et al. (2006), stated that the critical plants were more sensitive to water stress, which is reflected in the significant reduction in most of the growth parameters. Ibrahim et al. (2007) reported that the highest values of plant height, ear characteristics (length, diameter, and weight) as well as the ear and kernel yields of the corn plants per feddan (f.e: 1 feddan = 0.42 ha) in both seasons were obtained under the irrigation interval of 10 days, followed by 14, and 18 days. Aydinsakir et al. (2013) showed that different irrigation levels, when applied (full irrigation with no water stress and slight, mild, severe water stress, and no irrigation), have statistically significant effects on the yield components, such as the anthesis-silking interval, plant height, ear diameter, ear length, kernel number, and 100 kernels weight, except for the ear number. In addition, it was found that the cultivars had significantly different responses to water deficit. In another research, Jafari et al. (2012), showed that BC504 and BC652 produced the highest yields, and BC678 and NS504 produced the lowest yields under optimal, and stress conditions, respectively.

The aim of this study was to evaluate the effects of irrigation intervals at the critical stages of phonological, morphological, and physiological growth on yield and yield components of the four maize cultivars.

Materials and methods

Research studies were performed in 2015 and 2016 in a field experiment. The split-plot in the Randomized Complete Block Design (RCBD) was employed to conduct this study with three replications during a period of 2 years in the Islamic Azad University of Arak in the Markazi Province in Iran. Arak is the capital of Markazi province in Iran located with the latitude of 34.09 and the longitude of 49.69 at a mean elevation of 1748 m above sea level. Based on coupon divisions, this area is considered to be a desert temperate zone. The annual temperature difference is high. The average temperature and precipitation in this region are 18 °C and 255 mm respectively; July with an average temperature of 37 °C the warmest month and February with an average of -5 °C the coldest month. Weather data were collected from the weather station was located 1 km from the trial location. Average weather conditions at Arak area during the study are shown in *Table 1*. The soil at the trial site with its main characteristics presented in *Table 2*.

The irrigation intervals were as follows: I1: 7 days (control); I2: 10 days; I3: 12 days; I4: 14 days; and from the 3-leaf to 7-leaf stages the irrigation was withheld. After that, irrigation treated as the main factor was performed every I5: 10 days; I6: 12 days; I7: 14 days which was treated as the main factor. The cultivars included KSC 704(B73×MO17), KSC 703 (K47/3×MO17), KSC 705 (K3640/3×MO17), and KSC 706 (K3547/4×MO17) as sub-factors.

The land preparation was carried out by plowing, discing, land leveling, and furrowing before planting the seeds. The seeds were planted manually, and each plot contained 5 rows with a length of 4 m, and a distance of 0.5 m was maintained between each row. All plots were uniformly irrigated every 7 days for a month until the establishment of the seedlings (3-leaf stage). After matured physiological, selecting ten plants randomly from each subplot, some of the properties were studied, such as plant height, ear length, length of the non-inseminated ear, ear diameter, cob diameter, cob

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weight, number of ears per plant, number of kernels per plant, kernel yield, number of kernel rows per ear, number of kernels per row, 100 kernels weight, biological yield and harvest index.

Table 1. *Values of daily average air temperature (Ta, °C), rainfall (R, mm), relative humidity (RH, %)*

Year	Month	Ta	R	RH %
2015	June	26.72	0	15.57
	July	27.87	60	17.61
	August	26.65	0	17.9
	September	21.62	42	30.93
	October	13.29	42	40.08
	Average/Total	23.23	144	24.42
2016	June	26.41	0	22.66
	July	28.43	19.35	21.16
	August	25.94	0	17.12
	September	22.73	0	22.16
	October	16.04	37	29.56
	Average/Total	23.91	56.35	22.53

Table 2. *Some physicochemical properties of the soil at 0 to 30 cm*

Ec (dS/m)	2.9	**K(ava.) (ppm)**	435.2
pH	7.6	**Fe (ppm)**	3.4
OC%	2.34	**Zn (ppm)**	0.6
TNV%	11.75	**Clay %**	22.2
N%	0.24	**Silt %**	29.6
P(ava.) (ppm)	10.3	**Sand%**	48.2

The kernel yield was reported at moisture content of 14% and calculated in plant/g. To determine kernel yield, biological yield and harvest index, produced within two central rows in the field. Biological yield was recorded by weighing all the plants harvested from each plot and then converted into (plant/g). Then kernel yield and biological yield recorded on a dry weight basis. The harvest index was determined as follows:

$$HI = \left(\text{Economical yield} / \text{Biological yield}\right) \times 100$$

Data regarding 100 kernels weight was recorded by counting actual number of 100 kernels at random and then were weighed with electronic balance.

The analysis of the variance (ANOVA) and the comparison of the mean values were performed by the SAS statistical software, and the Duncan test both at 5% level.

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Results

The results of the ANOVA indicated that the simple effects of the treatments (irrigation interval, and cultivars) were statistically significant for all the studied traits at $p < 0.01$. In addition, all traits were subjected to treatments ($p < 0.01$) (*Table 3*). The data showed that withholding irrigation between the 3-leaf and 7-leaf stages resulted in height loss of the plant. According to the comparison of the mean values, the I1 and I7 treatments showed the highest (168.6 cm), and the lowest (93.43 cm) mean values of plant height, respectively. The height of the plants subjected to both irrigation intervals and withholding of water was dramatically lower than that of the plants under controlled irrigation. In addition, the irrigation intervals I5 and I6 showed 33% and 44% reduction in plant height, respectively, in comparison with those under controlled irrigation. The cultivars showed no significant effects on plant height, in relation to the treatments interaction, for controlled irrigation; KSC704 showed the highest plant height, however for the irrigation interval of 14 days and withholding irrigation, the highest mean height was obtained for KSC705 (*Fig. 1*).

Table 3. *Analysis of variance for studied traits*

S. o V.	Df	Plant height	Ear length	Non-inseminated ear length	Ear diameter	Cob diameter	Cob weight	Ear number per plant	Number of kernels per plant
Year	1	49050.01**	374.021**	3.262**	36.774**	23.118**	81.343	76.006**	34312.29
R(Y)	4	450.117	18.639	0.337	0.773	1.267	288.86**	0.685	168780.6
FactorA	6	15043.49**	11.489**	1.692**	2.15**	3.036**	149.88**	3.409**	428568.2*
YA	6	2030.964**	23.914	2.866**	1.312	1.039	184.051**	7.742**	1651658**
Error	24	470.72	10.984	0.38	0.628	0.705	41.267	0.837	173317.8
FactorB	3	96.743	15.575**	0.707*	0.438*	0.87**	21.61*	1.149	218175*
YB	3	317.763	8.708	0.569*	0.241	0.508*	61.335**	0.244	112731.4
AB	18	478.494**	11.91**	0.906**	0.396**	0.218	20.8**	1.033*	336976.7**
YAB	18	443.44**	11.676**	1.085**	0.253*	0.225*	14.057*	0.869	279362.9**
Error	84	176.431	3.968	0.207	0.129	0.133	8.363	0.569	78965.71
Cv%		11.45	12.53	30	8.89	12.66	24.1	23.7	29.3

S. o V.	Df	Kernel yield	Row number per ear	Kernel numberper row	100 kernels weight	Kernel number per ear	Biological yield	Harvest Index
Year	1	89980.82	193.887**	3347.054**	76.937	1108393**	1156633**	195837.3**
R(Y)	4	70990.24	2.383	76.441	146.852	9590.113	1958.957	34.224
FactorA	6	94868.23*	14.208**	152.993**	202.706*	65516.72**	7213.282**	97.215
YA	6	121417.9*	11.987**	147.108*	230.798*	50695.74*	19024.58**	67.4
Error	24	37775.28	2.396	51.851	79.159	14188.06	1756.094	72.816
FactorB	3	22430.87	2.094	41.086	55.826	5720.941	1684.432	161.348
YB	3	33326.71	2.935*	37.814	11.46	7168.151	10987.62**	106.921
AB	18	145399.87**	5.015**	34.675*	117.624*	13465.2**	13410.96**	72.761
YAB	18	81535.13*	1.928**	41.853**	178.599*	10299.35**	10812.17**	75.356
Error	84	29567.02	0.867	16.282	65.441	3924.427	907.529	68.523
Cv%		24.1	7.1	16.8	25.5	19.4	15.6	22.7

*, ** significant at the 0.05 and 0.01 probability levels, respectively

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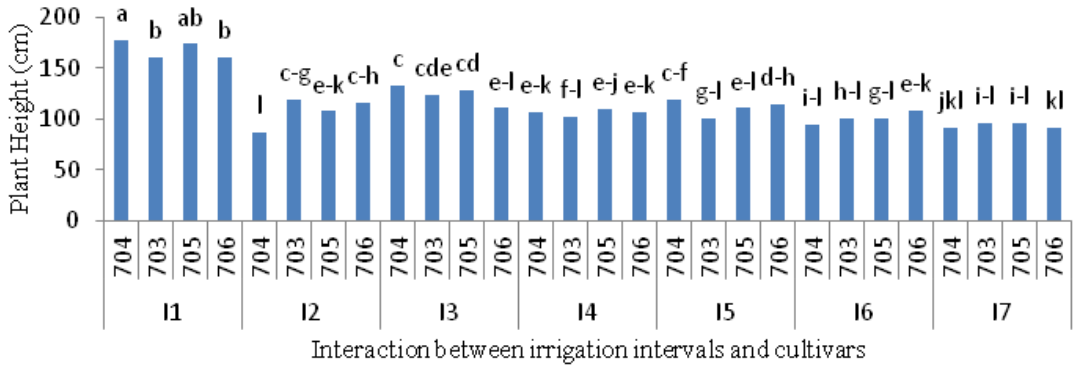


Figure 1. *Comparison of mean values of interaction treatments on plant height. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

A reduction of 5.8% in the ear length was observed in the case of I7 in comparison with I1 (controlled irrigation) (*Fig. 2*). The effects of interaction between irrigation and cultivars showed that the highest mean height (18.33 cm) and lowest mean height (12.86 cm) were obtained by I4V4, and I3V4, respectively. The non-inseminated ear length measured under controlled conditions was significantly lower than what was observed for the other irrigation intervals. The I6 treatment led to an increase of 9.6% in the non-inseminated ear length. In addition, KSC704 showed the lowest mean height (1.31 cm) among the cultivars. KSC706 demonstrated the lowest non-inseminated ear length for the 14 days irrigation interval along with withholding the irrigation effects between the 3-leaf and 7-leaf stages (*Fig. 3*).

Drought stress led to the reduction in the ear and cob diameter as compared to controlled irrigation. As drought stress increased, the ear and cob diameter significantly decreased. Among the cultivars, KSC705 showed the highest mean ear diameter (4.15 cm), and KSC706 showed the highest mean cob diameter (3.07 cm); however, the other cultivars showed no significant differences (*Figs. 4* and *5*). In the case of the I3 irrigation treatment, KSC703 showed the highest mean ear diameter, whereas KSC705 demonstrated the highest mean cob diameter. At the highest level of drought stress (I7), the highest ear diameter (4.39 cm), and cob diameter (3.07 cm) were obtained in KSC706.

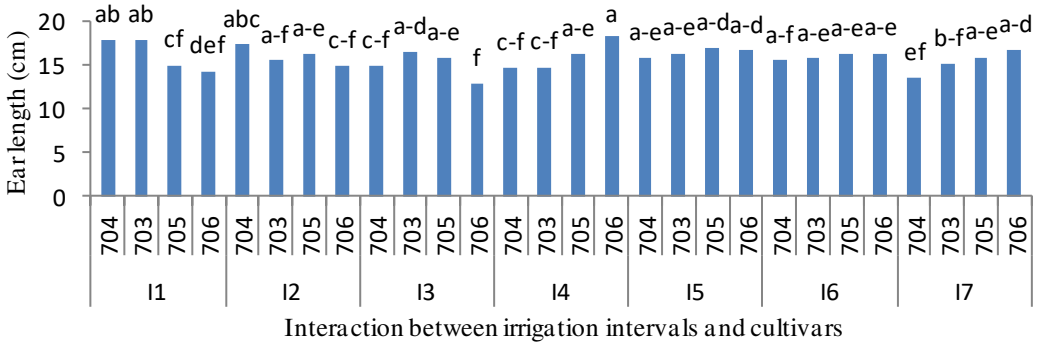


Figure 2. *Comparison of mean values of interaction treatments on ear length. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

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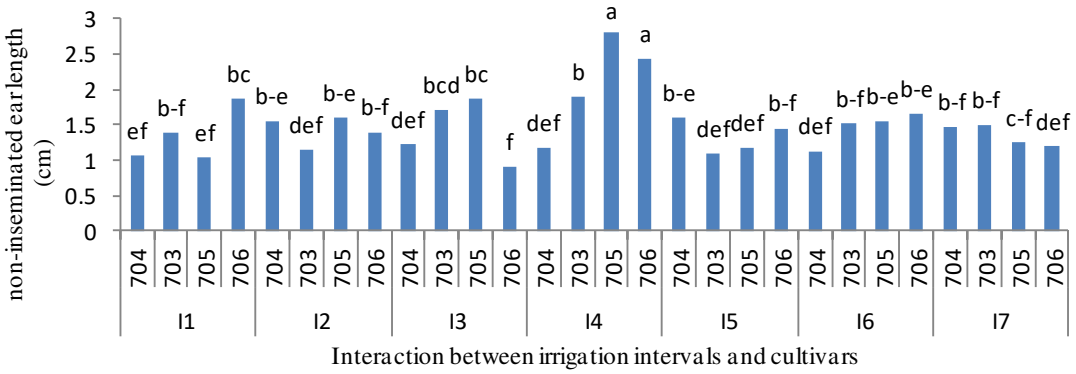


Figure 3. *Comparison of mean values of interaction treatments on non-inseminated ear length. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

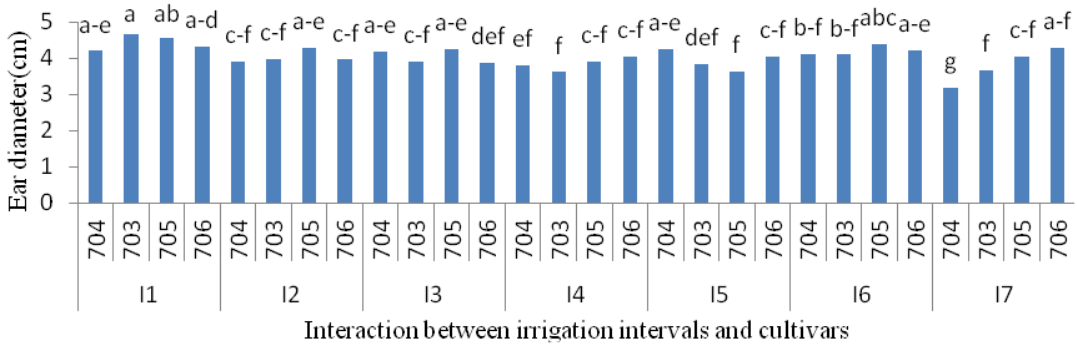


Figure 4. *Comparison of mean values of interaction treatments on ear diameter. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

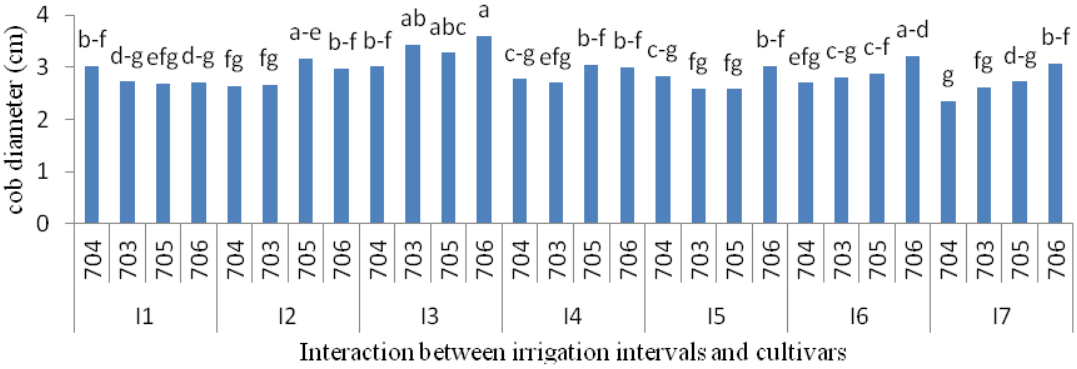


Figure 5. *Comparison of mean values of interaction treatments on cob diameter. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

The number of kernels decreased steadily from 1:176 per plant at I1 to 825 per plant in the I7 treatment. Among the cultivars, KSC705 yielded the highest mean value for the number of kernels per plant (1047.17). KSC704 yielded the highest number of

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kernels per plant as compared to the other cultivars for the 14 days interval while withholding irrigation (*Figs. 6, 7, 8, 9, 10,* and *11*).

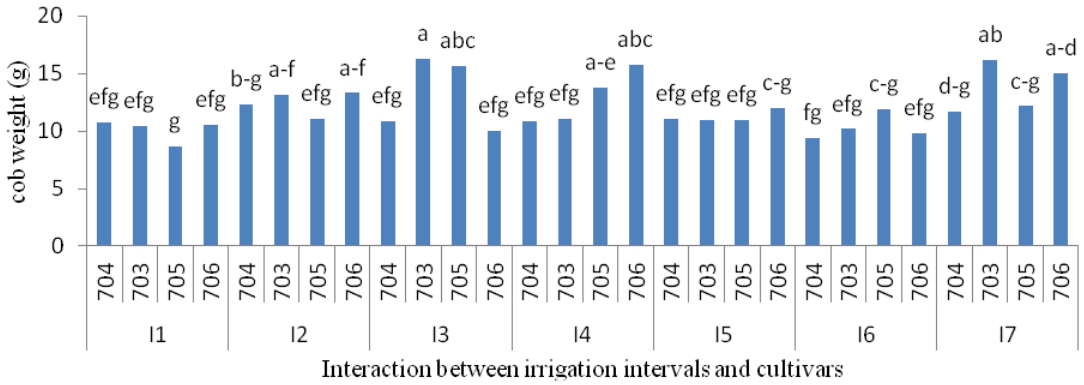


Figure 6. *Comparison of mean values of interaction treatments on cob weight. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

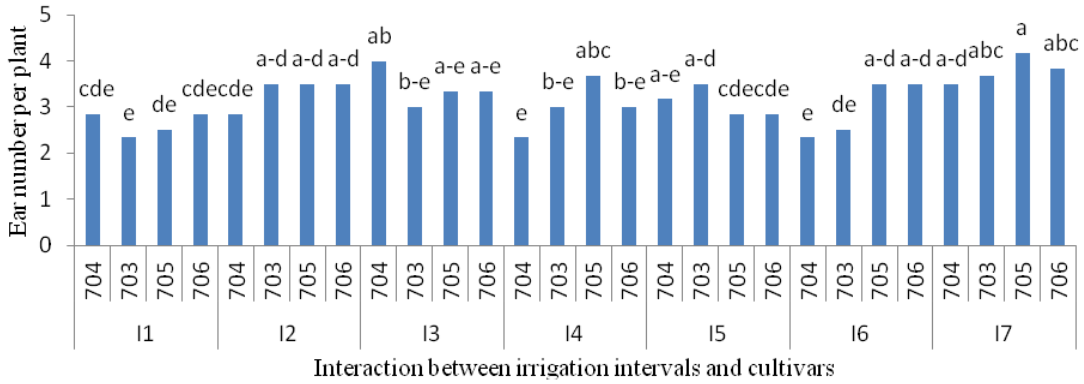


Figure 7. *Comparison of mean values of interaction treatments on Ear number per plant. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

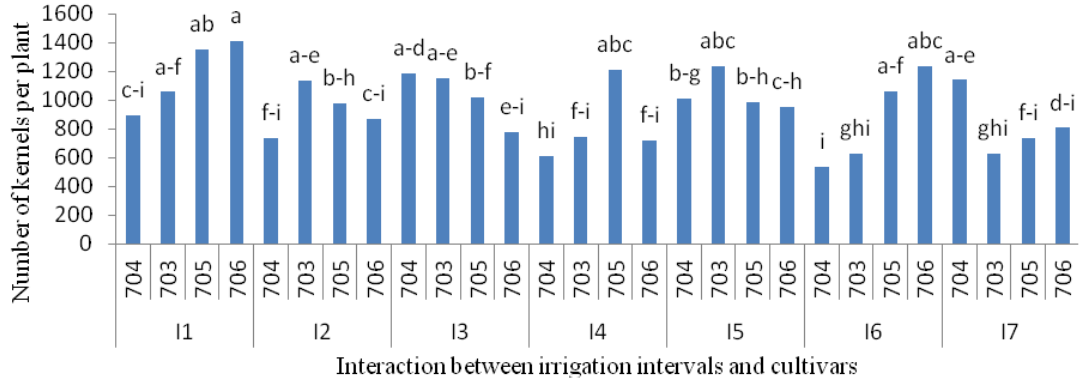


Figure 8. *Comparison of mean values of interaction treatments on Number of kernels per plant Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

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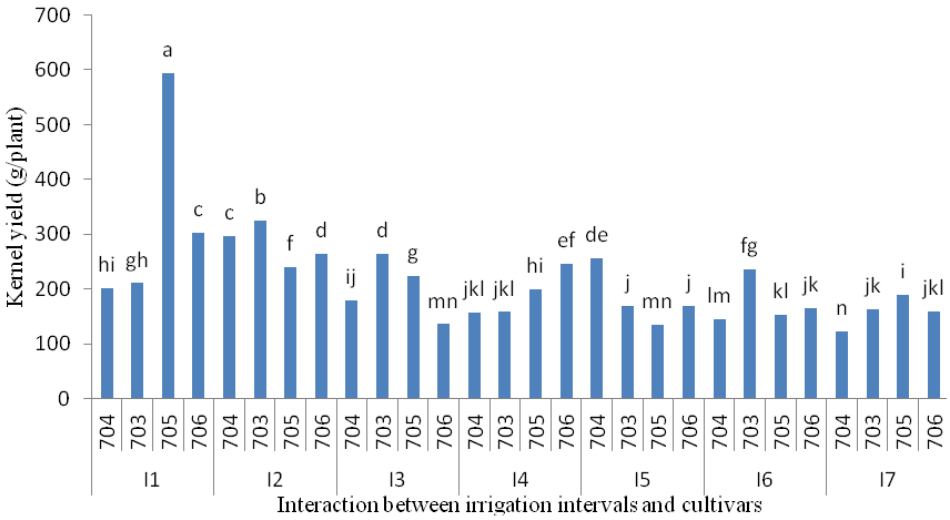


Figure 9. *Comparison of mean values of interaction treatments on kernel yield. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

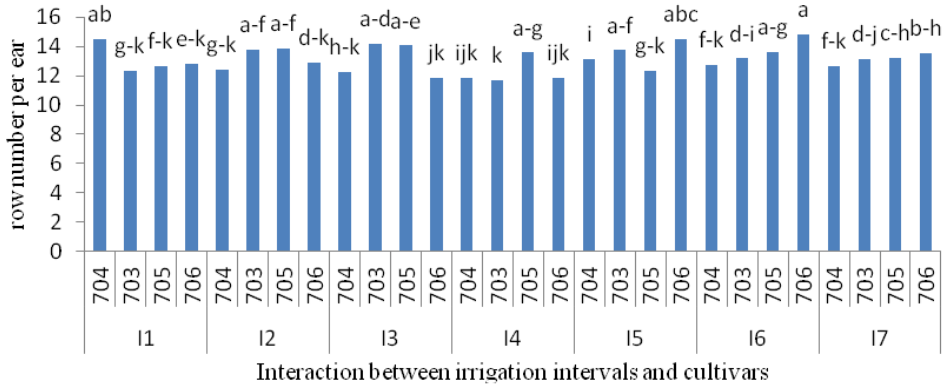


Figure 10. *Comparison of mean values of interaction treatments on row number per ear. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

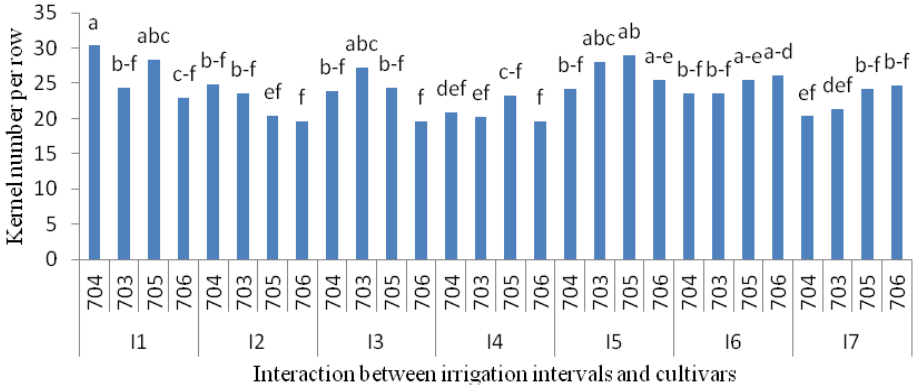


Figure 11. *Comparison of mean values of interaction treatments on kernel number per row. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

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The weight of 100 kernels of the severely stressed plants was the least compared to mildly stressed and well-watered plants. The irrigation interval of 14 days along with withholding the irrigation led to a significant reduction of 25% in the kernel weight compared to the kernel weight from plants under the controlled irrigation. The highest (24.27 g), and lowest (21.81 g) mean kernel weights were obtained for KSC703 and KSC705; however, KSC706: and KSC705 showed no significant differences (*Figs. 12* and *13*).

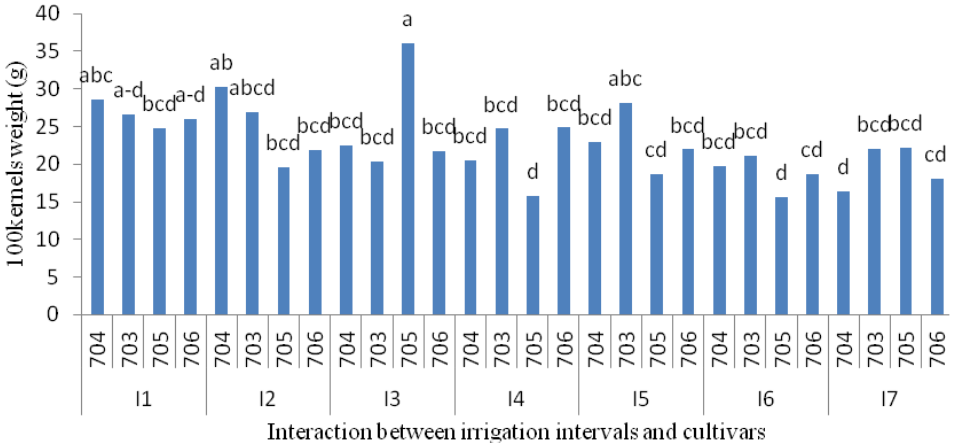


Figure 12. *Comparison of mean values of interaction treatments on 100 kernels weight (g). Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

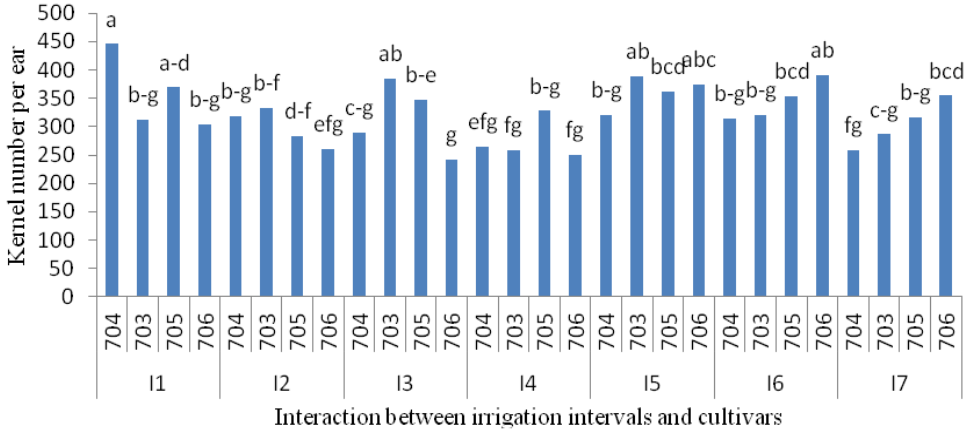


Figure 13. *Comparison of mean values of interaction treatments on kernel number per ear. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

All the treatments showed a reduction in yield and yield components in comparison with controlled irrigation but the 10-day, 12-day irrigation intervals showed a similar effect on the yield. Among the irrigation intervals, the highest mean yield (327.47 g/plant) was obtained under controlled irrigation (I1), whereas the lowest mean yield (158.7 g/plant) was obtained in the I7 interval (*Fig. 9*). As expected, there were

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some significant differences among the cultivars; KSC705 showed the highest mean yield (248.8 g/plant), whereas KSC704 showed the lowest mean yield (194.6 g/plant). Regarding the different irrigation intervals, the cultivars had different responses, such that, under the I1 and I7 treatments, the highest yields obtained from KSC705 were 594.3 g/plant, and 189.5 g/plant, respectively.

The biological yield represents the total dry matter at the time of harvest (*Figs. 14* and *15*).

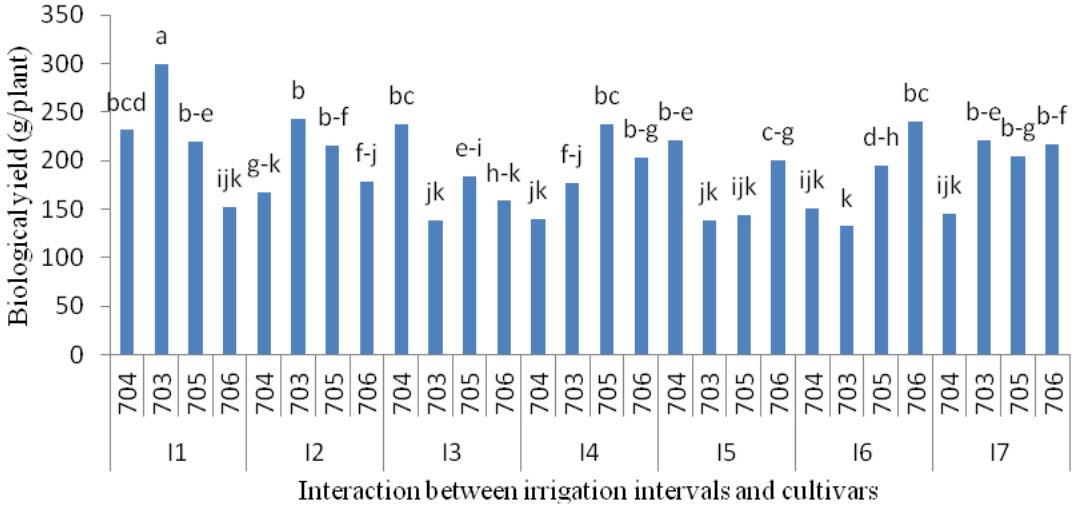


Figure 14. *Comparison of mean values of interaction treatments on biological yield. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

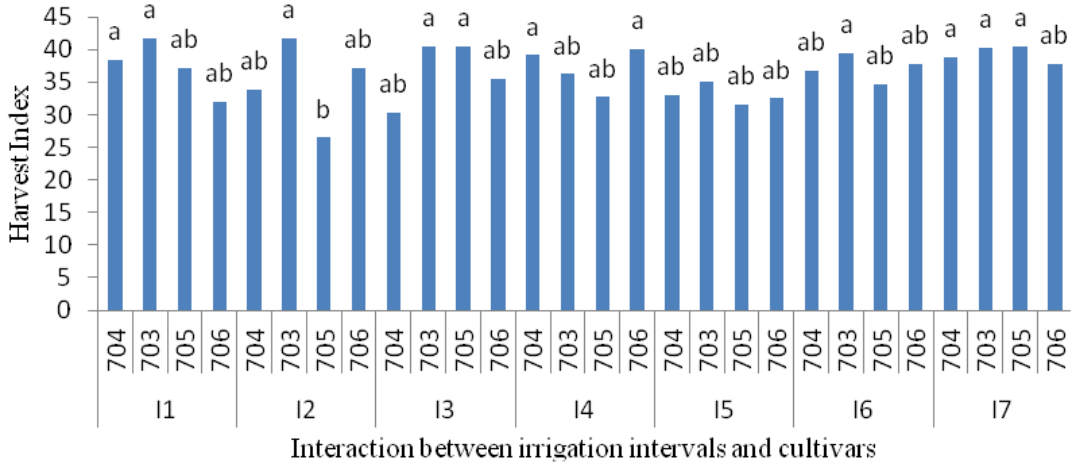


Figure 15. *Comparison of mean values of interaction treatments on harvest index. Mean values in each column marked by similar letters are not significantly different at the 5% probability level (Duncan 5%)*

The irrigation intervals I2, I3, I4, I5, I6, and I7 showed 10%, 20%, 16%, 21%, 20%, and 12% reduction in yield, respectively, as compared to the yield in controlled irrigation. A longer stay in the green period under optimum irrigation led to an increase in the

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biological yield. It seems that for hybrids with a shorter growth, the biological yield is less, as KSC705 and KSC704 had the highest (199.9 g/plant) and lowest (184.4 g/plant) mean yields, respectively.

Discussion

Under drought stress, the rate of water intake by the plant is not high enough to maintain the turgidity of the leaves, and resultantly, the dry yield per unit of water consumed decreases (Kramer, 1949). The reduction in the growth and yield parameters under the irrigation interval of 14 days may also occur due to the imbalance of soil–water–air relation under these conditions, which leads to a reduction in the photosynthetic activity as well as inducing adverse relations between the hormones and biological processes in all organs of the plant (Ibrahim et al., 2005). Badr et al. (2003) and Thalooth et al. (2006) stated that plants grown under water-stress conditions show significant reduction in all the photosynthetic pigment content compared to unstressed plants. In accordance with our results, Ibrahim et al. (2007) mentioned that corn plants showed significant reduction in yield when the soil moisture content was decreased under an irrigation interval of 18 days. This effect could be attributed mainly to the excessive water stress in the root-zone causing an imbalance in the plant's nutrition. These results are in concurrence with those reported by many investigators, such as Larson (1975), Navari-Izzo et al. (1990), Osman et al. (1994), Kramer (1995), Ibrahim et al. (2005), and Thalooth et al. (2006); they noticed that under water depletion, the total nitrogen content, protein content as well as amino acids decreased in *Zea mays* and *Helianthus annuus*. They related this effect to the unbalanced soil–water–air relations that lead to the reduction in the photosynthetic activity as well as the induction of adverse relations between hormones and biological processes in all organs of the plants. In addition, Badr et al. (2003) stated that the uptake of nutrients is closely linked to the water–soil conditions. It is expected that a decline in the available moisture in the soil might decrease the diffusion rate of nutrients from the soil matrix to the roots.

Conclusion

This study was performed to evaluate the effects of irrigation intervals and drought stress on yield and yield components of four maize cultivars. Our results demonstrated that all treatments showed a reduction in yield when compared to control irrigation; however, the irrigation intervals of 10 days and 12 days showed a similar effect. Controlled irrigation (I1) demonstrated the highest mean yield of 327.4 g/plant, whereas the lowest mean yield was 158.6 g/plant obtained under the I7 irrigation interval. As expected, there were significant differences between the cultivars; KSC705 and KSC704 showed the highest (199.9 g/plant) and lowest (184.4 g/plant) mean yields, respectively. Therefore, in comparing cultivar KSC704 with other cultivars under low irrigation conditions, KSC704 was affected by drought stress, while other cultivars showed less effect on irrigation conditions.

Irrigation methods contribute to increase the water use efficiency and decrease the amount of irrigation water each time and ultimately to increase the net profits. This experiment demonstrates the traits and indices have the highest correlation with yield and by these two factors identify sensitivity and tolerant cultivars are recognized.

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Irrigation intervals are effective on the performance and water use efficiency of different maize hybrids.

A professional management is required to get the best results from the cultivation of each product. Well-timed irrigation is an important factor meaning that the plant receives water at the right time and as it needs. With proper irrigation, the product is not affected by water stress, and water waste and energy losses are minimized. On the other hand, one of the basic steps in managing irrigation is right planning.

It is imperative to calculate the appropriate irrigation interval for other crops, which is suggested to be in different geographic latitudes.

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IMPACT OF TRAFFIC NOISE ON TIBETAN ANTELOPES: A PRELIMINARY EXPERIMENT ON THE QINGHAI–TIBET HIGHWAY IN CHINA

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Abstract. The traffic noise on a highway with a non-negligible negative impact on the behavior of wild animals has a great ecological impact on wildlife near the road area. To analyze the impact of traffic noise on Tibetan antelopes, field experiments were carried out to measure the traffic noise intensity and observe the behavior of the Tibetan antelope within 500 m of the Qinghai–Tibet Highway from Kunlun Mountain Pass to Wudaoliang on the Qinghai–Tibet Plateau, China. A relationship between different levels of traffic noise intensity and the behavior of Tibetan antelope was established by data analysis. Traffic levels were found to have the most significant effect on Tibetan antelope behavior within 150 m of the highway. The changes in the Tibetan antelope behavior before and after changes in noise mainly manifested as decreased foraging and increased movement. The results of this study can guide road construction and engineering measures to protect wildlife on the Qinghai–Tibet Plateau and other areas with high altitude.
Keywords: *road ecology, traffic noise, noise pollution, Tibetan antelope behavior, high altitude area*

Introduction

The traffic noise from a highway has a large ecological impact on wild animals near road area (Forman et al., 1998). Growing evidence has shown that traffic noise has a non-negligible negative impact on wild animals' behavior. The impact mainly manifests as avoidance of the road vicinity (Zande et al., 1980). Traffic noise may also obstruct acoustic communication among wild animals that depend on it, including frogs, birds, and mammals (Parris et al., 2009). The negative impact on wild animal fitness at the individual level may have severe population-level consequences because most of such wildlife are social animals. Thus, wildlife populations living near road areas exposed to traffic noise have a high potential for reduced reproductive success, which may ultimately break the local ecological balance.

The impact of roads on wildlife has drawn wide attention in recent years. A study in Canada measured the extent and type of relationship underlying the road-effect zones of a motorway with a high proportion of heavy-truck traffic for anuran species richness (Eigenbrod et al., 2009). Field experiments were performed to study the road-effect zones of ungulate wild animals in the USA (Gagnon et al., 2007). To analyze the effect of vehicular traffic on wild animals, a study in India estimated the road kills on state highways (Samson et al., 2016). New technologies have also been used in various studies. To analyze the effects of the highway transportation corridors on wildlife, a

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study used a geographic information system (GIS) to identify landscape attributes associated with species movement in Banff National Parka, Canada (Alexander et al., 2000). To locate accident points and acquire spatial statistics for analysis, a study utilized a GIS to research methods for mitigating wildlife–vehicle collisions in Spain (Diaz-Varela et al., 2011).

Most existing research on the impact of traffic noise on wildlife has mainly focused on birds. Field experiments have indicated a range of behavioral responses to urban noise in North America and Seville, Spain (Wood et al., 2006; Slabbekoorn et al., 2008). Traffic noise has also been shown to affect wildlife migration and reproduction. Parris and Schneider investigated the impact of traffic noise and traffic volume on two bird species in Australia, and found that traffic noise may reduce breeding success in noisy roadside habitats (Parris et al., 2009). The negative impact of traffic noise on avian reproductive success was analyzed in Netherlands (Halfwerk et al., 2011). However, there have been only a few studies that focused on the impact of traffic noise on mammals around the world.

The impact of traffic noise on wildlife has been extensively studied for various geographic topographies around the world. For instance, road kills were analyzed for state highways on Sigur Plateau, India (Gagnon et al., 2007). The effects of traffic noise on bird populations were studied for mountain forests in Idaho, USA (Mcclure et al., 2013). In China, the Qinghai–Tibet Plateau is a unique region with a high altitude and fragile ecological environment. Many wild animals, especially Tibetan antelope, are distributed around the Qinghai–Tibet Highway. The impact of roads on Tibetan antelope has received attention in recent years. Research has mainly focused on the road-effect zone (i.e., extent of significant ecologic effects from the edge of a road) and the impact on migration and protection measures of Tibetan antelopes (Lian et al., 2012; Lin et al., 2007). Traffic noise is known to have an impact on wildlife behavior. However, there has been no research on the impact of traffic noise on Tibetan antelopes and the extent of this impact.

In this study, the impact of different levels of traffic noise intensity on the population behavior of Tibetan antelopes was examined. Field experiments were performed to measure the traffic noise intensity and observe the behavior of the Tibetan antelope within 500 m of the Qinghai–Tibet Highway from Kunlun Mountain Pass to Wudaoliang on the Qinghai–Tibet Plateau, China. The relationship between different levels of traffic noise intensity and the behavior of Tibetan antelope was analyzed based on the traffic noise intensity data and corresponding proportions of different kinds of Tibetan antelope behaviors at different distances from the highway.

The remainder of the paper is organized as follows. First, the field experiments and data collection process are described in detail. Then the experimental results are analyzed, followed by a discussion and insights for future research.

Materials and Methods

Research Area

The research area was located at the junction connecting Hoh Xil National Nature Reserve and Sanjiang Yuan Nature Reserve: the crossing of Qinghai–Tibet Highway from Kunlun Mountain Pass (K2900, 4767 m ASL) to Wudaoliang (K3005, 4800 m ASL) (*Fig. 1*). The geographic structures of the research area include a plateau mountain and plateau grassland for a high-altitude environment. This is a favorable

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habitat for Tibetan antelope, especially from Chumar River to Wudaoliang. The migration season of Tibetan antelope is usually from June to August, so June was selected for surveying. Qinghai–Tibet Highway traverses the annual migration path of Tibetan antelopes. The geographic location of Qinghai–Tibet highway ensures that the main source of noise pollution is traffic. Thus, this 105 km long stretch of the highway from Kunlun Mountain Pass to Wudaoliang was selected for surveying.

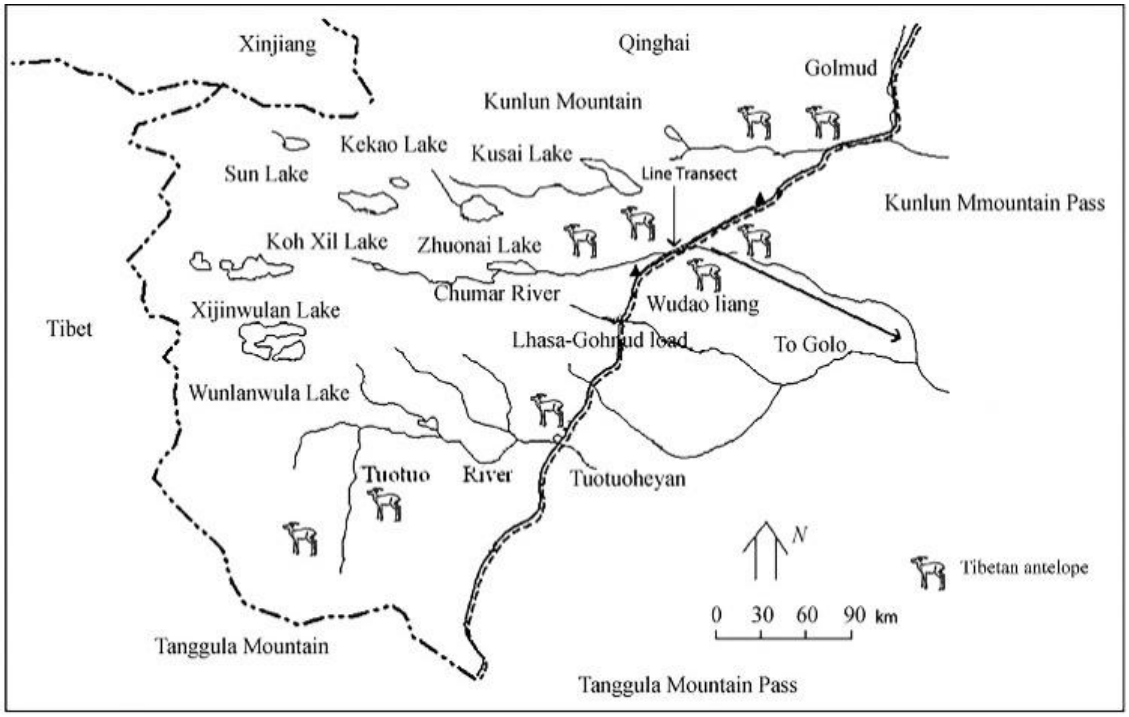


Figure 1. *Survey area*

Field Experiment

To analyze the impact of traffic noise on Tibetan antelope behavior, the influence of different levels of traffic noise intensity on the behavior of Tibetan antelope was measured. There are three key nodes in the research area along the migration path of Tibetan antelope: K2904 (i.e., the wildlife channel between the Kunlun Mountains and Qingshui River), K2965 (i.e., Chubei Channel), and K2998 (i.e., the channel between Chumar River and Hoh Xil). To determine the experiment site, these three nodes were surveyed three full daytime in June 1st and 3rd, 2016. The results showed that a large amount of Tibetan antelope was distributed on the east side of the highway at K2998 (*Fig. 2*). For further research, the hourly traffic volume at K2998 was collected by using the AXLELIGHT Roadside Laser Vehicle Classification System in daytime June 4th to 6th, 2016. The daily difference in traffic volume at K2998 was collected, and the results showed slight differences in the daily traffic volume. The traffic volume was greatest from 8:00 am to 10:00 am (126–138 pcu/h) and corresponds to the most unfavorable condition for Tibetan antelope. Therefore, the east side of Qinghai–Tibet Highway (K2998) from 8:00 am to 10:00 am was set as the experimental site for determining the impact of traffic noise on the behavior of Tibetan antelope.

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Figure 2. *Tibetan antelope population at K2998*

Noise Measures

To avoid adverse factors that may affect the accuracy of the experimental results, such as visual or aural interference for Tibetan antelope during the noise intensity measurement, the traffic noise intensity was measured before the observation of Tibetan antelope behavior. To avoid visual interference, observers did not appear in front of the Tibetan antelope but stayed in the test vehicle. With the aim of keep consistency in weather for observation, four sunny days without windy were selected to measure noise during the date from Jun 4th to Jun 11st, 2016, here it is worth to mention again that the observation time is identical four all the four discrete days for noise measures, from 8:00 am to 10:00 am.

The distance from the east roadside was demarcated along the highway by using a laser range finder. Within 500 m of the roadside, signs were marked every 50 m to acquire the approximate behavior of Tibetan antelope at different distances. The average noise intensity was measured with a noise meter having a resolution of 10 m. The average noise intensity at a certain distance during a certain time period was calculated as follows:

$$X_j = \sum \bar{x}_{ij} \qquad \text{(Eq.1)}$$

where, X_j is the level of average noise intensity at distance j during a certain time period and $\bar{x}_{ij}$ is the average noise intensity at distance j and time i during a certain time period. When a vehicle passed through the vertical intersection (i.e., shortest distance) between the experiment site and highway, the level of instantaneous noise was

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measured immediately, and the maximum value was selected as the maximum noise level during a certain time period.

Behavioral Observations

There are significant differences in the behavioral characteristics of Tibetan antelope depending on the research objective. Such behaviors can be classified as follows: foraging, vigilance, movement, rest, and others. *Table 1* presents the main behavioral variables.

Table 1. *Definitions of the behavioral variables for Tibetan antelope*

Behavior	Definitions of Behavioral Variables
Foraging	Standing or walking with the head below the horizontal line of the shoulder
Vigilance	Standing and watching a certain direction
Movement	Displacement of a single individual with the head parallel or above the horizontal line of the shoulder
Rest	Lying down on the ground
Others	Playing, breastfeeding, defecation, etc.

Field experiments for seven days were carried out to observe the behavior of the Tibetan antelope during the date from 12nd to 19th June, 2016. Based on the statistical data, the average driving speed on this road section was around 50 km/h. Thus, the test vehicle was driven back and forth in the experiment site at a cruise speed of around 50 km/h. Meanwhile, three observers sitting in the test vehicle observed the proportions of different behaviors exhibited by the antelope being passed by at different distances within 500 m of the east roadside with the help of a high-powered telescope.

Results

Level of Noise Changes at Different Distances from the Highway

In this study, 500 datasets were collected regarding the level of average noise intensity and instantaneous noise intensity at different distances within 500 m along the east roadside. The levels of the average noise intensity and maximum noise intensity were calculated at different distances away from the roadside from 8:00 am to 10:00 am. The noise level intensity was associated with different distances from the highway, as shown in *Figure 3*. The horizontal axis represents the distance from the highway, and the vertical axis represents the noise in decibels.

The levels of average noise and maximum noise within 500 m of the highway differed by 3.58–21.15 dB. The difference between them gradually decreased with increasing distance from the highway and then tended to a stable value. However, the two curves representing the levels of average noise and maximum noise showed a generally consistent trend (i.e., nonlinear negative correlation): the noise level decreased with increasing distance from the highway. The noise level decreased sharply with increasing distance up to 150 m from the highway, but the two curves declined gently beyond this range.

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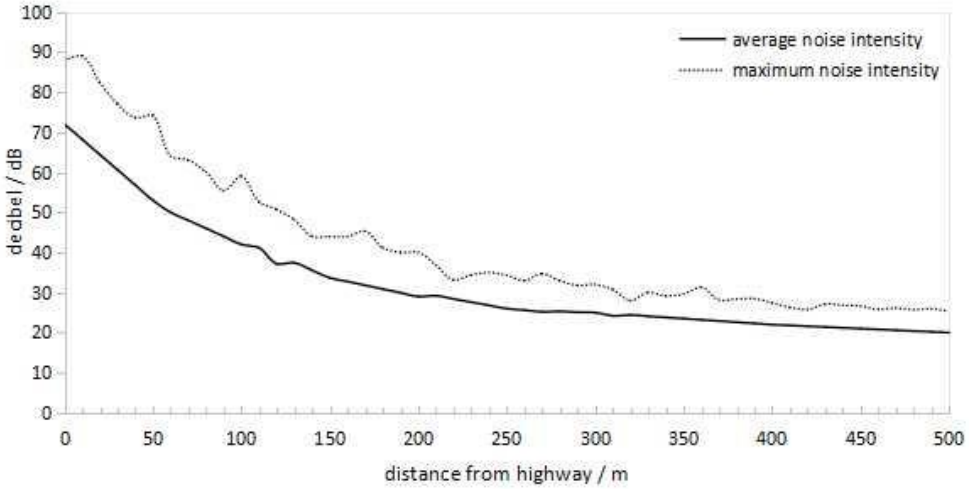


Figure 3. *Change in noise level with distance*

SPSS statistics 17.0 was used for statistical analysis to validate the data. Based on the normal distribution test method and the experimental data, two histograms were obtained, as shown in *Figure 4*. The horizontal axis represents the average noise and instantaneous noise in decibels, and the vertical axis represents the frequency that each decibel value occurred. The absolute values of the skewness and kurtosis were all less than 1. Therefore, the frequencies of the average and instantaneous noise intensity obeyed an approximately normal distribution.

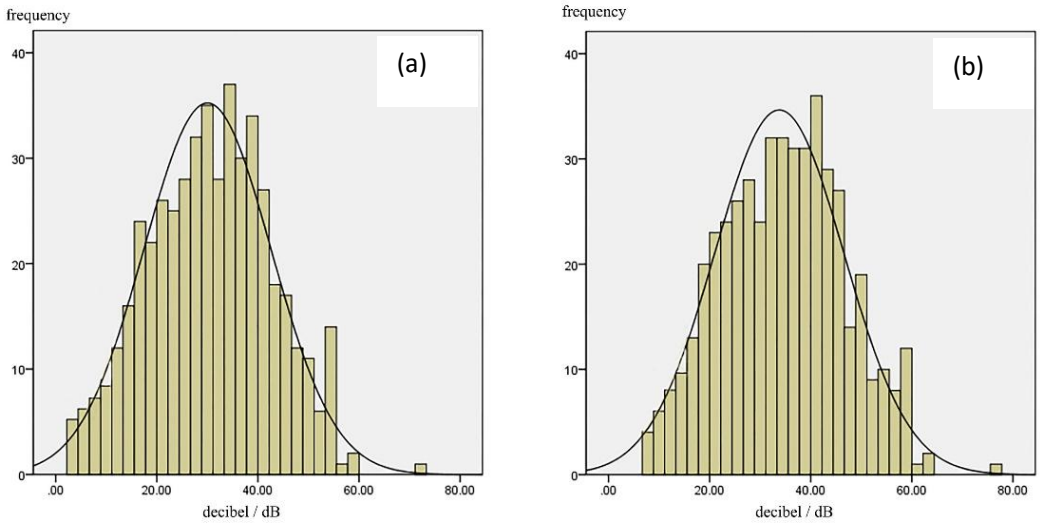


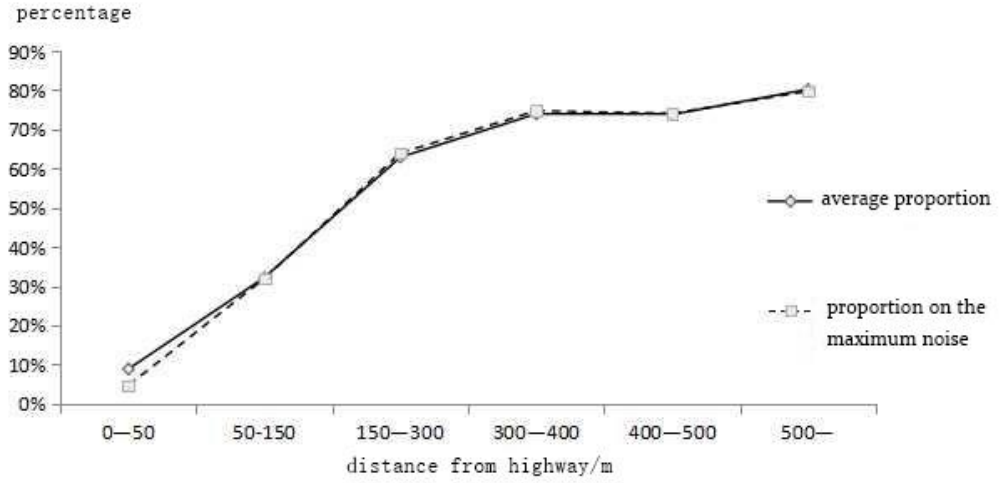
Figure 4. *Distribution of noise intensity data: (a) average and (b) instantaneous noise intensities*

Changes in Tibetan Antelope Behavior with Different Levels of Traffic Noise

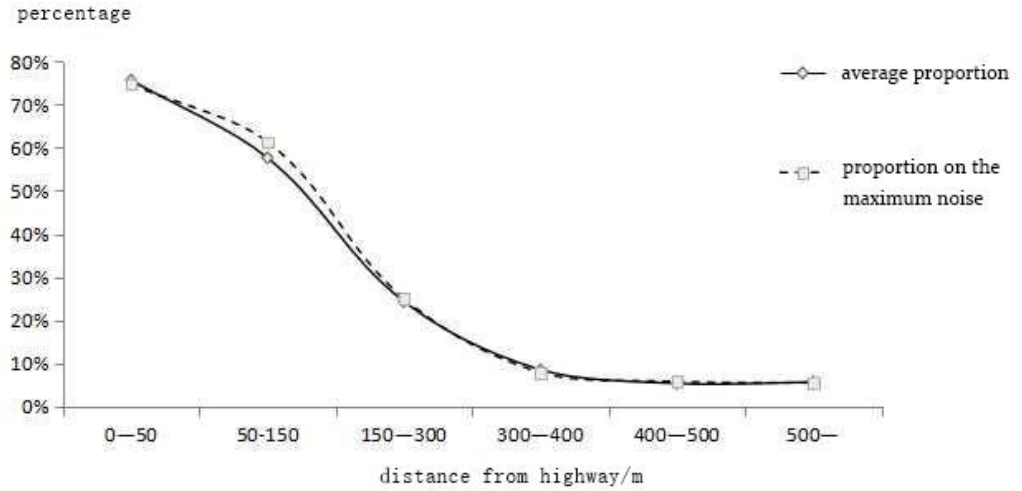
In this study, 1033 Tibetan antelopes were observed, and their behaviors such as foraging, vigilance, movement, rest, and others were recorded. To analyze the association between the Tibetan antelope behavior and noise level, the proportions of

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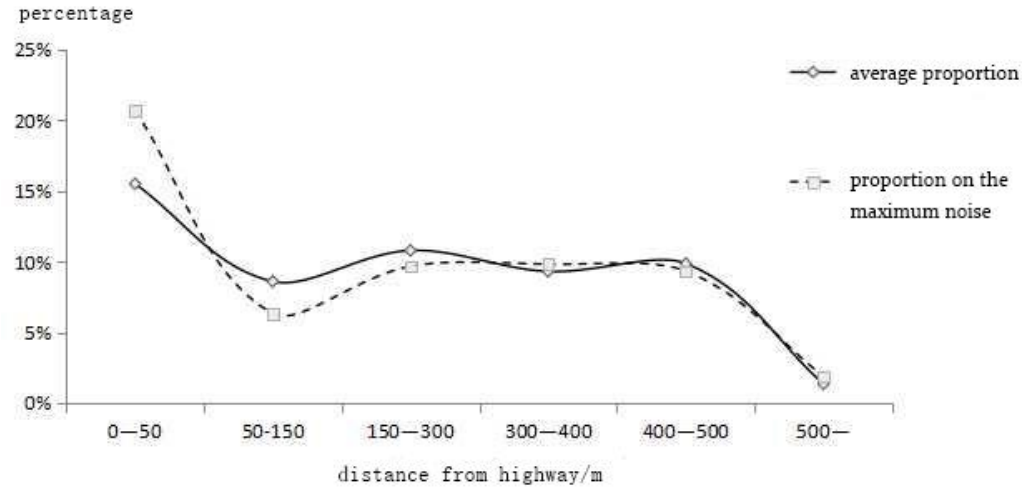
different behaviors affected by the different noise levels were plotted as shown in *Figure 5*. The horizontal axis represents the percentage of each behavior, and the vertical axis represents the distance from the highway.



(a) Foraging

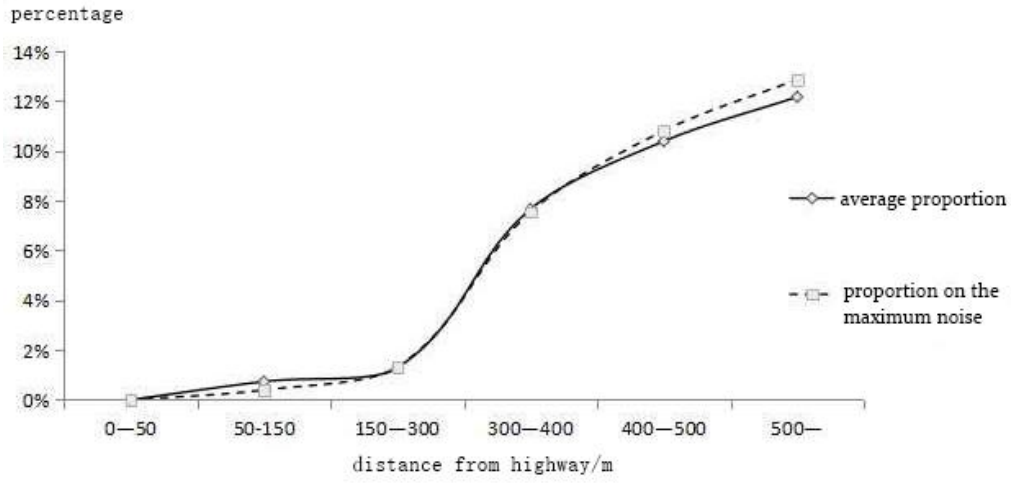


(b) Vigilance



(c) Movement

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(d) Rest and others

Figure 5. *Changes in Tibetan antelope behavior with different levels of traffic noise intensity*

The proportions of the four main types of Tibetan antelope behavior changed with the distance and level of traffic noise intensity. The analysis showed similarities in how each kind of behavior was affected by the level of traffic noise intensity. The percentage of each behavior for the average noise and maximum noise at the same distance from the highway were slightly different, which indicates different impacts of different levels of traffic noise intensity on all types of Tibetan antelope behavior. However, the changes in the proportions of Tibetan antelope behaviors due to the average noise and maximum noise were not significant.

As shown in *Figure 4(a)*, the proportion of foraging behavior before and after traffic noise showed a significant change (4.19%) 0–50 m from the highway. The proportion showed a comparatively small change (1.06%) 150–300 m from the highway. There were also changes in the proportion at 50–100 m and 300 m from the highway, but the changes were less than 1%. Therefore, the noise-effect zone of the highway for the foraging behavior of Tibetan antelope was 0–50 m from the highway.

As shown in *Figure 4(b)*, the most significant change (3.69%) in the proportion of vigilance behavior before and after traffic noise was at 50–150 m from the highway. Changes at 0–50 m and 100 m from the highway did not exceed 1%. Therefore, the noise-effect zone of the highway for the vigilance behavior of Tibetan antelope was 50–150 m from the highway.

As shown in *Figure 4(c)*, the proportion of movement behavior changed significantly (5.18%) before and after traffic noise at 0–50 m from the highway. There were relatively small changes of 2.27% and 1.12% at 0–50 m and 50–100 m, respectively, from the highway. However, the change was less than 1% at 100 m from the highway. Therefore, the noise-effect zone of the highway for the movement behavior of Tibetan antelope was 0–300 m from the highway with a particularly strong effect within 50 m.

As shown in *Figure 4(d)*, there were no significant changes in the proportions of rest and other behaviors before and after traffic noise at different distances from the highway. Thus, the noise-effect zone of the highway for the rest and other behaviors of Tibetan antelope could not be determined.

In conclusion, the main significant changes in the proportions of four kinds of Tibetan antelope behavior before and after traffic noise changed within 150 m from the

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highway. In other words, the noise-effect zone of the highway on the behavior of Tibetan antelope was 0–150 m from the highway.

At 0–50 m, the Tibetan antelope before and after traffic noise mainly exhibited decreased foraging behavior and increased movement behavior. In addition, the vigilance behavior fluctuated. When the vehicle was closer to the Tibetan antelope, some individuals stopped foraging and moved away from the highway, which caused the foraging behavior to decrease and movement behavior to increase. The vigilance behavior did not show a significant increase as expected but showed no irregular fluctuations. This is because the Tibetan antelope maintained a high degree of vigilance within 50 m from the highway with no radical reactions.

At 50–150 m, the Tibetan antelope behavior before and after traffic noise mainly exhibited decreased movement behavior and increased vigilance behavior. When the vehicle was closer to the Tibetan antelope population, some individuals stopped moving and exhibited vigilance behavior.

Discussion

The impact of traffic noise on wildlife has been extensively studied in other countries, although not for high-altitude areas like the Qinghai–Tibet Plateaus. In this study, 500 datasets for the average and instantaneous noise levels at different distances along the roadside within 500 m from the highway were collected, and 1033 Tibetan antelopes were observed in a field experiment.

The behavior of wild ungulates in the area of 0–500 m had a significant difference from 2000 m–3000 m from the highway (Ying et al., 2007). And the avoidance distance of Tibetan antelope is 286.31 m from the highway (Lian et al., 2012). In this study, the results indicate that the noise-effect zone for the behavior of Tibetan antelope was 0–150 m from the highway. This suggests that new highways built in high-altitude areas should be at least 500 m from the activity area of Tibetan antelopes to protect them from the noise-effect zone. More careful consideration of the impact of traffic noise on wildlife is important when planning a new highway. Measures that can be taken in the design phase include changing the pavement material or decreasing the designed speed of vehicles. For Qinghai–Tibet Highway, sound insulation measures may help with avoiding the negative effects of traffic noise on the migration and reproduction of the Tibetan antelope population. For instance, sound barriers can be set on both side of the route along the animal channel, and natural shelter and traffic signs can be set up to prompt drivers to slow down and ban the horn.

The present study was preliminary in nature because it was a short-term study. The long-term impact of traffic noise on wildlife was not considered. More year-round research is needed to better understand the impact of traffic noise on Tibetan antelope and other wildlife. And further research can focus on the performance of sound monitoring at different heights and distances from the highway.

Conclusion

In our study, the noise intensity level showed a nonlinear negative correlation with the distance from the highway. Different levels of traffic noise intensity were found to have different impacts on all types of Tibetan antelope behaviors. Traffic levels were found to have the most significant effect on Tibetan antelope behavior within 150 m of

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the highway. The Tibetan antelope before and after impacted by traffic noise mainly exhibited decreased foraging behavior and increased movement behavior.

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HIGH MITOCHONDRIAL DIVERSITY OF *APIS MELLIFERA* UNDER COI GENE FROM CHINA AND PAKISTAN

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Abstract. The genetic diversity of honeybee populations (240 individual) *Apis mellifera* from ten various locations of China and Pakistan was investigated by using COI (Cytochrome Oxidase Subunit I) gene. After screening of sequences, BioEdit was used for the alignment, and these aligned sequences were employed to MEGA-7 software, to find out the phylogenetic relationship with all available subspecies through Neighbor-Joining method. On the other hand, genetic divergence was calculated via Kimura-2-Parameter using MEGA7, whereas genetic diversity was calculated by DnaSP v5. Phylogenetic tree results divided all populations into seven subspecies within two groups and maximum genetic divergence was observed overall at 3.88%, followed by Pakistan at 2.65% while minimum divergence value (2.02%) was observed for China. Likewise, significant genetic diversity overall was observed with 23 mutations, confirmed with the probability value of Fu's *Fu* at 0.019. Country-wise observations revealed that Pakistan had much highly significant variability through Fu's *Fu* and *D* tests, along with 17 mutations whereas China had shown less diversity with ten mutations. Moreover, the calculation of nucleotide mismatch distribution had also confirmed genetic variability among all our sequenced data. Thus, our findings have revealed the strong genetic interaction and a significant genetic variation among all seven subspecies of *A. mellifera* from two different countries. These results could be more helpful to investigate the genetic relationship among other subspecies of *A. mellifera* from different ecological zones.
Keywords: *honeybee subspecies, mitochondrial DNA, A. m. ligustica, A. m. carnica, A. m. adami, A. m. macedonica, A. m. anatoliaca, phylogenetic analysis, ecological zones*

Introduction

Honeybee rearing is worldwide accepted for its commercial values, and Chinese people are dependent on reared animals, including honeybee, along the sides of various rivers in China. In earlier records of China, honeybee was known as Mi-feng (Li and Luo, 2003). Rearing of honeybees covers more than six million colonies across the country, and is dominated by two species, i.e., *Apis mellifera*, and *A. cerana*. Although, *A. cerana* is being replaced by *A. mellifera* in whole China due to transportation of *A. mellifera* (Yang, 1984), through natural mating and hybridization. Similarly, Pakistan is also involved in the rearing of *A. mellifera*, and has more than 0.3 million colonies along the entire country (Waghchoure-Camphor and Martin, 2008). Unfortunately, honeybees are facing mortality due to the random applications of pesticides worldwide (Husain et al., 2014; Williams et al., 2015), due to which all types of commercial products are being costly.

A wide range of subspecies of *Apis mellifera* inhabit in natural distribution and have differences on the basis of behavioral and morphological features (Koca and Kandemir, 2013; Tofilski, 2008). The evolutionary study for the characterization of the species of *Apis mellifera* (Linnaeus, 1758) was first demonstrated based on morphometric

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parameters (Ruttner et al., 1978). *A. mellifera* has almost 30 subspecies all over the world (Ruttner, 1988), and can be classified into four categories, i) lineage A covers African subspecies; ii) lineage C covers northern Mediterranean subspecies; iii) lineage M contains northern European subspecies and iv) lineage O includes subspecies from the Middle East (Garnery et al., 1992; Whitfield et al., 2006). The new sophisticated molecular techniques have made it easy to further confirm these groups more precisely (Wallberg et al., 2014). Furthermore, this classification has been expanded with the addition of two new lineages, e.g., lineage Y and Z cover Ethiopia (Franck et al., 2001) and Libya (Alburaki et al., 2013), respectively.

A number of studies have been carried out to identify *Apis mellifera* subspecies on the basis of geometric morphometrical features, using programs of automatic identification (Tofilski, 2008). Due to the importation of *A. m. carnica* and *A. m. ligustica* in Europe, the conservation of local subspecies or ecotypes has been damaged by gene flow and replacement over longer distances (De la Rúa et al., 2009; Jensen and Pedersen, 2005; Maul and Hähnle, 1994; Moritz et al., 2005; Peer, 1957). The two races of the Western honeybee are closely related and live in neighboring climatic regions, i.e., *A. m. carnica* Pollmann and *A. m. ligustica* Spinola (Ruttner, 1988). Mitochondrial DNA (mtDNA) is a commonly used approach to explore the genetic evolution and diversity of species due to its conserved gene content and nucleotide substitutions. The honeybees were being identified through the intergenic region of cytochrome oxidase subunit I and II (COI–COII), to find out the evolutionary relationships of honeybees (Chen et al., 2016; Magnus et al., 2014; Techer et al., 2017) under the length and sequence variations of mtDNA (Franck et al., 2000; Hall and Smith, 1991). Out of Hymenopteran subspecies, *A. m. ligustica* is first subspecies for which a complete mitochondrial genome was sequenced (Crozier and Crozier, 1993), and later on, many mitochondrial regions were selected and sequenced for the description of phylogenetic relationships (Cao et al., 2017; Magnus and Szalanski, 2010; Muñoz et al., 2012; Techer et al., 2017).

Even the mitochondrial genome information about the other regions is limited, but some particular regions are being employed regularly to differentiate evolutionary lineages and subspecies of honeybees, like 16S ribosomal DNA (rDNA) (Bouga et al., 2005; Kek et al., 2017), NADH dehydrogenase-2 (ND2) (Kandemir et al., 2006; Techer et al., 2017), and NADH dehydrogenase-5 (ND5) (Bouga et al., 2005; Martimianakis et al., 2011; Özdil and İlhan, 2012), as well as COI regions (Bouga et al., 2005; Kekeçoglu et al., 2009; Marino et al., 2002; Sheffield et al., 2009). As previous studies explored that mitochondrial DNA is transferred maternally and can face high range of polymorphism, therefore, this region of *A. mellifera* genome has been utilized for phylogenetic studies (Ilyasov et al., 2011; Kek et al., 2017; Radoslavov et al., 2017). Likewise, from mitochondrial study consisting two DNA segments, the highest genetic divergence values were observed in COI. For this purpose, different methods were employed to construct phylogenetic trees for COI region of mitochondrial DNA, such as Bayesian Inference; Maximum Parsimony and Neighbour-Joining (Kek et al., 2017; Martimianakis et al., 2011). The present study was designed to determine the mitochondrial diversity and phylogenetic relationships of *Apis mellifera* subspecies of China and Pakistan through COI gene.

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Materials and methods

Sample collection

We sampled a total 240 adult worker honeybees from three different ecological zones of China and Pakistan in year 2017 and immediately froze them in dry ice and brought the samples to the College of Bee Science of Fujian Agriculture and Forestry University, China (*Suppl. Table 1*).

Supplementary Table 1. *Sampling Sites from different Locations of China and Pakistan*

Country	Location	Colonies	Coordinates
China	JL *carnica*	24	43.1520°N,126.4371° E
	JL *ligustica*	24	43.1520°N,126.4371° E
	FZ	24	26.0745°N,119.2965°E
	FQ	24	25.7211°N,119.3843° E
	JM	24	31.0354°N,112.1994° E
	LY	24	29.0284°N,119.1722° E
	PH	24	30.702°N,121.0119° E
	TW	24	25.0330°N,121.5654° E
Pakistan	ID	24	33.7294° N, 73.0931° E
	FD	24	31.4187° N, 73.0791° E

JL = Ji-Lin, FZ = Fuzhou, FQ = Fu-Qing, JM = Jing- Men, LY = Long-You, PH = Ping-Hu, TW = Taiwan, ID = Islamabad, FD = Faisalabad

DNA extraction, PCR amplification and sequencing

Total nucleic acid was obtained from the thorax of honeybee samples by applying king Fisher^{tm}cell and tissue kit as per manufacturer's instructions. Several primers have been designed to amplify the COI gene. Polymerase Chain Reaction (PCR) amplification was conducted using the following primer, COI-F1: 5'-TTTGATTACTTCCTCCCTC-3' and COI-R1: 5'-GCACCTATTGAAAGAACA-3', designed on NCBI tool. The total volume 25 µL, containing, 2 µL DNA template, 1 µL each primer (forward and reverse), ddH_2O 8.5 µL and 12.5 µL 2X Easy Taq Super Mix Trans solution. The amplification program started with 94 °C for 4 min, followed by 36 cycles of 94 °C for 20 s, 56 °C for 30 s, 72 °C for 60 s, and a final step of 72 °C for 5 min. The amplified PCR products were visualized in 1.5% agarose gel stained with ethidium bromide and purified with E.Z.N.A® Gel Extraction Kit. After purification of PCR products, all samples were sent to a local company (Biomarker Pvt, Ltd.) for bidirectional sequencing (Islam et al., 2018a). COI sequences were analyzed and edited using BioEdit 7 (Hall, 1999; Islam et al., 2018b). The sequences were preliminarily aligned using Clustal X (Thompson et al., 1997) and subsequently aligned manually. Unique COI sequences of all samples were deposited in the NCBI GenBank database via accession numbers MF416679–MF416918.

Phylogenetic and genetic distance analysis

Along with our 240 sequences, 67 COI sequences of fourteen subspecies were collected from NCBI database, to represent all available subspecies as well as one sequence of *Apis florea* (JX982136) as an out-group member. The portion (738bp to

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831bp) of the COI5′ gene sequence utilized in our analysis existed between nucleotide 298-1150bp (COI complete sequence 1566bp) (Arias et al., 2008; Crozier and Crozier, 1993). MEGA-7 was used to perform phylogenetic analysis and nucleotide identity percentage within and among other major subgroups (Jan et al., 2017; Thompson et al., 1997). Likewise, genetic distances were also calculated by MEGA-7 under Kimura-2-Parameter (K2P) model.

Genetic diversity

For mitochondrial diversity, all indices were performed in DnaSP v5 (Librado and Rozas, 2009), under two neutrality tests [Fu's *Fs* (Fu, 1997) and Tajima's *D* (Tajima, 1989)]. For all blasted sequences of *A. mellifera* in the present study, a phylogenetic tree was constructed to understand the species network with relation to out-group member.

Results

Two hundred and forty honeybee samples were collected during 2015–2017 from various localities of China and Pakistan. The 240 COI-5' sequences (GenBank MF416679–MF416918) were blasted to determine the similarity index and identification of each isolate and are represented in the phylogenetic trees depicted in this study. Overall, there was 98%-100% nucleotide identity when all sequences were blasted in the NCBI database, along with zero percent E value.

Phylogenetic analysis

The phylogenetic tree grouped the entire collection of *A. mellifera* into two groups, and biggest group consists of individuals from all localities, except ID, along with seven subspecies. On the other hand, second group had covered only ID-sequences, along with two sequences from FD location and *A. m. ligustica* subspecies. The biggest group had shown that honeybees from all localities have good genetic interaction and diversity among the various colonies. On the other hand, it was observed that honeybee colonies from ID had least genetic diversity, due to which they had grouped into a single group that have little interaction with the colonies from FD. While two sequences of *A. florea* were kept as out-group member (*Fig. 1*). From all blasted sequences, it was observed that honeybee colonies from ID (Pakistan) have strong genetic interaction with each other as well as two colonies from FD (Pakistan) (*Suppl. Fig. 1*). Moreover, this group had presented resemblance up to 95%, as shown in *Figure 1*.

Colonies from other locations have huge genetic interaction among various colonies, due to enough diversity. As we know that FD colonies have different eco-geography, along with certain range of diversity, however, it was observed that most of the colonies built intermingled relationship with all Chinese colonies (*Fig. 1*). Likewise, a huge genetic interaction was also observed among all other Chinese colonies on the base of COI-5' DNA. Moreover, it was also observed that colonies from all locations had strong and random relationship with all seven subspecies in biggest group, like *A. m. ligustica*, *A. m. carnica*, *A. m. adami, A. m. macedonica, A. m. anatoliaca, A. m. caucasica* and *A. m. meda* (*Suppl. Fig. 1*). In short, our COI-5' DNA sequences had revealed that colonies from ID have minimum genetic variation and strong interaction with each other while all other colonies had enough variation, leading towards huge genetic interaction with *A. m. ligustica*.

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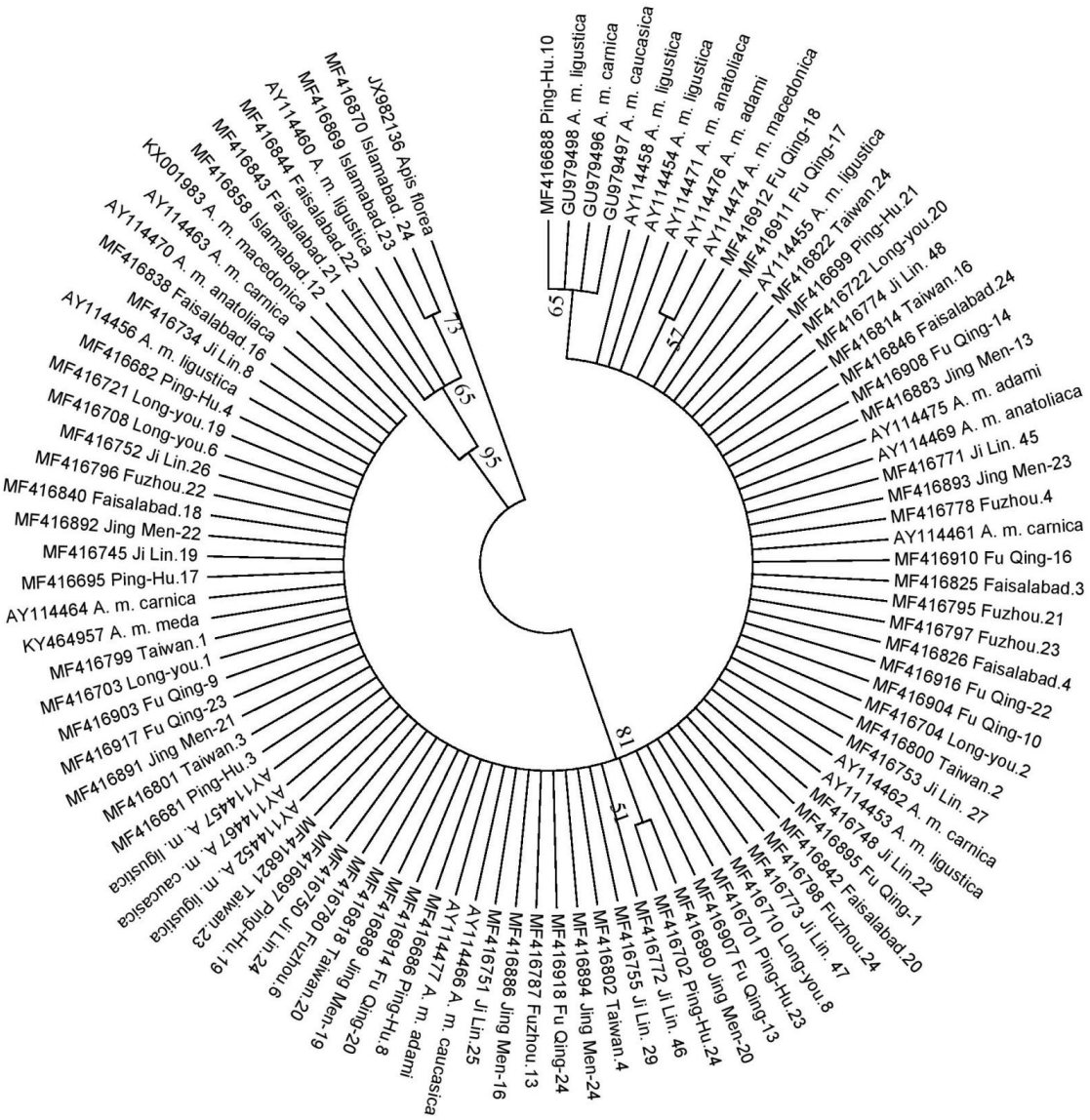


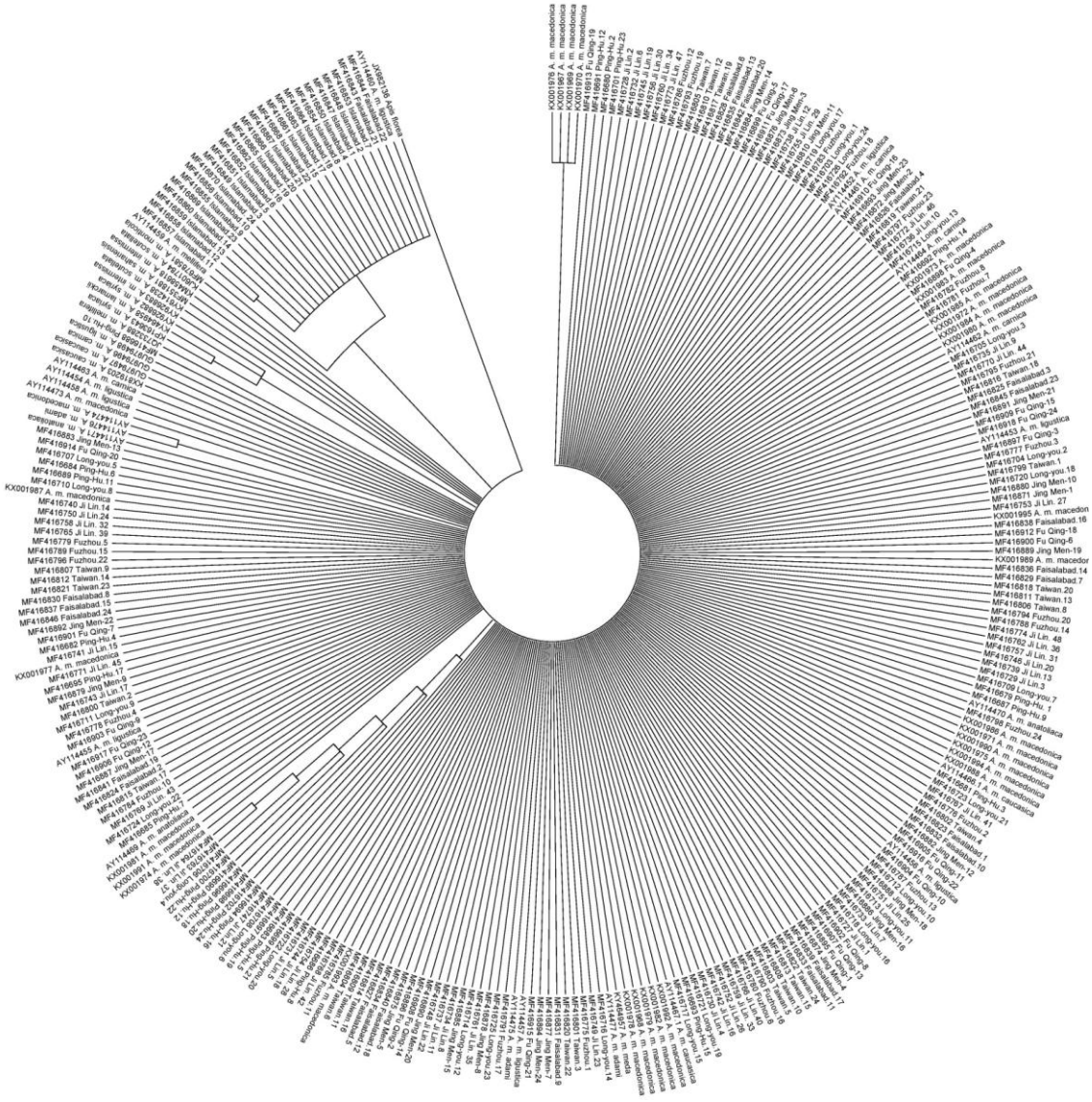
Figure 1. *Phylogenetic relationship of Apis mellifera sub-species in South-China and Pakistan. Phylogenetic tree was constructed through neighbor-joining method by using Kimura-2-Parameter model. For tree construction, we used 73 mtCOI-5' DNA sequences from our data collection, after choosing selected sequences, along with one complete mtCOI DNA sequence of A. florea (JX982136) as an out-group. Two main groups were developed after alignment. This tree shows percent similarity among different colonies from various locations. For both trees, bootstrap value was replicated 1000 times*

Genetic distance among subspecies

According to our sequenced database, pairwise maximum distances for our ten locations cumulatively range between 3.88%. As well, country-wise divergence was observed up to 2.65% and 2.02% for Pakistan and China, respectively. Moreover, maximum interspecific distance (2.36%) was observed for FD-location sequences, followed by PH-location (2.02%) whereas lowest value of maximum interspecific distance was observed for FQ-location and JL *carnica* sequences (0.26% for both). On

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the other hand, maximum intraspecific distance was observed for FD-location as well as FQ-location/JL *carnica*, which was up to 2.10% (*Table 1*).



Supplementary Figure 1. *Phylogenetic tree construction by MEGA 7. Phylogenetic tree was constructed for our 240 sequences and 67 sequences, taken from NCBI, representing 14 subspecies of Apis mellifera. On the other hand, A. florea (JX982136) was taken as out-group member. Tree was constructed through Neighbor-Joining Method under K2P model, and bootstrap value was 1000*

Genetic diversity

As colonies were collected from different locations, therefore, there must be some genetic variation based on their geographical zones. All sequences were subjected to the genetic diversity analysis, and diversity indices were calculated through two neutrality tests, i.e., *D* and *Fs* tests, based on pairwise nucleotide diversity. Maximum genetic diversity, based on Eta value, was observed in FD colonies, showing up to 14 mutations within all colonies, followed by PH location (12 mutations). The significance of genetic

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diversity was confirmed by both neutrality tests, which shown that FD and PH locations had significant variation based on the *p*-value of Fu's *Fs* test (0.016) and *p*-value of Tajima's *D* test (0.01), respectively. On the other hand, most of the Chinese colonies from various locations (FQ, JL *ligustica*, JL *carnica*, LY and TW) presented least value of genetic diversity (*Table 2*).

Table 1. *Distance divergence (%) determination by MEGA7 by using K2P model under pairwise deletion for honeybee colonies*

Colony	Sample size	Mean dist.	Maximum dist.
Overall	**240**	**0.46**	**3.88**
China	**192**	**0.08**	**2.02**
Pakistan	**48**	**1.10**	**2.65**
FD	24	0.34	2.36
FQ	24	0.07	0.26
FZ	24	0.07	0.38
ID	24	0.15	0.65
JL *carnica*	24	0.04	0.39
JL *mellifera*	24	0.05	0.26
JM	24	0.07	0.51
LY	24	0.12	0.42
PH	24	0.15	2.02
TW	24	0.03	0.38

Table 2. *Genetic diversity calculation of honeybee different colonies by DnaSP v5 through polymorphism data analysis*

Colony	n	Nucleotide diversity					Neutrality tests			
		S	k	Eta	Θ	π	Fu's *Fs*	*p*-value	*D*	*p*-value
Total	**240**	**44**	**2.429**	**23**	**0.00852**	**0.00412**	**4.539**	**0.019**	**-1.49450**	**0.10**
China	**192**	26	0.114	10	0.00256	0.00071	-1.850	0.110	-2.18819	0.01
Pakistan	**48**	**24**	**7.772**	**17**	**0.00499**	**0.01018**	**11.805**	**0.000**	**3.23453**	**0.001**
FD	24	18	2.232	14	0.00588	0.00322	6.230	0.016	-1.63342	0.10
FQ	24	3	n/c	n/c	0.00098	0.00069	n/c	n/c	-0.75696	0.10
FZ	24	4	0.159	1	0.00129	0.00065	-0.249	0.335	-0.6811	0.10
ID	24	10	0.562	4	0.00329	0.00141	0.141	0.292	-1.90540	0.10
JL *carnica*	24	5	n/c	n/c	0.00164	0.00038	n/c	n/c	-2.22353	0.08
JL *mellifera*	24	2	n/c	n/c	0.00065	0.00045	n/c	n/c	-0.66674	0.10
JM	24	5	0.159	1	0.00165	0.00072	-0.249	0.335	-1.63539	0.10
LY	24	4	n/c	n/c	0.00128	0.00109	0.000	0.000	-0.40369	0.10
PH	24	21	1.000	12	0.00663	0.00138	3.135	0.156	-2.89855	0.01
TW	24	3	n/c	n/c	0.00098	0.00033	n/c	n/c	-1.65313	0.10

Abbreviations: Eta = Total number of mutations, n = number of sequences, k = Average number of nucleotide differences, S = Number of segregating sites, Θ = nucleotide substitution rate, π = nucleotide diversity, Fu's *Fs* = A negative value of *Fs* is evidence for an excess number of alleles, whereas a positive is related to deficiency of alleles, and *D* is the Tajima test statistic (both *D* and Fu's *Fs* are used to single nucleotide polymorphism)

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Country based cumulative genetic diversity was determined, and colonies from Pakistan had shown maximum variation, representing 17 mutations among all colonies, whereas China had presented only ten mutations. High significance was noted for Pakistani colonies cumulatively, representing *p*-value 0.000 and 0.001 for Fu's *Fu* and *D* tests, respectively. However, overall cumulative genetic diversity of all sequences was significant at the utmost level, presenting 23 mutations, and this diversity was confirmed by Fu's *Fs* test (*Table 2, Fig. 2*).

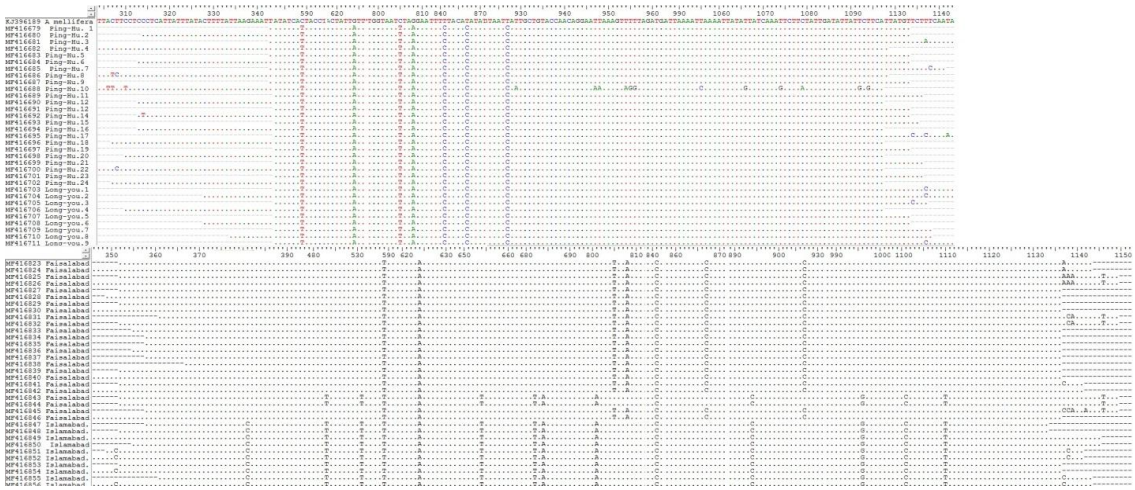


Figure 2. *Number of variable sites in COI region of our aligned sequences through BioEdit*

To confirm all the indices of genetic diversity, all blasted sequences were evaluated for population size changes, to calculate pairwise mismatch distribution for all sequences of subspecies. Overall and country-wise results had expressed a significant genetic variation for COI gene, but there was no population expansion of any subspecies in both countries (*Fig. 3*).

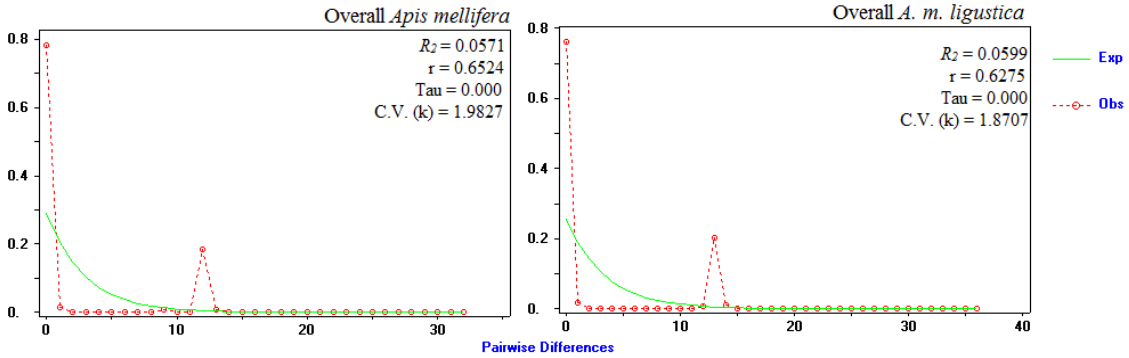


Figure 3. *Pairwise mismatch distributions for Apis mellifera sub-species, based on COI gene sequences by DnaSP v5. The X-axis shows the observed distribution of pairwise genetic variation, and the Y-axis represents the frequencies*

Discussion

The COI-5' DNA study was organized to characterize the genetic interaction and variability of *Apis mellifera* subspecies from two countries on a vast geographical zones.

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Therefore, we collected 240 colonies, and sequenced them to assess phylogenetic relationship among different colonies as well as their genetic diversity and interaction.

Phylogenetic relationship of all sequences has defined the genetic interaction among the colonies from various locations as well as diversity within the colonies of same location, based on COI DNA. The phylogenetic tree of *Apis mellifera* along with 14 referenced subspecies, presented two groups (*Suppl. Fig. 1*). The largest group has been consisted of populations from all localities, except ID, representing vast genetic interaction among all populations. However, *A. m. ligustica* and *A. m. carnica* were dominant in our sequences. On the other hand, populations from ID location also presented huge genetic interaction within locality and least diversity, represented strong relationship with *A. m. ligustica* (*Fig. 1*). Same trend of genetic interaction was observed by mitochondrial study of Martimianakis et al. (2011), in which they described that various subspecies of *A. mellifera* built relationship with each other from different localities. Such earlier studies support our results of genetic interaction among two different subspecies as well as different localities in this study. Furthermore, such phylogenetic relationship among different subspecies of *A. mellifera* was also described by other people across the world, based on the COI gene (Özdil and İlhan, 2012; Radoslavov et al., 2017) and other distinct genes (Ilyasov et al., 2016a, b; Techer et al., 2017).

In our study, all nucleotide substitutions were occurred between 298-1150bp. However, there were only seven main nucleotide substitution sites in our sequence whereas minor substitutions were observed at many sites of COI region. Due to such minor substitutions, colonies of both subspecies from all locations were clustered together in different groups (*Figs. 1* and *2*), except ID locations.

The genetic divergence and interaction for DNA sequences were explained by many researchers for different classifications of organisms, i.e., plant, animal, arthropod as well as prokaryotic species (Goraya et al., 2015; Islam et al., 2018a; Qasim et al., 2018a, b). Likewise, Radoslavov et al. (2017) explained the interaction and divergence of various *Apis* subspecies under various genes. Since, based on COI gene sequences, a comprehensive divergence was calculated for various localities as well as country wise, and all sequences were grouped into two groups, and determined their inter-specific maximum as well as interspecific mean distances by MEGA 7 (*Table 1*). The maximum interspecific distance (2.36%) was observed for FD colonies whereas maximum intraspecific distance was noted among FD and FQ colonies, also at 2.10%, because FQ colonies presented minor percent distance within the entire location. Likewise, maximum mean intraspecific distance was observed between FD and TW location, which was 0.31% only. However, overall highest mean value of divergence was observed for Pakistan (1.10%), while highest maximum value of divergence was observed for overall locations (3.88%) (*Table 1*).

Genetic variability indices were calculated up to various levels, like species, genus and family, to find out genetic diversity in the mitochondrial DNA. For this purpose, different genes were selected by several researchers, like ND5 (Ozdil and İIlhan, 2014) and COI-COII inter-genic region genes (Pinto et al., 2014; Rahimi, 2015). Therefore, for mitochondrial DNA diversity of our sampled *A. mellifera* colonies, mutations were calculated in the COI region of various locations (*Fig. 2; Table 2*). Most of the colonies from various locations had shown minor genetic diversity due to commercial rearing. However, FD location had shown maximum genetic variability within the colonies. Therefore, colonies from FD location have genetic interaction with all other colonies of whole collection (*Fig. 1*). A recent study of Özdil and İlhan (2012) confirms our results

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that there are many sequence mutations within and among the colonies. However, p-values of Fu's *Fu* and Tajima's *D* test for colonies of most locations are non-significant, which proves minor variation. On the other hand, genetic variability has shown significant impact for overall country wise colonies.

Conclusion

Conclusively, we can say that the honeybee colonies collected from all locations present a hybrid situation between *A. m. ligustica* and *A. m. carnica*, as both subspecies are capable of interbreeding and are dominant in our collection. Likewise, Wu et al. (2017) has described that *A. ligustica* has been originated from two different sources in Taiwan, which supports our hypothesis that there is some possibility of hybridization between different colonies. On the basis of hybridization, there are seven subspecies in our entire collection on the base of mitochondrial DNA. This mitochondrial characterization of honeybees from different localities of Asia further extends our understanding on the genetic diversity and interaction of honeybee based on COI gene, in spite of rearing in the confined environment, as compared to wild environment. Thus, on the base of our findings, we conclude that there could be many other interactions among honeybee colonies from various locations, based on more prominent traits, like honey production, royal jelly production, reproduction as well as behavior. Therefore, with respect of our results, we suggest the use of other genes to find out genetic interaction among different countries' colonies, to improve the potential of honey and royal jelly production, as well as to assess the genetic diversity based on other genes.

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REDUCING AMMONIA AND CHROMIUM CONCENTRATION IN BATIK WASTEWATER BY VETIVER (*CHRYSOPOGON ZIZANIOIDES* L.) GROWN IN FLOATING WETLAND

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Abstract. Waste of batik industry that is not well processed potentially contaminate the aquatic environment. Liquid waste concentration of 0%, 50%, 75%, and 100% was applied to vetiver (*Chrysopogon zizanioides* L.) grown in floating wetland system for treatment of batik industry liquid waste. Research was conducted in Akademi Kimia Analis (AKA) Bogor, Indonesia. This study aimed to determine the ability of vetiver for reducing the concentration of NH_3 and NH_4^+, TAN, BOD, COD, and total chromium. 50% of batik waste water was revealed to be the most suitable concentration treated by vetiver in floating wetland, since it could reduce not only ammonia, confirmed by biplot analysis, but also 40.29% total chromium concentration.
Keywords: *ammonia, batik, chromium, wastewater, wetland*

Introduction

The batik industry in Indonesia has been progressing very rapidly, especially after UNESCO designated Indonesian batik as a Masterpiece of Oral and Intangible Heritage of Humanity on 2 October, 2009. Raw materials for batik production such as chemicals in the form of dyes and supporting materials such as water used in the washing and rinsing process, potentially become batik liquid waste. Batik is mostly produced in home industries that do not have wastewater treatment. The batik liquid waste is directly disposed to canals or rivers, causing a negative impact on the environment. There are two types of dyes used in batik product, namely natural and synthetic. Natural dyes include indigosol and remazol black B (Holkar et al., 2014) and synthetic dyes are nitroso, nitro, azo, stilben, diphenyl methane, triphenyl methane, acridine, kinolin, indigoida, aminokinone, anine and indophenol (Al-kdasi, 2004). Various metal complex dyes (chromium, cobalt, copper and nickel complex dyes) are widely used in textile industries as coloring agent (Aksu and Balibek, 2010). The use of chromium metal in the textile dyeing process will have an impact on the environment and cause poisoning in waters (Akar and Tunali, 2006). The presence of chromium in waters is found in two forms: chromium valence III (Cr^{3+}) and Chromium VI (Cr^{6+}) ions. Chromium valence VI is more toxic than Chromium valence III as it is difficult to decompose.

Phytoremediation is a cost effective and ecofriendly technique to clean contaminated soil and wastewater. Phytoremediation is the use of green plants including forb grass and wood species to remove environmental contamination such as heavy metals, trace

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elements, organic compounds, and radioactive compounds in soil or water (Hegazy et al., 2011; Mojiri, 2012) to absorb pollutants. *C. zizanioides* exhibits good growth when grown on some types of wastes, such as fish farming wastes in aquaponic systems (Effendi et al., 2015a, b, c, 2016), crude oil contaminated waters (Effendi et al., 2017), tofu production wastewater (Seroja et al., 2018). It can improve the quality of the wastewater (Bedewe, 2010; Boonsong and Chansiri, 2008; Yeboah et al., 2015) and decrease Trichloroethylene (TCE) from contaminated soil by 98% (Janngam, 2010). In addition, *C. zizanioides* is able to absorb N and P (Darajeh, 2014), heavy metals, tolerant to herbicides and pesticides (Truong et al., 2010); to utilize tilapia cultivation waste (Delis et al., 2015), freshwater crayfish cultivation waste (Effendi et al., 2015b), and vaname shrimp aquaculture waste (Syafrudin, 2015); lowering chromium content in mud (Rodríguez et al., 2010). The research aimed at using vetiver plant in reducing NH_3, NH_4, TAN, BOD, COD, and total chromium of batik liquid waste in floating wetland system.

Materials and method

Liquid waste originated from batik industry in Jababeka Cikarang industrial area, Indonesia. Water quality analysis was conducted at the Laboratory of Environmental Test and Laboratory of Polytechnique of Akademi Kimia Analis (AKA) Bogor. The study used *C. zizanioides* as a pyhtoremediator plant. The treatments consisted of wastewater concentration of PL0 control (0% waste), PL50 (50% waste), PL75 (75% waste), and PL100 (100% waste) with triplicates. Into each batik liquid waste treatment was spiked Potassium dichromate as Cr^{6+} to increase chromium concentration up to 2 ppm. Concentration of the added chromium content refers to the Regulation of the Minister of Environment No. 5 of 2014 on waste of batik and textile industry of 0.5-1 ppm.

C. zizanioides are washed and placed in a plastic tube stored in a container (25 L) of water and nutrient solution of A & B mix consisting of N, P, K, Mg, B, Cu, Mn, Mo, and Fe. Each container comprised of 6 pots containing 3 clumps vetiver of each pot. The plants were acclimatized for 3 weeks in floating wetland system where the roots were submerged in the water (Darajeh, 2014) before used.

Water quality parameters of temperature, Total Suspended Solid (TSS), pH, total ammonia (NH_3), ammonium (NH_4), Total Ammonia Nitrogen (TAN), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and total chromium (in water, root, leaf) were determined. Measurements were taken weekly, referring to APHA (2012). NH_4^+ (*Eq. 1*) and NH_3 (*Eq. 2*) concentrations were calculated as follows (*Table 1*) (Strickland and Parsons, 1972).

$$NH_{4^+}(mg/l) = \frac{TAN}{1 + \text{antilog}(pH - pKa)}^{+} \quad \text{(Eq.1)}$$

$$NH_3(mg/L) = TAN - NH_{4^+} \quad \text{(Eq.2)}$$

Table 1. *Value of pKa according to value of temperature*

Temperature (°C)	5	10	15	20	25	30
pKa	9.90	9.73	9.56	9.40	9.24	9.09

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Statistical analysis was performed using ANOVA followed by Duncan test ($p < 0.05$). Correlation of Pearson and biplot analysis were also calculated using SAS software version 9.1.

Results and discussion

Growth performance of vetiver as phytoremediator of batik production wastewater is elaborated in detail and published elsewhere (Tambunan et al., 2018). Below water quality improvement is described following remediation process, considering especially nitrogen concentrations (TAN, NH_4^+, NH_3) and chromium reduction. Initial characteristic of batik wastewater is presented in *Table 2*.

Table 2. *Characteristics of inlet of batik wastewater (Tambunan et al., 2018)*

Parameter	Unit	Batik/textiles wastewater	Regulation of the Minister of Environment No 5, 2014
Temperature	°C	30	----
TSS	mg/L	268	50
pH	-	9.8	6.0 – 9.0
Total ammonia	mg/L	1.27	8.0
BOD	mg/L	966.83	60
COD	mg/L	2900.48	150
Total chromium	mg/L	2.34 (spike)	1.0

Temperature ranged from 28.10 to 29.10 °C. Based on statistical analysis, the temperature during the treatment was not significantly different, but observation time had significant effect on temperature ($p < 0.05$). The pH ranged between 7.30-9.76, pH on day T0-T28 was not significantly different ($p > 0.05$), however when compared with day T35-T49 it was significantly different ($p < 0.05$). According to Singh et al. (2014) plants effectively absorbed waste at pH of 6-9. pH 7-8 is the optimal pH for vetiver in removing BOD, while at pH < 5 vetiver performance in eliminating BOD ranged 10-40%. High pH (12) can inhibit vetiver performance (Darajeh, 2016).

BOD in all treatments ranged 1.80 – 3.68 mg/L, meanwhile before treatment BOD was 117.28 mg/L (PL50), undergoing reduction (97.76-98.47%) after treatment. Each treatment and observation time had significant effect on BOD ($p < 0.05$). *Figure 1* shows the fluctuations of the BOD during observation time. Drastic decline occurred on day T7 and T14, reducing BOD concentration occurred until T49. COD after treatment ranged 60.89-95.18 mg/L (initial range of 773.50-806.00 mg/L). Statistical analysis of each treatment and time of observation pointed out significant effect on COD ($p < 0.05$). It is shown in *Figure 2* that from T0 to the end of the observation for each treatment, COD showed the same pattern of decline, but from T14 to T21 increased from 152 mg/L to 262 mg/L, followed by a subsequent decrease at the end of the observation.

Total Ammonia Nitrogen (TAN) consists of unionized ammonia (NH_3) and ionized ammonia (NH_4^+). Ammonia (NH_3) is toxic to aquatic organisms, while ammonium (NH_4^+) is harmless and it is in a form that plants can harness as a source of nutrient (Effendi, 2003). The percentage of NH_3 in the waters decreased with decreasing pH and

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water temperature. TAN concentration increased from day T0-T7, then decreased drastically on day T14 (*Fig. 3*). Degradation of organic matter inhibits the rate of nitrification thus disrupting bacterial growth, inhibiting ammonia oxidation, and causing accumulation of TAN (Chen et al., 2005). Ammonia is water-soluble and very harmful to fish, and most tropical species that are generally more sensitive to ammonia (Effendi et al., 2015c; Wang and Leung, 2015). In this study the ammonia concentration increased on day 7, then decreased on day 14 drastically (*Fig. 4*).

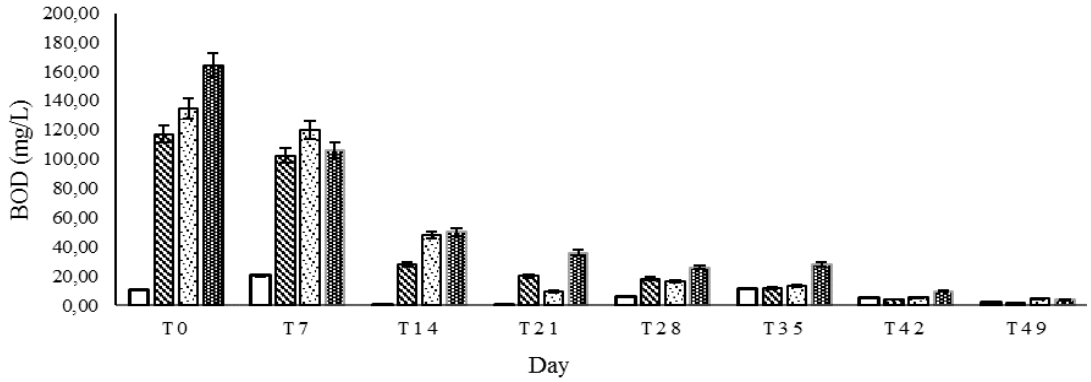


Figure 1. *BOD decrease during the observation period. Description: PL0 (without waste 0%), PL50 (50% waste), PL75 (75% waste), PL100 (100% waste)*

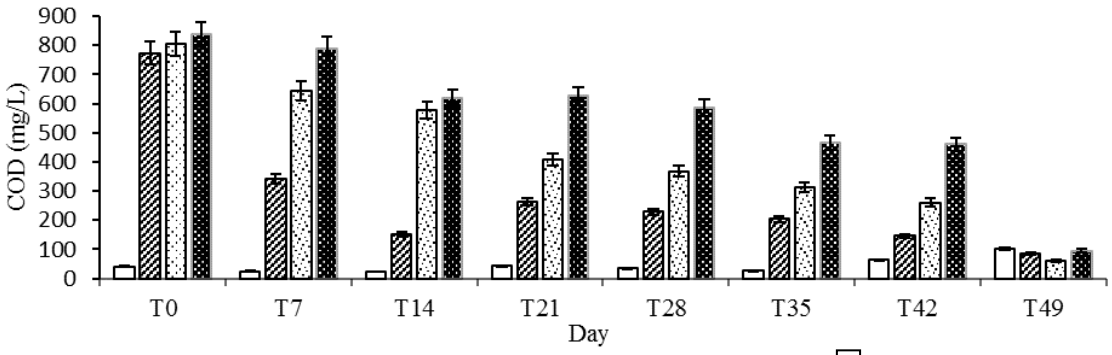


Figure 2. *COD decrease during the observation period. Description: PL0 (without waste 0%), PL50 (50% waste), PL75 (75% waste), PL100 (100% waste)*

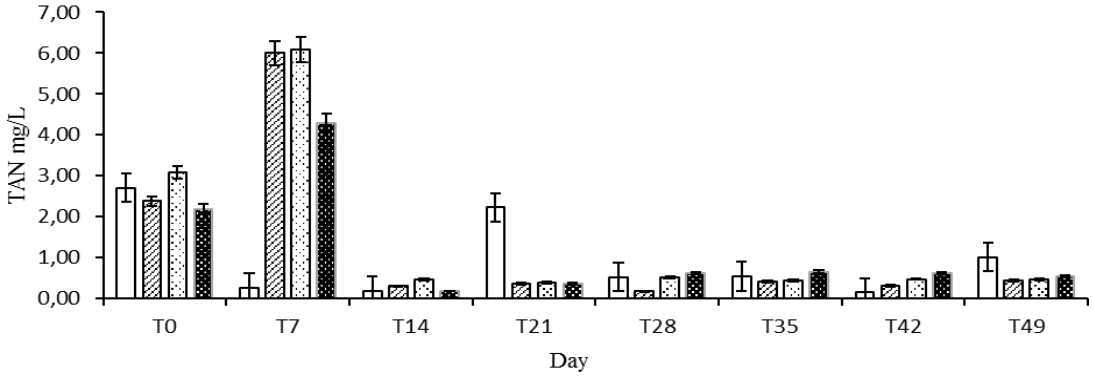


Figure 3. *Total ammonia nitrogen (TAN) reduction during observation period. Description: PL0 (without waste 0%), PL50 (50% waste), PL75 (75% waste), PL100 (100% waste)*

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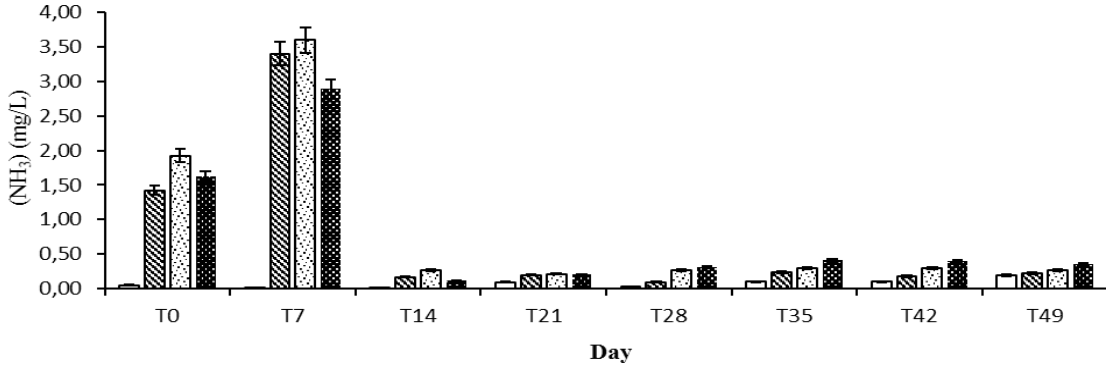


Figure 4. *Ammonia reduction during observation period. Description: ☐ PL0 (without waste 0%), ▨ PL50 (50% waste), ⊡ PL75 (75% waste), ▩PL100 (100% waste)*

The NH_3 concentrations at the end of the observations for the PL0, PL50, PL75 and PL100 treatments were 0.19 mg/L, 0.22 mg/L, 0.26 mg/L, 0.35 mg/L, respectively. Initial total ammonia concentration was 1.42-1.92 mg/L. The NH_3 difference occurred at day 14. Percentage of NH_3 removal in each treatment of PL50, PL75 and PL100 was 84.51%, 86.47% and 78.14%, respectively. The results of this study are not much different from the research of Effendi et al. (2015b) of 84.6% NH_3 removal in freshwater lobster cultivation waste using kale, but this result is higher than the result of Delis et al. (2015) with NH_3 removal percentage of 31.3-48.36%, and Syafrudin et al. (2015) of 37.14-43.83% NH_3 removal in shrimp farming waste in wetland system.

Figure 5 shows the NH_4^+ concentration which increased on day 7, then decreased until day 49. Decreasing NH_4^+ during the experiment indicated that the vetiver could absorb and utilize NH_4^+. High NH_4^+ fluctuations during the study were observed in PL0 (control), where day T0 and day T21 increased and decreased until day T49. The highest percentage of NH_4^+ degradation (82.57%) existed at PL75, greater than the result of research on shrimp farming waste in hydroponic system (Syafrudin et al., 2015).

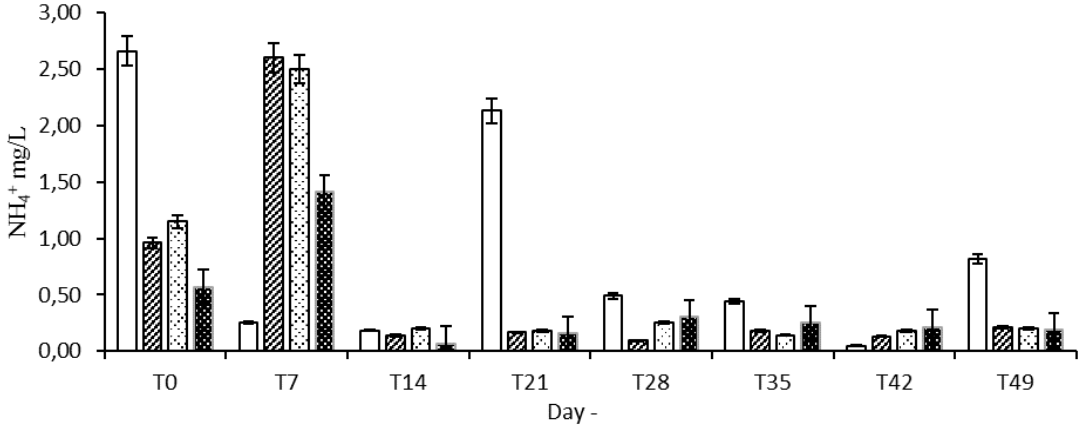


Figure 5. *Ammonium (NH_4^+) reduction during observation period. Description: ☐ PL0 (without waste 0%), ▨ PL50 (50% waste), ▨ PL75 (75% waste), ▩ PL100 (100% waste)*

Percentage of TSS decreases at PL50, PL75, and PL100 were -38%, -49%, and -72% respectively, indicating that an increase of TSS took place (*Table 3*). This is likely due

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to the addition of AB mix nutrients and remediation process with vetiver, so that TSS at the end of the observation (1183 mg/L) was greater than that at the beginning of the experiment (1673 mg/L). The higher the concentration of waste was the higher TSS concentration could be observed. The organic and inorganic compounds contained in the wastewater are bonded in suspended material (Effendi, 2003). Water quality parameters at PL100 generally had the smallest percentage of decline compared to those at PL50 and PL75.

Table 3. *Reduction percentage of all water quality parameters of each treatment*

Parameter	Unit	PL50		Reduction (%)	PL75		Reduction (%)	PL100		Reduction (%)
		Start	End		Start	End		Start	End	
TSS	mg/L	1183	1637	-38	1579	2346	-49	2127	3668	-72
pH	-	9.30	9.13	1.75	9.35	9.24	1.08	9.56	9.39	1.72
TAN	mg/L	2.38	0.44	81.69	3.07	0.46	85.01	2.19	0.54	75.52
NH_4^+	mg/L	0.96	0.56	77.50	1.15	0.60	82.57	0.57	0.39	67.17
NH_3	mg/L	1.42	0.22	84.51	1.92	0.26	86.47	1.62	0.35	78.46
BOD	mg/L	117.28	1.80	98.47	134.77	2.52	98.13	164.21	3.68	97.76
COD	mg/L	773.50	84.72	89.05	806.00	60.89	92.45	788.64	95.18	88.65
Total chromium	mg/L	1.07	0.64	40.29	1.52	0.99	34.87	2.17	1.98	8.85

TAN at each treatment was significantly different, but ammonium (NH_4^+) was not significantly different. Meanwhile, NH_3 concentration at PL0 was significantly different from NH_3 at PL50, PL75, and PL100. The percentage of NH_3 reduction for each treatment was PL50 (84.51%), PL75 (86.47%), and PL100 (78.46%). Fluctuation of TAN and ammonium at PL0 throughout the experiment might be related to the fact that the plant is naturally associated with bacteria living in its root submerged in the system.

The results of this study are still lower than the research using lettuce with percentage of NH_3 decrease of 90.1% (Effendi et al., 2015c), but higher than research using the combination of *Ipomea aquatica* and bacteria in the aquaponic system as much as 81% (Effendi et al., 2015d). Ammonia when discharged into the waters will disturb the life of aquatic biota such as fish (Effendi et al., 2015c). Meanwhile total chromium was significantly different in all treatments. In addition, total chromium reduction decreased with increasing waste concentration namely 40.29% (50% waste), 34.87% (75% waste), and 8.85% (100% waste). Bioremediation of chromium complex dyes in synthetic solutions and actual effluent was performed using growing *Aspergillus tamarii* in batch and continuous bioreactors. Maximum removal of chromium (94.00 ± 0.10 and 77.50 ± 0.10%) was obtained from synthetic solutions (100 mg/L) of acid brown 45 and acid blue 158 dyes, respectively in batch mode using *Aspergillus tamarii* (Ghosh et al., 2017). Chromium accumulation in root and leaf is presented in *Table 4*.

Plant growth parameters (clump, wet weight) correlated positively with nitrogen (TAN, NH_4, NH_3), BOD and COD with significant level of 0.05. This illustrated that plant might utilize nutrient resulting from organic substance decomposition. Meanwhile wet weight and Relative Growth Rate (RGR) correlated negatively with TSS (-0.761) and total chromium (-0.310), implying that both parameters disrupted the growth of plant (*Table 5*).

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Table 4. *Accumulation of Cr in vetiver (Tambunan et al., 2018)*

Parameter	Concentration (mg kg^{-1})			
	PL0	**PL50**	**PL75**	**PL100**
Root	0.45	18.28	27.17	33.52
Leaf	0.88	1.27	0.96	0.86
Total accumulation	1.33	19.56	28.12	34.37

Description: PL0 = vetiver control (*C. zizanioides*) without waste, PL50 = vetiver in 50% wastes + 50% water, PL75 = vetiver in 75% wastes + 25% water, PL100 = vetiver in 100% waste

Table 5. *Pearson correlation analysis of each parameter*

Parameter	Height	NH_3	NH_4	pH	Temp	COD	Total chromium	TSS	BOD	TAN	Clump	Wet weight	RGR
Height	1.000	-0.455	-0.299	0.081	-0.386	-0.633	-0.421	-0.040	-0.620	-0.487	-0.593	-0.219	0.936
NH_3	-0.455	1.000	0.130	0.373	0.172	0.706	0.460	0.044	0.628	0.682	0.453	0.251	-0.423
NH_4	-0.299	0.130	1.000	-0.597	0.231	-0.002	-0.309	-0.371	0.021	0.814	0.417	0.263	-0.263
pH	0.081	0.373	-0.597	1.000	-0.213	0.461	0.697	0.561	0.369	-0.221	-0.256	-0.211	0.052
Temp	-0.386	0.172	0.231	-0.213	1.000	0.199	0.147	-0.096	0.266	0.271	0.379	0.218	-0.266
COD	-0.633	0.706	-0.002	0.461	0.199	1.000	0.569	-0.023	0.903	0.413	0.687	0.420	-0.581
Total chromium	-0.421	0.460	-0.309	0.697	0.147	0.569	1.000	0.784	0.542	0.042	-0.054	-0.310	-0.500
TSS	-0.040	0.044	-0.371	0.561	-0.096	-0.023	0.784	1.000	-0.012	-0.247	-0.597	-0.761	-0.175
BOD	-0.620	0.628	0.021	0.369	0.266	0.903	0.542	-0.012	1.000	0.385	0.649	0.392	-0.573
TAN	-0.487	0.682	0.814	-0.221	0.271	0.413	0.042	-0.247	0.385	1.000	0.573	0.341	-0.441
Clump	-0.593	0.453	0.417	-0.256	0.379	0.687	-0.054	-0.597	0.649	0.573	1.000	0.775	-0.527
Wet weight	-0.219	0.251	0.263	-0.211	0.218	0.420	-0.310	-0.761	0.392	0.341	0.775	1.000	-0.133
RGR	0.936	-0.423	-0.263	0.052	-0.266	-0.581	-0.500	-0.175	-0.573	-0.441	-0.527	-0.133	1.000

The results of biplot analysis are shown in *Figure 6.*

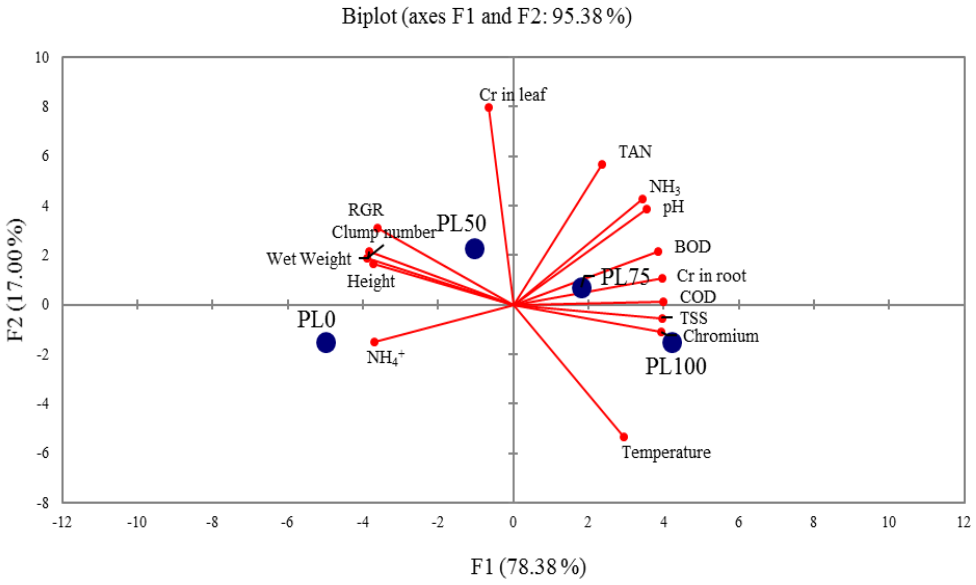


Figure 6. *Biplot analysis between each treatment and all parameters*

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Different treatments (PL0, PL50, PL75 and PL100) are illustrated by bold dots. These four points show the proximity of treatment with growth and water quality parameter. The parameters that have dominant proximity to PL0 and PL50 are mostly plant growth parameters (clump number, height, weight wet, RGR). This phenomena denoted that 50% waste was better for supporting the growth of plant compared to 75% and 100% waste. Meanwhile PL75 and PL100 were surrounded by water quality parameters (TAN, NH_4, BOD, COD, pH, TSS, chromium in water), as consequences of larger concentration of waste. Conversely, growth of plants in the form of plant height, wet weight and number of clumps become lower with increasing concentration of waste, since those parameters were away from PL75 and PL100 in biplot analysis.

Conclusion

In this particular research, 50% of batik waste water treated by vetiver in floating wetland was revealed as the most suitable concentration, resulting in better water quality parameter reduction, and total ammonia concentration reduction.

This research result provides simple promising biological treatment of batik production wastewater instead of chemical treatment. The application of this research in batik production wastewater location will soon be pursued.

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FATTY ACID, MINERAL AND PROXIMATE COMPOSITIONS OF VARIOUS GENOTYPES AND COMMERCIAL CULTIVARS OF SWEET ALMOND FROM THE SAME ECOLOGICAL CONDITIONS

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Abstract. This study was carried out on the fatty acid, mineral and proximate compositions of ten sweet almond genotypes and two commercial varieties named as Ferragnes and Ferraduel grown in the same ecological conditions known as the Firat riverside region during two consecutive years. In this context, the crude oil (Co), crude protein (Cp), total sugar (Ts), total ash (Ta) and moisture (Mo) contents from the almond genotypes and cultivars ranged from 48.69 to 55.8%, 20.41 to 25.82%, 2.92 to 5.27%, 3.29 to 4.66% and 2.52 to 3.75%, respectively. The genotypes and cultivars showed a varied mineral content as well. Potassium (K) was the main mineral in all genotypes and cultivars, ranging from 679.53 to 986.63 mg100 g^{-1}; phosphorus (P) was the second most abundant mineral, ranging from 584.57 to 697.31 mg100 g^{-1}; followed by magnesium (Mg) and calcium (Ca), ranging from 325.27 to 381.93 mg100 g^{-1}, 250.15 to 332.19 mg100 g^{-1}, respectively. Of the identified fatty acids, oleic acid (Oa) (69.76–72.02%) was the predominant fatty acid, followed by linoleic acid (La) (18.82–21.62%), palmitic acid (Pa) (5.62–7.76%), and stearic acid (Sa) (1.20-1.69%). Other fatty acids were present at trace amounts. The unsaturated (Ufa)/saturated fatty acid (Sfa) ratios ranged from 9.29 to 12.12. From the results of the present study, we can conclude that the kernels of almond genotypes and cultivars are being potential sources of valuable oil which might be used for edible and other industrial purposes.
Keywords: *nut, ecology, chemical content, ANOVA, Turkey*

Introduction

Sweet almond (*Prunus amygdalus* L.) is one of the important nut crops. It is cultivated and produced for multipurpose comparing to other commercial nuts in the world (Kester et al., 1991). Turkey is one of the significant countries in the world with respect to almond production. The total annual world almond production is about 3.214.303 tons. USA, Australia, Spain, Morocco, Iran and Syria produce 2.002.742,

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72.709, 202.339, 112.681, 147.863 and 88.841 tons of almond annually, respectively. Turkey ranks seventh in the world, producing 85.000 tons of almond (FAO, 2016).

The fruits such as almond, walnut, pistachios and hazelnut, in which classification the almond seeds are generally included, are very requested products and their destinations are the direct consumption after toasting, the confectionery industry and the production of cakes, sweets, and almond candies (Piscopo et al., 2010; Simsek, 2016; Simsek et al., 2017). These fruits are typically high in fat but their fatty acid composition is advantageous in relation to risk of coronary heart disease (Lei et al., 2014). From the pre-agricultural era to the present day, almonds have been significant part of the human diet, providing micronutrients, macronutrients, and various bioactive constituents (Simsek, 2016). Kernels of almond genotypes and cultivars have been used to combat important diseases of the heart and autoimmune system, rheumatoid arthritis, and cancer in recent years (Jenkins et al., 2002).

The chemical composition of almond fruit is of great importance to establish its nutritive value and its quality for the recent concern of consumers over ensuring a healthy life style (Nanos et al., 2002; Simsek and Kizmaz, 2017). Fatty acid, mineral and proximate compositions of almond, pistachio, walnut, hazelnut and chestnut kernels are nutritionally very valuable (Bliss, 1999). These chemical contents can be influenced by ecological conditions, location and technical and cultural practices (Askin et al., 2007).

The proximate composition of ten sweet almond genotypes in Diyarbakir region ranged from 43.50 to 54.81% crude oil (Co), from 21.18 to 32.90% crude protein (Cp), from 3.08 to 4.43% moisture (Mo) and from 2.54 to 4.42% total ash (Ta) (Simsek and Demirkiran, 2010). The fatty acid compositions of almond genotypes ranged from 48.80 to 88.40% oleic acid (Oa), from 11.30 to 33.20% linoleic acid (La), from 4.60 to 9.50% palmitic acid (Pa) and from 1.00 to 3.40% stearic acid (Sa) (Kiani et al., 2015). In addition, the mineral contents of almond cultivars ranged from 525.46 to 793.86 mg100 g^{-1} potassium (K), from 154.15 to 275.87 mg100 g^{-1} magnesium (Mg) and from 89.97 to 176.50 mg100 g^{-1} calcium (Ca) (Piscopo et al., 2010).

Many studies have been carried out especially dealing with the proximate, fatty acid and mineral compositions of almond genotypes grown in Turkey (Simsek and Demirkiran, 2010; Agar et al., 1998; Aslantas et al., 2001; Askin et al., 2007; Celik et al., 2010; Beyhan et al., 2011; Celik and Balta, 2011; Ozcan et al., 2011; Balta, 2013; Simsek and Kizmaz, 2017).

Hundreds of almond genotypes are grown in this location. They have grown heterozygously due to generative reproduction. So, the fruits that are handled may not be similar to each other as they can be. These genotypes have not been cultivated under regular care conditions. Ferragnes and Ferraduel almond varieties are also grown under good maintenance conditions. Because these varieties are vegetatively propagated, these have homozygote properties, the fruits obtained from these varieties are similar.

To our best of knowledge, no fatty acid, proximate and mineral composition studies on almond genotypes and cultivars have been reported in this area. Therefore, the present study is very significant. Therefore, the objectives of the present work were to determine the levels of significant fatty acid, mineral and proximate compositions in kernels of various genotypes and commercial cultivars of sweet almond. The study results are expected to contribute to breeding studies, commercial production, nutritional and technical applications, and healthy diets in future.

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Materials and methods

Materials

This study was carried out in the Firat riverside region of Hilvan district (Sanliurfa) of Southeast Turkey (FRH, 2018) in *Figure 1*. It has been stated that the soils in this region are sandy-loamy, calcified and / or humus and mostly rich in plant nutrients. In this area, a warm and temperate climate is prevalent. It receives more rainfall during winter time. Summers are generally hot and draught and as winters are temperate and wet and wet in Hilvan district with an altitude 591 m asl (CDO, 2018) and with an annual precipitation of 771.8 mm (Sanliurfa, 2018). In addition, temperature values of this region are given in *Table 1* (CDO, 2018). Summers are arid and very hot, winters are rainy and partly temperate. The area of the province which is 55 km away from Sanliurfa is 1.278 km^2 (IHI, 2018). In the Southeastern Anatolia Region, Şanlıurfa province is surrounded by an illegal district of Hilvan, with Adiyaman and Siverek in the north, Siverek in the east, Central district in the south, Bozova in the south, and Adiyaman in the west. The district lands located in the northeastern part of Şanlıurfa Plateau have a wavy land structure decreasing from the southeast to the southwest (IHI, 2018). The Firat riverside region of Hilvan district (Sanliurfa) of Southeast Turkey has a distinctive microclimatic environment derived from the Firat River. Ecological conditions around this river are similar mostly to those of Mediterranean region but are not similar to those of Sanliurfa province in the Southeast Anatolia region. In this ecology, there are also fruit trees such as almond, mulberry, pomegranate, figs, walnut, apricot, pistachio, cherry and forest trees such as pine, poplar and sycamore flourish (TSI, 2017). The fruits for determination of fatty acid, mineral and proximate compositions of ten genotypes and two commercial cultivars (Ferragnes and Ferraduel) of almond were harvested by hand during the years 2016-2017. After harvest, all samples of kernels were immediately dried and stored in their shells at room temperature until analysis.



Figure 1. *Map of districts in Sanliurfa province*

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Table 1. *Temperature parameters of Hilvan district of Sanliurfa province*

Climatical parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Avg. temp. (°C)	4.60	6.10	10.10	15.10	20.60	26.40	30.60	30.2	26.20	19.40	12.40	7.00
Min. temp. (°C)	1.00	2.20	5.30	9.50	14.10	19.50	23.50	23.10	19.10	13.30	7.30	3.10
Max. temp. (°C)	8.30	10.10	15.00	20.80	27.10	33.30	37.80	37.40	33.30	25.60	17.50	10.90

Since the characteristics of local almond genotypes and varieties were not affected by any year, the average values of 2 years were evaluated as a whole. Furthermore, chemical analysis for fatty acids, proximate acids and minerals is very expensive. Therefore, in a number of previous studies averages of a maximum of 2 years are evaluated on their contents. Therefore, samples of 30 g (10 g kernel x three replications) for every genotype or cultivar were taken from ten sweet almond genotypes and from two commercial varieties in each year.

Proximate composition of sweet almond genotypes and cultivars

The almonds were dried and stored in their shells at room temperature prior to analysis. The samples were finely grinded and their moisture (Mo) content was determined before the proximate analysis. Moisture (Mo) content was analyzed and calculated according to the methods given by the Turkish Standard Institute (AOAC, 1990). For crude oil (Co) analyses, the samples were homogenized and subjected to extraction for 6 h with petroleum ether (boiling range 30-60 °C) in a Soxhlet apparatus. The extracted total crude oil was dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure in a rotary film evaporator. Crude oil (Co) percentages of the genotypes and cultivars were determined by weight difference (AOAC, 1990). Total ash (Ta) content was determined in crucible to a muffle furnace at 900 °C for 8 h (AOAC, 1990). Total sugar (Ts) content was determined by the Anthrone method (Kaplankiran, 1984). The nitrogen content was estimated by the Kjeldahl method and was converted to crude protein (Cp) content by using the conversion factor 6.25 (AOAC, 1990).

Mineral composition of sweet almond genotypes and cultivars

The magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) contents were determined via Perkin Elmer 703 atomic absorption spectrophotometry, and the sodium and potassium (K) contents were measured via flame emission spectrometry. Phosphorus (P) was measured by colorimetric method (Saura Calixto and Canellās, 1982; Simsek and Kizmaz, 2017).

Fatty acid composition of sweet almond genotypes and cultivars

The crude oil (Co) contents were determined according to standard procedure (AOAC, 1990). For the preparation of fatty acid methyl esters (FAMEs) 0.4 g crude oil (Co) of the kernel samples were dissolved in 4 mL of isooctane and methylated in 0.2 mL 2 M methanolic KOH. Analysis of FAMEs was performed on an Agilent 6890

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series Gas Chromatography equipped with flame-ionization detector and a 60 m capillary column (internal diameter = 0.25 mm) coated with 0.25 μm of 50%-cyanopropyl-methylpolysiloxane (J&W Scientific, Folsom, CA, USA). Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1} and a split ratio of 1:10. The temperatures of the injector and detector were 250 °C and 260 °C, respectively. The oven was programmed to hold at 120 °C for a 5 min, and then to increase the temperature to 240 °C at a rate of 15 °C min^{-1} and to hold that temperature for 20 min (Basturk et al., 2007). FAMEs were identified by comparison of their retention times and equivalent chain lengths in terms of standard FAMEs (Supelco. 47885-U). FAMEs of samples were quantified according to their percentage areas (AOAC, 1990).

Statistical analysis of sweet almond genotypes and cultivars

Statistical analyses of proximate, fatty acid and mineral compositions of sweet almond genotypes and cultivars were performed in triplicate. All data were subjected to analysis of variance using JMP 5.0.1 (one-way ANOVA).

Results

Proximate composition of sweet almond genotypes and cultivars are shown in *Table 2.* According to this table, crude oil (Co), crude protein (Cp), total ash (Ta), moisture (Mo) and total sugar (Ts) contents of 10 almond genotypes and two commercial almond cultivars ranged from 48.69 (SAHI301) to 55.81% (FERRAGNES), from 20.41 (FERRAGNES) to 25.82% (SAHI301), from 3.29 (SAHI278) to 4.66% (SAHI6), from 2.52 (SAHI6) to 3.96% (SAHI324) and from 2.97 (FERRADUEL) to 5.27% (SAHI72), respectively.

Table 2. *Proximate composition of sweet almond genotypes and cultivars (%) (average 2016-2017)*

G & C	Mo (%)	Co (%)	Cp (%)	Ta (%)	Ts (%)
SAHI6	2.52f	51.13d	25.67a	4.66a	3.31gh
SAHI36	2.79ef	51.72cd	24.37b	3.47ef	3.57fg
SAHI72	3.39bc	52.10c	21.99e	3.30f	5.27a
SAHI132	3.21c	55.69a	20.54f	3.48ef	3.73efg
SAHI198	3.70ab	52.10c	22.94d	3.81de	3.89ef
SAHI232	2.87de	51.53cd	24.69b	3.63def	4.54cd
SAHI250	3.23c	48.93e	23.55c	4.52ab	5.07abc
SAHI278	3.41bc	52.10c	21.89e	3.29f	5.17ab
SAHI301	3.58b	48.69e	25.82a	4.21bc	4.13de
SAHI324	3.96a	51.80cd	23.40cd	3.92cd	4.63bcd
FERRADUEL	3.15cd	54.71b	21.74e	4.64a	2.97h
FERRAGNES	3.13cd	55.81a	20.41f	3.35f	5.08abc
LSD	0.40	0.77	0.60	0.37	0.56

*Statistically significant (at 0.05 level). Moisture: Mo, Crude Oil: Co, Crude Protein: Cp, Total Ash: Ta, Total Sugar: Ts, Genotypes & Cultivars: G & C

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The amount of the minerals studied in the sweet almond genotypes and cultivars are shown in *Table 3,* referred to non-dried matter. According to this table, among the genotypes and cultivars studied, SAHI36 genotype contained the highest potassium (K) (986.63 mg100 g^{-1}). The phosphorus (P), magnesium (Mg), and calcium (Ca) contents ranged from 584.57 (FERRADUEL) to 697.31 mg100 g^{-1} (SAHI36), from 325.27 (SAHI132) to 381.93 mg100 g^{-1} (SAHI324) and from 250.15 (SAHI250) to 332.19 mg100 g^{-1} (SAHI198), respectively. Sodium (Na), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) amounts ranged from 9.20 (SAHI301) to 16.06 mg100 g^{-1} (SAHI72), from 6.08 (FERRAGNES) to 10.62 mg100 g^{-1} (SAHI6), from 4.80 (FERRAGNES) to 9.53 mg100 g^{-1} (SAHI6), from 2.52 (SAHI36) to 4.76 mg100 g^{-1} (SAHI72) and from 2.02 mg100 g^{-1} (SAHI250) to 3.97 (SAHI232), respectively.

Table 3. *Mineral compositions of sweet almond genotypes and cultivars (mg 100 g^{-1}) (average 2016-2017)*

G & C	K	P	Mg	Ca
SAHI6	783.37e	590.12e	366.30ab	284.93bc
SAHI36	986.63a	697.31a	342.06ab	275.37cd
SAHI72	783.3e	591.16de	361.57ab	285.47bc
SAHI132	875.83bcd	594.31de	225.27c	189.63e
SAHI198	859.03cd	637.79abc	363.67ab	332.19a
SAHI232	947.76a	612.21cde	332.32a	285.87bc
SAHI250	842.25d	648.60bc	332.10b	250.15d
SAHI278	679.53f	616.04cde	353.21ab	312.25ab
SAHI301	900.57bc	671.14ab	342.04ab	281.78c
SAHI324	845.60d	668.43ab	381.93a	324.54a
FERRADUEL	903.30b	584.37e	375.10a	321.78a
FERRAGNES	879.43bcd	621.55cde	366.07ab	276.98cd
LSD	77.55	46.86	40.22	27.54

Continued…

G & C	Na	Fe	Zn	Mn	Cu
SAHI6	15.37ab	10.62a	9.53a	4.44ab	3.45ab
SAHI36	11.70f	8.49cd	7.59de	2.52e	3.59 a
SAHI72	16.06a	9.57b	8.70bc	4.76a	3.57a
SAHI132	9.58g	7.26ef	5.64g	4.00bc	2.70cd
SAHI198	13.50cd	6.14g	5.71g	4.07b	3.44ab
SAHI232	11.47f	8.58c	7.99d	2.87de	3.97a
SAHI250	13.01de	9.14bc	8.03cd	3.93bc	2.02e
SAHI278	10.96f	7.83de	6.92ef	2.54e	3.40ab
SAHI301	9.20g	9.56b	8.92ab	4.59ab	2.17de
SAHI324	11.96ef	7.18ef	6.79f	4.06bc	3.49ab
FERRADUEL	14.50bc	6.68fg	5.53g	3.40cd	2.93bc
FERRAGNES	10.92f	6.08g	4.80h	2.82de	2.89bc
LSD	1.06	0.71	0.69	0.68	0.6

*Statistically significant (at 0.05 level). Potassium: K, Phosphor: P, Magnesium: Mg, Calcium: Ca, Sodium: Na, Iron: Fe, Zinc: Zn, Manganese: Mn, Copper: Cu, Genotypes & Cultivars: G&C

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Fatty acid composition of promising almond genotypes and cultivars are shown in *Table 4*. According to this table, while oleic acid (Oa) content ranged from 69.76 (SAHI232) and 72.02% (SAHI250), linoleic acid (La) content changed from 18.82 (SAHI72) to 21.62% (SAHI232) in the samples. Palmitic acid (Pa) contents were found from 5.62 (SAHI132) and 7.76% (SAHI301) in the samples (*Table 3*). The stearic (Sa), myristic (Ma), palmitoleic (Paa) and linolenic acid (Lia) contents ranged from 1.20 (SAHI198) to 1.69% (SAHI72), from 0.27 (SAHI250) to 0.55% (FERRADUEL), from 0.19 (SAHI301) to 0.54% (SAHI72), and from 0.07 to 0.09%, respectively.

Table 4. *Fatty acid composition of promising almond genotypes and cultivars (%) (average 2016-2017)*

G & C	Pa	Sa	Ma	Paa	Oa	La	Lia
SAHI6	6.35cd	1.46abc	0.47ab	0.40abc	8.68b	19.91b-e	19.91b-e
SAHI36	6.26cd	1.57ab	0.47ab	0.21cd	8.52bc	19.89be	19.89b-e
SAHI72	6.94b	1.69a	0.40bc	0.54a	9.57a	18.82e	18.82e
SAHI132	5.62e	1.39 abc	0.34bc	0.20cd	7.62f	20.46b	20.46b
SAHI198	6.20d	1.20c	0.37bc	0.31bcd	8.09de	20.11bc	20.11bc
SAHI232	6.44cd	1.35bc	0.46ab	0.28cd	8.53bc	21.62a	21.62a
SAHI250	6.10d	1.23c	0.27c	0.31cd	7.91ef	20.00bcd	20.00bcd
SAHI278	6.18d	1.35bc	0.43ab	o.33bcd	8.29cd	19.68be	19.68b-e
SAHI301	7.76a	1.40abc	0.38bc	0.19d	9.73a	19.00de	19.00de
SAHI324	6.68bc	1.34bc	0.34bc	0.50ab	8.86b	20.04bcd	20.04bcd
FERRADUEL	6.99b	1.67a	0.55a	0.34bcd	9.55a	20.47b	20.47b
FERRAGNES	6.72bc	1.51abc	0.41abc	0.21cd	8.85b	19.06cde	19.06cde
LSD	0.46	0.31	0.15	0.19	1.13	1.11	0.01

*Statistically significant (at 0.05 level). Palmitic acid: Pa, Stearic acid: Sa, Myristic acid: Ma, Palmitoleic acid: Paa, Oleic acid: Oa, Linoleic acid: La, Linolenic acid: Lia, UFA: Unsaturated fatty acids, SFA: Saturated fatty acids, Genotypes & Cultivars: G&C

SFA, UFA and UFA/SFA ratios of sweet almond genotypes and cultivars are shown in *Figures 2, 3* and *4, respectively*. In this context, SFA, UFA and UFA/SFA ratios of samples ranged from 7.62 (SAHI198) to 9.73% (FERRADUEL), from 90.27 (SAHI301) to 92.09% (SAHI250) and from 9.29 (SAHI301) to 12.12% (SAHI132), respectively.

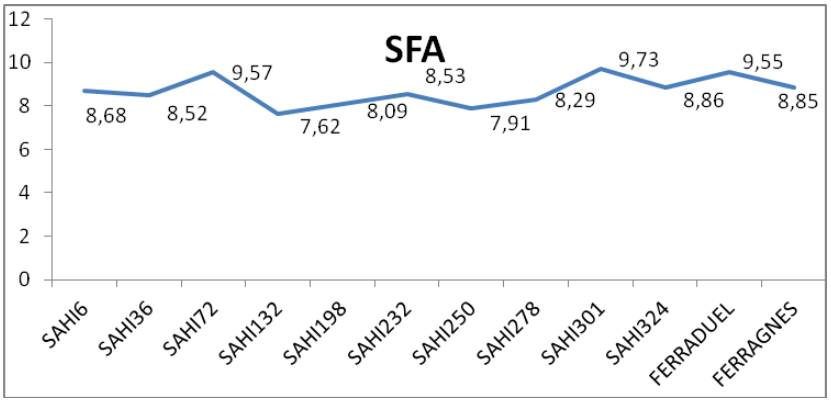


Figure 2. *The SFA ratios of sweet almond genotypes and cultivars (average 2016-2017)*

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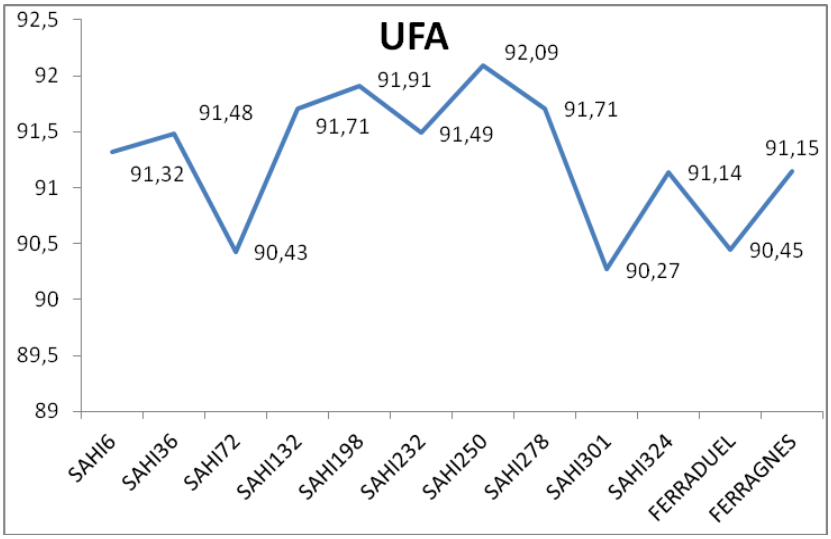


Figure 3. *The UFA ratios of sweet almond genotypes and cultivars (average 2016-2017)*

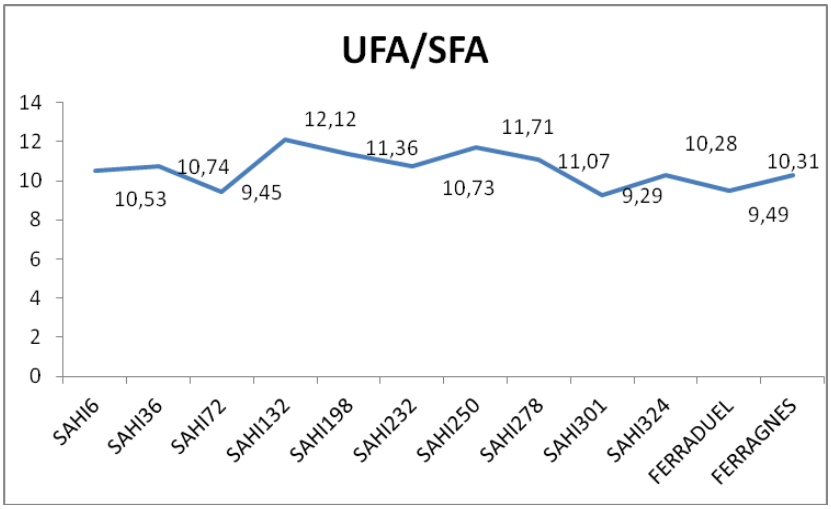


Figure 4. *The UFA/SFA ratio of sweet almond genotypes and cultivars (average 2016-2017)*

Discussion

Proximate composition of sweet almond genotypes and cultivars

This study was carried out on the almond genotypes and commercial varieties named as Ferragnes and Ferraduel grown in the same location known as the Firat riverside region during two consecutive years. In this study, proximate contents of the genotypes and varieties were compared at the same location. According to the results from the one-way ANOVA analysis, the differences between the proximate composition of the genotypes and/or the varieties were shown in different letters statistically and were presented in *Table 2*. In this context in this study, the samples of sweet almond genotypes and cultivars showed only a relatively small variation. Crude oil (Co) content is a relatively stable feature independent on the different almond genotypes and cultivars. All genotypes and varieties except for SAHI250 and SAHI301 genotypes are rich in crude oil because their crude oil content is greater than 50%. In various almond

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varieties that differed in genotypes, the oil (Co) contents were previously reported to range from 43.50 to 54.81% (Simsek and Demirkiran, 2010), from 52.08 to 57.49% (Agar et al., 1998), from 48.80% to 55.70% (Ozcan et al., 2011), from 52.00 to 56.00% (Barbera et al., 1984), from 53.00 to 62.00% (García-López et al., 1996), from 30.0 to 51.00% (Martins et al., 2000) and from 48.93 to 55.96% (Simsek and Kizmaz, 2017). In the present study, the oil (Co) contents were partly similar to those previously recorded. The almond kernel has high crude oil (Co) content, and the kernels do not constitute a bulky diet. Since all genotypes and varieties have crude protein values greater than 20%, they are protein rich. In various works, the crude protein (Cp) content varied from 22.50 to 25.80% (Barbera et al., 1984), from 18.00 to 25.00% (Soler et al., 1989), from 14.50 to 24.00% (Mbah et al., 2013) and from 20.81 to 25.99% (Simsek and Kizmaz, 2017). In the present study, the crude protein (Cp) contents agreed mostly well with earlier reports (Barbera et al., 1984; Soler et al., 1989; Mbah et al., 2013; Simsek and Kizmaz, 2017). Because the crude protein (Cp) content is high, the almond genotypes and cultivars may serve as dietary supplements for unhealthy people who require crude protein and suffer from hypertension. Sugar is found in both almond kernels and other plant tissues, and can be digested, absorbed and metabolised by humans to provide energy**.** In this study, because the genotypes SAHI232, SAHI250, SAHI278, SAHI301 and SAHI324, and the variety FERRAGNES have a sugar content greater than 4%, they are rich of sugar. In previous works, the total sugar (Ts) content ranged from 2.56 to 4.17% (Aslantas et al., 2001), from 3.10 to 5.30% (Romojaro et al., 1988), from 2.60 to 4.00% (Kazantzis et al., 2003), from 2.10 to 7.40% (Ahrens et al., 2005) from 2.10 to 5.50% (Amrein et al., 2005) and from 2.91 to 4.06% (Simsek and Kizmaz, 2017). In the present study, total sugar (Ts) content in harmony with earlier studies (Aslantas et al., 2001; Romojaro et al., 1988; Kazantzis et al., 2003; Ahrens et al., 2005; Amrein et al., 2005; Simsek and Kizmaz, 2017).

The importance of the moisture content of the kernels is great. Enzymatic activities in fruit with high moisture are higher than those in dry fruits. In general, the availability of moisture (Mo) encourages the growth of microorganisms and hence microbial spoilage of food, so it is not advisable to store almond kernels fresh. It has been determined that genotypes and varieties in this study do not have high moisture. In various studies, the moisture (Mo) contents changed from 3.08 to 4.43% (Simsek and Demirkiran, 2010), from 3.49 to 4.96% (Oliveira Sousa et al., 2011) and from 2.28 to 3.70% (Simsek and Kizmaz, 2017). In the present study, the moisture (Mo) content agreed with earlier data (Simsek and Demirkiran, 2010; Oliveira Sousa et al., 2011). The moisture (Mo) content should be reduced by drying to increase the safe storage time. The total ash (Ta) content was reported from 2.54 to 4.42% (Simsek and Demirkiran, 2010), from 2.74 to 3.05% (Ozcan et al., 2011), from 3.50 to 4.10% (Mbah et al., 2013), from 2.60 to 4.60% (Ahrens et al., 2005), from 2.30 to 3.70% (Ruggeri et al., 1998) and from 3.12 to 4.69% (Simsek and Kizmaz, 2017). Ash contents are in harmony with earlier reports (Simsek and Demirkiran, 2010; Ozcan et al., 2011; Mbah et al., 2013; Ahrens et al., 2005; Ruggeri et al., 1998; Simsek and Kizmaz, 20017). All genotypes and varieties except for SAHI250 and SAHI301 genotypes with respect to crude oil, all genotypes with respect to crude protein values and the genotypes SAHI232, SAHI250, SAHI278, SAHI301 and SAHI324, and the variety FERRAGNES with respect to sugar contents in this study are very important. Proximate acid compositions of almond genotypes and cultivars can change according to the genetic differences, maintenance and ecological conditions.

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Mineral composition of sweet almond genotypes and cultivars

In this study carried out on the almond genotypes and commercial varieties named as Ferragnes and Ferraduel grown in the same location known as the Firat riverside region during two consecutive years, the mineral composition of the genotypes and varieties were compared at the same location. According to the results from the one-way ANOVA analysis, the differences between the mineral contents of the genotypes and/or the varieties were shown in different letters statistically and were presented in *Table 3*. In this context, the most abundant minerals are potassium (K) and phosphorus (P), followed by magnesium (Mg) and calcium (Ca), while sodium, (Na) iron (Fe), zinc (Zn), manganese (Mg) and copper (Cu) are scarce. Among the minerals studied, potassium (K), the phosphorus (P), magnesium (Mg), and calcium (Ca) contents were the highest compared to the other minerals. However, all of the sodium (Na), iron (Fe), zinc (Zn), manganese (Mg) and copper (Cu) were only present at trace amounts. Our results are in agreement with the mineral contents for kernels of several almond genotypes and cultivars described in previous studies. In this context, Aslantas et al. (2001) found that mineral contents of selected almond genotypes naturally grown in Kemaliye district ranged from 98.50 to 187.00 mg100 g^{-1} for Calcium (Ca), from 360.80 to 513.40 mg100 g^{-1} for Magnesium (Mg), from 403.90 to 800.00 mg100 g^{-1} for phosphorous (P), from 1677.30 to 2051.10 mg100 g^{-1} for potassium (K). Ozcan et al. (2011) determined that mineral contents of five almond cultivars collected in Antalya and Mugla provinces ranged from 1315.00 to 1510.00 mg100 g^{-1} potassium (K), from 793.00 to 938.00 mg100 g^{-1} phosphorous (P), from 298.00 to 404.00 mg100 g^{-1} magnesium (Mg), from 183.00 to 294.00 mg100 g^{-1} calcium (Ca), from 29.00 to 38.00 mg100 g^{-1} sodium (Na), from 20.00 to 27.00 mg100 g^{-1} iron (Fe) and from 4.00 to 6.00 mg100 g^{-1} zinc (Zn), respectively. Simsek and Kizmaz (2017) determined that mineral contents of almond genotypes grown in Beyazsu district ranged from 646.27 to 925.13 mg 100 g^{-1} potassium (K), from 562.53 to 701.93 mg 100 g^{-1} phosphorous (P), from 217.13 to 367.27 mg 100 g^{-1} magnesium (Mg) and from 190.97 to 317.13 mg 100 g^{-1} calcium (Ca), respectively. Saura Calixto and Canellās (1982) observed that mineral composition of seven sweet almond varieties ranged from 649.00 to 824.00 mg100 g^{-1} potassium (K), from 462.00 to 595.00 mg100 g^{-1} phosphorous (P), from 239.00 to 280.00 mg100 g^{-1} magnesium (Mg), from 218.00 to 299.00 mg100 g^{-1} calcium (Ca), from 4.80 to 12.50 mg100 g^{-1} sodium (Na), from 3.10 to 4.40 mg100 g^{-1} iron (Fe) and from 0.90 to 1.30 mg100 g^{-1} copper (Cu). Barbera et al. (1984) reported that mineral composition of two almond varieties changed from 1546.00 to 1685.00 mg100 g^{-1} potassium (K), from 253.00 to 259.00 mg100 g^{-1} phosphorous (P), from 640.00 to 678.00 mg100 g^{-1} calcium (Ca), from 447.00 to 494.00 mg100 g^{-1} magnesium (Mg), from 24.30 to 25.80 mg100 g^{-1} copper (Cu). Saura Calixto et al. (1981) also reported that mineral contents of almond cultivars consisted of 766.00 mg100 g^{-1} potassium (K), 364.00 mg100 g^{-1} phosphorous (P), 227.00 mg100 g^{-1} magnesium (Mg) and 185.00 mg100 g^{-1} calcium (Ca). In general, the mineral contents in this study agreed with earlier reports (Barbera et al., 1984; Saura Calixto et al., 1981; Saura Calixto and Canellās, 1982; García-López et al., 1996; Aslantas et al., 2001; Ozcan et al., 2011; Simsek and Kizmaz, 2017). Considering macro and micronutrient values in this study, K, P, Ca and Mg (macro nutrients) and Na, Mn, Fe, Cu and Zn (micro nutrients) in all genotypes and varieties are important and statistically significant.

The minerals of almonds and other nuts are obtained from the soil and water applied during production. Thus, the mineral contents of almond kernels are affected by many

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ecological factors and agronomic practices including geographic location, soil composition, water source, irrigation regime, fertiliser components, and other aids to agronomic production. The mineral content can be influenced by plant genotypes, cultivars, maintenance and ecological conditions.

Fatty acid composition of sweet almond genotypes and cultivars

Fatty acid content is the most common feature for fat and oil characterization. It is related to oxidative stability as well as to some nutritional characteristics. In this study carried out on the almond genotypes and commercial varieties named as Ferragnes and Ferraduel grown in the same location known as the Firat riverside region during two consecutive years, the fatty acid compositions of the genotypes and varieties were compared at the same location. According to the results from the one-way ANOVA analysis, the differences between the proximate composition of the genotypes and/or the varieties were shown in different letters statistically and were presented in *Table 4*. Fatty acid composition of sweet almond genotypes and cultivars were presented in *Table 4*. Oleic (Oa) and linoleic acid (La) contents of sweet almond genotypes and cultivars were established as major fatty acids. The samples are rich in both oleic (Oa) and linoleic acids (La). However, they may be used as edible cooking and salad oils.

In previous works, Kiani et al. (2015) reported that some fatty acid contents of 40 wild-growing almond accessions ranged from 0.40 to 0.80% palmitoleic acid (Paa), from 1.00 to 3.40% stearic acid (Sa), from 4.60 to 9.50% palmitic acid (Pa), from 11.30 to 33.20% linoleic acid (La) and from 48.80 to 88.40% oleic acid (Oa). Askin et al. (2007) reported that some fatty acid contents in several almond genotypes ranged from 5.46 to 15.78% palmitic acid (Pa), from 0.36 to 2.52% palmitoleic acid (Paa), from 0.80 to 3.83% stearic acid (Sa), from 50.41 to 81.20% oleic acid (Oa) and from 6.21 to 37.13% linoleic acid (La). Celik and Balta (2011) determined that fatty acid contents of some almond genotypes ranged from 6.48 to 6.29% palmitic acid (Pa), from 0.41 to 0.64% palmitoleic acid (Paa), from 1.76 to 1.60% stearic acid (Sa), from 72.02 to 76.41% oleic acid (Oa) and from 18.92 to 14.71% linoleic acid (La). Balta (2013) reported that the fatty acid contents of seven almond genotypes ranged from 5.26 to 6.69% palmitic acid (Pa), from 0.33 to 0.45.00% palmitoleic acid (Paa), from 1.61 to 2.40% stearic acid (Sa), from 65.33 to 74.73% oleic acid (Oa) and from 17.36 to 25.17% linoleic acid (La). Martins et al. (2000) determied that fatty acid contents for twelve almond varieties ranged from 2.15 to 3.13% stearic acid (Sa), from 5.94 to 7.31% palmitic acid (Pa), from 17.52 to 29.89% linoleic acid (La) and from 58.96 to 69.68% oleic acid (Oa). Kodad and Socias I Company (2008) found that fatty acid contents of eight varieties and forty seven almond genotypes ranged from 63.00 to 78.00% oleic acid (Oa), from 12.00 to 27.00% linoleic acid (La), from 5.00 to 7.00% palmitic acid (Pa), from 1.20 to 2.50% stearic acid (Sa) and from 0.30 to 0.80% palmitoleic acid (Paa). Sathe et al. (2008) reported that fatty acid contents of eight almond cultivars ranged from 5.07 to 6.78% palmitic acid (Pa), from 57.54 to 73.94% oleic acid (Oa), from 19.32 to 35.18% linoleic acid (La) and from 0.04 to 0.10% linolenic acid (Lia). The results in this study are consistent with previous works (Kiani et al., 2015; Askin et al., 2007; Celik and Balta, 2011; Balta, 2013; Martins et al., 2000; Kodad and Socias I Company, 2008; Sathe et al., 2008).

Saturated fatty acid (SFA), unsaturated fatty acid contents (UFA) and UFA/SFA ratios in this study were presented in *Figures 2, 3* and *4,* respectively. Increased uptake of unsaturated fatty acids reduces risk of coronary artery disease. From this point of

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view, it is very important to prevent and treat chronic diseases such as cancer, because it is rich in unsaturated fatty acids, contain significant amounts of antioxidants and phytochemicals. In previous studies, Celik and Balta (2011) reported that fatty acid contents of twenty five almond genotypes ranged from 90.99 to 91.17% UFA, from 8.82 to 9.00% SFA and from 10.10 to 10.40 UFA/SFA, respectively. Balta (2013) found that fatty acid contents of almond genotypes ranged from 87.85 to 91.97% UFA, from 7.99 to 11.59% SFA and from 7.60 to 11.50 UFA/SFA. SFA and UFA compositions and UFA/SFA in the present study agreed with earlier studies (Celik and Balta, 2011; Balta, 2013). The use of samples with higher fatty acid levels might contribute to almond improvements directed at increasing nutritional value. In addition, fatty acid composition of kernel crude oils (Co) can vary widely among different plant genotypes and cultivars (Ozcan et al., 2011; Aitzetmüller, 1993), county (Sathe et al., 2008), irrigation status (Nanos et al., 2002), climatic conditions, maturity level and soil composition (Kodad and Socias I Company, 2008; Kornsteiner et al., 2006; Moayedi et al., 2011).

Conclusion

When evaluated statistically, the results in this study show that the sweet almond genotypes and cultivars have high levels of very valuable components. Fatty acids, proximate and mineral contents in particular almond kernel have a very important effect on health and can be included in the diet to improve human health. In this context, All genotypes and varieties except for SAHI250 and SAHI301 genotypes are rich in crude oil because their crude oil content is greater than 50%. Since all genotypes and varieties have crude protein values greater than 20%, they are protein rich. Because the genotypes SAHI232, SAHI250, SAHI278, SAHI301 and SAHI324, and the variety FERRAGNES have a sugar content greater than 4%, they are rich of sugar. It has been determined that genotypes and varieties in this study do not have high moisture. However, very few studies on minerals have thoroughly assessed different almond genotypes and cultivars in Turkey up to now. Considering macro and micro nutrient values, all genotypes and varieties are important and statistically significant. Almond has beneficial effects on human health since it contains a high level of unsaturated fatty acids / saturated fatty acids (UFA/SFA). UFA/SFA ratios of the genotypes SAHI132, SAHI198, SAHI250, SAHI278, and SAHI302 are greater than 11. Therefore, Adaptation studies should be performed in different ecological conditions with the genotypes SAHI132, SAHI198, SAHI232, SAHI250, SAHI278, SAHI301, SAHI302 and SAHI324, and variety FERRAGNES with other standard almond varieties for determination of commercial purposes and the food industrial applications. As a result, we believe that this study will be of considerable value in light of more detailed work.

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THE MERCURY (Hg) CONCENTRATIONS IN FEATHERS OF WILD BIRDS

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Abstract. In this study, mercury (Hg) concentrations were detected in feathers of 22 bird species from 10 different families living in terrestrial and aquatic ecosystems in Van Lake Basin in Turkey. The mercury rates in feathers were detected based on nutrition status and taxonomic classifications of bird species. Mercury levels were detected in 0.002 ± 0.007 mg/kg and 2.700 ± 0.560 mg/kg dry weight range. It was observed that there was a significant difference between the groups classified taxonomically ($p < 0.05$). The highest concentrations were detected in European Honey-buzzard (*Pernis apivorus*) 2.7 mg/kg, Lammergeier (*Gypaetus barbatus*) 0.45 mg/kg and Long-legged Buzzard (*Buteo rufinus*) 0.28 mg/kg species from Accipitridae family. And the lowest mercury concentration was detected in Golden Eagle (*Aquila chrysaetos*) from the same family (0.002 ± 0.007 mg/kg). Mercury was detected in the feather samples taken from the Egyptian Vulture, Lammergeier, Northern Lapwing, and Great Bustard, and these species were determined to be within different endangered categories according to the International Union for Conservation Nature (IUCN). Considering the trophic level (type of food) and systematic groups of the sampled species, it was determined that average mercury level was statistically significant in both cases. It was suggested that the mercury level of the carnivorous ones were higher than that of the herbivorous ones.
Keywords: *heavy metal, raptor birds, toxicology, endangered, East Anatolia, Turkey*

Introduction

Most heavy metals play an important role in the lives of living species. Heavy metals as an integral part of nature must be present in the structure of all living creatures in trace levels. If, these trace levels are exceeded, it shows toxic effect in the organism (Zolfaghari et al., 2007; Manjula et al., 2015).

As a result of developing technology and increasing human population, natural resources are consumed exceedingly and in consequence, overmuch waste is discharged into the nature. These wastes exceeding the bearing capacity of the nature accumulate in bodies of living species within the food web of the ecosystem through nutrition and environmental contact and consequently create risk (Nriagu and Pacyna, 1988; Dudka and Miller, 1999; Metcheva et al., 2010). The best indicator of a biological pollution of an environment is analyzing the creatures living in that environment and detecting toxic materials accumulated in their bodies.

Birds are ideal bio-indicator animals showing the best reaction about the structure of an ecosystem they live in. Analyzing these species located on the upper of the food web provides information on the status and condition of the related ecosystem (Burger, 1993). While, conducting these studies, present species should not be harmed in anyway. Therefore, analyzing the feathers taken from their bodies provides information on heavy metal rate of their environment. This does not only provide information on heavy metal concentrations in bird species but also the heavy metal concentrations in other living creatures under lower steps of the food web of birds which are foods of them (Battaglia et al., 2005).

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The metals taken by birds through nutrition during their growth are carried to their feathers through blood vessels and accumulate there by bonding with protein molecules (Burger, 1993; Dauwe et al., 2000; Goutner et al., 2001; Dauwe et al., 2003). The source of heavy metal concentrations in feathers is not only nutrition, but also some environmental factors such as contact with soil, water, atmospheric pollution and precipitations (Burger and Gochfeld, 1997; Movalli, 2000; Jasper et al., 2004; Muralidharan et al., 2004; Cardiel et al., 2011).

Using feathers for detecting heavy metals such as mercury provides advantages compared to other tissues (Furness et al., 1986, 1989; Burger, 1996; Movalli, 2000). These advantages are taking feather samples from living creatures without harming them, storing feathers easily and preserving them for a long time in a refrigerator without needing anything.

The purpose of this study is to determine the amount of mercury accumulated in structures of the birds located at an upper step of food web and living in different ecosystems of Van Lake Basin (Turkey) through nutrition. It is to reveal the differences between mercury concentrations by considering the birds' nutrition styles and places in systematic category.

Materials and methods

The feather samples used in this study were taken from 102 birds from 22 bird species. 22 bird species used in this study are the resident and migratory species living in aquatic and terrestrial ecosystems of Van Lake Basin (*Fig.1;* Adızel et al., 2010; Nergiz and Durmuş, 2017).

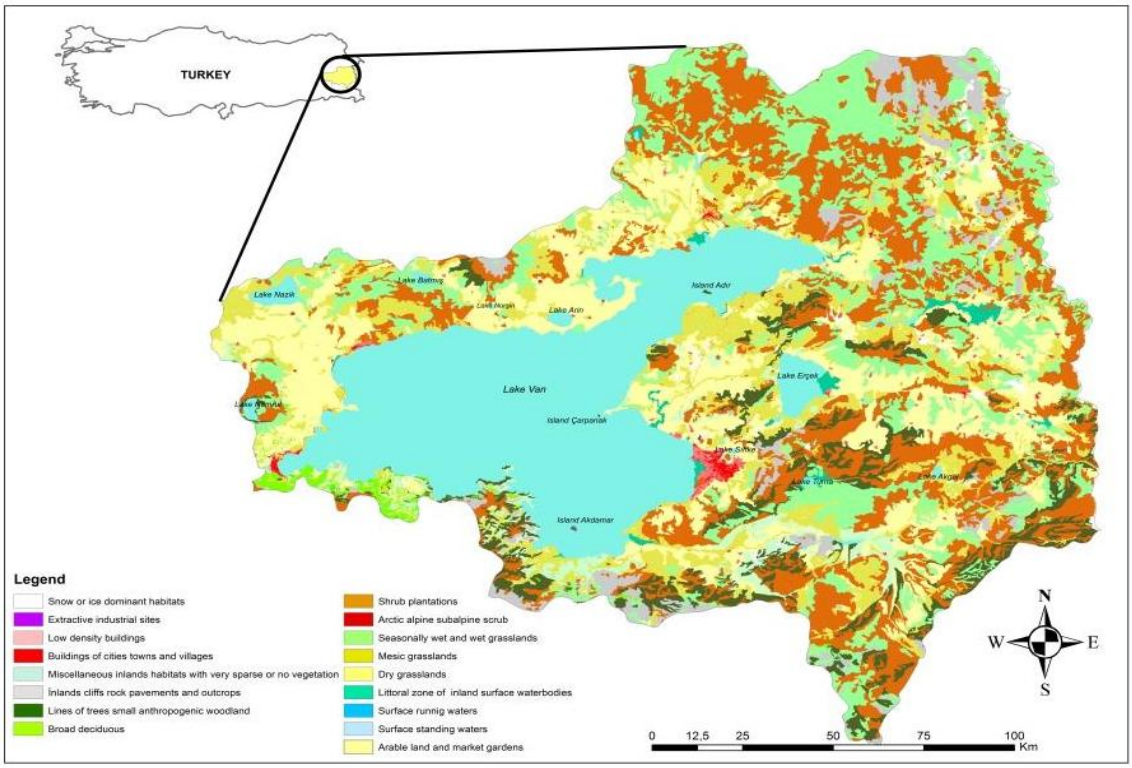


Figure 1. *Van Lake Basin and habitat types*

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These species were found injured or diseased in their ecosystems, taken to the Rehabilitation Center for Wild Animals of Van Yuzuncu Yil University, treated but then died. The feather samples were taken from these birds died between May 2016 and June 2017. Half of the sampled species were raptors.

Analysis of samples

The samples were transferred to the Science Application and Research Center of Van Yuzuncu Yil University for analysis. Feathers were washed with deionized water and acetone three times and then put in metal free polypropylene vials. All external pollution available in the feathers can be removed by washing the samples (Goede and Bruin, 1984).

The weights of the dry feather samples were detected and then they were put in an oven (70 °C) for 24 h. The dried feathers were cut with steel scissors and weighed on a precision scale at least 2 g (dry weight) from each sample and placed in glass containers. The prepared feather samples were burned with the tissue sample program in a microwave heating unit (Ethos Easy Advanced Microwave Digestion System, Milestone, 230/50 Hz, Italy) by adding 8 ml of 65% HNO_3 and 2 ml of H_2O_2 (Blust et al., 1988; Janssens et al., 2001). The 2 ml solution from the incinerated samples was diluted with distilled water to 10 ml and analyzed on the ICP-AAS (Thermo Scientific ICAP 600 series) instrument.

Calibration of the instrument was done with a certified solution (Multi element ICP QC standard solution-QCS-01-23E, Belgium). Mercury checked by National Institute of Standards and Technology (NIST) and Standard Reference Material (SRM). Accepted recoveries ranged from 95 to 105%.

Statistical analysis

The statistical analysis was done by the SPSS software (Version 23). Means and standard deviations were given in tables. We used one-way ANOVA and Tukey post-hoc comparison for evaluate effects of systematic categories and food manner on feather Mercury (Hg) levels.

Results and discussion

Average mercury concentrations in feather samples taken from 102 birds from 22 species and 10 families were taxonomically compared (*Tables 1–3*). Different levels of mercury were detected in all samples. According to the statistical analysis with 95% confidence range conducted in accordance with the trophic levels and taxonomic groups, it was observed that there were meaningful differences ($p < 0.05$).

The average mercury concentration varied from 0.002 mg/kg (Golden eagle) to 2.7 mg/kg-dry weight (European Honey-buzzard) (*Table 1*). The order of ten families from higher to lower Hg concentration is as follows: Accipitridae, Phasianidae, Otididae, Phoenicopteridae, Ardeidae, Falconidae, Strigidae, Anatidae, Ciconiidae, Charadriidae were compared (*Table 2*).

In the taxonomic evaluation conducted among the families, it was determined that the Accipitridae family had the highest mercury concentration while the Charadriidae had the lowest mercury concentration. It was observed that these differences between families were based on the nutrition and environmental differences between species.

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Table 1. *Mercury concentration (mg/kg dry weight) in feather of birds from eastern Turkey*

Family	Species common name	Scientific name	Number	Trophic level	Hg concentration mean ± std error
Accipitridae	European Honey-buzzard	*Pernis apivorus*	4	Carnivor	2.700±0.560
	Lammergeier	*Gypaetus barbatus*	3	Carnivor	0.450±0.370
	Egyptian Vulture	*Neophron percnopterus*	4	Carnivor	0.033±0.001
	Griffon Vulture	*Gyps fulvus*	4	Carnivor	0.019±0.002
	Western Marsh-harrier	*Circus aeruginosus*	5	Carnivor	0.050±0.033
	Long-legged Buzzard	*Buteo rufinus*	6	Carnivor	0.280±0.170
	Golden Eagle	*Aquila chrysaetos*	3	Carnivor	0.002±0.007
	White-tailed Eagle	*Haliaeetus albicilla*	4	Carnivor	0.015±0.001
Falconidae	Common Kestrel	*Falco tinnunculus*	5	Carnivor	0.054±0.007
Strigidae	Eurasian Eagle-owl	*Bubo bubo*	3	Carnivor	0.050±0.030
Ciconiidae	White Stork	*Ciconia ciconia*	4	Mix feed	0.012±0.001
Phoenicopteridae	Greater Flamingo	*Phoenicopterus roseus*	8	Invertebrate predator	0.130±0.460
Ardeidae	Grey Heron	*Ardea cinerea*	5	Mix feed	0.220±0.140
	Great Bittern	*Botaurus stellaris*	6	Mix feed	0.112±0.019
	Little Egret	*Egretta garzetta*	6	Mix feed	0.090±0.030
	Black-crowned Night-heron	*Nycticorax nycticorax*	7	Mix feed	0.040±0.003
Charadriidae	Northern Lapwing	*Vanellus vanellus*	3	Invertebrate predator	0.020±0.001
Phasianidae	Grey Partridge	*Perdix perdix*	5	Herbivor	0.150±0.020
Otididae	Great Bustard	*Otis tarda*	2	Herbivor	0.140±0.010
Anatidae	Common Teal	*Anas crecca*	6	Herbivor	0.020±0.002
	Ruddy Shelduck	*Tadorna ferruginea*	5	Herbivor	0.030±0.010
	Northern Pintail	*Anas acuta*	4	Herbivor	0.010±0.001

Table 2. *Mercury concentration (mg/kg dry weight) in the feathers according to families*

Family	Number	Mean	Std. d.	p	95% confidence interval for mean	
					Lower	Upper
Accipitridae	33	0.432	0.167	0.015	0.091	0.773
Phasianidae	5	0.146	0.036	0.001	0.101	0.190
Otididae	2	0.140	0.028	0.090	-0.114	0.394
Phoenicopteridae	8	0.131	0.046	0.026	0.021	0.240
Ardeidae	24	0.108	0.159	0.003	0.040	0.175
Falconidae	5	0.053	0.015	0.002	0.033	0.073
Strigidae	3	0.049	0.061	0.298	-0.102	0.201
Anatidae	15	0.016	0.014	0.001	0.007	0.023
Ciconiidae	4	0.012	0.003	0.005	0.006	0.017
Charadriidae	3	0.011	0.002	0.015	0.004	0.016

One-way ANOVA followed by Tukey's comparison between islands and water concentrations of metals. Significance level of $p < 0.005$ was used

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The highest Hg concentration determined in European Honey-buzzard (2.7 mg/kg), Lammergeier (0.45 mg/kg), and Long-legged Buzzard (0.28 mg/kg) belonging to Accipitridae family and Grey Heron (0.22 mg/kg-Ardeidae). Intermediate Hg concentration values were found in Grey Partridge (Phasianidae), Great Bustard (Otididae), Greater Flamingo (Phoenicopteridae), Great Bittern (Ardeidae), Little Egret (Ardeidae), Common Kestrel (Falconidae), Western Marsh-harrier (Accipitridae), Eurasian Eagle-owl (Strigidae), Black-crowned Night-heron (Ardeidae), Egyptian Vulture (Accipitridae), Ruddy Shelduck (Anatidae), Common Teal (Anatidae), Northern Lapwing (Charadriidae), and Griffon Vulture (Accipitridae) species; whereas the lowest Hg concentration values were found in White-tailed Eagle (Accipitridae), White Stork (Ciconiidae), Northern Pintail (Anatidae), and Golden Eagle (Accipitridae) species.

Birds of prey families (Accipitridae, Falconidae and Strigidae) have more than 2.5 times higher average Hg concentrations (overall average of average 0.178 mg/kg) than the rest of other families (overall average of average 0.08 mg/kg) (*Table 2*). With the exception of Strigidae and Otididae families, there was significant difference in other families ($p < 0.05$).

According to trophic level, species were separated into 4 classes as Carnivorous (41 samples belonging to 10 species), mix (vertebrate and invertebrate predator, 28 samples belonging to 5 species), invertebrate predators (11 samples belonging to 2 species) and herbivore (22 samples belonging to 5 species) (*Table 3; Fig. 2*).

Table 3. *Mercury concentration (mg/kg dry weight) in the trophic level of birds*

Family	Number	Mean	Std. d.	p	95% confidence interval for mean	
					Lower	Upper
Carnivore	41	0.358	0.136	0.012	0.082	0.633
Mix (vertebrate and invertebrate predator)	28	0.098	0.024	0.003	0.015	0.181
Invertebrate predator	11	0.095	0.028	0.025	0.035	0.152
Herbivore	22	0.056	0.013	0.001	0.027	0.085

One-way ANOVA followed by Tukey's comparison between islands and water concentrations of metals. Significance level of $p < 0.005$ was used

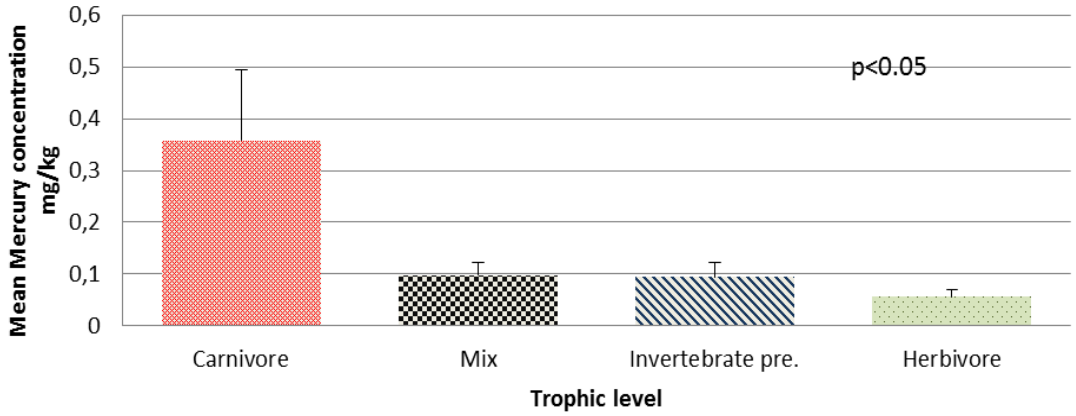


Figure 2*. Mercury (Hg) concentration in trophic levels*

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Accipitridae, Falconidae, and Strigidae families which eat vertebrates provided the highest mean mercury concentration in feathers (0.358 mg/kg), whereas the lowest mean Hg concentration value was found in the feathers of herbivorous birds (0.056 mg/kg).

Additionally, Mercury (hg) was detected in the feather samples taken from the Egyptian Vulture, Lammergeier, Northern Lapwing, and Great Bustard, and these species were determined to be within different endangered categories according to the International Union for Conservation Nature (IUCN). Related these species no work was done in Turkey. Egyptian Vulture listed in Endangered (EN) category in IUCN has mercury concentration of 0.033 mg/kg, Lammergeier and Northern Lapwing listed in Near Threatened (NT) have mercury concentrations of 0.45 mg/kg and 0.02 mg/kg, respectively. Great Bustard listed in Vulnerable (VU) category has mercury concentration of 0.14 mg/kg. In the literature Hg concentration was determined as 0.055-0.139 ppm (Xiuhua et al., 1998).

In the analysis and studies, it was observed that taxonomically different mercury (Hg) levels were influenced species-related factors such as age, gender and etc. and from environmental factors such as climate (Monteiro and Furness, 1995). However, the individuals taken as samples in our study were completely adults and the same gender so no difference was observed in specie-related individual variations. It was suggested that climatic factors and consequent migration activities as well as pubescence influence mercury concentration (Stewart et al., 1999; Mashroofeh et al., 2015). The species sampled in this study are the species which have not influenced from migration activities, yet, have been present in the region since last spring and the resident ones permanent in the field. Organic mercury is stored at the bottom of feathers grown post incubation period based on nutrition density (Zolfaghari et al,. 2007).

Several studies indicate that the mercury concentration in feathers vary based on types of nutrition and the environment where species are present (Braune and Gaskin, 1987; Cifuentes et al., 2003; Burger, 2013). Especially, the raptors at the top of food web are influenced more from bio-accumulation as they always have carnivorous food. Within this respect, they are more sensitive and under higher risk than other bird species.

In our study, mercury concentration was detected in the samples from all taxonomic groups. However, it was observed that the mercury concentration levels in Accipitridae family covering raptors has significantly higher mercury concentration compared to other families ($p < 0.05$; *Table 3*). In general, although, mercury concentration is high in raptors, the concentration was found the lowest in White-tailed Eagle and Golden Eagle species (*Table 1*). It is because that these species live in high-altitude natural areas and stay away from pollutive fields with anthropogenic source.

According to a study based on Family taxonomy which was conducted in Iran as the closest country to the study region, the highest mercury concentration was detected in Strigidae family (0.88 mg/kg) (Zolfaghari et al., 2007), in our study, the value for this family was 0.05 mg/kg. In the same study, average mercury concentration detected for the Accipitridae family was 1.25 mg/kg while, in our study, the value for this family was about two times higher and detected as 2.7 mg/kg. In both studies, the lowest concentration value was detected in the Charadriidae family. The reasons of basic differences between these two studies conducted in close regions are different numbers of sampled individuals and density of pollutant factors in nutrition environment of species.

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Mercury level also varies between the Carnivorous species based on types of nutrients. Concentration differences may be observed on the birds consuming high amounts of fish or the ones eating large fish. In the study, it was observed that the species of Ardeidae family have higher levels of mercury concentration compared with the members of another family which eats little fish (Burger, 2002). However, the level of mercury concentration detected in the members of Ardeidae family is around midlevel, not high.

The bird species feeding on primary producers such as vegetal nutrients, seeds, leaves, roots, fruits and algae are the group which are influenced the least from environmental pollutants (Zolfaghari et al.,2007; Behrooz et al.,2009). Parallel to other studies on this subject, the mercury concentration level was also found the lowest in the birds feeding on plants in our study (0.56 mg/kg). However, it was observed high in Great Bustard and Grey Partridge species feeding on seeds in agricultural fields and located in areas where agricultural pesticides are extensively used (*Table 1*).

Conclusion

The main purpose of this study is to determine the mercury level accumulated in feathers of the bird species living in terrestrial and aquatic ecosystems of Van Lake Basin located in the Eastern Region of Turkey. This study is the region's first heavy metal study conducted on the birds. In this study, the highest mercury concentration was detected in the Accipitridae family at the top of the food web (chain) with raptor species feeding in agricultural areas. Four raptor bird species that lives in rural habitats (*Gypaetus barbatus, Neophron percnopterus, Otis tarda, Vanellus vanellus*) were within the endangered species of the IUCN category.

With this study, it was suggested that the mercury level is correlated with nutrition style and habitat characteristics. While, the mercury level was found high for the regions of Van Lake Basin with agricultural activities and city pollution, it was determined that the species living in rural and high-altitude areas without pollution have lower mercury levels.

As the mercury concentrations detected in feathers reflect the amounts in their blood, they reveal the risk the species are exposed to. At the same time, it provides insight on other groups of the food web at lower steps of the birds.

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THE GENETIC RELATIONSHIP BETWEEN SOME STRAWBERRY CULTIVARS AND F1 POPULATION DERIVED FROM THESE CULTIVARS

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Abstract. In this study, while Osmanli strawberry, which has some superior characteristics like taste, aroma and male sterility that makes breeding studies easier, has been used as maternal parent, three local (Karacilek, Tuylu and Deli) cultivars in Turkey and three standard (Kabarla, Sweet Ann and Sweet Charlie) strawberry cultivars were used as paternal parents. Molecular analyses were conducted for determination of polymorphism levels by using 14 UBC-ISSR primers for the examination of 52 F1 genotypes that included selections as a result of hybridization studies, parents that were used in hybridization studies, and *Fragaria chiloensis*. 76 DNA bands were obtained in total and 60 of them were determined as polymorphic. The resulting data of the evaluation of ISSR-PCR bands, were analysed in Popgene32 and MEGA5.0 packaged software and the dendrogram was obtained according to the UPGMA method. The dendrogram was separated into two major groups at the 20% difference level, one is larger and the other is smaller. While *Fragaria chiloensis*, Tuylu-2 local cultivar, CC48 and CA97 F1 genotypes participated in the small group, the other F1 genotypes and parents participated in the larger group.
Keywords: *hybridization breeding, ISSR, molecular, Osmanli cultivar, strawberry*

Introduction

Strawberry is one of the rare fruit species that can be grown in large ecological zones, and it is known and grown in Turkey for a long time (Konarli, 1986). When the strawberry was classified botanically in the world, it was placed in *Rosales* family and *Fragaria* genus (Hancock and Luby, 1993). Most of strawberries that are grown commercially, belong *Fragaria x ananassa* species that was derived from hybridization of two local American species (*F. chiloensis* X *F. virginiana*) (Hancock, 1999).

More than 20 strawberry species with different ploidy levels were identified. *F. vesca* and *F. viridis* (2n = 2x = 14) are found as diploid species in the flora of Turkey. As well as these wild species, octoploid *F.* × *ananassa* were used for cultivation in Turkey for a long time, generating strawberry genetic resources. Osmanli and Arnavutkoy local cultivars were started to grown in limited areas because of cultivation of the new cultivars with higher quality and yield from another countries has become more common Osmanli cultivar that is known by its taste, colour and aroma, is only grown in a very small area in Eregli and Bartin regions in the West Black Sea Region of Turkey by few growers (Demirsoy and Serce, 2016). Dokuzoguz (1963) also reported that Eregli, Karsiyaka, and Kestel have specific characteristics comparing to the cultivars of Arnavutkoy, Eregli, Madame Moutot and Mangro, which are all called Osmanli or Arnavutkoy with small, oval-shaped, light-coloured fruits but have different other characteristics.

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Modern strawberry cultivation started in the 1960s in Turkey (Konarli and Philippe, 1968). Since the strawberry cultivation started, the main purpose is having a corner on the domestic and foreign market by doing high quality and early-grown cultivation. Besides the superior aromatic characteristics, profitable cultivation with local cultivars is not possible due to low yield, soft fruit flesh and small fruits. Although strawberry cultivars that is originated from another countries have earliness and high yield characteristics, they have middle level aromatic characteristics. Because of these reasons, breeding studies have being conducted that local cultivars have been used as parents since 1960. In the most of these studies, Osmanli strawberry, which have some superior characteristics (taste, aroma, the situation of fruit stalk, etc.) and male sterility that makes breeding studies easier, has been used as maternal parent (Kaska and Paydas, 1986; Ustun and Paydas, 1995; Paydas et al., 1996; Caglar and Paydas, 2002; Kiyga, 2009).

Modern breeding methods were developed especially after the beginning of the 20th century. In the last 30 years, 38 cultivars in Turkey and more than 900 cultivars in the world have been developed and registered (Della Strada and Fideghelli, 2011; Chandler et al., 2012; Turemis and Agaoglu, 2013; Faedi and Baruzzi, 2016).

The knowledge about genetic structure is important, especially when it is worked with wild species (Ahmadi and Bringhurst, 1991). Morphological, physiological and cytological characteristics were used at the beginning for the determination of genetic variation, afterwards, biochemical markers were developed. Furthermore, molecular studies are conducted recently (Scarano et al., 2002).

Molecular markers are derived from the DNA in the cell of the plants. Thus, molecular markers are almost 100% reliable for the determination of the diversity between plant populations and plant genotypes. Today, molecular markers are used commonly in plant taxonomy studies, breeding and evaluation of genetic resources (Gulsen and Mutlu, 2005).

DNA markers are more preferred than isoenzyme, biochemical and morphological markers in the studies about the determination of species and cultivars. PCR based DNA markers; AFLP Amplified Fragment Length Polymorphism), CAPS (Cleaved Amplified Polymorphic Sequences), ISSR (Inter Simple Sequence Repeats), SSR (Simple Sequence Repeats) and RAPD (Random Amplified Polymorphic DNA) were used on many studies about strawberry (Tyrkal et al., 2002; Kunihisa et al., 2003, 2005; Kashyap et al., 2005; Bassil et al., 2006; Milella et al., 2006; Serce et al., 2007). While CAPS, RFLP and SSR are codominant, AFLP, ISSR and RAPD are dominant molecular markers (Kafkas, 2006).

In ISSR (Inter Simple Sequence Repeats) method, one primer is used and it has two, three and four sequence repeat on it. This method is used commonly in the determination of DNA fingerprint and genetic relationships (Cekic et al., 2001; Arnau et al., 2002; Kuras et al., 2004; Debnath et al., 2008; Nunes et al., 2013).

Arnau et al. (2002) scanned eighteen ISSR primers and selected five of them. They used those primers for characterization of genotypes of 30 different strawberry (*F. x ananassa* L.) cultivars and they obtained 113 polymorphic bands.

Serçe et al. (2006) studied with 16 cultivars belong to *F. chiloensis, F. virginiana* and *Fragaria x ananassa*, and Osmanli, Karacilek, Tuylu and Arnavutkoy local cultivars. Although Osmanli strawberry was morphologically similar with *F. chiloensis* clones, it was observed in the dendrogram obtained by 13 SSR primers that Osmanli, Karacilek, Tuylu and Arnavutkoy cultivars were different with the other three species

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and generated different group. Moreover, researchers suggested that more primers should be used with the purpose of obtaining more reliable results.

The aim of this study was the determination of genetic relationships by using ISSR markers between 52 F1 genotypes that were selected according to their superior morphological, pomological and physiological characteristics, parents that were used in hybridization studies, and *Fragaria chiloensis* within the scope of 'Osmanli Strawberry Breeding' project that is conducted in University of Gaziosmanpasa. Obtained data were evaluated according to the UPGMA dendrogram and Principal coordinate analysis

Material and method

Material

In this study, within the scope of 'Osmanli Strawberry Breeding' project that is conducted at the University of Gaziosmanpasa in Turkey, 52 selected F1 genotypes, the parents (Osmanli and eleven pollinators) as well as *Fragaria chiloensis* for comparision were used in molecular analysis.

In the hybridization programme, while Osmanli strawberry cultivar which is morphologically male sterile was used as maternal parent, 8 local genotypes ('Osmanli-1', 'Osmanli-2', 'Osmanli-3', 'Osmanli-4', 'Tuylu-1', 'Tuylu-2', 'Karacilek-1', 'Karacilek-2') (*Table 1*), from Eregli district of Zonguldak province and 3 standard cultivars ('Kabarla', 'Sweet Ann' and 'Sweet Charlie') provided from private seedling companies were used as pollinators.

Table 1. *Coordinates belongs to local parents, and names of F1 genotypes*

Local parent			
Sample Code (ID)	**Altitude (m)**	**Latitude**	**Longitude**
Osmanli-1	220	41° 18.309K	031° 26.666D
Osmanli-2	267	41° 18.528K	031° 26.795D
Osmanli-3	237	41° 17.659K	031° 28.026D
Osmanli-4	152	41° 16.919K	031° 28.879D
Karacilek-1	220	41° 18.309K	031° 26.666D
Karacilek-2	237	41° 17.659K	031° 28.026D
Tuylu-1	237	41° 17.659K	031° 28.026D
Tuylu-2	152	41° 16.919K	031° 28.879D

F1 genotypes					
ID	**Parents**	**ID**	**Parents**	**ID**	**Parents**
CC-4	Osmanli3XSweet Charlie	DB-118	Osmanli4XKabarla	DC-63	Osmanli4XSweet Charlie
CC-35	Osmanli3XSweet Charlie	DB-119	Osmanli4XKabarla	DC-104	Osmanli4XSweet Charlie
CC-42	Osmanli3XSweet Charlie	DB-137	Osmanli4XKabarla	DC-126	Osmanli4XSweet Charlie
CC-48	Osmanli3XSweet Charlie	DA-1	Osmanli4XSweet Ann	DC-136	Osmanli4XSweet Charlie

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CC-60	Osmanli3XSweet Charlie	DA-4	Osmanli4XSweet Ann	CA-3	Osmanli3XSweet Ann
CC-64	Osmanli3XSweet Charlie	DA-6	Osmanli4XSweet Ann	CA-15	Osmanli3XSweet Ann
DB-7	Osmanli4XKabarla	DA-9	Osmanli4XSweet Ann	CA-23	Osmanli3XSweet Ann
DB-15	Osmanli4XKabarla	DA-40	Osmanli4XSweet Ann	CA-87	Osmanli3XSweet Ann
DB-35	Osmanli4XKabarla	DA-61	Osmanli4XSweet Ann	CA-97	Osmanli3XSweet Ann
DB-41	Osmanli4XKabarla	DA-87	Osmanli4XSweet Ann	CB-26	Osmanli3XKabarla
DB-48	Osmanli4XKabarla	DA-88	Osmanli4XSweet Ann	CB-86	Osmanli3XKabarla
DB-49	Osmanli4XKabarla	DA-95	Osmanli4XSweet Ann	CK1-9	Osmanli3XKaracilek1
DB-57	Osmanli4XKabarla	DC-7	Osmanli4XSweet Charlie	CK1-33	Osmanli3XKaracilek1
DB-68	Osmanli4XKabarla	DC-42	Osmanli4XSweet Charlie	DK1-24	Osmanli4XKaracilek1
DB-73	Osmanli4XKabarla	DC-44	Osmanli4XSweet Charlie	CC-2	Osmanli3XSweet Charlie
DB-88	Osmanli4XKabarla	DC-54	Osmanli4XSweet Charlie	CC-19	Osmanli3XSweet Charlie
DB-97	Osmanli4XKabarla	DC-60	Osmanli4XSweet Charlie	DB-69	Osmanli4XKabarla
DB-84	Osmanli4XKabarla				

Method

DNA isolation

DNA isolation was done from 1.5-2 cm length young leaf samples from each genotype according to the Mini-CTAB method belongs to Doyle and Doyle (1987).

PCR amplification

ISSR method was used because generated bands are reproducible (reliability) and have high polymorphism (scorability), and also, scanning large number of different locus is possible (Prevost and Wilkinson, 1999; Cekic et al., 2001).

For the determination of polymorphism levels, 14 primers were used that were selected from 100 UBC primers, which was used for '*Fragaria vesca'* before (*Table 2*) (Cekic et al., 2001). PCR method with constant annealing temperature that was used by Cekic et al. (2001) and Touchdown PCR method by Sargent et al. (2003) were modified and used. ISSR PCR reaction was done with 2 µL DNA (10 ng/µL) and 23 µL reaction composition [5 µL 10X PCR buffer solution, 2 µL 25 mM Mg^{+2}, 1.25 µL 2.5 mM dNTP, 0.1 µL Taq DNA Polymerase, 1 µL 0.5 µM primer and 13.65 µL PCR water]. PCR products were separated in 100V constant voltage during 2 hours by using 1 X TBE buffer as a gelling agent and the electrode buffer solution, and 2% agarose gel for electrophoresis. Afterwards, DNA bands were imaged under ultraviolet lights.

Data analyses

Data, which were used in the statistical analyses, were obtained by the method that is about scoring one (1) when there was ISSR band and scoring zero (0) when there was not ISSR band. Similarities and differences between genotypes were determined in the molecular level. Principal Coordinates Analysis (PCoA) was done by similarity coefficient. Data were analysed in Popgene32 version 1.32 (Population Genetic

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Analysis) and MEGA 5.0 (Molecular Evolutionary Genetic Analysis) packaged software and the dendrogram was obtained according to the UPGMA (Unweighted Pair-Group Method With Arithmetic Average) method (Parmaksiz and Ozcan, 2011). Furthermore, generating similarities/differences matrix between genotypes and Principal Coordinates Analysis (PCoA) were done by using NTSYS-pc ver. 2.10d (Numerical Taxonomy and Multivariate Analysis System) packaged software that was developed by Rohlf (2002).

Table 2. *ISSR primers*

Primer name	5' – 3' sequence	Degree of dissociation (C)	Primer name	5' – 3' sequence	Degree of dissociation (C)
807	AGAGAGAGAGAGAGAGT	50	888	BDBCACACACACACACA	52
808	AGAGAGAGAGAGAGAGC	53	889	DBDACACACACACACAC	52
810	GAGAGAGAGAGAGAGAT	50	890	VHVGTGTGTGTGTGTGT	52
811	GAGAGAGAGAGAGAGAC	53	891	HVHTGTGTGTGTGTGTG	52
835	AGAGAGAGAGAGAGAGYC	54	856	ACACACACACACACACYA	53
841	GAGAGAGAGAGAGAGAYC	54	826	ACACACACACACACACC	52
842	GAGAGAGAGAGAGAGAYG	55	844	CTCTCTCTCTCTCTCTRC	53

Results

Some DNA bands were not clear may be due to PCR gel imaging system or ethidium bromide. Therefore, scoring studies were done with clear DNA bands. 76 DNA bands were determined at the end of the study and 60 of them were polymorphic. Band number obtained from primers varied between 3 and 8, additionally, the average band number was 5.43 and the average polymorphic band number was 4.29. The highest band number was obtained from primer-810 (8 bands) and the lowest band number was obtained from primer-841 (3 bands). DNA bands, which were generated by primer-835, 841, 844 and 888, were observed as polymorphic. The lowest polymorphism rate was determined on primer-842 with 50%. The polymorphic band rate average was determined as 80.14% on 14 primers.

A dendrogram was obtained according to the UPGMA method (*Fig. 1*). The dendrogram was separated into two major groups at the 20% difference level, one is big and other is small. While *Fragaria chiloensis*, Tuylu-2 local cultivar, CC48 (Osmanlı-3 x Sweet Charlie) and CA97 (Osmanlı-3 x Sweet Ann) F1 genotypes participated in the small group, the other F1 genotypes and parents participated in the big group. It was observed that the big major group was separated again into groups with 17% level.

In this study, the highest similarity was determined between Osmanli-3 and Osmanli-4 with 3%, and the second highest similarity was determined between Sweet Charlie and Kabarla. It was also observed that Osmanli-1, Osmanli-2 and Osmanli-3 were in the same subgroup.

It is known that Scatterplot PCo1 and PCo2 analyses support the results of the distribution of dendrogram (Ozyurt, 2011). In this study, it was determined that the distribution of genotypes on the Scatterplot (*Fig. 2*) obtained with Principal

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Coordinates Analysis (PCoA), showed similarity with distribution on the dendrogram (*Fig. 1*). 'Osmanli-1 (OsmA)', 'Karacilek-2 (Kara-C)', 'Tuylu-2 (Tuylu-D)', 'CA-97' and '*Fragaria chiloensis* (FC)' were out of the high-density group according to Principal Coordinates Analysis 1 and 2. Furthermore, 'Karacilek-2 (Kara-C)', 'Tuylu-2 (Tuylu-D)', 'Sweet Charlie', '*Fragaria chiloensis* (FC)', 'CC-48', 'CA-97' and 'DB-97' were out of the high-density group according to Principal Coordinates Analysis 1 and 3.

Discussion and conclusion

76 DNA bands were determined at the end of the study and 60 of them were polymorphic. Cao et al. (2000) stated that 50 polymorphic bands are sufficient to determine similarities and differences between genotypes successfully. It was observed that 60 obtained polymorphic bands are sufficient to work with. The polymorphic band rate average was determined as 80.14% on 14 primers. Arnau et al. (2002) used 5 ISSR primers on characterization of 30 different strawberry (*F. x ananassa* L.) genotypes and it was stated that 113 polymorphic bands were obtained.

Kuras et al. (2004) used ISSR and RAPD marker methods for determination of genetic relationships between 24 strawberry cultivars, which were obtained by breeding program conducted in Poland. Moreover, these two marker methods were compared in the same study. 41 of 90 ISSR primers and 23 of 48 RAPD primers were found as polymorphic. As a result of the UPGMA analyses, genetic relationships were determined and the family tree was generated. As a result of comparison of marker methods, ISSR was found better than RAPD in terms of genetic relationships.

The dendrogram obtained from genotypes was separated into two major groups, one is big and other is small, and the separation was done successfully with 14 ISSR primers. Debnath et al. (2008) determined genetic relationship of 27 strawberry cultivars by using 17 ISSR primers, and they obtained 225 polymorphic bands. The results were evaluated with UPGMA analysis, and it was stated that genetic relationship between cultivars varied between 63-77%.

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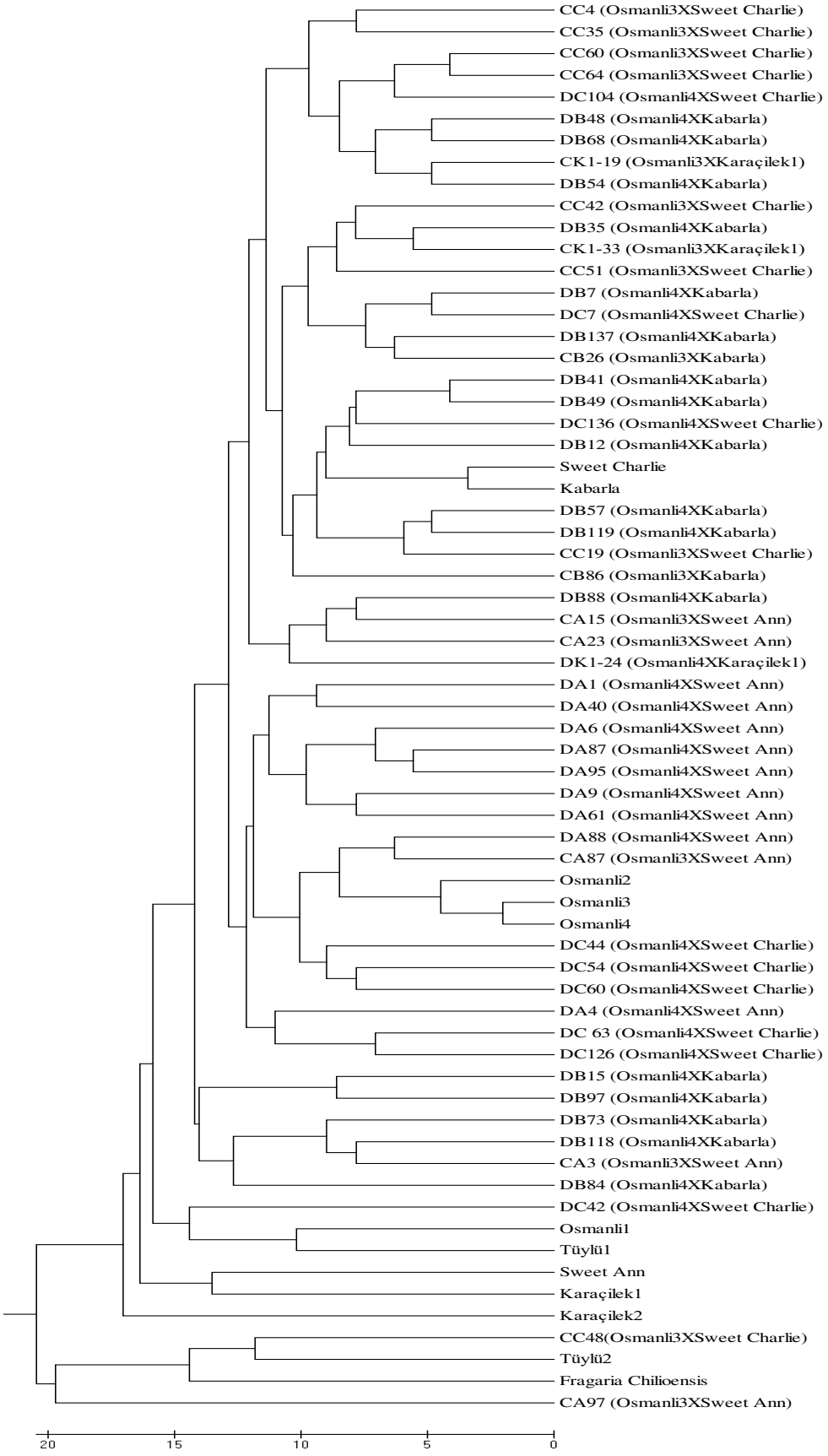


Figure 1. *Dendrogram obtained with cluster analysis*

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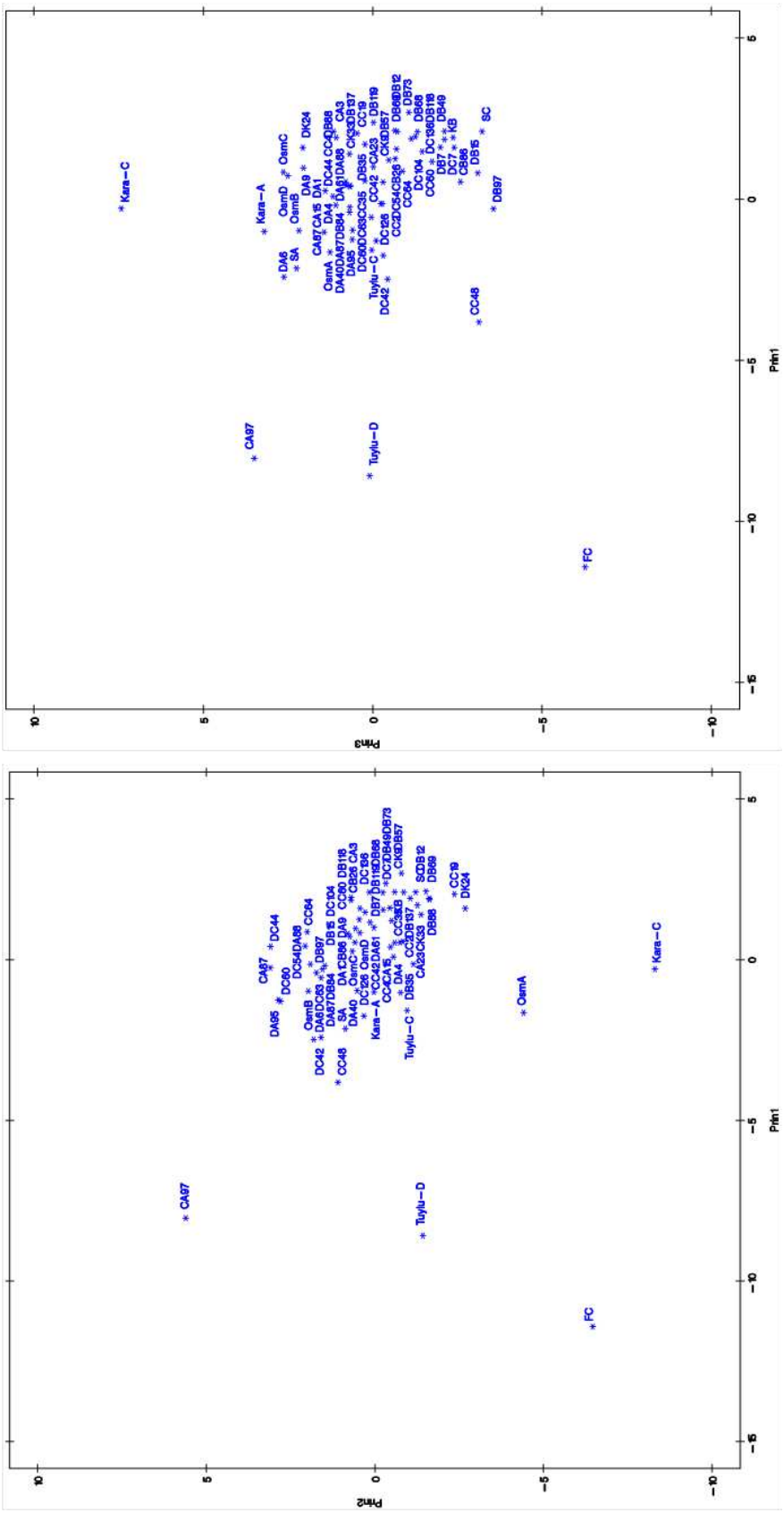


Figure 2. *Scatterplot of PCo2 vs PCO1 and scatterplot of PCo3 vs PCO1 obtained by principal coordinate analysis*

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In the literature, it was stated that 'Osmanli' local cultivar and *F. chiloensis* show similarity, however, Tuylu-2 genotype and *F. chiloensis* showed similarity with 14.00% in this study. All the local genotypes were collected from Eregli district in Black Sea Region, also, it was observed that Tuylu and Osmanli local cultivars showed similarity in terms of fruit structure. *F. chiloensis* and Tuylu local cultivars were in the same group, thus, it is thought that Osmanli local cultivar changed by the time due to cross pollination.

Selected F1 genotypes will be used in the further studies leading to obtain new strawberry cultivars. Specific molecular markers could also be generated using these F1 genotypes.

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A NOVEL STUDY OF THE INTERRELATIONSHIP OF SEASONALITY, SATELLITE DATA AND WEED COMPOSITIONAL CHANGES OF THE AGRO-ECOLOGICAL SYSTEM OF GUJRAT, PAKISTAN

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Abstract. The adverse effects of the weed flora on the agricultural yield are well documented. In an agro-ecosystem, weed species distribution pattern depends on both surrounding environment and farming practices. This first ever study was designed to compare and determine the weed species richness, distribution pattern and their ecologically meaningful groups in wheat and maize field of Gujrat, Pakistan. In this study, seasonal variation variable and satellite derived measurements like normalized difference vegetation index (NDVI), normalized difference moisture index (NDMI) and topographic wetness index (TWI) were calculated for each vegetation sample and their contribution towards explaining variations in the weed species data was tested by using direct unimodal ordination method. Phytosociological data was collected from 50 crop fields (25 each from wheat and maize fields during winter and monsoon season respectively) comprising of 250 sub-samples/plots (size 1 × 1 m) during 2016. The results of Monte Carlo testing and cluster analysis showed that there were four statistically significant weeds groups (i.e. 1. *Anagallis-Medicago-Phalaris* or AMP-Wheat weed group; 2. *Chenopodium-Convolvulus-Vicia* or CCV-Wheat weed group; 3. *Cyperus Malva-Amaranthus* or CMA-Maize weed group; 4. *Cannabis-Cynodon-Digera* or CCD-Maize weed group). A total of 60 weed species were recorded and the majority of them belongs to Poaceae and Amaranthaceae families. The majority of weed species flowered during monsoon season whereas therophytic life form and microphyllous leaf size categories were the most dominant. The highest mean weed diversity, NDVI and NDMI values were recorded for the weed communities of maize fields whereas the higher mean TWI value was recorded for the weed groups of wheat fields, suggesting strong linkage with the local climatic conditions. Canonical correspondence analysis (net effects) suggested that sum of local climatic conditions (seasonality variable) was found to be the most important in controlling the variations in the weed species data followed by longitude and altitude variables and least by the NDVI, NDMI and TWI. It is concluded that effective weed management activities are required to enhance the yield of these two important crops in the area. Thus, further studies related to determination of weed types and their distribution pattern by using factors like fertilization (type, timing, quantity), wheat and maize cultivar, land preparation (types, timing and intensities), soil management, pesticides (usage, type, quantity, quality), irrigation (type like rain-fed, tube-well, canals; timing, quantity), socio-economic and anthropogenic activities, and their relationship with the yield variable on temporal and spatial scales are recommended for this agro-ecosystem of the study area.

Keywords: *wheat and maize crops, normalized difference vegetation index, normalized difference moisture index, topographic wetness index, cluster analysis and ordination*

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Introduction

The weed species though might be useful as ecosystem services providing agents but due to their competitive nature (Anderson, 1996), their occurrence in a maintained agro-ecosystem is considered hazardous for agricultural yield. The control and management of weed species in such systems is desirable and practiced since centuries. Pakistan is a rapidly developing country and agriculture sector provides leading share in country's economy. A large number of factors like inappropriate land preparation, modest understanding about crop varieties, seed quality under varied micro-habitats amongst the farmers, water logging, salinity, fertilizers and pesticides types, their effectiveness including high cost and poor availability, agri-land fragmentation, small holdings, availability of water for irrigation and poor weed management are responsible of reduced agricultural production. The latter factor is most important and can reduce the crop yield from 15-50%, thus these are undesired plant species growing in any agro-ecosystem (Qureshi and Bhatti, 2001). They consume three to four time more soil nutrients than a weeds free crop. Their seeds becomes dormant and remained viable for many year, and their seed coat can resist adverse climatic and edaphic conditions (Qureshi and Bhatti, 2001). Similarly even some alien invasive weeds like *Parthenium hysterophorus* L. (Compositae) are threatening local biodiversity and agricultural production (Shabbir and Bajwa, 2006). The weed species synthesize and deliver different phytotoxins or allelochemicals in surrounding, thus causing increase in the costs of different cultural practices, decrease the effectiveness of agricultural tools, fertile lands and germination capability of crops seeds (Algandaby and Salama, 2016).

Agriculture is the major source of earning livelihood in Pakistan whereas wheat is a globally important Rabi cereal crop including Pakistan. It is cultivated at variety of micro-habitats from the sea level to high altitude mountains (Hussain et al., 2004). About more than 1/3rd of the world population is dependent on this crop for their dietary mainstay (Jakhar et al., 2005). But national losses in wheat yield due to weed infestation estimated about 28 billion/year in Pakistani rupees (Hussain et al., 2004). Wheat yield increases in the country during the last few decades but still its average yield/ha is far behind (only 30 to 35% of potential yield) than the developed countries (Hussain et al., 2007). Total global wheat production was about 724 million tons during 2017, whereas it was only about 25.5 million tons in Pakistan although cultivated on an area of 8494 million hectares. Thus, Pakistan average wheat yield was 2585 kg/ha, and was very low compared to European Union, India, China and Russian Federation (Iqbal et al., 2018).

Maize (*Zea mays* L.) is an annual, cross pollinated, short day plant. It was grown on an area of 132 million hectare with yield of 570 metric tons globally (Ashique et al., 1997). Within Pakistan, it is continuously gaining significant position in crop husbandry. This species has higher yield potential and complete its life cycle within short duration. During 2012-13, Pakistan maize yields was about 36.581 metric tons and cultivated on the total area of 0.981 million hectare, whereas during 2017, the production was about 5.8 million tonnes (FAO, 2017). Many weed species as tough competitors, compete with the maize crop for available resources (Malik et al., 2006; Ahmad et al., 2016a). Weed species can severely reduce maize production during initial stage of growth and ultimately effect yield. The loss of maize yield due to weed infestation was recorded quite high (about 16-40%) in Pakistan (Ahmad et al., 2016b).

By using satellite remote sensing (SRS) data, monitoring of vegetation change pattern can be conveniently understood and improve our knowledge about the spatial distribution and characteristics of vegetation of any area. Similarly, changes in the land

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use pattern can be used to investigate the relationship between population density and vegetation pattern changes on both spatial and temporal scale. The SRS data can illustrate the type, size and condition of the vegetation of any area. Sultana et al. (2014) found a significant correlation between the normalized difference vegetation index (NDVI) and yield behavior of the different wheat cultivars and nitrogen fertilization, and thus concluded that NDVI is a good tools for wheat yield prediction especially at crop maturity stage.

No record exists for such ecological exploration, and to fill this gap, this study was aimed to answer the following research questions for the first time from the study area. 1. What are the number of ecologically meaningful weed groups of wheat and maize crops, community types, composition and diversity? 2. What are the indicators species and their strength of association with their associated groups? 3. What about the leading weed families, genera, species, weeds flowering response (timing), life form and leaf size spectra? 4. How seasonality (wheat plots = winter = 1; maize plots = monsoon = 2), topographic and SRS variable are related amongst themselves, and with the weed species distribution pattern in the study area? Although this study, targeted the agro-ecological system of wheat and maize fields, the collected SRS data will be used to explore the pattern of relationship between SRS variables and weed distribution pattern (if any) instead of crop yield attributes in the study area. This study will serve as the first baseline survey and also be useful for future related agro-ecosystem explorations in the region.

Materials and Methods

Study Area

Gujrat district is located in the Punjab province, Pakistan and between two famous rivers, the Chenab and Jhelum. It is situated between the 73°33′ to 74°24′ E longitude and 32°24′ to 33°10′ N latitude. It is bounded by the Jhelum district towards the west and northwest, district Sialkot on the east, district Gujranwala on the southeast, district Mandi Bahauddin on the southwest, district Mirpur and Bhimber of Azad Jammu and Kashmir on the north and northeast (*Fig. 1*). It is further administratively divided into 4 tehsils (Sarai-Alamgir, Kharian, Gujrat and Jalalpur Jattan), 119 union councils and 1065 villages. The total land area of the district is 3192 km^2. The study area is comprised of many historic towns like Jalalpur Jattan, Lalamusa, Kharian, Sarai-Alamgir, Dinga and Kunjah. The total human population of the district is 2.756 million (i.e. Urban 827396; Rural 1928714; Household 442399), and comprising of 43% urban population. The human density is about 863 persons/km^2 (DCR, 2017), whereas population of Gujrat city is about 0.39 million. The district has moderate climate with hot summer and cool winter. The highest temperature during the peak of summer shoot up to 50°C whereas minimum temperature during winter peak might fall below 2°C. The average rainfall varies from 1000 mm to 500 mm in the different parts of the district, usually higher in the northern parts and decreases continuously towards the southern parts (Parvaiz, 2014). Monthly mean climatic data of temperature and rainfall variations of the study area is given in *Table 1*. The total area under cultivation was about 0.321 million hectare (25.68%), out of this, wheat was cultivated on about 158.2 hectare and its production was about 1.85 tons/ha. Maize crop was sporadically

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cultivated in the study area. A very small area (31000 ha) is being irrigated through canal irrigation system whereas majority of area under cultivation is always irrigated through tube wells (Anonymous, 2018).

The study area is represented by sub-tropical deciduous vegetation with *Acacia modesta* Wall., *Acacia nilotica* (L.) Delile, *Albizia lebbeck* (L.) Benth., *Cassia fistula* L., *Dalbergia sissoo* DC., *Melia azedarach* L., and *Morus alba* L. as indicator tree species, and *Datura innoxia* Mill., *Dodonaea viscosa* (L.) Jacq., *Justicia adhatoda* L., and *Ziziphus nummularia* (Burm.f.) Wight & Arn. were important components of shrub layer. Soil of the study area is poor in calcium carbonate (about 6%) and its texture varied from sandy loam to clayey loam, and located in the lower end of piedmont plains. The bedrock comprised of semi-consolidated and weakly cemented, coarse sandstone with underlying clay and shale layers (Ullah et al., 2007). These materials were deposited probably during Late Pleistocene and Subrecent periods. Application of advanced weed management activities for the crop protection were uncommon in the study area. Only 6% farmers claimed the use of these protocols especially the use of weedicides (Trade name: Buctril Super; Puma Super; Logran and Topik etc.) during different stages of crops growth and development. The majority of farmers belonging to remote areas are still using traditional weed management practices like timing and number of field ploughs, and weed up-rooting (by hand).

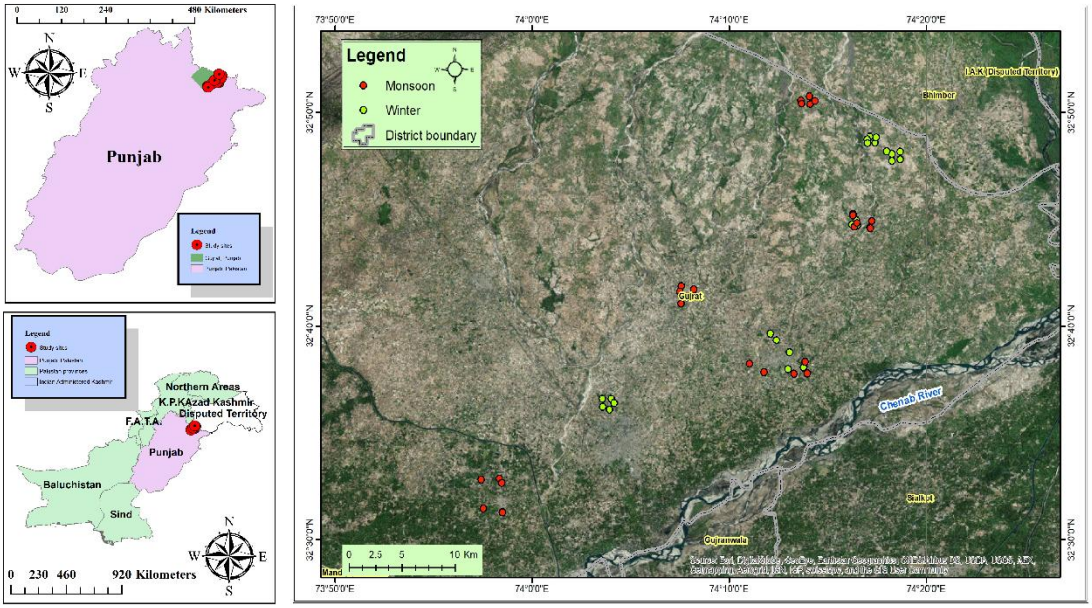


Figure 1. *GIS map of district Gujrat, Pakistan showing distribution of weeds samples collected from the wheat and maize crops (winter and monsoon seasons respectively) during 2016.*

Plant Collection and Sampling Protocols

The detailed floristic surveys were conducted during each month from January-December, 2016. The voucher specimens were collected, pressed, dried and mounted on standard sized herbarium sheets. The same were identified by using the available regional taxonomic literature and online floral databases (Stewart, 1972; Nasir and Ali, 1971-1995; Ali and Qaiser, 1995-2009; EFLORAS, 2012; TROPICOS, 2012). After

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identification, all the familial and species binomials were copied from the website; www.theplantlist.org (TPL, 2013) to attain global homogeneity with respect to updated taxonomic views (Khan et al., 2015; 2016). The same were deposited in the PMAS Herbarium of Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan for future references and record. The Raunkiaer's life form, leaf size, local names and flowering duration of each weed species were recorded and calculated for each weed community.

For vegetation sampling, stratified random method was adopted. For this, five different sites were randomly selected to record weed composition of each of bread wheat (during winter season) and maize crops (monsoon season) in the study area. Followed by this, at each site, five different fields were randomly selected and again, at each field, five plots/quadrats (size; 1×1 meter) were studied for collection of density, frequency and cover data of the weed species. The averaged values of these variable were then used to get their relative values, which were further used to get importance value index (IVI) value for each weed species (Iqbal et al., 2018). Thus, a total of 50 weed samples comprising of 250 plots [25 wheat weed samples: 5sites×5fields×5plots = 125 plots were studied during January-February and the same number of samples/subsamples were recorded from maize fields during July-August, 2016] were studied in the area.

Satellite Data

Satellite derived measurements related to 50 studied field locations of the study period, Landsat 8 (launched: 2013) satellite data was used that has spatial resolution of 30 m. Two instruments viz. operational land imager (OLI) and thermal infrared sensor (TIRS) are onboard at Landsat 8 satellite, and the satellite collects images of the Earth with a 16-day repeat cycle. It provides 8-band multispectral scenes at 30 meter resolution along with band-8 (Panchromatic; 15 meter resolution) and two Thermal infrared sensor bands including TIRS-1 and TIRS-2 as band-10 and 11 respectively at 100 meter resolution but are registered to and delivered with the 30-meter OLI data product as they were resampled to 30 meters to match multispectral bands. A median of the reflectance of all the images available for the study area and time period (after masking clouds) was created in Google Earth Engine and subsequent functional gradients/variables were derived from the median image. The reflectance values were corrected with respect to sun angle (Ganie and Nusrath, 2016). The following satellite measures were calculated;

The normalized difference vegetation index (NDVI) was calculated by using equation 1 as under;

$$NDVI = \frac{NIR - RED}{NIR + RED} \qquad \text{(Eq.1)}$$

Where, NIR=Near-infrared=band 5 (wavelength vegetation emits) and RED=band 4 (wavelength vegetation absorb).

The NDVI value ranges between -1 to +1. Higher NDVI value represent more abundance of live, healthy, green plants whereas small positive values (≤ 0.1) depicts non vegetated areas such as barren land, rocks, sand, urbanized area, snow, and negative values by water bodies.

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The normalized difference moisture index (NDMI) estimate the levels of moisture in the vegetation. The NDMI was calculated by using corrected value of the band 5 (NIR), being sensitive to the reflectance of leaf chlorophyll content to the band 6 (Short-wave infrared-1; SWIR-1) and sensitive to the absorbance of leaf moisture as communicated by Gao (1996) and represented as equation 2 as under;

$$NDMI = \frac{NIR - SWIR - 1}{NIR + SWIR - 1} \quad \text{(Eq.2)}$$

Here, NIR=Near-infrared=band 5 and SWIR-1=Short-wave infrared-1=band 6. NDMI value also ranges between -1 to +1 and the positive values represents moist areas and the negative ones to the non-moist features.

The location of sampling plots were determined by using Garmin, 76CSx handheld GPS device and two topographic variables namely altitude (meter) and topographic wetness index (TWI) were calculated through modelling 30 m digital elevation model (DEM) of the study area (download link; https://asterweb.jpl.nasa.gov/gdem.asp). The topographic wetness index (TWI) also known as compound topographic index (CTI) was developed by the Beven and Kirkby (1979), and shown in equation 3, to study the spatial scale effects on hydrological processes, flow paths, and biological processes like vegetation patterns and net primary productivity etc. The TWI was calculated as under as communicated by the Wilson and Gallant (2000).

$$TWI = \ln \frac{\alpha}{\tan \beta} \quad \text{(Eq.3)}$$

Where α is the local upslope area draining through a certain point per unit contour length and tan β is the local slope in radians. The TWI values were extracted from the sample point coordinate data using the distance-weighted average of the four nearest grid cells (bilinear interpolation) rather than a simple point extraction. ArcGIS was also used for mapping of bread wheat (winter) and maize (monsoon) crops weed samples distribution in the study area.

Table 1. *Mean Monthly rainfall and temperature variations in the study area for the period of 5 years from 2012-2016.*

Months	Min. Temperature °C	Max. Temperature °C	Rainfall (mm)	Rainy days
January	11.8	22.6	21.422	4.2
February	14.4	24	79.92	9.2
March	19.8	28.6	100.656	12.2
April	27.2	35.6	43.438	11.6
May	33.6	42	29.782	11.8
June	37	45.2	29.636	9.6
July	35.4	43.2	92.666	20.4
August	32.8	40.4	119.804	22.4
September	31	39.2	119.682	10.4
October	27.6	36.2	14.42	4
November	20.6	30.2	2.658	2
December	14.8	25.4	8.164	2.4

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Statistical Analyses

The decision about the number of ecological meaningful weed communities in the study area was determined by using the criteria as conveyed by Khan et al. (2017). For this, two to eight (2-8) possible groups were tested by using the Monte Carlo permutation test, and suitable level was selected, where mean p-value become minimized or significant species become maximized (McCune et al., 2002). The same appropriate grouping variable for the studied weed samples was further used in the hierarchical clustering (distance "relative euclidean"; linkage "ward") of studied samples and their associated weed species by using PC-ORD ver. 5 (McCune and Mefford, 2006), whereas the same for study sites was done by using R statistical software's (R Core Team, 2017). For this, package "decostand" was used to normalize vegetation data first followed by clustering through "hclust" package to use relative euclidean as distance method. For further confirmation, a non-parametric method (Multi-response permutation procedure "MRPP") as communicated by McCune et al. (2002) was used for pairwise comparasion of identified weed communities of wheat and maize crops and seasonality groups (i.e. winter and monsoon) to determine the significant difference with respect to their weeds composition. Indicator species analysis (Dufrêne and Legendre, 1997) was also performed to seek the indicator values of the weed species for the different groups defined by the cluster analysis. Species richness (Margalef, 1958), Shannon's diversity (Shannon and Weaver. 1949) Simpson's diversity index (1-D), Simpson's reciprocal diversity index (1/D) as conveyed by Magurran (1988) and evenness (Pielou, 1975) were calculated for each weeds community by using the "diversity(vegan)" package in R statistical software. The Kruskal-Wallis test was used to seek the significant difference in the weed communities with respect to their diversity, topographic and satellite-derived measurements like NDVI, NDMI and TWI variations. The direct unimodal ordination model (canonical correspondence analysis) was used to seek the weed species-environment relationship. The conditional or net terms effects of the studied variables and significance of constrained axes were tested by using the permutation test in Canoco ver. 5 (Ter Braak and Smilauer, 2012).

Results

A total of 60 weed species were recorded including 23 species in wheat, 28 in maize and 9 species as common in both types of fields in the study area. All these species (monocot 15; dicot 45) belongs to 26 plant families (monocot 3; dicot 23) and 52 genera (monocot 13; dicot 39) (*Table 2*). The leading plant family with maximum number of weed species was Poaceae (12 spp., 20%) followed by Amaranthaceae (7 spp., 11.67%), Compositae (6 spp., 10%), Leguminosae (5 spp., 8.34%), Solanaceae (4 spp., 6.67%), Euphorbiaceae (3 spp., 5%) and Convolvulaceae, Cyperaceae, Malvaceae with 2 species (3.34%) each. Similarly the leading genus was *Euphorbia* (3 spp., 5%) followed by *Amaranthus, Chenopodium, Cyperus, Dactyloctenium, Medicago* and *Solanum* with 2 species (3.34%) each (*Fig. 2*). The majority of weed species were found in the flowering stage during the month of August (42 spp., 70%) followed by July (41 spp., 68.34%), September (41 spp., 68.34%), June (35 spp., 58.34%), May (30 spp., 50%), April (27 spp., 45%), October (26 spp., 43.34%), March (24 spp., 40%), February (12 spp., 20%), January and November (8 spp., 13.34% each) and least during the month of December (8 spp., 13.34%) (*Fig. 3*). Based on Raunkiaer's life form, therophytes weed

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species were dominant (42 spp., 70%) in the study area followed by hemicryptophytes (9 spp., 15%), chamaephytes and lianas (3 spp., 5% each), geophytes (2 spp., 3.34%) and 1 weed species (1.67%) of nanophanerophyte. Similarly, leaf size spectra studies depicted that microphylls weed species were dominant (25 spp., 41.67%) in the study area followed by nanophylls (19 spp., 31.67%), leptophylls (11 spp., 18.34%) and least number of mesophyll (5 spp., 8.34%) weed species were recorded (*Fig. 8*).

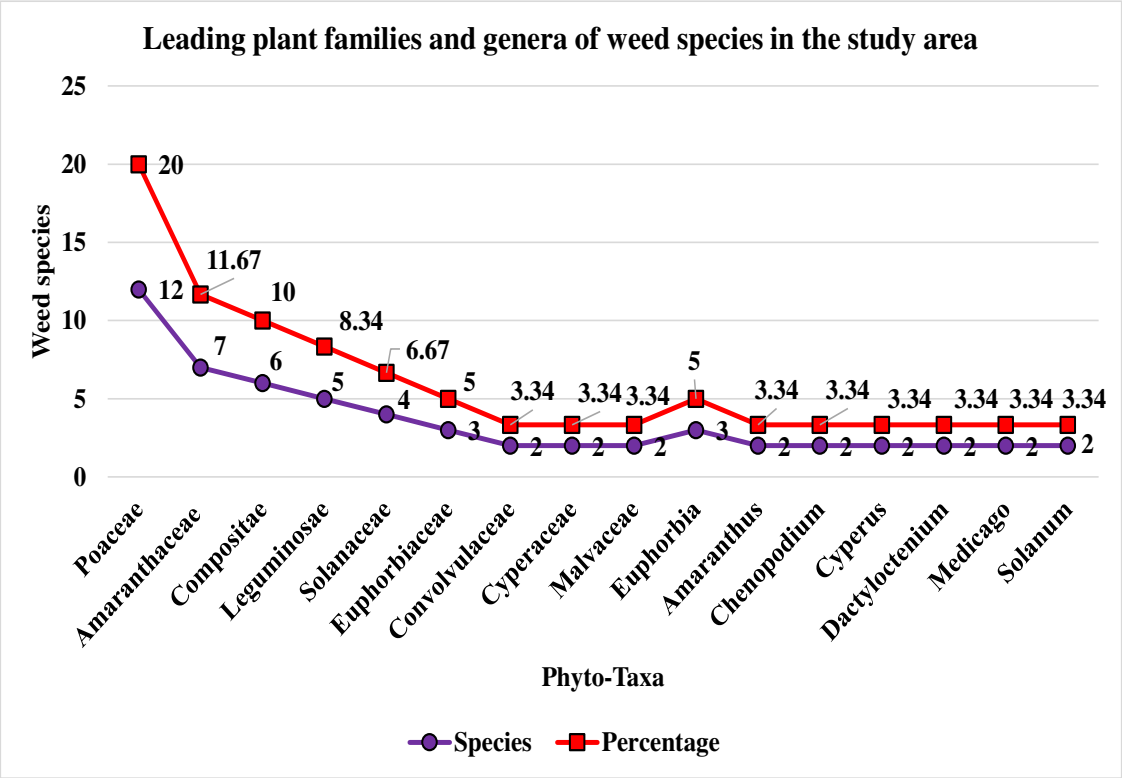


Figure 2. *Leading plant families and genera of weed species of wheat and maize crops of Gujrat, Pakistan.*

Indicator Species Analysis and Weed Communities of the Study Area

The number of ecologically meaningful weed groups were determined first by using Monte Carlo permutation test. The results for two to eight possible groups depicted that there were four (4) biologically meaningful weed communities of both wheat and maize crops in the study area. At fourth level, minimized mean p-value of 0.099 and maximized number of significant p-values or 32 significant indicator weed species were recorded (*Fig. 4*). For classification, and to seek relationship of 50 weed samples and their associated 60 weed species, hierarchical clustering dendrogram was developed. This not only showed that 25 wheat weed samples were sufficiently different from the same number of maize weed samples with respect to their weed species composition but also showed that each of both these major clusters were further comprised of reasonably different two minor clusters or weed communities (*Fig. 5*). Thus, we recorded a total of four significant weed communities in the study area, two each for wheat and maize crop fields. Our results in this regard do not match with any of related studies from Pakistan,

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as number of communities in this study are based on test statistics. The detail of each of wheat and maize communities is as under.

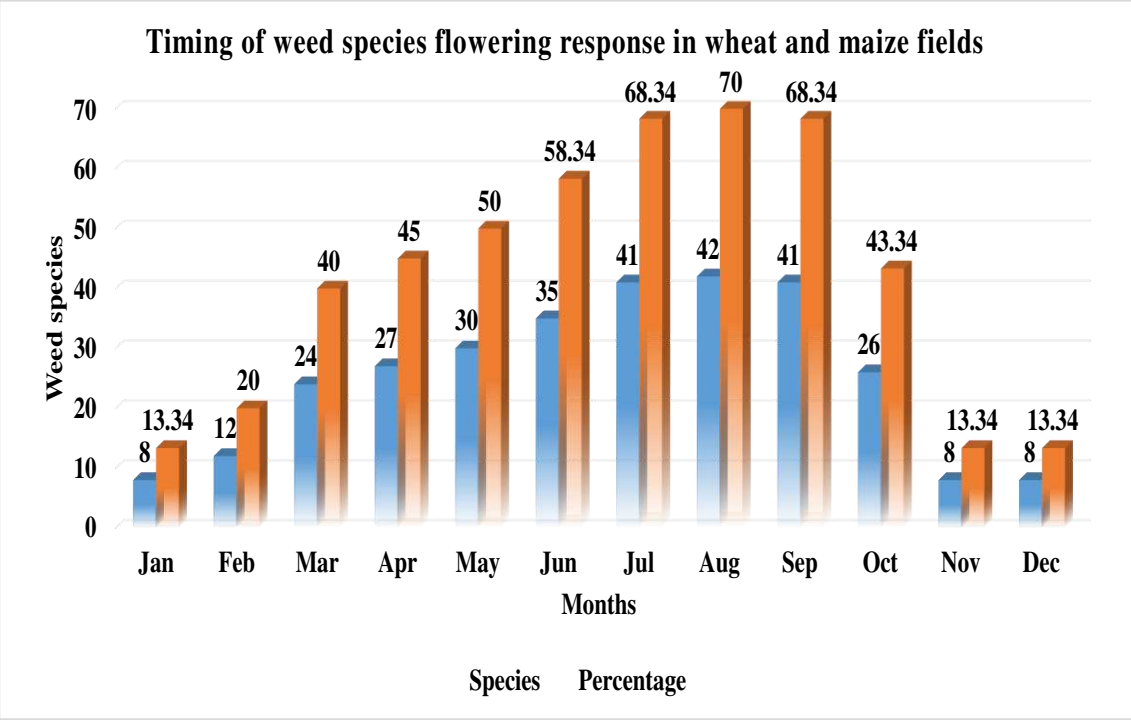


Figure 3. *Number and percentages of weed species found in flowering stage during the study period.*

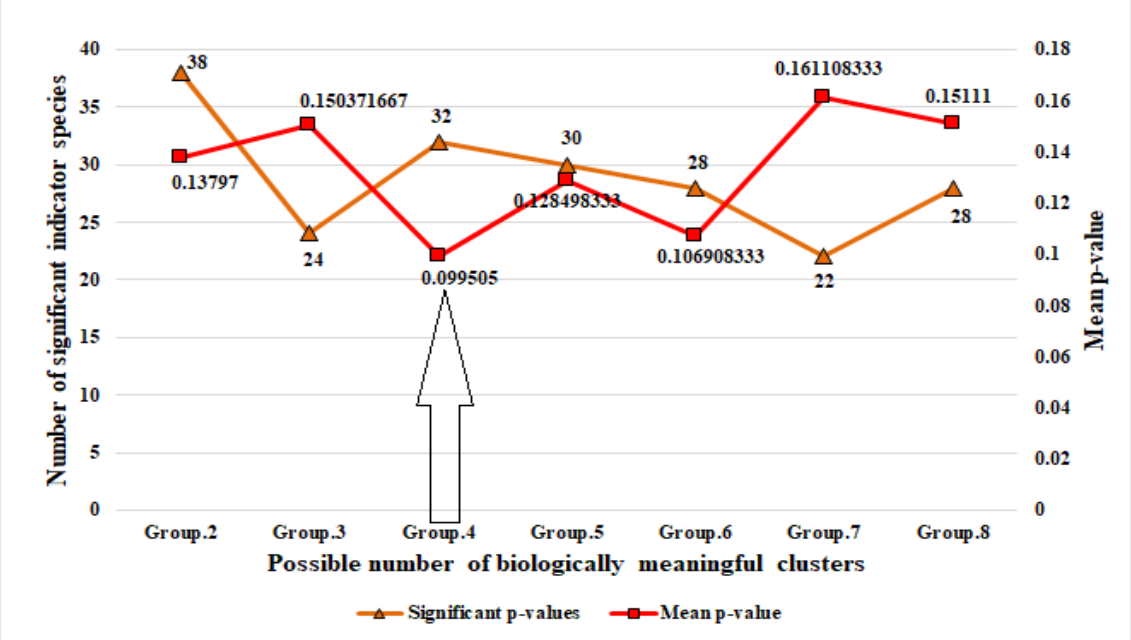


Figure 4. *Number of statistically significant indicator species and associated mean* p*-value variations for 2 to 8 possible vegetation groups/clusters.*

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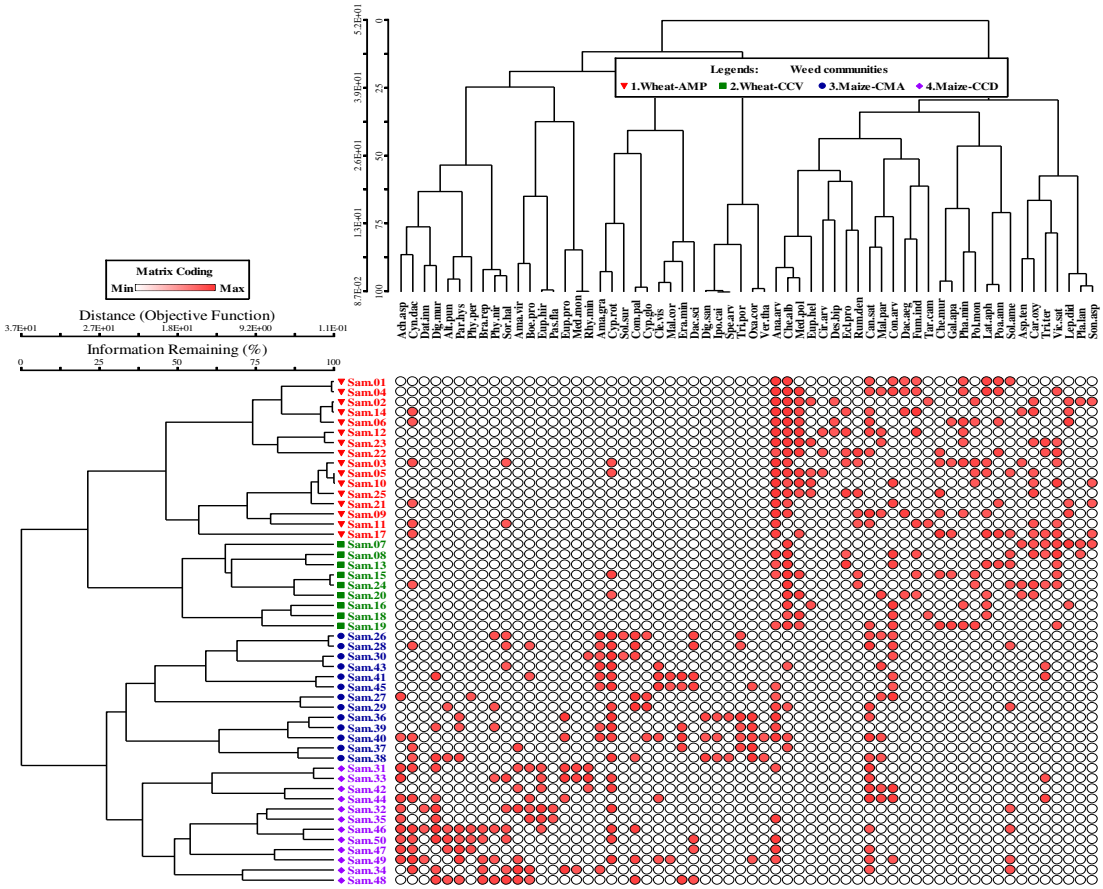


Figure 5. *Hierarchical clustering of weed samples and their associated plant species, showing four biologically meaningful clusters/groups.*

Wheat Fields Communities

Anagallis-Medicago-Phalaris (AMP) Weed Community

This, AMP weed community was recorded at 16 different wheat fields (samples) of Bhojpur, Ghaiyan, Jalalpur Jattan, Karianwala and Sabowal localities (*Figs. 5* and *6*). A total of 31 weed species including 15 indictor weed species were found associated with this group. There were 5 significant (p-value ≤ 0.05) indicator species in this community. This group was dominated by *Anagallis arvensis*, *Medicago polymorpha* and *Phalaris minor* species having mean indicator value of 24.8, 17.1 and 13.1 respectively, thus named as AMP group. The other co-dominant species were *Fumaria indica* (12.9), *Chenopodium murale* (12.4), *Lepidium didymum* (12.3), *Eclipta prostrata* (12.2), *Rumex dentatus* (12), *Poa annua* (11.8) followed by less common species like *Euphorbia helioscopia* (11.1), *Sonchus asper* (10.6) and *Dactyloctenium aegyptium* (10.5). The rare species of this group were *Cirsium arvense*, *Desmostachya bipinnata* and *Taraxacum campylodes* with mean indicator value of 8.8 for each (*Table 2*). The number of weed species in all 16 studied fields varied from 7-14 with average count of 10.25±1.64 species. Similarly the mean values of Margalef richness, Shannon's diversity (H′), Simpson's diversity, reciprocal Simpson's diversity and Pielou evenness of this community were 2.69±0.39, 2.08±0.18, 0.84±0.04, 6.66±1.36 and 0.9±0.05 respectively. It was studied at the mean elevation of 210.5±23.58 m a.s.l. which varied

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from 183-251 m a.s.l. It was located at latitude range of 32.61-32.82 DD (North) and longitude range of 74.06-74.32 DD (East). The mean NDVI, NDMI and TWI values were 0.38±0.05 (0.31-0.49), 0.15±0.05 (0.05-0.25) and 7.83±0.2 (7.47-8.15) respectively and found higher than the following weed community (CCV community) of wheat crop (*Fig. 7*). Raunkiaer's life form classification of weed species depicted the dominancy of therophytes (22 spp., 70.97%), followed by hemicryptophytes (4 spp., 12.91%), whereas geophytes and lianas were represented by 2 species each (6.46%) followed by chamaephytes (1 spp., 3.23%). Leaf size spectra showed the leading share of microphylls with 13 species (41.94%) followed by leptophylls and nanophylls with 8 species each (25.81%) whereas least number of mesophylls (2 spp., 6.46%) were recorded in this community (*Fig. 8*).

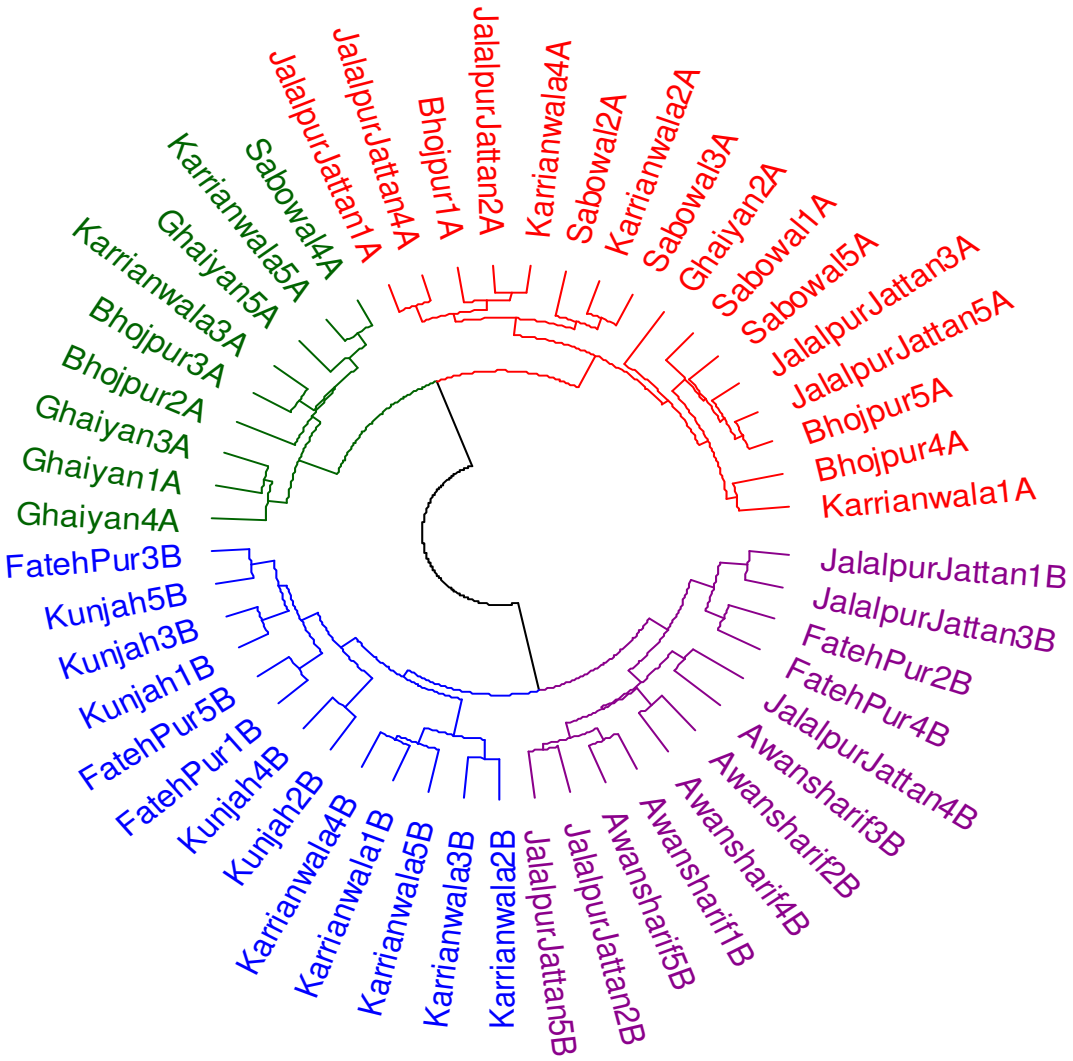


Figure 6. *Fan (polar) shaped hierarchical clustering dendrogram showing grouping pattern of study sites (Red=AMP; Green=CCV; Blue=CMA; Violet=CCD weed community).*

Chenopodium-Convolvulus-Vicia (CCV) Weed Community

This community was recorded at 9 different wheat field of Bhojpur, Ghaiyan, Karianwala and Sabowal localities (*Figs. 5* and *6*). It was comprised of 28 weed species

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including 11 indicators which further includes 3 significant indicators species. The maximum mean indicator value (IV) was recorded for *Chenopodium album* (23.2), *Convolvulus arvensis* (18.3) and *Vicia sativa* (15.9) hence named as CCV community. Co-dominants of this group includes, *Carthamus oxyacantha* (15.1), *Solanum americanum* (15.1), *Lathyrus aphaca* (13.9), *Tribulus terrestris* (13.8) and *Polypogon monspeliensis* (12.6) whereas *Galium aparine* (10.5), *Asphodelus tenuifolius* (10.4) were recorded as less common species. *Plantago lanceolata* (8.8) was measured as rare species of this group (*Table 2*). Average species count of this community was 8.34±1.7, and species number per sample/field ranges from 5 to 10. Values of Margalef richness, Shannon's diversity (H′), Simpson's diversity, reciprocal Simpson's diversity and Pielou evenness of this community were 2.34±0.4 (1.6-2.73), 1.93±0.22 (1.51-2.27), 0.83±0.05 (0.76-0.89), 6.09±1.55 (4.23-9.46) and 0.92±0.05 (0.84-0.98) respectively. It was recorded at an average altitude of 235.34±22.4 m a.s.l. (186-260 m a.s.l.) and located at latitude range of 32.61-32.82 DD (North) and longitude range of 74.06-74.32 DD (East). Similarly, mean NDVI, NDMI and TWI values were 0.34±0.06 (0.25-0.45), 0.12±0.05 (0.04-0.19) and 7.93±0.51 (7.41-8.81) respectively (*Fig. 7*). Life form categorization of 28 weed species of this community depicted the leading abundance of therophytes (21 spp., 77.78%) followed by hemicryptophytes (3 spp., 7.41%), lianas (2 spp., 7.41%) and 1 species (3.71%) each of chamaephytes and geophytes. Leaf size spectra results of this community again showed the dominancy of microphylls (12 spp., 42.86%), followed by leptophylls and nanophylls with 8 species each (28.58%) (*Fig. 8*).

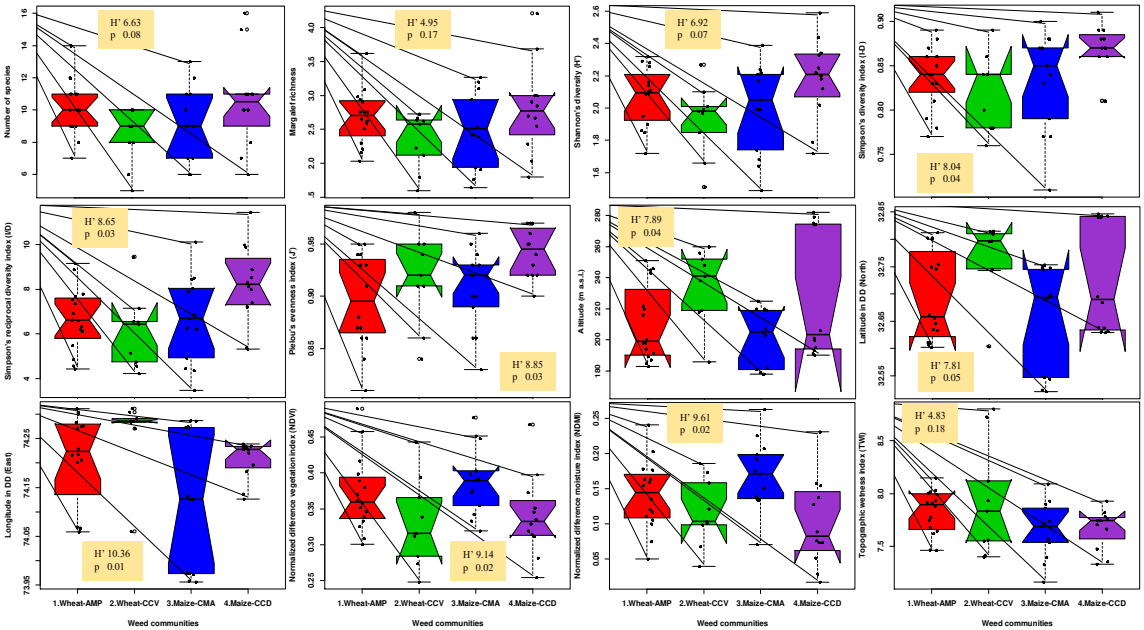


Figure 7. *Boxplots with Kruskal-Wallis H test and its associated p-values of environmental and biotic variables of weed communities in the study area*

Maize Fields Communities

Cyperus-Malva-Amaranthus (CMA) Weed Community

This significant group was studied at 13 different maize fields of Fatehpur, Karianwala and Kunjah localities (*Figs. 5* and *6*). It includes 34 weed species including 16 indicator

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and 10 significant indicator of this group. *Cyperus rotundus, Malva parviflora* and *Amaranthus graecizans* subsp. *silvestris* were leading three species with mean IV of 20.1, 15.3 and 13.4 respectively. Co-dominant species were *Commelina paludosa* (13.3), *Oxalis corniculata* (12.4) whereas, common ones were *Dactyloctenium scindicum* (11.8), *Trianthema portulacastrum* (11.4), *Cleome viscosa* (11.2), *Eragrostis minor* (11.2), *Cyperus glomeratus* (9.7) and *Malvastrum coromandelianum* (9). Less common and rare ones includes *Digitaria sanguinalis* (8.9), *Ipomoea cairica* (8.8), *Verbascum thapsus* (8.1) and *Spergula arvensis* (8), *Solanum surattense* (7.9) respectively (*Table 2*). Average number of species were 9.24±2.16 with range of 6 to 13 whereas Margalef richness, Shannon's diversity (H′), Simpson's diversity, reciprocal Simpson's diversity and Pielou evenness values of this community were 2.48±0.53 (1.64-3.27), 2.01±0.27 (1.49-2.39), 0.84±0.06 (0.71-0.9), 6.64±1.88 (3.48-10.11) and 0.91±0.04 (0.83-0.96) respectively. This group was studied at an average altitude of 201.24±18.2 m a.s.l. (178-225 m a.s.l.) and located at latitude range of 32.53-32.76 DD (North) and longitude range of 73.96-74.29 DD (East). Mean NDVI, NDMI and TWI values of this group were 0.39±0.05 (0.32-0.48), 0.17±0.05 (0.08-0.27) and 7.67±0.26 (7.17-8.1) respectively (*Fig. 7*). Results of Raunkiaer's life form classification of 34 weed species of this community suggested the maximum abundance of therophytes (23 spp., 67.65%), followed by the hemicryptophytes (5 spp., 14.71%), and 2 species (5.89%) each for chamaephytes, geophytes and lianas species. Leaf size spectra results showed the dominance of microphyllous species (16 spp., 47.06%), followed by nanophylls (11 spp., 32.36%), leptophylls (4 spp., 11.77%) and least species of mesophylls (3 spp., 8.83%) (*Fig. 8*).

Cannabis-Cynodon-Digera (CCD) Weed Community

This community was recorded at 12 different maize fields of Awan Sharif, Fatehpur and Jalalpur Jattan localities (*Figs. 5* and *6*). It was comprised 31 weed species including 18 indicator and 14 significant indicator species. Three leading species were *Cannabis sativa* (21.2), *Cynodon dactylon* (18.5) and *Digera muricata* (15.1), hence renamed as CCD community. Associated co-dominants were *Sorghum halepense* (14.7), *Achyranthes aspera* (14.5), *Boerhavia procumbens* (12.8), *Parthenium hysterophorus* (12.7), *Amaranthus viridis* (12.1), *Phyllanthus niruri* (11.9) and *Euphorbia hirta* (11.6), whereas less common were *Alternanthera pungens* (11.1), *Euphorbia prostrata* (11.1), *Brachiaria reptans* (10.4), *Rhynchosia minima* (9.8) and *Physalis peruviana* (9.7). The rare species of this group were *Datura innoxia* (8.8), *Medicago monantha* (8.8) and *Paspalidium flavidum* (8.2) (*Table 2*). Mean species count of this community was 10.5±2.76 with range of 6 to 16 weed species/sample, whereas Margalef richness, Shannon's diversity (H′), Simpson's diversity, reciprocal Simpson's diversity and Pielou evenness values of this community were 2.81±0.64 (1.8-4.21), 2.19±0.24 (1.72-2.59), 0.87±0.03 (0.81-0.91), 8.21±1.71 (5.33-11.44) and 0.95±0.03 (0.9-0.97) respectively. Mean elevation value was 230±39.83 m a.s.l. (190-282 m a.s.l.) and located at latitude range of 32.63-32.85 DD (North) and longitude range of 74.13-74.24 DD (East). Mean NDVI, NDMI and TWI values were 0.34±0.06 (0.26-0.47), 0.11±0.06 (0.02-0.24) and 7.68±0.18 (7.33-7.93) respectively (*Fig. 7*). Therophytes were recorded dominant (20 spp., 64.52%) in this community followed by hemicryptophytes (5 spp., 16.13%), chamaephytes and geophytes with 2 species (6.46%) each and one species (3.23%) each of lianas and nanophanerophytes. Leaf size categorization of weed species of this community depicted that microphylls were dominant (15 spp., 48.39%), followed by nanophylls (10 spp., 32.26%), and three species (9.68%) each of leptophylls and mesophylls (*Fig. 8*).

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Table 2. *Results of indicator species analysis and weed species attributes from the study area.*

Family	Species Name / Voch. No.	Abbrev.	MG	Obs. IV	M/IV	SD	p-value	LF	LS	FP	Local Name	S
Primulaceae	***Anagallis arvensis* L. (RK/MA-2693/16)**	***Ana.arv***	**1**	**65.7**	**24.8**	**5.1**	**0.0002**	**The**	**Nano**	**Feb-Mar**	**Billi Buti**	**3**
Leguminosae	***Medicago polymorpha* L. (RK/MA-2688/16)**	***Med.pol***	**1**	**46.7**	**17.1**	**5.9**	**0.0016**	**The**	**Nano**	**Mar-May**	**Maina**	**1**
Poaceae	***Phalaris minor* Retz. (RK/MA-2660/16)**	***Pha.min***	**1**	**26.8**	**13.1**	**5.6**	**0.0254**	**The**	**Nano**	**Mar-May**	**Dumbi Sitti**	**1**
Papaveraceae	*Fumaria indica* (Hausskn.) Pugsley (RK/MA-2653/16)	*Fum.ind*	1	20.5	12.9	5.95	0.1064	The	Lept	Mar-Jun	Pitpaprra	1
Amaranthaceae	*Chenopodium murale* L. (RK/MA-2698/16)	*Che.mur*	1	21.3	12.4	5.9	0.1024	The	Lept	Jan-Jul	Karund	1
Brassicaceae	*Lepidium didymum* L. (RK/MA-2706/16)	*Lep.did*	1	15.3	12.3	5.85	0.2272	The	Lept	Mar-Jun	Jangli Halian	1
Compositae	*Eclipta prostrata* (L.) L. (RK/MA-2701/16)	*Ecl.pro*	1	16	12.2	5.8	0.191	The	Micr	Jun-Sep	Bhangra	1
Polygonaceae	*Rumex dentatus* L. (RK/MA-2678/16)	*Rum.den*	1	19	12	5.72	0.119	Cha	Micr	May-Jun	Jangli Palak	1
Poaceae	*Poa annua* L. (RK/MA-2676/16)	*Poa.ann*	1	28.5	11.8	5.45	0.019	The	Lept	Apr-Sep	Gha, Ghaas	1
Euphorbiaceae	*Euphorbia helioscopia* L. (RK/MA-2681/16)	*Eup.hel*	1	23.6	11.1	5.5	0.033	The	Micr	Jan-Jul	Chatri-Dudal	1
Compositae	*Sonchus asper* (L.) Hill (RK/MA-2661/16)	*Son.asp*	1	13.2	10.6	5.55	0.2653	The	Micr	May-Oct	Dodak	1
Poaceae	*Dactyloctenium aegyptium* (L.) Willd. (RK/MA-2674/16)	*Dac.aeg*	1	18.4	10.5	5.52	0.0856	The	Nano	Jul-Oct	Madhana Gaas	1
Compositae	*Cirsium arvense* (L.) Scop. (RK/MA-2709/16)	*Cir.arv*	1	18.7	8.8	5.22	0.0572	The	Meso	Aug-Oct	Kandiyari, Leh	1
Poaceae	*Desmostachya bipinnata* (L.) Stapf. (RK/MA-2708/16)	*Des.bip*	1	18.7	8.8	5.2	0.0574	Hem	Micr	Jul-Oct	Dabh	1
Compositae	*Taraxacum campylodes* G.E.Haglund (RK/MA-2682/16)	*Tar.cam*	1	7.8	8.8	5.17	0.5079	Hem	Micr	Mar-Sep	Hund	1
Amaranthaceae	***Chenopodium album* L. (RK/MA-2702/16)**	***Che.alb***	**2**	**54**	**23.2**	**5.5**	**0.0004**	**The**	**Micr**	**Jun-Oct**	**Bathwa, Bathu**	**1**
Convolvulaceae	***Convolvulus arvensis* L. (RK/MA-2652/16)**	***Con.arv***	**2**	**23.8**	**18.3**	**5.4**	**0.138**	**Lia**	**Micr**	**May-Sep**	**Lailee, Hiran Padi**	**3**
Leguminosae	***Vicia sativa* L. (RK/MA-2655/16)**	***Vic.sat***	**2**	**43.6**	**15.9**	**6**	**0.0014**	**The**	**Lept**	**Mar-Aug**	**Rewari**	**1**
Compositae	*Carthamus oxyacantha* M.Bieb. (RK/MA-2710/16)	*Car.oxy*	2	24.1	15.1	5.76	0.074	The	Micr	Mar-Jun	Pohli	1
Solanaceae	*Solanum americanum* Mill. (RK/MA-2690/16)	*Sol.ame*	2	12.1	15.1	5.63	0.6509	The	Micr	Jan-Dec	Kach-Mach, Mako	3

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Family	Species Name / Voch. No.	Abbrev.	MG	Obs. IV	M/IV	SD	p-value	LF	LS	FP	Local Name	S
Leguminosae	*Lathyrus aphaca* L. (RK/MA-2680/16)	*Lat.aph*	2	20.9	13.9	5.81	0.1198	Lia	Lept	Feb-Apr	Jangli Matar	1
Zygophyllaceae	*Tribulus terrestris* L. (RK/MA-2664/16)	*Tri.ter*	2	8.8	13.8	5.67	0.8014	The	Nano	Jan-Dec	Bhakrra	3
Poaceae	*Polypogon monspeliensis* (L.) Desf. (RK/MA-2667/16)	*Pol.mon*	2	18.1	12.6	5.62	0.1478	The	Nano	Mar-Jul	Malhar	1
Rubiaceae	*Galium aparine* L. (RK/MA-2668/16)	*Gal.apa*	2	14.2	10.5	5.47	0.1848	The	Nano	Mar-Jul	Lahndra	1
Xanthorrhoeaceae	*Asphodelus tenuifolius* Cav. (RK/MA-2694/16)	*Asp.ten*	2	27.2	10.4	5.43	0.0124	The	Lept	Nov-Apr	Piyazi, Patakh	1
Plantaginaceae	*Plantago lanceolata* L. (RK/MA-2659/16)	*Pla.lan*	2	18.7	8.8	5.19	0.076	Hem	Micr	Jul-Sep	Isabgool	1
Cyperaceae	***Cyperus rotundus* L. (RK/MA-2662/16)**	***Cyp.rot***	**3**	**53.4**	**20.1**	**5.5**	**0.0004**	**Geo**	**Nano**	**Apr-Oct**	**Dheela**	**3**
Malvaceae	***Malva parviflora* L. (RK/MA-2705/16)**	***Mal.par***	**3**	**14.2**	**15.3**	**5.8**	**0.4713**	**The**	**Micr**	**Apr-Sep**	**Sonchal**	**3**
Amaranthaceae	***Amaranthus graecizans* subsp. *silvestris* (Vill.) Brenan (RK/MA-2669/16)**	***Ama.gra***	**3**	**55.5**	**13.4**	**5.7**	**0.0004**	**The**	**Micr**	**Jun-Sep**	**Lunak chellery**	**2**
Commelinaceae	*Commelina paludosa* Blume (RK/MA-2703/16)	*Com.pal*	3	32.4	13.3	5.67	0.0092	The	Meso	Aug-Oct	Kanjuna, Churra	2
Oxalidaceae	*Oxalis corniculata* L. (RK/MA-2673/16)	*Oxa.cor*	3	44.7	12.4	5.93	0.0012	Hem	Nano	Feb-Oct	Khatmit, Khatti	2
Poaceae	*Dactyloctenium scindicum* Boiss. (RK/MA-2686/16)	*Dac.sci*	3	20.4	11.8	5.38	0.0604	Hem	Nano	Sep-Dec	Madhana Gaas	2
Aizoaceae	*Trianthema portulacastrum* L. (RK/MA-2671/16)	*Tri.por*	3	46.2	11.4	5.62	0.0004	The	Nano	May-Oct	Itsit	2
Cleomaceae	*Cleome viscosa* L. (RK/MA-2657/16)	*Cle.vis*	3	25	11.2	5.52	0.0264	The	Micr	Jun-Aug	Gund-Buti, Bui	2
Poaceae	*Eragrostis minor* Host (RK/MA-2695/16)	*Era.min*	3	31.6	11.2	5.42	0.0084	The	Nano	May-Sep	Bharbhuri	2
Cyperaceae	*Cyperus glomeratus* L. (RK/MA-2679/16)	*Cyp.glo*	3	30.8	9.7	5.25	0.0062	The	Nano	Sep-Oct	Gooyaan	2
Malvaceae	*Malvastrum coromandelianum* (L.) Garcke (RK/MA-2656/16)	*Mal.cor*	3	12.1	9	5.04	0.2669	The	Micr	Jun-Sep	Gogi Booti	2
Poaceae	*Digitaria sanguinalis* (L.) Scop. (RK/MA-2696/16)	*Dig.san*	3	23.1	8.9	5.18	0.0296	The	Nano	Jul-Sep	Moti khabbal	2
Convolvulaceae	*Ipomoea cairica* (L.) Sweet (RK/MA-2670/16)	*Ipo.cai*	3	23.1	8.8	5.17	0.0296	Lia	Nano	Jul-Oct	Aair	2
Scrophulariaceae	*Verbascum thapsus* L. (RK/MA-2697/16)	*Ver.tha*	3	15.4	8.1	4.69	0.1518	The	Meso	Jun-Aug	Jangli Tumbaku	2
Caryophyllaceae	*Spergula arvensis* L. (RK/MA-2651/16)	*Spe.arv*	3	15.4	8	4.59	0.1384	The	Lept	Mar-Apr	Not Available	1
Solanaceae	*Solanum surattense* Burm. f. (RK/MA-2707/16)	*Sol.sur*	3	15.4	7.9	4.58	0.1432	Hem	Micr	Jan-Dec	Kundiyara, Mokri	2
Cannabaceae	***Cannabis sativa* L. (RK/MA-2685/16)**	***Can.sat***	**4**	**26.7**	**21.2**	**5.5**	**0.1522**	**The**	**Micr**	**Jul-Sep**	**Bhang**	**3**
Poaceae	***Cynodon dactylon* (L.) Pers. (RK/MA-**	***Cyn.dac***	**4**	**34.4**	**18.5**	**5.7**	**0.0168**	**Hem**	**Lept**	**Jan-Dec**	**Khabbal**	**3**

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Family	Species Name / Voch. No.	Abbrev.	MG	Obs. IV	M/IV	SD	p-value	LF	LS	FP	Local Name	S
	2663/16)											
Amaranthaceae	***Digera muricata* (L.) Mart. (RK/MA-2672/16)**	***Dig.mur***	**4**	**52.1**	**15.1**	**6.2**	**0.0002**	**The**	**Micr**	**Jul-Sep**	**Tandla saag**	**2**
Poaceae	*Sorghum halepense* (L.) Pers. (RK/MA-2665/16)	*Sor.hal*	4	34.8	14.7	5.73	0.009	Geo	Meso	May-Sep	Baru, Barwa	3
Amaranthaceae	*Achyranthes aspera* L. (RK/MA-2691/16)	*Ach.asp*	4	66.5	14.5	5.73	0.0002	Cha	Micr	Jul-Oct	Puth-Kanda	2
Nyctaginaceae	*Boerhavia procumbens* Banks ex Roxb. (RK/MA-2683/16)	*Boe.pro*	4	43.3	12.8	5.71	0.001	Hem	Micr	Aug-Sep	Sanati, Itsit	2
Compositae	*Parthenium hysterophorus* L. (RK/MA-2689/16)	*Par.hys*	4	29.9	12.7	5.77	0.0186	The	Nano	Apr-Oct	Sitara Buti	2
Amaranthaceae	*Amaranthus viridis* L. (RK/MA-2704/16)	*Ama.vir*	4	43.3	12.1	5.88	0.0016	The	Micr	Mar-Oct	Ghanyar, Cholai	2
Phyllanthaceae	*Phyllanthus niruri* L. (RK/MA-2692/16)	*Phy.nir*	4	18.6	11.9	5.61	0.1266	The	Lept	Aug-Sep	Bahupatra, Bhuiamla	2
Euphorbiaceae	*Euphorbia hirta* L. (RK/MA-2684/16)	*Eup.hir*	4	50	11.6	5.9	0.0006	The	Nano	Jul-Dec	Dudhli	2
Amaranthaceae	*Alternanthera pungens* Kunth (RK/MA-2666/16)	*Alt.pun*	4	24.8	11.1	5.5	0.021	The	Micr	Aug-Oct	Kanda, Leh	2
Euphorbiaceae	*Euphorbia prostrata* Ait. (RK/MA-2699/16)	*Eup.pro*	4	21.6	11.1	5.39	0.0592	The	Lept	Jan-Dec	Dudhli, Hazar Dani	2
Poaceae	*Brachiaria reptans* (L.) C.A.Gardner & C.E.Hubb. (RK/MA-2675/16)	*Bra.rep*	4	41.7	10.4	5.42	0.0012	The	Micr	Jun-Oct	Sair, Kandeeri	2
Leguminosae	*Rhynchosia minima* (L.) DC. (RK/MA-2687/16)	*Rhy.min*	4	20.7	9.8	5.37	0.047	Cha	Micr	Feb-Apr	Turvel	2
Solanaceae	*Physalis peruviana* L. (RK/MA-2677/16)	*Phy.per*	4	21.1	9.7	5.27	0.0416	The	Micr	May-Oct	Rasp berry	2
Solanaceae	*Datura innoxia* Mill. (RK/MA-2654/16)	*Dat.inn*	4	25	8.8	5.14	0.0164	Nan	Meso	May-Oct	Datura	2
Leguminosae	*Medicago monantha* (C.A.Mey.) Trautv. (RK/MA-2658/16)	*Med.mon*	4	25	8.8	5.13	0.0158	The	Nano	Mar-Apr	Jangli-Methi	2
Poaceae	*Paspalidium flavidum* (Retz.) A.Camus (RK/MA-2700/16)	*Pas.fla*	4	16.7	8.2	4.8	0.092	Hem	Nano	Jul-Oct	Kangna	2

Legends: MG: Maximum Group; Obs. IV: Observed Indicator Value; M/IV: Mean Indicator Value; SD: Standard Deviation; LF: Life Form (Cha: Chamaephytes; Geo: Geophytes; Hem: Hemicryptophytes; Lia: Lianas; Nan: Nanophanerophytes; The: Therophytes); LS: Leaf Size Spectra (Lept: Leptophylls; Nano: Nanophylls; Micr: Microphylls; Meso: Mesophylls); FP: Flowering Period; S: Seasonality (1: Winter/Wheat; 2: Monsoon/Maize; 3: Both Season/Crops), Dominant species of each group are in bold.

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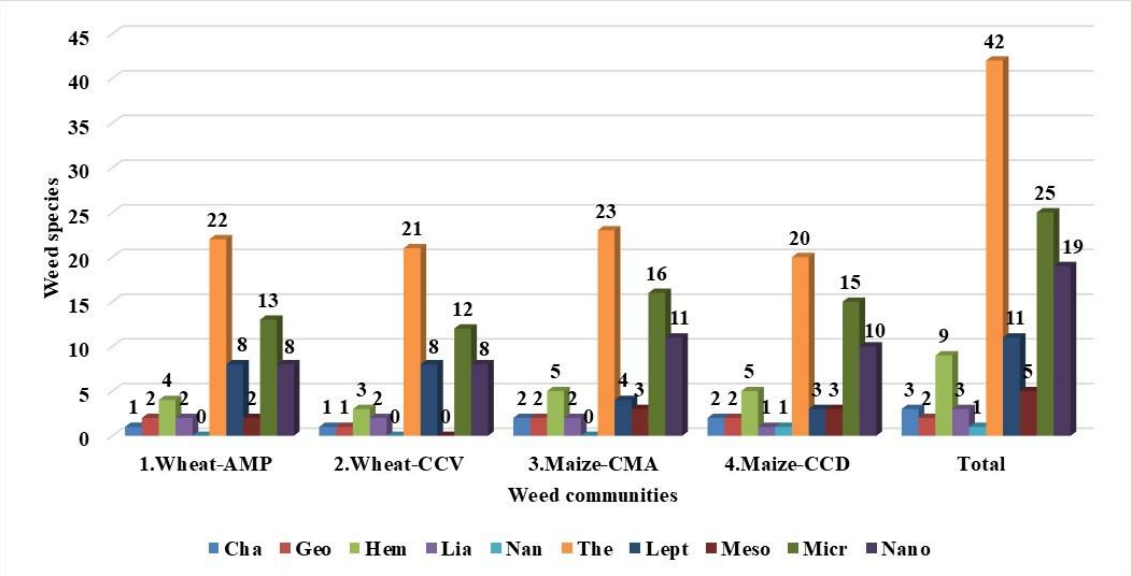


Figure 8. *Raunkiaer's life forms and leaf size spectra of weed communities of the agro-ecological system of the study area (for abbreviation see legends of Table 2).*

Seasonal and Weed Communities Significance

Pairwise comparasion of the two seasons (1. winter weed recorded in wheat fields; 2. monsoon weeds recorded in maize fields) and four weed communities were tested by using Multi-response permutation procedure (MRPP) test. The results depicted that all comparasion were significant (p-value ≤0.05) with respect to weed composition of either both winter and monsoon seasons, and for four weed communities. Similarly, A-values (within group agreement values) for all the comparasion were close to zero, thus depicting heterogeneity within group's equal's expectation by chance (*Table 3*).

Table 3. *Multi-response permutation procedure (MRPP) test results for pairwise comparasion of the seasonal and weed communities groups.*

Groups compared	T	A	p-value
Winter vs. Monsoon crop weeds	-24.376921	0.10575423	0.00000001
1.Wheat-AMP vs. 2.Wheat-CCV	-8.68390727	0.07680782	0.0000053
1.Wheat-AMP vs. 3.Maize-CMA	-15.84415686	0.15204394	0.00000002
1.Wheat-AMP vs. 4.Maize-CCD	-14.83208997	0.13589612	0.00000006
2.Wheat-CCV vs. 3.Maize-CMA	-11.16306984	0.12979608	0.00000077
2.Wheat-CCV vs. 4.Maize-CCD	-11.35771764	0.13157812	0.00000128
3.Maize-CMA vs. 4.Maize-CCD	-6.76188295	0.05187615	0.00000929
Overall	-21.086932	0.16229738	0.00000001

Canonical Correspondence Analysis (CCA)

The gradient length of compositional response data was more than 4 SD unit long, thus unimodal direct ordination model (CCA) was selected along with down weightage of rare species. It was used to seek species-environment relationship, contribution and

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ranking of explanatory variables towards explaining variations in the species data. A total of 7 variables including seasonality (1=samples studied during winter in wheat fields; 2=samples studied during monsoon in maize fields), longitude, latitude, altitude, NDVI, NDMI and TWI were tested for their influence on species response data (samples, 50; species, 60). Total variation in the species data were 4.87, whereas explanatory variable account for 1.5487 or 31.8% variations. Eigenvalues of CCA axis-1 and 2 were 0.73 and 0.25 respectively whereas species-environment relationship strength of the same was 0.9892 and 0.8912 respectively. Thus, first two constrained axes cumulatively explained 63.67% of fitted variations. Overall CCA model was significant thus further permutation test to seek the significance of explanatory variables and constrained axes (conditional or net term affects) were also performed. This depicted that four variables namely seasonality, longitude, altitude and latitude (in decreasing order) significantly (p-value <0.05) explained variations in the weed species data, whereas satellite derived variables were found non-significant in this regard. Out of 7, first three constrained axes were also found significant (*Table 4*). CCA graphical results (CCA biplot) for species and variables showed that seasonality gradient was the most important and clearly distribute weed species in the ordination space (right=winter/wheat weeds; left=monsoon/maize weeds; center=common weeds of both seasons) and strongly related with CCA axis-1 (*Fig. 9*). It was followed by the longitude (lower=high longitude species; upper= low longitude species) and related to CCA axis-2 and 3. The other important variables like altitude and latitude was found more related to CCA axis-4 (r = 0.74 and 0.75 respectively), followed by NDVI with axis-5 (r = 0.51), NDMI with axis-6 (r = -0.59) and TWI with axis-7 (r = 0.54).

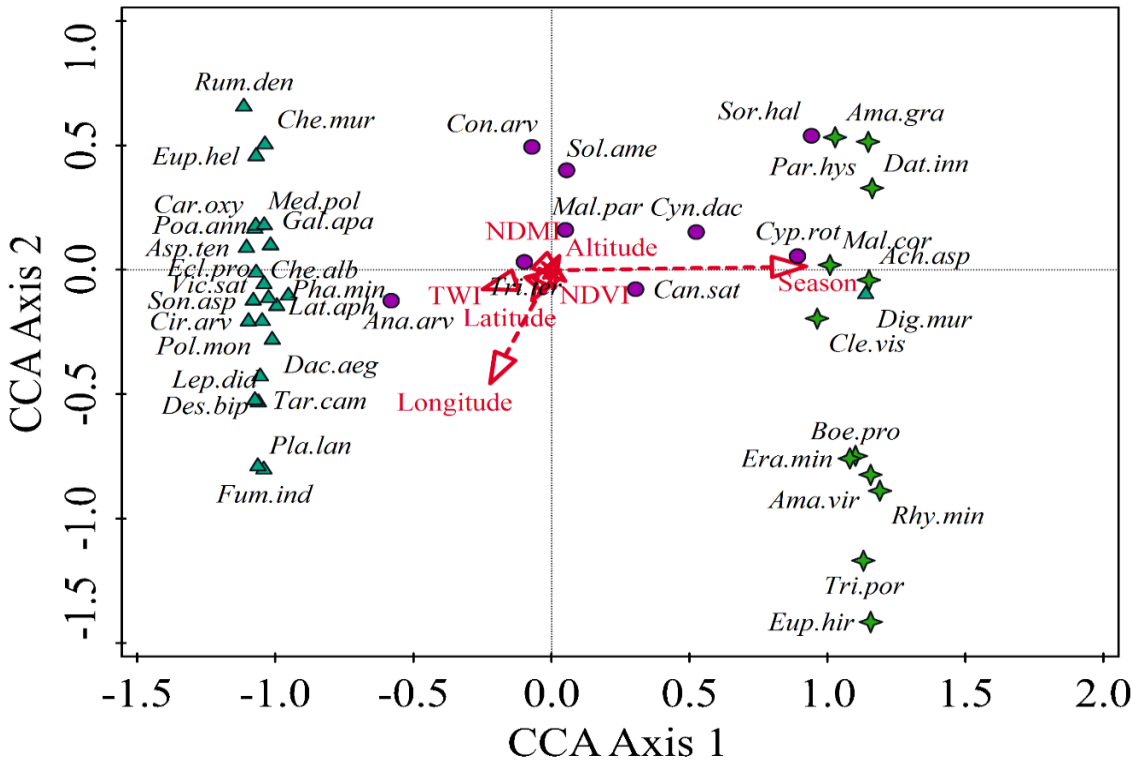


Figure 9. *Canonical correspondence analysis biplot showing the weed species-environment relationships and species groups based on seasonality variable viz. Wheat weeds (left), Maize weeds (right) and common of both crops/seasons (center).*

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Table 4. *Significance testing results for the explanatory variables and CCA axes.*

Variables/CCA-axes	Explains %	Pseudo-F	p-value
Seasonality	15	8.5	0.001
Longitude	3.8	2.2	0.001
Altitude	4.1	2.4	0.002
Latitude	3.9	2.4	0.001
NDVI	2	1.2	0.19
NDMI	1.8	1.1	0.264
TWI	1.2	0.8	0.878
Overall/CCA Model	**31.8**	**2.8**	**0.001**
CCA-Axis-1	14.82	7.3	0.001
CCA-Axis-2	5.18	2.7	0.001
CCA-Axis-3	3.99	2.2	0.007
CCA-Axis-4	2.94	1.7	0.093
CCA-Axis-5	2.11	1.2	0.533
CCA-Axis-6	1.65	1	0.771
CCA-Axis-7	0.95	0.6	0.977

Discussion

Phytosociological approaches are commonly used to assess the abundance, composition and classification of the weed species of agricultural crops (Anderson, 1996; Qureshi and Bhatti, 2001; Hussain et al., 2004; Jakhar et al., 2005; Hussain et al., 2007; Waheed et al., 2009; Iqbal et al., 2015; Iqbal et al., 2018). Such studies play an important role in the development of weed management to increase the agricultural yield. Additionally, invasiveness capabilities especially of the alien weeds can disrupt local biodiversity (Shabbir and Bajwa, 2006), thus their management is essential to avoid hazardous impacts especially on the crop yield. The satellite remote sensing (SRS) data can be used to study the different bio-physical features of planet earth like vegetation pattern, land cover changes and classification, agricultural yield, land surveying, hydrology and geology etc. For this, different measures like NDVI, NDMI and TWI can be calculated to assess the past, present and future scenario. Sultana et al. (2014) used NDVI values for prediction of wheat crop yield under varied experimental treatments like fertilization and cultivars quality. Thus use of these indices is common to seek vegetation pattern or in the prediction of crop yield pattern. Instead, this novel study was designed to use seasonality (micro-climatic variations) and satellite derived measurements for the exploration of their relationship with the weed distribution pattern of an agro-ecosystem (if any) of Gujrat, Pakistan.

A rich diversity of weed flora was recorded associated with the two important crops (wheat and maize) in district Gujrat, Pakistan. A total of 60 weed species were recorded and the majority of them belongs to Poaceae and Amaranthaceae families (*Fig. 2*). Iqbal et al. (2015) also recorded the dominancy of Poaceae weeds in the wheat crop from Malakand area of Pakistan. The majority of weed species flowered during monsoon season (July to August) due to micro-climatic suitability like higher temperature along with increased rainfall. Similar results were also recorded by Khan et al. (2018) from

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Muzaffarabad, Azad Jammu and Kashmir, Pakistan. Thus, during this season, therophytic weed species (42 spp., 70%) having microphyllous leaf size (25 spp., 42%) becomes dominant (*Fig. 8*). Similarly Kovačević et al. (2008) also concluded that therophytic weeds group was the dominant character from Herzegovina vineyard region.

Qureshi and Bhatti (2001), Hussain et al. (2004), Jakhar et al. (2005), Waheed et al. (2009), Iqbal et al. (2017) and Iqbal et al. (2018) recorded 5, 3, 3, 5, 5 and 5 weed communities respectively in the wheat fields whereas Ahmad et al. (2016a and 2016b) recorded 4 weed communities each from maize fields of different regions of Pakistan. The decision about the number of the weed communities in the aforementioned studies was made on physiognomic basis and no test statistics was employed. Thus, results of this study about the number of weed groups in both wheat and maize crops do not match with any earlier studies especially from related areas of Pakistan. By using the Monte Carlo testing, this study identified a total of four significant weed groups (*Fig. 4*), two each in wheat and maize fields, followed by hierarchical clustering for classification (*Figs. 5* and *6*) and indicator species analysis for species composition and their strength of association with the each group (*Table 2*). The same groups were further confirmed through MRPP testing (*Table 3*). Thus, weed species composition of each group of this study appeared distinct when compared with the earlier studies. However, the status of overall dominant weed species of both types of crops (i.e. *Anagallis arvensis*, *Medicago polymorpha*, *Phalaris minor*, *Chenopodium album*, *Convolvulus arvensis* and *Vicia sativa* weed species of wheat and *Cyperus rotundus, Malva parviflora*, *Amaranthus graecizans* subsp. *silvestris*, *Cannabis sativa*, *Cynodon dactylon* and *Digera muricata* weed species of maize fields) of this study was mostly comparable and remained the same. On the basis of seasonality difference, 9 weed species were found common in both wheat (winter season weeds) and maize (monsoon season weeds) fields. Out of these, four species named *Anagallis arvensis*, *Convolvulus arvensis*, *Solanum americanum* and *Tribulus terrestris* showed their maximum abundance in samples recorded from the wheat fields, thus appeared as indicator of the wheat communities. Similarly, based on abundance, the other five common weed species including *Cyperus rotundus*, *Malva parviflora, Cannabis sativa*, *Cynodon dactylon* and *Sorghum halepense* were become indicator of the maize communities (*Table 2*). The diversity values like Margalef richness, Shannon's diversity (H′), Simpson's diversity or Pielou evenness for each of weed community and their significance testing by using a non-parametric test (Kruskal-Wallis H test) was performed for the first time from Pakistan. These results depicted that longitude, NDVI, NDMI, reciprocal Simpson's diversity, Pielou evenness Simpson's diversity, altitude and latitude values (in decreasing order) of the studied fields of the four weed communities were significant (p-value ≤ 0.05). The highest mean weed diversity values were recorded for the maize field communities in monsoon season (*Fig. 7*). However, contribution of each studied variable for explaining variations in the species data was tested by using CCA ordination model. Results of CCA suggested that the local micro-climatic conditions (seasonality factor) were to be the most important in controlling the variations in the weed species data. By using CCA, Ahmad et al. (2016a) reported the importance of organic matter, potassium, sandy soil and farming practices as driving factor in controlling the weed distribution pattern

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in maize fields of Mardan, Pakistan. Similarly Iqbal et al. (2018) reported the importance of temperature, electrical conductivity, soil structure, organic matter, lime content, preceding crops, soil pH, herbicides usage, timing and quantity of manure as driving factor in wheat fields of Malakand, Pakistan.

No significant contribution of the NDVI, NDMI and TWI variables was recorded towards explaining the variations in the weed species data (*Table 4*). This depicted that crop health would be related to satellite derived measures undoubtedly but due to too much higher abundance of the wheat or maize individuals in comparison to their competing weed species individuals in the fields, these explanatory variables were not significant predictors of weed distribution pattern in any agro-ecosystem. The higher NDVI and NDMI values were recorded for the maize field communities in the monsoon season depicting higher greenness and moisture, whereas higher mean TWI value was recorded for the wheat fields, thus suggesting a strong linkage with the local micro-climatic conditions (*Fig. 7*). The overall variations of the SRS variables in the study area are also presented (*Fig. 10*).

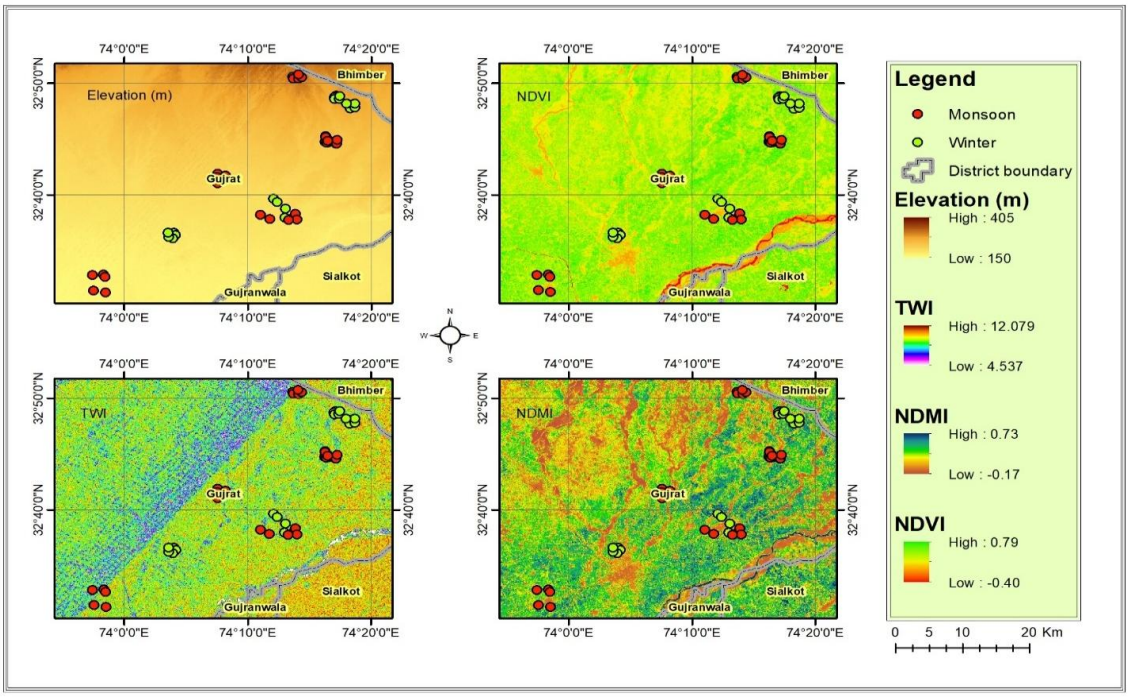


Figure 10. *Map showing satellite derived measurement variations in the study area of Gujrat, Pakistan during 2016.*

This study concluded that effective weed management activities are required to further enhance the yield of these two important crops in the area. Similarly, further studies related to determination of weed types and their distribution pattern by using factors like fertilization (type, timing, quantity), wheat and maize cultivar, land preparation (types, timing and intensities), soil management, pesticides (usage, type, quantity, quality), irrigation (type like rain-fed, tube-well, canals; timing, quantity), socio-economic and anthropogenic activities, and their relationship with the yield variable on temporal and spatial scales are recommended.

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NATIVE ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY COMPOSITION FROM BARTIN PROVINCE, TURKEY

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Abstract. The variety of the arbuscular mycorrhizal fungi (AMF) under the different forest stands is significant in terms of forest and rangeland ecosystems. In this study, dependent upon the morphological analyses, the diversity of the AMF within the pure oak forest (POF), fir-beech forest (FBF) stands, and gap in forest (GF) soils were compared in Bartın Province, Black Sea Region in northwestern of Turkey. The AMF community composition in POF, FBF and GF soil samples showed variation. Soil organic carbon, available P, number of AMF spores, species richness, diversity index, and dominance index were the main parameters differing among the three studied environments. In particular, the most prevalent "order" in these soils was Glomerales. The most frequent AMF genus in the soils of all these fields was *Glomus.* Varieties in AMF diversity were due to the: (1) changes in soil characteristics (e.g. soil available P and organic carbon); and (2) plant communities in the study fields. These findings support the detailed knowledge of AMF community compositions in soils from Turkey, and introduce new comprehension in order to analyze the ecological processes including the AMF species and native plant species in the northwestern Black Sea Region of Turkey.
Keywords: *Glomeromycota, native plant species, AMF diversity, AMF community composition, ecological processes*

Introduction

Temperate broadleaf and mixed forests constitute several ecoregions within the northwestern Black Sea Region, also referred to as temperate climate terrestrial biome. The temperate climate terrestrial biome was defined by Knudsen et al. (2017) as a "type of forest, which involves tall mature trees, shade-tolerant understory, small mature trees, saplings, suppressed juvenile canopy trees, low growing woody plants and herbaceous plants". This ecoregion is composed of variable vegetation types (usually, classified as four-layered vegetation structure). The ecoregion has dominant broadleaf tree species (e.g., *Quercus* spp. L., *Fagus* spp. L., *Acer* spp. L., *Betula* spp. L.) and coniferous tree species (*Pinus* spp. L., *Abies* spp. Mill., and *Picea* spp. Mill.). However, it is still unclear whether these vegetation types and soil properties have positive impacts on the native arbuscular mycorrhizal fungal (AMF) community (de Souza and Freitas, 2017).

Symbiosis between plant species and AMF is probably the oldest plant-microbes mutualistic relationship (Brundrett, 2002; Parniske, 2008). AMF are important biological component of soil and are closely associated with many ecosystem processes, including: (1) Plant community composition; (2) Soil microbial communities' structure;

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(3) Host-plant physiology; and (4) Ecosystem homeostasis (Rillig, 2004; Closa and Goicoeche, 2011; Ortiz et al., 2015; de Souza and Freitas, 2017). They can establish a symbiotic mutualistic connection (with the majority of terrestrial plant species), that is featured by the occurrence of intracellular structures such as arbuscules (Smith and Read, 2008; Hodge and Storer, 2014). AMF also occurs in several terrestrial ecosystems, from forests to grasslands, and from agroecosystems to deserts (Öpik et al., 2006).

In fact, AMF provides water and mineral nutrients (e.g. nitrogen and phosphorus) to the host-plant while plants provide energy (e.g. carbohydrates) to the AMF (Smith and Smith, 2011; Hodge and Storer, 2014). As reported by Rillig (2004), the changes in the physiology (e.g. root exudates, photosynthesis) of the host plant that are induced by the AMF and nutrient uptake, can be conveyed to the ecosystem processes. These ecosystem processes can directly and indirectly affect the diversity of the arbuscular mycorrhizal fungi (AMF) community and their function by altering (1) soil properties and (2) plant community composition (Clapp et al., 1998; de Souza et al., 2016a; Esch et al., 2017; de Souza and Freitas, 2017). AMF community diversity is positively correlated with the plant richness as reported by Zubek et al. (2013). Therefore, decreasing the diversity of plants hosting the AMF community, leads to the reduction in the growth and proliferation of AMF at the sites lacking diversity of host-plant community (Burrows and Pfleger, 2002; de Souza et al., 2016b;).

Development of arbuscular mycorrhizal fungi can strongly be influenced by the plant community composition (de Souza and Freitas, 2017). AMF sporulation and colonization have significantly been affected by the plant diversity (Zubek et al., 2013; de Souza et al., 2016a, b). AMF colonization diminishes due to the host-plant response, soil type and availability of the soil nutrient (Smith and Read, 2008). It is emphasized that the type of forest and host plant might be the determinants for influencing the AMF spore density and biodiversity. The highest AMF spore density along the year was registered from the pine-oak mixed stand, pure pine and oak stands and the agricultural land respectively in India (Chaturvedi et al., 2012).

We investigated the AMF community composition under the pure oak forest (POF), fir-beech mixed forest (FBF) and gap in forest (GF) from Bartın Province, in Black Sea Region at the northwest of Turkey, using morphological analyses. We hypothesized the existence of a diversification of AMF community within the areas that present a high variability of plant community regarding the trees, shrubs and herbs. Several studies were conducted about the interactions among AMF, soil characteristics, and plant community (de Souza et al., 2016a, b; de Souza and Freitas, 2017). In order to achieve our target, we investigated the soil samples collected from the root zones to analyze the soil characteristics and AMF community composition.

Materials and methods

Study sites

The study areas were located in Bartın Province, Turkey. The study areas which are about 61 km away from the city centre of Bartın Province, are located within the 41°32′ Northern latitudes and 32°50′ Eastern longitudes (*Fig. 1*). These areas are located in the northwestern Black Sea Region. The exposures of the pure oak stand and mixed fir-beech stand are South-East and North respectively. The gap in forest exposure is flat. The meteorological parameters used in this research were provided by the

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meteorological station of Bartın province. Relative humidity of the study areas ranges from 66.8% to 71.4% (Topay, 2003). The soil type is Ultisol according to the soil taxonomy of USDA (Kara et al., 2008; Palta et al., 2016). According to the Thornthwaite method, the study area is included into the humid mesothermal climate regime. Climatological data indicated that the annual average temperature is about 8.0°C. On the other hand, the average temperatures of the two hottest months, July and August, are 18.0 and 17.5°C, respectively. The mean annual precipitation in the study area is around 1534 mm (Şengönül et al., 2009).

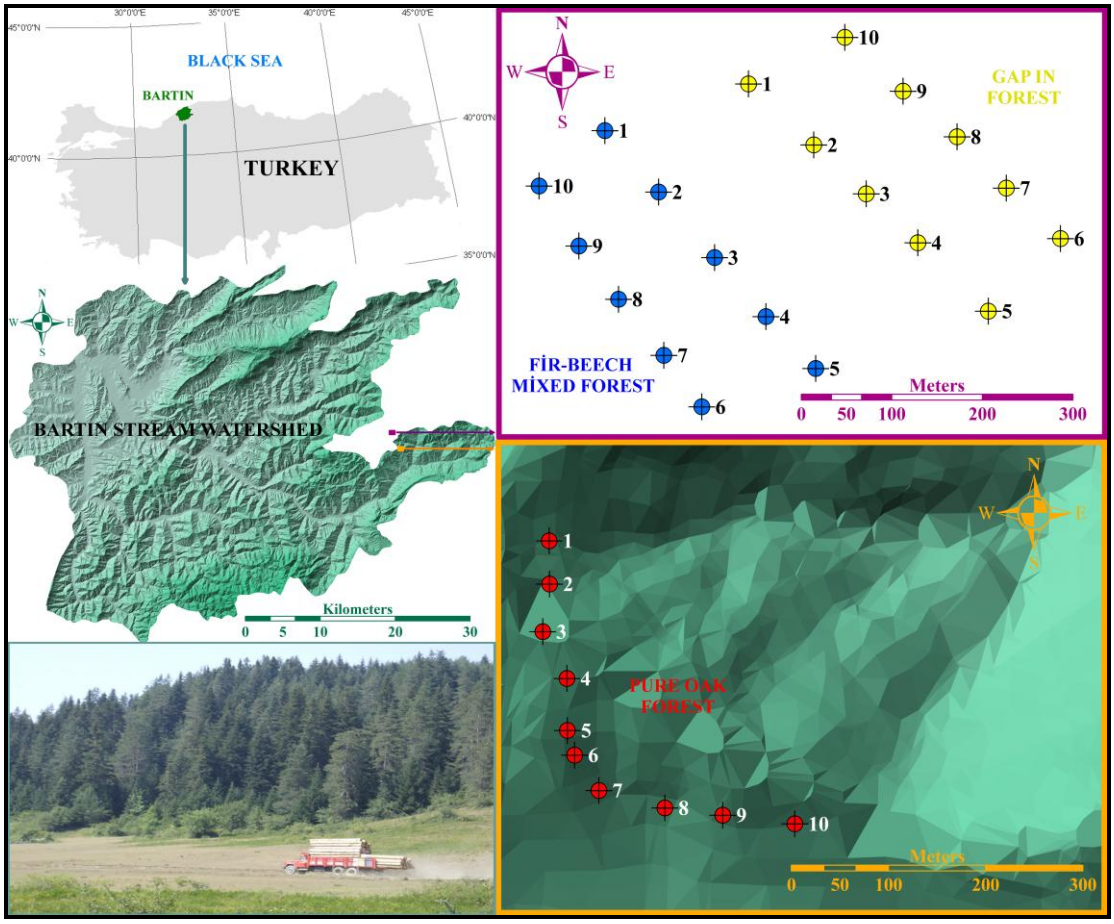


Figure 1. *Sampling points of pure oak, fir-beech mixed forest stands and gap in forest within Bartın Stream Watershed, Turkey. A field photograph is also attached to the figure*

Sampling was conducted on three different sites: (1) pure oak stand (1067 m asl.), (2) fir-beech mixed stand (1004 m asl.) and (3) gap in forest (976 m asl.). The following plant families were relatively common for the three areas: Fabaceae and Gramineae. The soil samples were collected from the rhizosphere of these plants. *Table 1* demonstrates the explicit presentation of the plant taxons and their families within the study fields.

Soil sampling

Soil sampling was conducted during June and July 2015 (dry season). In each area, ten different composite root zone samples that involve soil and root fragments, were

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randomly taken from the near drip line and beyond (0-30 cm deep) of different plant taxa. The root zone samples from each treatment were sieved by a 2-mm sieve, put in polyethylene bags, and stored at 4°C until analyses dates. Thus, we analyzed 10 samples (n = 10) for each studied area. In addition, along the dry season, the sampling was conducted since the fungal sporulation was estimated to be higher during that period in both arid and semi-arid ecosystems (Silva et al., 2014). The rhizosphere samples gathered from the study areas were separated into the portions in order to assess some of the chemical and physical characteristics of soil and AMF community.

Table 1. *Plant species where soil samples were taken, in a pure oak forest (POF), fir-beech mixed forest (FBMF), and gap in forest (GF) in the northwestern Black Sea Region of Bartın Province, Turkey*

Family	Plant species	POF	FBMF	GF
Apiaceae	*Sanicula europaea* L.		X	
Cyperaceae	*Carex sp.* L.		X	X
Dennstaedtiacae	*Pteridium aquilinum* (L.) Kuhn in Kerstin		X	
Fabaceae	*Dorycnium graecum* (L.) Ser	X		
	Lathyrus aphaca L.			X
	Lathyrus laxiflorus subsp. laxiflorus (Desf.) Kuntze	X		
	Lotus corniculatus L.	X		
	Sophora jaubertii Spach			X
Fagaceae	*Quercus petraea* [Matt.] Liebl.	X		
	Fagus orientalis Lipsky		X	
Lamiaceae	*Ajuga reptans* L.		X	
	Lamium purpureum L.		X	
Pinaceae	*Abies nordmanniana ssp. bornmülleriana* Mattf.		X	
Poaceae	*Anthoxanthum odoratum* L. subsp. *odoratum*			X
	Calamagrostis arundinaceae (L.) Roth	X		
	Dactylis glomerata L.	X		X
	Festuca sp. L.	X	X	X
	Hordeum violaceum Boiss. & Huet			X
	Poa bulbosa L.	X		
Plant species richness		8	7	8

Isolation and identification of arbuscular mycorrhizal fungi

Wet sieving methodology was referred during the isolation of the AMF spores (Gerdemann and Nicolson, 1963), which then was followed by the sucrose centrifugation (Jenkins, 1964). First of all, isolated spores were analyzed under water through a microscope. The spores were classified dependent upon their morphological characteristics. The species definition relied on the diagnosis based on Schenck and Perez (1990), and on scientific literatures about the new genera and families (i.e. Oehl et al., 2008; http://invam.caf.wvu.edu). For this study, we applied the classification suggested by Oehl et al. (2011), involving relatively recent identified new taxa (i.e. Goto et al., 2012; Sieverding et al., 2014). Furthermore, we investigated the AMF spore

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density and the species occurrence frequency (FO_i) of each AMF species. FO_i was computed based on this equation: $FO_i = n_i/N$, where n_i is the number of times an AMF species was examined and N is the total of AMF spores diagnosed from each treatment. Findings of FO_i was classified dependent upon Zhang's frequency of occurrence classification: dominant ($FO_i > 50\%$), most common ($31\% \leq FO_i \leq 50\%$), common ($10\% \leq FO_i \leq 30\%$) and rare ($FO_i < 10\%$) (Zhang et al., 2004; de Souza et al., 2016c).

Chemical and physical characteristics of soils

The standard methods were applied for the analysis of the soil chemical and physical characteristics. These standard methods include the hydrometer method (Bouyoucos, 1962) for the identification of the soil particle size distribution. The pH-meter (Rowell, 1994) was used for the identification of pH in 1:2.5 soil/water suspension. The electrical conductivity meter (Rhoades, 1982) was preferred for the determination of electrical conductivity in 1:5 soil/water suspension. The Walkley-Black wet oxidation method (Walkley and Black, 1934) and Kjeldahl method (Bremner and Mulvaney, 1982) was applied for the analysis of soil organic carbon and total nitrogen, respectively. The available phosphorus was determined based on Olsen et al. (1954). The Scheibler calcimeter method (Allison and Moodie, 1965) was practiced for identifying the $CaCO_3$ content.

Ecological and statistical analysis

In order to analyze the significance of the overall variation in spore density, and soil properties among studied areas, the one-way ANOVA was performed. Data sets were conveyed (arc sin square root for percentage variables and logarithmic for other variables), but the findings were shown in their original scale of measurement (means with standard deviation) (Zar, 1984). When appropriate, the averages were compared by the Duncan test ($p < 0.05$). AMF species richness, spore abundance, species occurrence frequency were also determined. Two ecological indices (Shannon and Simpson) were referred in this research (Shannon and Weaver, 1949; Simpson, 1949). Pearson correlation was also used to examine the relationships between AMF community composition and soil properties. The Statistical Package for the Social Sciences (SPSS), version 16.0 (SPSS 16.0, 2007) was applied for the statistical analyses.

Results

Arbuscular mycorrhizal fungi community composition

There were significant differences on number of AMF identified species ($F_{2,27} = 21.85$, $p < 0.001$), number of spores ($F_{2,27} = 28.45$, $p < 0.001$), diversity index ($F_{2,27} = 13.98$, $p < 0.001$) and dominance index ($F_{2,27} = 12.22$, $p < 0.001$) among the studied sites. It was recognized that the samples from gap in forest had included the highest AMF diagnosed species, with 7.0 identified species, followed by pure oak forest stand (2.2 AMF species) and fir-beech mixed forest stand (1.3 AMF species). The spore density of AMF was 7.6 spores/25 g soil in pure oak forest stand; 2.8 spores/25 g soil in fir-beech mixed forest stand; and 28.2 spores/25 g soil in gap in forest. Diversity (H, Shannon's index) and dominance (C, Simpson's index) in the pure oak forest stand, fir-beech mixed forest stand and gap in forest were 1.63 and 0.21; 1.12 and 0.37; and 2.79 and 0.08 respectively (*Table 2*).

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Table 2. *Average of AMF identified species (IS), number of spores (NS), diversity (H) and dominance index (C) of each site (mean ± SD, n = 10) for each analyzed parameter*

Parameter	POF	FBMF	GF
IS	2.20 ± 0.40^{b}	1.30 ± 0.10^{b}	7.00 ± 0.80^{a}
NS (25 g $soil^{-1}$)	7.60 ± 3.71^{b}	2.80 ± 1.98^{b}	28.20 ± 13.19^{a}
H	1.63 ± 0.32^{b}	1.12 ± 0.15^{b}	2.79 ± 0.28^{a}
C	0.21 ± 0.02^{b}	0.37 ± 0.03^{a}	0.08 ± 0.02^{c}

Pure oak forest stand (POF), fir-beech mixed forest stand (FBMF), and gap in forest (GF). Different letters mean significant differences by Duncan test ($p < 0.05$) after one-way ANOVA

Including all the study areas, 27 different AMF species were diagnosed belonging to 15 genera – *Acaulospora* (2), *Cetraspora* (1), *Claroideoglomus* (3), *Corymbiglomus* (1), *Dentiscutata* (3), *Diversispora* (2), *Entrophospora* (1), *Funneliformis* (2), *Gigaspora* (3), *Glomus* (2), *Racocetra* (1), *Rhizoglomus* (2), *Scutellospora* (2), and *Septoglomus* (1) (*Table 3*). Among the total AMF species, 2 (7.41%) were classified as most common species: *Glomus ambisporum* and *Glomus* spp.; 6 (22.23%) were classified as intermediate species: *Acaulospora capsicula, A. foveata, Corymbiglomus tortuosum, Funneliformis caledonium, Rhizoglomus aggregatum,* and *R. fasciculatum*; and 18 (70.36%) were classified as exclusive: *Cetraspora pellucida, Claroideoglomus claroideum*, *C. Lamellosum, C. Luteum, Dentiscutata erytrropus, D. heterogama, Dentiscutata spp., Diversispora eburnea, D. epigaea, Entrophospora infrequens, Funneliformis geosporum, Gigaspora albida, G. decipiens, G. gigantea, Racocetra verrucosa*, *Scutellospora calospora, Sclerocystis sinuosa, Scutellospora* spp., and *Septoglomus constrictum* (*Table 3*; *Figs. 2* and *3*).

Table 3. *Frequency of occurrence (FO_i) of AMF species of the pure oak forest stand (POF), fir-beech mixed forest stand (FBMF), and gap in forest (GF)*

AMF species	FO_i[1] (classification[2])			Species classification[3]
	POF	FBMF	GF	
Order Diversisporales				
Family Acaulosporaceae				
Acaulospora capsicula Błaszk.	-	4.7 (R)	9.9 (R)	Intermediate
A. foveata Trappe & Janos	9.2 (R)	-	3.5 (R)	Intermediate
Order Gigasporales				
Family Dentiscutataceae				
Dentiscutata erythropus (Koske & C. Walker) C. Walker & D. Redecker	-	-	1.0 (R)	Exclusive
D. heterogama (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl	-	-	1.0 (R)	Exclusive
Dentiscutata spp. Sieverd., F.A. Souza & Oehl	-	-	1.0 (R)	Exclusive
Family Diversisporaceae				
*Corymbiglomus tortuosum (*N.C. Schenck & G.S. Sm.) Błaszk. & Chwat	5.3 (R)	-	1.0 (R)	Intermediate

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Diversispora eburnea (L.J. Kenn., J.C. Stutz & J.B. Morton) C. Walker & A. Schüßler	-	-	1.0 (R)	Exclusive
D. epigaea (B.A. Daniels & Trappe) C. Walker & A. Schüßler	-	-	1.0 (R)	Exclusive
Family Gigasporaceae				
Cetraspora pellucida (T.H. Nicolson & N.C. Schenck) Oehl, F.A. Souza & Sieverd.	-	-	1.0 (R)	Exclusive
Gigaspora albida N.C. Schenck & G.S. Sm.	-	-	7.7 (R)	Exclusive
G. decipiens I.R. Hall & L.K. Abbott	-	-	1.0 (R)	Exclusive
G. gigantea (T.H. Nicolson & Gerd.) Gerd. & Trappe	-	-	6.6 (R)	Exclusive
Family Racocetraceae				
Racocetra verrucosa (Koske & C. Walker) Oehl, F.A. Souza & Sieverd.	-	-	1.6 (R)	Exclusive
Family Scutellosporaceae				
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	-	-	2.3 (R)	Exclusive
Scutellospora spp. C. Walker & F.E. Sanders	-	-	1.0	Exclusive
Order Glomerales				
Family Entrophosporaceae				
Claroideoglomus claroideum (N.C. Schenck & G.S. Sm.) C. Walker & Schüßler	-	-	2.1 (R)	Exclusive
C. lamellosum (Dalpé, Koske & Tews) C. Walker & A. Schüßler	-	-	1.4 (R)	Exclusive
C. luteum (L.J. Kenn., J.C. Stutz & J.B. Morton) C. Walker & A. Schüßler	-	-	1.6 (R)	Exclusive
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneider	-	-	1.0 (R)	Exclusive
Family Glomeraceae				
Funneliformis caledonium (T.H. Nicolson & Gerd.) C. Walker & Schüßler	10.5 (C)	-	2.0 (R)	Intermediate
F. geosporum (T.H. Nicolson & Gerd.) C. Walker & Schüßler	-	-	6.6 (R)	Exclusive
Glomus ambisporum G.S. Sm. & N.C. Schenck	32.9 (MC)	26.4 (C)	6.6 (R)	Generalists
Glomus spp. Tul. & C. Tul.	21.0 (C)	50.0 (D)	18.7 (C)	Generalists
Rhizoglomus aggregatum (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl	-	18.9 (C)	9.8 (R)	Intermediate
R. fasciculatum (Thaxt.) Sieverd., G.A. Silva & Oehl	21.1 (C)	-	6.0 (C)	Intermediate
Sclerocystis sinuosa Gerd. & B.K. Bakshi	-	-	1.6 (R)	Exclusive
Septoglomus constrictum (Trappe) Sieverd., G.A. Silva & Oehl	-	-	1.0 (R)	Exclusive

[1]$FO_i = n_i/N$, where n_i is the number of times an AMF species were encountered and N is the total of AMF spores observed from each study field; [2]Classification of AMF frequency of occurrence suggested by Zhang et al. (2004): R–rare (FO < 10%); C–common (10% ≤ FO ≤ 30%); MC–most common (31% ≤ FO ≤ 50%); and D–dominant (FO > 50%); and [3]Species classification suggested by Stürmer and Siqueira (2011) most common (present in all fields), intermediate (present in 2 fields) or exclusive (present in only one field).

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Chemical and physical properties of soils

All the chemical and physical properties of soil varied significantly among the studied areas. The highest values of organic carbon, available phosphorous (P), total nitrogen (N) and soil moisture were found in the gap in forest (GP). Soil pH was higher in the fir-beech mixed forest stand (FBMF) compared to the other treatments. For the physical properties, we found the highest values of sand, clay and silt in the pure oak forest (PO) stand (*Table 4*).

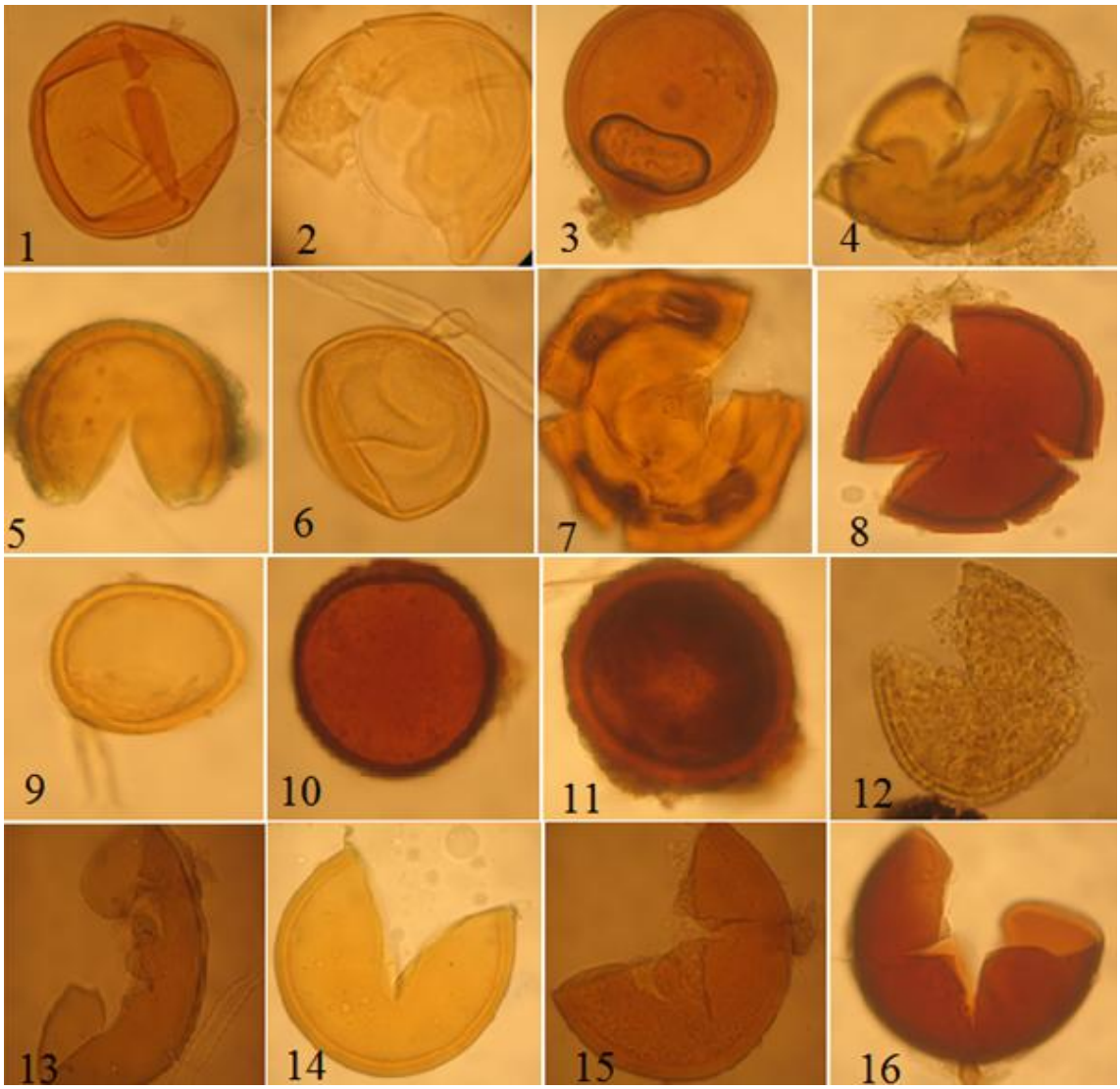


Figure 2. *AMF spores 1) Acaulospora capsicula (150 µm*), 2) Acaulospora foveata (280-360 µm), 3) Cetraspora pellucida (145 µm) 4) Claroideoglomus claroideum (110-150 µm), 5) Claroideoglomus lamellosum (150 µm), 6) Claroideoglomus luteum (140 µm), 7) Dentiscutata erythropa (250-300 µm), 8) Dentiscutata heterogama (140-150 µm), 9) Diversispora eburnea (100 µm), 10) Diversispora epigaea (135 µm), 11) Diversispora tortuosa (200 µm), 12) Enthrophospora infrequens (150-200 µm), 13) Funneliformis caledonius (200-290 µm), 14) Funneliformis geosporum (200-210 µm), 15) Gigaspora albida (220-240 µm), 16) Gigaspora decipiens (200-250 µm). *Diameter*

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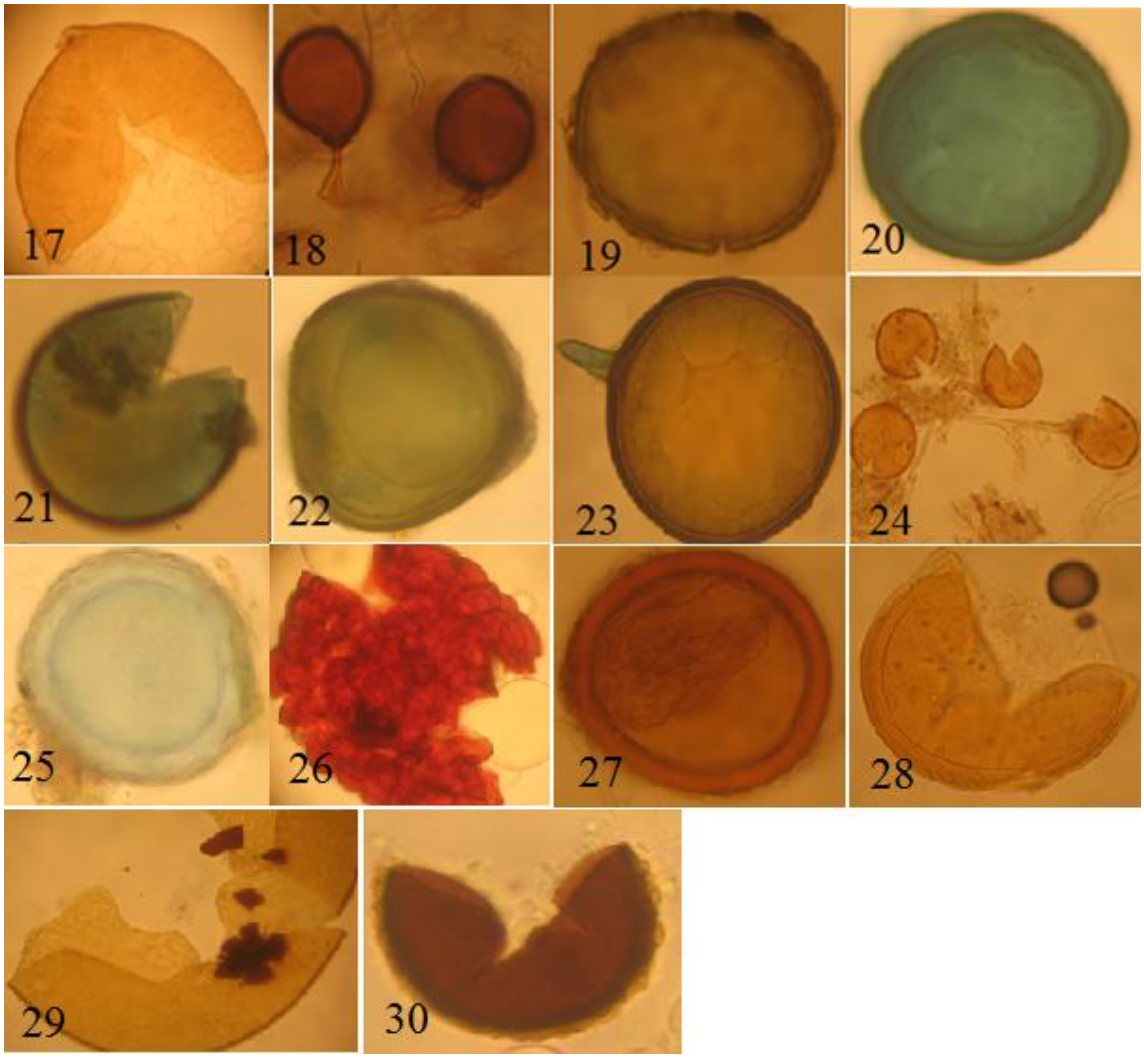


Figure 3. *17) Gigaspora gigantea (450-650 µm*), 18) Glomus ambisporum (50 µm), 19) Glomus spp. (90-100 µm), 20) Glomus spp. (80 µm), 21) Glomus spp. (60 µm), 22) Glomus spp. (120 µm), 23) Racocetra verrucosa (200 µm), 24) Rhizophagus aggregatus (50 µm), 25) Rhizophagus fasciculatum (90 µm), 26) Sclerosystis sinuosum (3 00-350 µm), 27) Scutellospora calospora (135 µm), 28) Scutellospora spp. (150-210 µm), 29) Scutellospora spp. (220 µm), 30) Septoglomus constrictum (50-70 µm). *Diameter*

Correlation analysis

According to the correlation analysis, a positive correlation was found between the AMF spore density, and organic carbon, available phosphorus, total nitrogen and the moisture of soil with *r-values*; 0.683, 0.534, 0.661 and 0.660, respectively (*Table 5*).

Discussion

Arbuscular mycorrhizal fungi

Our results indicate that the changes in arbuscular mycorrhizal community were led by differences in: (1) plant community composition among the gap in forest and the other two studied forested sites; and (2) soil properties among all the studied sites in the

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northwestern Black Sea Region, Turkey. In fact, these two factors may influence AMF community structure in different ways (Olsson et al., 2010; Zubek et al., 2016; de Souza and Freitas, 2017). In the pure oak forest and fir-beech mixed forest, both plant community and soil properties significantly affected the AMF diversity and dominance. These results are consistent with the former studies (Gehring, 2003; Heinemeyer et. al., 2004; Shukla et al., 2009; Shi et al., 2014), and support our hypothesis that a diversified AMF community is associated with the areas that present a high diversity of plant community involving trees, shrubs and herbs. As a consequence, areas with low diversity of plant species (pure oak forest and gap in forest) are likely to decrease mycorrhizal root colonization (Johnson, 2010; Shi et al., 2014).

Table 4. *Mean of physical and chemical soil characteristics (mean ± SD, n = 10) of the studied areas*

Soil characteristics	POF	FBMF	GF
Sand (%)	35.20(±6.51)^a	47.20(±3.32)^b	41.05(±7.51)^b
Clay (%)	27.30(±5.46)^a	18.60(±2.12)^b	20.05(±5.25)^b
Silt (%)	37.50(±3.95)^a	34.20(±2.35)^b	38.90(±3.60)^a
pH(H_2O)	5.09(±0.16)^a	5.33(±0.36)^a	5.30(±0.58)^a
Soil texture	Clay Loam	Loam	Loam
$CaCO_3$ (%)	0.26(±0.09)^a	0.29(±0.08)^a	0.27(±0.12)^a
Electrical conductivity (dS m^{-1})	0.03(±0.01)^a	0.03(±0.02)^a	0.06(±0.02)^b
Organic carbon (%)	5.18(±0.73)^a	5.46(±1.37)^a	8.10(±0.81)^b
Available P (mg/kg)	46.92(±14.48)^a	50.07(±8.18)^a	71.92(±16.98)^b
Total N (mg/lt)	0.39(±0.08)^a	0.41(±0.09)^a	0.56(±0.13)^b
Soil moisture (%)	33.02(±9.65)^a	26.53(±7.24)^a	43.37(±7.54)^b

The values in the same row that are followed by the different letter demonstrate significant ($p < 0.05$) differences between the means

Table 5. *Pearson's correlation coefficient values (r-values) for AMF spore density (SD), soil organic carbon (C_{org}), available phosphorous (AP), total nitrogen (TN), and soil moisture (SM) from the studied areas (n = 30)*

Variables	SD	C_{org}	AP	TN	SM
SD	-	0.683**	0.534**	0.661**	0.660**
C_{org}			0.637**	0.812**	0.375**
AP				0.709**	0.409*
TN					0.395*

Asterisks indicate significant differences by Pearson correlation analysis (*$p < 0.05$; **$p < 0.01$)

The variety in the AMF community structure among the study areas is due to the decreased vegetation species richness, decreased spore density, decreased biodiversity of arbuscular mycorrhizal fungi species (diversity index), and increased canopy closure of the stands. According to some particular studies also (e.g. Heinemeyer et al., 2004; Shukla et al., 2009; Shi et al., 2014), we hypothesize that the below factors may be drivers of the results observed for the AMF community composition. First, shade may have reduced the carbon allocation from shrubs and herbs by mycorrhizas. Thus,

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increasing canopy closure above understory may have reduced light intensity below understory and decreased the photosynthetic ability of host-plants (e.g. *Anthoxanthum odoratum* L, *Carex sp.* L., *Dactylis glomerata* L., *Festuca sp.* L., *Hordeum violaceum* Boiss & Huet., *Lathyrus aphaca* L., and *Sophora jauberii* Spach). Consequently, these two processes may have reduced AM colonization and spore abundance (Heinemeyer et al., 2004; Shukla et al., 2009).

Chemical and physical properties of soils

The results of soil analysis revealed that some of the soil chemical and physical characteristics altered within the study areas. The results observed in the Black Sea Region indicated that the soil pH(H_2O) of forest, tillage and rangeland ranged from 5.0 to 7.8 (Korkanç, 2003; Kara et al., 2008; Şengönül et al., 2009; Palta et al., 2012, 2013). Compatible with the findings of the former studies, our results indicated that the average soil pH(H_2O) values of the pure oak stand, the fir-beech stand and the gap in the forest, were 5.09, 5.33 and 5.30, respectively (*Table 5*).

The observed results demonstrated that the soil organic carbon contents ranged from 4.2 to 7.3% in the rangelands of the Black Sea Region (Okatan, 1987; Şengönül, et al., 2009; Palta et al., 2012, 2013). The average soil organic carbon contents were determined for the pure oak stand, the fir-beech stand and the gap in the forest as 5.18%, 5.46%, 8.10%, respectively (*Table 5*). In this study, the highest organic carbon content was found for the gap in the forest. This may be due to the exposure, slope and non-grazing in the study area. In addition, the gap in the forest field was relatively flat land but the stands were located on the sloping land. The toe of slope positions had superior soil organic carbon content due to the higher infiltration rate (Guzman and Al-Kaisi, 2011) and soil erosion (Gregorich et al., 1998). The highest number of forage legumes were found in the gap in the forest (Şengönül et al., 2009) and it was stated that legumes could supply nitrogen thanks to the biological fixation of N from the atmosphere (Monegat, 1991) and increased the rates of accumulation of both soil nitrogen and carbon (Knops and Tilman, 2000). In addition, it was stated that the soil organic carbon was influenced by the soil properties (Tisdall and Oades, 1982; Kay, 1998; Jones et al., 2004).

Conclusions

This research demonstrated that the arbuscular mycorrhizal fungi biodiversity was influenced by the stand types; the pure oak stand, mixed fir-beech stand and the gap in forest. It was showed that the higher AMF biodiversity and associated AMF spore density were determined for the gap in forest, pure oak forest stand and fir-beech mixed forest stand, respectively. Furthermore, AMF spore density was positively correlated with the organic carbon content, available phosphorus, total nitrogen and moisture of soil. The main results of this study overall indicate: (1) changes in plant community compositions and soil properties alter the AMF diversity and community structure; and (2) plant community composition below the understory can influence AMF biodiversity indicating that the overall vegetation diversity is significant for the AMF diversity. The studies on AMF are currently and relatively inadequate in the northwestern Black Sea Region. Therefore, similar researches should spatially be spread and favoured within the region in order to delineate the map of AMF distribution. Besides, genus of native AMF should be isolated

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and reproduced to be utilized for the prospective plantation studies. Consequently, sustainable management of the forest and rangeland interaction fields will be achieved.

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PRODUCER FIGHT AGAINST CROWN GALL DISEASE

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Abstract. *Agrobacterium vitis*-induced crown gall disease (tumor) is the greatest problem in vineyards. Much proceed has not been achieved in fight against crown gall disease. Producers either totally stub out the vines from already established vineyards or keep viticulture activities with palliative measures. This study was conducted for two years to investigate the effects of shoot regeneration on yield, quality and development of *A. vitis*-infected grapevines. Initially the grapevines with morphologic *A. vitis* symptoms were determined in experimental vineyard. Then, deep pruning (cutting) was performed 10-15 cm below the tumorous sections of the stems in 2010, 2011 and 2012 and shoot regeneration was stimulated. Data were gathered about yield, quality (number of clusters, cluster weight, berry weight, must TSS content, TA, maturation index) and vine growth parameters (number of buds, number of shoots, burst ratio, pruning weight). About 15% less development was observed in grapevines with shoot regeneration treatments in 2012, 25% in grapevines with shoot regeneration treatments in 2011 and 37% in grapevines with shoot regeneration treatments in 2010. With shoot regenerations in 2012, 2011 and 2010, yields decreased respectively by 49, 58 and 66%; cluster weights decreased respectively by 32, 37 and 47% and berry weights decreased respectively by 14, 19 and 21%.
Keywords: *pruning, Narince, yield, development, percent change*

Introduction

The yield and quality in viticulture are largely dominated by the rootstocks, training systems, irrigation methods, fertilization and relevant technical and cultural practices (Winkler et al., 1974; Çelik et al., 1998; Noar and Gal, 2002; Keller, 2010). Pests and disease management is also another factor significantly influencing yield and quality in viticulture. It was reported that without the proper control practices, 66% yield loss was caused by powdery mildew (Çetinkaya and Onoğur, 2006) and 10-15% yield loss was caused by dead-arm (*Phomopsis viticola*) (Anonymous, 2013). Pest and diseases result in significant changes in number of clusters, cluster weights, berry weights and physico-chemical attributes of the berries (Pool et al., 1984; Stummer et al., 2003; Akgül et al., 2017).

Pests and disease prevention and control measures are not either taken or such measures are uneconomic in vineyards. Common pests and diseases include bacterial crown gall (crown gall disease) (*Agrobacterium vitis* or *Rhizobium vitis*), Eutypa *(Eutypa lata)*, viral infections (*Grapevine Leafroll, Grapevine Fanleaf)* and phylloxera pest (*Viteus vitifoliae*). Crown gall (*A. vitis*) disease is a bacterial disease (Ophel and Kerr, 1990). Disease progress is closely related to climate conditions. The disease result is significant yield and quality losses in grapevines (Schroth et al., 1988; Çelik et al., 2000; Diana and Dejeu, 2011; Akgül et al., 2016).

A. vitis is transported through xylem and phloem transportation vessels (Lehoczky, 1971; Burr et al., 1998). The bacterium usually causes tumor formations over grapevine stem. The disease also encountered in roots and shoots (Lehoczky, 1971; Burr et al., 1987; Keller, 2010). The tumors formed over various parts of the plant limit water and nutrient transport throughout the plant. Then vine growth and development, yield and quality decreases in time (Schroth et al., 1988; Ferreira et al., 1992; Keller, 2010; Diana

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and Dejeu, 2011). Scrapping the tumors over the stem and 5-7% Bordeaux mixture applications over the lesions (Saygılı et al., 2008) may prolong grapevine life for couple years, but cannot prevent ultimate deaths (Burr and Katz, 1984; Schroth et al., 1988).

In present research region, 75.35% of the vineyards are infected with *A. vitis* (Durak et al., 2017). Therefore, farmers are still making productions with generally infected grapevines. Some producers cut *A. vitis*-infected grapevines 10 cm below the tumors and apply high concentration (7-8%) Bordeaux mixture over the lesion. Then, adventive buds over the stem shoot out and new shoots are formed (*Figs. 1* and *2*). Such a practice is shortly defined as shoot regeneration.

This study was conducted to investigate the effects of shoot regeneration treatments on grape yield, quality and development of *A. vitis*-infected grapevines.

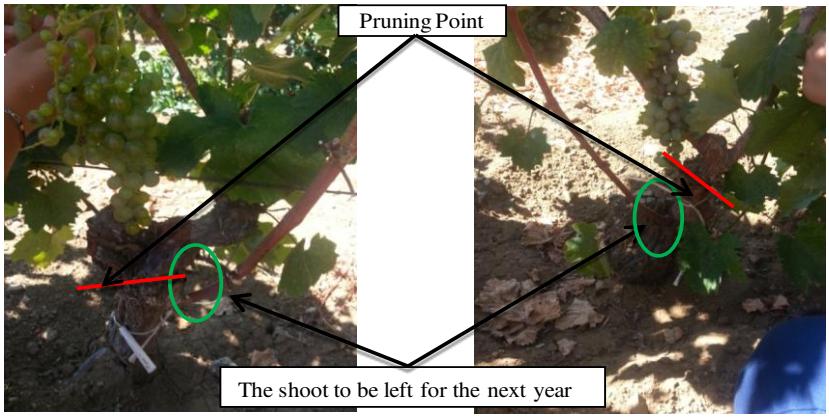


Figure 1. *The grapevines to be shoot regenerated in the next year: pruning locations and the shoots to be left*



Figure 2. *Shoot regenerated in 2013 (harvest time of 2013)*

Materials and methods

Experiments were conducted in a producer vineyard (Tokat-Turkey; located at 40° 37' 10.99" N, 36° 43' 27.87" E) in 2013 and 2014. Narince/1103 Paulsen grape cultivar planted at 3.0 x 1.5 m planting density in 2000 was used as the plant material. Narince is a significant white wine cultivar of Turkey. International *Vitis* code of the cultivar is 8351. Bilateral cordon system was used as training system. Dry farming was practiced

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without any irrigations. Standard care practices were performed throughout the experiments.

The first *A. vitis* symptoms were observed by the owner in 2008. From the year 2010, severe/deep pruning was performed 10-15 cm below tumorous section of the grapevine stem (*Figs. 1* and *2*). This process was kept on in 2011 and 2012. New shoots of the grapevines were long-pruned (10-12 buds) in the second year. The grapevines with shoot regeneration treatments were marked in each year. Brief information about the grapevines considered in this study is provided in *Table 1*.

Table 1. *Stem regeneration year, stem diameter and number of grapevines*

Stem regeneration year	Stem diameter (mm)	Number of grapevines
Non-tumorous (2000)	78.0-82.0 mm	6
GY-1 (year 2012)	13.0-19.0 mm	6
GY-2 (year 2011)	15.0-20.0 mm	6
GY-3 (year 2010)	15.0-22.0 mm	6

Experiments were conducted in 2013 and 2014. Therefore, the time elapsed between shoot regeneration and yielding years in grapevines with stem regeneration in 2010, 2011 and 2012 was expressed as: 1-2 years (GY-1) (for 2012), 2-3 years (GY-2) (for 2011) and 3-4 years (GY-3) (for 2010).

In 2013, pathogen existence and diagnosis analysis was performed in experimental vineyard. *A. vitis* pathogen was diagnosed in all of the shoot-regenerated grapevines. For diagnosis; DNA extractions were performed in accordance with Benlioğlu et al. (1998) and PCR tests were performed in accordance with Szegedi and Bottka (2002). Within the same vineyard, the grapevines without tumor development and with negative analysis results were considered as control vines.

Methods

Development, yield and some quality parameters were measured on grapevines.

Development values

Number of buds: The buds left over the grapevines were counted after pruning (*Eq. 1*).

Number of shoots: The shoots in each grapevine were counted.

$$\text{Burst ratio (\%)} = \left(\frac{\text{Number of bursted buds}}{\text{Total number of buds}}\right) \text{x } 100 \qquad \text{(Eq.1)}$$

Pruning weight (kg/vine): Pruning was performed after defoliation and 1-year-old wood (shoot) were weighted.

Yield and quality values

Harvest was performed at The Soluble Solid (TSS) contents of between 19.0-23.0% (Rieger, 2006).

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Yield (kg/vine): The grapes harvested from each vine were weighted with a digital balance (Densi PC-300) to get the yield per vine.

Number of clusters (cluster/vine): Clusters were counted at harvest.

Cluster and berry weights (g) were determined with a digital balance (Precisa BJ 1200C); TSS contents were determined with a refractometer (Atago Master-93H); must pH values were determined with a pH meter (WTW Inolab pH 7310); titratable acid (TA) (g/l) was measured in accordance with Cemeroğlu (1992); maturation index was calculated as TSS/TA ratio.

Statistical analysis

Experiments were designed in randomized plots with six replications. Descriptive statistics for the continuous variables were presented as Mean and Standard Error of Mean while count and percentages for categorical variables. One-way ANOVA was used to compare group means. LSD multiple comparison test was performed to identify different group means followed by ANOVA. Statistical significance level was considered as 5% and SPSS (ver: 13) statistical program was used for all statistical computations.

Percent change: Percent change in investigated parameters indicates positive or negative changes in data as compared to the control. Percent changes were calculated by using *Equation 2*.

$$\text{Percent Change} = \left(\frac{\text{Control}}{\text{Treatment}} - 1\right) \text{x } 100 \qquad \text{(Eq.2)}$$

Results and discussion

Development values

In observations made at the period in which inflorescences were started to be seen, number of buds left per vine, number of shoots and pruning weights were significantly different in both years, but the differences in bursting ratios were not found to be significant. Pruning weight is a significant indicator of vine growth and development and the values were higher in healthy grapevines. Pruning weights proportionally decreased with the shoot age. In other words, the grapevines with earlier shoot regeneration were more influenced by disease severity. Such a case then regressed vine development (*Tables 2* and *3*). When the vine growth in healthy vines are assumed to be 100, the development values in 2013 and 2014 were respectively 19-15% less in GY-1, 26-25% less in GY-2, 41-37% less in GY-3.

Table 2. *Effects of shoot regeneration on vine growth and development (year 2013)*

Shoot regeneration year	Number of buds	Number of shoots	Bud burst (%) (*Eq. 1*)	Pruning weight (kg/vine)
Control	25.5±2.1 a	22.3±2.4 a	88±2.8 a	2.29±0.3 a
GY-1	21.2±0.8 bc	17.7±1.1 b	84±3.2 b	2.08±0.3 ab
GY-2	19.7±1.2 c	15.8±1.4 b	81±2.6 bc	1.78±0.4 bc
GY-3	22.0±0.9 b	16.8±1.1 b	77±3.2 c	1.580±0.4 c
$LSD_{(0.05)}$	1.7	2.1	3.7	0.4

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Table 3. *Effects of shoot regeneration on vine growth and development (year 2014)*

Shoot regeneration year	Number of buds	Number of shoots	Bud burst (%) (*Eq. 1*)	Pruning weight (kg/vine)
Control	26.5±2.7 a	23.0±2.2 a	87±1.8	3.33±0.4 a
GY-1	24.7±1.7 b	21.2±1.1 b	86±2.6	2.70±0.2 b
GY-2	24.5±2.5 b	21.2±2.7 b	86±2.7	2.46±0.3 b
GY-3	21.2±1.7 c	17.8±1.4 c	85±2.8	1.98±0.4 c
$LSD_{(0.05)}$	2.9	2.9	NS	0.4

Yield and quality values

The grapevines subjected to shoot regenerations due to *A. vitis* had significantly different yield and quality values from the healthy vines. With regard to yield, number of cluster, cluster weight, berry weight and maturation index values, while healthy vines were placed in one group, shoot-regenerated vines were placed in two different groups. The lowest values were observed in the oldest shoot-regenerated (year 2010) vines (*Tables 4* and *5*).

Table 4. *Effects of shoot regeneration on grapevine yield and quality (year 2013)*

Treatment	Yield (kg/vine)	Number of clusters	Cluster weight (g)	100 berry weight (g)	TSS (%)	pH	TA (g/l)	Maturation index
Control	6.57±0.7a	21.3±2.3a	308±15a	309±12a	21.6±1.1	2.84±0.12ab	5.82±0.14b	35.5±2.5a
GY-1	3.48±0.3b	15.2±1.7b	229±18b	236±35b	19.5±1.5	2.64±0.21c	7.21±0.84a	27.5±4.6b
GY-2	2.79±0.4c	13.3±1.2bc	210±29b	251±32b	19.6±0.8	2.91±0.15a	6.63±0.54a	29.7±2.9b
GY-3	2.18±0.5c	13.0±1.4c	166±25c	226±13b	19.4±1.2	2.66±0.22bc	7.05±0.71a	27.8±2.8b
$LSD_{(0.05)}$	0.65	2.1	26.7	28.3	NS	0.2	0.8	4.0

Table 5. *Effects of shoot regeneration on grapevine yield and quality (year 2014)*

Treatment	Yield (kg/vine)	Number of clusters	Cluster weight (g)	100 berry weight (g)	TSS (%)	pH	TA (g/l)	Maturation index
Control	8.82±0.7a	25.5±1.8a	347±25a	305±26a	2.30±0.7a	3.39±0.2	6.39±0.3	33.5±2.8
GY-1	4.40±0.5b	18.7±2.1b	217±16b	295±42ab	20.76±1.2a	3.56±0.1	7.80±1.2	27.3±4.7
GY-2	3.71±0.7bc	18.7±2.5b	201±38b	275±22ab	19.04±1.5b	3.60±0.1	7.55±1.0	25.9±5.2
GY-3	3.11±0.9c	16.8±11b	184±47b	261±39b	20.36±1.6ab	3.56±0.1	6.85±1.4	31.3±5.7
$LSD_{(0.05)}$	0.9	2.3	43.7	35.5	1.4	NS	NS	NS

When the shoot-regenerated vines were compared with the healthy vines, it was observed that yield values in 2013 and 2014 respectively decreased by 47-50% in GY-1, 58-58% in GY-2 and 67-64% in GY-3.

According to two-year averages, grape yield of healthy vines was 7.70 kg, cluster weight was 328 g, 100 berry weight was 307 g and pruning weight was 2.81 kg/vine. In shoot-regenerated grapevines, grape yields varied between 2.65-3.94 kg, cluster weights varied between 175-223 g, 100 berry weights varied between 244-266 g and pruning weights varied between 1.78-2.39 kg/vine. Regression analyses revealed continuous

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decrease in yield, cluster weight, berry weight and pruning weights with the years. Such decreases were found to be significant in all four parameters (*Fig. 3a-d*).

According to two-year averages, as compared to control treatments, percent change in yield and quality parameters of *A. vitis*-infected oldest shoot-regenerated grapevines was quite greater than the other vines (*Fig. 4*).

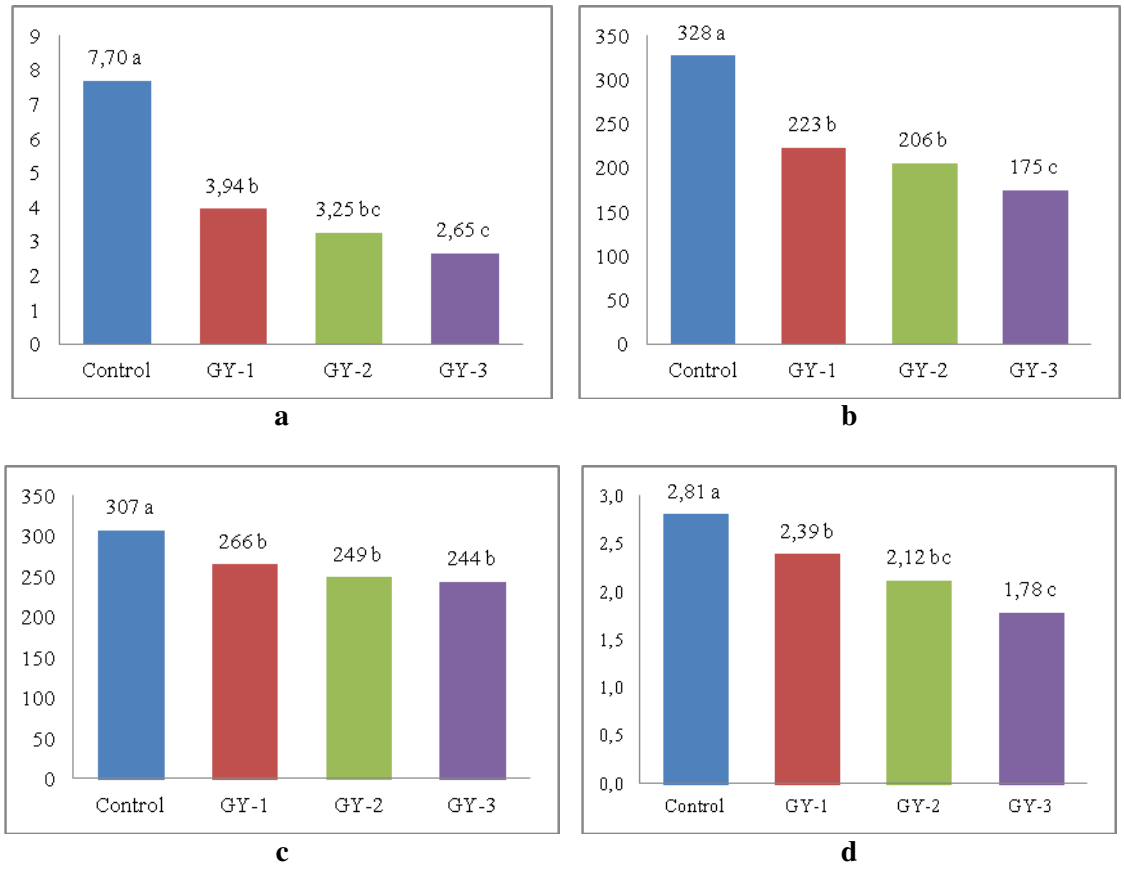


Figure 3. *a: Average yield values based on shoot regeneration years (kg/vine) ($LSD_{0.05}$ = 0.8). b: Average cluster weights based on shoot regeneration years (g) ($LSD_{0.05}$ = 24.1). c: Average 100 berry weights based on shoot regeneration years (g) ($LSD_{0.05}$ = 26.1). d: Average pruning weight based on shoot regeneration years (kg/vine) ($LSD_{0.05}$ = 0.41)*

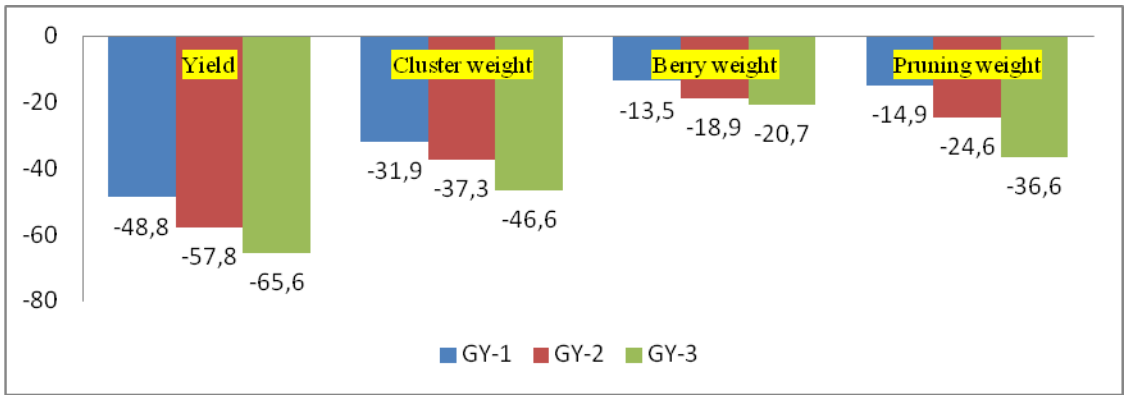


Figure 4. *Percent change in yield and quality parameters of shoot-regenerated grapevines as compared to the control vines (Eq. 2)*

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Present findings on *A. vitis*-induced decreases in yield, growth and development of grapevines comply with the results of Schroth et al. (1988) and Ferreira et al. (1992). Negative trends in changes in infected grapevines (yield, number of clusters, cluster weight, berry weight, TTS and TA) were mostly resulted from growth weakening effect of *A. vitis*.

Pruning weight is a significant indicator of grapevine growth and development (Noar and Gal, 2002; Çelik and Çağdaş, 2007; Keller, 2010). With the weakening in vine growth and development, especially carbohydrate mechanism of the grapevines is negatively influenced (Ağaoğlu, 2002; Keller, 2010) and resultant tumors significantly limit or restrict nutrient transport through various parts of the plants (Lehoczky, 1971; Burr et al., 1998).

Conclusion

Producers usually try or employ various methods to overcome the problems they experienced in viticulture practices. Significant *A. vitis*-induced decreases in yield and quality parameters were also reported by the growers. Instead of totally stubbing already established vineyard and waiting 4-5 years to reestablish the new one, deep pruning for shoot regeneration and to rejuvenate the grapevines may provide a practical solution for *A. vitis*-infected grapevines. However, such practices can only serve a short-term solution, may prolong vine life and allow growers to get yields for couple more years. *A. vitis*-infected vines ultimately end up with total die outs. For now, in present fight, the winner is still *A. vitis* …

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ESTIMATION OF FOREST STAND PARAMETERS BY USING THE SPECTRAL AND TEXTURAL FEATURES DERIVED FROM DIGITAL AERIAL IMAGES

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Abstract. Remote sensing data are important data sources in the management and planning of forest ecosystems. In this study, we aimed to estimate forest stand parameters using the spectral (*Average Brightness Value*) and textural features (*Standard Deviation of Gray Levels, Entropy, Contrast, Correlation, Homogeneity*) derived from digital aerial images. Study was carried out in Adıyaman Forestry Operation Directorate in Turkey. Relationships between image features and stand parameters (diameter at breast height-DBH, mean height, stand volume, basal area, and number of trees) were tested by using Pearson's correlation coefficient. Image features exhibiting the highest correlation with stand parameters were modelled by a linear regression analysis. The validity of the models developed was then tested using the leave-one-out cross-validation method. The '*Contrast*' values derived from the infrared band showed the highest correlation with DBH and mean height, the '*Contrast*' values from the red band showed the highest correlation with stand volume and basal area, and the '*Homogeneity*' values from the infrared band showed the highest correlation with the number of trees. The adjusted coefficients of determination (R^2_{adj}) of the estimation models were calculated 0.48 for DBH, 0.38 for mean height, 0.41 for stand volume, 0.45 for basal area and 0.43 for tree number. The relative root mean square error (RMSE%) values were for each parameters 11.91%, 23.57%, 20.96%, 15.81% and 16.20%, respectively.
Keywords: *forest inventory, remote sensing, digital aerial image, forest properties, image features*

Introduction

Effective management and planning of forest ecosystems requires up-to-date and reliable data. Remote sensing data have great potential as an auxiliary data source in estimating forest structural variables (Kayitakire et al., 2006). Forest inventory for forest management planning is traditionally based on the combination of field measurement and aerial photographs. In this method, forest stands are delineated on the basis of aerial photographs and forest stand parameters (e.g. volume, basal area, density) are calculated using some empirical formulas after necessary measures on the sample plots. Aerial photographs are widely used in mapping forest stands. However, as this approach usually necessitates visual photo interpretation, its success depends on the experience of interpreter. Therefore, it is time-consuming and subjective particularly on large areas (Gong et al., 1999; Tuominen and Pekkarinen, 2005; Morgan and Gergel, 2013; Balenovic et al., 2015). Moreover, field measurements are the most widely used data source in forest inventory. Periodical field measurements yield the most accurate results. Nevertheless, its cost, time-consuming and labour-intensive nature removes these advantages (Müllerová, 2005; Gonzalez-Benecke et al., 2014).

Apart from these disadvantages of traditional methods, improvements in computer technologies and satellite images have caused a gradual transition from traditional methods to semi-automatic and automatic methods (Hájek, 2008; Balenovic et al.,

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2015). Multispectral remote sensing images produce reliable digital data for the automatic estimation of forest stand parameters. Automatic image analysis techniques also provide a rapid estimation of the forest stand parameters through these images (Hayitakire et al., 2006; Huang et al., 2013). Taking advantage of satellite image data in forest inventory has increased interest since the first satellite of LANDSAT program launched in 1972. With the advances in technology, the production of sensors which provide high resolution and multispectral image has raised more interest (Franco-Lopez et al., 2001; Katila et al., 2001). Several studies carried out so far have focused on using multispectral satellite images such as Landsat, SPOT, IKONOS, QuickBird and WorldView to estimate forest stand parameters. For instance, Hyyppä et al. (2000) evaluated SPOT XS, SPOT Pan and Landsat TM satellite images in terms of the estimation accuracy of stand volume, mean tree height and basal area. Ozkan (2006) investigated the possibilities of estimating the forest stand parameters (number of trees, stand volume, basal area, mean diameter and mean height) with the spectral features derived from SPOT-5 satellite data. In another study carried out by Kayitakire et al. (2006), the estimation capabilities of IKONOS satellite image in spruce forest stands for five main forest variables (age, top height, circumference, stand density and basal area) was evaluated. Hall et al. (2006) developed a forest stand volume model using the relation between forest structural features and spectral features from Landsat ETM+ image. Similarly, Ozdemir and Karnieli (2011) evaluated the determination of forest structural parameters using image textural features derived from WorldView-2 satellite image. The obtained results indicated that satellite images can be used for the estimation of forest structural features on large areas. However, the accuracy of estimations models failed to satisfy at sample plot or forest stand level. Therefore, their suitability is thought to be low in forest management planning studies.

Recent studies have focused on estimating forest stand parameters using digital aerial images due to the less accuracy of satellite images (Kayitakire et al., 2006; Balenovic et al., 2015). Aerial photographs are an important data source for sample plot or forest stand level forest inventory (Morgan et al., 2010). Over the last 30 years, there has been a transition from analogous photogrammetry to digital photogrammetry as analogous aerial photographs are replaced with digital aerial images (Balenovic et al., 2015). In addition to high spatial resolution, RGBIR (Red, Green, Blue, Infrared) bands and digital data flow of digital aerial images provide a great advantage in estimating forest stand parameters and producing forest stand maps (Hájek, 2008). While some of the forest stand parameters (such as tree height, crown diameter, crown closure) can be measured directly from digital images, some others can be calculated using empirical formulas or estimated using image features. For the estimation of forest stand parameters, spectral and textural properties of remote sensed images are the most important characteristics (Zhou and Sun, 1995; Mäkinen et al., 2006; Ozdemir and Karnieli, 2011).

In addition to image spectral features, textural features can also be calculated through original pixel values. The spectral features can be defined as average reflection value of an image window (Anttila, 2005). 'first-order texture' which includes statistical variation measurements and 'second-order texture' which includes *Grey Level Co-occurence Matrix (GLCM)* proposed by Haralick et al. (1973) are the most widely used textural features of digital images to define (Ozdemir et al., 2008; Ozdemir and Donoghue, 2013). Recently, *GLCM* Textural features have been widely used (Levesque and King, 2003; Coburn and Roberts, 2004; Tuominen and Pekkarinen, 2005;

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Kayitakire et al., 2006; Maltamo et al., 2006; Hájek, 2008; Ozdemir and Karnieli, 2011; Tuominen and Haapanen, 2011; Sarker et al., 2012; Ozdemir et al., 2012; Ozdemir and Donoghue, 2013; Ozkan, 2014; Walner et al., 2014; Lu et al., 2016; Ozkan et al., 2016; Ozkan et al., 2017). Nevertheless, these studies are mostly carried out using satellite images. It is stated that the textural features can be an important explanatory variable to estimate the stand parameters from high-spatial resolution images such as digital aerial images. And also textural features as well as spectral features can improve the estimation accuracy (Tuominen and Pekkarinen, 2005; Anttila, 2005; Packalén, 2009). Nevertheless, estimation of forest stand parameters using spectral and textural features of digital aerial images has not been studied adequately. Further studies are still necessary for operational using of spectral and textural features in forest management planning.

Effective use of digital aerial images in forest management planning relies on the relation between the scale of target object (tree, forest stand, etc.) and spatial resolution of digital aerial image (Falkowski et al., 2009). Target objects are much larger than image pixel size and a single pixel does not contain significant data when considering target objects. Therefore, related parameter should be extracted from a larger area than a single pixel. For example, although forest stand is defined as a homogenous area in forest management practice, forest stand is not an entirely homogenous object in terms of image features. Forest stands in remote sensed images comprise of a composition of various spectral classes such as small gaps, shadowy and sunny crown. Object-based image analysis is considered as an important approach for extracting significant information from high spatial resolution remote sensed images (Antunes et al., 2003; Huang et al., 2013). On forest stand level, image segmentation which is the first step in object-based image analysis, is a useful algorithm that increase accuracy of forest stand parameters estimation (Anttila, 2005).

The purpose of this study is to predict forest stand parameters using the spectral and textural features derived from digital aerial image. To achieve this goal, firstly, the relations between forest stand parameters and image features were analyzed and investigated to determine which one of them are suitable options for estimation models. Then, linear regression analysis utilized for estimation models with considering image features and also validity of the models were tested using adjusted coefficient of determination (R^2_{adj}), root mean square error (RMSE) and relative root mean square error (RMSE%).

Materials and methods

Study area

Study area is located between 37°59’29”–37°24’02” north latitude and 38°31’30”–37°25’44” east longitude in Şanlıurfa Regional Directorate of Forestry, Adıyaman Forestry Operation Directorate in Turkey (*Figure 1*). The study area covers 439621 hectares which general land comprise of 105771 hectare forested land. Dominant tree species of study area is Brutian pine (*Pinus brutia Ten.*) and Oak (*Quercus brantii var. persica, Quercus libani var. angustifolia, Quercus infectoria var. boissieri, Quercus cerris var. variegata*). Besides these, Juniper (*Juniperus excelsa var. excelsa, Juniperus oxycedrus var. oxycedrus*) species are found at high altitude areas. Oak stands are managed as coppice and juniper stands are located fragmentary. This study covers Brutian pine plantations.

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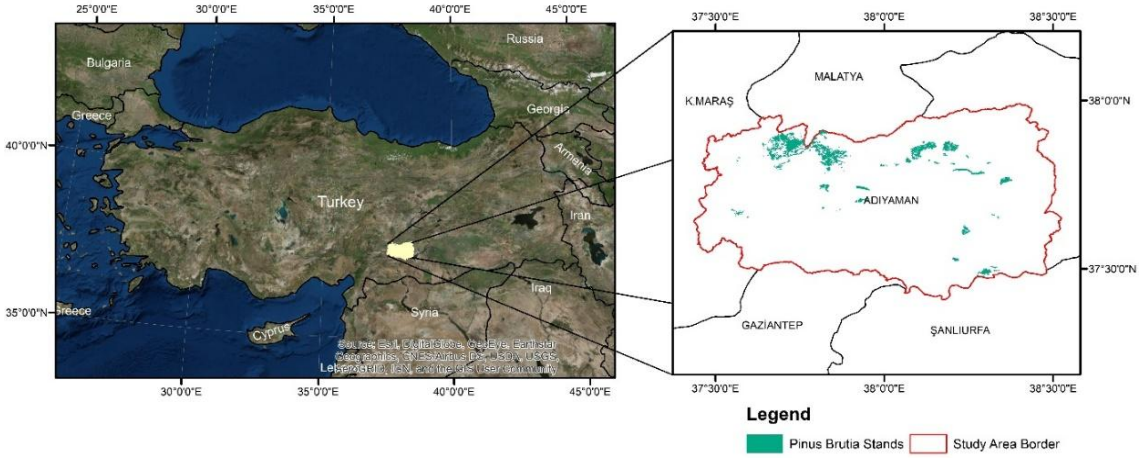


Figure 1. *Geographical location of study area*

Digital aerial imagery

Aerial images used in the study are totally comprised of four bands which are Red (580-700 nm), Green (480-630 nm), Blue (410-540 nm) and Infrared (690-1000 nm) bands. These images are taken as stereo from altitude of 7200 meters, 70 % forward and 30 % side overlap, by airborne using UltraCamX (Microsoft, Vexcel Imaging GmbH) digital aerial camera which has 7.2 µm physical pixel size, in 2011. For each stereo image pair, geometric calibration and radiometric correction utilized in ERDAS LPS (Hexagon Geospatial) software using interior (focal length, coordinates of the principal point) and exterior (position and rotation of the camera) orientation parameters of raw images.

Vegetation indexes were developed using the original bands in order to provide more detailed data concerning the areas covered with vegetation in multi-channel images, (Jensen and Lulla, 1987). In the present study, 3 vegetation indexes (Difference Vegetation Index-DVI; Infrared Percentage Vegetation Index-IPVI; Normalized Difference Vegetation Index-NDVI) were used (*Table 1*).

Table 1. *Vegetation indexes used in the study*

Vegetation index	Equation	Author
DVI	$NIR - RED$	Tucker 1979
IPVI	$\frac{NIR}{NIR + RED}$	Crippen 1990
NDVI	$\frac{NIR - RED}{NIR + RED}$	Rouse et al. 1974

Besides, Principal Component Analysis (PCA) was applied to digital image in order to form a new data group including more information (Jensen and Lulla, 1987) by pushing similar channels. PCA can be repeated more than once with the purpose of increasing

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detail, new data group which are produced in this way are also named as PC1, PC2. However, many studies have indicated that PC1 involves the most details for remote sensing data (Sena et al., 2002; Çam et al., 2015). PCA image, vegetation indexes and original bands used in the study are shown in *Figure 2*.

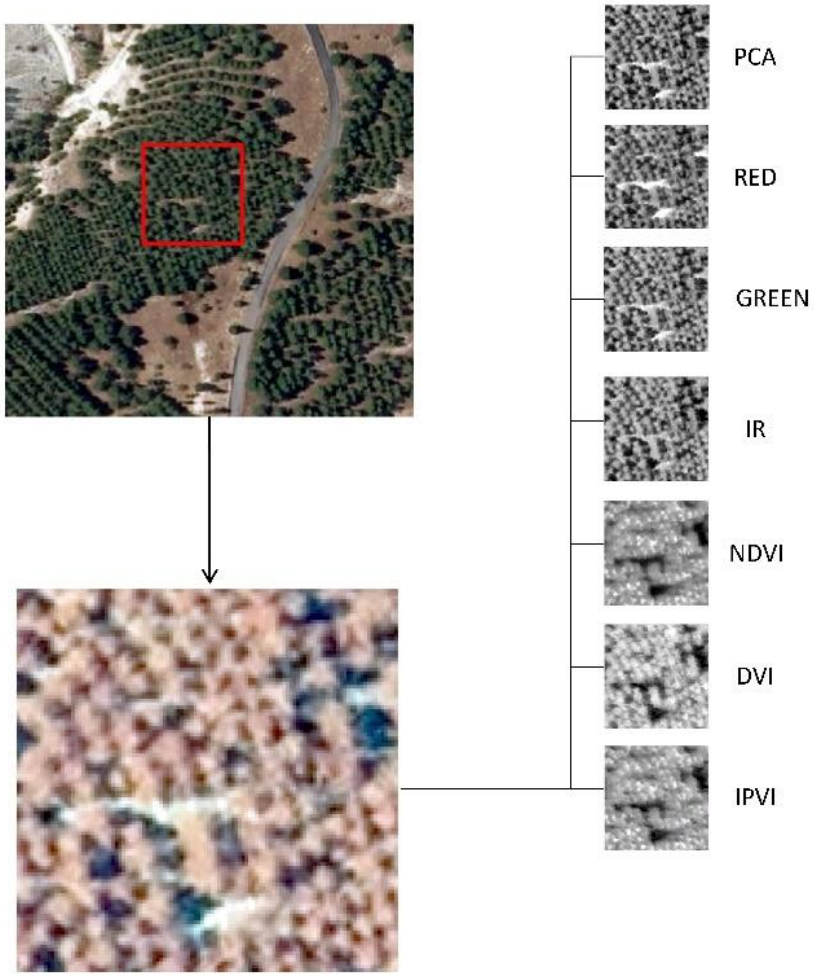


Figure 2. *Original bands, vegetation indexes and PCA image*

Field data

Field data were used with the purpose of revealing the relation between forest stand parameters and image features. Fieldwork was carried out in sampling clusters of four plots which had size of 50 x 50 m during July-August 2013. Sampling clusters, as 20 items in total, were randomly chosen from image objects formed with image segmentation process. Sample plots in sampling clusters were located as 50 m distance from the center to center. The centers of sample plots were located with the distance of 25 m from the center of sampling clusters to North, South, East and West directions. Sample plots were established as 400 or 600 m^2 circle (*Figure 3*).

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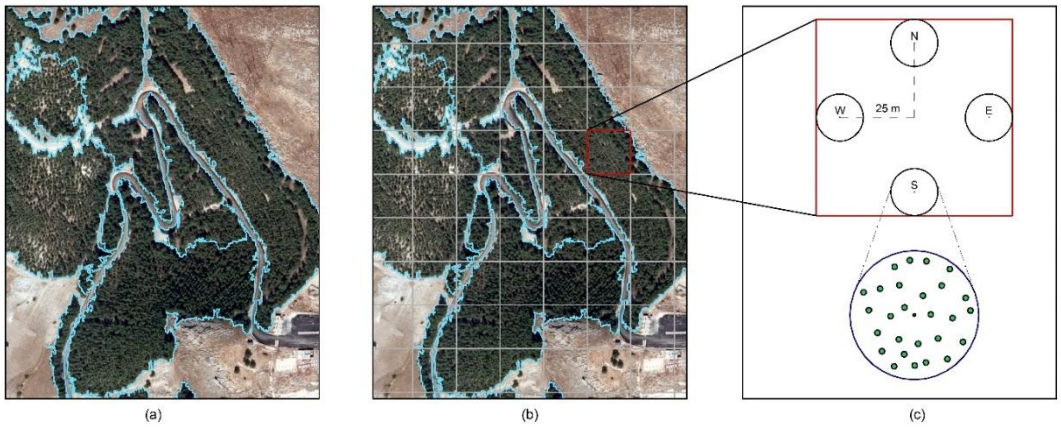


Figure 3. *a) Multi-resolution segmentation b) Chessboard segmentation c) Sample plots*

The size of sample plots were determined considering stand development age and crown closure. The measurements were carried out considering conventional forest inventory rules in totally 80 sample plots. Diameter of all trees inside the sample plot with the diameter at breast height (DBH) more than 8 cm, the height (m) and age according to the %40 rule were measured. Stand volume (m^3/ha), basal area (m^2/ha), number of trees (stems/ha), DBH (cm) and mean height (m) of each sample plot were calculated using the equations given in *Table 2*. For sampling clusters, forest stand parameters per hectare were determined as the average of (4) sample plots in each sampling cluster. Descriptive statistics related to forest stand parameters determined with field measurements are presented in *Table 3*.

Table 2. *Equations to calculate forest stand parameters*

Stand parameters	Equation	Author
Volume	$\ln V = \ln a_1 + a_2 \ln d_{1.3} + a_3 \ln h$ * $a_1 = -2.077466 \quad a_2 = 1.676818 \quad a_3 = 0.845096$	Usta (1991)
DBH	$d_g = \sqrt{\frac{4*G}{\pi * N}}$	Husch et al. (1993)
Basal Area	$BA = \frac{\pi}{4} \sum d_{1.3}{}^2$	Kalıpsız (1999)
Mean Height	$h = h_{weise}$	Kalıpsız (1999)

*where $d_{1.3}$ were diamater at breast height, G were basal area in cm^2, N were number of trees per hectare, h were height of tree

Table 3. *Descriptive statistics of ground measurement data*

	DBH (cm)	Mean height (m)	Stand volume (m^3/ha)	Basal area (m^2/ha)	Number of trees (stems/ha)
Mean value	16.92	8.16	45.55	10.39	487.63
Minimum value	13.14	4.35	24.36	6.51	345.75
Maximum value	21.77	12.70	70.23	15.28	681.25
Standart deviation	2.65	1.93	11.93	2.14	99.74

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Image segmentation

Sampling clusters obtained by image segmentation are stated to be a better estimation unit than sample plots (Anttila, 2005; Hyvönen et al., 2005). The segmentation is an algorithm which creates meaningful objects in an image by grouping the individual pixels according to their spatial and spectral features and is performed using the scale parameter and homogeneity criteria (Benz et al., 2004). The main objective of this process is to obtain more meaningful sub-objects from the heterogeneous structure of images (Blaschke et al., 2014; Li et al., 2015). A number of segmentation techniques have been developed (Definiens, 2006). In this study, image segments were firstly obtained from digital aerial image by using Multi-resolution Segmentation algorithm on forest stand level. For this purpose, the image segmentation process was implemented by testing different scale and color/shape parameters which represents the forest stands in the study area. As a result of visual evaluations, the most suitable image objects were obtained by using scale parameter: 175, color: 0.7 and shape: 0.3. Then, chessboard segmentation approach was implemented to achieve image windows representing ground sampling clusters into the obtained image objects. In this way, the size of image objects was segmented into 50 x 50 m cells (*Figure 3*).

Extraction of the image features

In the study, spectral and textural features of digital aerial image were used for the estimation of forest stand parameters (*Table 4*).

Table 4. *Image features used in the study*

Image Features		**Relationship model***
Average brightness value		$\frac{1}{\#P_v}\sum_{(x,y)\in P} C_k(x,y)$
First-order	*Standard Deviation of Gray Levels*	$\sigma_k(v) \coloneqq \sigma_k(P_v) = \sqrt{\frac{1}{\#P_v}\left(\sum_{(x,y)\in P} {c_k}^2(x,y) - \frac{1}{\#P_v}\sum_{(x,y)\in P} C_k(x,y)\sum_{(x,y)\in P} C_k(x,y)\right)}$
Second-order	*GLCM Contrast*	$\sum_{i,j=0}^{N-1} P_{i,j}(i-j)^2$
	GLCM Entropy	$\sum_{i,j=0}^{N-1} P_{i,j}(-lnP_{i,j})$
	GLCM Correlation	$\sum_{i,j=0}^{N-1} P_{i,j}\left[\frac{(i-\mu_i)(j-\mu_j)}{\sqrt{({\sigma_i}^2)({\sigma_j}^2)}}\right]$
	GLCM Homogeneity	$\sum_{i,j=0}^{N-1} \frac{P_{i,j}}{1+(i-j)^2}$

*Parameters (Definiens 2006)
i is the row number, j is the column number, P_v is the cluster of pixels of the image object, # P_v is the total number of current pixels in P_v, (x,y) is the coordinates of pixels, $c_k(x,y)$ is the pixel value, Pi,j is the normalized value in the cell i, j, N is the number of columns and rows, μi and μj represent the average of the row i and column j, σi and σj represent the standard deviation of the row i and column j

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Average Brightness Value (ABV) of pixels in an image window corresponding sampling clusters was taken into consideration as spectral property. The first-order (*Standard Deviation of Gray Levels-SDGL*) and second-order texture features were used as textural features as well. The first-order texture features are calculated using statistical properties of pixel brightness values in an image area. Furthermore, the second-order texture features are calculated based on *Grey Level Co-occurence Matrix (GLCM)*. *GLCM* is a tabulation of how often different combinations of pixel grey levels occur in an image object (Harralick et al., 1973). According to *GLCM*, many textural features can be determined which are categorized in three main groups as *Contrast, Orderliness* and *Descriptive statistics* (Ozdemir and Karnieli, 2011). *Homogeneity (HOM), Contrast (CON), Correlation (COR) and Entropy (ENT)*, which habe been used widely in previous studies, were chosen to be used in the current study. Image features were derived from 3 digital bands (R, G, NIR), PCA and 3 vegetation indexes (NDVI, DVI, IPVI). Image features were extracted from image objects which segmented into 50 x 50 m cells by Definiens image processing software

Statistical analysis

SPSS 16 (SPSS Chicago, IL, USA) statistical software was used to perform statistical analysis. Pearson Correlation analysis was used for the determining the statistical relation between forest stand parameters and image features. Pearson correlation coefficient can be used in the cases in which the data have a normal or near-normal distribution. The normality of the distribution of the variables were tested based on Shapiro-Wilk method. Variable was regarded as having normal distribution when P>0.05 (Kalaycı, 2006; Kayitakire et al., 2006).

The linear regression analysis was utilized to develop forest stand parameter estimation models, using image features derived from the digital aerial image. Forest stand parameters which were calculated based on field measurements were considered as dependent variable and image features which were derived from image windows were considered as independent variable in the regression analysis. In the regression analysis, one of the important issues is the variable choice when there are a large amount of predictor variables (Shinzato et al., 2016). For choising the proper variable, the highest correlation coefficient between forest stand parameters and image features was considered. By Kayitakire et al. (2006) a simple linear model of the form $y = b_0 + b_1x$ has been proposed to present a simple and interpretable model, emphasizing that there are no theoretical functional forms for the forest variable estimation models using textural features.y refers to forest stand parameters, x refers to image features, b_0 and b_1 refer to coefficients of the model. In the present study, this approach was adopted to model relation between forest stand parameters and image features. Each forest stand parameter was evaluated separately and a different model was developed for each one. Then, the validity of the estimation models was tested using the leave-one-out cross-validation test. In this method, one sampling cluster from dataset was eliminated and a model was developed using left sampling cluster. The forest parameters of eliminated sampling cluster were predicted according to the model that was not include leaved out. The predicted values for 20 sampling clusters were obtained by repeating the same process for each sampling clusters. Then, the adjusted coefficient of determination (R^2_{adj}), the root mean square error (RMSE) and the relative root mean square error (RMSE%) values were calculated by comparing predicted values with ground truth data.

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Results

Correlation analysis

Data should exhibit a normal distribution to reveal the direction and power of a linear relationship between image features and forest stand parameters by performing a correlation analysis. According to the normal distribution test conducted, it was determined that the all forest stand parameters obtained from field measurements in the sample plots exhibited a normal distribution. Following the normal distribution test, a relationship between the image features and forest stand parameters was evaluated by Pearson's correlation coefficient.

As a result of the evaluation performed, statistically significant relationships were found between DBH, mean height, stand volume, basal area, and the number of trees calculated based on the sampling clusters and brightness and textural features derived from a digital aerial image. The relationships between the forest stand parameters and image features are presented in *Table 5*.

Table 5. *Pearson correlation coefficients between image features and forest stand parameters (best correlation)*

	DBH	**Mean height**	**Stand volume**	**Basal area**	**Number of tree**
a) Correlation coefficients between stand parameters and image features derived from infrared band					
ABV	-0.196	-0,056	-0,071	-0,059	0,285
SDGL	-0,065	-0,007	-0,044	0,164	0,234
HOM	0,658**	0,506**	0,442	0,431	-0,679**
CON	-0,708**	-0,641**	-0,524*	-0,508*	0,668**
COR	0.690**	0,627**	0,558*	0,578**	-0,577**
ENT	-0.030	-0,088	-0,136	-0,112	0,010
b) Correlation coefficients between stand parameters and image features derived from red band					
ABV	-0,221	-0,154	-0,126	-0,092	0,245
SDGL	-0,427	-0,373	-0,401	-0,276	0,314
HOM	0,641**	0,631**	0,637**	0,643**	-0,455
CON	-0,604**	-0,626	-0,663**	-0,692**	0,322
COR	0,535*	0,597*	0,600**	0,648**	-0,322
ENT	-0,163	-0,096	-0,082	-0,032	0,247
d) Correlation coefficients between stand parameters and image features derived from DVI					
ABV	-0,002	0,122	0,062	-0,033	0,097
SDGL	-0,381	-0,397	-0,393	-0,315	0,179
HOM	0,364	0,384	0,354	0,388	-0,253
CON	-0,322	-0,307	-0,394	-0,432	0,161
COR	0,301	0,282	0,369	0,411	-0,152
ENT	-0,393	-0,451*	-0,452*	-0,459*	0,248
d) Correlation coefficients between stand parameters and image features derived from PCA					
ABV	-0,277	-0,182	-0,153	-0,113	0,320
SDGL	0,064	-0,005	0,030	0,108	0,013
HOM	0,636**	0,591**	0,492*	0,508*	-0,567**
CON	-0,607**	-0,650**	-0,612**	-0,652**	0,398
COR	0,600**	0,634**	0,605**	0,652**	-0,387
ENT	-0,654**	-0,612	-0,568**	-0,565**	0,519*

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

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The relevant table shows the image features having the best correlation with the forest stand parameters. In summary, the highest correlation between DBH and image features was obtained with the *CON* values derived from the infrared band ($r=0.708$, $p<0.01$). A significant and negative correlation was found between the *CON* value derived from the red band and DBH ($r=0.604$, $p<0.01$). A significant and positive correlation was observed between the *HOM*, *COR* values derived from the red and infrared bands and DBH ($r=0.641$, 0.535 and 0.58, 0.690 $p<0.01$, respectively). Besides, significant relationships were determined between the *CON*, *HOM, COR*, and *ENT* values derived from PCA and DBH. In addition to these, relationships between other textural features and brightness values and DBH were generally weak. The highest correlation between the mean height and the image features was also calculated with the *CON* values derived from the infrared band ($r=0.641$, $p<0.01$). While significant relationships were observed between the *HOM, CON, COR* values derived from the red band and the *CON, ENT, COR* values derived from PCA and the mean height, the relationships between the mean height and other image features (*ABV* and *SDGL*) were generally weak.

Significant correlations were also calculated between the stand volume and some textural features. On the other hand, it was observed that relationships between the stand volume and brightness values were weak. The highest correlation with the stand volume was calculated with the *CON* values derived from the red band ($r=0.663$, $p<0.01$). Furthermore, stand volume had significant positive relationships with the *HOM, COR* values and significant negative relationships with *CON, ENT* values derived from red band, infrared band and PCA. Similarly, while the basal area exhibited significant correlations with some textural features, exhibited a weak correlation with brightness values. The highest correlation for the basal area was obtained with the *CON* values derived from the red band ($r=0.692$, $p<0.01$). The highest correlation in the number of trees was obtained with the *HOM* values derived from the infrared band ($r=0.679$, $p<0.01$). Moreover, it was determined that there was a significant and positive correlation between the number of trees and the *CON* values derived from the infrared band and the *ENT* values derived from PCA ($r=0.668$, 0.519, $p<0.01$). Apart from these, it was understood that the relationships between the *ABV, SDGL, COR* values and the number of trees were generally weak.

Estimation of forest stand parameters

In order to estimate the forest stand parameters based on the image features, the relationship between the forest stand parameters and the image features was modeled by a linear regression analysis. According to the results of the correlation analysis, various textural features exhibited a statistically significant correlation with the forest stand parameters. The image features to be used as independent variables of the model were selected from those having the highest correlation with the forest stand parameters. Accordingly, the *CON* values derived from the infrared band were modeled as the best indicators of DBH and mean height. The *CON* values derived from the red band were the independent variables of the model as the best indicators of the forest stand volume and basal area. The *HOM* values derived from the infrared band were used as independent variables of the model estimating the number of trees. The accuracy of the estimates provided by the regression models was explained by the R^2_{adj}, RMSE, and RMSE% values. The R^2_{adj}, RMSE, and RMSE% values related to the regression models developed for each forest stand parameter and the estimates obtained from the models are presented in *Table 6*.

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As seen from *Table 6*, the model coefficients were found as statistically significant ($p<0.001$) in the models developed to estimate DBH, mean height, stand volume, basal area, and the number of trees. Upon evaluating the results, it was found out that the model providing the highest estimation accuracy among the estimation models was the model developed to estimate DBH. The adjusted coefficient of determination (R^2_{adj}) of the relevant model was calculated to be 0.48. Accordingly, the linear regression model, in which the *CON* values derived from the infrared band were used as independent variables, estimated 48% of the change in DBH. In terms of the estimation accuracy, this model was followed by estimation models developed to calculate the basal area, number of trees, and stand volume. The estimation accuracy of the model developed for the mean height was relatively low compared to the others. The R^2_{adj} values of these models calculated using all datasets were found to be 0.45, 0.43, 0.41 and 0.38, respectively. Upon comparing the DBH values estimated from the model with the ground truth DBH values, RMSE was calculated to be 2.02 cm (RMSE 11.91%). Similarly, upon comparing the other stand parameters estimated from the developed models with the ground truth data, RMSE was calculated to be 1.92 m for the mean height, 9.55 m^3/ha for the stand volume, 1.64 m^2/ha for the basal area, and 79.01 stems/ha for the number of trees. RMSE% was found to be 23.57% for the mean height, 20.96% for the stand volume, 15.81% for the basal area, and 16.20% for the number of trees.

Table 6. *Estimation models based on image textural features of forest stand parameters and their statistical summaries*

Stand parameters	Estimation model	R^2_{adj}	RMSE	%RMSE	p-value
DBH	25.296-0.017**CON*_IR	0.48	2.02	11.91	0.000
Mean height	14.658-0.013**CON*_IR	0.38	1.92	23.57	0.002
Stand volume	71.986-0.064**CON*_RED	0.41	9.55	20.96	0.001
Basal area	15.334-0.012**CON*_RED	0.45	1.64	15.81	0.001
Tree number	834.271-4965.119**HOM*_IR	0.43	79.01	16.20	0.001

Discussion

Our study goal was to reveal the potential of spectral and textural features derived from digital aerial image in predicting forest stand parameters. The results of correlation analysis revealed that there were statistically significant correlations especially between the image textural features and forest stand parameters. DBH, among the forest stand parameters, exhibited the highest correlation with the *CON* values derived from the infrared band ($r=0.708$, $p<0.01$). Furthermore, significant correlations were observed between some textural features (*CON, HOM, COR, ENT*) derived from the red, infrared bands, and PCA, and the DBH. Again, while there were significant correlations between the textural features such as *CON, HOM, COR*, and *ENT* with the mean height, the highest correlation was obtained with the mean height and the *CON* values derived from the infrared band ($r=0.641$, $p<0.01$). Significant correlations were observed between the stand volume and basal area and some textural features. The highest correlation with the stand volume and the basal area was achieved with the *CON* values derived from the red band ($r=0.663$, 0.692, $p<0.01$). While the number of trees had significant correlations with the textural features such as *HOM, CON*, and *ENT*, it exhibited the highest correlation with the *HOM* values derived from the infrared band ($r=0.679$, $p<0.01$).

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According to these results, while the correlations between *ABV* and *SDGL* derived from the digital image and forest stand parameters were generally weak, *GLCM* textural features gave the best results for the estimation of the forest stand parameters. Therefore, based on the correlation coefficient values, the *CON* property derived from the infrared band was the best indicator in the estimation of DBH and mean height parameters. While the *CON* property derived from the red band was the best indicator in the estimation of the stand volume and basal area, the *HOM* property derived from the infrared band was the most appropriate indicator in the estimation of the number of trees. Furthermore, it was revealed that the original bands of the digital aerial image (RED, IR) were more appropriate than vegetation indexes and PCA for the estimation. Significant differences were also found between the vegetation indexes and PCA. PCA was found to have a better correlation with the forest stand parameters than the vegetation indexes.

The results of our study are consistent with the relevant literature. For example, in the study conducted by Tuominen and Pekkarinen (2005), the estimation accuracy of the forest properties (DBH, height, basal area, and volume) was examined by using the spectral and textural features derived from the digital aerial image. As a result of the study, textural features generally exhibited higher correlations with forest properties than spectral features. Again in the same study, differently from the present study, image features derived from the original image bands generally exhibited a weaker correlation with forest properties. Kayitakire et al. (2006) evaluated the ability to estimate forest properties based on the textural features of a 1 m resolution IKONOS-2 satellite image. It was stated that there was a very high correlation (R^2= from 0.76 to 0.80) between the top height, circumference, stand density, and age and *GLCM* textural features (*COR* and *CON*). A weak correlation (R^2=0.48) was determined between the basal area and textural features. In a study conducted by Ozdemir et al. (2008) on natural forests using ASTER satellite images, it was determined that there was a significant correlation between the tree size diversity based on the basal area and *HOM*. In a study conducted to estimate the tropical forest properties from a SPOT-5 satellite image using textural features, Castillo-Santiago et al. (2010) revealed that textural features may be a determinant of forest properties. In another study conducted by Ozdemir and Karnieli (2011), it was aimed to estimate the forest structural parameters by using the image textural features derived from a WorldView-2 satellite image. As a result of the study, it was stated that there were significant correlations between the first-order and second-order textural properties and structural parameters. It was expressed that the strongest correlation was calculated between the *CON* derived from the red band and the Standard Deviation of Diameters at Breast Heights (R^2=0.56) and between the *ENT* derived from the blue band and the basal area (R^2=0.53). In a study conducted by Ozkan et al. (2017) in urban forests using Landsat-8, ASTER, and RapidEye satellite images with different resolutions, it was emphasized that image textural features can be a good indicator for evaluating the structural diversity of urban forest stands.

Our results showed that textural features is likely a better indicator than spectral features in estimating stand parameters from digital aerial images. The second-order textural features had more advantageous than first-order textural features. According to the results of the leave-one-out cross-validation analysis, the R^2_{adj} values of the linear regression models developed for the estimation of forest stand parameters varied between 0.38 and 0.48. The DBH and the mean height estimation models developed

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based on the *CON* property derived from the infrared band had 0.48 and 0.38 R^2_{adj}, 2.02 cm and 1.92 m RMSE. The forest stand volume and the basal area models developed using the *CON* property derived from the red band had 0.41 and 0.45 R^2_{adj}, 9.55 m^3/ha and 1.64 m^2/ha RMSE. The estimation model of the number of trees developed based on the *HOM* property derived from the infrared band had 0.43 R^2_{adj}, 79.01 stems/ha RMSE.

Due to the lack of studies on the estimation of forest stand parameters based on textural features from digital aerial images, the results of the present study could not be directly compared with the relevant literature. Therefore, studies in which estimation models were developed based on satellite images were used in the comparison of results. In the study conducted by Tuominen and Pekkarinen (2005), the DBH, height, basal area, and volume were attempted to be estimated by using spectral and textural features (such as *COR, CON, ENT*) derived from color-infrared aerial photographs. In the study in which forest stand parameters were estimated using the k-NN method, the RMSE values of the estimation model based on a single image property were calculated to be 8.54 cm for DBH, 6.69 m for height, 8.40 m^2/ha for basal area, and 78.75 m^3/ha for volume. In the study, it was also stated that the use of textural features improved the estimation accuracy. Kayitakire et al. (2006) modeled the forest stand parameters including the age, top height, circumference, stand density, and basal area using the image textural features derived from 1-m resolution IKONOS-2 imagery. The estimation errors of the models were estimated by using the leave-one-out cross-validation method. In the model in which the *CON* property was used, the R^2 value for the basal area was calculated to be 0.35, and in the model in which the *COR* property was used, the R^2 value for the height was calculated to be 0.76. Wunderle et al. (2007) tried to develop models that best describe forest stand parameters such as the diameter at breast height, height, stem density, and basal area, using the spectral and textural features derived from a pan-sharpened SPOT-5 image. It was emphasized that the first-order (standard deviation) and second-order (correlation) textural features explain the change in forest stand parameters better in the models developed with the stepwise regression analysis. In a study conducted by Ozdemir and Karnieli (2011), forest stand parameters were modelled as the function of textural features derived from the original bands of a WorldView satellite image. The models were developed by using the linear regression analysis. The second-order texture features (*CON* and *ENT*) gave the best result for the DBH, basal area, and stand volume and the first-order texture features (*SDGL*) gave the best result for the number of trees. The R^2 values of the most appropriate model were calculated to be 0.67 for DBH, 0.54 for the basal area, 0.42 for the stand volume, and 0.38 for the number of trees. Wallner et al. (2014) modelled forest stand parameters based on spectral and textural features derived from a RapidEye image. The R^2 values of the developed models were calculated to be in the range of 0.37-0.55 for the mean diameter, 0.40-0.58 for the basal area, 0.30-0.41 for the stem number, and 0.42-0.63 for the volume.

In the literature on the subject, there are mostly studies using spectral features derived from digital aerial images and usually satellite images. In a study conducted by Hyypä et al. (2000), estimation models were developed for the mean height, basal area, and stem volume using aerial images and SPOT PAN. As a result of the study, it was observed that the model based on the aerial image (R^2= 0.34, 0.48, 0.48) provides a higher estimation accuracy than SPOT PAN (R^2= 0.17, 0.38, 0.35). Mallinis et al. (2004) revealed that R^2_{adj} values varied between 0.24-0.31, 0.29-0.32, and 0.20-0.32,

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respectively, in the estimation of volume, basal area, and number of trees with models developed based on the spectral features of a Landsat TM satellite image. Peuhkurinen et al. (2008) reported RMSE values to be 52.2 m^3/ha, 5.6 m^2/ha, and 3.1, respectively, in the estimation of stand volume, basal area, and mean height estimation by using the Ikonos satellite image and k-MSN method. In a study conducted by Günlü et al. (2013), the R^2_{adj} values of the regression models in which the spectral feature derived from a Quickbird satellite image was an independent variable, varied between 0.19 and 0.70 for the forest stand volume. In another study conducted by Günlü et al. (2014), the correlation between spectral features and some forest stand parameters was modeled by using a Pan-Sharpened IKONOS satellite image. The R^2_{adj} values of the best models were determined to be 0.41 for the stand volume, 0.43 for the basal area, and 0.45 for the dominant height. Çil et al. (2015) modelled the tree number, stand volume, and basal area as a function of spectral features derived from Göktürk-2, Rasat, Landsat-8 and the digital aerial image. The R^2_{adj} values of the model developed based on the aerial image were found to be 0.23, 0.48, and 0.55, respectively, for the relevant parameters. The R^2_{adj} values of the model developed based on satellite images varied between 0.34 and 0.67. It was also stated in the study that the original bands gave better results than the vegetation indexes and ratio images.

As a result, the adjusted coefficient of determination values of the estimation models developed for forest stand parameters varied between 0.38 and 0.48 and these values are in line with the literature results. However, these values do not seem to be sufficient in terms of practical forest management. In order for estimation models to be transferred to the practical application, the estimation accuracy should be improved. Therefore, further studies on this subject can evaluate other *GLCM* textural features of the contrast, orderliness, and descriptive statistics groups and models using different image features together can be developed. Another alternative is the combined use of digital aerial images and airborne laser scanning (ALS) data. Studies conducted by Mohammadi et al. (2017) and Jayathunga et al. (2018) revealed promising results for the modeling of forest stand parameters with the combination of digital aerial images and ALS data. Furthermore, the study we conducted was carried out in a conifer plantation forest. The results of the previous studies show differences according to tree species. There is a need for this study to be conducted on different forest types.

Conclusion

The most important result of this study, which was conducted to estimate forest stand parameters based on the spectral and the textural features derived from digital aerial images, is that the second-order texture features gave the best results compared to the spectral and first-order textural features. Among the second-order textural features, '*Contrast*' exhibited better performance than the others. The models developed by using the '*Contrast*' property enabled the better estimation of DBH, mean height, stand volume, and basal area. Nevertheless, the model developed by using the '*Homogeneity*' property allows better estimation of the tree number. Another result that can be obtained from this study is that the original bands of digital aerial image is more capable than the bands obtained by the combination of the original bands in estimating the forest stand parameter. Textural features derived from the infrared band exhibited better performance for DBH, mean height, and tree number, and textural features derived from the red band exhibited better performance for the stand volume and the basal area. For

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the operational use of the method in forest management planning, further studies are required to improve the estimation accuracy. Furthermore, future studies should be conducted in pure and complex forest stands in different ecological regions to better understand the potential digital aerial photos.

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SHRIMP AND FISH CATCH LANDING TRENDS IN SONGKHLA LAGOON, THAILAND DURING 2003-2016

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Abstract. Time series analysis techniques and Seasonal Autoregressive Integrated Moving Average (SARIMA) models were used to analyze monthly fish and shrimp catch landing trends recorded for Songkhla shallow lagoon in Thailand (2003-2016). Autocorrelation (AC) and Partial Autocorrelation (PAC) functions were calculated to build seasonal ARIMA models. These models were well-chosen for explaining the time series and forecasting future catch landings. It is found that both fish and shrimp catch landings tend to fluctuate steadily. The fish catch from 2017 to 2020 is steadily increasing on the average catch for the period 2003-2016 by up to 36.06%, while the shrimp catch is decreasing by around 15.47% for the same period. This study demonstrates the importance of undertaking detailed studies of ecological and economic sustainable development to support the comprehensive fisheries management policy for Songkhla Lagoon. The present study shows an effective tool for making accurate forecasts; it also helps in decision making about, and fisheries management of the Songkhla Lagoon.
Keywords: *seasonal ARIMA models, artificial neural networks, time series forecasting, fishery trend, Songkhla lagoon*

Introduction

Time series analysis of fishery landings plays a vital role in fisheries management and decision making due to its capacity for demonstrating the trends and seasonality patterns of the data (Koutroumanidis et al., 2006; Tsitsika et al., 2007). In the fishery field, time series analysis qualifies for forecasting because it expresses past patterns and projects into the future (Stergiou et al., 1997).

Forecasting has been applied to all sectors of the economy. During the last three decades, forecasting has been rapidly developing in the field of fisheries, in describing fishery units (Murawski et al., 1983) and the state of fisheries' resources and management (Fox, 1970; Pauly, 1989; Sparre et al., 1989; Stergiou and Petrakis, 1993). Various statistical models have been used to analyze trends and to forecast fish catches.

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Significant regression, univariate and multivariate time series models were used to forecast monthly and annual marine fisheries' catches (Stergiou and Christow, 1996; Stergiou et al., 1997) of Loliginid and Ommastrephid (Georgakarakos et al., 2002, 2006).

The forecasting of fisheries' landings has been studied by many researchers using the Autoregressive Integrated Moving Average (ARIMA) methodology. So far, this method has been successful in describing and forecasting fishery dynamics of broadly different species-significantly, demersal and pelagic species (Stergiou, 1990; Stergiou and Christow, 1996; Stergiou et al., 1997; Tsitsika et al., 2007), squid (Pierce and Boyle, 2003), mackerel (Lloret et al., 2000; Punzón et al., 2004), loliginid and ommastrephid (Georgakarakos et al., 2002, 2006).

Songkhla Lagoon is one of the two lagoons in the world that has Irrawaddy dolphins, an endangered species. The lagoon's multitude of flora and fauna species makes it rich in biodiversity and it provides resources for local fisheries' fishermen all year around. The livelihood of the more than 1.9 million people of the 25 districts located in the three provinces of Southern Thailand rely on this fishery resource. The lagoon provides both economic benefits and nutrition for the local people. It is not only an important source of livelihood for men, but also for women, who do fish processing.

Small-scale fishing has been the main livelihood for local fishermen around Songkhla Lagoon for many generations. However, as in many places in the world, the numbers of fish have been declining sharply. Many fishermen of Songkhla Lagoon are wondering whether they can survive in fishing or if they must convert to another occupation. Many of them are moving to other fishing areas or changing their work.

For the effective socio-economic and environmental management of Songkhla Lagoon, and in aiming for sustainable development of fisheries' resources, it is vital to know the seasons and the trends of fish catch in the future, as well as the factors that affect any changes in these. Forecasting fish catch trends in Songkhla Lagoon is necessary.

There is a growing body of literature that recognizes the importance of statistical models in forecasting as well as in analyzing fish catches in Songkhla Lagoon over the last few decades. Significantly, Chesoh and Lim (2008) used a linear regression model for forecasting fish catches during 1977-2006. The structure of the fish community was analyzed using a clustering model (Chesoh and Choonpradub, 2011), the method for analyzing fish assemblage distribution (Chesoh and Choonpradub, 2009). However, historical time series fish catch data usually cover a long time, which includes seasonal and non-seasonal periods. The seasonal ARIMA model, therefore, is widely used for time series forecasting and resolves the problems of season and non-season of the data by describing the autocorrelations of the data.

Fish catch landing data depends not only on human activities, but also very much on the weather. Normally this data changes over time. Artificial neural network (ANNs) models were used in this study to compare with SARIMA models because ANNs have some advantage over other forecasting models. ANNs are powerful nonlinear regression techniques (Bishop, 1995; Ripley, 1996; Titterington, 2010). Nowadays, ANNs have been used widely in time series forecasting due to their ability to approximate various nonlinearities in the data (Zhang, 2003). The significant advantage of ANNs is no prior assumption of the model form is required in the model building process; moreover, the network model is mainly determined by the characteristics of the data.

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This study used monthly fish catch landings in Songkhla Lagoon data from 2003 to 2016 to evaluate the accuracy of SARIMA and ANNs models. The better model was chosen to forecast the fish and shrimp catch landing trends in Songkhla Lagoon to 2020.

Materials and Methods

Study site and sample collection

The total of fish and shrimp catch landings were collected for Songkhla Lagoon every month during 2003-2016 by the National Institute of Coastal Aquaculture (NICA), Thailand *(Fig. 1)*.

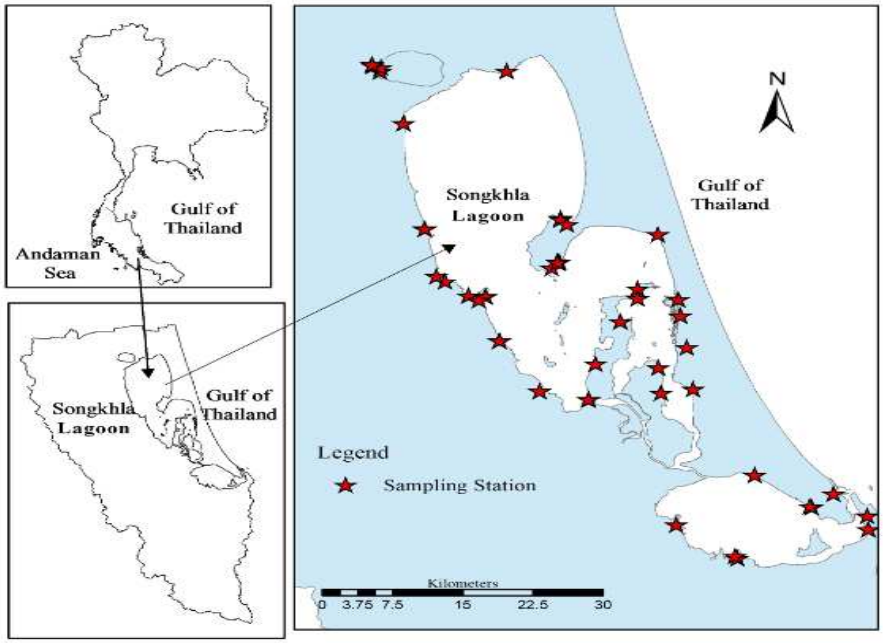


Figure 1. *Sampling stations in Songkhla Lagoon*

During 14 years of data collecting, 21 shrimp species and 158 fish species were collected every month in 37 stations around Songkhla Lagoon. The main types of fishing gear that have been used in Songkhla Lagoon include traps, set-bag nets and gill nets; and fishing activities that use such gear affect fish stock dynamics and the ecosystem (Chesoh and Choonpradub, 2011).

Time series forecasting models

SARIMA models

A time series $\{Z_t \mid t = 1, 2, ..., k\}$ is built using the SARIMA $(p, d, q)(P, D, Q)_s$ process with the mean μ of the Box and Jenkins (1976) time series model if

$$\varphi(B)\phi(B^s)(1-B)^d(1-B^s)^D(Z_t-\mu) = \theta(B)\Theta(B^s)a_t \qquad \text{(Eq.1)}$$

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where, Z_t denotes the observed value at time t, t=1, 2,…, k and a_t is the estimated residual at time t (*Eq. 1*); *p, d, q* denote the order of autoregressive (AR), degree of differencing or moving average (MA) of the non-seasonal part of the model to reach to stationary; *P, D, Q* are autoregressive (AR), order or moving average (MA) of the seasonal part of the model; *s* denotes the number of periods of the season;

$\varphi(B) = 1 - \varphi_1 B - \varphi_2 B^2 - ... - \varphi_p B^p$, $\phi(B^s) = 1 - \phi_1 B^s - \phi_2 B^{2s} - ... - \phi_p B^{ps}$,

$\theta(B) = 1 - \theta_1 B - \theta_2 B^2 - ... - \theta_q B^q$, and $\Theta(B^s) = 1 - \Theta_1 B^s - \Theta_2 B^{2s} - ... - \Theta_Q B^{Qs}$

are polynomials in *B* of degree *p, q, P* and *Q; B* is the backward shift operator.

The SARIMA model includes four basic steps:

- Identification of the SARIMA $(p,d,q)(P,D,Q)_s$ structure.
- Estimation of the unknown parameters.
- Goodness-of-fit tests on the estimated residuals (Box and Jenkins, 1976; Makridakis,1990).
- Forecast future outcomes based on the known data.

The a_t should be independently and identically distributed as normal random variables with mean = 0 and variance σ^2. The roots of $\varphi(Z) = 0$ and $\theta(Z) = 0$ should all lie outside the unit circle. The SARIMA model should use at least 50, or preferably 100 observations (Box and Jenkins, 1976). This data includes more than 100 months, which is adequate for a proper time-series analysis (Tsitsika et al., 2007).

The autocorrelation (ACF) and partial autocorrelation functions (PACF) of a series together are the most powerful tool usually applied to reveal the correct values of the model parameters. The ACF gives the autocorrelations calculated at lags 1, 2 and so on, while PACF gives the corresponding partial autocorrelations, controlling for autocorrelations at intervening lags. Parameter estimation of tentative models was determined using maximum-likelihood methods. The results included the parameter estimates, standard errors, estimate of residual variance, standard error of the estimate, natural log likelihood Akaike's information criterion (AIC), and Schwartz's Bayesian criterion (SBC). Model selection was based on the minimization of AIC and SBC. These criteria were descriptors of the model's parsimony as they simultaneously account for the model's fit onto the observed series alongside the number of parameters used in the fit.

Building SARIMA models

Monthly fish and shrimp catch landing data during 2003-2016 in Songkhla Lagoon were divided into two data sets. The training data set was applied for the period (January 2003 to September 2015), and was transformed by taking the natural logarithm. The transformed data sets then were White Neural Network, tested for nonlinearity by the package "nonlinearityTseries" in R (Garcia, 2015). Both transformed shrimp and fish tests had p value >0.05, which means that there is no evidence to reject the null hypothesis of linearity (Varvey and Leybourne, 2007). The transformed training data of both shrimp and fish catch landings were tested for normality by using the Shapiro-Wilk normality test. The shrimp data test results were w=0.95967, p values > 0.05 and the fish data test results were (w= 0.9843, p values >0.05). Therefore, the study had evidence to confirm that both transformed data sets

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were of normal distribution (Royston, 1995). Then, the data sets were tested the stationarity by the Box-Ljung test, Augmented Dickey-Fuller Test and KPSS Test. All these stationary test results had evidence to confirm that the transformed data of fish and shrimp catch landings were non-stationary time series; therefore, we can apply seasonal ARIMA models for forecasting shrimp and fish catches in the short term.

However, to get forecasts for shrimp and fish catch landings back to the original unit, the forecasted data were back-transformed after using a fitting model by taking exponentiation of the coefficients and the forecast is then the mean of the catches.

Twelve last months of data (October 2015 to September 2016) were used to validate the models.

The data was strongly seasonal and obviously non-stationary; therefore, seasonal differencing was used. The seasonally differenced monthly shrimp and fish catch landing data are shown in *Fig. 2*.

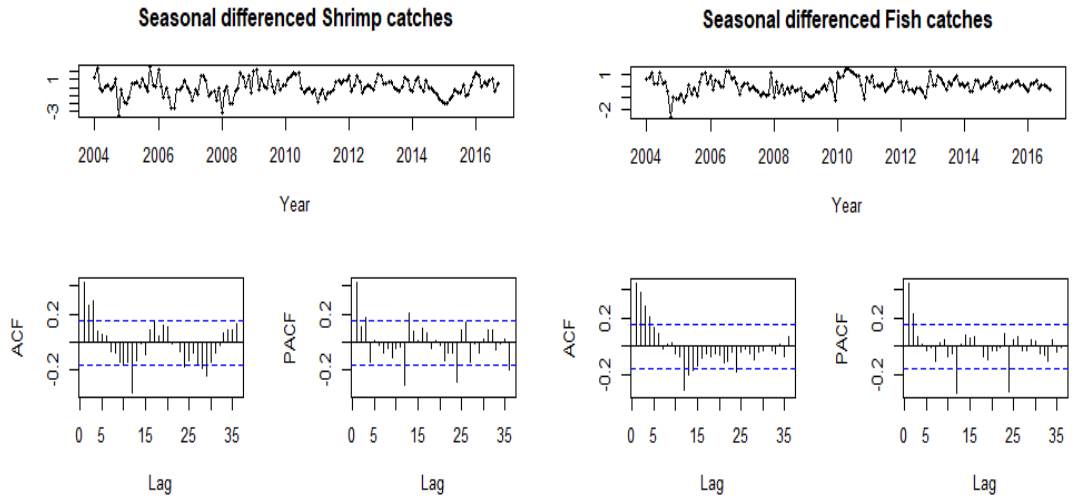


Figure 2. *First differencing log monthly shrimp and fish catches landings data*

The ACF and PACF of the transformed data sets showed spikes at periodic lags 12 and 24 *(Fig. 2)*, which declined slowly, indicating that seasonal differencing was required to achieve stationary. Abrupt fluctuations in the seasonal ACF and PACF had been leveled out.

Based on the first differencing correlograms of autocorrelation (ACF) and partial autocorrelation (PACF) of natural log transformed data of shrimp and fish catch landings, this result was combined with the Akaike information criterion (AIC) to determine the best model. The values for SARIMA $(p,d,q)(P,D,Q)_s$ models were applied as follows:

- Shrimp catch landing, the SARIMA model $(1,1,2)(0,0,3)_{12}$ was considered as the most appropriate, and due to this model passing all required checks (all the spikes are now within the significant limits), the residuals appear to be white noise. A Ljung-Box test also shows that the residuals have no remaining autocorrelations; additionally, this model has the smallest AIC and RMSE and gives the smallest mean error (ME) (0.0456). This statement is shown in *Fig. 3*.

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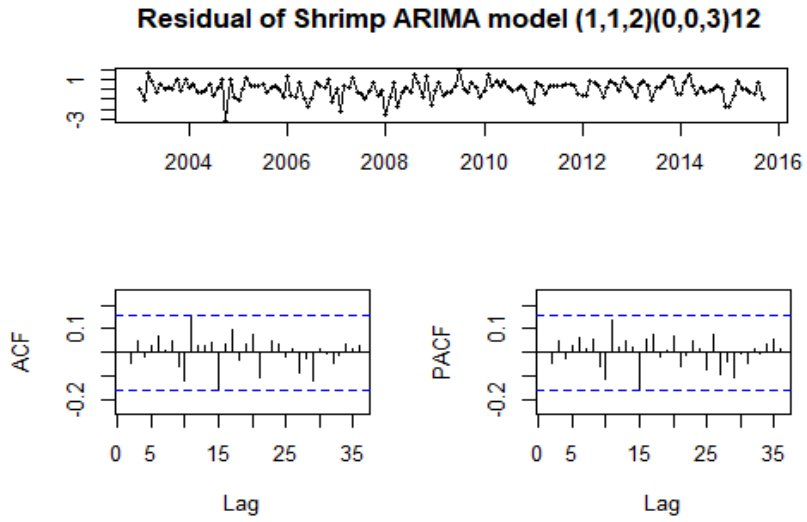


Figure 3. *Residual of shrimp seasonal ARIMA model* $(1,1,2)(0,0,3)_{12}$

- For fish catch landings, the seasonal ARIMA model $(1,1,1)(1,0,1)_{12}$ was considered as the most appropriate, due to this model having passed all requried checks (all the spikes are now within the significant limits, and the residuals appear to be white noise). A Ljung-Box test also shows that the residuals have no remaining autocorrelations. Jarque Bera Test result with X-squared = 4.0196, df = 2, p-value = 0.134 means that the data is consistent with having skewness and excess kurtosis zero; as well as this, this model has the smallest AIC and RMSE and gives the smallest of mean errors (ME) (0.018). This declaration is shown in *Fig. 4*.

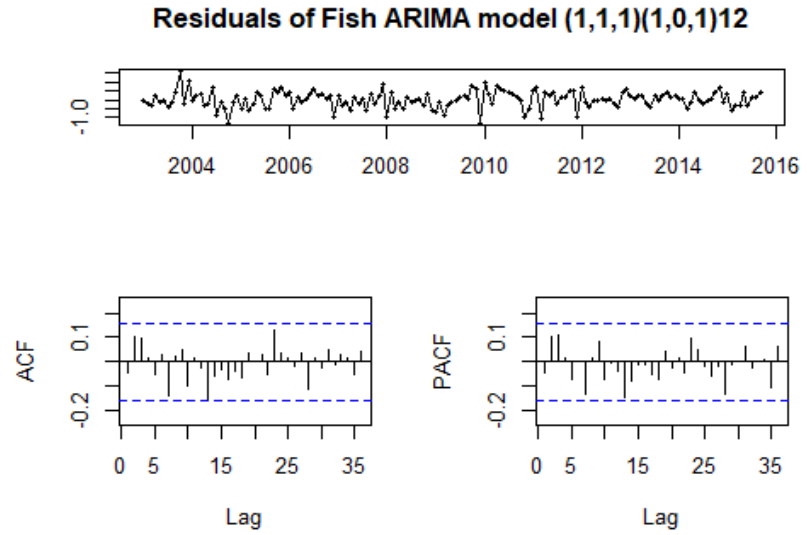


Figure 4. *Residual of fish seasonal ARIMA model* $(1,1,1)(1,0,1)_{12}$

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Artificial neural networks (ANNs)

In this study we used single hidden layer feed forward network, this is the model form that is the most widely used for time series modeling and forecasting (Zhang et al., 1998). The model is described by a network of three layers of simple processing units connected by acyclic links. The relationship between the output (y_t) and the inputs $(y_{t-1}, y_{t-2}, ..., y_{t-p})$ follows the mathematical equation:

$$y_t = \alpha_0 + \sum_{j=1}^{q} \alpha_j g(\beta_{0j} + \sum_{i=1}^{p} \beta_{ij} y_{t-i}) + \varepsilon_t \quad \text{(Eq.2)}$$

where, $\alpha_j (j = 0,1,2,...,q)$ and $\beta_{ij} (i = 0,1,2,..., p; j = 1,2,...,q)$ are the model parameters, often called the connection weights; p is the number of input nodes; and q is the number of hidden nodes. The logistic function is often used as the hidden layer transfer function:

$$g(x) = \frac{1}{1 + \exp(-x)} \quad \text{(Eq.3)}$$

Thus, the ANN model (2) in fact performs a nonlinear functional capturing from the past observations $(y_{t-1}, y_{t-2}, ..., y_{t-p})$ to the future value y_t ; therefore, y_t can be written:

$$y_t = f(y_{t-1}, y_{t-2}, ..., y_{t-p}, w) + \varepsilon_t \quad \text{(Eq.4)}$$

where, w is a vector of all parameters and f is a function identified by the network structure and connection weights. Hence, the neural network is equivalent to a nonlinear autoregressive model. The *Eq. 2* implies one output node in the output layer which is typically used for one step ahead forecasting.

Building ANN model

Like SARIMA model building, the training data set was natural logarithm transformed to get the best results and was used to build the ANN model, then the testing data was used to validate the model. While the SARIMA model uses one sample for model identification, estimation and evaluation, the ANNs estimated model is usually evaluated using a separate hold-out sample that is not displayed to the training process (Zhang, 2003). The model with the smallest mean squared error (MSE) will be chosen. The authors used the package nnfor (Nikolaos, 2017) from R software to create the ANNs model. The forecast statistical packages from R software were used to formulate the SARIMA models (Hyndman, 2017). All graphics and statistics were implemented by R software (R Core Team, 2018).

The best ANNs model was chosen to forecast shrimp fit with 5 hidden nodes and 20 repetitions, series modelled in differences: D1, univariate lags: (1,2,3,4,5,6,7,10) due to its smallest MSE (0.0078). The procedure demonstration is expressed in *Fig. 5*.

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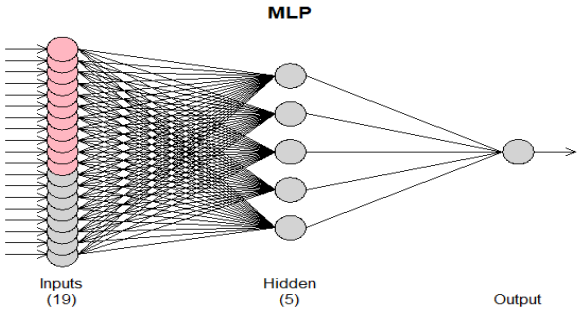


Figure 5. *Structures of the best fitted Artificial neural network model for shrimp*

The best ANNs model was chosen to forecast fish fit with 5 hidden nodes and 20 repetitions. Series modelled in differences: D1, univariate lags: (1, 2, 3, 4, 5, 6, 7, 8, 12) due to its smallest MSE (0.0372) is shown in *Fig. 6*.

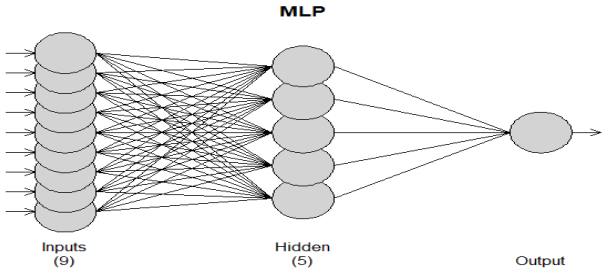


Figure 6. *Structures of the best fitted artificial neural network model for fish*

Results

Comparison between the SARIMA and ANNs models

To estimate the accuracy of the two models, the authors calculated the percentage improvement of SARIMA and ANNs models through Mean Absolute Error (MAE). The percentage MAE was calculated on the average over the validation samples of fish and shrimp catch landings from October 2015 to September 2016. The equation to measure percentage of MAE is as below:

$$MAE(\%) = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{|y_i - \hat{y_i}|}{y_i}\right)x100\% \qquad \text{(Eq.5)}$$

where, $|y_i - \hat{y_i}|$ is absolute difference between prediction and actual observation; n is the number of observations. The optimum model with the smaller percentage of MAE will be chosen for forecasting. The percentage MAE of SARIMA and ANNs models are shown in *Table 1*.

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Table 1. *The percentage MAE values of shrimp and fish catch landings from chosen SARIMA models and ANNs during the period October 2015 to September 2016*

Months	Shrimp catches landings (tons)	SARIMA model prediction (95% CI)-mean		Neural Network prediction		Fish catches landings (tons)	SARIMA model prediction (95% CI)-mean		Neural Network prediction	
		Amount (tons)	|% error|	Amount (tons)	|% error|		Amount (tons)	|% error|	Amount (tons)	|% error|
Oct 2015	60.52	47.22	21.97	45.75	24.41	114.50	76.83	32.90	101.33	11.50
Nov 2015	180.93	68.43	62.18	149.99	17.10	114.50	79.03	30.98	99.77	12.86
Dec 2015	41.5	37.41	9.85	19.00	54.22	63.27	71.54	13.07	66.26	4.73
Jan 2016	29.01	28.07	3.24	29.48	1.62	61.07	73.51	20.37	71.75	17.49
Feb 2016	34.68	29.18	15.85	20.57	40.69	54.19	62.78	15.85	70.31	29.75
Mar 2016	97.09	59.77	38.44	22.19	77.14	49.27	65.96	33.87	63.96	29.82
Apr 2016	129.39	60.17	53.50	24.69	80.92	59.03	64.26	8.86	44.87	5.84
May 2016	75.72	55.97	26.08	31.14	58.87	69.49	69.42	0.10	73.55	19.80
Jun 2016	78.32	38.56	50.77	10.60	86.47	60.29	64.20	6.49	72.23	22.81
Jul 2016	76.79	39.11	49.07	32.93	57.12	63.40	69.02	8.86	77.86	19.66
Aug 2016	50.44	51.59	2.28	31.66	37.23	63.28	69.65	10.07	75.72	5.70
Sep 2016	39.17	43.67	11.49	34.55	11.79	74.96	75.11	0.2	79.23	8.94
MAE (%)			**28.73**		**45.63**	**MAE (%)**		**15.14**		**16.70**

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As can be seen from *Table 1*, the ANNs models used for forecasting shrimp and fish catch have higher percentage MAE than SARIMA models, especially in the shrimp data set. These results are clearly described by the amount of the fish and shrimp catch in validated data sets. These are shown in *Fig. 7*.

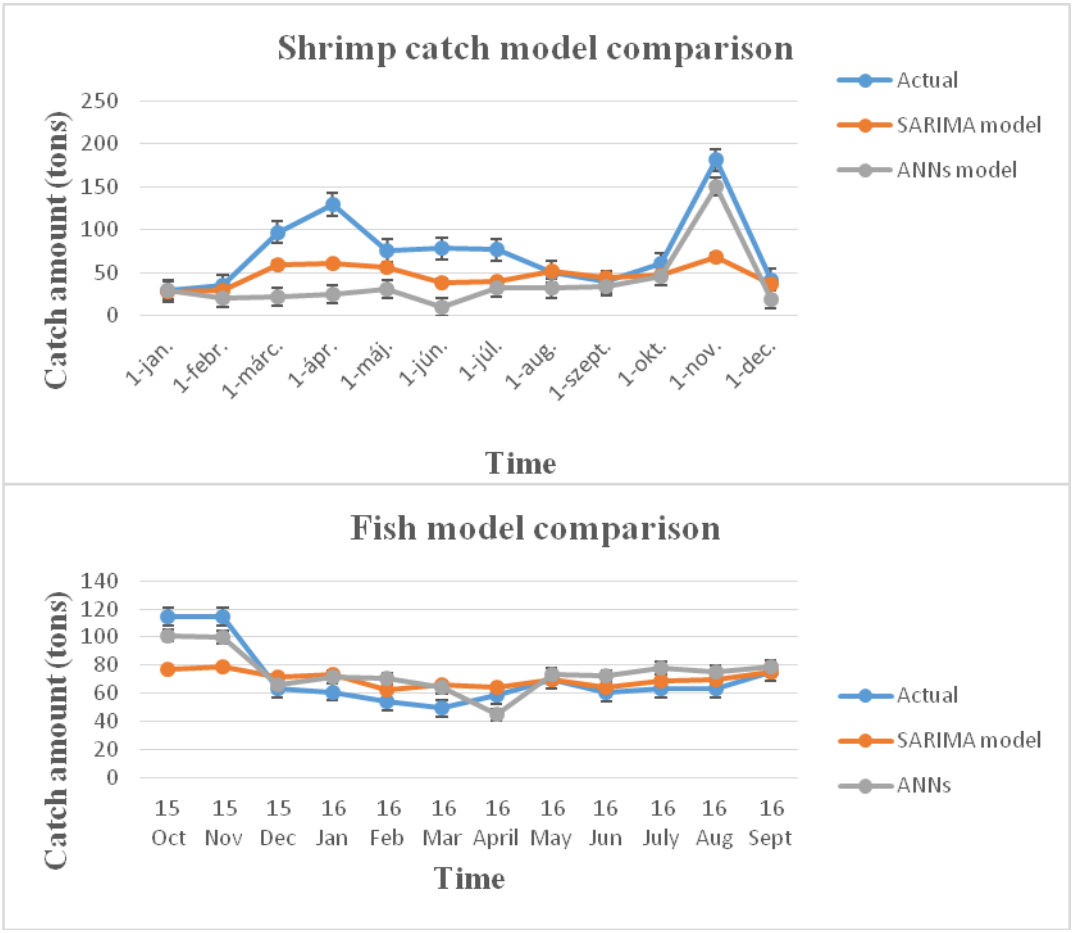


Figure 7. *Fish and shrimp model comparisons*

In both the shrimp and fish models, the 'forecast' line for the SARIMA model has the smaller distance, along with the Actual line, than the ANN model *(Fig. 7)*. Therefore, this study uses SARIMA models for forecasting shrimp and fish catch landings in Songkhla Lagoon until 2020.

Shrimp and fish catch landing trends in Songkhla Lagoon during 2016-2020

The monthly catch landings of fish and shrimp groups during 2003-2016, fits and forecasts for coming years, that were produced by the best fitting and best seasonal ARIMA forecasting model per group species, are shown in *Fig. 8*. Overall, the amplitude and the duration of the between–month fluctuations are adequately described and forecasted by the model with few exceptions.

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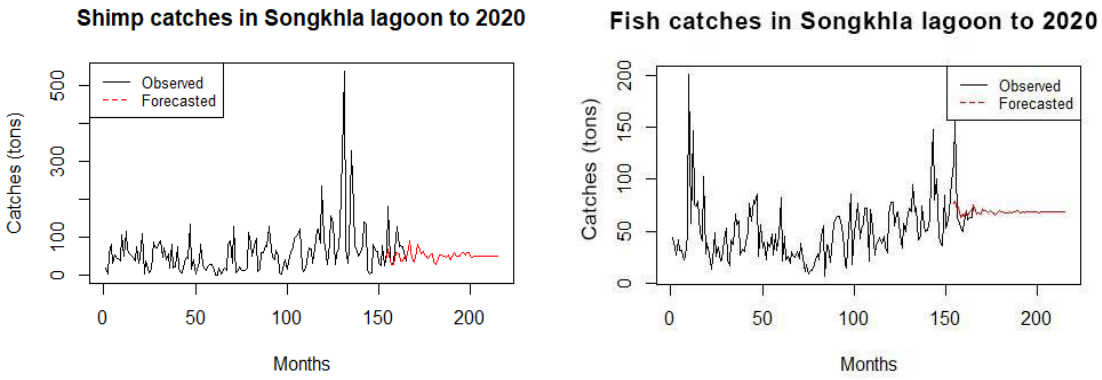


Figure 8. *Shrimp and fish catch forecasted in Songkhla Lagoon to 2020*

The result of shrimp and fish catch landings to 2020 is shown in the *Fig. 8*. It can be seen clearly that, during the period between 2017 and 2020, the shrimp catch landing in Songkhla Lagoon fluctuates at around 53 tons per month, compared with the mean catch (62.5 tons per month) during 2003-2016. Moreover, the fish catch will be around 68 tons per month, compared with the mean catch of around 49 tons during the period 2003-2016. The detailed trends of fish and shrimp catch landings in Songkhla Lagoon until 2020 are clearly described in *Fig. 9*.

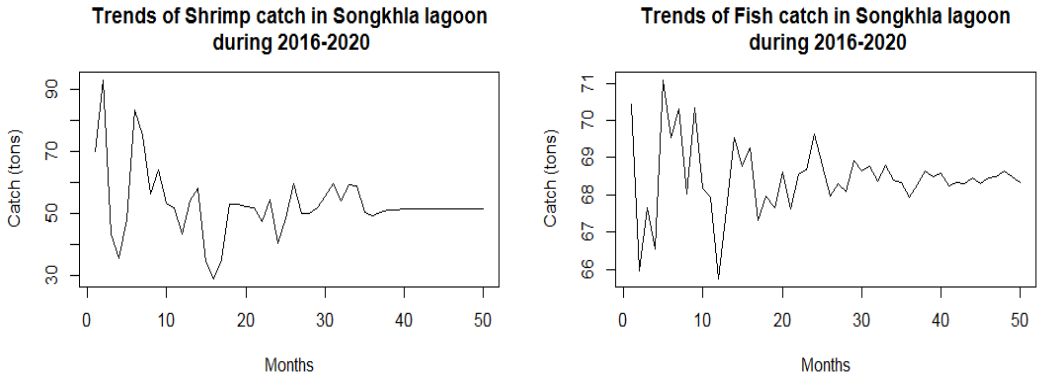


Figure 9. *Shrimp and fish catch forecasted for Songkhla Lagoon during 2016- 2020*

In *Fig. 9*, the fluctuations of the shrimp and fish catch landings during 50 upcoming months are somehow similar. During the two first years (from month 1 to month 24), the catch amount fluctuated sharply between months; after that, the catch amount was more stable. The difference between the lowest and highest catch amounts of shrimp are significant bigger than for fish. The lowest shrimp catch was January 2018 (28.95 tons) and the highest catch in November 2016 (92.94 tons), the difference being approximately 64 tons. The lowest fish catch was September 2017 (65.75 tons) and the highest fish catch was February 2017 (71.08 tons), with a difference of only 5.33 tons.

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Seasonal factor

The overview of monthly shrimp and fish catch landings in Songkhla Lagoon during 2003-2016 are presented in *Fig. 10*.

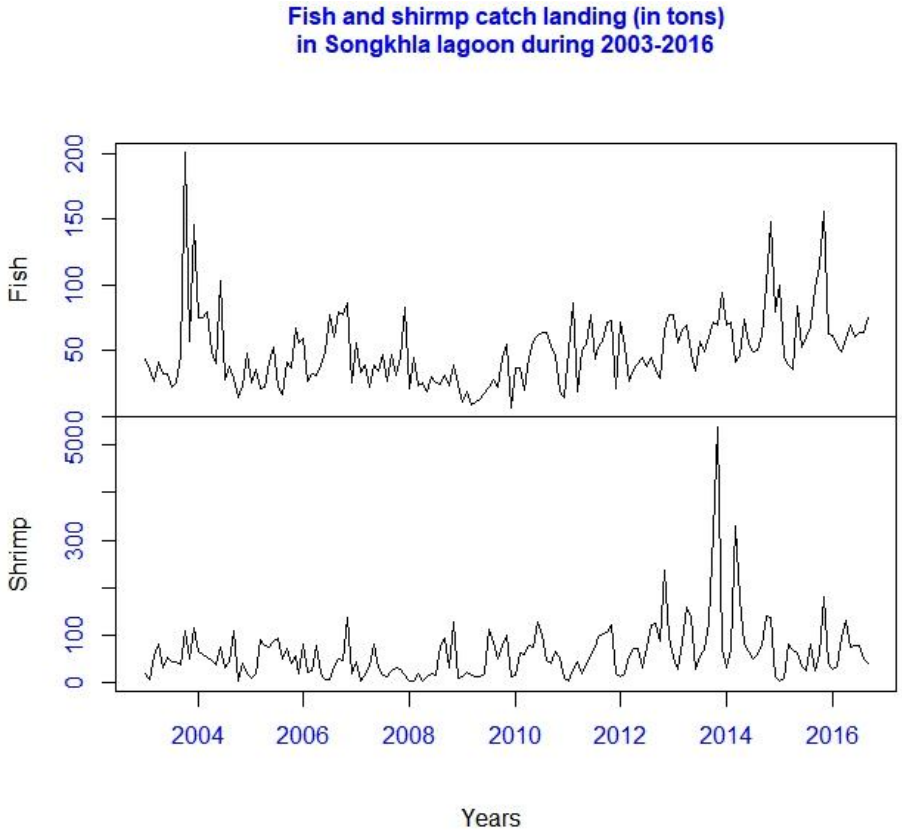


Figure 10. *Monthly shrimp and fish catch landings in Songkhla Lagoon during 2003-2016*

As shown in *Fig. 10*, the lowest shrimp catch landing was in January 2008, with only 1.77 tons; and the highest catch amount was in November 2013, the shrimp catch reached a peak with 537.41 tons. The mean shrimp catch was estimated at around 62.56 tons. The results obtained for the monthly fish landings were also shown in *Fig. 2*, when the most significant catch amount topped 200.78 tons in October 2003; and the lowest in this 2003-2016 period was in December 2009 (6.96 tons). The average monthly catch was around 50.31 tons for this period.

Using Seasonal Factors is the way to analyze data to reveal regular recurring changes associated with the calendar. A seasonal factor measures the percentage amount when, on average, a month is higher or lower than normal (Austin, 1981). From *Fig. 10*, it can be seen that the size of the seasonal fluctuations in both shrimp and fish time series data seems to be roughly constant over time, and does not depend on the level of the time series. Thus, to know exactly the seasonal trend of monthly shrimp and fish catches, the study estimated seasonal factors affecting shrimp and fish for the particular month from January to December. These factors are the same for each particular month, each year. Before monsoon season in Southern Thailand, December, is the best season for both shrimp and fish catching, which is shown by the high seasonal factors (from September

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to November). However, during monsoon season (December to February), the seasonal factors for shrimp catch landings are significant low while the factors for fish catch are really low during the dry season (March to May). The details for these claims are given in *Table 2* and *Fig. 11*. November provides the best seasonal factors for both shrimp catch landings (about 0.88) and fish (about 0.31); while the lowest seasonal factor for shrimp catch (about -0.96) is in January, and for the fish catch (about -0.36) in March, indicating that there seems to be a peak in both shrimp and fish catch landings in November with the bottom for shrimp catch in January, and for the fish catch in March in each year.

Table 2. *Seasonal factors for shrimp and fish catch in Songkhla Lagoon during 2003-2016*

Months	Shrimp seasonal factors	Fish seasonal factors
January	-0.96	0.09
February	-0.76	0.03
March	0.20	-0.36
April	0.14	-0.23
May	0.13	-0.03
June	-0.10	0.09
July	-0.005	-0.08
August	0.30	-0.10
September	0.41	0.09
October	0.33	0.19
November	0.88	0.31
December	-0.59	-0.01

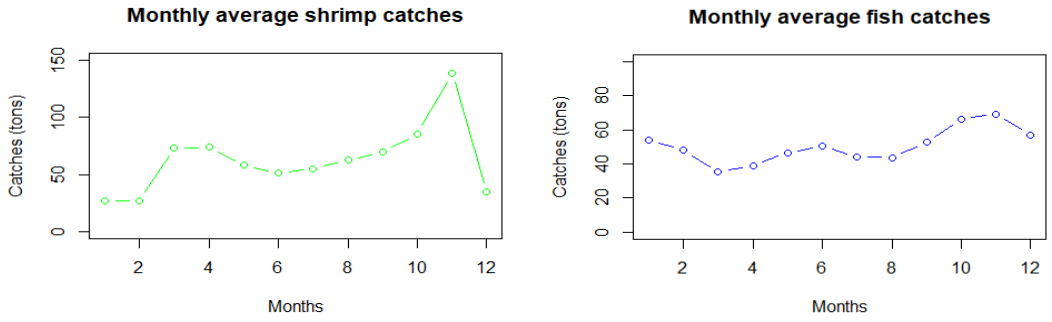
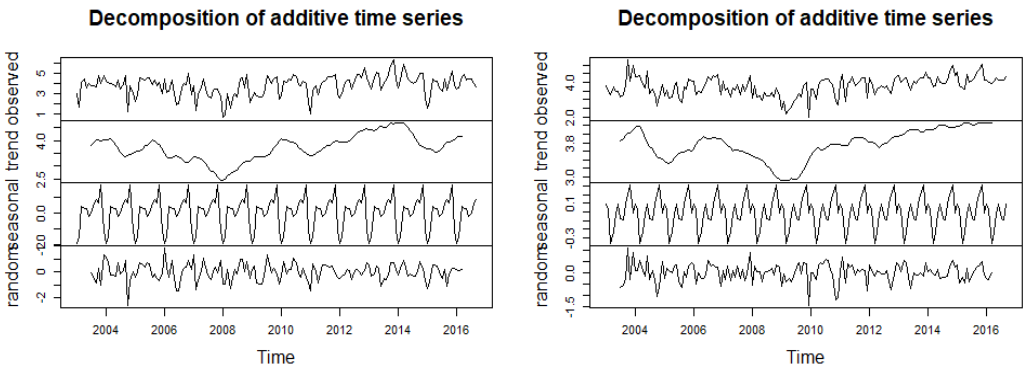


Figure 11. *The average catch of fish and shrimp per month in Songkhla Lagoon during 2003-2016*

According to the data for the total fish and shrimp catch from January to December for January 2003 through to the first 9 months of 2016, the total catch in months of the two species was different with reference to both the amount and the time of catch in the year. The amount of shrimp caught was lowest in January (estimate 27.06 tons) and highest in November, up to 138.55 tons. The amount of fish caught was lowest in March (only 35.13 tons) and highest in November, around 69.36 tons. In general, there was a sharp fluctuation in the average catch between months in year, particularly with reference to shrimp catch landings.

The trend and season of monthly shrimp and fish catch landings is shown clearly in *Fig. 12*.

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a.Shrimp catches b.Fish catches

Figure 12. *Trend and season of monthly shrimp and fish catch landings in Songkhla Lagoon during 2003-2016*

Discussion

Model fitting and forecasting performances

In this study, the percentage improvement figures for SARIMA and the ANN model for shrimp catch are significantly different (16.9%). In contrast with this result, the improvement figures for fish are very little different (1.56%). In the past, the percentage improvement of ARIMA and the ANN model has been different according to the data set and the period of the forecasting, which was confirmed by Khashei and Bijari (2011) when they used sunspot, Canadian lynx, and exchange rate data sets to compare percentage improvements of ARIMA and the ANN model. Almost all the results showed the better percentage improvement for ANNs over the ARIMA model at different levels. However, when they forecasted 67 points ahead from sunspot data set, percentage MAE of ANNs model is 3.32% bigger than ARIMA model. Again, this statement was proved by Tseng et al. (2002), when the SARIMA model of machinery production time series showed the better result compared to the neural network (NN) with 60 historical forecasting items of data, but the SARIMA model's results were not as good as NN when they forecast 36 points ahead.

In the past, Stergiow (1989) found two suitable ARIMA models to forecast pilchard (*sardina pilchardus*) catches up to 12 months ahead with mean errors (ME) of 14.6% and 12% for the two models. Suitable SARIMA models were found in this study for shrimp and fish with mean errors of 4.56% and 1.83%.

Univariate SARIMA models satisfactorily predicted the total fish and shrimp catch landings. So far, linear regression models developed for total fish catch in Songkhla Lagoon have illustrated little forecasting power (the percentage improvement was 34.6%) (Chesoh and Lim, 2008). In the present study, the univariate SARIMA models that were built show good performance in terms of explained variability and predicting power. The results demonstrate a strong autoregressive character of the time series, i.e. the fish catch landing value at month t depends on the catch at month t-12. SARIMA models provided satisfactory forecasts that were close to the recorded fish and shrimp catch values. The seasonal ARIMA models were found not to be appropriate for both shrimp and fish catch landing, suggesting that the catch landings of fish and shrimp had a different statistical structure. The present findings also imply the clear existence of a

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seasonal pattern in the monthly fish/shrimp catch landing, which is shown both in the original data and seasonal factor *(Table 2 and Fig. 11).*

Seasonal fish and shrimp catch landing

There are major peaks for seasonal fish and shrimp catch landing in the rainy season (from September to November), however, they quickly reduce in December-January. The seasonal cycle is most likely related to the nature of the lagoon ecosystem. In the monsoon seasons (October-January), there is intrusion of sea water from the Gulf of Thailand into the lagoon through a short narrow channel (about 8m in depth) in the outer section. Where the most intensive fishing activities occur, the average depth is very shallow, 1.0-1.5 m. The dry season in southeast Thailand extends from February to April. Most of the brackish and saltwater fish populations migrate to the open sea, whereas the freshwater fish in the upper lake migrate to deeper pools. This phenomenon is consistent with the seasonal migration of some fishermen to find work in the cities (Chesoh and Lim, 2008). The fishing is more intense during the first months of the monsoon season and decreases in December, due to such heavy rain and flooding that the fishermen cannot go fishing. The seasonal catch of shrimp and fish during 2003-2016 was different: while the shrimp catch decreased sharply from December to February, the fish catch decreased significant in the dry season, mostly concentrated in March to May, and before the rainy season (July and August). During the dry season, the fish from Songkhla Lagoon migrate back to the sea (the Gulf of Thailand) for breeding, spawning and larval nursing (Choonhapran, 1996; Chesoh and Samphantharaga, 2004). This claim is also confirmed by the composition of the first finding species found in both Songkhla Lagoon and Sathing Pra (a coastal area in Songkhla province that the author is studying). The seasonal shrimp catch landing is somehow different from the seasonal fish catch, the explanation coming from the effect of the restocking program during this period.

Factors involved in the seasonal variation in fish and shrimp catches may be due to regional climate, especially the effect of the monsoon season in the Southern Thailand, for during these months, the catch in coastal provinces decreases sharply (Komontree et al., 2006), and the catch in the lagoon tends to increase. Seasonal difference effects on monthly fish catch also have been found in previous studies (Stergiou and Christou, 1996; Komontree et al., 2006). Even though the fish catch is very low during March to May, the shrimp catch tends to increase which may be due to the effect of seasonal difference, as the annual closure of the fishing area of the Gulf of Thailand from February 16 to May 15 does not affect the fish catch in the lagoon. The changes of season lead to the changes in zooplankton (Brysiewicz et al., 2006), which would be one of the reasons for the difference in fish and shrimp catch between seasons.

Fish and shrimp catch trends in Songkhla Lagoon

In the monthly fish and shrimp catch landings until 2020, both shrimp and fish catch fluctuated sharply during the two first years of forecasting, then they fluctuated steadily. The shrimp catch reduced to 52.88 tons, by around 15.47% of the catch average during 2003-2016. In contrast with the shrimp catch, the fish catch increased to 68.45 tons, estimated at a 36.05% increase in comparison with the mean fish for the same period in 2003-2016. This result reflects the reality of fishing activities in Songkhla: the general trends of shrimp catch reduce due to the decrease in the number of juvenile shrimps from the stocking program.

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During the past four decades, only two papers have reported the annual catches in the main Songkhla Lagoon. The first study was conducted during 1984-1986 (Tonkwinas et al., 1986) and the second during 1994-1995 (Choonhapran et al., 1996). These studies reported the total annual fish catch during 1994-1995, a decrease of 22% compared to the catch in 1984-1986. The monthly report of total fish catches during 1977 to 2006 was 219.9 tons (Chesoh and Lim, 2008).

Conclusion

Knowing the seasons and forecasting both play an important role in fisheries' management, as they constitute the first steps of the planning and decision-making process (Stergiou and Christow, 1996; Stergiou et al., 1997). The facts of the good performance of the final ARIMA models applied to shrimp and fish catch landings in this paper in terms of short term forecasting of up to 12 months in advance, as well as the results that the models provided for insight into the seasonal components of the time series, all justify their use in fisheries' management. Accurate forecasts for 1-2 years may provide useful information to fishery policy makers as well as fishermen. For small scale fishing, the conditions for fishing do not seem to change much inside the lagoon and there are limited budgets for organizing the usual surveys, so SARIMA models can be considered as suitable tools to forecast the persistence of fish and shrimp catches. The performance of the models could be improved by additional information about environmental factors (e.g. temperature, salinity…) and other factors (market prices and biological information) put into the multivariate models.

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Conflict of interest. The authors definitely confirm that there is no conflict of interest concerning this article.

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CHEMICAL CONSTITUENTS OF THE VOLATILE OILS OF *STACHYS CRETICA* SUBSP. *ANATOLICA* AND *STACHYS LAVANDULIFOLIA* FROM SEYDISEHIR, TURKEY

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Abstract. This study was conducted to determine volatile oil components for leaf and flowers of *Stachys cretica* subsp. *anatolica* Rech. and *Stachys lavandulifolia* Vahl. in different reaping periods, such as pre-flowering, flowering, and post-flowering by gas chromatography mass spectroscopy (GC-MS) after solid phase micro extraction (SPME). To achieve this aim, leaf and flower samples were collected from Tınaztepe Cavern province of Seydisehir, in 2015 and 2016. A total of 62 components were determined for *Stachys cretica* subsp. *anatolica*, with benzaldehyde (46.34%), β-caryophyllene (11.23%) and (E)-2-hexenal (8.50%) being the main components. Additionally, 55 volatile components were found for *S. lavandulifolia,* and the main components were β-phellandrene, myrcene, and α-pinene at 27.71%, 11.56% and 11.20% respectively.
Keywords: *Stachys, SPME, (E)-2-Hexenal, Benzaldehyde, β-phellandrene, Turkey*

Introduction

Turkey is situated at the intersection of different climate zones due to its geographical location. Therefore, it is rich in plant species and diversity. Turkey is also an important germplasm for the plants in the Lamiaceae family, which is the number one plant species traded across the world that has an important place in alternative medicine (Anonymous, 1996; Arslan et al., 2000; Ozkan, 2007). The members of the Lamiaceae family represented by 45 genera and more than 546 species in Turkey are vital for the pharmacology and perfumery industry thanks to their volatile and aromatic oil content (Secmen et al., 2000).

The economic value of the Lamiaceae family members is very high, as they contain the aromatic compounds and essential oils. Some taxa in the *Pogestemon* and *Lavandula* genera are used in the perfumery industry while *Thymus*, *Mentha* and *Origanum* species are used in the food industry (Watson and Dallwitz, 1978; Clive and Stace, 1980; Baytop, 1991; Ozkan, 2007). Furthermore, several species in the family (*Stachys*, *Sideritis*, *Salvia*, *Lamium*, *Ajuga*, *Teucrium*, *Thymus*, *Phlomis* etc.) are known to be used for therapeutic purpose (Baytop, 1984, 1991; Ozkan, 2007).

The genus *Stachys* from the Lamiaceae family, which was the research material, in this study, comprises nearly 200 taxa. The members of *Stachys* L. are found almost everywhere in the world except Australia and New Zealand. The Balkans, South and East Anatolia, Caucasians, Northwest Iran and north of Iraq are the regions where these species grow intensively (Bhattacharjee, 1974; Davis, 1988). They are represented by 121 taxa in Turkey with an endemism rate of 43.4%.

Stachys species are medicinal plants used by people for the treatment of diseases. *Stachys annua* is used to heal insomnia and menstrual irregularities; *S. sylvatica* is used as antispasmodic, diuretic, tonic and astringent agent, *S. officinalis* is used as tonic and

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for the treatment of insomnia, headache and cough, while *S. palustris* is used externally to heal wounds (Steinmetz, 1954; Garnier et al., 1961).

Stachys cretica is perennial with basal sterile rosettes. Flowering stems are 25-100 cm, erect, they can be branched or unbranched, usually densely or sparsely adpressed greyish tomentose, rarely patent-pilose with glandular hairs being less frequent than eglandular hairs. Basal leaves are oblong-spathulate, 4-10 x 1-3.5 cm, obscurely crenulate to faintly crenate, apex obtuse sometimes acute, attenuate or rarely rounded to subcordate at base, petiole 2-8 cm. Cauline leaves are like basal but gradually become smaller above, shortly petiolate to subsessile or sessile. Floral leaves are oblong-lanceolate to lanceolate, 1-6 x 0.5-1.2 cm, the same lenght or longer compared to verticillasters. Additionally, verticillasters usually remote throughout, rarely approximate above, 10-16-flowered. Brachteoles ovate-lanceolate to lanceolate, 5-16 mm, herbaceous, not spinescent. Pedicels 1-2.5 mm. Calyx sub-bilabiate, subcampanulate to tubular, 12-16 mm, densely tomentose, teeth subequal, ovate to lanceolate, 1/3-1 x tube; mucro 1-3 mm. Corolla rose-pink, 18-20mm, tube subexserted. Nutlets obovoid, 2 x 1.5 mm, smooth (Davis, 1982).

S. lavandulifolia is suffrutescent perennial with basal rosettes of sterile shoots. Flowering stems numerous, 10-30 cm, sparsely to densely pilose, sometimes absent, rarely white-tomentose with unequal stellate hairs. Basal leaves subsessile to sessile, oblong-lanceolate to oblanceolate, 2-6 x 0.4-1.5 cm, entire to faintiy serrate, attenuate at base, cauline similar. Floral leaves oblong to ovate, 1.2-2 x 0.25-0.8 cm, shorter than verticillasters. Verticillasters (at least lower ones) usually remote, (2-)4-6-flowered. Brachteoles few, linear, 2-3 mm, hirsute. Pedicels 1.5-2 mm. Calyx ± regular, subcampanulate, patently pilose, tip softly spinescent. Corolla purple to mauve, 13-15 mm, tube subincluded. Nutlets obovoid, 2.5-3 x 2-2.5 mm (Davis, 1982).

Stachys species are becoming increasingly important due to the aromatic compounds and essential oils they contain. In this study, we analyzed the volatile compounds in the leaves and flowers of *Stachys cretica* subsp. *anatolica* and *S. lavadulifolia* species at three different vegetation stages, pre-flowering, flowering and post-flowering.

Materials and Methods

The material of the study conducted in 2015 and 2016 comprised of *Stachys cretica* subsp. *anatolica* and *S. lavandulifolia* samples collected during pre-flowering, flowering and post-flowering stages from two different sampling sites in Konya-Seydisehir, Tınaztepe Cavern province that is situated N 37° 14', E 31° 55', and 1525 m elevation (*Figure 1*). Samples of *S. cretica* subsp. *anatolica* were collected in April, May, and June of both 2015 and 2016 at pre-flowering, flowering, and post-flowering stages. Additionally, *S. lavandulifolia* samples were collected in May, June, and July of both years.

The leaves and flower samples that were collected were put into paper packages and transferred to the laboratory the same day to avoid exposure to sunlight. After the collected plant materials were dried at room temperature (25°C), and flower and leaf samples were subjected to solid phase microextraction (SPME). 2 g of samples were placed into a 10 mL vial. After incubation for 30 min at 60°C, SPME fibre was pushed through the headspace of a sample vial to absorb the volatiles and then inserted directly into the injection port of the GC-MS (Shimadzu 2010 Plus GC-MS with the capillary column, Restek Rxi®-5Sil MS 30 m x 0.25 mm, 0.25 μm) at a temperature of 250°C for

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desorption (5 min) of the adsorbed volatile compounds. The constituents were identified using retention times of standard substances by aligning mass spectra with the data given in the Wiley, NIST Tutor, FFNSC library.



Figure 1. *Sampling area for Stachys materials*

Results

The volatile compounds in the leaves and flowers of *Stachys cretica* subsp. *anatolica* and *S. lavandulifolia* species were collected at pre-flowering, flowering, and post-flowering stages were analyzed by SPME (solid phase microextraction) method. With SPME method, 62 different volatile compounds were identified in *Stachys cretica* and 55 in *S. lavandulifolia*. These results are shown in *Table 1*. (E)-2-Hexenal, Benzaldehyde, and β-caryophyllene *Stachys cretica* were identified as the main compounds of *Stachys cretica*. Specifically, it contained 7.82% (E)-2-Hexenal, 40.54% Benzaldehyde, and 8.36% β-caryophyllene at the pre-flowering stage; 8.50% (E)-2-hexena, 46.34% Benzaldehyde and 11.23% β-caryophyllene at the flowering stage; and 6.62% (E)-2-Hexenal, 41.80% Benzaldehyde and 10.93% β-caryophyllene at the post-flowering stage. Additionally, α-pinene, myrcene and β-phellandrene were found to be the main compounds of *Stachys lavandulifolia*. Specifically, these flowers contained 10.70% α-pinene, 10.98% myrcene and 26.12% β-phellandrene at the pre-flowering stage; 11.20% α-pinene, 11.56% myrcene and 27.71% β-phellandrene at the flowering stage; and 10.90% α-pinene, 10.25% myrcene and 26.06% β-phellandrene at the post-flowering stage (*Table 1*).

Table 1. *Chemical Constituents of the Volatile Oils of Stachys cretica subps. anatolica and Stachys lavadulifolia in different reaping periods*

			Stachys cretica			*Stachys lavandulifolia*		
	Retention Time	**Components**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**
1.	1.370	Isobutanal	0.33	-	-	0.03	0.06	0.07
2.	1.440	2-Methylpropenal	-	-	-	-	0.03	0.02
3.	1.517	3-Methyl-2-butanone	0.16	-	-	-	-	-
4.	1.531	2-Ethylbutanal	-	-	-	0.05	0.03	0.04

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			Stachys cretica			*Stachys lavandulifolia*		
	Retention Time	**Components**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**
5.	1.559	2-methylfuran	0.20	-	-	-	0.01	-
6.	1.638	3-methylfuran	-	-	-	0.01	0.01	-
7.	1.887	2-Butenal	1.22	0.89	1.38	0.15	0.22	0.20
8.	1.929	3-Methylbutanal	0.44	0.19	-	-	-	-
9.	2.009	2-Methylbutanal	0.37	-	-	0.11	0.10	0.09
10.	2.235	1-Ethylcyclopropanol	-	-	-	0.02	0.04	0.01
11.	2.325	Pentanal	0.69	0.70	0.77	0.25	0.22	0.18
12.	2.357	2-ethyl-Furan	0.54	-	-	0.23	0.23	0.20
13.	2.892	(E)-3-Penten-2-one	0.30	0.22	0.34	0.02	0.05	0.04
14.	3.032	3-Methylenepentane	-	-	-	-	0.01	0.08
15.	3.181	(E)-2-Pentenal	0.22	-	-	0.05	0.08	0.03
16.	4.101	n-Hexanal	3.98	1.02	1.47	0.68	0.74	0.71
17.	5.408	Ethyl 2-methylbutyrate	-	0.19	-	-	-	-
18.	5.514	(E)-2-Hexenal	7.86	8.50	6.62	4.24	3.45	4.82
19.	5.589	cis-3-Hexene-1-ol	0.44	0.33	0.27	0.68	0.34	0.29
20.	5.906	(E)-2 -Hexenol	0.40	0.27	-	-	-	-
21.	6.028	n-Hexanol	1.31	0.46	0.29	0.06	0.06	-
22.	6.612	Heptan-2-one	0.35	-	-	-	-	-
23.	7.025	Heptanal	1.29	0.70	0.67	-	-	-
24.	7.294	Sorbaldehyde	-	0.31	0.37	0.05	0.09	0.07
25.	7.837	α-thujene	-	-	-	1.98	1.06	1.92
26.	8.069	α-pinene	2.12	1.09	1.22	10.70	11.20	10.90
27.	8.482	Propenylbenzene	3.14	1.64	2.02	2.98	2.07	2.02
28.	8.944	cis-2-Heptenal	0.47	0.22	-	0.11	0.13	0.08
29.	9.043	Benzaldehyde	40.54	46.34	41.80	0.05	0.10	0.04
30.	9.647	2-β-pinene	1.60	0.85	1.05	5.56	5.02	5.87
31.	9.767	Sabinene	-	-	-	4.88	4.11	4.12
32.	9.873	Vinyl amyl ketone	-	-	0.24	0.65	0.70	0.72
33.	9.909	Vinyl amyl carbinol	1.70	1.52	1.17	-	-	-
34.	10.023	6-Methyl-5-hepten-2-one	0.95	0.79	0.70	-	-	-
35.	10.208	2-Pentylfuran	1.30	0.78	0.76	-	-	-
36.	10.202	Myrcene	-	-	-	10.98	11.56	10.25
37.	10.705	n-Octanal	1.15	0.97	0.78	-	-	-
38.	10.744	α-phellandrene	-	-	-	3.36	2.43	3.20
39.	10.968	(E,E)-2,4-Heptadienal	-	0.46	0.58	-	-	-
40.	11.352	p-Dichlorobenzene	-	-	-	0.09	0.18	0.12
41.	11.491	α-terpinene	-	-	-	0.85	1.07	1.99
42.	11.458	Cymene	0.46	-	-	-	-	-
43.	11.649	dl-Limonene	2.95	1.57	1.20	0.15	0.15	0.12
44.	12.005	3-Octen-2-one	0.68	0.79	0.89	-	-	-
45.	12.134	Benzeneacetaldehyde	-	0.39	0.40	-	-	-
46.	12.139	β-phellandrene	-	-	-	26.12	27.71	26.06
47.	12.331	Cis-Ocimene	-	-	-	4.71	4.98	4.52
48.	12.370	trans-β-Ocimene	-	-	-	0.54	0.75	0.66
49.	12.763	(E)-2-Octenal	0.36	0.45	0.43	-	-	-
50.	13.065	γ-terpinene	-	-	-	0.18	0.23	0.11
51.	13.205	3,5-Octadien-2-one	-	0.31	0.43	-	-	-

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			Stachys cretica			*Stachys lavandulifolia*		
	Retention Time	**Components**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**	**Pre-flowering (%)**	**Flowering (%)**	**Post-flowering (%)**
52.	14.417	Linalool	-	0.39	-	-	-	-
53.	14.590	n-Nonanal	1.90	1.26	1.82	-	-	-
54.	15.510	.+/-.-4-Acetyl-1-methylcyclohexene	0.25	-	-	-	-	-
55.	15.841	2,4,6-Octatriene, 3,4-dimethyl-	-	-	-	0.57	0.73	0.69
56.	16.311	2,4,6-Octatriene,2,6-dimethyl-, (E,Z)-	-	-	-	0.42	0.60	0.62
57.	15.916	3-Methyl-2-butenyl pentanoate	1.19	0.74	0.64	-	-	-
58.	17.824	Methyl salicylate	0.30	0.71	0.74	0.15	0.22	0.21
59.	18.452	n-Decanal	0.48	0.53	0.49	-	-	-
60.	19.457	Pentylallyl butyrate	-	0,24	-	-	-	-
61.	19.578	hexyl 2-methylbutanoate	0.80	0.81	0.81	-	-	-
62.	22.573	1-Undecene, 1-ethoxy-	0.55	0.66	0.57	0.25	0.22	0.20
63.	23.315	Bicycloelemene	-	-	-	0.55	0.62	0.58
64.	23.450	n-Octyl isobutyrate	-	-	0.64	-	-	-
65.	23.506	α-cubebene	0.80	1.14	0.65	-	-	-
66.	24.479	α-copaene	0.84	1.41	1.66	0.07	0.10	0.12
67.	24.744	β-bourbonene	1.04	1.61	1.09	0.28	0.49	0.33
68.	24.949	β-elemene	0.59	-	1.44	0.19	0.26	0.27
69.	25.264	β-cubebene	-	-	-	0.09	0.12	0.10
70.	25.431	6,10-Dimethylundecan-2-one	-	0.34	-	-	-	-
71.	26.076	trans-Caryophyllene	8.36	11.23	10.93	-	-	-
72.	26.283	α-amorphene	-	-	-	0.31	0.23	0.14
73.	26.458	Octyl 3-methylbutanoate	1.32	1.36	2.69	-	-	-
74.	26.803	α-bergamotene	-	-	-	0.42	0.48	0.36
75.	26.970	(+)-Aromadendrene	-	-	-	0.12	0.18	0.19
76.	27.108	(E)-β-farnesene	2.03	2.42	4.84	-	-	-
77.	27.359	Epi-bicyclosesquiphellandrene	-	-	-	0.25	0.24	0.17
78.	27.501	β-sesquiphellandrene	-	-	-	0.76	0.98	0.90
79.	27.670	Neoalloocimene	-	-	-	0.18	0.20	0.13
80.	27.971	Germacrene-D	0.52	0.82	0.59	6.84	6.37	6.32
81.	28.089	2-Phenylethyl 2-methylbutanoate	-	0.78	0.55	-	-	-
82.	28.202	β-selinene	-	-	0.45	-	-	-
83.	28.438	Bicyclogermacrene	0.72	0.94	0.98	5.52	5.96	5.80
84.	28.548	α-muurolene	-	0.36	0.44	-	-	-
85.	28.998	γ-cadinene	-	-	-	0.19	0.21	0.12
86.	29.176	δ-cadinene	-	0.30	0.27	-	-	-
87.	29.645	α-panasinsen	-	-	-	2.10	2.28	2.84
88.	30.954	Spathulenol	-	-	-	0.22	0.29	0.36
89.	31.105	Caryophyllene oxide	-	-	0.32	-	-	-
90.	40.239	Curcumene	0.79	-	1.54	-	-	-

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Discussion and Conclusions

Stachys cretica subsp. *anatolica* was found to have 62 different volatile compounds, with (E)-2-Hexenal (8.50%), Benzaldehyde (46.34%), and β-caryophyllene (11.23%) being its main compounds.

Ozkan et al. (2005) found that the main component of *Stachys cretica* subsp. *mersinaea* was α-curcumene (34.10%). In another study, Ozturk et al. (2009), identified 37 components of *Stachys cretica* subsp. *smyrnaea* using GC-MS analyses, with β-caryophyllene (51.0%), germacrene-D (32.8%), a-humulene (3.1%), Δ-cadinene (2.1%), and Δ-elemene (2.1%) being its main components. β-caryophyllene was found to be the main compound in both studies. The results for the other compounds was different from our findings. The other main compounds identified in our study were (E)-2-Hexenal and Benzaldehyde.

Goren et al. (2011) identified 24 main components of two *Stachys cretica* subsp. *cassia* and *S. cretica* subsp. *garana*, and 27, 26 and, 23 components of *S. cretica* subsp. *lesbiaca*, *S. cretica* subsp. *kutahyensis* and *S. cretica* subsp. *symrnaea* respectively. As results, germacrene-D (27.8%), δ-elemene (14.9%), and β-caryophyllene (8.9%) were identified for *Stachys cretica* subsp. *cassia*, however α-cadinol (16.4%), verbenol (11.4%) and dodecanoic acid (8.9%) were found for *S. cretica* subsp. *garana*. Additionally, germacrene-D, β-caryophyllene, and α-cadinol were found to be the main components of *S. cretica* subsp. *lesbiaca* at 13.9%, 12.5%, and 7.4% respectively. In the same study, they found germacrene-D (38.9%), β-caryophyllene (14.8%), and caryophyllene oxide (8.7%) to be the main components of *S. cretica* subsp. *symrnaea* and germacrene-D (28.1%), τ-muurolol (9.3%) and cubenol (8.8%) to be the main components of *S. cretica* subsp. *kutahyensis*. β- caryophyllene in both studies. The results for the other compounds differed from our findings. Goren et al. (2011) identified germacrene-D as the other dominant compound. The other main compounds identified in our study were (E)-2-Hexenal and Benzaldehyde.

Through SPME analysis, *Stachys lavandulifolia* was found to have 55 different volatile compounds, with α-pinene (11.20%), myrcene (11.56%), and β-phellandrene (27.71%) being its main compounds.

In another study conducted in Iran by Feizbaksh et al. (2003), 55 components of *Stachys lavandulifolia,* which is endemic to Iran, were identified, and α-pinene (20.1%), β-pinene (12.1%), and spathulenol (7.2%) were found to be its main components. Moreover, α-pinene was found to be the main compound in both analyses. Their results for the other compounds differed from our findings. Feizbaksh et al. (2003) found that the other dominant compounds were β-pinene and spathulenol while the other main compounds identified in our study were myrcene and β-phellandrene.

The studies of Javidnia et al. (2004) found that germacrene-D (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%), and Z-β-ocimene (5.8%) are the main components of *Stachys lavandulifolia*, however, Morteza-Semnani et al. (2005) identified 4-hydroxy-4-methyl-2-pentanone (9.3%), α-pinene (7.9%), and hexadecanoic acid (5.2%). In both studies, α-pinene was found to be the main compound. The result for the other compounds differed from our findings. Morteza-Semnani et al. (2005) found that the other dominant compounds were 4-hydroxy-4-methyl-2-pentanone and hexadecanoic acid. The other main compounds identified in our study were Myrcene and β-phellandrene.

Meshkatalsadat et al. (2007) reported that the main components of *Stachys lavandulifolia* are α-pinene (25.66%), myrcene (17.33 %), β-phellandrene (21.96 %),

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and β- caryophylene (14.3 %). In another study, Sajjadi ve Amiri (2007) determined the components as α-pinene (27.25, 25.66, 8.52%), myrcene (17.33, 9.33, 23.85%), and β-phellandrene (21.96, 37.49, 12.58%) for pre-flowering, flowering, and post-flowering periods of *S. lavandulifolia,* respectively. These studies supported our results.

Among 37 compounds of *Stachys lavandulifolia* Vahl. subsp. *lavandulifolia,* Iscan et al. (2012) found β-phellandrene (27%), α-pinene (18.5%), and germacrene-D (13%) to be the major components. In both studies, β-phellandrene and alpha-pinene were found to be the main components. In their study, germacrene-D was another dominant compound. The other main compounds identified in our study were myrcene and β-Phellandrene. Additionally, Pirbalouti et al. (2013) determined α-thujone (0.3%–32.3%), α-pinene (trace to 37.3%), myrcene (0.5%–15.9%), β-phellandrene (1.1%–37.9%), germacrene-D (0.4%–11.3%), Δ-cadinene (trace to 11.6%) and 1, 4-methano-1 H-indene (trace to 10.1%) to be the main components of *S. lavandulifolia*. These studies also supported our results.

In conclusion, *Stachys cretica* subsp. *anatolica* and *Stachys lavandulifolia*, the leaves and flowers of which were collected from the field, had the highest efficiency in terms of volatile compounds when they were collected during the flowering stage. The findings of this study inform the merchants and local people about the collection time to prevent random plant collection and potential economic losses.

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EFFECT OF CROP ROTATION AND TILLAGE SYSTEM ON THE WEED INFESTATION AND YIELD OF SPRING WHEAT AND ON SOIL PROPERTIES

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Abstract. A field experiment was conducted to evaluate the effect of crop rotation and tillage system on the yield and weed infestation of spring wheat as well as on the chemical properties of soil and number of earthworms. The first experimental factor was the cropping system: 1) crop rotation A: pea – spring wheat – durum wheat, 2) crop rotation B: pea – spring barley – spring wheat, 3) cereal monoculture: spring barley – spring wheat – durum wheat, whereas the second one was the tillage system: a) conventional (CT), b) reduced (RT), and c) no-tillage (NT). Spring wheat produced a higher grain yield in crop rotation A than in crop rotation B and cereal monoculture. Its higher yield was also achieved in the CT system than in the NT and RT systems. A higher number and weight of weeds was noted in the cereal monoculture compared to crop rotations A and B. Greater weed infestation occurred also in RT and NT systems than in the CT system. In soil samples from crop rotations A and B, analyses demonstrated higher contents of organic C and total N and a lower C/N ratio than in the cereal monoculture. The increased contents of organic C and total N in the soil were also affected by NT and RT systems, then by the CT system. Also the number of earthworms in soil m^{-2} was significantly higher in RT and NT than in CT system.
Keywords: *cropping system, earthworms, organic carbon, soil tillage, weeds*

Introduction

Crop rotation and soil tillage exert a significant influence on crop yield, weed infestation, and soil properties. Cereals cultivation in a monoculture contributes to increased weed infestation of the crop (Poggio, 2005; Woźniak and Soroka, 2015), deteriorated plant health (Hernandez-Restrepo et al., 2016), and consequently to a decreased crop yield (Nevens and Reheul, 2001; Sieling et al., 2005). The extent of yield decrease varies and depends on multiple factors including species and cultivar, weather course, fertilization, plant protection, and soil tillage (Hernanz et al., 2014). However, these factors act comprehensively, therefore have different effects on the productivity of plants (Shahzad et al., 2016). As demonstrated by De Vita et al. (2007) and by Montemurro and Maiorana (2014), in warm and arid areas higher yields of cereals are achieved in the no-till than in the conventional tillage system, whereas in moderately humid conditions better effects are obtained in the conventional than in the no-till system (Lahmar, 2010; Woźniak and Soroka, 2014).

Other outcomes of cereals cultivation in the monoculture include adverse changes in the soil including mainly loss of organic matter and organic carbon (Maillard et al., 2016), as well as suppressed enzymatic and biological activity of the soil (Kretzschmar and Monestiez, 1992; Woźniak and Kawecka-Radomska, 2016). In general, it may be

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stated that cereal monoculture leaves little diversified post-harvest residues, which leads to a reduction in the number of soil saprophytes (Olsson and Alström, 2000; Balota et al., 2004). Soil properties are also determined by its tillage systems. Many studies have demonstrated the no-till system to increase organic carbon content (West and Marland, 2002; Tabaglio et al., 2008; Ernst and Emmerling, 2009), to stabilize soil structure (Madari et al., 2005; Celik et al., 2012), to prevent soil erosion (Jordan et al., 2000), and to increase the enzymatic activity of soil and the number of earthworms in the soil (Woźniak and Kawecka-Radomska, 2016). A higher number of earthworms is indicative of the good condition of the soil environment (Lenart and Sławiński, 2010). Earthworms intensify organic matter decomposition in the soil, take part in the formation of mineral-organic colloids, regulate water-air balance in the topsoil (Laossi et al., 2010), and even indirectly alleviate the soil-borne diseases of plants induced e.g. by *Gaeumannomyces graminis* var. *tritici* (Clapperton et al., 2001).

This study aimed to evaluate the effect of crop rotation and tillage system on the yield and weed infestation of spring wheat as well as on soil properties and the number of earthworms.

Material and Methods

Location and design of the experiment

An exact field experiment was established in 2007 at the Experimental Station Uhrusk (51°18'10"N, 23°36'45"E), belonging to the University of Life Sciences in Lublin, south-eastern Poland, whereas data presented in this manuscript was collected in the years 2015–2017. The experiment was established with the method of complete sub-blocks (6×25 m) in 3 replications. The first experimental factor was the cropping system: 1) crop rotation A: pea – spring wheat – durum wheat, 2) crop rotation B: pea – spring barley – spring wheat, 3) cereal monoculture: spring barley – spring wheat – durum wheat, whereas the second one was the tillage system: a) conventional (CT), b) reduced (RT), and c) no-tillage (NT). In the CT system, a shallow ploughing (at a depth of 10-12 cm) and pre-winter ploughing (25-30 cm) were applied under all crops. In RT, both ploughing treatments were replaced by cultivation, whereas only glyphosate (4 L ha^{-1}) was applied in NT. A cultivator and a tillage set were used on all plots in the spring time.

Soil and agroclimatic conditions

The soil the experiment was established on was Rendzic Phaeozem (IUSS Working Group WRB, 2015) with the composition of sandy loam and with silty fraction content of 24.4% and dusty fraction content of 13.3%, with a slightly alkaline pH (pH_{kCL}=7.2), high contents of available forms of phosphorus (127 mg P kg^{-1} d.m.) and potassium (223 mg K kg^{-1}), and a medium content of magnesium (69 mg Mg kg^{-1}).

The annual sum of atmospheric precipitation on the study area reached 620 mm, including over 380 mm in the period since March till August (since the sowing to harvest of spring wheat). The highest average monthly sums of precipitation accounted for 71 mm in May, 73 mm in June, 84 mm in July, and 72 mm in August, whereas distribution of average daily air temperatures was as follows: 14.0 °C in May, 17.1 °C in June, 19.4 °C in July, and 18.4 °C in August.

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Fertilization and protection of plants

In all study years, spring wheat and other crops cultivated in crop rotations were sown between the 1st and 5th of April. Spring wheat of Sonett cultivar was sown at the density of 450 seeds m^{-2}. It was fertilized with nitrogen in a total dose of 140 kg N ha^{-1} applied in the following terms: 50 kg N ha^{-1} before sowing, 40 kg N ha^{-1} at the tillering stage, 30 kg N ha^{-1} at the shooting stage, and 20 kg N ha^{-1} at the earing stage. Fertilization with phosphorus and potassium was applied before wheat sowing in doses of 35 kg P ha^{-1} and 99 kg K ha^{-1}. Spring wheat was protected against fungi with the following fungicides: flusilazole + carbendazim at the 32-33 stage in Zadoks scale (Zadoks et al., 1974), and propiconazole + fenpropidin at the 53-54 stage. Weed infestation was reduced by applying a herbicide with a.i. mecoprop + MCPA + dicamba at the 23-24 stage.

Yield components and statistical analysis

The following traits were evaluated: 1) grain yield (t ha^{-1}), 2) 1000 grain weight (g), 3) spike number m^{-2} before harvest, 4) grain weight per spike (g), 5) number and air-dry weight of weeds (g m^{-2}), 6) species composition of weeds (m^{-2}), 7) organic C content in the soil (g kg^{-1}) (with Tiurin method); 8) total N content in the soil (g kg^{-1}) (with Kjeldahl method), 9) C/N ratio in soil samples, and 10) number of earthworms in the soil (m^{-2}). Grain was harvested using a Wintersteiger plot harvester, 1000 grain weight was determined by measuring out 2×500 grains, the number of spikes m^{-2} was calculated twice from the surface area of 0.5×1.0 m of each plot, whereas grain weight per spike was established based on 40 spikes collected at random. Weed infestation was evaluated with the botanical-gravimetric method at the waxy maturity stage (stage 83-84), by determining species composition of weeds and air-dry weight of weeds collected from m^2 of a plot. This area was marked at random (twice) using a frame 0.5×1.0 m in size. The air-dry weight of weeds was determined by collecting all weeds from the frame area, removing their root system, and keeping them in a well-ventilated room until constant weight has been achieved (Woźniak and Soroka, 2015). The number of earthworms m^{-2} was calculated in each study year at the end of May, by manual picking of the earthworms and calculating their number in 2 soil samples collected from the surface area of 0.25×1.0 m and depth of 0.30 m from each plot (Woźniak and Kawecka-Radomska, 2016).

Results obtained were elaborated statistically with the method of the analysis of variance (ANOVA) using Statistica PL software (StatSoft Poland). The significance of differences between mean values was evaluated with the HSD Tukey's test at $P<0.05$.

Results

Grain yield and its components

The highest grain yield was determined for spring wheat sown in crop rotation A, and a significantly lower one for wheat grown in crop rotation B (by 9.1%) and cereal monoculture (by 23%) – *Table 1*. Significant differences in grain yield occurred also between crop rotation B and cereal monoculture. Grain yield was also affected by soil tillage. Its higher value was achieved in the CT than in the NT and RT systems (by 9.8% and 19.2%, respectively). It was also demonstrated that in crop rotations A and B

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a higher yield of wheat was obtained in the CT than in the RT and NT systems, whereas in cereal monoculture – in the NT and CT compared to the RT system. The evaluation of variance analysis components indicated that grain yield was affected to a greater extent by CS than by TS (*Table 2*).

Table 1. *Effect of cropping system and tillage system on grain yield of spring wheat and its components (mean from 2015-2017)*

Cropping system (CS)	Tillage system (TS)			Mean
	[a]CT	RT	NT	
	Grain yield (t ha^{-1})			
[1]A: p – sw – dw	7.26	6.08	6.11	6.48
[2]B: p – sb – sw	6.74	5.26	5.65	5.89
[3]C: sb – sw– dw	5.23	4.17	5.56	4.99
Mean	6.41	5.17	5.78	-
$HSD_{0.05}$ for CS = 0.30; TS = 0.30; CS x TS = 0.72				
	1000 grain weight (g)			
A: p – sw – dw	49.3	46.0	46.2	47.2
B: p – sb – sw	48.3	44.6	45.5	46.2
C: sb – sw – dw	43.5	40.3	44.3	42.7
Mean	47.0	43.7	45.3	-
$HSD_{0.05}$ for CS = 1.44; TS = 1.44; CS x TS = 3.43				
	Spike number m^{-2}			
A: p – sw – dw	551	505	528	528
B: p – sb – sw	533	478	492	501
C: sb – sw – dw	476	448	455	460
Mean	520	477	492	-
$HSD_{0.05}$ for CS = 12.2; TS = 12.2; CS x TS = ns				
	Grain weight per spike (g)			
A: p – sw – dw	1.32	1.20	1.16	1.23
B: p – sb – sw	1.27	1.10	1.15	1.17
C: sb – sw – dw	1.10	0.93	1.22	1.08
Mean	1.23	1.08	1.18	-
$HSD_{0.05}$ for CR = 0.06; TS = 0.06; CR x TS = 0.15				

[1]Crop rotation A: p – sw – dw (pea – spring wheat – durum wheat), [2]Crop rotation B: p – sb – sw (pea – spring barley - spring wheat), [3]Cereal monoculture: sb – sw – dw (spring barley – spring wheat – durum wheat), [a]CT – conventional tillage, RT – reduced tillage, NT – no-tillage,
ns – not significant, $P<0.05$

Cropping and tillage systems affected the values of grain yield components. The 1000 grain weight was significantly higher in crop rotations A and B than in the cereal monoculture. More plump grain was also produced by wheat in the CT than in NT and RT systems. Significant differences in 1000 grain weight occurred also between NT and RT systems. Based on variance analysis components, it may be concluded that the 1000 grain weight depended to a greater extent on CS than on TS.

The number of spikes m^{-2} was significantly higher in crop rotation A than in crop rotation B (by 27 spikes m^{-2}) and cereal monoculture (by 68 spikes m^{-2}). Differences in spike number m^{-2} were also observed between crop rotation B and the cereal monoculture. A higher spike number m^{-2} was also demonstrated in the CT system than in the NT (by 28 spikes m^{-2}) and RT (by 43 spikes m^{-2}) systems. The value of this yield component was affect to a greater extent by CS than by TS.

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Grain weight per spike was significantly higher in crop rotations A and B than in the cereal monoculture. A higher grain weight per spike was also produced by wheat grown in the CT and NT systems compared to the RT system, however the evaluation of variance analysis components indicated that CS and TS had a similar effect upon this trait.

Table 2. *Analysis of variance for grain yield and its components*

Specification	Value	[a]CS	TS	CS x TS
Grain yield	*F*	79.56	54.25	9.76
	P	**	**	**
1000 grain weight	*F*	34.51	18.03	3.14
	P	**	**	*
Spike number m^{-2}	*F*	103.03	42.14	1.83
	P	**	**	ns
Grain weight per spike	*F*	17.46	19.32	10.29
	P	**	**	**

[a]CS – cropping system, TS – tillage system, **P*<0.05, ***P*<0.01, ns – not significant

Number, air-dry weight, and species composition of weeds

Wheat cultivation in the cereal monoculture caused an increase in weed number m^{-2} (by ca. 30%), compared to crop rotations A and B (*Table 3*). The number of weeds m^{-2} was also higher in plots cultivated in the RT system than in the CT and NT systems.

Table 3. *Effect of cropping system and tillage system on the number of weeds and air-dry weight of weeds in spring wheat crop (mean from 2015-2017)*

Cropping system (CS)	Tillage system (TS)			Mean
	[a]CT	RT	NT	
	Number of weeds m^{-2}			
[1]A: p – sw – dw	12.4	41.1	26.2	26.6
[2]B: p – sb – sw	13.9	38.6	28.1	26.9
[3]C: sb – sw– dw	31.3	52.1	30.5	38.0
Mean	19.2	43.9	28.3	-
$HSD_{0.05}$ for CS = 8.8; TS = 8.8; CS x TS = ns				
	Air-dry weight of weeds (g m^{-2})			
A: p – sw – dw	39.1	106.1	82.7	76.0
B: p – sb – sw	36.1	95.2	73.1	68.2
C: sb – sw – dw	84.6	140.8	82.4	102.6
Mean	53.3	114.0	79.4	
$HSD_{0.05}$ for CS = 22.9; TS = 22.9; CS x TS = ns				

[1]Crop rotation A: p – sw – dw (pea – spring wheat – durum wheat), [2]Crop rotation B: p – sb – sw (pea – spring barley - spring wheat), [3]Cereal monoculture: sb – sw – dw (spring barley – spring wheat – durum wheat), [a]CT – conventional tillage, RT – reduced tillage, NT – no-tillage,
ns – not significant, *P<0.05*

Likewise, the air-dry weight of weeds was higher in the cereal monoculture than in crop rotations A and B as well as in plots cultivated in the RT than in the CT and NT systems. Greater weight was also produced by weeds in the NT than in the CT system. The evaluation of variance analysis components indicated that the number and air-dry weight of weeds were to a greater extent affected by CS than by TS (*Table 4*).

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Cropping and tillage systems influenced also the species composition of weeds (*Table 5*).

Table 4. *Analysis of variance for weed infestation parameters*

Specification	Value	[a]CS	TS	CS x TS
Number of weeds	*F*	7.21	26.63	1.13
	P	**	**	ns
Air-dry weight of weeds	*F*	8.10	23.10	1.47
	P	**	**	ns

[a]CS – cropping system, TS – tillage system, *P<0.05, **P<0.01, ns – not significant

In crop rotation A, prevailing weed species included: *Papaver rhoeas* and *Avena fatua*, on crop rotation B: *Avena fatua* and *Fallopia convolvulus*, whereas in the cereal monoculture: *Avena fatua* and *Stellaria media*. In addition, a higher number of weed species occurred in crop rotations A and B than in the cereal monoculture. A greater diversity of weed species was also observed in RT plots, a lesser one in CT plots, and the least one in NT plots.

Table 5. *Dominant weed species in spring wheat per m^2*

Weed species	Tillage system (TS)		
	[a]CT	RT	NT
Crop rotation A (Pea – spring wheat – durum wheat)			
Papaver rhoeas L.	2.5	4.3	3.3
Avena fatua L.	1.4	12.1	4.6
Stellaria media (L.) Vill.	1.2	4.3	4.0
Fallopia convolvulus (L.) A. Löve	1.2	1.8	2.2
Galium aparine L.	1.1	2.2	5.4
Other species	5.0	16.4	6.7
Number of weed species	19	24	16
Crop rotation B (Pea – spring barley - spring wheat)			
Avena fatua L.	3.0	10.9	4.8
Fallopia convolvulus (L.) A. Löve	2.2	5.6	2.1
Stellaria media (L.) Vill.	2.0	5.0	5.3
Galium aparine L.	0.8	4.3	7.8
Consolida regalis Gray.	0.8	3.3	2.2
Other species	5.1	9.5	5.9
Number of weed species	18	25	14
Cereal monoculture (Spring barley – spring wheat – durum wheat)			
Stellaria media (L.) Vill.	4.5	7.1	4.8
Avena fatua L.	4.3	12.4	3.7
Galium aparine L.	3.6	4.5	8.6
Amaranthus retroflexus L.	3.0	3.3	4.3
Galeopsis tetrahit L.	1.2	4.0	3.9
Other species	14.7	20.8	5.2
Number of weed species	13	21	11

[a]CT – conventional tillage, RT – reduced tillage, NT – no-tillage

Chemical properties of soil and number of earthworms in soil

The content of organic C in soil sampled from crop rotations A and B was higher by 13.3-16.3% than in soil samples from the cereal monoculture (*Table 6*). Its content was

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also by 15.3-18.7% higher in soils from NT and RT plots, compared to soil samples from CT plots.

Table 6. *Effect of cropping system and tillage system on chemical properties of soil and number of earthworms (0-30 cm)*

Cropping system (CS)	Tillage system (TS)			Mean
	[a]CT	RT	NT	
	Organic C (g kg^{-1} d.m.)			
[1]A: p – sw – dw	7.93	9.60	9.83	9.12
[2]B: p – sb - sw	7.67	8.83	9.90	8.80
[3]C: sb – sw - dw	6.87	8.10	7.93	7.63
Mean	7.49	8.84	9.22	-
$HSD_{0.05}$ for CS = 0.90; TS = 0.90; CS x TS = ns				
	Total N (g kg^{-1} d.m.)			
A: p – sw – dw	0.74	0.87	0.90	0.84
B: p – sb - sw	0.69	0.87	0.86	0.80
C: sb – sw - dw	0.67	0.76	0.75	0.73
Mean	0.70	0.83	0.84	-
$HSD_{0.05}$ for CS = 0.06; TS = 0.06; CS x TS = ns				
	C/N ratio			
A: p – sw – dw	12.4	13.3	13.1	12.9
B: p – sb - sw	13.1	14.0	13.5	13.5
C: sb – sw - dw	16.8	17.3	19.5	17.9
Mean	14.1	14.9	15.3	-
$HSD_{0.05}$ for CS = 1.38; TS = ns; CS x TS = ns				
	Number of earthworms m^{-2}			
A: p – sw – dw	12.7	24.3	23.7	20.2
B: p – sb - sw	15.3	26.0	24.7	22.0
C: sb – sw - dw	12.0	24.0	24.3	20.1
Mean	13.3	24.8	24.2	-
$HSD_{0.05}$ for CS = ns; TS = 4.3; CS x TS = ns				

[1]Crop rotation A: p – sw – dw (pea – spring wheat – durum wheat), [2]Crop rotation B: p – sb – sw (pea – spring barley - spring wheat), [3]Cereal monoculture: sb – sw – dw (spring barley – spring wheat – durum wheat), [a]CT – conventional tillage, RT – reduced tillage, NT – no-tillage,
ns – not significant, P<0.05

Likewise, total N content was higher in the soil sampled from crop rotation A and B than from the cereal monoculture as well as from NT and RT plots compared to CT plots. In crop rotations A and B, the C/N ratio was lower than in the cereal monoculture. In turn, the C/N ratio was not affected by tillage systems, though its lower value was determined in soil samples from CT compared to NT plots. The number of earthworms in soil m^{-2} was significantly higher in RT and NT than in CT system. The evaluation of variance analysis components indicated that contents of organic C and total N and number of earthworms in the soil were affected to a greater extent by TS than by CS, whereas the value of C/N ratio – by CS (*Table 7*).

Table 7. *Analysis of variance for chemical properties of soil and number of earthworms*

Specification	Value	[a]CS	TS	CS x TS
Organic C	*F*	9.92	13.43	0.75
	P	**	**	ns
Total N	*F*	9.48	19.26	0.90
	P	**	**	ns

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C/N ratio	*F*	49.94	2.79	1.46
	P	**	ns	ns
Number of earthworms m^{-2}	*F*	2.12	6.86	1.03
	P	ns	*	ns

[a]CS – cropping system, TS – tillage system, *P<0.05; **P<0.01; ns – not significant

Discussion

The reduced grain yield in the monoculture results from increased weed infestation (Woźniak and Soroka, 2015; Shahzad et al., 2016) and from crop infestation with fungal diseases, mainly these of stalk base and roots (Hernandez-Restrepo et al., 2016). In addition, some adverse changes occur in the soil under cereal monoculture including especially decreased contents of organic matter and organic C (Maillard et al., 2016), suppressed enzymatic activity of soil (Woźniak and Kawecka-Radomska, 2016), but also a reduced number of earthworms (Kretzschmar and Monestiez, 1992; Laossi et al., 2010). It may be speculated that this is due to little diversified post-harvest residues left from this type of cultivation, which leads to reduced numbers of soil saprophytes and increased counts of soil pathogens (Olsson and Alström, 2000; Balota et al. 2004). In this context, interesting findings are provided by a work of Clapperton et al. (2001) on the role of earthworm in the indirect reduction of soil-borne diseases of plants induced by *Gaeumannomyces graminis*. In our experiment, a higher number of earthworms in soil m^{-2} was determined in RT and NT than in the CT system. Likewise research conducted by Maillard et al. (2016) and by Nevens and Reheul (2001), our study showed a decrease in contents of organic C and total N in the soil sampled from the cereal monoculture, which consequently caused a less favorable C/N ratio than in crop rotations A and B. The higher contents of organic C and total N were also found in soil samples from NT and RT plots compared to CT plots. Similarly to findings reported by Poggio (2005), Shahzad et al. (2016), and by Woźniak and Soroka (2015), wheat cultivation in the monoculture resulted in increased weed infestation of the crop, i.e. weed number increase by ca. 30% and weed weight increase by over 33%, compared to crop rotations. As a result, grain yield of spring wheat grown in the monoculture was lower than in crop rotations. The reduction in yield was due to a lower number of spikes m^{-2}, a lower grain weight per spike, and a lower 1000 grain weight than in crop rotations. Higher wheat grain yields were also obtained in the CT system than in the NT and RT systems, which also resulted from higher values of yield components in this system of soil tillage.

Conclusion

To recapitulate, cultivation of spring wheat in the cereal monoculture led to unbeneficial changes in soil properties including mainly decreased contents of organic C and total N. Spring wheat cultivation in the cereal monoculture resulted also in a significant increase in the number and air-dry weight of weeds, compared to crop rotations. Soil properties were also influenced by its tillage system. In soil sampled from RT and NT plots analyses demonstrated higher contents of organic C and total N, and the number of earthworms in soil m^{-2}, compared to CT plots. In turn, wheat cultivation in RT and NT systems caused a significant increase in the number and weight of weeds compared to the CT system. In a consequence, spring wheat grown in the cereal

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monoculture produced a significantly lower grain yield than in crop rotation A and B. Yield decrease resulted from a lower number of spikes m^{-2}, lower grain weight per spike, and a lower 1000 grain weight than in the crop rotations. The higher yield of wheat was also demonstrated in the CT than in the NT and RT systems, which was due to higher values of yield components in this system of soil tillage.

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EFFECT OF ZINC AND IRON FOLIAR APPLICATION WITH TWO LEVELS OF IRRIGATION WATER ACIDITY ON THE QUANTITATIVE AND QUALITATIVE PARAMETERS OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract. In addition to enhancing wheat's photosynthesis, growth and ability to cope with stress, the foliar application of zinc and iron can play an effective role in improving consumer nutrition. Therefore, the aim of this study was investigation of zinc and iron foliar application in irrigation water acidity on wheat (*Triticum aestivum* L.). This experiment was conducted as a triple factorial using a randomized complete blocks design with three replications in Tehran, Iran in 2014-15 and 2015-16. The treatments were two irrigation water acidity (pH value = 8.14 and 5), and the foliar application of iron and zinc sulfate (0, 2.5, and 5 g L^{-1}). The results revealed that the most grain yield was produced at a pH value = 5, with 5 g L^{-1} zinc application. The main cause for increasing the grain yield was enhancement of spike length, tillers and grains per plant. The greatest grain protein content was measured at a pH value = 5, with a 5 g L^{-1} zinc application. The maximum grain iron content was calculated at a pH value = 5, with 5 g L^{-1} zinc usage. The highest grain zinc content was obtained at a pH value = 5, with 5 g L^{-1} zinc consumption. The maximum zinc utilization efficiency was observed for the iron control treatment with 5 g L^{-1} zinc consumption. Therefore, the treatments that were irrigated with water with an acidic pH of 5 along with 5 g L^{-1} zinc and iron foliar application had the highest yield and the best qualitative traits.
Keywords: *grain yield, harvest index, micronutrient nutrition, protein percentage, utilization efficiency*

Introduction

Agricultural production in the twenty-first century has to go along and result in increased food security and it has to be less dependent on fairly rare resources such as agricultural lands, water, fossil fuels, and non-renewable energies (Uphoff, 2012; Pazouki et al., 2017). Wheat crops are grown in a wide range of environments that affect overall performance, particularly grain yield and end-use quality (Yadi et al., 2016). According to the official statistics released by FAO, the wheat cultivation area in the world during the past years has been between 230 million hectares (FAO, 2016). The last set of global statistics showed that the grain yield production was 750 million tons (FAO, 2016). Iran has 6.2 million hectares of wheat field area and 12.3 million tons of wheat production (Ministry of Jihad-e-Agriculture of Iran, 2016).

However, staple grains, such as wheat often contain suboptimal quantities of micronutrients, especially zinc and iron, and most of this content is removed by milling (Borrill et al., 2014). The World Health Organization estimates that approximately 25% of the world's population suffers from anemia (WHO, 2008); and that iron-deficiency anemia led to the loss of over 46,000 disability-adjusted life years (DALYs) in 2010 alone (Murray and Lopez, 2013). An estimated 17.3% of people worldwide are at risk of inadequate zinc intake (Wessells and Brown, 2012), and zinc-deficiency leads to the

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estimated annual deaths of 433,000 children under the age of five (WHO, 2009). By taking the geological experiments in Iran into consideration, it becomes clear that 37% of the lands under irrigated wheat cultivation have problems regarding zinc deficiency (40%), manganese deficiency (25%), and supply of copper, which can be absorbed equals 24% (Ministry of Jihad-e-Agriculture of Iran, 2016). World statistics also show that approximately 50% and 30% of the soils under grain cultivation all around the world have problems regarding the deficiency of available zinc and iron, respectively (FAO, 2016).

When nutrition is not properly absorbed in the soil, the foliar application of different elements is an efficient way for solving the problem of the deficiency of these elements (Cakmak, 2008). Based on the studies of many researchers, it was revealed that there are wide variations in grain iron and zinc contents in the wild relatives of modern wheat, and it was found that the contents can significantly exceed those found in modern elite cultivars (Cakmak et al., 2000; Monasterio and Graham, 2000). This natural variation can be utilized for wheat bio-fortification by zinc and iron (Uauy et al., 2006), which was originally identified for increasing the protein content in wild emmer (*Triticum turgidum* ssp dicoccoides). In isogenic lines, the zinc and iron content of grain increased by 12 and 18%, respectively (Distelfeld et al., 2007). Zhang et al. (2010) revealed that the iron content of grain had increased significantly from the control treatment to ferric citrate, ferric citrate plus $ZnSO_4$ and $FeSO_4$ application, respectively. Moreover, the grain zinc and the iron content were increased by using zinc and iron fertilizer resources in comparison to the control treatment. Other researchers have shown that micronutrients spraying, particularly zinc, iron, and manganese on leaves, leads to significantly increased wheat grain yield (Wissuwa et al., 2008). Mohammad et al. (2000) reported that zinc and iron application with different methods increased grain yield in comparison to control, and using zinc by the spraying method, maximum grain yield, and zinc concentration were obtained in the grain; using the soil and the spraying method recorded the highest grain yield and grain iron content. Given that micronutrients increase crop production and also influence human well-being and health, one of the simple and economical ways for achieving self-sufficiency and a healthy society is through the addition of micronutrients, especially iron and zinc, to the soil or consuming it through foliar application. In this was, in addition to enhancing the production of crops, the concentration of micronutrients in agricultural crops, including wheat, which is a central part of the food that Iranian people eat, can be increased. By taking into account issues such as the high costs of supplying nutrients from chemical resources, deficiency of nutrients in basic soils, paying attention toward finding an effective and economical method, and replacing the current methods with it with the purpose of fulfilling the needs of agricultural plants, and enriching the diets of humans and animals, seems necessary. Therefore, the aim of this study was to implement the foliar application of zinc and iron in irrigation water with an acidic and a basic pH value on the yield and the qualitative traits of wheat.

Material and Methods

Description of the site

The study was conducted in the University of Shahed, Tehran Province, Iran in 2014-15 and 2015-16. The experimental farm is geographically situated at 35°, 33' N latitude,

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and 51°, 20' E longitude. Tehran is located between mountainous region and plains. Three factors of the Alborz mountain range, the desert, and the western wet winds play an important role in the climate of Tehran. Different weather conditions have been formed in the various regions of Tehran due to its special geographical location. The climate of the Tehran Province is warm and dry in the dry area, cold and semi-humid in the mountain range, and cold with longer winters in high mountains. This experiment was carried out in a warm and dry area. Local weather data during the rice growing period were collected daily from the synoptic meteorological station nearest to the paddy fields (*Table 1*). In addition, the soil properties at a depth of 0-30 cm are detailed in *Table 2*.

Table 1. *Description of climatic parameters in the experiment region*

Month	Year	Min. temp. (°C)	Max. temp. (°C)	Evaporation (mm)	Rain (mm)	Mean humidity (%)
Sep.-Oct.	2014-15	10.2	18.1	78.2	28.9	65
	2015-16	9.6	19.3	81.6	44.6	70
Oct.-Nov.	2014-15	10.5	20.1	54.4	65.6	72
	2015-16	10	19.1	49.2	54.8	68
Nov.-Dec.	2014-15	5.4	16.3	15.1	69	62
	2015-16	2.5	10.1	13.2	57	71
Dec.-Jan.	2014-15	4.2	11.5	8.1	78	63
	2015-16	3.1	13.2	12.1	18	72
Jan.-Feb.	2014-15	1.2	8.6	14.3	48	58
	2015-16	0	11.1	25.8	36	50
March.-Apr.	2014-15	4.5	12.2	32.5	18	55
	2015-16	3.3	12	39.2	15	71
Apr.-May	2014-15	6.9	16.3	65.8	38	65
	2015-16	6	15.1	58.2	25	66
May-Jun.	2014-15	18.1	24.1	98.3	12	57
	2015-16	20.2	25.1	108.6	14	56
Jun.-Jul.	2014-15	18.1	25	125.6	8	63
	2015-16	23.1	26.3	168.6	12	65

Table 2. *Soil properties of experiment site in Tehran region*

Depth (cm)	Soil texture	EC (dS m^{-1})	CEC (%)	pH	Organic carbon (%)	Phosphorus availability (mg kg^{-1})	Potassium availability (mg kg^{-1})	Iron availability (mg kg^{-1})	Zinc availability (mg kg^{-1})
0-30	Sandy loam	3.8	11.5	7.8	0.52	13	159	2.78	4.8

Description of the experiment

The investigated wheat cultivar in this experiment was Pishtaz. This experiment was conducted as a triple factorial using a randomized complete blocks design with three replications. The treatments were as follows: two irrigation water acidity (water of the area with a pH value = 8.14 and water with a pH value = 5, which were created by sulfuric acid), and the foliar application of iron sulfate fertilizer (0, 2.5, and 5 gr L^{-1}), and zinc sulfate fertilizer (0, 2.5, and 5 gr L^{-1}).

Nitrogen, phosphorus, and potassium were added to the soil based on the soil test. In terms of fertilizer application, a basal dose of 75 kg P_2O_5 ha^{-1} was applied in the form of

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triple super phosphate. Nitrogen was applied in the form of urea (115 kg N ha^{-1}). Potassium was applied in the form of potassium sulfate (75 kg K_2O ha^{-1}). Phosphorus and potassium were applied as basal dressing. Nitrogen application was split: 20% at planting stage, 25% of top-dressed at the early tillering stage, and 25% of nitrogen was applied at the booting stage. The planting row spaces were 12×2 cm^2 at a seed rate of 250 kg per hectare. Standard agronomic practices were followed throughout the growing season. It is necessary to note that the experimental site was used for wheat and corn cultivation in the previous crop year. To control the weeds, herbicide Topic was applied at 1 litre per hectare, once on narrow leaf weeds, and simultaneously, herbicide Granestar was applied at 25 gram per hectare on wide leaf weeds. The protection operation for fighting pests and diseases was performed according to the recommendations of the region.

Evaluation traits

Quantitative parameters

During the growth period, after the removal of marginal effects in each site, traits were randomly measured according to the standard evaluation system. Thus, 10 plants were randomly selected from each experimental plot and their average was analyzed.

Data on agronomical traits such as spike length, number of tillers per plant, number of grains per plant, grain yield, and harvest index were recorded. Tillers per plant were reordered by counting the number of tillers per plant in each experimental plot. Grain yield in each experimental plot was determined at the physiologically matured stage and then converted into kg ha^{-1}.

Qualitative parameters

The dry digestion method was used for measuring the zinc and the iron in the plant samples. The dried plant samples were crushed and made into powder at 70℃ so that they would pass through a 20-mesh sieve (of 0.86 centimeter diameter). Moreover, in order to measure the protein content of the grain, the micro Kjeldahl method was used and nitrogen was measured, and by multiplying the amount of nitrogen by 5.7, protein content was calculated. The protein content per hectare was calculated from the product of the protein percentage multiplied by grain yield (Martre et al., 2003; Samonte et al., 2006). To determine the qualitative properties related to the iron and the zinc elements, the following instructions were applied (Dobermann, 2005; Fan et al., 2004).

Iron and zinc uptake by the grain was achieved by the element content in the grain multiplied by the grain yield. The iron and zinc uptake by straw (stem and leaf) was achieved by the element content in the straw (stem and leaf) multiplied by straw yield. The efficiency utilization of the elements was calculated from the ratio of grain weight to the amount of the absorbed element by the plant. This index was expressed in kilograms per kilogram. By determining the amount of iron and zinc uptake by grain and shoot, the harvest index was calculated through the amount of element absorbed by the grain to the total amount of absorbed element, which are expressed as percentages.

Statistical analysis

After normalization and the Kaiser-Meyer-Olkin (KMO) test, the data analyzed by the statistical analysis software (SAS) and the comparison of averages were calculated by least significant differences (LSD) tests at a 5% probability level.

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Results

In order to evaluate the studied traits in the experiment, the obtained data were analyzed using the variance homogeneity test by the Bartlett method. The results showed that the traits were not significant in Bartlett's test. Therefore, data analysis was performed based on compound analysis.

Quantitative parameters

Data concerning quantitative parameters is presented in *Table 3*. The combined statistical analysis of the data revealed that all the quantitative parameters (spike length, number of tillers per hill, number of grains per plant, grain yield, and harvest index) were significantly statistically ($P \leq 0.01; 0.05$) affected by water acidity (pH) and iron (Fe) foliar application; furthermore, except for the harvest index, all the quantitative parameters were significant statistically ($P \leq 0.01$) on zinc (Zn) treatment and plant height, spike length, the number of tillers and grains per plant, and grain yield were significant at the 1% probability level under the effect of the year (*Table 3*). At double interaction of year and water acidity, spike length, and the number of tillers per plant were significant statistically at the 1% and the 5% probability level.

Table 3. *Compound mean square of quantitative traits of wheat under pH of water, iron and zinc fertilizer treatment*

S.O.V.	DF	Spike length	Number of tiller per plant	Number of grain per plant	Grain yield	Harvest index
Year (Y)	1	9.94^{**}	1.52^{**}	2034.68^{**}	4039352.40^{**}	0.26^{ns}
Y (R)	4	0.86	1.22	442.61	562159.80	5.31
pH (P)	1	30.12^{**}	5.16^{**}	1885.26^{**}	11368403.56^{**}	42.10^{*}
Iron (F)	2	10.04^{**}	2.21^{**}	733.65^{**}	38575517.38^{**}	598.93^{**}
Zinc (Z)	2	2.05^{**}	0.50^{**}	193.63^{**}	2749586.78^{**}	18.45^{ns}
Y×P	1	0.68^{**}	0.04^{*}	0.02^{ns}	172688.02^{ns}	22.50^{ns}
Y×F	2	0.02^{ns}	0.005^{ns}	2.54^{ns}	302234.81^{ns}	5.06^{ns}
Y×Z	2	0.01^{ns}	0.007^{ns}	0.80^{ns}	45581.84^{ns}	2.79^{ns}
P×F	2	0.26^{**}	0.06^{**}	1.43^{ns}	4183529.95^{**}	217.80^{**}
P×Z	2	0.21^{*}	0.003^{ns}	2.72^{ns}	70537.00^{ns}	7.41^{ns}
F×Z	4	0.03^{ns}	0.01^{ns}	2.80^{ns}	16411.09^{ns}	4.15^{ns}
Y×P×F	2	0.003^{ns}	0.001^{ns}	2.59^{ns}	212483.79^{ns}	23.19^{ns}
Y×P×Z	2	0.008^{ns}	0.0004^{ns}	0.51^{ns}	15786.14^{ns}	0.01^{ns}
Y×F×Z	4	0.03^{ns}	0.001^{ns}	0.03^{ns}	4382.00^{ns}	0.66^{ns}
P×F×Z	4	0.04^{ns}	0.01^{ns}	7.32^{*}	47437.17^{ns}	12.10^{ns}
Y×P×F×Z	4	0.004^{ns}	0.0002^{ns}	0.14^{ns}	12917.94^{ns}	3.72^{ns}
Error	68	0.04	0.01	2.37	157858.10	14.91
C.V. (%)	-	3.19	3.09	2.70	8.84	9.75

ns, * and **: non significant and significant in 5 % and 1 % probability level, respectively

Moreover, spike length, the number of tillers per plant, grain yield, and harvest index were significant at the 1% probability level under the interaction of water acidity and iron (Fe). Only the spike length was significant at the 5% probability level under the interaction of water acidity and the zinc fertilizer (*Table 3*). A mean comparison showed that the at the P×F interaction, the greatest spike length was obtained at a pH value = 5 for 2.5 and 5 g L^{-1} iron (7.14 and 7.45 cm, respectively). The lowest spike length (5.35 cm) was obtained at a pH value = 8.14 and iron control treatment (*Table 4*).

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Table 4. *Double interaction pH of water and iron fertilizer application on quantitative traits of wheat*

Interaction	Spike length (cm)	Number of tiller per plant	Grain yield (kg ha^{-1})	Harvest index (%)
P_1F_1	5.35 c	2.40 c	3509 e	38.72 b
P_1F_2	6.08 ab	2.63 bc	4009 d	38.77 b
P_1F_3	6.56 b	2.82 b	4999 c	43.12 a
P_2F_1	6.58 b	2.75 b	3371 f	31.89 c
P_2F_2	7.14 a	3.08 ab	5074 b	41.24 ab
P_2F_3	7.45 a	3.32 a	6018 a	43.73 a

*: Values within a column followed by same letter are not significantly different at LSD (P≤0.05)
* P_1 and P_2 are pH of irrigation water including 8.14 and 5 respectively
F_1, F_2 and F_3 are iron fertilizer including 0, 2.5 and 5 g L^{-1} respectively

With attention to the interaction of water acidity and zinc fertilizer, it was found that the maximum spike length was produced at a pH value = 5 with 2.5 and 5 g L^{-1} zinc application. The minimum spike length had been observed at a pH value = 8.14 with control treatment and 2 g L^{-1} Zn usage (*Table 5*).

Table 5. *Double interaction of pH of water and zinc fertilizer on qualitative and quantitative parameters of wheat*

Interaction	Spike length (cm)	Grain protein content (%)	Protein yield (kg ha^{-1})	Grain zinc uptake (kg ha^{-1})
P_1Z_1	5.71 c	8.49 c	335.35 e	38.58 d
P_1Z_2	5.96 c	8.86 bc	370.88 d	82.21 c
P_1Z_3	6.33 abc	9.24 abc	408.24 cd	113.49 bc
P_2Z_1	6.89 ab	10.43 ab	473.50 c	61.35 cd
P_2Z_2	7.06 a	11.06 a	538.28 ab	135.30 b
P_2Z_3	7.22 a	11.50 a	596.10 a	190.94 a

*: Values within a column followed by same letter are not significantly different at LSD (P≤0.05)
* P_1 and P_2 are pH of irrigation water including 8.14 and 5 respectively
Z_1, Z_2 and Z_3 are zinc fertilizer including 0, 2.5 and 5 g L^{-1} respectively

With regard to the mean comparison results, with 5 g L^{-1} zinc fertilizer application, the number of tillers per plant was increased compared to 0 and 2.5 g L^{-1} zinc application (*Table 6*). In terms of the double interaction of the year and the water acidity, it was found that the maximum number of tillers per plant (3.19 tillers) was obtained in the first year for a pH value = 5. The minimum number of tillers per plant (2.51 tillers) was obtained in the second year for a pH value = 8.14 (*Table 7*). Moreover, at the double interaction of water acidity and the iron (Fe) fertilizer, the most tillers per plant (3.32 tillers) was obtained for a pH value = 5, with 5 m L^{-1} iron application. The minimum tillers per plant (2.40 tillers) was produced at a pH value = 8.14 and a control treatment (*Table 4*). At the triple interaction of water acidity, and iron (Fe) and zinc (Zn) application, the highest number of grains per plant was observed for $P_2F_2Z_3$ (64.68 grains) and at a pH value = 5, with 5 g L^{-1} of iron consumption, and all three zinc fertilizer levels equaling 63.51, 65.37, and 67.56 grains, respectively (*Table 8*).

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Table 6. *Mean comparison quantitative traits of wheat under pH of water, iron and zinc fertilizer treatment*

Treatment	Spike length (cm)	Number of tiller per plant	Number of grain per plant	Grain yield (kg ha^{-1})	Harvest index (%)
Year					
2014-15	6.83 a	2.95 a	61.36 a	4690 a	39.63 a
2015-16	6.23 b	2.71 b	52.68 b	4303 b	39.53 a
LSD 0.05	0.08	0.03	0.59	152.58	1.48
pH					
8.14	6.00 b	2.61 b	52.84 b	4172 b	40.20 a
5	7.06 a	3.05 a	61.19 a	4821 a	38.96 a
LSD 0.05	0.08	0.03	0.59	152.58	1.48
Fe					
0	5.96 c	2.58 c	52.28 c	3440 c	35.30 c
2.5 g L^{-1}	6.61 b	2.86 b	57.50 b	4542 b	40.01 b
5 g L^{-1}	7.01 a	3.07 a	61.27 a	5508 a	43.43 a
LSD 0.05	0.10	0.04	0.72	186.87	1.82
Zn					
0	6.30 c	2.71 c	54.49 c	4220 c	38.80 a
2.5 g L^{-1}	6.51 b	2.86 b	57.52 b	4497 b	39.74 a
5 g L^{-1}	6.78 a	2.94 a	59.04 a	4772 a	40.20 a
LSD 0.05	0.10	0.04	0.72	186.87	1.82

*: Values within a column followed by same letter are not significantly different at LSD (P≤0.05)

Table 7. *Double interaction of year and pH of water on qualitative and quantitative parameters of wheat*

Interaction	Spike length (cm)	Number of tiller per plant	Iron utilization efficiency (kg kg^{-1})	Iron harvest index (%)	Straw zinc content (%)	Zinc harvest index (%)
Y_1P_1	6.38 ab	2.71 ab	18.91 b	61.71 a	14.27 ab	56.06 a
Y_1P_2	7.28 a	3.19 a	10.21 c	63.69 a	18.12 a	58.36 a
Y_2P_1	5.62 b	2.51 b	23.12 a	62.47 a	11.69 b	57.39 a
Y_2P_2	6.83 a	2.91 ab	10.32 c	62.45 a	18.18 a	55.64 a

*: Values within a column followed by same letter are not significantly different at LSD (P≤0.05)
Y_1 and Y_2 are experiment period including 2014-15 and 2015-16 years respectively
* P_1 and P_2 are pH of irrigation water including 8.14 and 5 respectively

The mean comparison shows that with 5 g L^{-1} zinc foliar application, the grain yield was enhanced to 13.08 and 6.12% in comparison to 0 and 2.5 g L^{-1}, respectively. The main cause for increasing the grain yield in the 5 g L^{-1} zinc foliar application involved the enhancement of spike length, and the number of tillers and grains per plant in this treatment (*Table 6*). At the double interaction of water acidity and iron consumption, it was found that the most grain yield (6,018 kg ha^{-1}) was produced at a pH value = 5, with 5 g L^{-1} zinc foliar application. The least grain yield (3,371 kg ha^{-1}) was obtained at a pH value = 5 and control treatment (*Table 4*). Moreover, at water acidity and iron interaction, the highest harvest index was observed at both pH levels, with 5 g L^{-1} iron foliar application (43.12% and 43.73%, respectively). The least harvest index (31.89%) was calculated at pH value =5 and the control iron treatment (*Table 4*).

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Table 8. *Triple interaction pH of water, iron and zinc fertilizers on grain per plant and iron harvest index of wheat*

Interaction	Number of grain per plant	Iron harvest index (%)
$P_1F_1Z_1$	46.27 c	57.82 b
$P_1F_1Z_2$	48.75 bc	54.12 bc
$P_1F_1Z_3$	49.92 bc	52.23 c
$P_1F_2Z_1$	51.63 abc	63.80 ab
$P_1F_2Z_2$	52.81 abc	64.69 ab
$P_1F_2Z_3$	54.97 abc	62.77 ab
$P_1F_3Z_1$	53.57 abc	67.54 a
$P_1F_3Z_2$	58.87 ab	66.57 a
$P_1F_3Z_3$	58.75 ab	69.24 a
$P_2F_1Z_1$	52.95 abc	62.22 ab
$P_2F_1Z_2$	57.43 ab	62.07 ab
$P_2F_1Z_3$	58.36 ab	61.59 ab
$P_2F_2Z_1$	58.98 ab	62.86 ab
$P_2F_2Z_2$	61.91 ab	61.96 ab
$P_2F_2Z_3$	64.68 a	62.14 ab
$P_2F_3Z_1$	63.51 a	64.43 ab
$P_2F_3Z_2$	65.37 a	65.46 ab
$P_2F_3Z_3$	67.56 a	64.90 ab

*: Values within a column followed by same letter are not significantly different at LSD ($P \leq 0.05$)
* P_1 and P_2 are pH of irrigation water including 8.14 and 5 respectively
F_1, F_2 and F_3 are iron fertilizer including 0, 2.5 and 5 g L^{-1} respectively
Z_1, Z_2 and Z_3 are zinc fertilizer including 0, 2.5 and 5 g L^{-1} respectively

Qualitative parameters

The combined variance analysis of the qualitative parameters indicated that all qualitative parameters except the zinc harvest index showed a significant difference under the effect of water acidity (pH value) and iron (Fe) for foliar application in 1% and 5% probability levels, respectively (*Table 9*). Moreover, some qualitative parameters including grain protein content, protein yield, grain iron uptake, grain and straw zinc uptake, zinc utilization efficiency, and zinc harvest index had significant differences ($P \leq 0.01$) under the effect of zinc (Zn) foliar application. In addition, all the qualitative parameters, except iron harvest index, zinc utilization efficiency, and zinc harvest index, were significant at the 1% probability level on year effect (*Table 9*). At the double interaction of year and water acidity straw iron content, the iron utilization efficiency and the harvest index, and the straw zinc content and the zinc harvest index were statistically significant. Moreover, at year and iron double interaction, protein yield, grain iron uptake, and grain zinc uptake were significant statistically (*Table 9*). At the interaction of year and zinc foliar application, only the grain zinc uptake was significant statistically with a 1% probability level. In addition, at the double interaction of water acidity and iron fertilizer grain protein content, the protein yield, grain iron uptake, iron utilization efficiency, iron harvest index, grain zinc uptake, zinc utilization efficiency, and zinc harvest index were significant statistically at a 1% probability level (*Table 9*). At the double interaction of water acidity and zinc foliar application, it was found that the grain protein content, protein yield, and the grain zinc uptake were significant statistically. In iron and zinc double interaction, certain parameters, including iron harvest index, grain zinc uptake, zinc utilization efficiency, and zinc harvest index showed significant differences in the 1% and the 5% probability levels.

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Table 9. *Compound mean square of quantitative traits of wheat under pH of water, iron and zinc fertilizers treatment*

S.O.V.	DF	Grain protein content	Protein yield	Grain iron uptake	Iron utilization efficiency	Iron harvest index	Grain zinc uptake	Zinc utilization efficiency	Zinc harvest index
Year (Y)	1	32.17**	201462.26**	29589.27**	126.29**	1.56^{ns}	12490.15**	6.00^{ns}	13.14^{ns}
Y (R)	4	1.58	5671.68	2733.65	31.63	43.53	215.20	28.14	38.98
pH (P)	1	122.75**	730361.23**	460832.06**	3119.76**	26.13*	70506.26**	451.19**	2.00^{ns}
Iron (F)	2	2.69**	511619.03**	356838.14**	505.02**	584.20**	56869.68**	58.33**	13.15^{ns}
Zinc (Z)	2	7.41**	86012.68**	4071.10**	0.13^{ns}	8.69^{ns}	94798.10**	1365.46**	1441.10**
Y×P	1	0.09^{ns}	160.90^{ns}	172.02^{ns}	113.70**	26.88*	20.45^{ns}	13.58^{ns}	110.11**
Y×F	2	0.01^{ns}	7190.24*	3939.56**	11.69^{ns}	1.61^{ns}	1098.05**	1.13^{ns}	5.04^{ns}
Y×Z	2	0.03^{ns}	2134.89^{ns}	117.99^{ns}	0.52^{ns}	1.97^{ns}	889.08**	0.37^{ns}	2.07^{ns}
P×F	2	2.35**	1011447.26**	14980.85**	410.31**	268.31**	6076.16**	44.64**	71.17**
P×Z	2	0.25*	5619.06*	374.11^{ns}	4.26^{ns}	5.10^{ns}	6754.66**	12.14^{ns}	12.86^{ns}
F×Z	4	0.02^{ns}	986.85^{ns}	349.88^{ns}	2.01^{ns}	14.25*	4968.46**	100.69**	51.64**
Y×P×F	2	0.0003^{ns}	915.96^{ns}	533.38^{ns}	16.05*	0.67^{ns}	11.32^{ns}	6.29^{ns}	0.06^{ns}
Y×P×Z	2	0.005^{ns}	424.69^{ns}	96.99^{ns}	0.62^{ns}	1.23^{ns}	0.50^{ns}	0.97^{ns}	3.44^{ns}
Y×F×Z	4	0.003^{ns}	59.92^{ns}	41.19^{ns}	0.25^{ns}	3.51^{ns}	23.40^{ns}	0.65^{ns}	2.06^{ns}
P×F×Z	4	0.022^{ns}	102.61^{ns}	237.49^{ns}	2.60^{ns}	13.19*	324.23^{ns}	4.82^{ns}	21.88^{ns}
Y×P×F×Z	4	0.01^{ns}	181.86^{ns}	25.18^{ns}	0.51^{ns}	3.75^{ns}	9.71^{ns}	0.98^{ns}	3.76^{ns}
Error	68	0.06	1562.64	321.39	5.66	5.58	174.34	7.57	11.47
C.V. (%)	-	2.39	8.71	9.41	15.21	3.77	12.74	7.71	5.96

ns, * and **: non significant and significant in 5 % and 1 % probability level, respectively

Table 10. *Double interaction of pH of water and iron fertilizer on quantitative parameters of wheat*

Interaction	Grain protein content (%)	Protein yield (kg ha^{-1})	Grain iron uptake (kg.ha^{-1})	Iron utilization efficiency (kg kg^{-1})	Iron harvest index (%)	Grain zinc uptake (kg ha^{-1})	Zinc utilization efficiency (kg kg^{-1})	Zinc harvest index (%)
P_1F_1	8.88 b	311.43 e	53.77 e	28.74 a	54.72 b	52.71 e	37.76 a	58.35 a
P_1F_2	8.81 b	355.60 d	109.96 d	19.47 b	63.75 ab	73.60 d	37.18 a	55.89 b
P_1F_3	8.90 b	447.44 c	211.59 bc	14.83 c	67.78 a	107.97 c	38.23 a	55.93 b
P_2F_1	10.54 ab	357.33 d	140.45 c	10.39 cd	61.96 ab	74.15 d	31.13 b	55.66 b
P_2F_2	10.87 ab	552.38 b	247.93 b	11.02 cd	62.32 ab	135.60 b	34.03 ab	56.50 ab
P_2F_3	1.57 a	698.17 a	378.87 a	9.38 d	64.93 ab	177.84 a	35.74 ab	58.83 a

*: Values within a column followed by same letter are not significantly different at LSD (P≤0.05)
* P_1 and P_2 are pH of irrigation water including 8.14 and 5 respectively
F_1, F_2 and F_3 are iron fertilizer including 0, 2.5 and 5 g L^{-1} respectively

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Table 11. *Mean comparison of quantitative parameters of wheat under pH of water, iron and zinc fertilizers treatment*

Treatment	Grain protein content (%)	Protein yield (kg ha^{-1})	Grain iron uptake (kg ha^{-1})	Iron utilization efficiency (kg kg^{-1})	Iron harvest index (%)	Grain zinc uptake (kg ha^{-1})	Zinc utilization efficiency (kg kg^{-1})	Zinc harvest index (%)
Year								
2014-15	10.47 a	496.92 a	206.98 a	14.56 b	62.70 a	114.40 a	35.44 a	57.21 a
2015-16	9.38 b	410.53 b	173.88 b	16.72 a	62.46 a	92.89 b	35.92 a	56.51 a
LSD 0.05	0.09	15.18	6.88	0.91	0.91	5.07	1.06	1.30
pH								
8.14	8.86 b	371.49 b	125.11 b	21.01 a	62.09 b	78.10 b	37.72 a	56.72 a
5	10.99 a	535.96 a	255.75 a	10.26 b	63.07 a	129.20 a	33.64 b	57.00 a
LSD 0.05	0.09	18.18	6.88	0.91	0.91	5.07	1.06	1.30
Fe								
0	19.71 c	334.38 c	97.11 c	12.11 c	58.34 c	63.43 c	34.45 b	57.01 a
2.5 g L^{-1}	9.84 b	453.99 b	178.95 b	15.25 b	63.04 b	104.60 b	35.61 b	56.20 a
5 g L^{-1}	10.24 a	572.80 a	295.23 a	19.57 a	66.36 a	142.91 a	36.99 a	57.38 a
LSD 0.05	0.11	18.59	8.43	1.12	1.11	6.21	1.29	1.59
Zn								
0	9.46 c	404.42 c	180.01 c	15.62 a	63.11 a	49.97 c	42.53 a	49.65 c
2.5 g L^{-1}	9.96 b	454.58 b	190.01 b	15.71 a	62.48 a	108.75 b	33.92 b	59.45 b
5 g L^{-1}	10.37 a	502.17 a	201.27 a	15.60 a	62.15 a	152.22 a	30.59 c	61.48 a
LSD 0.05	0.11	18.59	8.43	1.12	1.11	6.21	1.29	1.59

*: Values within a column followed by same letter are not significantly different at LSD (P≤0.05)

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At the triple interactions of year, water acidity, and iron consumption, only the iron utilization efficiency demonstrated a significant difference of 5% in the probability level. Moreover, in the triple interaction of water acidity, iron, and zinc foliar application, it was found that the iron harvest index and the straw zinc content statistically revealed significant differences at 1% and 5% probability levels (*Table 9*).

At the double interaction of water acidity and the iron foliar application, the greatest grain protein content (11.57%) was observed at a pH value = 5, with a 5 g L^{-1} zinc foliar application. The least grain protein content (8.88, 8.81, and 8.90%) was observed at a pH value = 8.14 in the all the three zinc fertilizer levels (*Table 10*). Moreover, the double interaction of water acidity and zinc foliar application revealed that the maximum grain protein contents (11.06 and 11.50%) were obtained at a pH value = 5, with 2.5 and 5 g L^{-1} zinc foliar applications. The minimum grain protein content (8.49%) was observed at a pH value = 8.14 and control treatment (*Table 5*). At the double interaction of the year and the iron foliar application, the highest protein yield (629.69 kg ha^{-1}) was produced in the first year for 5 g L^{-1} iron consumption. The least protein yield (305.72 kg ha^{-1}) was obtained in the second year for control treatment (*Table 10*). Moreover, at the double interaction of water acidity and iron foliar application, the greatest protein yield (698.17 kg ha^{-1}) was produced for a pH value = 5 and 5 gL^{-1} iron consumption. The least protein yield (335.35 kg ha^{-1}) was observed at a pH value = 8.14 and control treatment (*Table 5*).

The mean comparison of the treatments showed that the grain iron uptake was increased in 5 g L^{-1} zinc foliar application compared to other levels (*Table 11*). At the double interaction of the year and the iron foliar application, the most grain iron uptake was obtained in the first year for 5 g L^{-1} iron consumption (323.45 kg ha^{-1}). The least grain iron uptake (105.11 kg ha^{-1}) was recorded in the second year for the control treatment (*Table 12*). In addition, at the double interaction of water acidity and iron foliar application, the highest grain iron uptake (378.87 kg ha^{-1}) was observed at a pH value = 5, with a 5 g L^{-1} iron foliar application, but the least grain iron uptake (335.35 kg ha^{-1}) was observed at a pH value = 8.14 and control treatment (*Table 5*).

Table 12. *Double interaction of year and iron fertilizer on qualitative traits of wheat*

Interaction	Protein yield (kg ha^{-1})	Grain iron content (%)	Grain iron uptake (kg ha^{-1})	Grain zinc uptake (kg ha^{-1})
Y_1F_1	363.04 bc	29.90 c	105.11 d	68.60 c
Y_1F_2	498.02 ab	39.56 b	192.38 bc	115.48 b
Y_1F_3	629.69 a	55.20 a	323.45 a	159.12 a
Y_2F_1	305.72 c	27.14 c	89.11 e	58.26 d
Y_2F_2	409.96 b	36.48 b	165.51 c	93.72 bc
Y_2F_3	515.92 ab	49.82 ab	267.01 b	126.69 ab

*: Values within a column followed by same letter are not significantly different at LSD ($P \leq 0.05$)
Y_1 and Y_2 are experiment period including 2014-15 and 2015-16 years respectively
F_1, F_2 and F_3 are iron fertilizer including 0, 2.5 and 5 g L^{-1} respectively

Moreover, at the triple interaction of the year, water acidity, and the iron foliar application, the greatest iron utilization efficiency was produced in both years for a pH value = 8.14 and control treatment. The least iron utilization efficiency was, however, calculated in both years for a pH value = 5, with 5 g L^{-1} iron foliar application (*Figure 1*). In addition, at a triple interaction of water acidity, iron, and zinc foliar application,

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the largest iron harvest index was recorded at a pH value = 8.14, with 5 g L^{-1} iron foliar application, and all the three zinc consumption levels (67.54, 66.57, and 69.24%) respectively. The least iron harvest index (52.23%) was obtained at a pH value = 8.14 and iron control treatment with 5 g L^{-1} zinc foliar application (*Table 8*).

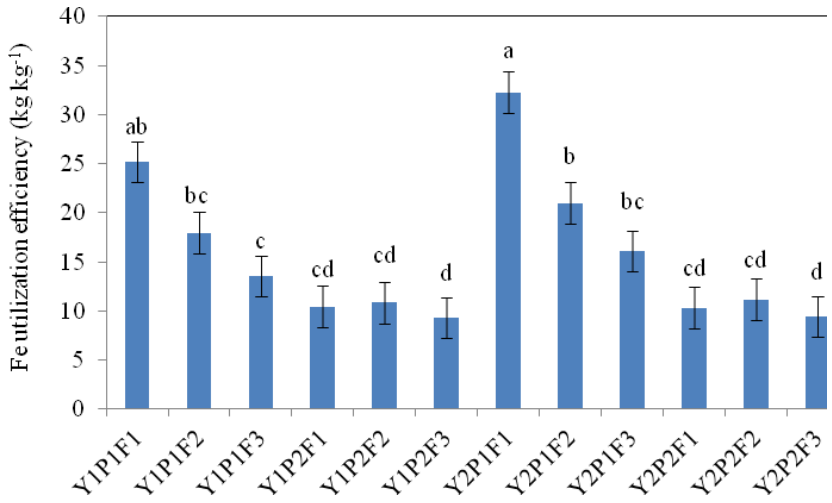


Figure 1. *Triple interaction of year, pH of water and iron fertilizer on iron utilization efficiency*
Y_1 and Y_2 are experiment period including 2014-15 and 2015-16 years respectively
** P_1 and P_2 are pH of irrigation water including 8.14 and 5 respectively*
F_1, F_2 and F_3 are iron fertilizer including 0, 2.5 and 5 g L^{-1} respectively

According to the double interaction of year and water acidity, the highest grain zinc uptake (159.12 kg ha^{-1}) was recorded in the first year with 5 g L^{-1} iron consumption, but the least grain zinc uptake (68.60 kg ha^{-1}) was obtained in the first year for control treatment (*Table 12*). At the double interaction of water acidity and iron consumption, the greatest grain zinc uptake (177.84 kg ha^{-1}) was recorded at pH value = 5, with 5 g L^{-1} iron usage, but the least grain zinc uptake was observed at pH value = 8.14 for the control treatment (*Table 10*). Moreover, at the double interaction of water acidity and the zinc foliar application, the highest grain zinc uptake (190.94 kg ha^{-1}) was calculated at pH value = 5, with 5 g L^{-1} zinc consumption; but the least grain zinc uptake was recorded at pH value = 8.14 for the control treatment (*Table 5*). In addition, at the double interaction of the iron and zinc foliar application, the maximum grain zinc uptake (208.23 kg ha^{-1}) was produced for 5 g L^{-1} iron and zinc consumption, but the least grain zinc uptake (32.16 kg ha^{-1}) was observed for the control treatment (*Table 13*). According to the double interaction of water acidity and the iron foliar application, the greatest zinc utilization efficiency was calculated at a pH value = 8.14 for all the three iron consumption levels equaling 37.76, 37.18, and 38.23 kg ka^{-1}, respectively. The least zinc utilization efficiency (31.13 kg kg^{-1}) was observed at pH value = 5 for the control treatment (*Table 10*). At the double interaction of iron and zinc foliar application, the maximum zinc utilization efficiency (47.11 kg kg^{-1}) was obtained for the iron control treatment with 5 g L^{-1} zinc consumption. The least zinc utilization efficiency (30.55 kg kg^{-1}) was obtained for 2.5 g L^{-1} iron usage with 5 g L^{-1} zinc consumption, and 5 g L^{-1} iron and zinc consumption (*Table 13*).

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Table 13. *Double interaction of iron and zinc fertilizers on quantitative parameters of wheat*

Interaction	Iron harvest index (%)	Grain zinc uptake (kg ha^{-1})	Zinc utilization efficiency (kg kg^{-1})	Zinc harvest index (%)
F_1Z_1	60.01 ab	32.16 e	38.01 b	48.13 c
F_1Z_2	58.09 ab	64.40 cd	33.79 c	59.36 ab
F_1Z_3	56.91 b	93.73 c	31.54 cd	63.54 a
F_2Z_1	63.33 a	50.63 d	42.46 ab	48.25 bc
F_2Z_2	63.33 a	108.47 bc	33.81 cd	59.49 ab
F_2Z_3	62.46 a	154.70 b	30.55 d	60.84 ab
F_3Z_1	65.99 a	67.10 cd	47.11 a	52.57 b
F_3Z_2	66.01 a	153.38 b	34.16 c	59.50 ab
F_3Z_3	67.07 a	208.23 a	29.69 d	60.07 ab

*: Values within a column followed by same letter are not significantly different at LSD (P≤0.05)
F_1, F_2 and F_3 are iron fertilizer including 0, 2.5 and 5 g L^{-1} respectively
Z_1, Z_2 and Z_3 are zinc fertilizer including 0, 2.5 and 5 g L^{-1} respectively

According to the double interaction of year and water acidity, the zinc harvest index in the first year for both the pH levels were 56.06 and 58.36%, and in the second year, both the pH levels were 57.39 and 55.64% (*Table 7*). At the double interaction of water acidity and iron foliar application, the highest zinc harvest index was recorded at the pH value = 8.14 for the control treatment (58.35%) and pH value = 5, with 5 g L^{-1} iron consumption. The least zinc harvest index was calculated at a pH value = 8.14 for 2.5 and 5 g L^{-1} (55.89 and 55.93%), and pH value = 5 and control treatment (55.56%), respectively (*Table 10*). Moreover, at the double interaction of iron and zinc foliar application, the greatest zinc harvest index (63.54%) was observed in the iron control treatment with 5 g L^{-1} zinc consumption. The minimum zinc harvest index (48.13%) was recorded in the control treatment (*Table 13*). Furthermore, at double interaction of year and zinc foliar application, grain zinc uptake in both years was increased with 2.5 and 5 g L^{-1} zinc consumption compared to control treatment that the highest amount was observed with 5 g L^{-1} zinc consumption (*Figure 2*).

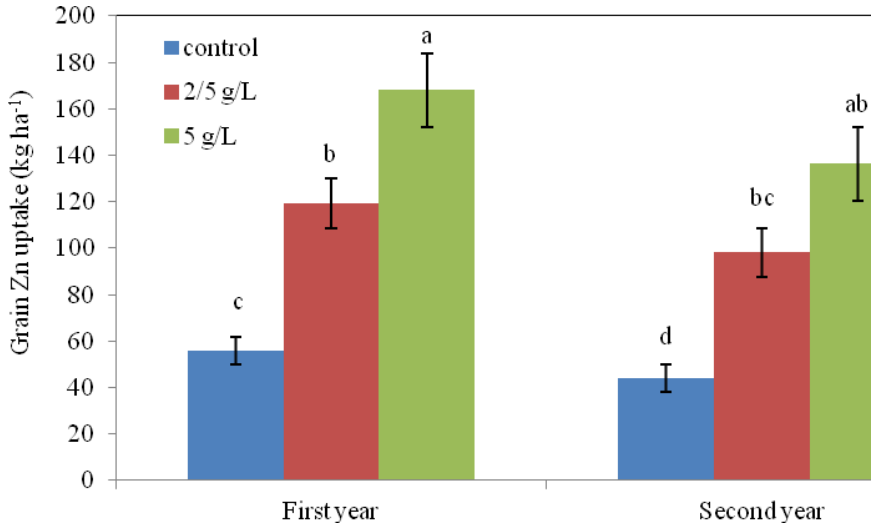


Figure 2. *Double interaction of year and zinc fertilizer on grain zinc uptake*

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Discussion

Low soil reaction is associated with a number of adverse chemical and biological properties that cause problems for plant growth. Chemical pH-dependent changes limit the accessibility of essential elements and increase the availability of toxic metals, such as aluminum and manganese (Holier and Rutherglen, 2005). Zinc seems to increase plant height by increasing the CO_2 assimilation, increasing photosynthesis (Thalooth et al., 2006), and increasing the water use efficiency (Khan, 2004). Mohammad et al. (2000) reported that zinc and iron application with different methods increased grain yield in comparison to control, and using zinc by the spraying method, maximum grain yield, and zinc concentration were obtained in the grain; using the soil and the spraying method recorded the highest grain yield and grain iron content. The presence of a factor, such as acidity, which restricts access to nutrients in the soil, reduces the expected use of fertilizers. Under these conditions, the nutrient elements for plants can be provided by the use of a leaf (Altındişli et al., 2004). Foliar application is the spraying of nutrients on leaves and stems, and their absorption from these places (Kapoor et al., 2003). Leaf consumption can guarantee plant access to nutrients to achieve high yields. Ecologically, foliar fertilization is more acceptable because lower quantities of nutrients are provided for quick consumption by the plant (Stampar et al., 2008).

There is a negative interaction between iron intake and zinc concentration in the grain. When the amount of zinc absorbed by the soil is low, it is possible that the iron application intensifies the zinc deficiency. The absorption of elements such as iron, zinc, magnesium, phosphorus, and molybdenum depends on the acidity of the soil. In addition to the above elements, the nitrogen uptake by the root also depends on the acidity of the soil; thus nitrogen uptake in alkaline soils has a higher limitation than in acidic soils (Nyachiro and Briggs, 1994). Iran's irrigated soils have severe micronutrient deficiencies, especially zinc and iron, for reasons such as calcareous soils, un-carbonated irrigation water, low organic matter, and excessive consumption of phosphate fertilizers. Sadana et al. (2006) concluded that iron soil and foliar application increased the growth and the yield of wheat compared to control treatment, and the amount of iron in grains and straw increased. According to some researchers, zinc foliar application increases photosynthesis, promotes plant growth, improves nitrogen uptake, grain protein and grain yield, and zinc deficiency leads to poor grain formation (Thalooth et al., 2006). In other research for rice plant, the most zinc content in grain and straw were produced with 40 kg zinc per hectare (Yadi and Dastan, 2012). The reduction of wheat yield in alkaline acidity has been attributed to many factors. In addition, in alkaline acidity, the plant's access to nitrogen compounds is very low; reducing the amount of nitrogen utilized by soil is the main factor for reducing the grain yield. The acidity of alkali causes changes in the permeability of the membrane of the rooted cells and prevents the entry of elements such as iron and zinc into the plant (Nyachiro and Briggs, 1994).

Conclusion

The application of micronutrient fertilizers affected the yield and the absorption of iron and zinc on wheat and increased the net production. An increase in acidity leads to the precipitation of nutrients (reduction of solubility) and a decrease in the soil's ability to absorb the elements; this is why the plant consumes more energy and decreases the pH of the environment surrounding the root of the plant so that it would be able to

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absorb the necessary nutrients. Therefore, the energy that is consumed for growth and production under normal conditions is consumed for absorbing the necessary nutrients at higher acidities. On the other hand, a severe reduction of acidity is very harmful because as the solubility of the elements increase, the plant becomes more toxic, which is in itself a limiting factor and the plant copes with the oxidative stress by consuming energy. Therefore, this research should be conducted in the other region to determine the best finding for extensions.

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EFFECTS OF IRRIGATION AND NITROGEN ON YIELD AND WATER PRODUCTIVITY IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) AND COWPEA (*VIGNA UNGUICULATA* L.) IN NORTH OF IRAN

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Abstract. Water is known as a critical and rare source that humans and ecological systems depend on. With growing population and developing technologies in many countries, availability to this critical source has been decreased. This research was conducted in a split plot experiment in a randomized complete block design with 3 replications in Iran (the north of Iran, Astaneh-ye-Ashrafiyeh). The main treatments were irrigation with 40, 60, 80 and 100% water requirement using water balance and direct method and the second treatments were 0 and 30, 60 and 90 kg N ha^{-1}, and two bean cultivars were tested (local Dehsari and Cowpea). Seed yield in 2016 and 2017, in the case of irrigation with100% water requirement and 60 kg N ha^{-1} was higher in common bean cultivar than in Cowpea and yields were 3486 and 3646 kg ha^{-1}, respectively. The highest water use productivity in seed yield in 2016 and 2017 was in common bean cultivar, and in the case of irrigation with 100% water requirement and 60 kg N ha^{-1} fertilizer added, levels were 0.70 and 0.69 kg m^{-3}, respectively.
Keywords: *fertilizer, plant production, water requirements, water productivity, yield component*

Introduction

Agricultural sections, known as the largest consumers of water, create many challenges in producing foods with less consumed water. In recent years, competitions for getting more water sources among industry, agriculture and urban sections have increased, therefore, the optimum water consumption is a priority for the agricultural industry. Legumes, after cereals, are the second main food. The protein content in legumes is two or three times higher than in cereals and Iran is the second largest producer of legumes. Cowpeas are summer crops which grow in African tropical and subtropical regions (Jean-Martial et al., 2013). Cowpea is cultivated in north of Mexico and south-west of America and since they are resistant to high temperature, they have known as an important crop for hot regions (Jean-Martial et al., 2013; Singh, 2004; Hall et al., 2003; Clavel et al., 2005; Shardendu et al., 2011). Common bean contains 20-25% protein and 50-56% carbohydrate, and comparing to cereal and starch crops, it contains 2-3 and 10-20 times more protein, respectively (Carmona-Garcia et al., 2007). Water is a critical parameter which influences these crops significantly (Kotchoni and Bartels, 2003). Drought stress is known as one of the main reasons for crop loss which leads to 50% decrease in efficiency (Singh, 2007). Water shortage stress resulted in the

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decrease of growth of the crops and decreased the efficiency (Korir et al., 2006). About 60% of beans experienced alternative droughts and/or droughts for the end of the season (Beebe et al., 2008). The vegetative growth period of crops is an important and long period in crop cultivation (Baker and Rosenqvist, 2004). The decrease of this period in beans resulted in significant changes in growth and biological functions (Baker and Rosenqvist, 2004). Nitrogen is a critical element and is involved in producing the chlorophyll molecule that plays an important role in photosynthesis (Fageria and Santos, 2008). One-third of the world's most cultivated lands faces a shortage of water for agriculture, which is characterized by climate change and the increasing population will make this problem more serious in the future. Understanding the detrimental effects of drought on plant processes and mechanisms of drought tolerance in crop species, particularly those adapted to dry conditions will help to improve their agronomic performance by incorporating the superior traits into new species or cultivars (Clavel et al., 2005; Shardendu et al., 2011). Therefore, identification of plants resistant to dryness or dehydrated condition with proper performance and their resistance mechanisms is one of the most important solutions to combat drought stress. Due to limited water resources and low rainfall in the Country, the prospect is uncertain for agricultural production. According to forecasts, with the warming of the Earth's crust in the Future, the water requirement of plants will increase and the use of water resources will be very limited. Increasing competition for obtaining water and supplying water needs for growth of products should be done with regular irrigation to prevent irrigation from drought and flooding, and provide a basis for optimizing water use (Aujla et al., 2005).

Water productivity is defined as the ratio of dry matter production to water use (Anyia and Herzog, 2004). water productivity might not provide much information about the competitive or yield advantage of one particular species over another because improved water productivity may actually restrict growth. However, it is one trait that has been studied a great deal because it can give an idea of the variation amongst genotypes in ability where water is limited. Nitrogen plays a critical role in synthesis of proteins and nucleic acids and the shortage of nitrogen is easily seen in old leaves (Fageria and Santos, 2008). This research aims to evaluate the effect of nitrogen and water requirement on bean crops in the region of the study.

Materials and methods

This study was conducted in north of Iran (Astaneh-ye-Ashrafiyeh) located in latitude 37 and 29 min and longitude 49 and 95 53 min, 3 m below sea level on average in 2016 and 2017. In 2016 and 2017 rainfall was 65.7 and 76 mm, respectively. The meteorology and soil physicochemical traits are shown in *Table 1* and *2*. This research was implemented in split plot and random complete block in Astaneh-ye-Ashrafiyeh and all experiments were repeated 3 times. The main variables were irrigation with 40, 60, 80 and 100% water requirement, 0, 30, 60 and 90 kg nitrogen added per hectare and two bean varieties were tested (local Dehsari and Cowpea). In a plot, two rows were omitted and 12 crops were chosen randomly to evaluate the seed yield. The beans were removed and dried in oven with 70 °C for 48 h. After drying, the samples were weighed with precision of 0.01 and converted to kg ha^{-1}. Cowpea was harvested in several turns and the average of 6 turns was evaluated. The drainage of soil moisture was considered as irrigation variable and the water requirement was considered to be 100% of

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irrigation. Other variables were calculated based on irrigation value. In order to achieve 100% irrigation, the soil moisture near crops root was calculated using *Equation 1*.

$$d_n = (\theta_{Fc} - \theta_i)\ \rho_b D_r \quad \text{(Eq.1)}$$

The soil moisture to height of root showed the farm capacity. The duration of irrigation was calculated based on receiving moisture by soil. The θ_{Fc} is moisture percent of cultivation (%w), θ_i is soil moisture (%w), ρ_b is density (gr cm^{-3}) and D_r is effective root height (cm). The efficiency of water was determined by dividing seed yield on consuming water (Passioura, 2006). The irrigation and consumed water in each sample during growing phase is shown in *Table 3*.

Water productivity (WP; *Eq. 2*) is the seed yield (kg) per water use (m^3):

$$WP\ (kg\ m^{-3}) = \frac{\text{seed yield } (kg)}{\text{water use } (m^3)} \quad \text{(Eq.2)}$$

Variance analysis and average comparison were conducted by MSTATC software (Duncan test 5%) and all figures were drawn in Excel software.

Table 1. *Information on meteorological data*

During growth	Maximum temperature (°C)		Minimum temperature (°C)		Average humidity (%)		Rain (mm)	
	2016	2017	2016	2017	2016	2017	2016	2017
June	27.3	28.4	17.3	18.4	70.1	59.8	11.3	12.5
July	41.9	31.9	20	19	52.3	50	30.7	33.2
August	29.5	28.9	18.8	20.2	67.9	62.3	0	6.3
September	28.4	27.3	18.5	19.2	66.7	64.1	23.7	24

Table 2. *Characteristics of soil in the study area*

Soil depths (cm)	Particle size distribution (%)				EC (%)	Total nitrogen (%)
	Sand	Silt	Clay	Organic carbon		
0-20	47	32	21	0.65	0.646	27.1
20-40	46	34	20	0.71	0.653	28.5

Table 3. *The amount of water use in each treatment in 2016 and 2017*

Year	2016	2017	2016	2017
Water requirements	Amount of irrigation (mm)		Amount of water use (mm)	
40%	162	175	366.7	251
60%	243	262.5	308.7	338.5
80%	324	350	389.7	426
100%	403.1	436.2	468.8	512.2

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Results

Seed yield

The relations between bean crops and nitrogen fertilizer in seed yield in 2016 and 2017 were significant in level 5% and other changes were observed in level 1% (*Tables 4* and *5*). The highest seed yield in two bean species in 100% water requirements and in 2016 and 2017, were 1700 and 1776 kg ha^{-1} (*Fig. 1*). The highest seed yield in 30 and 60 kg N ha^{-1} fertilizer in 2016 was 1076 and 1173 kg ha^{-1} (*Fig. 2*). The highest seed yield in 30 and 60 kg N ha^{-1} fertilizers in 2017 were 1107 and 1209 kg ha^{-1} (*Fig. 3*). The interaction between bean crops and fertilizer in normal beans and in 60 kg N ha^{-1} fertilizer were 2400 and 2512 kg ha^{-1} (*Fig. 4*). The highest seed yield in 2016 and 2017 in 100% water requirements and 60 kg N ha^{-1} fertilizers were 2169 and 2253 kg ha^{-1} (*Fig. 5*). The highest seed yield in 2016 and 2017 in 100% water requirements and 60 kg N ha^{-1} fertilizers for common bean were 3486 and 3646 kg ha^{-1} (*Table 6*).

Table 4. *Mean squares form the combined ANOVA for agronomic traits*

Source	df	Seed yield (kg ha^{-1})		100-seed weight (g)		Number of pods per shrub	
		2016	2017	2016	2017	2016	2017
Replication	2	143677^{ns}	130645^{ns}	197^{ns}	190.1^{ns}	1.57^{ns}	2.26^{ns}
Water requirements	1	2240842^{ns}	3135651^{ns}	4067^{*}	3390^{ns}	19579	18984^{ns}
Error	2	277146	291045	173	167.6	1.91	2.09
Nitrogen	3	8182123^{**}	9159561^{**}	885^{**}	880^{**}	195	318.6^{**}
Water requirements×nitrogen	3	3206147^{**}	3390614^{**}	702.8^{*}	556	20.6^{**}	5.41^{**}
Varieties	3	855459^{**}	924025^{**}	37.29^{*}	35.26	16.1^{**}	16.9^{**}
Water requirements×varieties	3	136747^{*}	159486^{*}	70.49	68.3^{**}	7.68^{**}	9.91^{**}
Nitrogen×varieties	9	389196^{**}	425595^{**}	64.94^{**}	66.1^{**}	5.32^{**}	7.02^{**}
Water requirements×nitrogen×varieties	9	199775^{**}	213990^{**}	31.46^{**}	31.2^{*}	3.14^{**}	2.74^{**}
Error	60	41673	42530	12.72	13.58	0.562	0.699
CV (%)		12.4	12.6	12.1	12.8	2.19	3.32

ns = non-significant; * and ** = significant at 5% and 1% probability level, respectively

Table 5. *Mean squares form the combined ANOVA for agronomic traits*

Source	df	Plant height (cm)		Water productivity on seed yield (kg m^{-3})	
		2016	2017	2016	2017
Replication	2	3082.4^{ns}	2986.1^{ns}	0.013^{ns}	0.010^{ns}
Water requirements	1	56654^{*}	56197.4^{*}	0.008^{ns}	0.038^{ns}
Error	2	2884.9	2731.9	0.024	0.020
Nitrogen	3	2661.9^{**}	3768.6^{**}	0.15^{**}	0.158^{**}
Water requirements×nitrogen	3	403.23^{**}	98.09^{**}	0.205^{**}	0.184^{**}
Varieties	3	232.01^{**}	204.33^{**}	0.051^{**}	0.046^{**}
Water requirements varieties	3	116.64^{**}	122.59^{**}	0.003^{ns}	0.004^{ns}
Nitrogen×varieties	9	55.21^{**}	64.85^{**}	0.015^{**}	0.014^{**}
Water requirements×nitrogen×varieties	9	51.78^{**}	52.77^{**}	0.010^{**}	0.010^{**}
Error	60	6.92	7.51	0.002	0.002
CV (%)			3.93	6.7	6.6

ns = non-significant; * and ** = significant at 5% and 1% probability level, respectively

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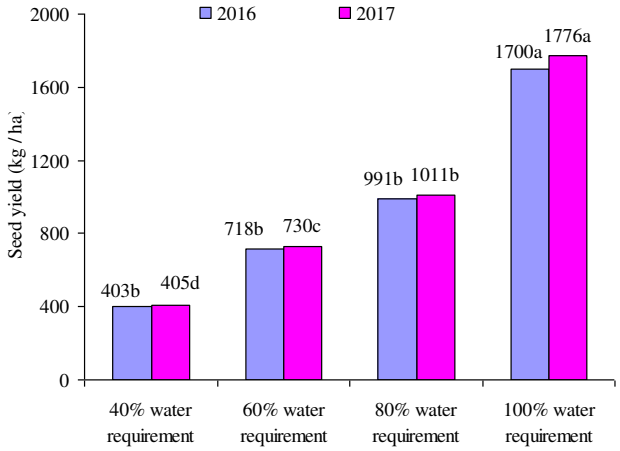


Figure 1. *Effect of irrigation on seed yield in bean cultivars in 2016 and 2017*

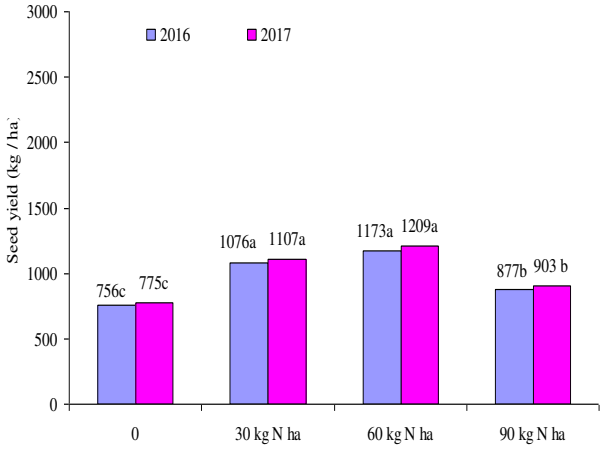


Figure 2. *Effect of nitrogen on seed yield in bean cultivars in 2016 and 2017*

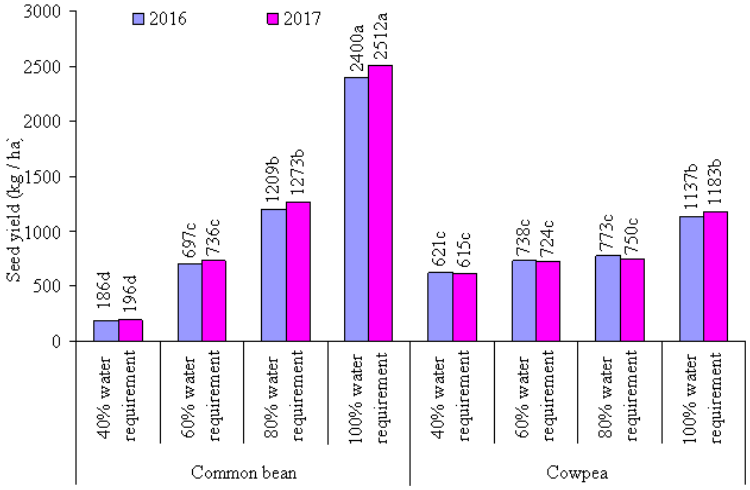


Figure 3. *Interaction of bean cultivars and irrigation on seed yield in bean cultivars in 2016 and 2017*

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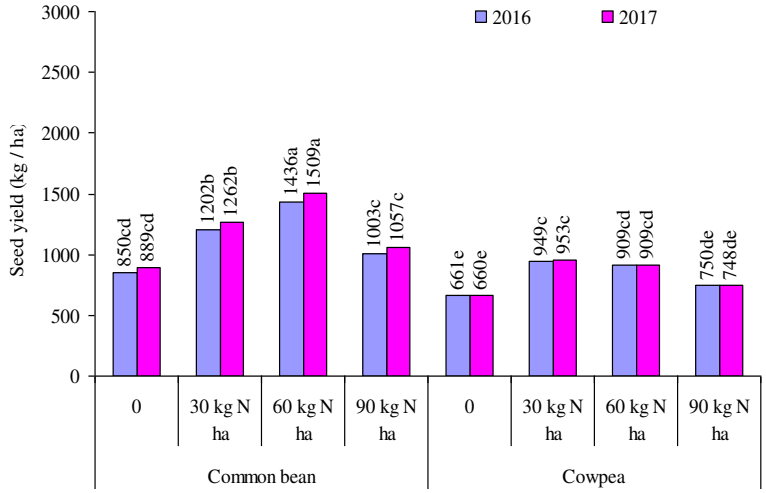


Figure 4. *Interaction of nitrogen and cultivars on seed yield in 2016 and 2017*

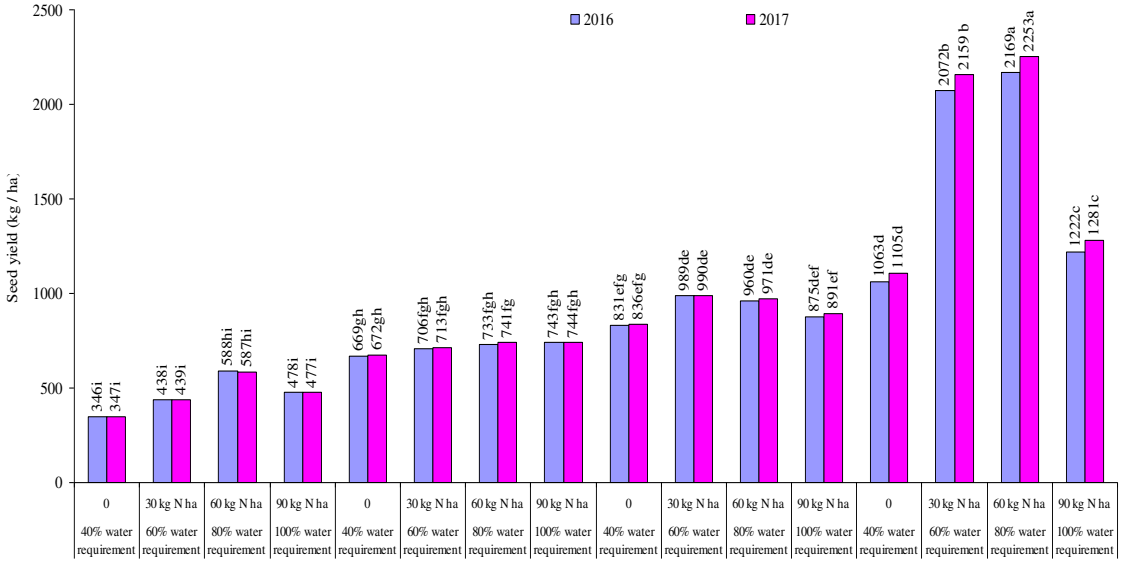


Figure 5. *Interaction of nitrogen fertilizer and irrigation on seed yield in 2016 and 2017*

Weight of 100 seeds

The total weight of 100 seeds in 2016 and 2017 were significant in 5% and other variables were significant in 1% (*Table 4*). The weight of 100 seeds in 2016 and 2017 were 34.7 and 36.1 g (*Fig. 6*). The highest weight of 100 seeds in 100% water requirement were 37.4 and 36.7 g in 2016 and 2017 (*Fig. 7*). The highest weight of 100 seeds in 2016 and 2017 with 100% water requirement were normal (*Fig. 8*). The highest weight of 100 seeds in 2016 and 2017 in 60 kg N ha^{-1} fertilizer were 31.1 and 30.2 g (*Fig. 9*). The highest Weight of 100 seeds, considering species and Nitrogen fertilizer, in 2016 and 2017 were 40.2 and 36.6 g in 60 kg N ha^{-1} (*Fig. 10*). The highest weight of 100 seeds in 100% water requirement and 60 kg N ha^{-1} fertilizer in common bean in 2016 and 2017 were 64.2 and 66.7 g (*Table 6*).

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Table 6. *Mean squares form the combined ANOVA for agronomic traits*

WP (kg m^{-3})		Number of pods per plant		Plant height (cm)		Weight of 100 seed (g)		Seed yield (kg/ha)		Nitrogen (kg ha^{-1})	Water requirement (%)	
2017	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**			
0.06o	0.06l	8s	7r	32.8q	37.1p	31.5d	32.2d	155l	146j	0	40%	Common bean
0.08o	0.08l	8qrs	8pqr	37.0opq	38.9op	27.3d-j	28.1d-h	205l	195j	30		
0.08no	0.08l	8rs	8qr	36.0pq	38.5op	31.3de	32.0d	204l	193j	60		
0.08no	0.09l	8qrs	8qr	37.3opq	39.0op	26.1d-k	27.1d-i	218l	209j	90		
0.19j-m	0.20jk	8pqrs	8n-r	38.3n-q	40.6nop	29.9def	31.3def	670ijk	635hi	0	60%	
0.22h-m	0.22h-k	9opqr	9m-q	39.5m-p	41.8mno	28.8d-g	29.9def	758ijk	718hi	30		
0.23g-l	0.23g-k	9op-s	8m-r	39.3m-p	42.0l-o	29.6def	31.3def	800ij	753hi	60		
0.20i-m	0.21h-k	9opqr	8m-r	40.5mno	42.6l-o	28.0d-h	29.0d-g	715 ijk	683hi	90		
0.27e-i	0.27fgh	9opq	9l-p	42.4mn	44.1lmn	29.4def	30.4def	1170fgh	1125fg	0	80%	
0.30d-g	0.30efg	10nop	9k-o	42.6mn	44.7lmn	30.6de	31.6de	1299ef	1240ef	30		
0.31c-f	0.32def	10nop	9k-n	42.9m	45.5lm	29.4def	30.6def	1385def	1313def	60		
0.28d-h	0.28fgh	10mno	9k-n	43.0m	46.2l	29.4def	30.7def	1237efg	1156fg	90		
0.29d-g	0.30efg	12hij	11hi	60.0l	62.6k	43.2c	45.7c	1561de	1495de	0	100%	
0.52b	0.54b	12hij	11hi	60.9l	64.1k	49.5b	52.3b	2784b	2657b	30		
0.69a	**0.70a**	15c	14cd	75.2jk	78.9i	**64.2a**	**66.7a**	**3646a**	**3486a**	60		
0.39c	0.40c	13ghi	12gh	60.1l	63.3k	47.1bc	48.6bc	2058c	1963c	90		
0.19klm	0.21h-k	8qrs	8o-r	71.3k	72.7j	25.8d-l	26.0e-j	457kl	462ij	0	40%	Cowpea
0.23g-l	0.27f-i	10mno	9klm	88.6gh	91.8fg	19.3m	19.7k	573jk	579hi	30		
0.34cd	0.38cd	11klm	10jk	84.1i	88.5g	21.1klm	21.7ijk	825ij	834gh	60		
0.25f-k	0.28fgh	10lmn	10kl	77.8j	83.2h	22.8h-m	23.7g-k	604ijk	610hi	90		
0.21i-m	0.24g-k	11jkl	11ij	88.8g	91.0fg	25.4e-l	25.7f-j	683ijk	697hi	0	60%	
0.23g-l	0.26f-j	12ijk	11hij	92.4fg	96.2de	20.4klm	20.8jk	739ijk	754hi	30		
0.22h-m	0.25f-j	12g-j	12ghi	89.5g	94.8ef	21.1klm	21.7ijk	713ijk	728hi	60		
0.23g-l	0.26f-i	12hij	12ghi	84.1hi	90.4g	21.8i-m	22.7h-k	760ijk	775hi	90		
0.15mn	0.17k	14cde	14de	98.3de	101.4c	20.7klm	20.9jk	625ijk	644hi	0	80%	
0.21i-m	0.24g-k	14def	14def	97.0de	101.6c	27.4d-i	27.9d-h	857hij	884gh	30		
0.19i-m	0.22h-k	13efg	13fg	95.8def	101.9c	20.4klm	21.1jk	802ij	827gh	60		
0.17lm	0.20ijk	13efg	13fg	94.0ef	101.7c	20.2lm	21.0jk	714ijk	736hi	90		
0.18klm	0.19jk	13fgh	13ef	98.2d	95.3ef	21.5j-m	21.2jk	875hij	841gh	0	100%	
0.33cde	0.35cde	**19a**	**20a**	**122.3a**	**116.5a**	30.0def	29.4def	1643d	1580d	30		
0.26e-j	0.28fgh	17b	18b	113.2b	106.3b	24.2f-m	23.5g-k	1296ef	1246ef	60		
0.18klm	0.20ijk	15cd	15c	108.3c	100.3cd	23.0g-m	22.2ijk	916ghi	881gh	90		

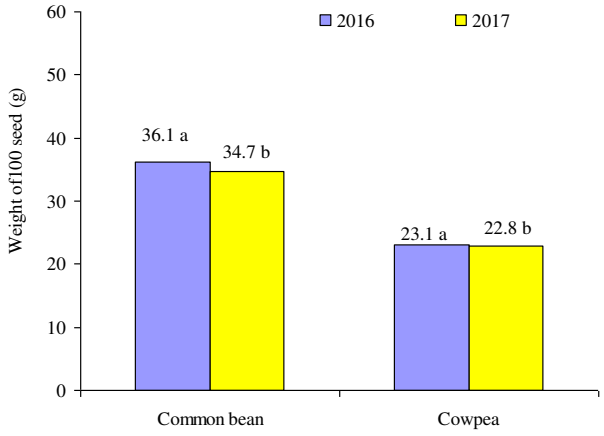


Figure 6. *Weight of 100 seed in bean cultivars in 2016 and 2017*

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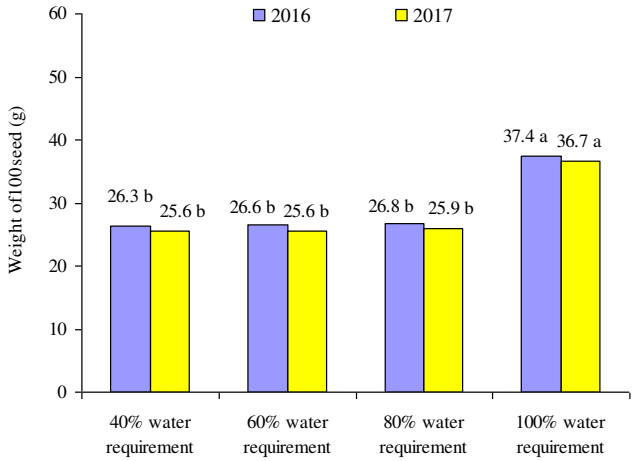


Figure 7. *Effect of irrigation on weight of 100 seed cultivars in 2016 and 2017*

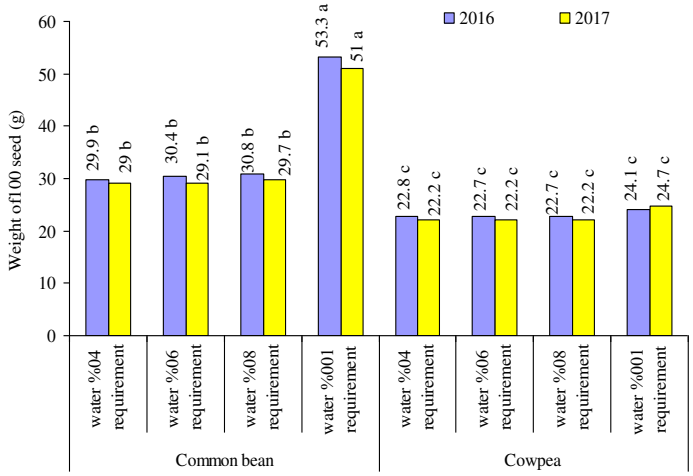


Figure 8. *Interaction of nitrogen and cultivars on weight of 100 seed in 2016 and 2017*

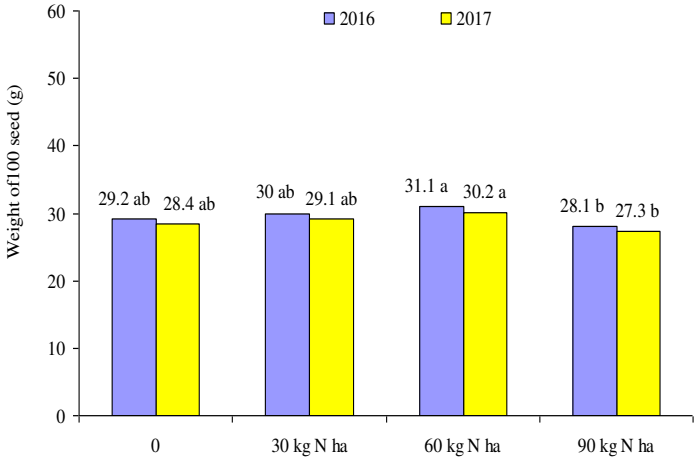


Figure 9. *Effect of nitrogen on weight of 100 seed in bean. cultivars in 2016 and 2017*

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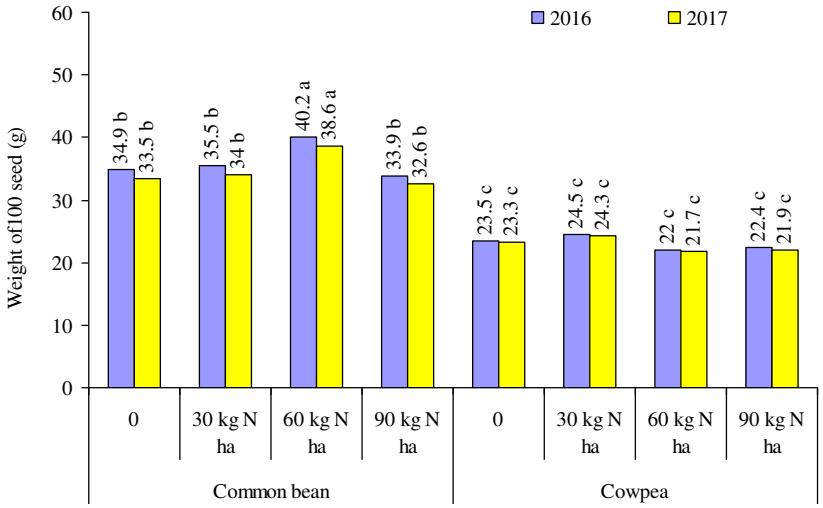


Figure 10. *Interaction of nitrogen fertilizer and cultivars on weight of 100 seed in 2016 and 2017*

Number of pods per plant

The effect of varieties was not significant during two years but in other references it was 1% (*Table 4*). The highest Number of pods per plant varieties in 100% water requirement in 2016 and 2017 were 31 and 32 (*Fig. 11*). The highest number of pods in 2016 and 2017, in 100% water requirement for Cowpea were 43 and 45 (*Fig. 12*). The highest number of pods in 2016 and 2017, in 30 kg N ha^{-1} were 27 and 26 (*Fig. 13*). The highest number of pods in reaction of varieties and Nitrogen fertilizer in 2016 and 2017 were 42 and 41 in Cowpea and in 30 kg N ha^{-1} (*Fig. 14*). The highest number of pods in 2016, in 60% and 80% water requirement and 30 and 60 kg N ha^{-1} were 36 and 35 (*Fig. 15*). The highest number of pods in 2017, in 60% and 80% water requirement and 30 and 60 kg N ha^{-1} in Cowpea were 37 and 36 (*Fig. 15*).

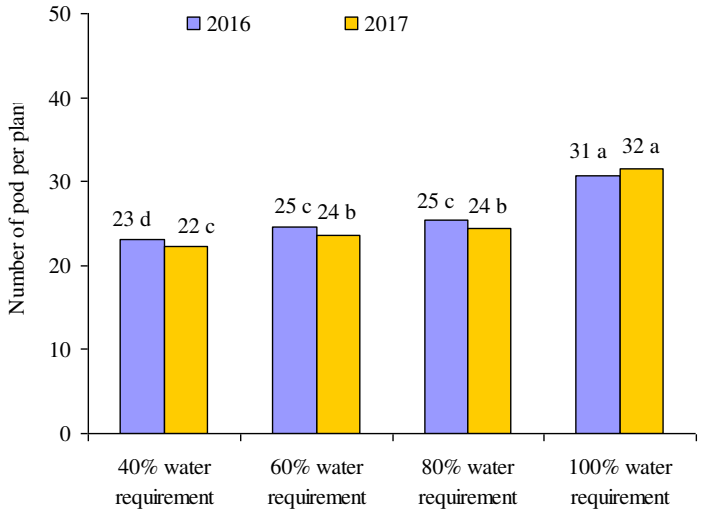


Figure 11. *Effect of irrigation on number of pods per plant in bean*

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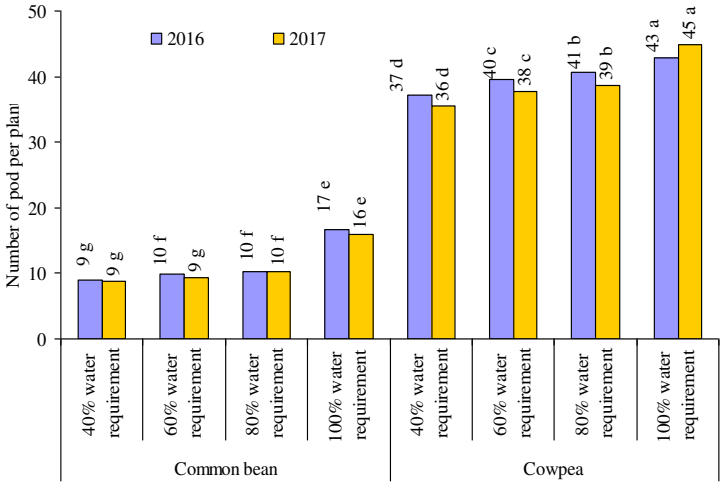


Figure 12. *Interaction of irrigation and cultivars on number of pods per plant in 2016 and 2017*

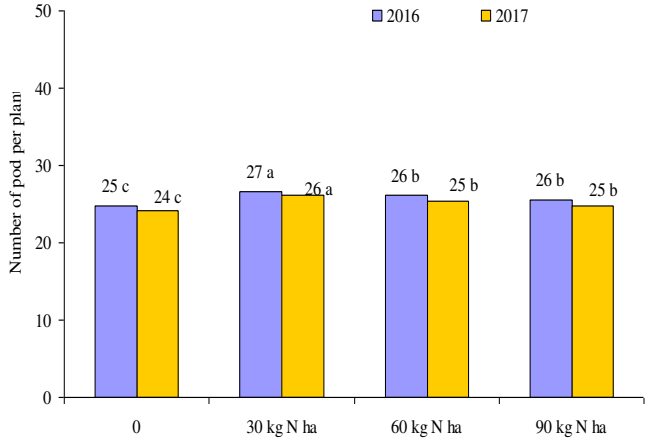


Figure 13. *Effect of nitrogen fertilizer on number of pods per plant in bean*

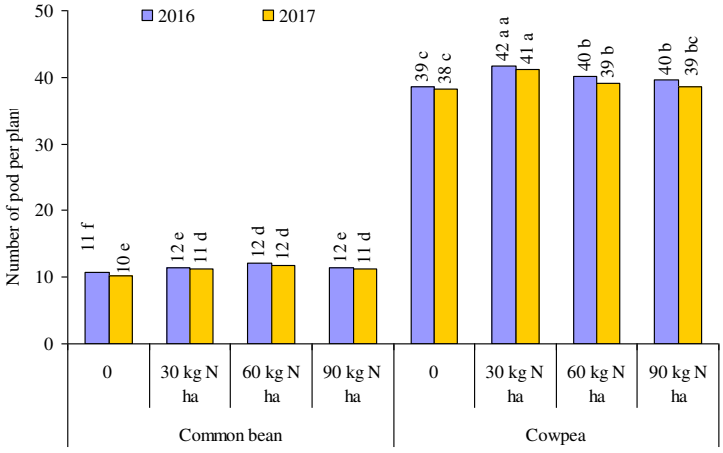


Figure 14. *Interaction of nitrogen fertilizer and cultivars on number of pods per plant in 2016 and 2017*

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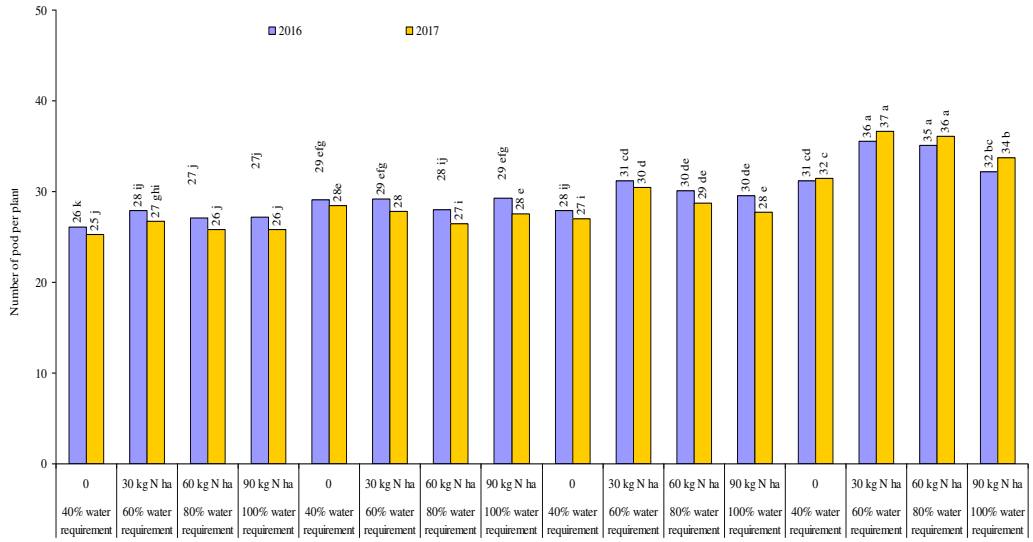


Figure 15. *Interaction of nitrogen fertilizer and irrigation on number of pods per plant in 2016 and 2017*

Plant height

Plant height was significant during 2 years in 5% and other changes were significant in 1% (*Table 5*). The maximum height of Cowpea plant in 2016 and 2017 were 95.8 and 94 cm (*Fig. 16*). The maximum Plant height in bean varieties in 100% water requirement were 87 and 88.9 cm in 2016 and 2017 (*Fig. 17*). Maximum plant height in bean varieties in 100% water requirement and Cowpea varieties were 104.6 and 110.6 cm in 2016 and 2017 (*Fig. 18*). The maximum plant height in 2016 in 30 and 60 kg N ha^{-1} was 74.5 cm (*Fig. 19*). The maximum plant height in 2016 in 30 and 60 kg N ha^{-1} fertilizer were 72 and 72.5 cm (*Fig. 19*). Maximum Plant height in relations of varieties and Nitrogen fertilizer during 2 years in 30 kg N ha^{-1} were 101.5 and 100.1 cm (*Fig. 20*). Maximum plant height in 2016 in 100% water requirement and in 30 and 60 kg N ha^{-1} were 98.2 and 95.7 cm (*Fig. 21*). Maximum plant height in 2017 in 100% water requirement and in 30 and 60 kg N ha^{-1} fertilizer were 100.9 and 99 cm (*Fig. 21*). Maximum plant height in 2016 and 2017 in 100% water requirement and 30 kg N ha^{-1} fertilizer were 116.3 and 122.2 cm for Cowpea (*Table 5*).

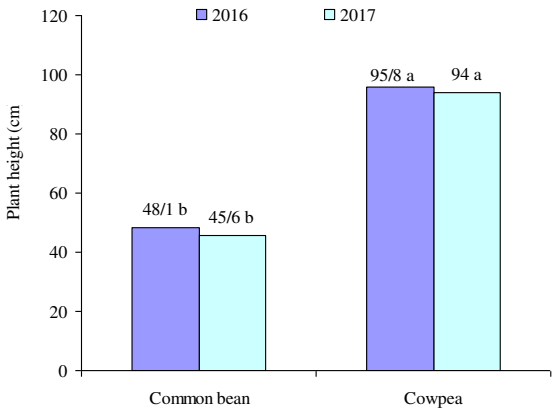


Figure 16. *Plant height in bean cultivars in 2016 and 2017*

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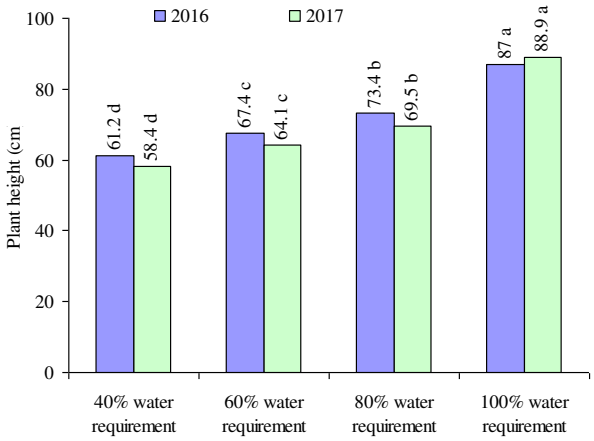


Figure 17. *Effect of irrigation on plant height in bean*

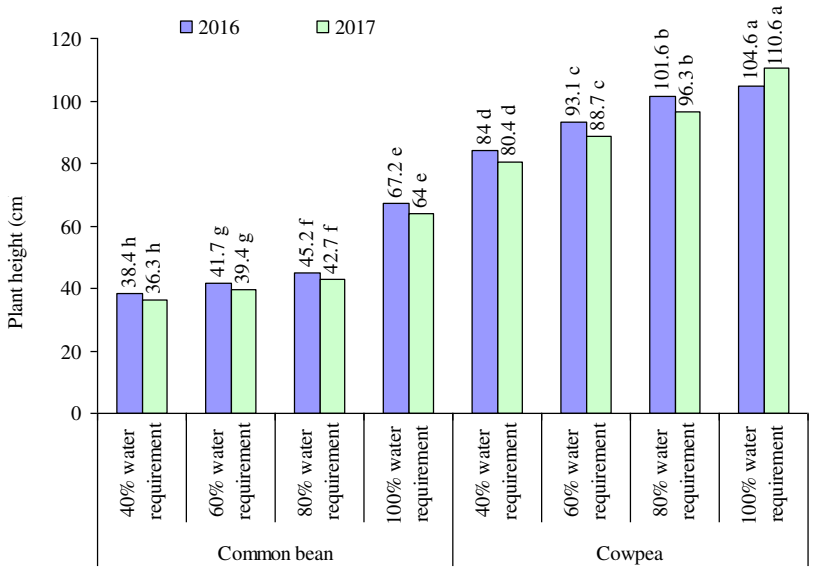


Figure 18. *Interaction of irrigation and cultivars on plant height in 2016 and 2017*

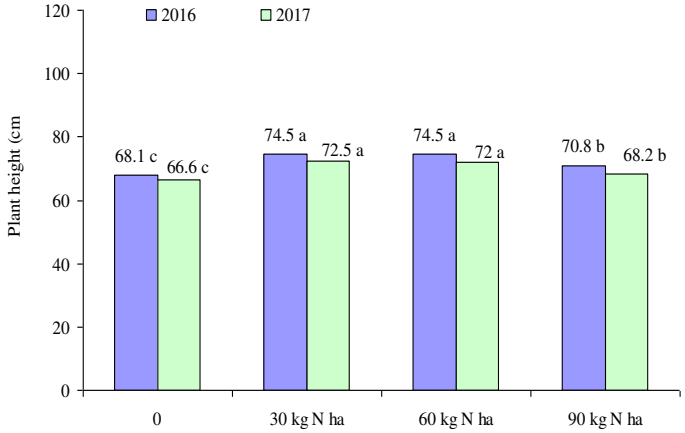


Figure 19. *Effect of nitrogen fertilizer on plant height in bean cultivars in 2016 and 2017*

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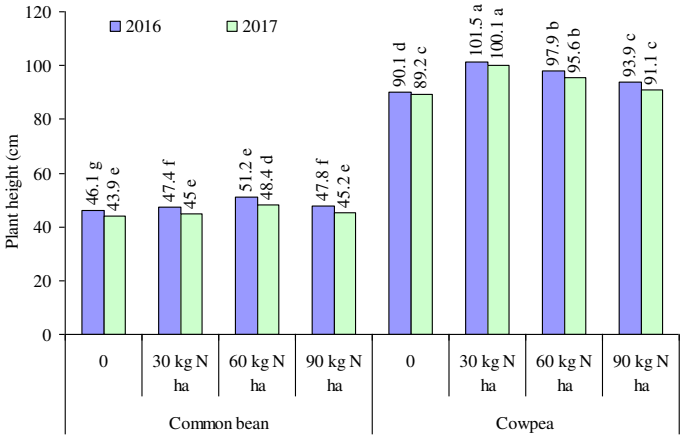


Figure 20. *Interaction of nitrogen fertilizer and cultivars on plant height in 2016 and 2017*

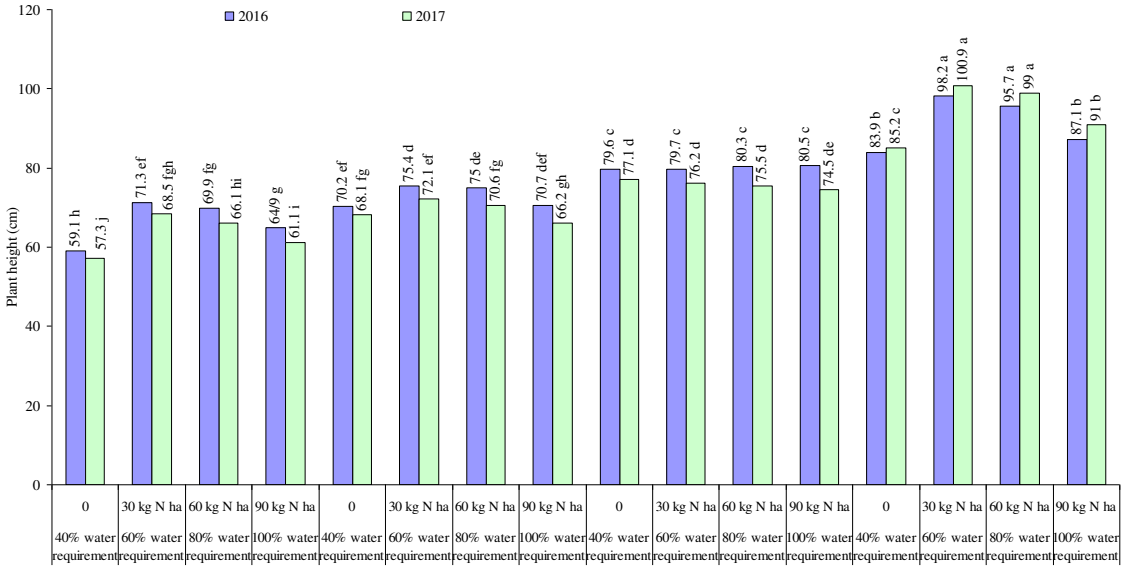


Figure 21. *Interaction of nitrogen fertilizer and irrigation on plant height in 2016 and 2017*

Water productivity (WP)

The Water productivity on seed yield regarding to bean varieties and interaction of varieties and fertilizer in 2016 and 2017 were not significant. However, the other sources were significant in 1% (*Table 5*). The highest efficiency of water consumption in 2016 and 2017 in 100% water requirements were 0.36 and 0.34 kg m^{-3} (*Fig. 22*). The highest efficiency of water consumption in 2016 and 2017 for normal beans in 100% water requirement were 0.48 and 0.47 kg m^{-3} (*Fig. 23*). The highest efficiency of water consumption in 2016 and 2017 in 60 kg N ha^{-1} fertilizer were 0.31 and 0.29 kg m^{-3} (*Fig. 24*). The highest efficiency of water consumption in 2016 and 2017 was observed in 30 and 60 kg N ha^{-1} fertilizer and 100% water requirement (*Fig. 25*). The highest Water productivity in 2016 and 2017 was observed in common bean, 100% water requirement and 60 kg N ha^{-1} fertilizer (*Table 6*).

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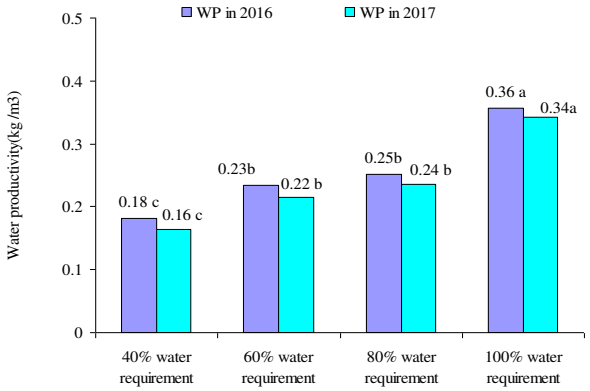


Figure 22. *Effect of irrigation on WP in 2016 and 2017*

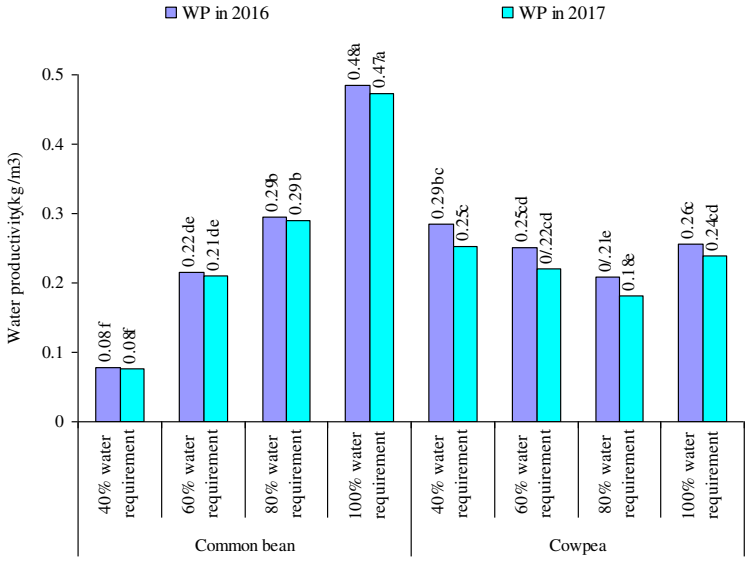


Figure 23. *Interaction of irrigation and cultivars on WP in 2016 and 2017*

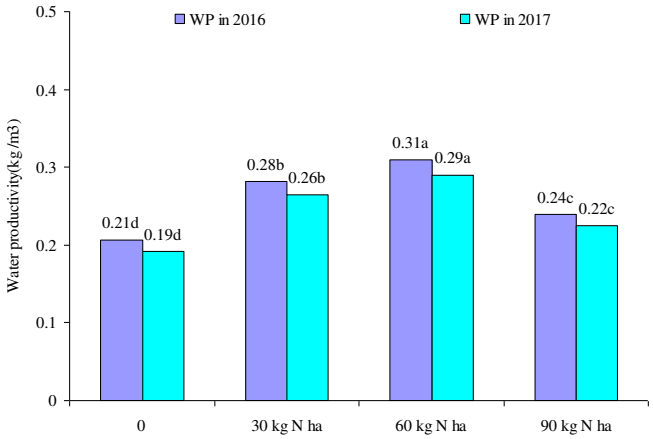


Figure 24. *Effect of nitrogen fertilizer on WP in 2016 and 2017*

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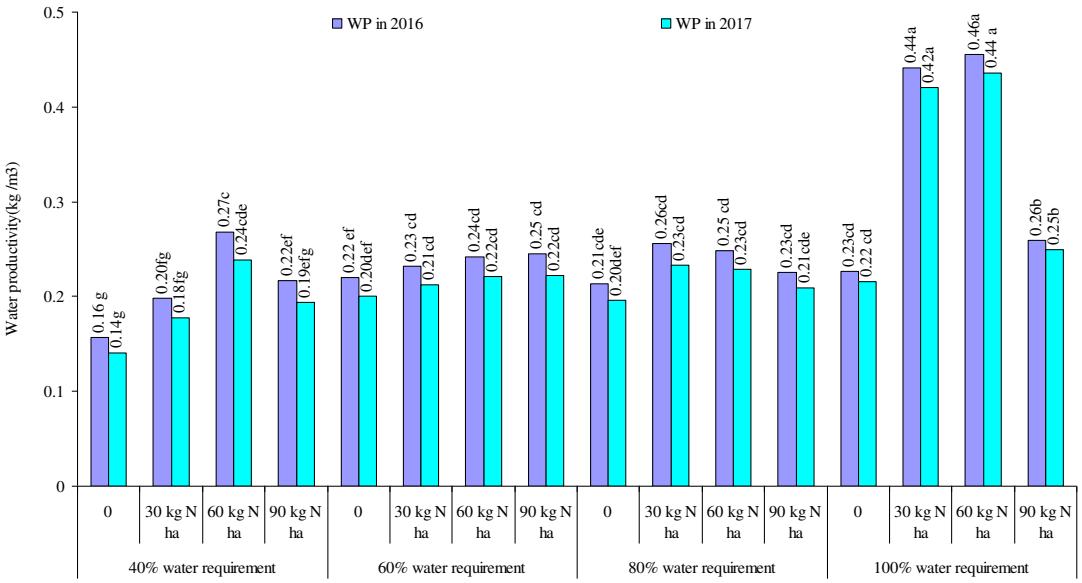


Figure 25. *Interaction of irrigation, nitrogen fertilizer and cultivars on WP in 2016 and 2017*

Discussion

Beans are sensitive to soil condition and drought damage and their water consumption increases with age. Irrigation is very important in water stress and showed direct effect on yield (Korir et al., 2006). Increase amount of Nitrogen to a determined level resulted in increase of yield (Singh, 2007). The results showed that moisture stress resulted in decrease of seed weight. Nitrogen influenced all elements of seeds such as number and weight. Shortage of Nitrogen in plants resulted in decrease of efficiency (Lawlor, 2001). According to Marschner (1995), high amount of Nitrogen in crops made them more sensitive to diseases.

Beans were sensitive to water shortage before and during flowering stage. In this step, shortage of water prevented the evolution of inflorescence and decreased the number of young pods and seeds (Singh, 2007; Lizana et al., 2006). High level of Nitrogen had no effect on number of pods. Authors reported the loss of legume yield in drought related to decrease of pods in crops (Singh, 2007). Long term water stress decreases the crop height. Availability of nitrogen influenced cell division and enlarged leaves (Singh, 2007). Water shortage is the most limiting factor in bean and Cowpea production, and improving agricultural Water productivity is essential for the increased leguminous demand. Conventional irrigation cannot be sustained in arid and semi-arid areas because of the rapid depletion of water resources. It is believed that conventional irrigation is a luxury use of water and can be reduced without much effect on economic yield. Methods that may cut down irrigation are of considerable interest and should be explored. Physiological water-saving irrigation strategies especially temporal and spatial deficit irrigation have great potential to practice in Leguminous production in arid and semiarid areas. It is well established that plant Seed production depends on the amount of water used for growth as well as on water productivity. The estimation of water productivity suggests that the amount of seed produced is equal to the water used during growth multiplied by Water productivity, which thus implies that the amount of Seed increases with increasing water productivity for constant water use. Therefore,

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Water productivity would be of particular interest in situations where growth is affected as a result of limiting water availability. The increased water productivity of bean and cowpea under water stress is attributed to seed production being reduced less by drought than water use.

Conclusion

The results showed that water shortage has negative influence on crop yield. The highest seed yield required 100% water and the lowest value was 40% in two varieties. The highest loss of seed yield in drought showed that supplying enough water is necessary to ensure photosynthetic elements to seeds by producing leaves and green coverage. The efficiency of water in common bean was more proper than in Cowpea. Finally based on results, it is suggested that normal beans are in priority of cultivation in studied region.

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DESIGN AND OPERATION ANALYSIS OF DRIP IRRIGATION SYSTEMS USED IN THE IRRIGATION OF CHERRY *(PRUNUS AVIUM* L.*)* ORCHARDS IN ISPARTA-ULUBORLU REGION, TURKEY

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Abstract. In this study, the existing drip irrigation systems of 20 cherry (*Prunus avium* L.) orchards in Isparta-Uluborlu (Turkey) region where cherry production is done intensively were evaluated with selected by purposive sampling method; were examined in terms of design and operation; problems related to the subject were determined. For this reason, drip and under tree micro sprinkler irrigation system was re-projected for each enterprise, elements of both irrigation systems compared with the present applications, and suggestions for both irrigation systems and irrigation scheduling are developed. As a result, it has been found that the system elements generally had not been designed properly, and the system layouts and operations were insufficient in all of the existing drip irrigation systems considered. Suggestions for both irrigation systems and irrigation scheduling are developed.
Keywords: *cherry orchard, Prunus avium L., drip irrigation, under tree micro sprinkler irrigation, irrigation scheduling*

Introduction

Almost all new cherry orchards established in Turkey for export are established by the 0900 Agricultural variety of saplings. In general, the harvest of the export grade of 0900 Agricultural variety begins in the first week of May in Izmir province the early season and ends in the last days of July in Konya-Hadim and Nigde-Ulukisla districts as the late season (Taner, 2001). Parallel to the developments in cherry exports, cherry production, in many cities begun to increase, in recent years; though leading provinces in cherry production are Izmir, Manisa, Afyon, Bursa, Konya, Amasya, Isparta, and Denizli in Turkey (*Table 1;* Anonymous, 2016a). Denizli and Izmir are early; other provinces are mid-season cherry production regions.

Isparta province is an important cherry production region for export with high-quality mid-season production. It is in the 6th rank in mid-season production regions in terms of yield. In 2016, cherry production was 55657 tons, the total number of trees was 1197822, and average yield per tree was 46 kg. The major cherry producing areas are the districts of Senirkent and Uluborlu (*Table 2;* Anonymous, 2016b). Fresh cherries are the leading agricultural product that provides export income for the Isparta province. 60% of agricultural products in 2007 were provided by cherries. The value of cherry exports was $5.7 million in 2005, $7.9 million in 2006, and amounted to $10.14 million in 2007 (Anonymous, 2009a).

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Table 1. *Data of leading provinces in cherry production in Turkey*

Province in Turkey	Area of collective fruit trees (ha)	Total fruit yield (t)	Fruit yield per tree (kg tree^{-1})	Total number of trees
Izmir	12097	46574	15	3033963
Manisa	9886	46648	20	2325630
Afyon	4215	403387	63	645942
Bursa	6250	32468	23	1435434
Konya	6664	55426	33	1662921
Amasya	2529	25008	34	739425
Isparta	5427	55657	46	1197822
Denizli	3787	22695	33	686015
Nigde	2519	23386	46	507116
Kutahya	2769	35152	28	1235223
Others regions	28603	216249	24	7784421
Turkey	84745	599650	25	21313912

Table 2. *Distribution of Isparta cherry production according to districts*

District	Area of collective fruit trees (ha)	Total fruit yield (t)	Fruit yield per tree (kg tree^{-1})	Total number of trees (number)
Center	675	6993	30	149100
Aksu	7	48	42	1452
Atabey	300	1720	25	61120
Egirdir	61	375	35	11435
Gelendost	54	4188	35	89300
Gonen	140	603	35	64265
Keciborlu	226	910	33	44100
Senirkent	1750	22700	33	302500
Sutculer	31	274	35	11670
Sarkikaraagac	175	1157	30	45700
Uluborlu	1760	14905	33	353100
Yalvac	172	961	30	41000
Yenisarbademli	75	823	35	23080
Isparta	5427	55657	46	1197822

It is expected that the climate changes expected to occur as a result of global warming will have adverse effects on water resources as well as agricultural production. Depending on the expected changes in climate, such as temperature, precipitation, relative humidity and wind, the water consumption of the plant, which is important in terms of irrigation, and the feeding of the groundwater also change, and those affect the water resources negatively (Anonymous, 1995). In addition to the problems of climate change and drought that may be experienced in relation to this, rapid population

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increase, increase in demand for water resources, the tendency of water usage to change gradually according to sectors, decrease in the water quality, and so on increase the importance of the utilization of water resources. According to the results of the assessment made by the World Meteorological Organization (WMO) among 87 member countries, Turkey is among the 74 countries affected by drought (WMO, 1992).

It is possible to predict the pressure over water resources occurred by the impacts of factors such as current growth rate and change of water consumption habits. Therefore, it is necessary to use water resources rationally to leave for healthy and sufficient water to the future generations of Turkey. This means the utilization of water should be planned and in the most efficient way.

In Turkey, both the consciousness and education levels of producers and excessive irregularity of irrigation studies and extreme and faulty water use due to the problems in transferring the results of those studies to the producers have negative effects on soil, environment, plants and water resources in particular. It was found in a study conducted in Sanlıurfa-Harran irrigation that about 5 to 7 times of the needed water was supplied to irrigation and that the groundwater levels were higher than 150 cm, which is considered as the threshold of danger (Tekinel et al., 2001).

Turkey is the first sweet cherry producer cherry production with 494000 tons of sweet cherry a year (Anonymous, 2009b). Isparta-Uluborlu region has an important place in our country in terms of production quantity and product quality. In recent years cherry producers have started to prefer drip irrigation instead of surface irrigation methods. However, these orchards, which are established on the basis of traditional farming, are experiencing problems in designing and operating drip irrigation systems which are subsequently constructed.

In studies conducted to monitor and evaluate the existing drip irrigation systems. It was observed that farmers had generally preferred the drip irrigation system with low initial capital cost, thus planning, layout and operation of a system had been unsuitable and water distribution had not been uniform required. It was also found that drippers had rapidly clogged due to inconvenient filtration and poor water quality (Pitchford, 1979; Fry, 1985). In a few research to evaluate some drip irrigation systems in Turkey (Turkey), the system elements had not been designed properly, the filtration of irrigation water to prevent emitter clogging had not been supplied by installing an adequate control head, and the system layouts and operations were insufficient in all drip irrigation systems considered nine farms in the Antalya region (Yildirim and Orta, 1993), and it also found that drip irrigation systems were not projected as suitable according to project criteria, sufficient control unity was not established, excessive irrigation water was applied and planning and managing of the systems were not done as suitable with regard to current conditions in Sultanhisar and Eskisehir region (Ayran, 2009; Harman, 2013).

In this study, the existing drip irrigation systems of 20 cherry (*Prunus avium* L.) orchards in Isparta-Uluborlu (Turkey) region where cherry production is done intensively were evaluated with selected by purposive sampling method; were examined in terms of design and operation; problems related to the subject were determined. For this reason, drip and under tree micro sprinkler irrigation system was re-projected for each enterprise. Elements of both irrigation systems compared with the present applications. And suggestions for both irrigation systems and irrigation scheduling are developed.

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Materials and methods

The research was carried out in 20 cherry (*Prunus avium* L.) orchards in Isparta-Uluborlu (Turkey) region (*Fig. 1*), selected by purposive sampling method, among the enterprises where drip irrigation method is applied.



Figure 1. *The placement of Isparta and Uluborlu in Turkey*

During the research, a survey study was conducted primarily to determine the existing irrigation application skills and levels of the selected producers. The characteristics of the existing irrigation systems in all enterprises (dripper flow and distance, lateral pipe diameter and length, number of lateral pipes running in a row and to be supplied water at the same time, manifold pipe diameter and length, main pipe diameter and length, pipe materials) and control unit factors (filter system, gravel filters, hydro cyclone, sieve-disc filter, pressure regulator, fertilizer tank volume, manometer, valve etc.), were determined according to the measurements and observations conducted.

However, for all enterprises, the data that is essential for project design of the drip and micro sprinkler irrigation method has been obtained. For this purpose, some important characteristics of the enterprise's soil (texture class, field capacity, wilting point and volume weights) for irrigation was determined according to Demiralay (1993) and Tuzuner (1990). Deteriorated and intact soil samples were taken from different layers (0-30, 30-60, 60-90 and 90-120 cm) from the test site. The infiltration rates of the soil of the test area were determined according to the principles given by Gungor and Yildirim (1989) with double cylinder infiltrometer measurements. The dimensions of the enterprise areas were measured by steel tape meters and slopes with the aid of a leveling instrument. The flow rate of the water source was measured by the volume-time method and the sufficiency for cultivation was examined.

In order to determine the irrigation water needs of the cherry plants cultivated in the examined enterprises, firstly the values of the plant water consumption are determined. Climate data of the region where the enterprises are located were taken from Isparta Meteorology Directorate and the values of plant water consumption were determined by the Penman-Monteith method for the process in plant vegetation period. For this, ten-day-long average values of the climate data between 1990 and 2010 were taken into account (*Table 3;* Anonymous, 2011), the reference plant water consumption was calculated, and actual plant water consumption values are obtained by correcting the obtained values with the plant coefficient. Plant coefficients were calculated according to a previous study (Evans et al., 1996).

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Table 3. *Some climatic values of Uluborlu District, including a ten-day long average*

Date	Average steam pressure (h Pa)	Average temperature (°C)	Average wind speed (m s^{-1})	Average relative humidity (%)	Sunrise time (h)
01-10 May	898.66	13.7	2.75	56.30	9.60
11-20 May	899.43	15.7	2.33	52.46	9.60
21-31 May	898.48	17.5	2.49	55.99	9.40
01-10 June	898.97	18.4	2.50	55.24	9.60
11-20 June	898.78	20.2	2.41	51.11	11.30
21-30 June	898.34	21.4	2.50	49.41	11.00
01-10 July	897.10	22.7	2.32	47.46	11.30
11-20 July	897.64	23.0	2.35	49.19	11.30
21-31 July	896.76	23.3	2.45	46.77	11.60
01-10 August	896.25	23.5	2.35	49.26	11.20
11-20 August	897.64	23.1	1.93	48.25	11.10
21-31 August	898.37	21.6	1.83	48.19	10.90
01-10 Sept	899.23	18.8	1.91	52.90	9.90
11-20 Sept	900.18	17.7	1.90	53.55	9.30
21-30 Sept	900.99	15.9	2.28	60.54	8.20
01-10 Oct	901.92	14.9	2.25	59.75	8.00
11-20 Oct	902.00	13.3	2.37	64.14	6.20
21-31 Oct	902.37	10.5	2.33	66.57	6.20

For each enterprise, a re-drip and under micro sprinkler irrigation system has been designed. In determining the engineering characteristics of the system, the principles given in Yildirim (2008) were utilized. The equations used for the under tree micro sprinkler irrigation system's project design are summarized below.

Wetted area (P)

$$P = \frac{\alpha^{\circ}}{360^{\circ}} \frac{\pi D^2}{4 S_a S_s} \quad \text{(Eq.1)}$$

Heading wetting diameter (D)

$$D = \sqrt{\frac{360\ 4\ S_a S_s\ 0{,}30}{\alpha \pi}} \quad \text{for P = 0.30} \quad \text{(Eq.2)}$$

$$D = \sqrt{\frac{360\ 4\ S_a S_s\ 0{,}50}{\alpha \pi}} \quad \text{for P = 0.50} \quad \text{(Eq.3)}$$

Precipitating rate (Pr_y)

$$Pr_y = \frac{360^{\circ}}{\alpha} \frac{4\ q}{\pi D^2} \quad \text{(Eq.4)}$$

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Evapotranspiration for micro irrigation methods (T)

$$T = ET \frac{Ps}{95} \quad \text{(Eq.5)}$$

Amount of maximum net irrigation water ($d_n max$)

$$d_n max = d_k \; Ry \; D \; \frac{p}{100} \quad \text{(Eq.6)}$$

Amount of total irrigation water (d_t)

$$d_t = \frac{d_n}{E_a} \quad \text{(Eq.7)}$$

Maximum irrigation interval (SA_{max})

$$SA_{max} = \frac{dn_{max}}{T} \quad \text{(Eq.8)}$$

Amount of net irrigation water (d_n)

$$d_n = T\,(SA) \quad \text{(Eq.9)}$$

Irrigation duration (Ta)

$$T_a = \frac{1000\; d_t}{q\; N_d} \quad \text{(Eq.10)}$$

Maximum number of operating units (N_{max})

$$N_{max} = \left(\frac{T_g}{T_a}\right)(SA) \quad \text{(Eq.11)}$$

Reference evapotranspiration for Penman-Monteith method

$$ET_0 = \delta/(\delta + \gamma^*)(Rn - G)\; \frac{1}{\lambda} + \gamma/(\delta + \gamma)u^2 \left(\frac{900}{t + 275}\right)(e_a - e_d) \quad \text{(Eq.12)}$$

where P is wetted area (%), D is head wetting diameter (m), S_a is tree spacing (m), S_s is row spacing (m), Pr_y is precipitating rate (mm h^{-1}), α is wetting angle, q is head flow (l h^{-1}), T is evapotranspiration for micro sprinkler irrigation method (mm day^{-1}), ET is evapotranspiration (mm day^{-1}), Ps is ratio of the area shaded by the plant to the total area (%), $d_n max$ is amount of maximum net irrigation water (mm), d_k is available water holding capacity (mm/m), Ry is allowable part of d_k, D is watering soil depth (mm), Ea is water application efficiency (%), SA_{max} is maximum water application interval (days), SA is irrigation interval (days), T_a is irrigation duration (h), d_t is amount of total

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irrigation water (mm), N_d is number of head in a decare area (pieces da^{-1}), N_{max} is maximum number of operating units, T_g is daily irrigation duration (h day^{-1}), ET_o is reference evapotranspiration (mm day^{-1}), δ is slope of the vapor pressure curve (kPa $°C^{-1}$), γ* is modified psychometric constant (kPa/°C), Rn is net radiation on the plant surface (MJ m^{-2} day^{-1}), G is heat flow in the ground (MJ m^{-2} day^{-1}), λ is evaporation latent heat (MJ /kg^{-1}), u^2 is equivalent of wind speed at 2 m height (m s^{-1}), t is average temperature (°C), e_a is saturated vapor pressure at average air temperature (kPa) and e_d is actual vapor pressure at average air temperature (kPa) in *Equations 1-12*.

Results and discussion

The average values of soil texture, field capacity, wilting point, volume weight and available water holding capacity of the soil samples taken from the enterprises obtained from the physical analyzes and the values of infiltration rate measured by the double cylinder infiltrometer are given in *Table 4*. The soil texture class was generally clayey, loamy and sandy-loamy and the infiltration rate values of the enterprise's soil ranged from 6.5 mm/h to 18.1 mm/h.

Table 4. *Some physical properties of the enterprise's soil*

Orchard number	Soil texture class	Infiltration rate (mm h^{-1})	Field capacity (%)	Wilting point (%)	Volume weight (g cm^{-3})	Available water holding capacity (mm 120 cm^{-1})
1	L	12.4	26.10	12.32	1.40	230.68
2	SL	18.1	18.99	8.58	1.49	185.51
3	L, SL	13.0	22.14	11.88	1.58	194.84
4	L, CL, SICL	9.0	28.53	18.48	1.64	197.48
5	L, SIL	10.6	25.92	13.53	1.49	220.79
6	SL	16.0	18.81	9.02	1.53	179.74
7	L	12.5	26.55	12.76	1.40	230.84
8	CL	6.9	31.68	22.44	1.69	187.39
9	CL	7.0	29.79	20.79	1.78	192.51
10	L	11.0	26.19	13.64	1.46	219.50
11	L, SCL	10.8	27.36	16.17	1.45	194.71
12	L	12.0	25.65	12.65	1.44	223.25
13	C, CL	6.5	27.99	19.80	1.85	181.33
14	SCL, C	9.0	27.90	19.14	1.81	190.27
15	L	12.0	24.84	12.65	1.43	209.55
16	L, SICL	9.5	26.10	12.98	1.40	220.81
17	SL	17.0	21.06	9.35	1.26	177.06
18	CL	7.0	29.34	20.13	1.76	193.96
19	SL, CL	14.0	22.68	9.79	1.35	208.82
20	SL	16.0	21.87	11.11	1.42	183.67

C: clayey, L: loamy, SL: sandy loam, SCL: sandy clayey loam, SICL: silt clayey loam, SIL: silt loam

The results of the survey study to determine the existing irrigation application skills and levels of the producers of cherry farming in the enterprises and the factors of the

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existing drip irrigation systems in the enterprises (dripper flow and distance, lateral pipe diameter and length, number of lateral pipes running in a row and to be supplied water at the same at the same time, manifold pipe diameter and length, main pipe diameter and length, pipe materials, etc.) and the results of measurements and observations made on the elements of the control unit (filter system, gravel filter, hydro cyclone, sieve-disk filter, pressure regulator, volume of fertilizer tank, manometer, valve etc.) are given in *Table 5*.

Table 5. *Features of the existing drip irrigation systems in enterprises*

Irrigation system parameters	Enterprise number																			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Area (ha)	1.0	0.23	4.8	0.5	0.95	0.95	4.8	1.45	0.47	1.45	1.0	3.3	0.63	2.0	0.63	0.9	2.9	0.55	1.05	2.4
Tree distance (m)	8	8	7	5	5	8	6	6	6	7	7	8	6	7	7	7	5	5	7	7
Row distance (m)	8	9	6	10	8	6	6	5	6	7	7	8	6	7	7	7	3	3	6.5	6
Land slope (%)	0.1	0.5	0.1	1	0.1	4	1.5	2	5	2	0.5	0.5	0.5	1.5	2	3.5	2	1	0.2	4
Fertilizer tank volume (l)	50	-	150	50	-	100	100	50	50	50	100	50	50	50	100	100	100	50	50	50
Filter	+	-	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+
Main pipe material	PE	PE	PVC	PE	PE	PVC	PVC	PVC	PE	PE	PE	PVC	PE	PVC	PE	PE	PE	PE	PVC	PVC-PE
Main pipe diameter (mm)	75	75	110	75	75	110	110	110	90	90	90	110	90	110	90	90	90	90	110	75-110
Main pipe length (m)	50	55	120	50	50	85	240	0	65	105	100	223	45	88	150	152	225	145	109	246
Manifold pipe diameter (mm)	75	50	110	50	63	75	90	90	50	63	63	63-110	63-90	75-110	75	63	50-90	90	90	75
Manifold pipe length (m)	65	42	110	50	50	75-110	200	110	110	70	200	416	75	190	50	50	55-145	48	115	77
Lateral pipe diameter (mm)	16	16	20	16	16	16	16	16	16	16	16	16	16	16	20	16	16	16	16	16
Lateral pipe length (m)	75	56	282	50	100	60	30	100	50	50	50	48-100	55	63	75	45	45	45-62	40	42-78
Lateral pipe number of a tree line (number)	2	4	2	4	5	4	2	2	2	4	4	4	4	4	4	4	2	4	4	2
Dripper range (m)	0.5	0.33	0.5	0.4	0.5	0.4	0.4	0.5	0.2	0.5	0.5	0.33	0.5	0.5	0.4	0.5	0.4	0.4	0.5	0.33
Operating pressure (m)	15	15	18	18	15	15	18	12	12	15	10	15	20	10	10	15	20	20	25	20
Dripper flow ($l\ h^{-1}$)	2	3	2	2	2	2.4	2	2.2	2	4	4	4	3	4	2	1.6	2	2	4	2
Irrigation interval (days)	7	7	10	8	5	8	7	7	15	15	-	7	7	12	7	7	8	7	7	5
Irrigation duration (h)	6	13	12	12	6	12	24	12	8	8	8	10	6	8	10	7	12	10	10	9
Applied irrigation water amount (mm)	45.9	95.9	13.5	126	220	19.5	59.3	37.8	432.1	21	32.5	88.8	31.5	28.6	56	139.9	104.4	172.3	113.6	82.4
Wetted area (%)	15	26	17	38	60	24	16	20	8	34	34	20	40	34	35	34	19	38	34	11

As can be seen from *Table 5*, the enterprises participated in the research have a size ranging from 0.23 to 4.8 ha. Eleven of the enterprises (55%) are small (<1.0 ha) family businesses. Operation flows in almost all of the enterprises are sufficient to allow the irrigation systems work efficiently.

It has been determined that the diameters of the main pipes used in the twelve enterprises (60%) in the research (1, 2, 7, 8, 9, 10, 13, 14, 15, 16, 18 and 19) are oversized. This situation increases the initial installation costs of the systems. In addition, in three enterprises (15%) (3, 5 and 11) the employed main pipe diameters were less than the required value. Side pipe diameters are larger than the required values in twelve enterprises (60%) (1, 2, 7, 8, 12, 13, 15, 16, 17, 18 and 20) and smaller in seven enterprises (35%).

It was stated that if the wetted area percentage (p) is less than 30%, in the case of using two laterals for each tree row in the irrigation of the fruit trees according to the

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drafting criteria of the drip irrigation system project, a micro-sprinkler system for this area will be more suitable (Anonymous, 2011). Seven of the farms (35%) surveyed in the research had two lateral laid along each plant row, but the wetted areas did not reach the desired level in any of them. In the other thirteen enterprises (65%), irrigation is carried out by 4-5 laterals laid along each tree row. In this case, the cost of the first installation of the irrigation system increases, as well as problems in terms of management occurs. However, in the nineteenth of the enterprises (95%) (except for the operation of 15) the lateral pipe diameters are in required ranges.

Besides, there is no problem in the operation of the irrigation system except for the 3rd enterprise. However, there is a problem in the operation of the irrigation system in the 3rd enterprise. Sufficient amount of irrigation water in sufficient time to apply the appropriate irrigation program to the enterprises located in the area where this enterprise is also located, cannot be supplied by the legal entity with the duty of operating the water mains. The infrastructure of the irrigation system has not been set up properly to provide enough water for agricultural land. It is not believed that the dispute between the company, which established the irrigation system that is located here, and the legal entity of the village can be resolved by the courts, either.

In the examined cherry orchards, the plant row spacing ranges from 5 to 8 m. Dripper spacing ranges from 0.2 to 0.5 m between the laterals used in existing irrigation systems. Therefore, 4-5 laterals in each plant row should be laid in order to obtain a sufficient level of wetting in the root area of the plant. a sufficient wetting zone in the plant root area cannot be established in 50% of the enterprises surveyed. The irrigation interval is longer than necessary for all enterprises except the 5th and 20th (90%). In other words, irrigation practices are not done in the form required by the drip irrigation. As is known, the plant root area is kept moist at the appropriate level by giving a little water at short intervals by drip irrigation method. In addition to this, although the irrigation duration is favorable in four enterprises (20%) (1, 4, 12 and 13), more irrigation periods are being implemented in ten enterprises (50%) (2, 3, 6, 7, 8, 14, 15, 17, 19 and 20), which in turn, causes excess irrigation water to be used in the mentioned enterprises. In the other six enterprises (30%) (5, 9, 10, 11, 16 and 18), less irrigation water is given to the plants because of the less irrigation time applied.

Similar to this research findings, in a few research to evaluate some drip irrigation systems in Turkey (Turkey), the system elements had not been designed properly according to project criteria (Ayran, 2009; Harman, 2013), the filtration of irrigation water to prevent emitter clogging had not been supplied by installing an adequate control head, and the system layouts and operations were insufficient in all considered drip irrigation systems (Yildirim and Orta, 1993).

While *Table 6* gives information on irrigation systems that are re-projected, considering the characteristics of soil, plant and water resources for each enterprise examined whereas the recommended irrigation scheduling at the meetings held with the producer is summarized in *Table 7*. Moreover, the hypothetical irrigation scheduling to be applied if the irrigation was continued with the existing irrigation system in the enterprises is given in *Table 8*.

It was suggested that in the pressurized system; the main pipeline should be constructed from PVC pipes and the manifold pipelines from PE pipes while the T pipelines should be installed as buried in the ground (Kekec, 2006; Yilmaz, 2011). And it was concluded that it would be more appropriate to place the control unit at a single point and at the start of the main pipeline for easier operation in micro irrigation

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systems and it would be more appropriate to place the required sand separator and sand-gravel filter tank at the beginning of the water distribution network in the case of collective micro-irrigation systems. Also, the control unit elements in individual irrigation systems in each operation should be formed of the fertilizer tank, sieve filter, and pressure regulator, if necessary (Yildirim, 2008). In the project of the reconstructed irrigation system for the surveyed enterprises, the main and manifold pipelines were designed according to the principles proposed by Kekec (2006) and Yilmaz (2011) whereas mass irrigation system and selected control unit elements (except pressure regulator) for individual gardens were made according to the principles recommended by Yildirim (2008). The possibility of applying drip irrigation method for each enterprise unit in the survey was examined by taking the principles given in Yildirim (2008) into account. Since the plant and soil characteristics of the enterprises do not allow the installation of the drip irrigation system by laying two laterals along each plant row, it is decided that the more suitable irrigation method for the operators is under tree micro-sprinkler irrigation and the proposed irrigation system is designed accordingly.

Table 6. *The parameter of under tree micro sprinkler irrigation systems*

Irrigation system parameters	Enterprise number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Head flow ($l\ h^{-1}$)	160	300	40	200	160	160	200	90	90	200	200	250	90	160	200	160	100	100	200	200
Head wetting diameter (m)	6.5	7	5	5.5	5	5.5	5	4.5	4.5	5.5	5.5	6.5	6.5	5.5	5.5	5.5	3	3	5	5
Number of operating unit	1	1	3	1	1	1	2	1	1	1	1	2	1	1	1	1	3	1	1	3
Lateral pipe length (m)	75	56	50	50	50	60	60	65-70	50	50	50	56	55	63	75	70	25-90	45-62	62.5	48-60
Lateral pipe diameter (mm)	20	25	16	16	16	20	25	16	16	25	25	20	16	25	25	20	20-25	16	20	20
Manifold pipe length (m)	65	42	75-90	50	100	72	66-67	100	110	70	200	80	75	190	50	50	65-125	48	80	77
Manifold pipe diameter (m)	75	63	50-63	75	75	75	75	75	63	75	110	63	63	90	90	50	75	50	90	90-110
Number of operating unit to be supplied at the same time	1	1	1	1	1	1	3	1	1	1	1	2	1	1	1	1	2	1	1	1
Main pipe diameter (mm)	75	63	90	75	75	75	110	75	90	75	110	90-110	75	110	90	75	90-110	48	62.5	264
Wetted area (%)	46	47	41	47	43	42	48	47	39	43	43	46	39	43	43	43	41	41	38	41

Table 7. *Irrigation scheduling for the under tree micro sprinkler irrigation systems*

Enterprise number	May			June			July			Augustus			September		
	Ta (h)	SA (days)	dt (mm)	Ta (h)	SA (days)	dt (mm)	Ta (h)	SA (days)	dt (mm)	Ta (h)	SA (days)	dt (mm)	Ta (h)	SA (days)	dt (mm)
1	10.5	10	56.7	10.6	6	57.5	9.0	4	48.9	10.6	4	57.2	10.9	12	58.9
2	5.2	8	45.4	5.5	5	47.9	5.6	4	48.9	4.9	3	42.9	5.6	10	49.1
3	4.5	9	51.3	4.2	5	47.9	4.3	4	48.9	3.7	3	42.9	4.3	10	49.1
4	6.3	9	53.1	5.7	5	47.9	5.8	4	48.9	5.1	3	42.9	5.8	10	49.1
5	5.6	9	51.0	5.2	5	47.9	5.3	4	48.9	4.7	3	42.9	5.9	11	54.0
6	5.1	7	39.7	4.9	4	38.3	4.7	3	36.7	5.5	3	42.9	5.0	8	39.2
7	5.6	11	63.3	5.0	6	57.5	5.4	5	61.1	5.0	4	57.2	5.2	12	58.9
8	7.1	8	45.4	7.5	5	47.9	7.7	4	48.9	6.8	3	42.9	7.7	10	49.1
9	6.7	7	42.9	9.0	6	57.5	5.8	3	36.7	6.7	5	42.9	6.2	8	39.2
10	5.4	9	51.0	5.1	5	47.9	5.2	4	48.9	4.5	3	42.9	5.2	10	49.1
11	5.0	8	47.8	4.0	4	38.3	3.9	3	36.7	4.5	3	42.9	4.7	9	44.2
12	6.9	10	58.7	6.8	6	57.5	5.8	4	48.9	6.8	4	57.2	6.4	11	54.0

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13	6.4	8	40.4	6.0	4	38.3	5.8	3	36.7	4.5	2	28.6	6.2	8	39.2
14	6.2	8	46.7	5.1	4	38.3	4.9	3	36.7	5.7	3	42.9	5.9	9	44.2
15	9.0	9	51.0	8.4	5	47.9	8.6	4	48.9	7.6	3	42.9	9.5	11	54.0
16	6.8	9	51.0	6.4	5	47.9	6.5	4	48.9	5.7	3	42.9	7.2	11	54.0
17	2.6	7	41.5	2.4	4	38.3	2.3	3	36.7	1.8	2	28.6	2.5	8	39.2
18	7.9	8	45.4	6.7	4	38.3	6.4	3	36.7	7.5	3	42.9	7.7	9	44.2
19	3.5	7	39.7	3.4	4	38.3	3.2	3	36.7	3.8	3	42.9	3.9	9	44.2
20	3.5	7	39.7	3.4	4	38.3	3.2	3	36.7	3.8	3	42.9	3.4	8	39.2

Ta: irrigation duration; SA: irrigation interval; dt: amount of total irrigation water

Table 8. *Irrigation scheduling for the existing irrigation systems*

Enterprise number	May			June			July			Augustus			September		
	Ta (h)	SA (days)	dt (mm)	Ta (h)	SA (days)	dt (mm)	Ta (h)	SA (days)	dt (mm)	Ta (h)	SA (days)	dt (mm)	Ta (h)	SA (days)	dt (mm)
1	8	10	45	8	5	45	8	4	45	10.6	5	45	8	10	45
2	7	20	110	7	15	110	7	12	110	7	12	110	7	15	110
3	8.2	12	57.5	8.2	6	57.5	8.2	5-6	57.5	8.2	7	57.5	8.2	10	57.5
4	5.5	12	58	5.5	6	58	5.5	5	58	5.5	5	58	5.5	12	58
5	7.2	9	43.3	7.2	5	43.3	7.2	4	43.3	7.2	5	43.3	7.2	9	43.3
6	5	12	52.9	5	7	52.9	5	5	52.9	5	8	52.9	5	13	52.9
7	7	14	67.9	7	7	67.9	7	7	67.9	7	9	67.9	7	14	67.9
8	5.5	13	55.2	5.5	7	55.2	5.5	6	55.2	5.5	7	55.2	5.5	10	55.2
9	2.5	28	113.2	2.5	15	113.2	2.5	11	113.2	2.5	19	113.2	2.2	24	113.2
10	7.5	9	43	7.5	5	43	7.5	4-5	43	7.5	6	43	7.5	9	43
11	5	7	57.3	5	6	57.3	5	6	57.3	5	7	57.3	5	12	57.3
12	6-6.5	14	65.7	6-6.5	9	65.7	6-6.5	6	65.7	6-6.5	8	65.7	6-6.5	14	65.7
13	9.3	12	53.4	9.3	6	53.4	9.3	5	53.4	9.3	7	53.4	9.3	9	53.4
14	10	13	56	10	6-7	56	10	5-6	56	10	6	56	10	9	56
15	6	12	61.6	6	7	61.6	6	6	61.6	6	8	61.6	6	11	61.6
16	9.2	9	65	9.2	5	65	9.2	4	65	9.2	6	65	9.2	10	65
17	5	12	52.1	5	5-6	52.1	5	5-6	52.1	5	6	52.1	5	15	52.1
18	8.5	7	57	8.5	6-7	57	8.5	5	57	8.5	8	57	8.5	14	57
19	5.5	13	61.4	5.5	7	61.4	5.5	5-6	61.4	5.5	7-8	61.4	5.5	8-9	61.4
20	3.5	12	54	3.5	6	54	3.5	6	54	3.5	7	54	3.5	10	54

Ta: irrigation duration; SA: irrigation interval; dt: amount of total irrigation water

Conclusion

As a result, if it is desired to receive the expected benefits and an increase in the irrigation application efficiency from irrigation systems in the enterprise; the importance of assigning the design and construction of the drip irrigation systems, which require knowledge and experience, to professionals, and to firms with the required expertise has been stated.

During the design of the irrigation systems projects, the soil characteristics of the enterprise (texture, field capacity, wilting point, volume weight, available water holding capacity texture, infiltration rate etc); plant characteristics (characteristics of rootstocks and root systems used, planting distances, development and yield of trees, etc.), and characteristics of water resources (flow, quality etc) should be taken into account; designing the project for each enterprise must be done individually, and specific to the enterprise.

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After the irrigation system in the enterprises has been designed and installed in accordance with the criteria mentioned above; training of the operators on the operation of the irrigation system to by experts, and the necessity to apply the appropriate irrigation program considering the climate values of the regions where the enterprises are located, should be provided.

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EVALUATION OF RECOVERY FROM ACUTE LUNG INJURY INDUCED BY INTRATRACHEAL INSTILLATION OF ZINC OXIDE NANOPARTICLES

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Abstract. Zinc oxide nanoparticles (ZnO-NPs) can potentially cause adverse health effects because they are highly soluble and widely used. We evaluated the effect of ZnO-NPs in rat lung. ZnO-NPs were administered via intratracheal instillation to Sprague Dawley rats at three different dosages (1, 5, and 10 mg/kg body weight). Macrophage numbers, total cell count, and total protein concentrations in bronchoalveolar lavage fluid (BALF) highly increased at day 1 in a dose-dependent manner relative to those in the control. Macrophage numbers and total protein concentrations were still elevated at day 14 relative to baseline, whereas total cell number continued to increase. These results indicate that inflammation had not fully resolved by day 14 in rat lungs. Furthermore, a significant increase in IL-6 (but not TNF-α) was observed in 5 and 10 mg/kg dose groups at day 1, which was likely caused by a ZnO-NP-treatment-induced increase in polymorphonuclear leukocytes. Histological examination indicated that multifocal acute inflammation occurred in the ZnO-NP-treated groups and progressed to chronic inflammation by day 14. A partial recovery of lung injury was observed within 14 days after exposure. In conclusion, the results of this study were consistent with those of previous sub-chronic exposure studies.
Keywords: *nanomaterials, pulmonary toxicity, safety, lung burden, BALF*

Introduction

Nanotechnology, as defined by the International Standards Organization (ISO), is a technology incorporating materials which must be less than 100 nm in size in at least one axis. As it is cutting edge technology, many industries have striven to innovate in the nanotechnology field in order to overcome physical limitations and improve economic efficiency (Mousavi and Rezaei, 2011, Wetzel et al., 2003). Therefore, nanotechnology has been widely applied in medicines, electronics, and fabrics, and has rapidly spread in consumer products, including cosmetics and personal care goods (McKiernan et al., 2013, Sawhney et al., 2008, Kaur and Agrawal, 2007). Given this, the safety of nanoparticle products must been properly assessed (Nohynek et al., 2010). Metal nanoparticles such as silver, titanium, zinc, and aluminum are widely used. For

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example, zinc nanoparticles (Zn-NPs) and titanium nanoparticles (Ti-NPs) are currently used in sunscreen cosmetics for the prevention of ultraviolet (UV) radiation-induced dermal diseases, including carcinoma and burns (Monteiro-Riviere et al., 2011, Smijs and Pavel, 2011, Nohynek and Dufour, 2012). However, previous studies have shown that metal nanoparticles induced DNA damage and oxidative stress in keratinocytes (Shukla et al., 2011), hepatoma cells (Kim et al., 2009) and lung epithelial cells (Limbach et al., 2007).

Zinc is an essential mineral with important physiological roles in the human body, and many diseases such as diarrhea (Hambidge, 2000), anorexia (Hambidge et al., 1972), and pneumonia (Bhutta et al., 1999) have been associated with zinc deficiency. It has been reported that zinc oxide nanoparticles (ZnO-NPs) are distinct from other metal oxide NPs as they have high solubility (Zhang et al., 2012). The increased production and use of ZnO-NPs enhances the probability of exposure, causing elevated concerns regarding their potential for unintended adverse health effects. Due to their extremely small size, NPs have a very high probability of cellular uptake and interactions with biological molecules and tissues. Indeed, exposure to NPs is not only providing advantageous opportunities, but also potential risks (Zhang et al., 2014).

Therefore, it is imperative to characterize the environmental health and safety aspects of exposure to ZnO-NPs. However, research investigating the toxicity of ZnO-NPs is far behind nanotechnology advances. In vivo results indicate ZnO-NP exposure via inhalation poses the most important hazard. However, risk assessments, such as exposure assessments and dose-response relationships of ZnO-NP exposure, are relatively scarce. In a previous study, a single intratracheal instillation of rats with 50-70 nm ZnO-NPs and 1000 nm ZnO microparticles (1 and 5 mg/kg Body Weight (BW) respectively) resulted in potent inflammation (Sayes et al., 2007).

Although studies on Zn nanoparticles have been conducted for several decades (Fosmire, 1990, Xia et al., 2008, Buerki-Thurnherr et al., 2013), information about the effects of ZnO-NPs exposure in the rat lung and its recovery have not been reported in detail. Therefore, the objective of this study was to evaluate the recovery at 14 days from acute lung injury induced by intratracheal instillation of ZnO-NPs in 3 different doses (1, 5, and 10 mg/kg BW). We performed intratracheal instillations of ZnO-NPs in Sprague-Dawley rats, and the rats were autopsied at 1 or 14 days after installation. Inflammation responses, Zn burden, and histopathological observations were examined in the collected rat lungs. This study was carried out to examine recovery after intratracheal instillation of ZnO-NPs in the rat lung.

Materials and Methods

Preparation of Zn-NPs

ZnO-NPs used in this study were purchased from Sigma-Aldrich (Sigma-Aldrich, St Louis, MO, USA). ZnO-NPs were dispersed in sterile saline and prepared via 10 min sonication before starting the experiment. Particle size was measured by transmission electron microscopy (TEM). To investigate the state of dispersion of ZnO-NPs when placed into saline as vehicles, samples were analyzed by Dynamic light scattering (DLS).

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Animals and Animal Experimental Design

Pathogen-free male Sprague-Dawley (SD) rats aged 7 weeks were purchased from Orient Bio Inc (Seongnam, Korea). Animals were acclimatized for at least 1 week prior to starting the study. Rats were randomized into 4 groups (n = 10 individuals per group) with a vehicle control group (treated with sterile saline). The doses for intratracheal instillation were selected based on a previous study by Warheit et al (Warheit et al., 2009). The treatment procedures for each group were as follows: animals were exposed to 1 (low exposure group), 5 (middle exposure group), or 10 mg/kg body weight (high exposure group) doses of ZnO-NPs by intratracheal instillation. The control group was exposed to saline. Rats were sacrificed at 1 or 14 days after instillation. All animal experiments were performed according to the Guidelines for Care and Use of the National Institute of Environmental Research.

Acute Pulmonary Analysis

To investigate acute lung injury, bronchoalveolar lavage fluid (BALF) was obtained by lavaging the lungs five separate times with 3 ml of calcium- and magnesium-free phosphate buffer solution (PBS, pH 7.4) with the rat deeply anesthetized by isoflurane. The samples were centrifuged at 1,500 rpm for 10 min. The cell-free supernatant of the first lavage was kept separate from other samples for biochemical analysis. The recovered cells from all lavages were resuspended in PBS. The total number of cells (TC) in the BALF were counted with a Vi-Cell ® XR analyzer (Beckman Coulter, Brea, CA, USA), and cell differential tests were performed using cytospin preparations (Shandon, Pittsburgh, PA, USA) stained with Diff-Quick staining solution (Fisher Scientific, Swedesboro, NJ, USA). BALF cells (300/rat) were differentially counted as polymorphonuclear leukocytes (PMN) using light microscopy. The total protein (TP) in the BALF supernatant was quantified using a BCA protein assay kit (iNtRON Biotechnology, Seoul, Korea), and the activity of lactate acid dehydrogenase (LDH) in the BALF was measured by using a QuantiChrom Lactate Dehydrogenase Kit (BioAssay Systems, Hayward, CA, USA). The levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in the BALF were estimated by commercially available enzyme linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA), which were performed based on the manufacturer's instructions.

Zinc Burden in Lungs

Lungs were collected under anesthesia and frozen at -20°C overnight. Then, the samples were weighed, and the zinc burdens in the lungs were quantified with inductively coupled plasma mass spectrometry (ICP-MS, Varian 820-MS, Australia) after microwave-assisted digestion of the samples with HNO_3 using a microwave digestion system.

Histopathological Examination

For histopathological analysis, lungs were collected and fixed in 10% neutral buffered formalin. Then, the tissues were embedded in paraffin, sectioned (3-5 μm in thickness), and stained with hematoxilin and eosin (H&E). Stained sections were examined by two toxicological pathologists. If there was a discrepancy in diagnosis

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between the pathologists, the final consensus would be made by discussing the disagreements under dual microscopy (Olympus Co., Tokyo, Japan).

Statistical Analysis

Analysis of variance (ANOVA) tests and Student's t-tests (GraphPad, San Diego, CA, USA) were used to compare test groups with the control group.

Results and Discussion

Characterization of ZnO-NPs

The average primary sizes of ZnO-NPs were measured by TEM, demonstrating a variety of nanoparticle shapes approximately 200 nm in size, including aggregations (*Fig. 1A*). DLS analysis showed that ZnO-NPs were agglomerated, and the dispersion of ZnO-NPs in saline was observed over a range of 100 to 500 nm in a dose-dependent manner (*Fig. 1B*). In the present study, the DLS results were broader in range than the TEM observations regarding primary particle size. This could be explained by the results of a previous study, which showed higher surface absorbed protein concentrations when zinc oxide was diluted in water rather than in PBS (Wells et al., 2012). The above findings illustrate that when surface-absorbed protein concentrations increase, ZnO-NPs preferentially form agglomerations. This likely indicates why particle sizes measured using DLS were increased compared to TEM.

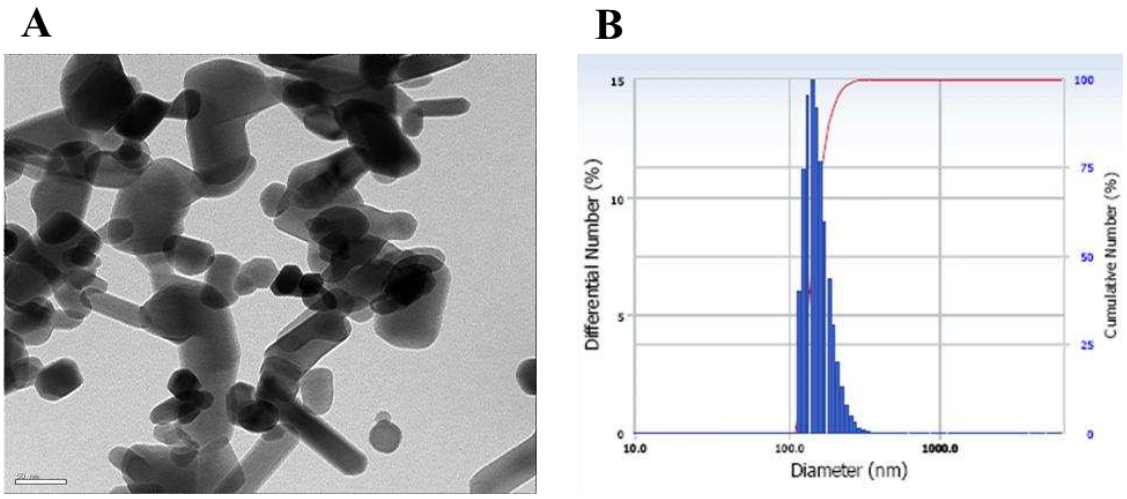


Figure 1. *Characterization of exposure conditions. (A) TEM image of Zn-NPs. Scale bar: 50 nm. (B) DLS analysis of Zn-NPs in saline.*

Body and Lung Weight

The body weights of the rats were measured from the ZnO-NP exposure day until 14 days after exposure. Body weights increased steadily after treatment in all groups, and no statistical differences between the treatment groups and the control group were observed (*Fig. 2A*). The relative lung weights of the rats were also measured to identify

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the effect of ZnO-NPs, and no significant differences were found between treatment groups or between days after exposure (*Fig. 2B*).

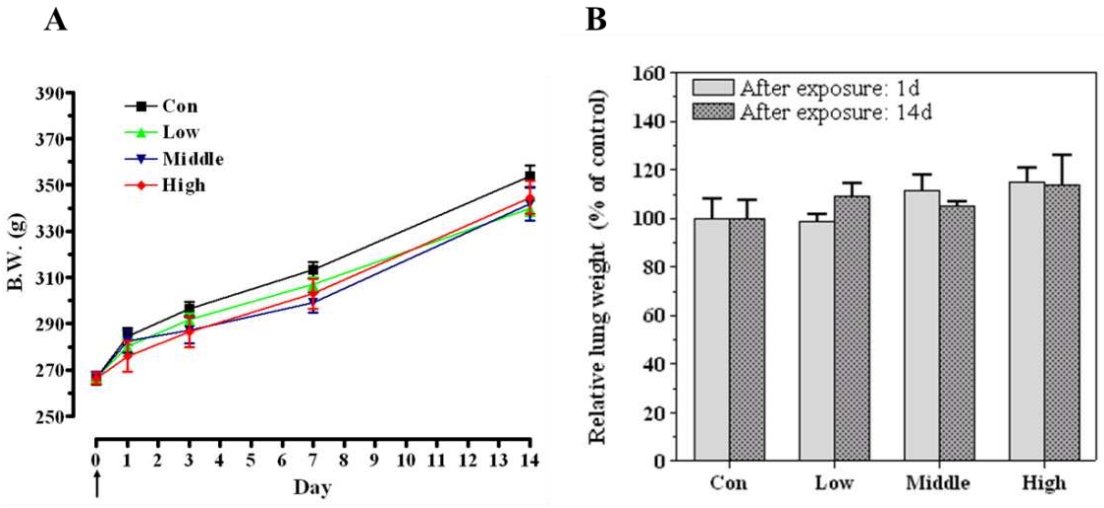


Figure 2. *Effect of ZnO-NPs on body and relative lung weights in rats. (A) Body weight change. (B) Relative lung weight. Data was presented as mean ± S.E.M.*

ZnO-NP Exposure Effects on BALF Cytology

Cell morphology changes were observed in a dose-dependent manner when intratracheal instillation of ZnO-NPs was performed in rats (*Fig. 3A*). 1 day after ZnO-NPs instillation, BALF macrophage presence was increased in a dose-dependent manner. This increase persisted until day 14, although the number of macrophages present at day 14 was lower than that of day 1, suggesting that ZnO-NP effects still exist after 2 weeks of non-exposure. Likewise, the total number of cells in BALF increased in a dose-dependent manner after ZnO-NP instillation, and persisted even at day 14 after treatment. Indeed, total cells at day 14 were even higher than at day 1 (*Fig. 3B*). PMNs also increased at day 1 after ZnO-NP instillation in a dose-dependent manner, but no difference was detected by day 14 (*Fig. 3C*). By comparing the ZnO-NP-exposed groups with the control group results in both day 1 and day 14 rats, we can say that recovery after 14 days did occur. In a previous report, rats were exposed to 1 or 5 mg/kg of ZnO particles, and lung samples were collected after 1 day, 1 week, 1 month, and 3 months after intratracheal instillation (Sayes et al., 2007). We were able to confirm the increase reported by Sayes et al. in total cell numbers at the dose of 5 mg/kg at 1 day after instillation. In our study, an additional dosage of instillation was held for 10 mg/kg. In this dosage treated group, the cell number remained increased even after 14 days of recovery. Regarding the proportion of PMNs in total BALF cells, an increase was observed at 1 day after instillation at a dose of 5 mg/kg, but a return to baseline was observed after 14 days. However, an increase in the proportion of PMNs has been reported to be induced by the same dosage at 1 week after instillation (Sayes et al., 2007). Therefore, by combining the previous study and the present report, we were able to identify that intratracheal instillation at a dose of 5 mg/kg was performed in rats, the proportion of PMNs recovered between 1 and 2 weeks after treatment.

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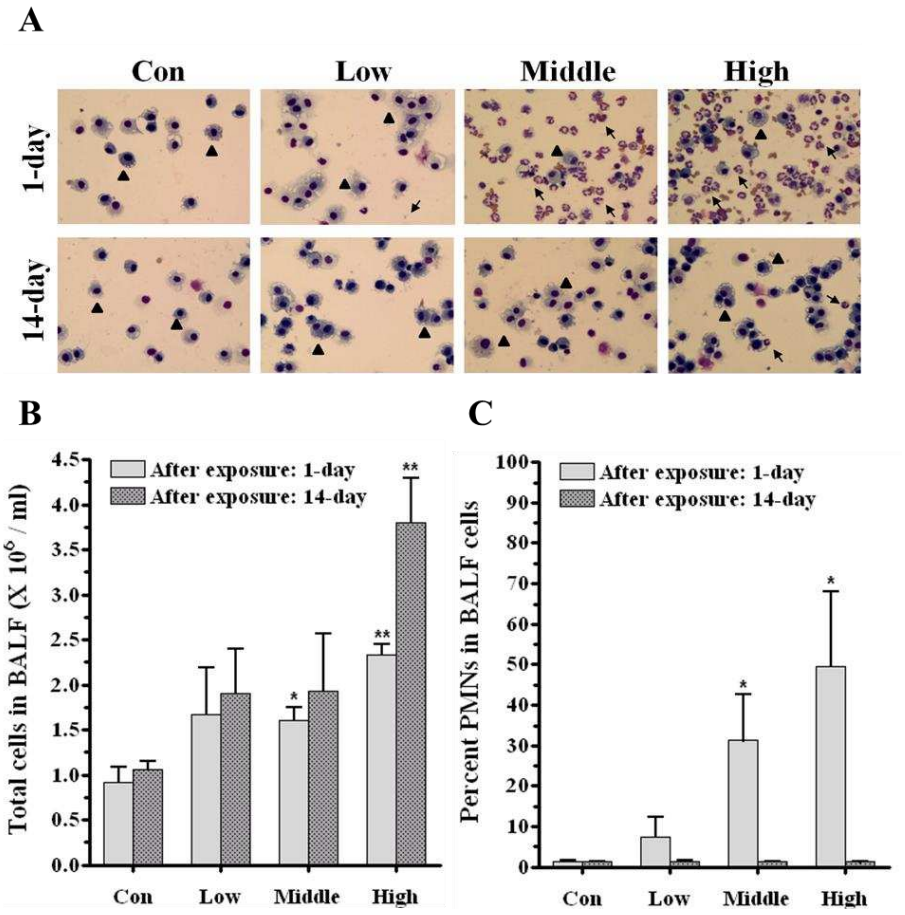


Figure 3. *Bronchoalveolar lavage fluid (BALF) cell analysis after instillation of ZnO-NPs. (A) Morphology of BALF cells. (B) Total cell counts. (C) Percent of PMNs in total BALF cells. Data was presented as mean ± S.E.M. * = P < 0.05 and ** = P < 0.01 vs. control.*

Effect of ZnO-NPs on BALF Biochemical Parameters

BALF was collected and analyzed to assess pulmonary inflammation at days 1 and 14 after exposure to ZnO-NPs. The total protein concentration in BALF increased after treatment in a dose-dependent manner (*Fig. 4A*). The magnitude of increase was higher on day 1 than day 14, although a slight increase was still present at day 14. This result indicates that inflammation was not fully resolved by 14 days after treatment in rat lung cells.

LDH is a cytoplasmic enzyme which is present in essentially all major organ systems. LDH levels in BALF were measured in the control and treatment groups, and no differences were observed, except in the 10 mg/kg dose treatment group at day 1 after instillation (*Fig. 4B*). Increases in extracellular LDH concentrations, which may be caused by cell lysis or cell membrane damage, is used to detect cell damage or cell death (Lott et al., 1986, Click, 1969). Therefore, analysis of BALF LDH concentrations is a potentially useful tool for evaluating lung tissue damage. The increase in LDH caused by high dose (10 mg/kg) ZnO-NP intratracheal instillation observed after day 1 indicated significant cytotoxicity. However, after 14 days, the LDH level had decreased to that detected in the control group. In a previous report, LDH levels were also found to be recovered at 1 week after ZnO-NP exposure at the doses of 1 and 5 mg/kg (Fukui et al, 2015). In our study, this phenomenon was not only observed in these two dosages,

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but also at a higher dose (10 mg/kg). Therefore, the recovery of LDH levels appears to occur between day 1 and day 14 in rat lungs.

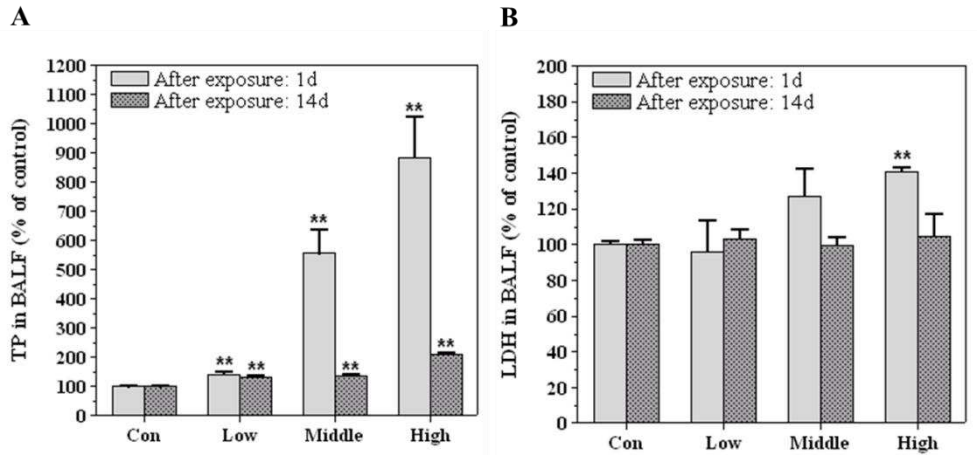


Figure 4. *Analysis of biochemical markers in BALF. (A) Change of total protein concentration in BALF. (B) Level of lactate dehydrogenase (LDH) in BALF. Data was presented as mean ± S.E.M. * = P < 0.05 and ** = P < 0.01 vs. control.*

IL-6 is a second wave cytokine, and it has been reported that serum levels of IL-6 increased in lung cancer patients in relation to the intensity of the acute phase response (Yanagawa et al., 1995). Previous studies showed that TNF-α and IL-6 were produced in the airway or BALF during mycoplasmal infection in pigs, and lung inflammation was observed when the production of these cytokines was increased (Asai et al., 1993, Thacker et al., 2000). Therefore, in this study, the levels of inflammatory cytokines, including IL-6 and TNF-α, were determined in BALF to examine the inflammatory response to ZnO-NP exposure (*Fig. 5*). IL-6 levels were increased in middle and high dose treatment groups at day 1 (*Fig. 5A*). However, IL-6 levels in treated rats were the same as control rats at day 14 after instillation. This result indicates that the rat lung was able to recover IL-6 production during the 14 days after treatment. IL-6 enhances endothelial cell expression of leukocyte adhesion molecules and thereby causes leukocyte accumulation in the lung (Gabay, 2006, Barnes et al., 2011). In a previous study reported by Cicco et al investigated the ability of PMN to secrete IL-6 (Cicco et al., 1990). Thus, the increase of IL-6 in the middle and high-dose treated groups at day 1 may be caused by the increase of PMNs in ZnO-NP-treated animals, as PMNs showed the same trend as IL-6. Although IL-6 is mostly produced by macrophages, PMNs have also been reported as predominant sources of IL-6 in lung inflammation (Zhou et al., 1994). Therefore, we were able to confirm that the increase in PMN seems to be related to the observed increase in inflammatory cytokine levels in the BALF.

TNF-α is a cell signaling protein involved in systemic inflammation, and is one of the cytokines that make up the acute phase reaction (Koj, 1996). It is well known that the dysregulation of TNF production is implicated in various diseases, such as Alzheimer's disease (Swardfager et al., 2010), and inflammatory bowel disease (Brynskov et al., 1994). Therefore, we measured the concentration of TNF-α in the BALF. No significant differences between ZnO-NP-treated and control groups were detected at either day 1 or day 14 after exposure (*Fig. 5B*). A previous study examining IL-6 and TNF-α level in BALF after sub-acute or sub-chronic ZnO-NP exposure had

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noted that lower concentrations of IL-6 and TNF-α were found in the sub-chronic study compared to the sub-acute study (Lampinen et al., 2004). Furthermore, they also found that IL-6 and TNF-α increased immediately after acute exposure (day 1) and progressively decreased with time (day 14), which was similar to the result observed as in the chronic exposure. Therefore, the IL-6 levels found at day 14 in this study showed a similar pattern to the sub-chronic exposure.

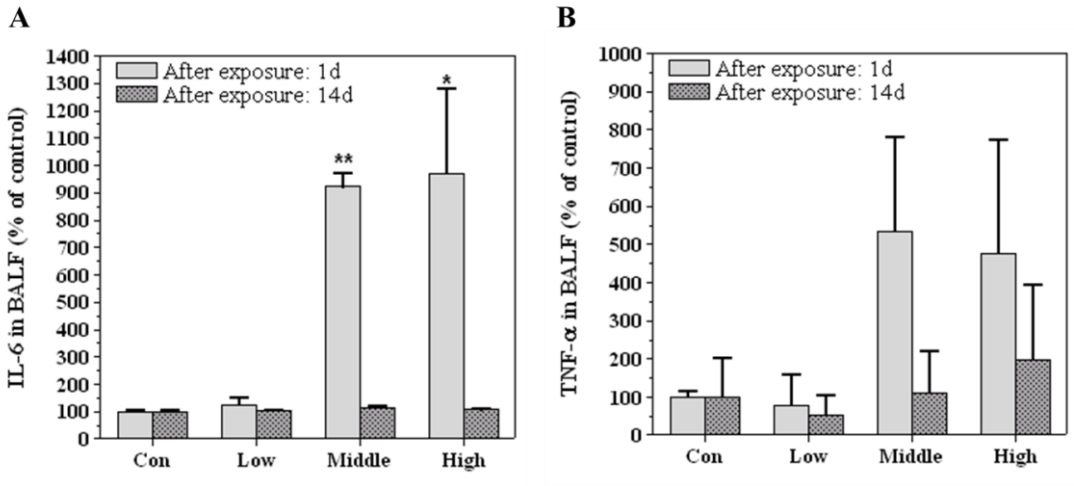


Figure 5. *Effect on secretion of cytokine in BALF after exposure of ZnO-NPs. (A) Level of IL-6 in BALF. (B) Concentration of TNF-α in BALF. Data was presented as mean ± S.E.M. * = P < 0.05 and ** = P < 0.01 vs. control.*

Zinc Burden

Zn content in the lung showed different results in relation to the treatment dosage (*Fig. 6*). Rats sacrificed after day 1 showed a significant increase in all the treatment groups (P < 0.05 for low and middle dose treated groups, P < 0.01 for the high dose treated group). However, by day 14, a significant increase in lung zinc burden was only observed in rats treated with a 5 mg/kg dose. These results indicate that Zn burden was dependent on ZnO-NP exposure quantities, and that Zn burden decreases with time.

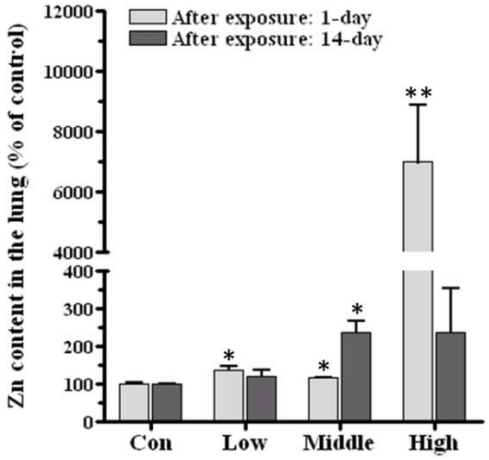


Figure 6. *The burden of zinc in the lung after intratracheal instillation of Zn-NPs. Data was presented as mean ± S.E.M. * = P < 0.05 and ** = P < 0.01 vs. control.*

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Histopathological Findings

Histopathological changes were observed in the lungs of rats from the control and treatment groups at day 1 after ZnO-NP exposure (*Fig. 7*). At this timepoint, multifocal acute inflammation was observed near the terminal bronchioles and alveolar ducts in all ZnO-NP exposed groups, however no inflammatory lesion was observed in the control group.The inflammation was particularly severe in the high dose group. In the high dose group, pulmonary edema was also often observed. Pervascular infiltration of inflammatory cells was detected in the ZnO-NP exposed groups in a dose dependent manner, showing 20%, 40%, and 60% incidence rate in the low dose, middle dose, and high dose exposure groups respectively. Inflammatory cell infiltration near the bronchiole was also observed when inflammatory foci were detected, suggesting that they were related to ZnO-NP exposure.

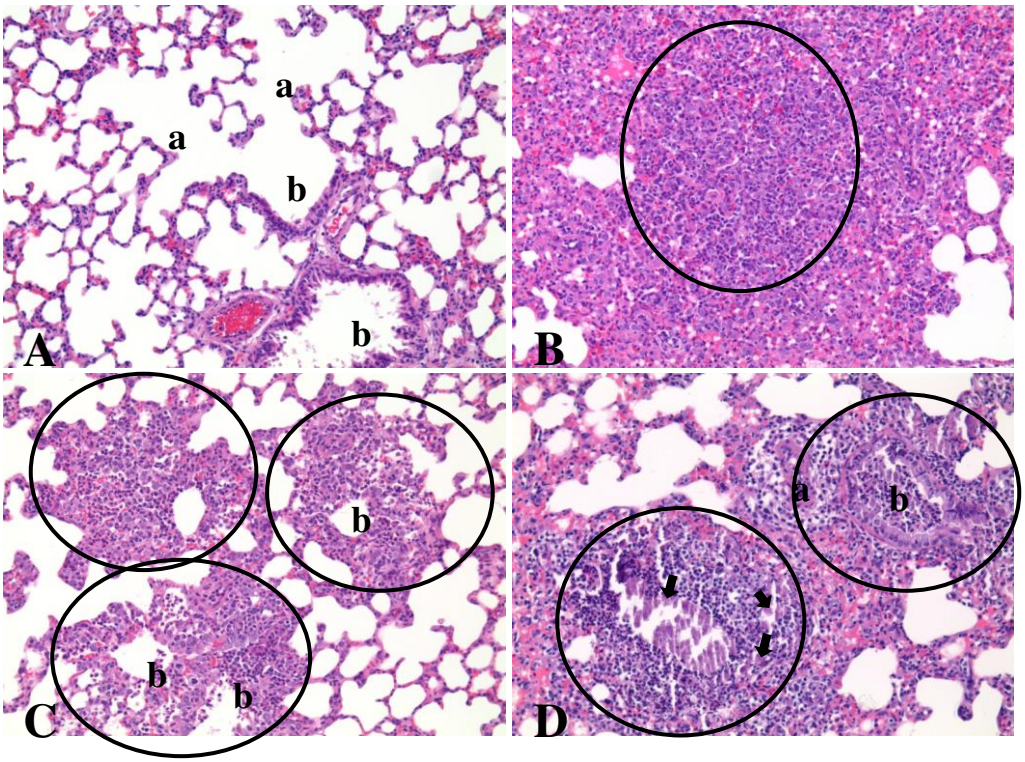


Figure 7. *Histopathology of lungs from rats treated with intratracheal ZnO-NPs at day 1 after treatment. Presence of acute bronchopneumonia, as characterized by neutrophil infiltration around the terminal bronchioles with type II alveolar cell hyperplasia, is marked with circles (B, C, and D). Dark brown foreign materials were often present in the lesion and bronchiolar lymina (arrows in D). a, arteriole; b, terminal bronchioles; ad, alveolar ducts. A = Control; B = Low Dose; C = Middle Dose; D = High Dose. H&E. Magnification=X200 for all.*

Histopathological changes were also observed in the lungs of rats from the ZnO-NP treatment groups at day 14 after the exposure (Fig. 8). Chronic histiocytic alveolitis, at the terminal bronchioles and alveolar ducts, was observed at rates of 40%, 60%, and 40% in low, middle, and high dose treatment groups respectively (Fig. 8). The presence of inflammatory cells may be explained as a chronic inflammatory mechanism, where the acute purulent alveolitis observed at day 1 has become chronic due to the passing of

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time. In contrast with the results from day 1, we were not able to observe purulent inflammation or pulmonary edema at the bronchiole at day 14, and this could be considered as a recovery. In low, middle, and high dose of ZnO-NP exposure groups, lymphocyte infiltration near the blood vessels were observed as 40%, 80%, and 80% respectively, showing a trend to dosage, although statistically significant difference among the groups were not observed.

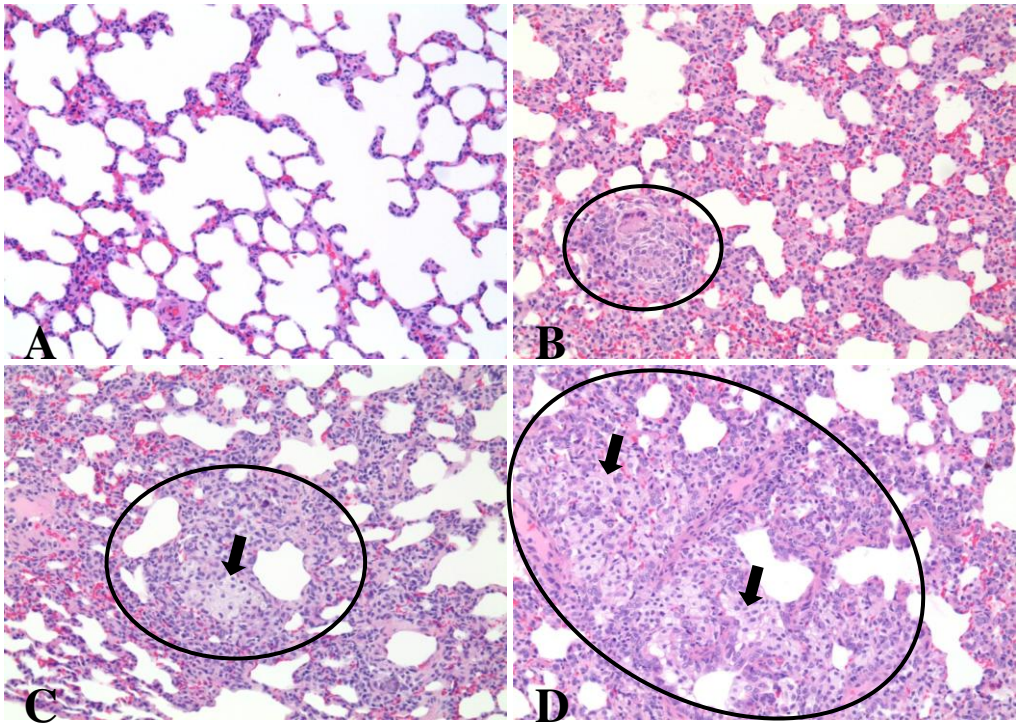


Figure 8. *Histopathology of lungs from rats treated with intratracheal ZnO-NPs at day 14 after treatment. Multifocal histiocytic pneumonitis, as characterized by massive infiltration of foamy macrophages(arrows), in the lungs of low, middle and high dose groups are marked with circles (B,C and D). a, arteriole; b, terminal bronchioles; ad, alveolar ducts. A = Control; B = Low Dose; C = Middle Dose; D = High Dose. H&E. Magnification=X200 for all.*

In previous studies, histopathological observations revealed serious pulmonary inflammation and alveolar wall thickening in the lungs of treated mice (Chang et al., 2012, Saptarshi et al., 2015). Although the experimental animal used was different, similar results were observed in our ZnO-NP exposure experiments. In this study, we observed histopathological differences between day 1 and day 14 after treatment, which suggests some recovery after exposure. Acute bronchopneumonia, as characterized by neutrophil infiltration around the terminal bronchioles with type II alveolar cell hyperplasia, was observed in the ZnO-NP treated groups at acute phase (marked with circles in Fig. 7). Multifocal histiocytic pneumonitis was identified at day 14 (Fig. 8), showing that full recovery had not yet occurred by this timepoint, and suggesting that acute inflammation had shifted to chronic infilammation. In the lesions, infiltration of foamy macrophages was frequently detected (arrow in Fig. 8), and macrophage size increased dose-dependently.

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Conclusion

In the present study, we investigated pulmonary toxicity and histopathology of the lungs of rats exposed to ZnO-NPs by intratracheal instillation. The number of macrophages were decreased at day 14 compared to day 1, although they were still higher than baseline levels, suggesting that the effect of ZnO-NPs still existed at 2 weeks after exposure. The total number of BALF cells increased in a dose-dependent manner after treatment, and continued to increase to day 14 after treatment. However, proportion of PMNs returned to baseline levels between 1 and 2 weeks after treatment with 5 mg/kg of ZnO-NPs. LDH levels did recover between day 1 and day 14. Levels of IL-6 increased significantly in rats given 5 or 10 mg/kg doses at day 1, while IL-6 levels were stable by day 14. Based on these results, we can say that recovery was present at day 14 after exposure, which was observed in previous sub-chronic exposure studies. Furthermore, we noted that intratracheal instillation of ZnO-NPs caused multifocal acute purulent inflammation in pulmonary parenchyma. The findings of this study suggest that acute exposure to ZnO-NPs may have deleterious effects in cells, which may lead to tissue damage and possible chronicization of pathological conditions. For further research, examination held after more than 14 days may be required to determine the long-term effects which may be caused by ZnO-NPs through ITI.

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COMBINED EFFECT OF CHICKPEA CULTIVATION AND TYPE OF FERTILIZER ON GROWTH, YIELD AND MINERAL ELEMENT CONCENTRATION OF CORN (*ZEA MAYS* L.)

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Abstract. The aim of this study was to assess effects of prior cultivation in combination with bio-fertilizers and the use of combined fertilizers and on corn (*Zea mays* L.) yield and soil efficacy. A strip-plot randomized complete block design with three replications was used in 2015 at the research farm of Payam-e Nour University in Arak, Iran. Treatments included the pre-cultivation with chickpeas and lying fallow. The fertilizer treatments were no organic fertilizer (control); manure containing 60 kg N per ha [FYM]; vermicompost containing 60 kg N per ha [VC]; FYM + 3000 kg chickpea waste/ha [M]; VM+M; FYM+M+ bio-fertilizer (Celolitec Kalchero) and phosphate-solubilizing bacteria [B] + azetobacter; vermicompost containing 60 kg N/ha + 3000 kg chickpea waste/ha + Celolitec Kalchero and; VC+M+B). The results showed that the effect of pre-cultivation with chickpeas on plant height, fresh forage yield, leaf phosphorus, leaf protein, organic carbon, available soil nitrogen and available soil phosphorus were significant. The effect of fertilizer type on all traits (except grain width) was significant. The highest phosphorus (5.53 mg/g), grain protein (10.57%), length of corncob (17.17 cm), grain width (2.18 mm), grain length (9.48 mm), fresh forage yield (22436.7 kg/h) and raw ash (6.60 mg/g) were obtained with the VC+M+B treatments. Leaf phosphorus and fresh forage yield after pre-cultivation with chickpeas were higher than for the fallow pre-treatment.
Keywords: *grain yield, biofertilizer, chickpea, vermicompost, fallow, organic matter*

Introduction

Corn is the world's third most important dual-purpose crop (grain and forage). It plays an important role in human and animal nutrition. Corn forage used for livestock feed provides a high amount of energy, is not harmful and is free of substances such as prussic acid and oxalic acid which are found in plants such as sorghum. It also can be consumed by livestock at all stages of growth (Dahmardeh et al., 2009; Hollander et al., 2007).

Recent attention has focused on the quality and health of the soil in order to produce sustainable crops. Many countries have encouraged the use of natural inputs out of growing concern for the safety of food products (Hollander et al., 2007). The frequent use of chemical fertilizers and lack of soil conservation in ways that are destructive to soil organisms eventually leads to loss of vitamins and other beneficial compounds in plants which are essential to their production (Hiltbrunner et al., 2007).

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Currently, the fertilization system focuses on the supply of a few macronutrients, although plants need at least 13 types of mineral nutrients which come from the soil. This is another reason for the imbalance in plants that are fed mineral fertilizers (Atiyeh et al., 2000).

Manure and vermicompost are widely used organic fertilizers. In addition to the usual benefits of compost, vermicompost is a source of organic substances, improves the water-holding capacity of the soil, increases absorption of nutrients and has a hormone-like effect on the plant (Bachman and Metzger, 2008; Campitelli and Ceppi, 2008). Vermicompost is finer and better in texture than compost and has nutrient forms that are easily absorbed by plants (Roy et al., 2010). Studies have shown that the presence of plant growth regulators in vermicompost improves plant growth (Atiyeh et al., 2000). A study on chickpeas showed that the application of three tons of vermicompost per hectare significantly increased the biomass over that of the control (Kapoor et al., 2002). Zaller (2007) showed that vermicompost significantly increased the biomass of tomato varieties.

Biological fertilizer is organic fertilizer derived from animals, plants and other types of waste that includes microorganisms that act in relation to the fixation of nitrogen, phosphorus and other nutrients in the soil (Kumar et al., 2014). One way to achieve sustainable agriculture is the use of microorganisms, which play an important role in the nutrition of plants (Abdel-Aziez et al., 2016).

Currently, phosphate-solubilizing microorganisms (PSMs) are used as biological fertilizer to increase yield and maintain soil health (Khan et al., 2010). Studies have shown that PSMs dissolve phosphorus that is fixed in the soil and improves plant yield (Gull et al., 2004). Research also showed that PSMs increase the germination, absorption of nutrients, plant height and number of branches, nodules, biomass and chickpea yield over that of the control (Rudresh et al., 2005).

One element that limits crop yield is nitrogen. Product waste, especially from legumes, which have a symbiotic relationship with the bacteria of the rhizobium family can improve soil nitrogen. The aim of this study was to investigate the effect of pre-cultivation with chickpeas and the application of phosphorus and nitrogen dissolvent and chickpea waste to increase the efficiency of vermicompost and manure for corn and soil efficacy.

Materials and methods

This experiment was done in 2015 on the research farm of Payam-e Nour University in Arak, Iran (34° N and 50° E at 1757 m in elevation). This experiment was a strip-plot randomized complete block design with three replications. The region has a cold steppe climate, an average rainfall of 250-350 mm, a maximum temperature of 40 °C and a minimum temperature of -7 °C (for more detail, see *Table 1*).

The pre-cultivation treatments were 1- chickpeas and 2- lying fallow. The organic fertilizers were as follows: 1- control (without fertilizer); 2- manure containing 60 kg N/ha (FYM); 3- vermicompost containing 60 kg N/ha (VC); 4- manure containing 60 kg N/ha + 3000 kg chickpea waste/ha (FYM+M); 5- vermicompost containing 60 kg N/ha + 3000 kg chickpea waste/ha (M+VM); 6- manure containing 60 kg N/ha + 3000 kg chickpea waste/ha + bio-fertilizer (Celolitec Kalchero) + phosphate-solubilizing bacteria + azotobacter (B+M+FYM); 7- vermicompost

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containing 60 kg N/ha + 3000 kg chickpea waste/ha + Celolitec Kalchero + phosphate-solubilizing bacteria (Barvar 2) + azotobacter (VC+M+B).

Table 1. *Soil physical and chemical properties of field*

EC (dS/m)	pH	Saturation Percentage	Available P (ppm)	Available K (ppm)	Total N (%)	Total C (%)	Soil texture
1.73	7.9	27.05	12.1	302	0.052	0.52	Sandy loam

Each plot consisted of four rows 6 m in length. The distance between rows was 35 cm and the plant spacing was 10 cm. The density of the plants was 28.5 plants/m^2. Before applying the treatments, soil sampling was done from different parts of the farm and the results are shown in *Table 2*.

Table 2. *Monthly meteorological data of the experimental site (Arak-2014)*

Climate variable	J	F	M	A	M	J	J	A	S	O	N	D
Precipitation (mm)	5.2	46.9	50.2	33.5	11.6	2.1	6	0.4	3.7	51.6	49.1	34.5
Mean temperature (°C)	3.6	5.8	8.1	14.9	20.8	27.1	28.5	26.9	21.4	16.5	6.6	-0.5
Maximum temperature (°C)	14.8	18.4	21.6	32.4	40.2	39.8	37.8	33.6	30.6	20.6	20.2	17
Minimum temperature (°C)	-8.8	-6.6	-6.6	1.6	6.2	13.6	15	15.4	8.4	5	-4.2	-8.8

In order to prepare the field for planting, fertilizer was added to the soil in the amount required for each treatment. It was mixed with the soil using a disc to a depth of 30 cm. The corn variety used in this experiment was the single-cross 704. Barvar 2 is a phosphate-solubilizing fertilizer containing *Pantoea agglomerans* strain (P5) and *Pseudomonas putida* strain (P13) that was used in some treatments. The date of planting was 6 June, which is the usual time for the region. During the growth season, irrigation was carried out as needed and weed control was done manually. The protein content of grain was determined by Kjeldahl method (N × 6.25, wet basis) (Wang et al., 2016).

Phosphorus contents of corn leaf and soil were determined using spectrophotometer (UVD 2960). Dried material was burned in a muffle furnace at 550°C and used to determine the raw ash content (Theuretzbacher et al., 2014).

At the early dent, after removing the half-a-meter margins from both sides of the planting lines and two lateral lines, harvesting was carried out near the soil surface. In each plot, six plants were selected. After measuring the growth characteristics of plant height, length, width and grain thickness, diameter and ear length and fresh yield, the plants were transferred to the laboratory and the amount of plant nutrients in them was determined. The protein content was determined as by Kjeldahl at N = 6.25. Phosphorus was estimated using a spectrophotometer. SAS (ver. 11) software was used to analyze the variance of data. LSD 0.05 values were used for comparison of means.

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Results

The results showed that the effects of prior cultivation on plant height, fresh forage yield, leaf phosphorus, leaf protein, organic carbon, available soil nitrogen and available soil phosphorus were significant at the 1% level. The effect of fertilizer type on grain length, ear length, plant height, fresh forage yield, phosphorus, leaf and seed protein content, organic carbon, available soil nitrogen and available soil phosphorus were significant at the 1% level and on grain thickness, corncob diameter, crude fiber and crude ash content at the 5% level. The interactions of prior cultivation and fertilizer type with plant height, organic carbon, available soil nitrogen and available soil phosphorus were significant at the 1% level (*Tables 3* and *4*).

Table 3. *Analysis-of-variance of corn traits under different fertilizer and prior cultivation treatments*

		MS							
S.O.V.	**d.f.**	**Grain length**	**Grain width**	**Grain thickness**	**Corncob length**	**Corncob diameter**	**Plant height**	**Fresh forage yield**	**Leaf phosphorus**
Replication	2	0.0512	0.1608	0.1545**	0.0535	0.3848	2.08	60016.7*	0.0196
Prior cultivation (a)	1	0.4588	0.0952	0.0415	0.0841	0.8204	443.92**	722859/5**	1.6521**
Error (a)	2	0.0588	0.2804	0.0042	0.0067	1.3188	0.86	2759-5	0.0020
Fertilizer (b)	6	0.8739**	0.0894	0.0420*	0.7442**	1.4968*	2114.19**	6116643/6**	10.3000**
Error (b)	12	0.0236	0.01874	0.0110	0.0110	0.3763	4.20	5630.6	0.0097
a*b	6	0.0394	0.2043	0.0001	0.0020	0.0804	18.76**	10437/3	0.0833
Error	12	0.0877	0.2139	0.0183	0.0106	0.4048	2.52	7945.6	0.0116
C.V.	-	0.98	7.46	0.98	0.61	2.98	1.35	0.42	2.99

*Significant at $P \leq 0.05$
**Significant at $P \leq 0.01$

Table 4. *Analysis-of-variance of corn traits under different fertilizer and prior cultivation treatments*

		MS						
S.O.V.	**d.f.**	**Leaf potassium**	**Grain protein**	**Raw fiber**	**Raw ash**	**Soil organic carbon**	**Available soil nitrogen**	**Available soil phosphorus**
Replication	2	1.60	0.4570	0.0202	0.0416	143211/3	911.9	24123.6
Prior cultivation (a)	1	508.22**	5.6467	0.0229	0.0293	1217320.9**	2981.2**	32121.2**
Error (a)	2	0.29	0.3553	0.0023	0.0113	2312345/0	499.5	7111.2
Fertilizer (b)	6	1707.60**	21.8756**	0.0072^{ns}	0.1906*	11598411.9**	1212.1**	32219.9**
Error (b)	12	0.68	0.1868	0.0061	0.0282	1168564/6	321.0	5049.0
a*b	6	4069**	0.0461	0.0006	0.0006	1214320.9**	1001.2**	12321.1**
Error	12	0.45	0.2312	0.0202	0.0119	431153/4	291.3	4025.6
C.V.		0.98	5.75	0.38	1.72	11.9	8.3	11.4

*Significant at $P \leq 0.05$
**Significant at $P \leq 0.01$

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Grain characteristics

The largest values for grain length and grain thickness were obtained from the treatments containing three fertilizers. Then next largest values were for the two-fertilizer treatment followed by a single fertilizer. The lowest amount was observed in the control treatment (without fertilizer). The length and thickness of the grains, respectively, were 14% and 12% for VC+M+B, 12% and 11% for FYM+M+B, 11% and 11% for VC+M, 11% and 9% for FYM+M, 8% and 7% for VC, 10% and 6% higher for FYM greater than for the control without fertilizer. In general, grain yield and grain thickness were higher in the compost treatments than in the manure treatments, but the difference between these was not significant.

Ear characteristics

The greatest lengths and diameters of corncobs were found in the three-fertilizer treatments and the lowest in the control treatment (without fertilizer). The length of the corncob in both the three-fertilizer treatments (VC+M+B and FYM+M+B) increased by 6% and in the FYM+M, VC+M, FYM and VC treatments by 5% when compared to the control (*Table 4*). The treatments containing compost resulted in greater corncob lengths than those with manure at all levels of fertilization, but the difference was not significant. All fertilizer treatments increased the corncob diameter over that of the control (*Table 5*).

Table 5. *Mean comparisons of corn traits under different fertilizer*

Treatments	Mean			
	Grain length (mm)	**Grain width (mm)**	**Corncob length (cm)**	**Corncob diameter (mm)**
CO	8.31^{e}	1.92c	16.12^{d}	20.24^{b}
FYM	9.12^{cd}	2.04^{bc}	16.91^{c}	21.32^{a}
VC	8.98^{d}	2.06^{ab}	16.92^{c}	21.34^{a}
FYM+M	9.20^{bc}	2.10^{ab}	16.98^{bc}	21.44^{a}
VC+M	9.26^{bc}	2.12^{ab}	17.02^{bc}	21.52^{a}
FYM+M+B	9.33^{ab}	2.15^{ab}	17.10^{ab}	21.70^{a}
VC+M+B	9.48^{a}	2.18^{a}	17.17^{a}	21.67^{a}

CO: control; FYM: manure containing 60 kg N/ha; VC: vermicompost containing 60 kg N/ha; M: 3000 kg chickpea waste/ha; N: bio-fertilizer (Celolitec Kalchero) + phosphate-solubilizing bacteria + azotobacter

Plant height

The plant height at all levels of fertilization except for VC+M, was higher with chickpea pre-cultivation than with no pre-treatment (6% increase in plant height over the control). The greatest plant height with and without cultivation was observed for VC+M+B and the lowest was for the control (*Table 8*).

Yield of fresh forage

The highest fresh forage yield was obtained for VC+M+B and FYM+M+B (15% and 14% increase over the control, respectively). The VC+M and FYM+M combinations

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increased 13% and 11%, respectively, over the control and the VC and FYM treatments increased 9% and 4%, respectively, over the control (*Table 6*). The use of vermicompost in all fertilizer treatments increased the yield over that of the manure treatment. The prior cultivation treatment significantly increased the yield of forage by 361 kg over the fallow treatment (*Table 6*).

Table 6. *Mean comparisons of corn traits under different fertilizer*

Treatments	Mean			
	Fresh forage yield (kg/ha)	**Leaf phosphorus (mg/g)**	**Grain protein (%)**	**Raw ash (mg/g)**
CO	19473.3^{f}	1.96^{g}	5.17^{e}	6.08^{e}
FYM	20220.0^{e}	2.52^{f}	7.01^{d}	6.23^{de}
VC	21220.7^{e}	2.70^{e}	7.54^{d}	6.30^{cde}
FYM+M	21586.7^{d}	3.64^{d}	8.72^{c}	6.35^{bcd}
VC+M	21991.7^{c}	3.89^{c}	9.32^{b}	6.45^{abc}
FYM+M+B	22163.3^{b}	4.95^{b}	10.16^{a}	6.52^{ab}
VC+M+B	22436.7^{a}	5.53^{a}	10.57^{a}	6.60^{a}

CO: control; FYM: manure containing 60 kg N/ha; VC: vermicompost containing 60 kg N/ha; M: 3000 kg chickpea waste/ha; N: bio-fertilizer (Celolitec Kalchero) + phosphate-solubilizing bacteria + azotobacter
Within columns, means followed by the same letter do not differ significantly ($p \leq 0.05$)

Leaf phosphorus

This trait was strongly influenced by the type of fertilizer used. The amount of phosphorus of in the leaves of the VC+M+B and FYM+M+B treatments increased by 182% and 152%, respectively. For the VC+M and FYM+M treatments, respectively, it increased by 98% and 86% and in the VC and FYM treatments by 38% and 28%, respectively (*Table 6*). Fertilizer treatments containing vermicompost showed significantly higher levels of phosphorus over treatments containing manure (*Table 6*). The difference between the fallow and pre-cultivation with chickpeas was significant in terms of leaf phosphorus content. The chickpea treatment showed an 11% increase in leaf phosphorus over the fallow treatment (*Table 7*).

Table 7. *Mean comparisons of corn traits under different prior cultivation treatments*

Treatments	Mean	
	Fresh forage yield (kg/ha)	**Leaf phosphorus(mg/g)**
Fallow	21291.81^{b}	3.39^{b}
Pre-pea cultivation	21553.18^{a}	3.80^{a}

Within columns, means followed by the same letter do not differ significantly ($p \leq 0.05$)

Grain protein

The highest protein content in the seeds was observed in the three-fertilizer treatments. The lowest protein content was observed in the non-fertilized (control) treatment. Grain protein content in the VC+M+B and FYM+M+B increased by 104%

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and 97%, respectively. In the VC+M and FYM+M treatments, it increased 80% and 67%, respectively, and in the VC and FYM treatments, it increased 46% and 36%, respectively, over the control (*Table 6*). There was no significant difference in this trait between the vermicompost and manure treatments.

Raw ash

The highest raw ash content was observed in the three-fertilizer treatment of VC+M+B (9% increase compared to control); however, the difference was not significant from the FYM+M+B (7% increase compared to control) and VC+M (6% increase compared to control). The lowest ash content was observed in the control treatment (*Table 6*).

Soil properties

The traits of soil organic carbon, available soil nitrogen and available soil phosphorus were strongly influenced by the simple effects of fertilizer type and pre-cultivation and the interaction of these treatments was also significant ($p < 0.01$; *Table 4*). Pre-cultivation with chickpeas at almost all levels of fertilizer increased the levels of organic carbon, available soil nitrogen and phosphorus because of the effects of chickpea cultivation that remained in the soil. Soil organic carbon is one of the most important factors for soil and production capacity and is a major reason for the higher values of most traits in the pre-cultivation with chickpea treatment over the fallow treatment. Fertilizers contained higher amounts of organic carbon compared to the control. The highest available soil nitrogen levels were 0.887% and 0.844% in the VC+M+B and FYM+M+B treatments, respectively, in the pre-cultivation with chickpea treatment (*Table 8*).

Table 8. *Interaction effect of fertilizer and prior cultivation on corn height and soil properties*

Pre-cultivation	Fertilizer treatment	Plant height (cm)	Organic carbon (%)	Available soil N (%)	Available soil P (ppm)
Fallow	CO	85.6 ^{j}	0.503 ^{e}	0.524 ^{h}	12.4 ^{g}
	FYM	98.2 ^{h}	0.531 ^{d}	0.642 ^{g}	13.8 ^{f}
	VC	104.5 ^{g}	0.532 ^{d}	0.670 ^{g}	13.9 ^{f}
	FYM+M	112.6 f	0.548 ^{c}	0.708 ^{fg}	14.7 ^{cd}
	VC+M	125.3 ^{d}	0.546 ^{c}	0.738 ^{ef}	14.8 ^{cde}
	FYM+M+B	132.6 ^{c}	0.550 ^{c}	0.722 ^{ef}	15.1 ^{cd}
	VC+M+B	138.4 ^{b}	0.554 ^{c}	0.741 ^{def}	15.3 ^{bc}
Chickpea	CO	92.8 ^{i}	0.492 ^{f}	0.538 ^{h}	12.4 ^{g}
	FYM	106.3 ^{g}	0.553 ^{c}	0.724 ^{ef}	14.2 ^{ef}
	VC	113.9 ^{f}	0.572 ^{b}	0.759 ^{de}	14.4 ^{def}
	FYM+M	119.8 ^{e}	0.589 ^{a}	0.796 ^{cd}	15.1 ^{cd}
	VC+M	124.0 ^{d}	0.570 ^{b}	0.803 ^{bc}	15.9 ^{ab}
	FYM+M+B	140.6 ^{b}	0.597 ^{a}	0.844 ^{ab}	16.3 ^{a}
	VC+M+B	145.3 ^{a}	0.588 ^{a}	0.887 ^{a}	16.5 ^{a}

CO: control; FYM: manure containing 60 kg N/ha; VC: vermicompost containing 60 kg N/ha; M: 3000 kg chickpea waste/ha; N: bio-fertilizer (Celolitec Kalchero) + phosphate-solubilizing bacteria + azotobacter

Within columns, means followed by the same letter do not differ significantly ($p \leq 0.05$)

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The highest available soil phosphorous was found in the three-fertilizer combination under pre-cultivation (16.5 and 16.3 ppm. respectively), dual fertilizer (15.9 ppm) and VC+M+B under fallow conditions (15.3 ppm). These results show how the strong synergistic effects of various fertilizers in the soil to improve its physical and nutritional status. Other researchers have reported vermicompost as a soil organic modifier for increased nitrogen and phosphorus absorption (Sekar and Karmegan, 2010).

Discussion

Based on the results of this experiment, the combination of fertilizers that included organic fertilizer, corn residue and biofertilizer achieved the high yield. It appears that the increase in absorption of nutrition by the plant in this type of treatment and subsequent improvement in growth conditions led to the increased yields.

Manure and vermicompost alone managed to improve the nutritional status of the plant and, subsequently, increased the relative yield compared to the control, but this increase was lower than for the combined fertilizer treatments. Darzi and Haj-Sihedadi (2012) reported that the use of high levels of vermicompost in dill cultivation significantly increased the yield and plant height. Other researchers reported an increase in the physical, chemical and biological properties of soil and improved plant nutritional conditions that were attributed to the increase in the duration of vegetative and reproductive growth, the better use of growth resources and an increase in yield components and grain yield (Arankon et al., 2004). Roesti et al. (2006 improvement of the soil fertility, prevention of the leaching of nitrogen, an increase of biological activity and improvement of the soil structure as resulting from the use of vermicompost. Vermicompost application has been found to stimulate soil microorganisms, maintained the supply of nutrients to the plant and increase yield (Roy and Singh, 2006). The use of vermicompost, in addition to supplying nutrients from worm residue, improves the soil biological conditions and increases the microorganism activity in the soil (Anwar et al., 2005). An increase in phosphorus uptake in the soil was found to increase soil nitrogen efficiency and ultimately result in better plant growth (Zubigala et al., 2002).

Simultaneous use of organic fertilizers, plant residue and biofertilizer increased the absorption of elements from the soil, proper growth of the plant and produced the highest biological yield and seed yield in corn. In many other studies, PSMs have also increased the phosphorus concentration in the soil and plant and ultimately improved yield (Gull et al., 2004). Research has also shown that the use of these microorganisms increased and improved the germination ability, absorption of elements, plant height, number of branches, biomass and yield of chickpeas compared to the control treatment (Rodrish et al., 2005).

These biofertilizers increase nutrient availability and produce growth hormones by increasing nitrogen efficiency. Gull et al. (2004) observed that phosphate-solubilizing bacteria increased the number of nodules in chickpeas by 50% and increased N fixation and concentration in the plant. The application of phosphorus solubilizing bacteria increased the phosphorus concentration, which increased nitrogen efficiency, which increased development of the plant shoot, root and leaf area and, ultimately, the yield (Soltani et al., 2010). Mokhtari and Besharati (2013) also showed that the use of phosphorus-soluble bacteria significantly increased the concentration of nitrogen, phosphorus, potassium, iron, and zinc compared to the control, which significantly increased the yield over that for the absence of bacteria.

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In the current study, pre-cultivation with chickpeas had positive effects on phosphorus soil availability and absorption, which increased the corn forage yield. Other studies have also found a positive effect of the adding of plant residue, especially legumes either by reversing the residue of the previous crop or adding residue to the field on soil characteristics, nutrient reserves in the soil and plant tissues and yield (Wilhelm et al., 2004). Fischer et al. (2002) showed that the return of plant residue is effective in providing more nitrogen and increasing the yield of corn and wheat, especially in the long-term, along with a more gradual decrease in soil fertility and degradation of yield. This makes the procedure economically advantageous.

Conclusion

As a conclusion the effect of pre-cultivation with chickpeas on more plant traits were higher than fallow. The highest phosphorus, grain protein, length of corncob, grain width, grain length, fresh forage yield and raw ash were obtained with the VC+M+B treatments. In more traits there were a synergistic effects of pre-cultivation with fertilizers compared with fallow plots.

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ECOSYSTEM MODELING OF THE RESETTLED MARITIME AREA OF THE BAY OF BENGAL, BANGLADESH THROUGH WELL-ADJUSTED ECOPATH APPROACH

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Abstract. This study was selected to assemble the current information in order to establish a mass-balanced ecosystem model within the resettled maritime limit zone of the Bay of Bengal, Bangladesh through ECOPATH approach covering over 90000 km^2. A total of 19 functional groups were considered demonstrating all trophic levels in the food web where the estimated trophic interactions between the groups were varied from 1 (primary producers and detritus) to 3.45 (sharks). The ecotrophic efficiency (EE) of most of the consumers was >0.80; indicating a largely exploited ecosystem and high energy transfer from lower to higher trophic levels. Moreover, the gross efficiency (0.0018) and transfer efficiency (11.12%) of the whole system symbolize the 'developing systems' with somewhat maturity. Ecosystem's overhead (64.6) and ascendancy (35.4) also designate the ecosystem's stability. Thus, this study concludes that the resettled maritime area of BoB reserves significant backup strength to face stress situations having capacity to rapid restoration to the original states.
Keywords: *Ecopath with Ecosim (EwE), Bay of Bengal (BoB), ecological groups, maritime ecosystem, mass balance*

Introduction

Ecosystems are superbly balanced although they are reasonably complex, non-linear and their structures are composed by the interconnection of living and non-living bodies with their habitat. In every portion of an ecosystem, has a significant role to play and if any alteration is occurred to any portion of a particular ecosystem, the whole ecosystem of that area can also be altered. This is not only dynamic but also hierarchically ascended. Ecosystems structures and processes are associated across the spatial and temporal balances. Due to their complication and the array of negative and positive response across balances, the predictability of these structures is inadequate (Gunderson, 1999). Sustainable usage of ecosystem components is unlikely in absence of a better understanding of balancing ecosystem dynamics that foster ecosystem capability (Bengtsson et al., 2003). Coastal and marine ecosystems are among the largest aquatic ecosystems of the world, covering 71% of the earth and fish populations are one of the most vital parts of these systems (BOBLME, 2011). Therefore, management approach that is based on fisheries ecosystem has reaped consideration for maintaining long-term sustainability to balance ecosystems (ICES, 2000). In recent, the ability of marine ecosystems to produce fish for supporting human interest is extremely degraded by

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overfishing, excessive trawling, and loss of spawning and nursing grounds (McGlade et al., 2002). Noticeable limitation exists between single and multispecies fisheries management, which does not consider a comprehensive assessment of changes in ecosystem arrangement, and functions linked with the interactions of individuals (Mace, 2001; Pikitch et al., 2004). The concept of ecosystem-based fishery management needs the improvement of tools to gain perceptions in ecosystem functioning and the effect of fishing on ecosystem structure. Some of the critical issues like interspecies interactions within an ecosystem, effects of the enormous environmental and climatic alterations along with the fishing impacts should be incorporated to formulate an ecosystem based management strategy in any habitat (Browman et al., 2004; Pikitch et al., 2004).

Recent improvements in constructing multispecific ecosystem based modeling approach of aquatic ecosystems named "Ecopath with Ecosim (EwE)" are successfully used to evaluate the structure and ecosystem effects of fishing (Polovina, 1984; Christensen and Pauly, 1992). This approach provides a base for feasible and applied simulation model having actual predictive power that can be performed more rapidly and rigorously than ever before (Christensen and Pauly, 1993; Walters et al., 1997; Christensen and Walters, 2004). The construction of such mass-balance models of ecosystems are based mainly on food consumption, diet composition, biomass and mortality estimates. The ECOPATH packages of software have now been improved (Walters et al., 1997, 1999) through including ECOSIM (simulation module) and ECOSPACE (spatial module). These new modules have not only upgraded the quantitative command of the approach but also permitted qualitatively new inquiries.

Recently, Bangladesh has been settled maritime boundary with neighboring states Myanmar and India up to 200 nautical miles from the coastline comprising about 121110 km^2 (MoFA, 2014), whereas coastal and shallow shelf waters constitute about 20% and 35% respectively, the rest covers deeper waters (Khan et al., 1997). The entire shelf area of Bangladesh (up to 200 m depth contour) covers about 66440 km^2 and from the coastline to 10 m (0-10 m) depth zone comprises 24000 km^2 (*Table 1*). Nevertheless, in the context of fisheries resources, from the coastline to 200 m depth zone plays a significant role as it almost covers four major fishing grounds of Bangladeshi waters and also produces most of the marine catch (Lamboeuf, 1987; Khan et al., 1997). The declared areas of four major fishing grounds (*Fig. 1b*) are 'South Patches' and 'South of South Patches' covering an area about 6200 km^2. In addition, 'Middle Fishing Ground' contains 4600 km^2 area (*Table 1*) and 'Swatch of No Ground' covers 3800 km^2 (Khan et al., 1997). Among them 'Middle ground' and 'South patches' have been declared as 'Fish sanctuaries' in the Bay of Bengal. Still, the detailed ecological and oceanographic data of these areas are limited to determine the suitability of these areas as fish sanctuary.

In the context of the recent settlement of the maritime boundary, 'Blue Economy' recently turned into a buzzword for sustainable development, particularly in highlighting the vision of SDG (Sustainable Development Goals) for Bangladesh. The Government of Bangladesh emphasized those marine and coastal fisheries resources based blue economy might act as a driver for sustainable development, designating development not only for today but also for the upcoming years (Shamsuddoha and Islam, 2016). Moreover, this new relocated vast area could not be accessible to fishing due to the lack of vessel capability and suitable fishing technology (Hossain et al., 2014). The Bay of Bengal (BoB) defined as a temperately productive ecosystem among the world's 64 Large Marine Ecosystems (Hussain and Hoq, 2010) has great potential

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for fisheries because of the enormous nutrient input from the river flow of the Ganges and Brahmaputra (ESCAP,1988). A total of 475 species of finfish, 36 of shrimps, 15 types of crabs, 5 varieties of lobster, and over 300 species of mollusks were recorded (FRSS, DoF). The fishes which are presently exploited consist mainly of the shallow water estuarine species and some mid-water species. The average total marine industrial catch over the last decade (*Table 1*) was 540592 Mt (DoF, 2016). Functionally, marine fisheries are sub-divided into industrial and artisanal fisheries. The average production of the last ten years shows that the industrial fishery based on trawl fishery (shrimp and fish trawl) contributes only 5% of the total marine captures and the artisanal fisheries (mainly based on Set bag net and Gillnet fisheries) contributes 95%.

Table 1. *The geographic location and covered areas of the major fishing grounds of the EEZ of the Bay of Bengal, Bangladesh (Khan et al., 1997)*

Depth zone (m)	Area (km^2)	Fishing grounds	Location	Depth
≤ 10	2400	South Patches	(Lat. 20°50'N to 21°40'N;	10-40 m (depth)
10-24	8,400	(3400 km^2)	Long. 91°00'E to 91°50'E)	
25-49	4,800	South of South Patches	(Lat. 20°50'N to 21°40'N;	10-100 m (depth)
50-74	5,580	(2800 km^2)	Long. 91°00'E to 91°50'E)	
75-99	13,410	Middle Ground	(Lat. 20°50'N to 21°20'N;	10-100 m (depth)
100-199	10,250	(4600 km^2)	Long. 90°00'E to 91°00'E)	
All shelf	66,440	Swatch of No Ground	(Lat. 21°00'N to 21°25'N;	10-100 m (depth)
		(3800 km^2)	Long. 89°00'E to 90°00'E)	

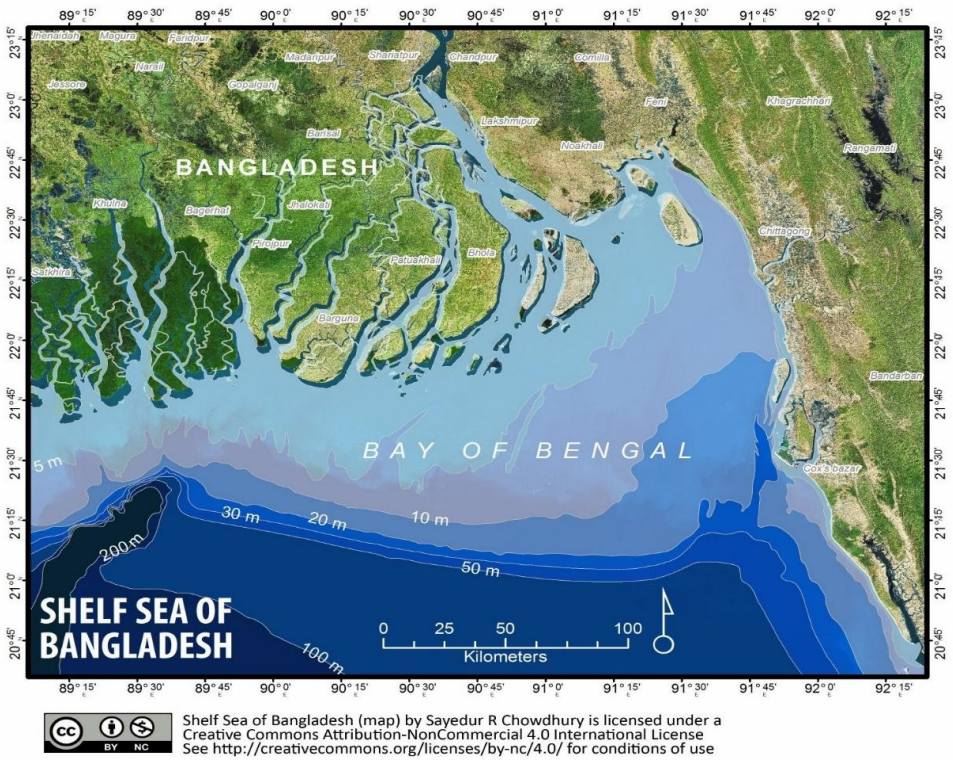
Several surveys and studies have been piloted to assess and estimate the potentialities of marine fisheries resources of Bangladesh since 1970 to 1980 by the collaboration of National and International agencies, but no fresh assessment is available. Limited reports are found focusing on biological, environmental and resource management concerns of the Bay of Bengal (BoB) (Hossain, 2003; Huntington et al., 2007; Hussain and Hoq, 2010), but such pieces of evidences are insufficient to consider and manage of our marine resources systematically. Conversely, in the near future, there is a possibility of dropped off the fisheries role on people's livelihoods as the stocks of various fish and shrimps are showing diminishing trends due to effects of climatic change, water pollution and fishing pressure (BOBLME, 2011). In recent, Mustafa (2003) made an attempt to describe the ecosystem features of BoB based on trawl catch data but overlooked the most important artisanal fishing areas and Ullah et al. (2012) described the shallow coastal areas not exceeding the 10 m in depth. Therefore, the maritime waters of the BoB, Bangladesh remain to be one of the most unfocused areas in the world, thus limiting the exploitation, exploration, conservation and management of its marine, though this zone is recognized as one of the world's ample and diversified ecosystems considered by greater productivity (Islam, 2003). All of these studies considered only short-term available data that not elucidate long-term outputs properly. Hence, attempts are made by authors to drive a new study concerning management of the maritime resources of the BoB, Bangladesh using the ecosystem scheme. The EwE (ECOPATH with ECOSIM; version 6.5) routine package was used in the current study to develop the mass-balance ecosystem model. The aim of this study is to illustrate the interaction between the diverse ecological groups and to define the flow of energy in the maritime area of Bay of Bengal, Bangladesh. In addition, another aim of this study is to measure the existing capability of the maritime ecosystem of BoB, Bangladesh to cope with the emerging "blue economy" pressure.

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Materials and methods

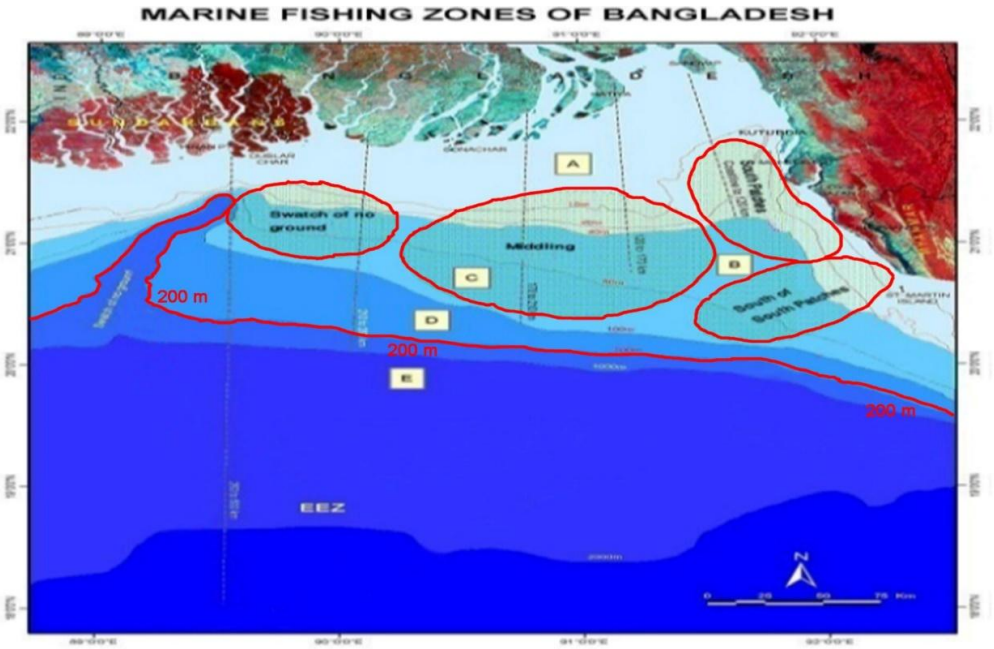
Study areas

The Bangladesh part of the Bay of Bengal is located between 20° N and 22°30' N latitude, and 89◦ E and 92°30' E longitudes (*Fig. 1a*). The current study mainly focuses on the ecosystem structures of the major fishing grounds of the BoB, which lies from the baseline to 200 m depth zone (*Table 1*) occupying about 90000 km^2 that covers not only 24000 km^2 of inshore area but also 66440 km^2 of all shelf areas. The demarcation line is used to illustrate the depth zone and fishing grounds in the current study (*Fig. 1a, b*). Nevertheless, this area of both offshore and inshore water covers more than 90% of fishing activities in the country (DoF, 2015a) and thus, this model can be designated as 'resettled maritime ecosystem of Bay of Bengal, Bangladesh'. The bottom topography of the shelf sea is usually coarse sandy and muddy with some rocky blotches, but the shallow inshore areas are dominated by muddy bottom. Moreover, the shelf zone down to about 150 m seems to be quite smooth with very limited obstacles to bottom trawling whereas due to existence of precipitous slope trawling is not possible into the waters deeper than 180 m (Khan et al., 1997). The oceanographic features in BoB mainly depend on three key factors: (i) precipitation, (ii) wind direction; and (iii) mass siltation from the river discharges (Lamboeuf, 1987) that have a strong influence on the abundance and distribution of fishes too. The annual average temperature is about 26°C and the yearly average rainfall of the BoB, Bangladesh is approximately 3800 mm where over 80% precipitation is received from June to September (DoEF, 2015). Hence, these areas are treated as a sole homogenous area as they cover a wide range of marine habitats. However, this zone has been carefully chosen for the present study because of the availability of fisheries landing data.



a

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b

Figure 1. ***a.*** *Total shelf area of the Bay of Bengal, Bangladesh highlighting the depth zones (Sayedur, 2014).* ***b.*** *Existing marine capture fisheries zones of Bangladesh focused on the major fishing grounds area*

Overview of ECOPATH model (version 6.5)

The software 'ECOPATH' was designed for analyzing trophic dynamic interactions within the fisheries resources systems (Christensen and Pauly, 1992, 1995). This scheme is based on the previous work, which was first demonstrated by Polovina (1984), and it has been widely applied to aquatic systems after successive improvements (Christensen and Pauly, 1993; Pauly et al., 2000). Ecopath is basically a large spreadsheet that is instantaneously keeping track of all the individuals or species and entire feeding interactions occurring within the ecosystem (Christensen and Pauly, 1993). This approach designates an ecosystem at steady-state for a certain period and assumes mass balance in production of any specified prey that is equivalent to the biomass consumed by predators in combination with the estimated captured biomass (e.g. in fisheries) and any other exports from the system, i.e. (*Eq. 1*):

$$B_i \left(P / B\right)_i EE_i = Y_i + \sum\left(B_j\right) \left(Q / B\right)_j DC_{ij} + EX_i \qquad \text{(Eq.1)}$$

B_i represents the biomass of prey i group; P/B_i is the production/biomass ratio of that group; EE_i is the ecotrophic efficiency. Y_i symbolizes the yield (or catch rate of fisheries) of i groups; B_j is the biomass of predator j group and Q/B_j is the food export or consumption per unit biomass of j, DC_{ji} is the fraction of prey i in the diet of j and EX_i is the export of i. When the input values of the parameters of the model are delivered, ECOPATH assesses the missing parameters for each group within the model, e.g. the annual biomass production, the annual biomass consumption or ecotrophic efficiency for each functional groups of the ecosystem.

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Model construction

Fisheries landing data

Existing marine capture fisheries of BoB are categorized into two major sub-divisions, i.e. industrial trawl captures and artisanal captures which put up about 95% of the total marine landings. The term 'industrial captures' indicate 'large-scale' which mainly focus on demersal fish and penaeid shrimps in the offshore waters. Artisanal consist of a number of diverse categories of traditional fishing gears and crafts i.e. gillnets, set bag nets (SBN), longline, trammel net, purse seine etc (*Table 2*).

Table 2. *Gear wise landed total marine catches (metric ton) since 2005-06 to 2014-15 (10 years) of Bangladesh*

Year	Fishing types (fleets/gears)						Total marine catch (MT)
	Industrial trawl catch	*Gillnet	*Set bag net (SBN)	*Longline	*Trammel net	*Others (Purse seine)	
2005-06	34084	264627	153687	16224	7399	3789	479810
2006-07	35391	265668	154736	15325	12883	3435	487438
2007-08	34159	274526	159043	13856	12466	3523	497573
2008-09	35429	296634	154164	16724	9680	2013	514644
2009-10	34182	297332	149142	18373	12608	5645	517282

*Fishing gears that used for large-scale commercial catch having different net mesh size (Gillnet, Trammel net and Purse seine consist of fine twin nylon net while Longline consist of hooks and rope lines and Set bag net having sack like bag at it's cod end)

Annual catch data of the last 10 years (from 2005-06 to 2014-15) from the Fisheries Resource Survey System (FRSS) of Department of Fisheries (DoF) of Bangladesh were considered in this study (*Table 2*). Due to the scarcity of an efficient data collecting and recording system, it is not possible to find the total species-wise annual landing data but group-wise data in FRSS. In addition, some of the reference reports (Mustafa, 1999; Nabi, 2007) and reports from the NGO organizations (eg. FAO country data) were also considered for this study.

Ecological or functional groups

Functional groups were characterized based on similarities in feeding habits, bulk body mass, life history parameters, physiological behavior and spatial distributions to keep homogeneous characteristics throughout the species within a group (Yodzis and Winemiller, 1999). More than 82 fish species and 20 types of shrimps and crustaceans are regularly collected by FRSS (DoF, 2015b), thus, over 77 taxonomic families of fish, shrimps, crustaceans, mollusks were included in this study. Representative species were selected based on their significance to fisheries and available information in the statistics (*Table 3*).

Greater preference was given to collect data from those pieces of literatures having local and regional data for every group when life history parameters, diet composition, food consumption, habitat and other information were considered for the modelling (*Table 4*). Where diet data were not available, information from similar ecosystems was

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considered. Fishbase (www.fishbase.org; Fröese and Pauly, 2006) has also been applied to link up the gaps whenever possible. Data sources of non-fish groups are given in *Table 5*.

Table 3. *Ecological or functional groups used in ECOPATH model including estimated total catch and percent composition of the maritime ecosystem of BoB, Bangladesh*

Ecological or functional group	Major families or species	Total catch (MT)	Catch composition (%)	Explanation (habitat/fishing type)
1. Sharks	Carcharihinidae Squalidae Sphyrnidae Rajidae	3245	0.97	Down to 30 m depth Mostly Longline, SBN and Gillnet
2. Rays	Rhinobatidae Dasyatidae	2005	0.001	Down to 30 m depth Mostly Longline, SBN
3. Diadromous (Pelagic)	*Tenualosa ilisha* *Tenualosa toli*	215500	39.8	0-100 m depth, mostly by Gillnet
4. Minor Pelagics	Muglidae Gobidae *Setipinna spp* *Coilia dusumieri* *Gudusia chapra* Other Clupeids (Sardines) Engraulidae (Anchovies) Exocoetidae (Flying fish)	17530	3.2	Down to 10 m depth Mostly SBN, Gillnet and few catch by trawls and purse seines
5. Medium Pelagics	Scombridae *Scomberomurus guttatus* *Rastrelliger kanagurta* Other Mackerals *Euthunnus affinis* *Parastromateus niger* *Megalaspis cordyla* *Pterotolithus maculatus* *Decapterus spp.*	29352	5.4	10-100 m depth Mostly Gillnet and few catch by trawls
6. Minor Mesopelagics	Leiognathidae	6240	1.15	10-100 m depth Mostly Gillnet catch
7. Medium Mesopelagics	*Lepturacanthus savala* *Pampus chinensis* *Pampus argenteus* Trichiuridae	27843	5.15	10-100 m depth Mostly SBN and Gillnet, slightly by trawls
8. Medium Demersal	*Lates calcarifer* Lutjanidae (Snappers) Synodontidae (Bombay duck) Sciaenidae (Jew fish)* Pomadasidae	141434	27.7	0-100 m depth Mostly SBN and Gillnet, slightly by trawls and trammel net *Longline also used in fishing

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	Johnius argenteus *Polynemus indicus* *Acanthopagrus latus* Ariidae* Lethrinidae *Eleutheronema spp.* *Otolithes spp* *Protonibea diacanthus* *Otolithoides spp.*			
9. Minor Demersal	Nemipteridae *Harpodon nehereus* *Saurida tumbil* Serranidae (Groupers) *Agryrops spinifer* Sparidae *Cynoglossus spp* Tetradontidae Priacanthidae *Tricanthus spp* Platycephalidae	47528	8.8	0-100 m depth Mostly SBN and Gillnet, slightly by trawls
10. Penaeid Shrimp	*Penaeus monodon* Other Penaeids *Metapenaeus monceros* *Metapenaeus spinulatus* *Parapenaeopsis stylifera*	34523	6.4	0-90 m depth Mostly by SBN and major target species by shrimp trawls
11. Other Shrimp	*M. rossenbergii** *Ascetes indicus*	9019	1.67	0-40 m depth, Mostly by SBN *Estuarine catch
12. Other crustaceans	Lobstars Crabs	2017	0.37	10-50 m depth and Intertidal zone
13. Cephalopods	Loligo Sepia Octopus	4356	0.8	10-100 m depth Mostly by catch of various gear and some catch by Squid jiggers
14. Mollusks	Bivalves			
15. Benthos	Annelids, Polychaetes, worms	-	-	0-40 m depth
16. Aquatic Invertebrates	Meiobenthos, Jelly Fish etc	-	-	0-50 m depth
17. Zooplankton	Copepods etc.	-	-	
18. Phytoplankton				
19. Detritus				

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Basic parameters of fish

Estimation of biomss (B): The biomass of each ecological group per unit area in the habitat area (i.e. t/km^2) was estimated in average by using Gulland (1971) formula of $B = Y/F$, where Y represents the annual yield of each group and F symbolizes the coefficient of fishing mortality. Biomass of sharks and other unexploited groups, i.e. benthos, aquatic invertebrates, zooplankton, phytoplankton, and detritus were obtained from various reference data (*Table 5*). In addition, biomass of other crustacean, gastropod and cephalopod groups were obtained from Arreguin-Sanchez et al. (1993) and Christensen and Pauly (1993).

Estimation of production/biomass (P/B): Estimation of this ratio is equivalent to the total mortality (Z) (Pauly et al., 2000) as it was really hard to assess directly. Consequently, this parameter was calculated by obtaining the sum of the fishing (F) and natural (M) mortalities while the entry of *P/B* ratios is optional. The estimated *P/B* values and other population parameters are given in *Table 4*.

Table 4. *Life history parameters of the functional fish groups that were selected for ECOPATH model in the maritime ecosystem of BoB, Bangladesh*

Representative species	L_{inf} (cm)	K (/yr)	Z (/yr)	F (/yr)	M (/yr)	E	References
1. Sharks							
Scoliodon laticaudus	74	0.68	3.91	2.94	0.97	0.75	Mathew et al. (1997)
2. Rays							
Rhinobatus granulosus	97	0.38	2.56	1.02	1.44	0.4	Mohamed et al. (2005)
3. Diadromous (Pelagic)							
Tenualosa ilisha	60.0	0.82	3.77	2.49	1.28	0.66	Amin et al. (2002)
Ilisha filigera	35.0	0.75	3.37	1.95	1.42	0.58	Mustafa (1999)
4. Minor Pelagics							
Coilia dusumieri	16.8	1.30	2.61	1.60	1.01	0.61	Mustafa (1999)
Gudusia chapra	11.03	1.72	3.43	1.7	1.73	0.49	Islam (2005)
Setipinna spp	17.33	1.8	8.34	5.33	3.01	0.64	Nabi (2007)
Escualosa thoracata	12.20	1.2	9.84	7.29	2.55	0.74	Nabi (2007)
B. mcclellandii	11.03	0.83	3.53	1.48	2.06	0.42	Nabi (2007)
5. Medium Pelagics							
Rastrelliger kanagurta	27.8	0.9	4.92	3.21	1.71	0.65	Mustafa (1999)
Parastromateus niger	41.0	0.59	3.05	1.66	1.39	0.54	Mustafa (1999)
Megalaspis cordyla	38.5	0.54	2.86	1.44	1.11	0.51	Mustafa (1999)
6. Minor Mesopelagics							
Leiognathus eqqulus	28.0	1.08	3.2	2.5	0.7	0.78	Haque (1998)
7. Medium Mesopelagics							
Lepturacanthus savala	108.0	0.75	2.96	1.72	1.24	0.58	Haque (1998)
Pampus chinensis	38.1	0.67	2.56	1.23	1.33	0.48	Haque (1998)
Pampus argenteus	29.8	0.53	2.40	1.10	1.3	0.46	Haque (1998)
8. Medium Demersal							
Lates calcarifer	87.5	0.6	1.62	0.95	0.67	0.58	Haque (1998)
E. tetradactylum	38.1	0.18	4.4	3.5	0.85	0.87	Islam et al. (1993)
Polynemus pradiseus	20.5	0.48	4.38	3.17	1.21	0.72	Nabi et al. (2007)
Sillaginopsis panijus	43.3	0.38	3.6	2.7	0.86	0.76	Islam et al. (1993)

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9. Minor Demersal							
Harpadon nehereus	29.4	1.5	4.18	3.01	1.17	0.72	Mustafa et. al. (1998)
10. Panaeid shrimp							
Penaeus monodon	31.5	0.95	4.75	1.74	3.01	0.37	Mustafa (1999)
M. Monoceros	17.9	1.5	6.51	2.70	3.81	0.42	Mustafa (1999)
11. Other shrimp							
M. rosenbergii	35.5	0.34	8.5	4.57	3.94	0.54	Islam et al. (1993)
12. Other crustaceans							
Lobstars (*P. polyphagus*)	58.0	0.43	7.9	1.67	6.23	0.21	Islam et al. (1993)
Crabs (*S. serrata*)	96.5	0.55	6.9	2.41	4.49	0.35	Islam et al. (1993)
13. Cephalopods							
Squid (Loligo)	44.7	0.21	3.5	1.02	2.48	0.29	Islam et al. (1993)

Table 5. *Production-consumption data sources for non-fish groups*

Ecological groups	**Production/biomass (*P/B*)**	**Consumption/biomass (*Q/B*)**	**Ecotrophic efficiency (*EE*)**
Sharks	Mathew and Devraj (1997)	Mohamed et al. (2005)	Computed by ECOPATH
Rays	Mustafa (1999)	Calculated in this study	Computed by ECOPATH
Penaeid Shrimp	Calculated in this study	Calculated in this study	Computed by ECOPATH
Other Shrimp	Calculated in this study	Calculated in this study	Computed by ECOPATH
Other Crustaceans	Calculated in this study	Arreguin-Sanchez et al. (1993)	Computed by ECOPATH
Cephalopods	Calculated in this study	Christensen and Pauly (1993)	Computed by ECOPATH
Gastropods	Fishbase (2006)	Guénette (2013)	Computed by ECOPATH
Benthos	Silvestre et al. (1993)	Silvestre et al. (1993)	Computed by ECOPATH
Aquatic Invertebrates	Guénette (2013)	Guénette (2013)	Computed by ECOPATH
Zooplankton	Arreguin-Sánchez et al. (1993)	Arreguin-Sánchez et al. (1993)	Computed by ECOPATH
Phytoplankton	Computed by ECOPATH	-	Fixed Value

Estimation of relative food consumption (Q/B): Consumption/biomass (*Q/B*) ratio of each group was calculated through the following empirical relationship suggested by Palomares and Pauly (1999):

$$log\ Q/B = 7.964 - 0.204\ log\ W_{inf} - 1.965.T + 0.083.A + 0.532.h + 0.398.d$$

where, W_{inf} represents the asymptotic weight that can be calculated from the asymptotic length L_{inf} (one of the VBGF parameter found from ELEFAN routine of FiSAT II package) and length–weight relationships (LWR) of the representative species derived

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from various sources (*Table 4*). *T* symbolizes the average annual temperature of that habitat which is expressed as 1000/(*Tc* + 273.1). Here *Tc* represents the annual average temperature of sea surface (26°C) (DoEF, 2015). *A* signifies the ratio of the square of the caudal fin height and its surface area, and *h* and *d* are binary variables demonstrating the feeding class of the fish species, i.e. for detritivore ($h = 0$, $d = 1$) and herbivore ($h = 1$, $d = 0$) and for carnivore ($h = 0$, $d = 0$). The aspect ratio of the caudal fin (*A*), which is the indication of metabolic activity, was collected mostly from the FishBase (Fröese and Pauly, 2006) and also from the laboratory works that were conducted in the Population Dynamics laboratory of Bangladesh Fisheries Research Institute (BFRI) during the data collection period. For other functional groups, *Q/B* was assembled from various literatures (*Table 5*).

Diet composition of every group

Still today, there are no complete investigations about trophic interactions of the coastal and marine ecosystem of Bangladesh. Only limited works have been done on diet composition of fishes in this area (Mustafa and Mansura, 1994; Mazid, 1998). Most of the studies are qualitative in nature where diet items of fish are frequently lumped together. Due to the deficiency of available data on diet composition of functional groups considered, Mustafa (2003), Fishbase (www.fishbase.org) data (Fröese and Pauly, 2006) and the study of Mohamed (2010) were used to complete the diet matrix of this study (*Appendix 1*).

Ecotrophic efficiency (EE)

It provides the fraction of the yield of a group that is consumed within the system (i.e. transported through the trophic web) or caught by any fishery. In most cases, maintenance of the input values that provide the output fraction of EE between 0 and 1 are not possible as EE by definition, its fraction lies between 0 and 1. The EE is used to be computed from additional parameters in the Ecopath model since there is no field calculation to assess this parameter (Christensen et al., 2000).

Estimation of pedigree index

It categorizes the given input sources of an Ecopath through computing the type of source data on which it is based and specifying the probable uncertainty associated with the input. The main principles used here is that input estimated from native data (i.e. from the area covered by the model in question) as a rule is better than the data from elsewhere, be it a guesstimate, derived from other models or derived from empirical relationships. Three scales meet the above principles here, firstly for biomass assessing, second one for the estimating of *P/B* and *Q/B* and the rest for diet composition that are varied between 0 to 1. When the score is close to 0 that indicates the used input data is not rooted within local data, whereas value close to 1 means that are fully rooted within local data. The measure of fit (t^*) is also computed to define how well rooted the given model is in local data.

Evaluating the model through mass balancing method

The parametrization of Ecopath is based on its master equation (*Eq. 1*) which requires mass-balancing states of the functional groups. For this reason, the input

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parameters need to be adjusted in a style to maintain the ecotrophic efficiency (EE) values less than 1. This practice depends on the understanding of which adjustments need to be done (Kavanagh et al., 2004). Primarily, input parameters like biomass, production-consumption rate, food consumption and fishing catch records were set into the basic input tools sheet of the package. Biomass accumulation value was taken as zero as the model was considered to describe an average for one year. Initial running of the routine provided the *P/Q* ratio and EE, but some of them were not balanced as EE values were greater than 1 which pointed out their higher demand in relation with sustainability. Initially EE values were found in case of diadromous pelagic (0.86), minor pelagic (0.96), medium mesopelagic (0.89), minor demersal (0.98), medium demersal (0.99), Gastropods (0.68) and benthos (1.26). On the other hand, some groups showed the exceeded value (one) of respiration/assimilation, which is not possible because respiration value cannot be exceeded from assimilation (Christensen et al., 2000). Thus, new run continues with the revised entries of biomass and diet composition values of the previous run, till to the achievement of mass balance. New run was also performed to obtain EE and gross efficiency values of all groups as it should be less than one. In order to get mass-balance, diet matrix needs to be adjusted because of its variation and instability of food sources and feeding habits throughout the groups. The foremost output of Ecopath modeling is the assessment of trophic levels which sometimes may be beyond expectation. In this case, one must check the diet matrix of the input data and also compare the data with the trophic levels of the same or similar species data of FishBase.

Results

The basic estimates of the mass-balanced models of maritime eosystem of BoB, Bangladesh are given in *Table 6*. Life-history parameters and production-consumption data of non-fish groups are also presented in the *Tables 4* and *5*. Moreover, pre-balancing diagnostics of the maritime ecosystem BoB, Bangladesh model are presented in *Figure 2*.

Table 6. *Input values and estimated parameters (bold) achieved after mass-balancing using auto-mass balance routine of ECOPATH model of BoB, Bangladesh*

Group name	TL	*B (t/km²)*	*P/B (yr^{-1})*	*Q/B (yr^{-1})*	*EE*	*P/Q*	*R/A*	*NE*	*OI*
1. Sharks and Rays	**3.45**	0.42	3.50	12.8	**0.16**	**0.173**	**0.66**	**0.34**	**0.49**
2. Rays	**3.03**	0.76	0.55	8.6	**0.68**	**0.206**	**0.63**	**0.37**	**0.61**
3. Diadromous (Pelagic)	**2.02**	1.11	3.77	13.1	**0.77**	**0.287**	**0.64**	**0.36**	**0.11**
4. Minor Pelagics	**2.27**	1.56	5.57	25.8	**0.58**	**0.216**	**0.73**	**0.27**	**0.27**
5. Medium Pelagics	**2.34**	0.42	3.76	18.9	**0.80**	**0.198**	**0.75**	**0.25**	**0.31**
6. Medium Mesopelagics	**2.31**	1.38	3.04	10.6	**0.92**	**0.286**	**0.64**	**0.36**	**0.26**
7. Minor Mesopelagics	**2.36**	1.05	3.2	12.8	**0.81**	**0.25**	**0.69**	**0.31**	**0.27**
8. Medium Demersal	**2.80**	1.3	3.5	13.1	**0.86**	**0.267**	**0.66**	**0.33**	**0.48**
9. Minor Demersal	**2.54**	1.65	4.18	14.1	**0.46**	**0.296**	**0.63**	**0.37**	**0.43**
10. Penaeid Shrimp	**2.40**	0.74	5.63	22.8	**0.99**	**0.247**	**0.69**	**0.31**	**0.33**

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11. Other Shrimp	**2.59**	0.82	8.5	29.2	**0.80**	**0.291**	**0.63**	**0.36**	**0.49**
12. Other crustaceans	**2.34**	0.76	7.4	25.4	**0.46**	**0.291**	**0.63**	**0.36**	**0.31**
13. Cephalopods	**2.65**	0.7	3.5	22.9	**0.98**	**0.153**	**0.81**	**0.19**	**0.43**
14. Gastropods	**2.85**	0.85	5.3	23.5	**0.29**	**0.225**	**0.72**	**0.28**	**0.50**
15. Benthos	**2.44**	1.69	6.8	66.34	**0.69**	**0.103**	**0.87**	**0.12**	**0.35**
16. Aquatic Invertebrates	**2.34**	13	11	45	**0.24**	**0.24**	**0.69**	**0.31**	**0.26**
17. Zooplankton	**2.11**	27	34	119	**0.61**	**0.286**	**0.64**	**0.36**	**0.11**
18. Phytoplankton	**1**	58	100	0	**0.57**		**-**	**-**	**0**
19. Detritus	**1**	9.8	3.5	12.8	**0.15**		**-**	**-**	**0**

TL = trophic level, B = biomass, P/B = production rate, Q/B = consumption rate, EE = ecotrophic efficiency, R/A = respiration-assimilation ratio, NE = net efficiency and OI = omnivory index

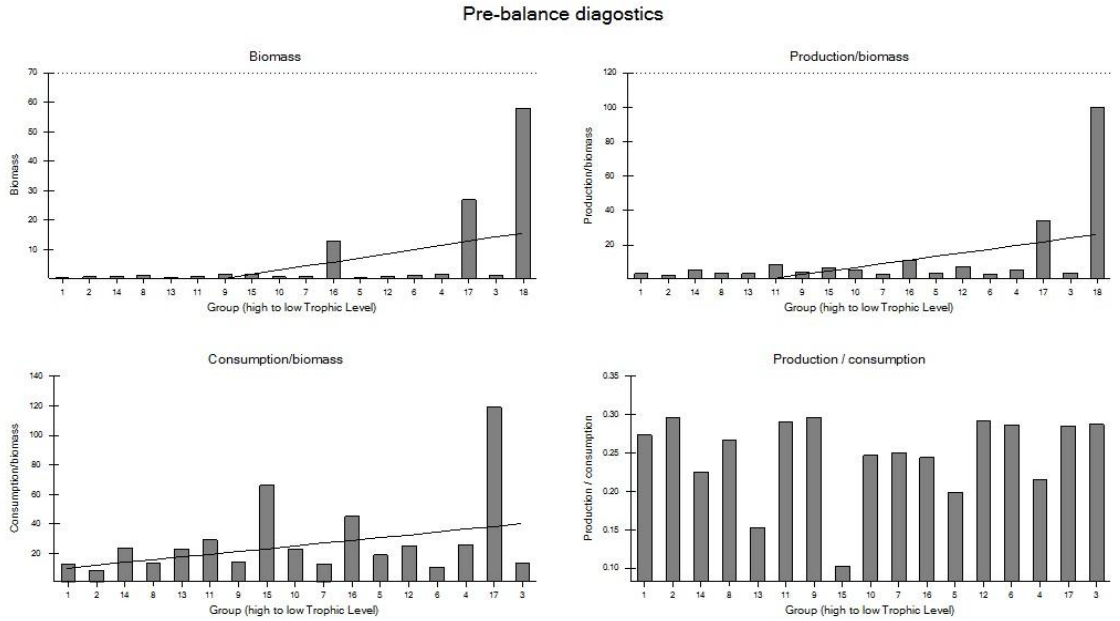


Figure 2. *The figure represents the pre-balancing diagnostics of basic inputs for construction of mass-balanced marine ecosystem model of Bay of Bengal, Bangladesh*

Mean trophic level was found 2.53, with the highest value obtained from sharks (3.45) and rays (3.04) groups followed by medium demersal (2.80) and gastropods (2.85), while the lowest value was obtained from phytoplankton (*Table 6*). In case of estimating ecotrophic efficiency, penaeid shrimps showed the higher EE value (0.99) followed by cephapods (0.98) while diadromous pelagic showed lower value (0.202) and except minor pelagics and demersal all fish groups showed higher EE values (EE > 0.8). Benthos showed the highest rate of respiration–assimilation ratio (0.87) whereas shrimps and crustaceans showed lower. In this model, the maximum omnivory index was found in the apex predators like rays group (0.61) followed by sharks (0.49). The highest *P/Q* values was found in minor demersal group (0.296) while net efficiency was obtained from the rays and minor demersal groups (0.37). Most of the fish groups showed the lower fishing mortality rate over the predation mortalities. In addition,

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maximum prey overlaps were detected for benthos and zooplankton groups. The relative and absolute results of flows and biomasses are showed in *Table 7* and *8*. In case of cycles and pathways, mean length of pathways from prey to predator was found 6.33 and the total number of pathway was 680.

Table 7. *Tropic level flows from primary producers and detritus (t/km^2/year)*

Trophic level \ Flow	Consumption by predators	Export	Flow to detritus	Respiration	Throughput
Tropic level flows from primary producers					
IX		0.000	0.000	0.000	0.000
VIII		0.000011	0.000006	0.000064	0.000120
VII		0.000273	0.000104	0.00138	0.00264
VI		0.00447	0.00192	0.0331	0.0584
V		0.0979	0.0416	0.469	0.868
IV		1.476	0.374	5.064	11.38
III		18.29	1.786	92.33	152.4
II		264.8	2.952	1154	1895
I	0.000	3317	0.000	2483	0.000
Sum	0.000	3601	5.155	3735	2060
Tropic level flows from detritus					
IX		0.000	0.000	0.000	0.000
VIII		0.000	0.000	0.000	0.000
VII		0.000011	0.000006	0.000062	0.000122
VI		0.000313	0.000097	0.00126	0.00249
V		0.00416	0.00183	0.0269	0.0476
IV		0.0804	0.0375	0.445	0.826
III		1.389	0.367	4.454	9.641
II		15.85	4.956	64.18	115.7
I	0.000	200.7	3603	0.000	0.000
Sum	0.000	218.0	3609	69.11	126.3

Table 8. *Transfer efficiencies (%) for each TL of the maritime ecosystem of the Bay of Bengal, Bangladesh*

Source \ Trophic level	II	III	IV	V	VI	VII	VIII	IX
Producer	12.17	12.53	10.11	9.451	6.528	8.563		
Detritus	7.873	10.01	8.490	7.444	9.865			
All flows	11.89	11.21	10.000	9.348	6.664	8.548	8.226	
Proportion of total flow originating from detritus: 0.30								
Transfer efficiencies (calculated as geometric mean for TL II-IV)								
From primary producers: 11.09%								
From detritus: 9.93%								
Total: 11.02%								

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The resource biomass assembly of the BoB, Bangladesh maritime ecosystem model points out that it is predominantly a low trophic level driven ecosystem. However, the assessed pedigree index of the BoB maritime ecosystem was found 0.631 (*Table 9*) that conformed the developing stage of overall quality of an Ecopath approach as discussed by Christensen et al. (2005). In this study, Shanon diversity index of the maritime ecosystem of BoB was found 1.55 that was also within a satisfactory level (*Table 9*).

Table 9. *The overall system statistics of the maritime ecosystem of BoB (no unit for indices and ratios)*

Parameter	Value	Units
Sum of all consumption	4149.14	t/km^2/year
Sum of all exports	3613.73	t/km^2/year
Sum of all respiratory flows	2186.27	t/km^2/year
Sum of all flows into detritus	3803.94	t/km^2/year
Total system throughput	13753.08	t/km^2/year
Sum of all production	6933.04	t/km^2/year
Mean trophic level of the catch	2.53	
Gross efficiency (catch/net p.p.)	0.0018	
Calculated total net primary production	5800	t/km^2/year
Total primary production/total respiration	2.65	
Net system production	3613.73	t/km^2/year
Total primary production/total biomass	51.24	
Total biomass/total throughput	0.008	/year
Total biomass (excluding detritus)	113.2	t/km^2
Total catch	10.5	t/km^2/year
Connectance Index	0.325	
System Omnivory Index	0.285	
Ascendancy	35.4	
Overhead	64.61	
Ecopath pedigree index	0.631	
Measure of fit, t*	3.25	
Shannon diversity index	1.56	
Throughput cycled (excluding detritus)	328.2	t/km²/year
Predatory cycling index	5.34	% of throughput without detritus
Throughput cycled (including detritus)	403	t/km²/year
Finn's cycling index	2.93	% of total throughput
Finn's mean path length	2.47	

In addition, in the flow diagram of the systems, the greatest flows were observed from phytoplankton to zooplankton and from detritus to aquatic invertebrates, benthos, and others that are presented in *Figure 3*. The direct and indirect impacts throughout the groups including their exploiting gears are also shown in *Figure 4*.

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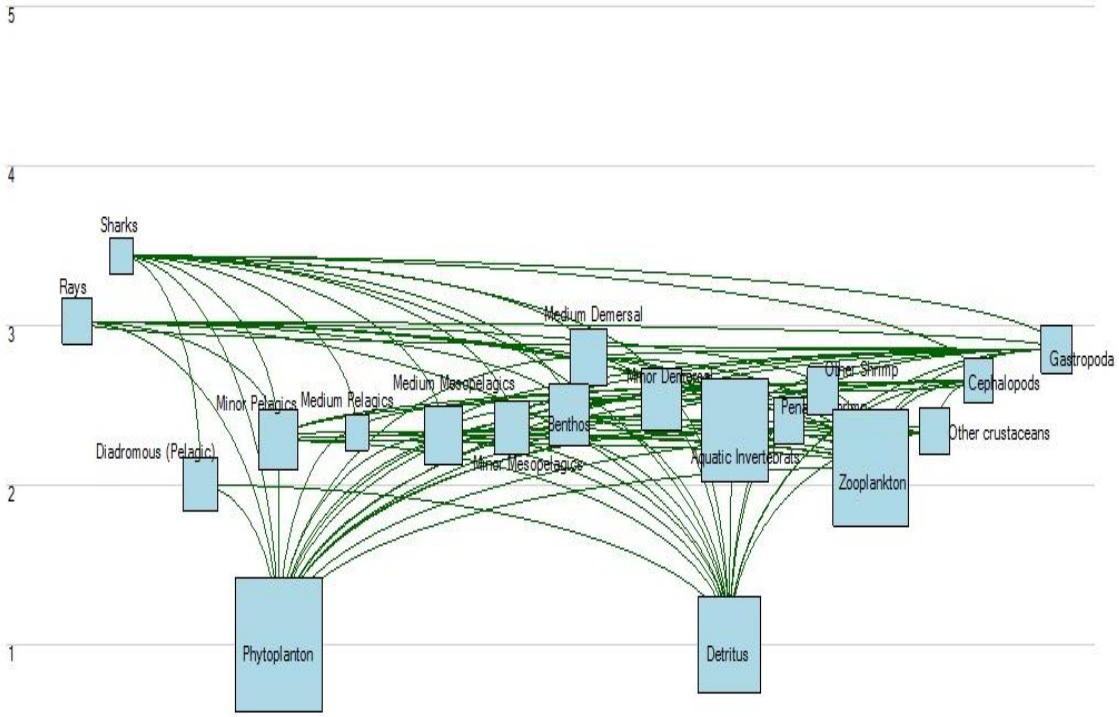


Figure 3. *Flow diagram of trophic relationships in the marine ecosystem of the Bay of Bengal, Bangladesh*

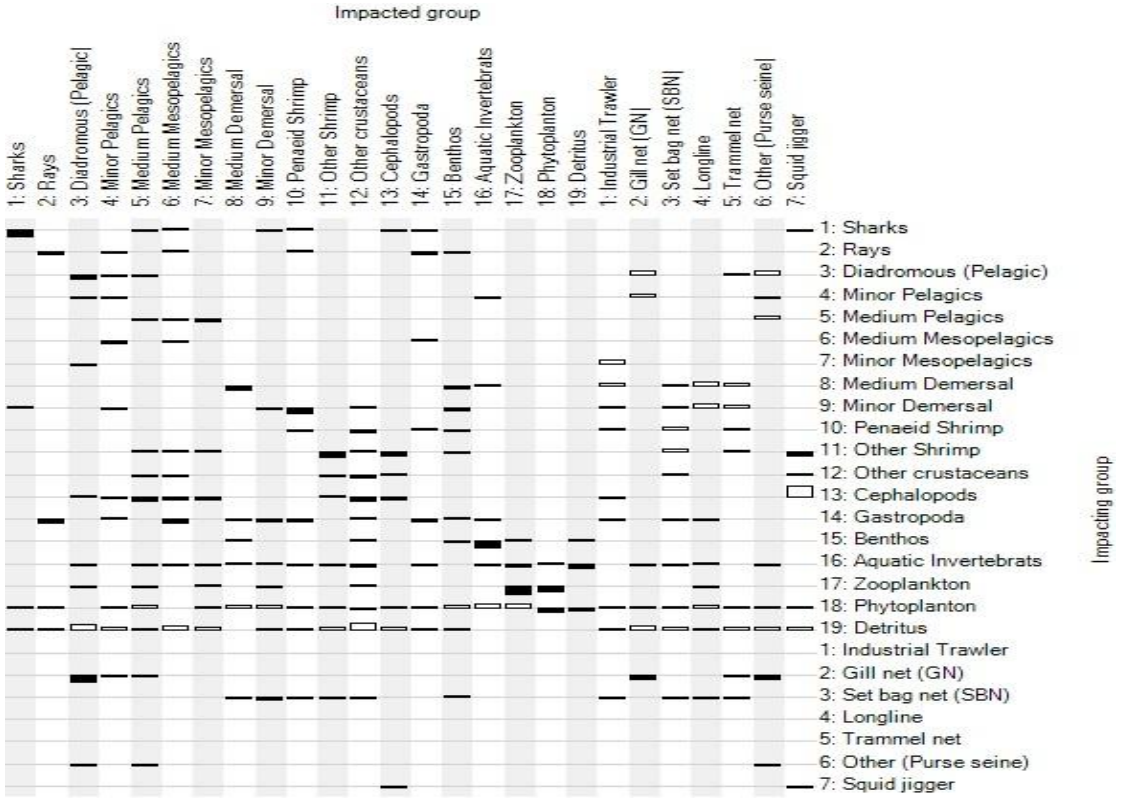


Figure 4. *The diagram illustrates the direct and indirect impact of the groups and fishing fleets mentioned at the upper and right-side of the histograms (rows). The upward bars represent positive impacts and downwards reveal negative impacts*

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The summary of the total system statistics of the BoB maritime ecosystem model is given into the *Table 9*. Finally, comparative study of the functional characteristics of maritime ecosystem model of BoB, Bangladesh with some other marine ecosystem models are also presented in the *Table 10*.

Table 10. *Comparison of the maritime ecosystem of BoB, Bangladesh with other ecosystems*

Name of the ecosystems	Throughput	Catch/PP	PP/B	B/T	Net sys prod.	Omni. index	Ascendency	Cycling index	Path length
British Columbia Shelf[a]	1237.0	-	21.1	0.18	4106	0.14	40.1		2.03
Yacutan[a]	2362	0.003	27.4	0.036	370	0.134	44.0	2.8	2.84
Sarawak, Malaysia[b]	1414	0.004	19.37	0.02	273	0.22			
Brunei, SE Asia[a]	1816	0.0008	28.6	0.018	300	0.201	29.4	16.3	2.8
N. Gulf of Mexico[a]	1790	0.0002	7.0	0.015	19	0.195	39.1	2.1	3.03
San Pedro Bay, Leyte, Phillipinnes[c]	183960	0.0011	46.81	0.008	1879.8	0.29			
Peru 70 (upwelling)[a]	18800	0.0017	87.5	0.012	14709	0.169	38.1	8.7	3.63
Bering Sea 80's[d]	5692	0.0021	4.9	0.050	-356	0.157	30.9	11.1	3.51
Karnataka Arabian Sea[e]	11522	0.0016	29.9	0.012	904	0.299	33.0	6.03	2.81
Coastal Ecosys. of BoB[f]	2628	0.0015	14.69	0.026	264.24	0.224	38.7	10	2.58
Marine Ecosys. of BoB*	13753	0.0018	51.24	0.008	3614	0.285	35.4	2.93	2.47

a. Christensen and Pauly (1993); b. Garces et al. (2003); c. Campos (2003); d. Trites et al. (1999); e. Mohamed et al. (2005); f. Ullah et al. (2012); *Present study

Discussion

The relative biomass of the ecological groups of this study initially estimated from the Ecopath model that revealed considerable differences due to three issues. Firstly, all groups were not equally captured through all the gears and same catchability was not found in every gear types. Therefore, some may be under or over-exploited in the survey. Secondly, Ecopath computes the biomass by the data of given catch. So, the larger the catch, the greater the biomass estimate. Lastly, the estimated catch data was mostly on group-wise rather than species-wise which may led to comparatively diverge catch value for different species. The estimated biomass of the BoB maritime ecosystem varied from 0.42 to 1.65 mainly for targeted catch groups i.e. demersal fishery and lowest for the shark groups due to absence of large predators.

Diet matrix preparation for this ECOPATH model was the most difficult task due to lack of earlier studies on feeding ecology. In order to make the study realistic some stomach content studies and diet isolations have been done. But most of the qualitative data provided by external studies were converted into a quantitative form for preparing the diet matrix. Although this model contains many groups (19 groups), the diet matrix

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comprises some low proportions. In fact, these values are hard to determine from field data. This nature might be significantly influenced the ultimate result.

Production-consumption (*P/Q*) rate of less than 0.3 may assist as a diagnostic feature in a balanced model. In most cases, the *P/Q* values of all possible functional groups range from 0.05 to 0.3, but some exceptions may happen in case of coral reefs, fish larvae, nauplii, bacteria and other minute, fast-growing organisms. The present study supports this concept for all groups which was 0.103 to 0.296. Demersal groups showed higher *P/Q* ratio than most of the pelagic groups (*Table 6*), this may be due to higher consumption rate of benthic communities and some predatory behavior of few demersal species. Nevertheless, pelagic species are typically herbivores and generally dependent on phytoplankton and detritus.

One of the most significant feature of an ecosystem is to assess ecotrophic efficiency (EE) (Christensen et al., 2000) which is mainly the fraction of production consumed by predators. In the BoB model, the EE values varied extensively from 0.24 to 0.99 (*Table 6*). Normally, just over the zero value points out the 'group' was not consumed until now by any other groups throughout the system while close or equal to 1 designates a heavily preyed group, where there are no individuals left to die of old age. Only sharks (0.16) showed lower EE value which was realistic and expected as for the top predators (Christensen et al., 2000). In addition, in both cases of phytoplankton and detritus, the EE values were less than 1.0 that was settled with the systems proposed by Christensen and Pauly (1992) that indicates more producers would have entered than left. However, the estimated EE value of detritus (0.15) was the lowest among the groups probably due to absence of typical primary consumers in the ecosystem. Out of all targeted groups, the estimated EE value of penaeid shrimps and cephalopods were shown the highest values over 0.9 (*Table 6*) that were likely the consequence of greater fishing pressure and predation. Similar reports were found from Islam et al. (1993) that penaeid shrimps were highly exploited throughout the maritime waters of BoB.

Mean trophic level (TL) is the vital index of the complete exploitation level of fisheries groups low in the food web and its effect on predator and prey species. Fishing down the marine food web, in which fishing fleets gradually target species minimum in the food web, may or may not be the reason for decline in global mean trophic levels of catches. The BoB maritime ecosystem had a mean trophic level of 2.53, with the highest 3.45 (sharks) and lowest being 1 (phytoplankton and detritus) (*Fig. 3*). Except the predators' maximum group biomass and ecological production occupied the place at around TLIII (*Table 6*). Ecopath generally computes trophic levels higher than IV (Ulanowicz, 1995). A total of 9 TLs were counted in this study (*Table 8*), which may be due to the cannibalistic characters of the peak trophic levels (Haputhantri et al., 2008). Therefore, the trophic accumulation routine in Ecopath gathered the 19 groups in a normal food chain with ten trophic levels where primary producers (TLI) comprised detritus and phytoplankton (*Table 7*). The result displayed that the maximum flow of the system was transferred from secondary consumers mainly composed of zooplankton group followed by TLI where phytoplankton was the single contributor. This output reveals the significance of zooplankton for the activation of trophic level of the BoB maritime ecosystem which is dominated by lower trophic level of fisheries resources as reported by Hossain (2003). For each group, the flow to the detritus contains non-absorption rate of diet and those components of the group that die of old age, diseases, etc. The detritus flow indicates that almost every functional group of the BoB maritime

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ecosystem are severely exploited by either excessive fishing pressure or predation along with other mortalities, i.e. cannibalism (*Table 7*).

Gross efficiency of the system was 0.0018 indicates less crowd of target fisheries in the food chain. Actually increasing trend of gross efficiency index represents the fisheries 'development' which is generally much lesser than 1.0 as globally it is about 0.0002. This results is almost similar with the by Mohamed et al. (2005) and Ullah et al. (2012). In addition, the average transfer efficiency (TE) was 11.02% where flows from phytoplankton were demonstrated more essential than flows from detritus demonstrating a significant planktonic food chain throughout the maritime ecosystem of BoB (*Table 8*). Actually, transfer efficiency acts as an index of the significance of detritus in a system. The TEs for TLII, as suggested by Ryther (1969), are 15% for marine areas, but the most common range is 10–20% (Odum, 1971; Barnes and Hughes, 1988). However, the accounted of all flows was 11.89% (*Table 8*) which covers the above range demonstrating ecosystem maturity (Odum, 1971). In addition, estimated TEs of the trophic levels III, IV and V of this study were within the standardize range of 10–20% for coastal zones (Odum, 1971; Barnes and Hughes, 1988). Hence, the existence of some dominating fish groups having higher EE value around TL III may be the basis of the higher TE in TLIII.

Mixed trophic impacts may be direct or indirect i.e. as for a prey; a group causes a positive impact on others while as a direct predator, impact is negative. In the current observation, zooplankton, phytoplankton, and detritus were showed to have a positive impact on most other groups. The impact was greater on their direct consumers, i.e. most of the pelagic fishes depend on either phytoplankton or detritus or both. Negative impacts were due to benthos or zooplankton as a consumer of aquatic invertebrates, phytoplankton, and detritus or as a competitor for the same food source. Phytoplankton and detritus were shown a significant positive impact on pelagic groups and moderately positive on some of the demersal groups whereas barely any impact was found on penaeid shrimps and cephalopods (*Fig. 4*). The system package designated that the impact on detritus would be negative since detritus normally accompanied by phytoplankton in the regimes of several primary consumers. Thus, upsurge of phytoplankton would stimulate primary consumers to forage more detritus. Zooplankton had significant negative impact on themselves due to the existence of greater quantity of carnivorous zooplankton and relatively low negative impact on phytoplankton, which also indicates the existence of herbivory zooplankton in that ecosystem. Remarkable positive impact of detritus was observed on most of the functional groups whereas detritus had neither positive nor negative impact on itself in that ecosystem. Comparable results are also observed in other ecosystems (Christensen and Pauly, 1993). Fishing fleets also negatively affected several groups i.e. pelagic groups were negatively impacted by Gill net whereas demersal by SBN operations. Thus, it can be concluded that increased fishing pressure by gillnet and SBN in the BoB maritime ecosystem would have generated more negative impacts on fish groups, especially on the demersal and predators.

Total system throughput (*TST*) is the sum of all flows (e.g. total consumption, total respiration, total export and total flows to detritus) in a system which represents the 'size' of the entire system in relation to flow (Ulanowicz, 1986). The estimated *TST* for BoB was relatively higher 13753 t/km^2/year (*Table 9*) but consistent with tropical marine ecosystems with much turnover. This result was also similar (*Table 10*) with the

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studies of San-Pedro Bay, Phillipines by Campos (2003) and Karnataka, India by Mohamed et al. (2005).

The ratio of total primary production and total respiration (*PP/R*) is considered by Odum (1971) to be a significant ratio to describe the 'maturity' of an ecosystem. Previously, a system needs to be developed for being balanced as the production is expected to exceed respiration that provides a ratio greater than 1. Finally, in 'mature' systems, the *PP/R* ratio should be 1; when energy is constant that is roughly balanced by maintenance (Odum, 1971). The *PP/R* value was found 2.65 which was higher than 1, thus, it is concluded that the BoB marine ecosystem is not so developed or still in developing stage. The net system production value of 3613.7 t/km²/year was assessed for the BoB maritime ecosystem, which again points out the BoB is still developing but also little bit matured comparing with the other studies. Generally, system production is greater in immature systems and nearby zero in maturity stages. The net system production value of British Columbia shelf ecosystem were also provided 4106 t/km^2/year (*Table 10*) which was greater than the current value. This may be happened due to absence of large predators as well as lower trophic level as this system completely based on TLII to TLIII. Moreover, the ratio between a system's primary production (*PP*) and total biomass (*B*) was 51.24, which was higher and also indicates some sorts of immaturity. Production exceeds respiration largely in immature systems and therefore it will influence the system's *PP/B* ratio which dimension is per unit time, and it can take any positive value.

The available ecosystem energy flow directly supports the total system biomass which can be expected to rise maximum for the utmost maturity stages of a system (Odum, 1971). The ratio of *B/TST* is directly proportional to the system maturity, where the estimated value tends to be low during the ecosystem development stage and increases as a purpose of maturity (Christensen, 1995). From the present study, system biomass/throughput ratio was found 0.008 being lower compared with those given by Ullah et al. (2012); Mohamed et al. (2005); Garces et al. (2003), which showed the status of comparative maturity of the ecosystem (*Table 10*). The yield of a group throughout a system is size-specific that has been revealed that the inverse of a group's production/biomass ratio is a degree of size (Christensen and Pauly, 1993). However, the estimated total biomass (excluding detritus) 113.2 t/km^2 was higher than the study of Ullah et al. (2012) but lower than the value of Mohamed et al. (2005), as their study based on the adjacent area of BoB. But the total catch (10.5) was comparatively higher than the above studies which may be due to excessive artisanal fishing pressure in Bangladeshi waters.

The trophic flows to detritus were observed highest for zooplankton and smallest for top predators. For each group, the flow to the detritus contains non-absorption rate of diet and those components of the group that die of old age, diseases, etc. The detritus flow indicates that almost every functional group of the BoB maritime ecosystem are severely exploited by either excessive fishing pressure or predation along with other mortalities, i.e. cannibalism (*Table 7*). The cycling matter and energy flow is considered as a vital process of any active natural ecosystems (Odum, 1969). The percentage of 'cycling index' is the fraction of an ecosystem's throughput that was developed by Finn (1976). It was basically intended to calculate Odum's (1969) system maturity. However, its explanation was not as simple as originally conceived that intensify recycling as a system matures. Detritus played a supreme role in all flows (cycled) in the BoB model where the total cycled flow was 328.2 t/km^2/year. The Finn's cycling index was

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obtained 2.93%, which was lower compared with the study by Mohamed et al. (2005) for Karnataka, India and Ullah et al. (2012) for coastal ecosystem of BoB. Similar close output was reported by Christensen and Pauly (1993) for Yacatan and Northern Gulf of Mexico. Actually, Finn's cycling index gives the proportion of flow in a system that is recycled compared with total system throughput and immature systems give lower value while matured provide higher. Finn's mean path length is the measurement of the mean quantity of groups that a unit of flux will experience from its entry into the system until it leaves the system. Over-all 680 pathways was obtained from the BoB maritime ecosystem and the mean length of pathways was computed as 6.33, was found a little bit higher when compared with the range (2.86–4.95) reported by Baird and Ulanowicz (1993) for the four estuaries. Nevertheless, the lower number of pathways proves the simplified feature of that ecosystem and it is cycled through detrital pathways.

The connectance (CI) and system omnivory index (SOI) of the BoB maritime ecosystem was 0.322 and 0.285 respectively. In fact, the CI can be predictable and associated with maturity, which can be define by the ratio of 'the quantity of actual links with the number of possible links'. The connectance index is mostly determined by the level of taxonomic aspect used to characterize prey groups. On the other hand, the system omnivory index is proposed as a substitute which is defined as the mean omnivory index of all consumer's weight-mass by the logarithm of each consumer's consumption. The SOI is a measurement of how the diet interactions are scattered between trophic levels. The SOI is stimulated by apparent drawbacks of the CI. In the development stage of an ecosystem, CI would be close to 1 in most systems and increase maturity reduce the value of connectance. Moreover, a prey having the same 'score' in case of connectance index whether it supports 1, 10 or 100% of its higher trophic's diet.

Another important fact is ascendancy, which is a measurement of system growth, and development of network links whereas proportion of a system's capacity not considered. When the system's capacity is considered, it is known as system's overhead, which was the reserved energy of an ecosystem (Ulanowicz and Norden, 1990). The relative values of ascendancy (35.4) and system's overhead (64.6) of the maritime ecosystem of BoB indicated the ecosystem's stability and some sorts of maturity. This proved that maritime ecosystem of BoB have significant backup strength also have the capacity to control any pressure situations through fast reformation to the original stages.

Conclusion

The software outputs indicate that the maritime ecosystem of BoB is steadily reaching maturity, although it is still considered to be in a developing phase. Excessive artisanal fishing pressure is the main hindrance to develop ecosystem quality. One of the major challenges for this multispecies ecological modelling was the scarce of studies on the feeding ecology of the various functional groups. Therefore, further studies should be required in order to get more specific estimations for a multispecies approach to improve basic inputs of the system and also improve the catch landing statistics of the national record.

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Conflict of interest. We declare that there is no conflict of interest.

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APPENDIX

Appendix 1. *Estimates of diet consumption for ecological or functional groups in the maritime ecosystem of Bay of Bengal, Bangladesh*

	Prey \ predator	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Sharks	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Rays	0	0.14	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0
3	Diadromous (Pelagic)	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Minor Pelagics	0.005	0.05	0	0	0.031	0.071	0	0	0.04	0	0	0	0.076	0	0	0	0
5	Medium Pelagics	0.06	0	0	0	0	0	0	0	0	0	0	0	0.03	0	0	0	0
6	Medium Mesopelagics	0.042	0	0	0	0.054	0	0	0	0	0	0	0	0.08	0.08	0	0	0
7	Minor Mesopelagics	0.08	0	0	0	0.085	0	0	0	0	0	0	0	0.055	0.01	0	0	0
8	Medium Demersal	0.067	0	0	0	0	0	0	0.1	0	0	0	0	0	0.05	0	0	0
9	Minor Demersal	0.182	0.04	0	0	0	0	0	0	0	0	0	0	0	0.05	0	0	0
10	Penaeid Shrimp	0	0	0	0	0	0	0	0	0.065	0	0	0.009	0	0.08	0	0	0
11	Other Shrimp	0	0.05	0	0	0	0	0	0	0	0	0.13	0.052	0	0	0	0	0
12	Other crustaceans	0	0	0	0	0	0	0	0	0	0.034	0	0.01	0.08	0	0	0	0
13	Cephalopods	0.134	0	0	0	0	0	0	0	0	0	0.06	0	0	0	0	0	0
14	Gastropoda	0.05	0.08	0	0	0	0	0	0	0	0	0	0	0	0.02	0	0	0
15	Benthos	0.06	0.1	0	0	0	0	0	0.155	0.081	0.046	0.048	0.02	0.01	0	0	0	0
16	Aquatic Invertebrats	0	0.1	0	0.125	0	0	0	0.119	0.152	0.049	0.018	0	0.118	0.03	0.175	0	0
17	Zooplankton	0	0.1	0	0.1	0.1	0.2	0.321	0.216	0.072	0.2	0.171	0.177	0.05	0.25	0.181	0.31	0.1
18	Phytoplankton	0.1	0.18	0.3	0.327	0.45	0.239	0.315	0.285	0.338	0.358	0.109	0.014	0.217	0.2	0.388	0.55	0.9
19	Detritus	0.05	0.16	0.7	0.448	0.28	0.49	0.364	0.125	0.252	0.313	0.464	0.718	0.284	0.18	0.256	0.14	0
20	Import	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	Sum	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
22	(1 - Sum)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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CARBON DYNAMIC SHIFTS WITH LAND USE CHANGE IN MARGALLAH HILLS NATIONAL PARK, ISLAMABAD (PAKISTAN) FROM 1990 TO 2017

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Abstract. Land use change (LUC) particularly the conversion of forest to other land uses, determines the scale of forest carbon shifts. Here, remotely sensed data Multispectral Satellite Images (MSI) of the study area for the year 1990, 2000, 2010, 2017 and geographic information system (GIS) tools were used to analyze the land use change patterns. For biomass calculation and related forest carbon losses in the Margallah Hills National Park of Pakistan the field inventory data were collected by random forest sampling from 1990 to 2017 . Our results showed that during the period from 1990 to 2017, the area under dense forest decreased by 11.14% (0.41% yr^{-1}), while the area under open forests increased by 4.28% (0.15% yr^{-1}). Similarly, agricultural land and settlements increased by 53.17% (1.96% yr^{-1}) and 67.78% (2.51% yr^{-1}) respectively, during the same period. The conversion of forests into other land uses resulted in the reduction of carbon value. In 1990, carbon loss is estimated at 5531.5 $MgC \cdot yr^{-1}$ (7.22%) of the total carbon pool. Overall, land use change released 154 GgC (5.70 $GgC \cdot yr^{-1}$), highlighting that land use change significantly alters carbon dynamics of the Park. Land use change and related carbon losses indicating the magnitude of anthropogenic disturbances in the Park. The preservation and rehabilitation of degraded forest as well as reforestation of the converted forests are necessary for forest carbon conservation meeting emissions reduction obligations of the Kyoto Protocol.

Keywords: *remote sensing, forest loss, satellite images, land use change, carbon loss*

Introduction

Land Use Change (LUC) is the modification of the earth's surface due to human activities which result in the disturbance of the Earth's natural ecosystem and environmental processes (Lambin, 1997). The availability of LUC data has had an important role in advancing research and providing long-lasting solutions to environmental and ecological policymakers (Zhao et al., 2004; Ullah et al., 2016; Mukete et al., 2017). For instance, LUC has become the key issue in many disciplines like forestry, agriculture, environmental sciences, geology and hydrology (Weng, 2001). Additionally, LUC has been greatly applied in studies relating to soil degradation, cropland loss, urban expansion, desertification and deforestation within the past three decades. LUC has been known to cause long-term effects on forest

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ecosystem functions such as a net decrease in forest area globally from 31.6% to 30.6% since 1990 (FAO, 2015).

The application of MSI in LUC studies has gained precedence in detecting and estimating both spatial and temporal dynamics of forest cover changes. In our study remote sensing data at periodic intervals and Geographic Information System (GIS) provide information for land use change analysis, modeling, and management (Sudhakar Reddy et al., 2008; Kennedy et al., 2009). For instance, using LANDSAT data and digital analysis the detection of land degradation due to waterlogging and soil salinization occurring over a 10-year period in China was enabled(Gao and Liu, 2010). Similarly, Rembold et al. (2000) used LANDSAT TM data between 1972 and 1994 to estimate land cover changes in central Ethiopia. This is because monitoring through satellite images is not only cost-effective and fast but also provides reliable land use and land cover change detection information (Martin and Tracy, 2007). Within the past three decades, various change detection techniques using satellite imagery have been developed including post-classification comparison (PCC), image differentiation, principal component analysis (PCA) and vegetation index differencing (Lu et al., 2004). Among these, many studies have found the post-classification comparison to be the most accurate procedure especially as it represents the nature of changes as they occur. This method is also thought to reduce problems associated with multi-temporal images recorded under different atmospheric and environmental conditions (Yuan et al., 1999).

Forests are the largest sources of global terrestrial carbon sink, while LUC is the key factor which determines the extent of carbon sink over time (Pan et al., 2011). Forests have a key role in carbon sequestration which is distorted by LUC and land management activities (Abatzoglou et al., 2017). From 1985 to 1990 LUC and land management activities released over 156 PgC into the atmosphere, particularly in tropical areas where deforestation was observed to have caused approximately 60% of these emissions (Houghton, 2003). Since the 1980s, LUC in Forest land and grassland has been responsible for forest carbon sink in North America and Europe (Nabuurs, 2013). LUC associated with deforestation and land degradation emits CO_2 and other greenhouse gases and therefore it emerged as a burning issue in the scientific community (Thompson et al., 2011). However, in a recent economic survey, LUC would affect over 36% of the United States land area. This is attributed to increased urban land and where urban developmental factors such as culture and socio-economic would be major contributors (Radeloff et al., 2012). In addition, there are also chances and risks of forest conversion into new land uses including agriculture and settlements (Searchinger et al., 2008). Furthermore, concerns about climate change have forced many countries to monitor terrestrial carbon. The Inter-Governmental Panel on Climate Change (IPCC) guidelines for national greenhouse gases strictly requires monitoring of carbon pool through the effect of land-use change and wood removal (IPCC, 2006).

LUC and their associated impacts on forest cover have been widely addressed by the United Nations Framework Convention on Climate Change (UNFCCC). The UNFCCC has also developed guidelines for member countries to measure LUC and related activities. Pakistan as a member of the UNFCCC and IPCC has to follow the proposed greenhouse gas emissions and removal related guidelines. Only about 4% of Pakistan's total land area is under forest which is under threat from deforestation (Cochard and Dar, 2014).

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In Pakistan, most studies focused on sequestered carbon in different forest types such as in subtropical forests (Nizami, 2012) and in forest plantations (Ahmad and Nizami, 2015; Ahmad et al., 2015; Saeed et al., 2016). Similarly, studies were conducted about the carbon stock in Oak Scrub Forest of Sheringal Valley Dir Kohistan (Khan et al., 2015), and the carbon stock in Chichawatni irrigated plantation in Pakistan (Muhammad et al., 2017). Most of these studies evaluated carbon stocks in particular land use types (Ahmad and Nizami, 2015), while others focus on land use change and deforestation patterns (Hassan et al., 2016). However, none of these studies has been carried out on LUC and their related carbon losses in protected areas. In order to determine LUC and carbon induced emissions in a protected area in Pakistan, the present study in Margallah Hills National Park (MHNP) of North West of Islamabad aims to determine LUC and carbon induced emissions in a protected area in Pakistan.

MHNP has subtropical broad-leaved evergreen, subtropical chir pine forests and rich in flora and fauna. It is one of the best-protected areas in Pakistan providing medicinal plants, economic and aesthetic values to surrounding communities and the country. However, in the past three decades, land use change and deforestation have ultimately released considerable amounts of carbon into the atmosphere. This study, therefore, presents a comparative analysis of the temporal LUC and forest carbon-loss estimation in MHNP. The main objectives of the study includes the pattern of LUC in the MHNP during the period 1990, 2000, 2010 and 2017, their impacts on carbon dynamics and to analyze the major drivers of LUC in the MHNP.

Materials and methods

Study area

The study area is located at the foothills of the Himalaya Mountains around the north east of Islamabad, the MHNP (73°7'3.32"E, 33°41'59.61"N and 73°1'34.07"E, 33°45'2.87"N) (WWF, 2009) covers an estimated area of 17,386 hectares (ha) and composed of diverse ecological resources MHNP is dominated by *Acacia modesta, Quercus incana, Pinus roxburghii, Dodonea viscosa, Olea ferruginea* and *ziziphus mauritiana* and also have plant spp having medicinal value like *Lantana camara* (Asma et al., 2009). This park has about 1700 flowering species, more than 50 species of grasses 53 species of ferns and it is also rich in wildlife having common leopard, jackal, rhesus monkey, barking deer, porcupine, foxes and wild boars (WWF, 2009). The MHNP with the altitude of 469-1560 m, as shown in *Figure 1,* is made up of rugged slopes and gullies and the soil are characteristically composed of limestone (Butt et al., 2015). Temperature ranges between -3.9 °C in January to 41.6 °C in June and average annual rainfall ranges between 245 and 1142 mm (PMD, 2015).

Data acquisition

Satellite images (LANDSAT) were obtained from United States geological survey (USGS) (http:// glovis.usgs.gov), for March 1990, 2000, 2010 and 2017. The images were selected based on quality and minimum cloud cover, see *Table 1.* In addition to using satellite images, ancillary data were collected which included topographic maps, aerial photographs, and ground truth data. In March 2017 using GPS, ground truth data in the form of reference points were collected which was used for image analysis and accuracy assessment.

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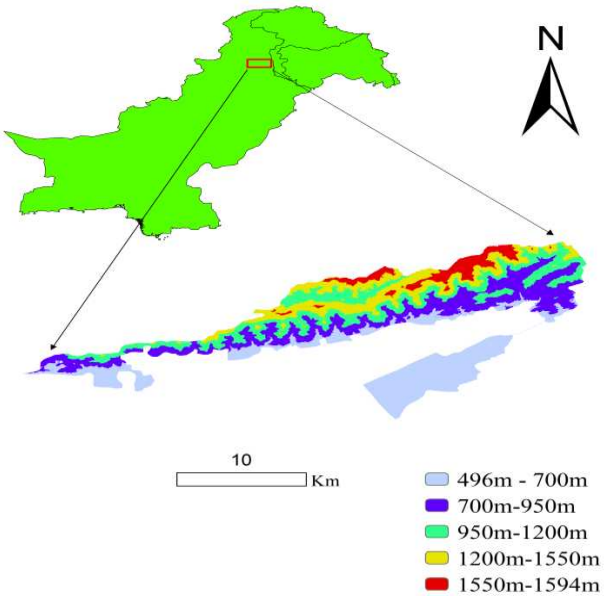


Figure 1. *Map of Pakistan and elevation map of Margallah Hills National Park (MHNP)*

Table 1. *Satellite sensors specification*

Sensor	Year	Band/color	Resolution	Source
Landsat 8 OIL imagery	2017	Multispectral	30 m	USGS, glovis
Landsat 5 TM imagery	2010	Multispectral	30 m	USGS, glovis
Landsat 7 ETM imagery	2000	Multispectral	30 m	USGS, glovis
Landsat 5 TM imagery	1990	Multispectral	30 m	USGS, glovis

Preprocessing of satellite imagery

Image pre-processing was preceded by the acquisition of topographic maps at the scale of (1:50,000) for the study area, obtained from a survey of Pakistan. The map was first scanned and exported into the processing software ArcGIS 10.2 and ENVI 5.1. Image pre-processing was necessary before the classification and change detection, for establishing a relationship between acquired data and biophysical processes (Abd El-Kawy et al., 2011). To correct the geometric distortion of the satellite image geometric correction was performed from the topographic sheet as well as from the GPS by selecting 20 Ground Control Points (GCP's) around the study area. Then the geometrically corrected image was used to clip the region of interest (ROI). After that, the image was classified into five different classes such as dense forest (DF), open forest (OF), agricultural land (AL), settlements (SM), and water bodies (WB) with corresponding classes by assigning per-pixel signatures. On the bases of different digital number (DN), different colours were assigned to each landscape to differentiate them. Later on, Atmospheric, radiometric and topographic corrections were performed using ArcGIS 10.2 and ENVI 5.1. Training samples for each predetermined class were selected by delimiting polygons and spectral signatures for each land cover type derived from satellite imagery. Supervised classification was performed by using a maximum likelihood algorithm. To improve the quality of classification and to classify the un-classed pixels, post classification smoothing was performed.

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Accuracy assessment

The accuracy of the classified maps was crosschecked by 200 random GCPs. GCPs were randomly selected after which the user and producer accuracy were calculated for each LUC. Finally, the Kappa statistics were calculated by the observed and expected using *Equation 1* (Chandra and Ghosh, 2006).

$$k = \frac{\text{Observed} - \text{Expected}}{1 - \text{Expected}} \quad \text{(Eq.1)}$$

Kappa statistics for 1990, 2000, 2010, and 2017 were 0.90, 0.91, 0.87, and 0.91 respectively.

Land-use change detection map

Post-classification change detection technique was performed by using ENVI 5.1. LUC detection map was developed by using the classified map of 1990 and 2017; the map of 1990 was used as the base map (*Fig. 5*). In an urban environment, the post classification has been used by various researchers effectively due to its efficiency in location, nature, and rate of change detection (Hardin et al., 2007). The annual rate of change in each land use type was measured according to Puyravaud (2003; *Eq. 2*):

$$r = \{\frac{1}{(t_2 - t_1)}\} x \ln(\frac{A_2}{A_1}) \quad \text{(Eq.2)}$$

where, A_1 and A_2 are the areas of land use type at different time t_1 and t_2

Carbon inventory

Biomass carbon was assessed by field inventory in dense forest (DF) and open forest (OF). A total 150 sample plots of 20 × 30 m were taken randomly in the study area (75 plots in DF and 75 in OF). In each sample plot, the diameter of all trees greater than 4 cm was measured at breast height (DBH). The height of 20 trees per species was recorded, sufficient to construct DBH/height function of a specific plot. Abney's level was used to measure the height of the trees. The volume of all trees present in the sampling sites was measured using *Equation 3* (Newbery, 2009), and total biomass $kg \cdot ha^{-1}$ was calculated by multiplying biomass with the wood density kg m^{-3} of particular species using IPCC (2006) proposed guidelines.

$$\text{Volume of tree } (m^3) = (\frac{\pi}{4}) \times d^2 \times h \times f \quad \text{(Eq.3)}$$

where d^2 is the square of DBH, *h* is height and *f* is the form factor. Biomass of tree was calculated by using stem volume (m^3) and multiplying it by the wood density kg m^{-3}. The wood density values were confirmed by previous research and reports. Biomass expansion factor (BEF) for subtropical forests of Pakistan was used to determine the biomass of entire forests (*Eq. 4;* Haripriya, 2002; IPCC, 2006).

$$\text{Biomass } (kg) = \text{Volume}(m^3) \times \text{Wood density } (kg \cdot m^{-3}) \quad \text{(Eq.4)}$$

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For determining the biomass, carbon values of understory vegetation (USV) sub-plot of 4 × 4 m was established in each sample plot. First, the vegetation in each plot was harvested, their fresh weight was recorded, and all the harvested materials were put into the labeled bags for further analysis. Then, the samples were dried in an oven at 72 °C for 48 h and their dried weights were measured for biomass. Similarly, litter, dead wood and cones present in the sub-plot were also collected, and followed the procedure mentioned above for USV for biomass carbon assessment. The soil samples were collected from the selected plots at the depth of 0-15 cm and 15-30 cm with the help of soil auger and core sampler in three replications. Each soil sample was weighed and labelled in the field. Core sampler of 0.0001256 m^3 (diameter = 4 cm, height = 10 cm) was used to measure the bulk density of each soil sample. The soil samples were brought to the laboratory for further analysis. To measure the soil organic content the method of soil oxidizable organic carbon given by Walkley (1934) and Rayment (1992) was used. Carbon was determined by using total plant biomass convertible factor that is the representative of average biomass content of the plant. According to this convertible factor, the carbon content is 50% of the total plant biomass, this factor is used worldwide (Brown and Lugo, 1982; Saugier et al., 2001). The carbon values in the soil were (MgC ha^{-1}) measured by multiplying the bulk density, carbon concentration and the thickness of the layer (Ahmad and Nizami, 2015).

Carbon loss assessment

The deforestation resulted in the conversion of forestland into AL and SM. Similarly it also resulted in the conversion of DF to OF. We assess the net loss of carbon value associated with deforestation. Change in carbon is determined by *Equation 5* (Zheng et al., 2011)

$$\Delta C = TFC_{t2} - TFC_{t1} \quad \text{(Eq.5)}$$

The annual rate of carbon loss is determined by *Equation 6:*

$$\Delta C = \frac{(TFC_{t2} - TFC_{t1})}{(t_2 - t_1)} \quad \text{(Eq.6)}$$

ΔC = Carbon loss or gain
TFC_{t2} = Total forest carbon at time t2
TFC_{t1} = Total forest carbon at time t1

Results

Land uses and Land use change

Our image analysis results showed that in 1990 the major land uses in the study area were a dense forest (DF), open forest (OF), agricultural land (AL), settlements (SM), and water bodies (WB). The land-use change statistics (*Table 2*) showed that from 1990 to 2017, the area under DF and WB were decreased, while we saw increase in the area under OF, AL, and SM as shown in *Figures 2, 3, 4* and *5,* respectively. In 1990, the area under DF was 55.87% and it was reduced to 49.55% in 2017. The conversion of DF to other land uses resulted in a 6.32% decrease in the area under DF. Our results of LUC

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pattern analysis for three different periods (1990-2000, 2000-2010 and 2010 to 2017) are presented in *Table 3.*

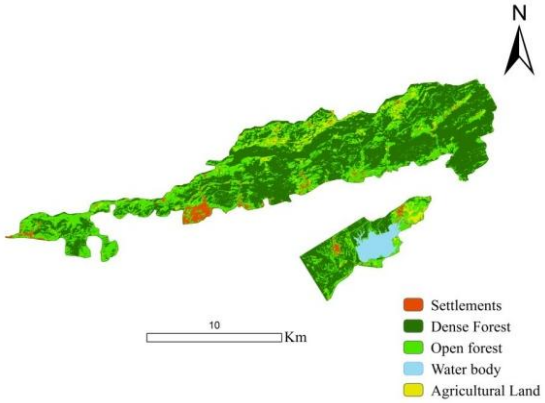


Figure 2. *Land use cover map of Margallah Hills National Park (MHNP) for the year 1990*

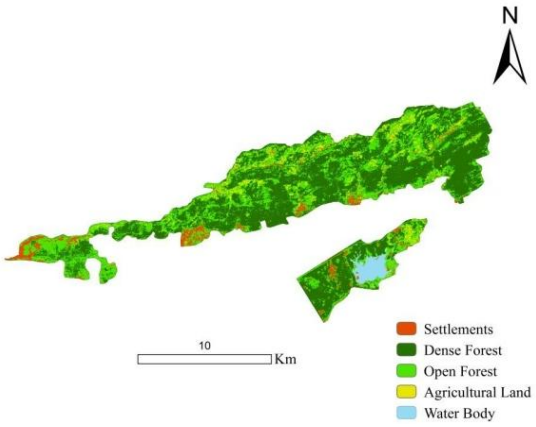


Figure 3. *Land use cover map of Margallah Hills National Park (MHNP) for the year 2000*

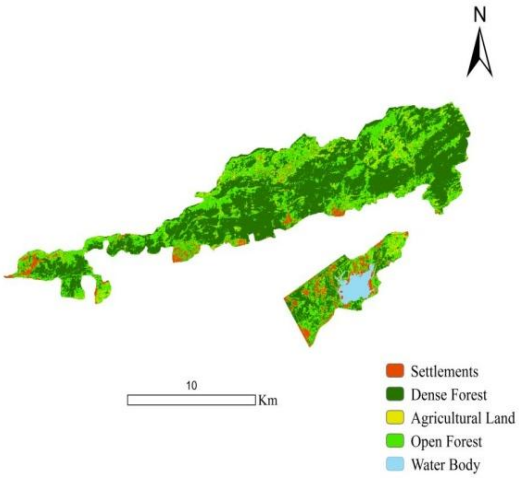


Figure 4. *Land use cover map of Margallah Hills National Park (MHNP) for the year2010*

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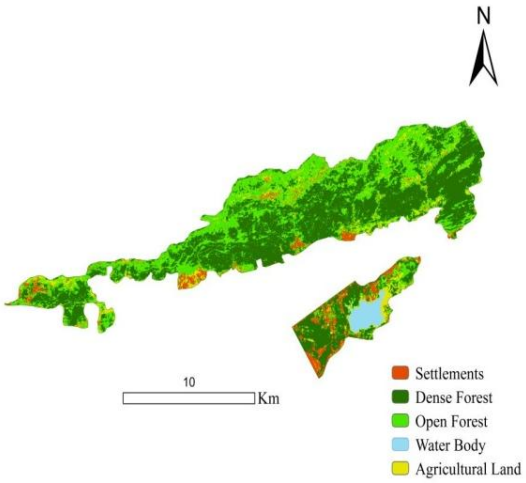


Figure 5. *Land use cover map of Margallah Hills National Park (MHNP) for the year 2017*

Table 2. *Major land uses and LUC*

Land uses	1990		2000		2010		2017	
	Area (ha)	% Area	Area (ha)	% Area	Area (ha)	% Area	Area (ha)	% Area
DF	9697.1	55.87	9256	53.23	8989.2	51.7	8616.6	49.55
OF	5712.3	32.8	5892.7	33.52	5907.9	34.33	5957	34.26
AL	770.9	4.43	841.9	4.83	1020.6	5.86	1180.8	6.78
SM	662.5	3.8	760.5	4.37	940.3	5.4	1111.6	6.39
WB	544.2	3.1	534.9	3.07	528	3.03	520	2.99
Total	17387	99.97	100	17286	99.02	17386	100.32	17386

Table 3. *Periodic/annual pattern and trend of land uses and LUC*

Land use categories	1990-2000 change (%)	Annual change (%)	2000-2010 change (%)	Annual change (%)	2010-2017 change (%)	Annual change (%)
Dense vegetation	-4.54	**-0.453**	-2.88	**-0.288**	-4.14	**-0.591**
Open forest	+3.15	**+0.315**	+0.257	**+0.025**	+0.831	**+0.118**
Agricultural land	+9.21	**+0.92**	+21.22	**+2.12**	+15.69	**+2.22**
Settlements	+14.79	**+1.47**	+23.64	**+2.36**	+18.21	**+2.2**
Water body	-1.7	**-0.17**	-1.2	**-0.12**	-1.5	**-0.12**

The results showed that during the period 1990-2000, DF decrease by 4.54% (0.453% yr^{-1}), during 2000-2010, DF reduced by 2.88 (0.288% yr^{-1},) and 4.14% (0.591% yr^{-1}), respectively as shown in *Table 3*. Apart from a decrease in area under DF, major changes were also observed for WB, OF, AL and SM (*Table 4* and *Fig. 6*). Our results showed that a total of 1268.78 ha forest land (DF, OF) has been converted into other land uses from 1990 to 2017 and Indicating the increasing scale of anthropogenic pressure on forest resources of the Park as shown in *Figure 7*.

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Table 4. *LUC statistics from 1990 to 2017*

Land use type	Land use change to other land use	Area change (ha) (1990-2017)	Total area change (ha)
Agricultural land	Open forest	345.4200	335.69
	Dense forest	47.9700	
	Settlement	42.3000	
Dense vegetation	Agricultural land	40.1	2242.94
	Open forest	1,944.9000	
	Settlement	257.9400	
Open forest	Agricultural	528.0300	2291.22
	Settlements	442.7100	
	Dense forest	1,320.4800	
Settlements	Agricultural land	69.3000	276.84
	Open forest	180.0000	
	Dense forest	27.5400	
Water body	Dense forest	2.3400	136.35
	Open forest	14.4000	
	Settlements	1.5300	
	Agricultural land	118.0800	

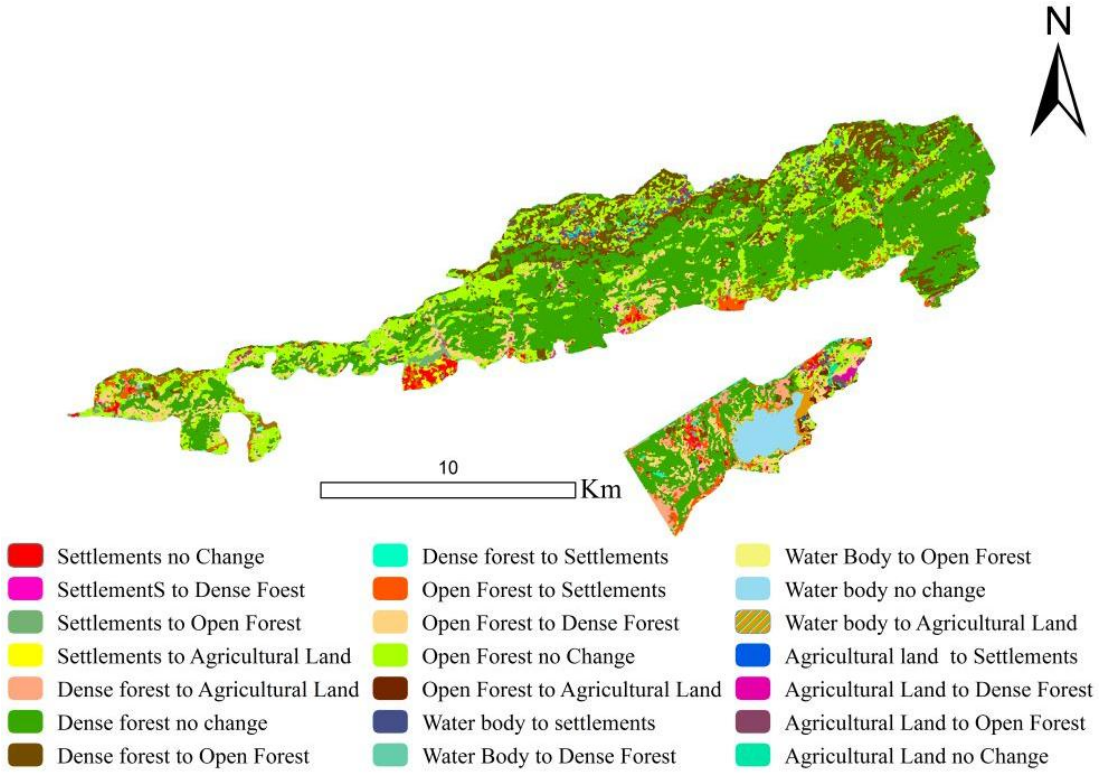


Figure 6. *Statistical land classes change in Margallah Hills National Park (MHNP) from 1990 to 2017*

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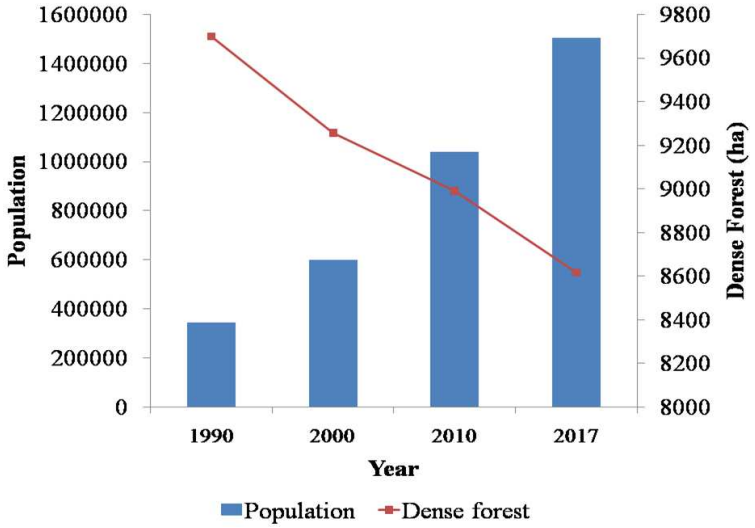


Figure 7. *Increase in population of Islamabad city*

Carbon dynamics and carbon losses

The estimated carbon value recorded in DF is 164.14 ± 8.96 MgC ha^{-1}, whereas it is in 94.62 ± 8.75 MgC ha^{-1} in OF (*Table 5*). In each forest, the higher value of carbon is recorded for upper storey vegetation (UPSV) followed by soil, understorey vegetation and litter dead wood. The results of the total carbon stock (*Table 6*) showed that in 1990 the total forest carbon stock was 2131 GgC. Similarly, the total forest carbon stock was reduced in 2000, 2010 and 2017 i.e. 2076 GgC, 2034 GgC, and 1987 GgC respectively. These findings revealed the decreasing trend in carbon value associated with LUC. Taken together as a whole the present results indicated that LUC accounted for 154 GgC loss from 1990 to 2017.

Table 5. *Carbon pool comparison*

Carbon pool	Dense forest (MgC·ha^{-1})	Open forest (MgC·ha^{-1})
Upper storey	105.69 ±22.58	40.78 ±24.55
Understorey	2.3 ±0.27	1.81 ±0.41
Litter/dead wood	1.13 ±0.32	0.96 ±0.22
Soil	54.97 ±12.67	51.06 ±9.81
Total carbon	164.14 ±8.96	94.62 ±8.75

Table 6. *Total stored carbon*

	Dense forest (ha)	Open forest (ha)	Carbon DF (GgC)	Carbon OF (GgC)	Total carbon (GgC)
1990	9697.1	5712.3	1591	540	2131
2000	9256	5892.7	1528	557	2076
2010	8989.2	5907.9	1475	559	2034
2017	8616.6	5957	1414	573	1987

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Discussion

Land use and land cover change

Natural forests are mostly located in the temperate and subtropical regions of Pakistan where LUC and deforestation are continuous processes. The increase in SM and AL from 1990-2017, indicate the scale of LUC in the park. We found that in the last 27 years SM has increased by 67.78% as compared to our base year 1990. Between 1990 and 2017 SM for Islamabad increased from 16,281 to 51,039 ha as shown in *Figure 8*. This increase is attributed to the migration of people from villages to cities for better health facilities, education, and job opportunities. Also, settlement expansion (built-up areas) around Rawal Lake, have caused an increase in SM in and around the park (Hassan et al., 2016). Similarly, due to unsustainable agriculture practiced by local villagers, AL expanded from 770.9 ha in 1990 to 1180.8 ha in 2017.

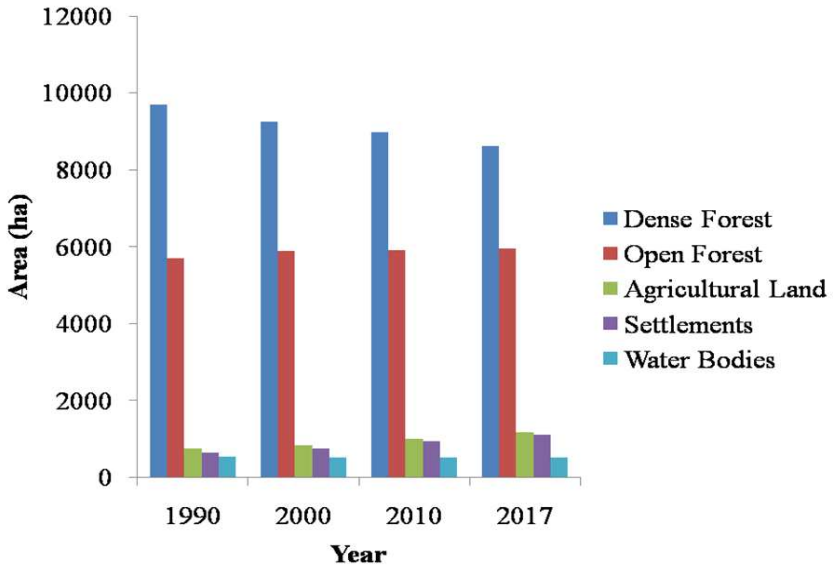


Figure 8. *Temporal change in classes*

Increase in settlements from 10 villages in 1990 to over 20 villages in 2017 may have induced AL expansion in the MHNP due to population pressures (Khalid et al., 2015). Related studies also show settlements to have increased by 23% in the MHNP between 1992 and 2012 (Hassan et al., 2016). The periodic and annual rates of increase in the OF, AL, and SM were notably higher between 2010 and 2017. This may be due to an increase in population between 2010 and 2017 per km^2 of Islamabad (PBS, 2017). This increase in population around the park causes major shifts in LUC. Overall, an average annual loss in DF for MHNP is 0.42% which is much higher than the 0.21% average annual deforestation from terrestrial protected areas of the Philippines (Apan et al., 2017).

For instance, the DF in the base year 1990 was 9697.1 ha and decreased to 8616.69 ha in 2017, as shown in normalized difference vegetation index (NDVI) map (*Fig. 9*). This decreasing trend in DF is similar to that reported by Malik and Husain (2003) which was 59.4% in 1990 and decreased to 56.3% in 1998. The conversion of DF into OF in and around the park could be attributed to unregulated and illegal logging

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by local villagers for fuelwood, livestock grazing, and forest fires. As elsewhere around the globe, forest fires are a regular part of MHNP especially during the drier months of summer (Chanthalath et al., 2017). These fires usually damage the DF more severely as compared to OF. The continuous reduction in the area under DF and increase in areas under OF, AL, and SM showed DF to have been mainly converted into OF, AL, and SM.

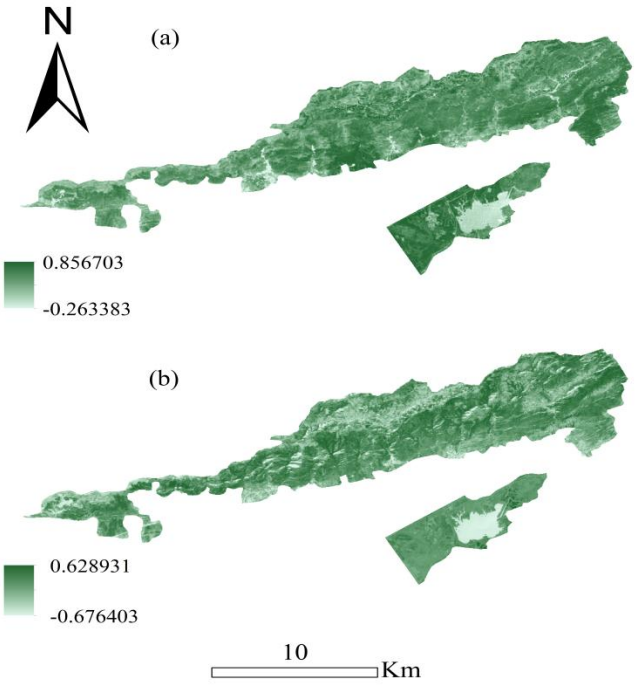


Figure 9. a. *Normalized difference vegetation index (NDVI) for(a) 1990 and (b) 2017*

Carbon dynamics and carbon losses

The calculated value of carbon varied between 94.62 ± 8.75 $MgCha^{-1}$ in OF to 164.14 ± 8.96 MgC ha^{-1} in DF with average carbon stored in forestland being 129 ± 8.85 MgC ha^{-1}. Similar results were reported for carbon stock in Ghoragali, Pakistan (subtropical/chir pine) (Nizami, 2012). Similarly, our present estimates are also inconsistent with the reported value by Rana et al. (1989) from the Himalayan region of India.

The conversion of forestland to other land uses resulted in carbon loss from 2131 GgC for 1990 to 1977 GgC in 2017. Same results were previously reported which showed loss of 32.7 PgC from the protected areas of Brazil Amazonia in 2014 (Nogueira et al., 2018). Similarly, in the protected areas of US from 1700-2005 more than 3 million km^2 of forest land, grassland and shrub land were converted to agricultural land that caused 10,607 TgC loss (Lu et al., 2018). This reduction in carbon supports the fact that the transformation of forestland to other land uses reduces the carbon storage potential of forestland. Deforestation, agricultural and settlement expansion attributed to population growth, resulted in forest conversion, hence decrease in carbon stock. Over these different periods, the highest rate of carbon losses was

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recorded for the period 2010-2017 as shown in *Table 7*. This period also coincided with the maximum of population growth and human migration within the study area. According to the population statistics data (PBS, 2017) between 2010 and 2017, 0.422 million people migrated to Islamabad as compared to 0.245 million during 1990-2000. The expansion in AL and SM usually occurred near the old settlements, where local people grow crops. Although this LUC affects carbon loss, the intensity is much higher where DF is converted into SM, AL, or OF (Zheng et al., 2011). Within the past seven years (2010-2017) the rate of annual DF loss calculated was -0.59% much higher than in the first and in the second decade. This is due to an increase in hiking trails, roads, and recreational spots in and around the park thus directly affecting natural forest cover and carbon storage.

Table 7. *Carbon loss*

	Total carbon loss/gain (MgC)		**Net carbon loss (MgC)**	**Net annual carbon loss ($MgC \cdot yr^{-1}$)**
	DF	**OF**		
1990-2000	-72386.71	+17071.13	-55315.57	-5531.55
2000-2010	-43783.21	+1438.36	-42344.84	-4234.48
2010-2017	-61145.52	+4646.30	-56499.22	-8071.31

The population of Islamabad increased from 343,000 to over 1,502,000 from 1990 to 2017. These demographic factors in conjunction with other environmental and socioeconomic factors constitute major driving forces of LUC in and around MHNP (PBS, 2017). Additionally, in 1990 the population living in villages around Margallah Hills was estimated at about 20,000 but this increased to over 90,000 in 2017 distributed across 20 villages (Khalid et al., 2015). This demographic pressure is also a major threat to the sustainability of these natural ecosystems (Khan and Khan, 2009; Ali and Nyborg, 2010; Cochard and Dar, 2014). This demographic pressure also drives the expansion of built-up areas and agricultural land, as well as wood extraction (fuelwood, artisanal and commercial logging), which are drivers of forest cover change and degradation of natural ecosystems (Moffat et al., 1998; Agudelo-Vera et al., 2011). Other studies have also shown that anthropogenic activities are major threats to global forest cover (Rokityanskiy et al., 2007; Zomer et al., 2008; Ahmad and Nizami, 2015; Saeed et al., 2016). Historically, this increase in population and other underlying factors particularly in developing countries have been observed to be the most important driving force of LUC (Rosero-Bixby and Palloni, 1998). According to (Lambin et al., 2003), these underlying drivers of LUC are poverty, population growth, and socio-economic situation. Increased population often results in the migration of people in search of farmlands and open forests are easier to convert to agricultural land (Meli and Meli, 2015). Rural communities around the MHNP have witnessed an unregulated and uncontrolled urban expansion which has pushed these communities into forested lands of the area. Stone quarrying for cement making is also a contributor to carbon loss because Margallah hills have a high quality of limestone, shale, and sandstone used for cement production (Muhammad et al., 2013). There are over 700 stone quarries around the MHNP with dire consequences for the surrounding vegetation, plant and animal species, namely 38 species of mammals and over 600 plant species in the MHNP (Dawn News, August 23, 2010). In a related study, Ali et al. (2006) observed wood harvesting

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for commercial and household consumption, forest disease, and unregulated harvesting to be main drivers of deforestation in the watershed area of the MHNP.

Forest fires regularly occur in the MHNP due to fallen dry needles of chir pine in summer and dry season around May. According to de Groot et al. (2009), about 40.9% to 53.9% of carbon is lost annually due to forest fires depending on the intensity of forest fire. Most of the forest fires that occurred in subtropical forests of Pakistan are accidental due to recreational activities and negligence. Increasing trends of forest surface fires have been observed in subtropical forests of Pakistan (Nafees and Asghar, 2009). Forest fires are known to reduce soil carbon, soil erosion, reduction in understory vegetation and soil bareness (Jaiswal et al., 2002). The grazing and trampling of livestock severely affect vegetation regeneration thus also contributing towards LUC. Khalid et al. (2015) found that MHNP has to bear the pressure of 12,000 and 13,000 domestic livestock grazing.

Conclusion

The knowledge obtained from this study may serve as an input into the rehabilitation and management of degraded forest ecosystems in Pakistan and in the world. Furthermore, the MHNP is located in Islamabad and near Rawalpindi, which are Pakistan's most important twin cities with high demographic pressures. The present Study highlighted the temporal loss of forest carbon sink with the land use change in MHNP. The study also discussed the major drivers of land use change and carbon loss in MHNP i.e. stone crushing for cement making, fuel wood for commercial, population pressure, AL and SM expansion with detrimental effects on the ecosystem of the park. Moreover, the building of new infrastructures in the twin cities has resulted in a decrease in the forest area. These factors coupled with weak governmental policies, and inadequate land use management plans, facilitate a reduction in forest biomass and ultimately carbon sequestered in the MHNP. Deforestation control through improved forest management, appropriate forest conservation policies, development of land use management plans and urbanization regulations will help curtail, curb and reverse this deforestation process. This study provides results and recommendations to researchers for future studies focusing on especially the effects of land use change on wildlife habitat of MHNP and the effects of carbon loss on the local environment of MHNP. Additionally, an improvement in the national forest monitoring capacity and the implementation of REDD+ arrangement and sustainable land and forest management approaches can control the current forest cover loss, and carbon loss resulting from LUC.

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LOCAL ECOLOGICAL KNOWLEDGE IN *ANGKLUNG PAGLAK* OF *USING* COMMUNITY OF BANYUWANGI, INDONESIA

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Abstract. *Angklung paglak* is a traditional musical instrument made of bamboo of *Using* (an ethnic community of Banyuwangi). This study was conducted to reveal the Local Ecological Knowledge (LEK) in *angklung paglak* manufacturing and its correlation to bamboo conservation. The data collecting was done through documentation, participant observations, and interviews. For ensuring the validity and reliability of the data, cross-referenced and repeated information methods were used. The local knowledge in *angklung* making includes selecting *benel* (*Gigantochloa atter*) and *ori* (*Bambusa arundinacea*) as *angklung* materials, the age of the bamboo (more than three years old), the best month for logging since August to October, and the air drying the bamboo without exposing it to the direct sunlight in upright position. The belief includes logging on a particular day and a taboo for logging in December to February (sprouting period). Meanwhile, the local practice is reflected in the selective felling. The LEK found in the study- knowledge, belief and practice, contributes to the bamboo preservation. The whole process of *angklung* making teaches people to preserve and to live with nature, which reflects an ecological human characteristic. The perseverance and the ecological awareness are the keys to make a high-quality *angklung paglak*.
Keywords: *ethnoecology, musical instrument, bamboo conservation, local wisdom, bamboo management*

Introduction

Local people play an important role in conserving and managing natural resources as well as environment (Chaiphar, et al., 2013). Their interaction with nature has influenced the way of thinking, behaving, expressing and appreciating their culture (Keraf, 2002). Local people develop a system of local knowledge as a representative of the adaptation processes to their environment, including knowledge, belief, tradition, practices, and view of life (Vandebroek et al., 2011). Local knowledge can be unique because it differs in each community or country (Jasmine et al., 2016). The accumulation of knowledge, practices, and belief in the relationship of living things (including human) and environment is defined as Local Ecological Knowledge (LEK) (Berkes et al., 2000). LEK itself has contributed to the biodiversity conservation through ecosystem management and sustainable biodiversity which are experienced by local people. The persistent practices of natural resources by the local people are the basic knowledge of a complex ecological system (Gadgil et al., 1993).

Most *Using* (also *Osing,* an ethnic community of Banyuwangi in East Java Indonesia) are farmers for which the rice fields are their main resource and valuable property. The agricultural tradition that has been created by them is then passed to the next generation and is still being practiced by today's *Using* people. Anthropologically,

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culture is defined as human's creations which are obtained by acquiring or learning (Koentjaraningrat, 2015). One of the agricultural cultures of the *Using* people is a musical instrument called *Angklung Paglak*. This instrument is made of bamboo and played at the harvest time. The aim of playing it is to tell people that one's rice is about to be harvested. It also motivates the workers. The term *paglak* is derived from the name of a hut used by *Using* farmers to rest and oversee the field. People usually play the *angklung* in this *paglak* (Saputro et al., 2015); its height is 5-10 meters so that the sound can be heard by people around. The *angklung* players stand on the *paglak* and they will climb to it through the stairs of a single bamboo culm in which each internode is equipped with a bar for footing.

The *angklung* manufacture requires special knowledge and skill to produce a set of a melodious one. A set of *angklung paglak* consists of two pieces, each is made of 13-15 bamboo tubes (13-15 tones). The makers exceptionally pay attention to the quality; even they select the bamboo culm carefully. The tubes must be unbreakable and the tone must be stable. The belief in specific days and months to log bamboo trees also becomes the consideration in manufacturing to this day.

LEK can be learnt through holistic approach by using ethnoecology study. This approach analyzes how LEK, the history, the social and ecological function, as well as the relation to other dominant cultures are obtained in the local society (Ruiz-Mallén et al., 2012). Ethnoecology is a study of knowledge and management of the local people which is related to the ecological interactions (Martin, 2001). The purpose of the present study was to reveal the LEK of *Using* people in making *angklung paglak* through an ethnoecology study. The LEK covers knowledge, belief, practices of managing bamboo during the process of *angklung* manufacture which leads to bamboo conservation.

Methodology

Banyuwangi is a region in East Java Province, Indonesia. It is located at 7°43'- 8°46' South latitude and 113°53'- 14°38 East longitude (Badan Pusat Statistik Kabupaten Banyuwangi, 2015). Banyuwangi is a province located in the eastern area of East Java (*Fig.1*). This study was conducted in Glagah, Banyuwangi, specifically in three villages, namely Kemiren, Glagah, and Tamansuruh. In these three villages, there are *angklung* makers and musicians, and the people who are still playing it during their field ritual. *Using* community of Kemiren often present *angklung* art performances for tourists. Of all *Using* communities, only those living in Kemiren hold still strongly to the culture of *Using*, thus Kemiren was declared as an *Using* tourism village by the government of East Java Province in 1995 (Indiarti, 2015).

This study employed descriptive qualitative approach and based on the standard method of anthropology research (wide-ranged individual interviews, deep discussions, and participants observations) (Ripunjoy & Indira, 2012; Choudhary et al., 2008). Data collecting was done through documentation, participant observations, and interviews (Bungin, 2012; Albuquerque et al., 2014a). The researcher acted as the instrument and the data collector. The documentation technique was conducted through reviewing literature of researches, journals, and books related to *Using* culture, especially *angklung paglak*. The participant observation was done by involving the researcher into the whole manufacturing process of *angklung paglak*, beginning from the bamboos logging until the harmonizing. The interview was done through direct communication

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with some informants. The interview was in the form of semi-structured individually and Focus Group Discussions (FGDs) (Choudhary et al., 2008).

Individual dept-interview was conducted through three stages namely preliminary, primary, and closing. The preliminary interview was carried out by introducing the researchers to the informants, explaining the research description generally, asking the informants' willingness or ensuring the importance of that informant's role in research that would be undertaken. In the primary interview, the research data collecting was done by semi-structured interview and directly observing the informant's activity; this interview was averagely conducted three times. In the close interview, the validity and reliability were conducted using cross-referenced and repeated information methods. Focus Group Discussion was executed twice by involving the main informant and recommendation-informant. The interview was conducted using interview guideline. There was an open discussion in every interview and it was recorded using a recorder as permitted by the informant and then the field journal was made.

The informants consist of main and recommended informants who were obtained through purposive and snowball sampling techniques (Bungin, 2012; Albuquerque et al., 2014b) . The purposive sampling technique was used to choose the main informants, while the snowball sampling technique was used to choose the recommended informants. The main informants were 61 and 57 years old-elders of *Using* in Kemiren Village. The recommendation-informants were the maker as well as the player of *angklung paglak* (local expert) and *Using* society. Based on the recommendation from both elders, the local experts are chosen from two people living in Kemiren Village and Tamansuruh Village and four people from *Using* society in Kemiren Village. According to the local expert from Tamansuruh Village, two other local experts were found namely one person is from Glagah Village, Glagah Sub-District and another one is from Mangir Village, Rogojampi Sub-District. The recommendation informants are 37-74 years old. All informants are the original residents of their village (they were born and still live in those villages). Based on the observation and preliminary interview results, the selected *angklung* makers are from Tamansuruh and Glagah Villages. The data analysis was done qualitatively. The data validity and reliability tests were done by using cross-referenced and repeated informations (Albuquerque et al., 2014b).

Results

Angklung paglak is played to amuse and motivate people who are harvesting their rice. Unluckily, the *Using* people now rarely play it when harvesting their rice. The *angklungs* were played instrumentally following the pentatonic *slendro* of Banyuwangi; it is different from the *Sundanese angklung* whose notes refer to the Western diatonic (Syaiful, 2015). The *Using* people call one piece of *angklung* as *ancak angklung*. One *ancak* contains 13-15 tones (13-15 bamboo tubes) which are connected using a strap and attached to a wooden bar (*Fig. 2*). The *angklung* set consists of two pieces, so that it needs two people to play; one as the main player and another one is the backing player (*Fig. 3*). Mostly, the *angklung* performance is followed by two *kendang* players; they use small size *kendang*. The most played songs are *Lemar-lemir, Sekar Gadhung, Tetho Lelung, Gondoriyo, Kembang Jeruk, Lebak-lebak* which are considered as old songs, while the new songs are *Hoya-hoya, Jaran Ucul, Ojo Cilik Ati* (Syaiful, 2015).

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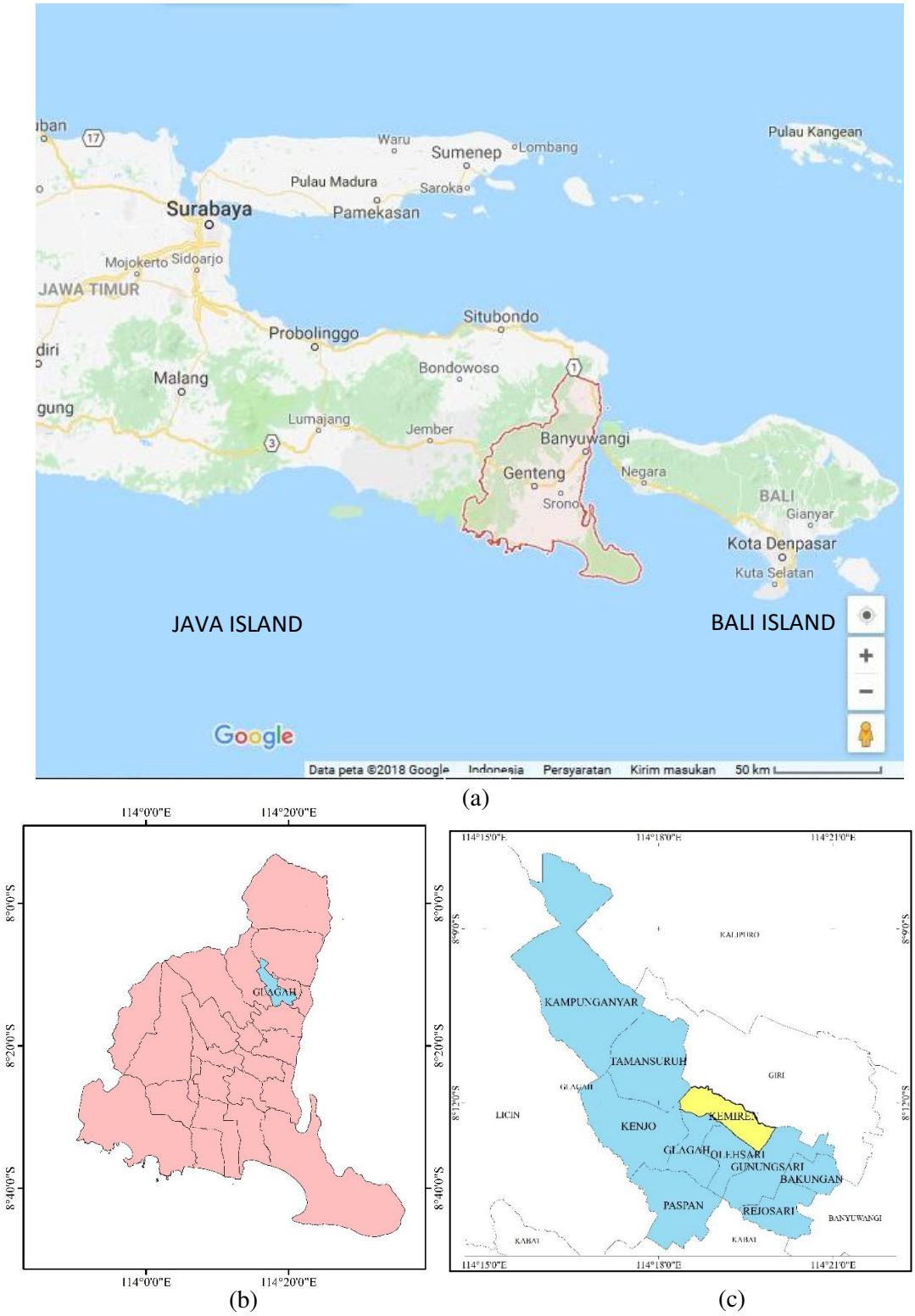


Figure 1. *Map of Banyuwangi Regency Location (a) Glagah Sub-District (b) Kemiren Village (c)*

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(a) (b)

Figure 2. *Photographs of angklung paglak. The original type of angklung paglak (a) and the modified one (b)*



Figure 3. *Angklung paglak performance in a custom ceremony. The musicians consist of two angklung players and two kendang players*

Based on the participant's observation and interview, it is known that there were several stages in the process of *angklung paglak* manufacture, among others (1) bamboo culm selection, (2) bamboo logging, (3) drying, and (4) *angklung* harmonizing. The principle in its manufacture is to produce a quality *angklung* that has melodious and stable tone and unbreakable bamboo tubes. This principle is inherited by the ancestors.

Bamboo culm selection

Using people used *benel* (*Gigantochloa atter*) and *ori* (*Bambusa arundinacea*) bamboos to make *angklung*. The knowledge of both types of bamboo is originated by their ancestors hereditarily. The *benel* bamboo is used more frequently than the ori one due to the gentle sounds produced and the durable tone stability. Meanwhile, *ori* is used because of its high tone sound. However, there are some people also make a set of

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angklung from both *benel* and *ori*, in which tones 1-10 are from *benel* for producing bass sound, while the higher pitch tones 11-15 are from *ori*. The difference in selecting the type of bamboo used is related to everybody's interest in the resulted tone. Nevertheless, generally, people recognize that the sound of *benel* bamboo is the most tunable for *angklung*.

Angklung makers buy bamboo from bamboo clump owners and do not take the wild one. The bamboo is selected by the makers them selves since it is only they who know the characteristics of potential bamboos (*Fig. 4a*). The makers use bamboos of more than three years old with the erect culm. In this selection stage, there are two methods used to choose the culm; the first includes a bamboo that is more than three years old but still alive and the second includes a dead (dry) which they call the *glagaran.* The alive bamboo is characterized by its yellowish culm and over grown lichen, and is still leafy, coloured mostly yellow. While bamboo *glagaran* is characterized by the yellow and leafless culm. The maker who uses *glagaran* claims that based on his experience, it will not any further shrink making the sound stable (does not need to be harmonized frequently). Meanwhile, those who choose the alive culm also claim that the *angklung* tube does not easily break. Until today, both ways to select the culm are still utilized by the *angklung* makers that finally become the special characteristic of *angklung.* However, they should maintain the principle of producing a high-quality *angklung* (the sound is tunable and the tube does not easily break.

Bamboo logging

Using people believe that *Pahing* (one of the Javanese days) in August, September, and October is the best day to log a bamboo tree. However, the maker who uses *glagaran* may log in June up to October. *Using* people mark December up to February as a time when a shoot bamboo sprouts or rainy season comes. They believe that during the rainy days the bamboo "breastfeeds", this time the shoot bamboo sprouts, so *Using* people will not log the bamboo during those times. Based on the *Using* people's experience, the bamboo logged during the rainy days will be easily powdered due to the insects, the culm will get softer and cannot be used to make an *angklung*. Thus, the sound will be less melodious, unstable, and they need more time to dry the culm. The *angklung* makers are very selective in logging the bamboo and never choose the wild ones. There is a belief that bamboo growing near a river will need a longer time to dry, so they do not use such kind of bamboo. Bamboo that grows near a river produces less melodious sound, thus the tones tend to be unstable. Generally, they will test the potential tone of the culm by hitting it and listening to the sound produced.

Bamboo drying

Angklung makers who use live bamboo need to dry the bamboo before cutting it into pieces. After logging the bamboo culms, they will air dry the culm for about three months, without exposing it to the direct sunlight. Culm drying is done upright with the base position of the culm is below (*Fig. 4b*). Whenever they think that the culms are dry enough, the culms are then cut at every two nodes. *Glagaran* needs not drying. After logging *glagaran* culms, the maker cuts them at every two nodes and immediately continues with the harmonizing process.

The ancestors of *Using* people used to store the bamboo tubes on *pogo* (a rack made of a small sized bamboo which are arranged well). The *Pogo* is placed under the roof of their kitchen and above the stove, so that the bamboos were exposed to smoke.

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However, today's *angklung* makers prefer not doing so because the bamboo will turn black and it might influence the aesthetic. Based on their experience, there is no difference between bamboo stored on *pogo* or elsewhere.

Harmonizing bamboo tones

One of the bamboo tube sides is firstly cut with certain length using a *buding* (a type of a big knife). The bamboo tube is harmonized by shaving it with a knife to get the right tone according to its potential (*Fig. 4c*). *Using* people call the knife as *ladeng lanang* because it is usually used by men (*Fig. 4d*). This is the most difficult stage of all because they need to concentrate and be meticulous in listening to the tone produced. The setting of the first tone (the third low note) is the most important step in this stage. The meticulousness in listening to the tones is believed to be related to the art talent of the makers so that they can produce a melodious set of *angklung* (*Fig. 4e*). The bamboo tubes that have formed an *angklung* (13-15 tone) are perforated for the holes of strap. The fault position of the hole may affect the tone generated by the bamboo tubes. The strap used to tie and arrange *angklung* tubes is a plastic rope (*Fig. 4f*), but the *Using* ancestors used a rattan rope in the past. The simple form of the wood buttress without additional ornament eases the angklung players to bring it to the *paglak*. Nowadays, the shape of the wood buttress is made by request.



Figure 4. *The angklung paglak manufacturing: Bamboo logging, done by the maker using saw and* buding *(a); Bamboo drying, the culm (alive bamboo) is air dried without being exposed to direct sunlight in upright position (b); Harmonizing angklung tones use ladeng lanang (a knife usually use by Using men), done by shaving the bamboo tube to get the right tone according to its potential (c); traditional tool used to manufacture angklung (ladeng lanang to shave the bamboo tube,* buding *to cut bamboo's branch, saw to log and cut the bamboo into pieces) (d); a set of angklung paglak tubes as the result of angklung harmonizing (e); and the arrangement of angklung tubes using plastic rope at the wood buttress (f)*

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Discussion

The art of angklung paglak

Nowadays, *Using* farmers who play *angklung paglak* or invite the musicians during harvest time are farmers who get a testament (*weluri*) from their ancestors. *Weluri* in *Using* can be assumed as messages or attitude inherited by the ancestors to their descendants. Now, *angklung paglak* is mostly played during art performances for tourism, traditional ritual such as *Ider Bumi* and *Tumpeng Sewu* in Kemiren, welcoming guests, and at event of *Using* family. Some musicians are often invited to perform in some international art performances. *Angklung paglak* is also sold and purchased by various buyers with different social background such as academicians, musicians, art lovers, and *Using* community outside of Banyuwangi. This is one of the reasons why *angklung* makers do not want to dry the bamboo on the *pogo*. *Angklung* selling also stimulates the makers to modify the wooden buttress to beautify the *angklung*, including the shape of buttress, added curvings and other ornaments.

LEK in angklung paglak manufacture

The ancestors of an ethnic community have proven to have done a local knowledge-based conservation (Tamalene and Al Muhdhar, 2017). The *angklung* manufacture is still based on *weluri* from ancestors. The principle of manufacturing a good *angklung* is still preserved. The *angklungs* are made carefully even though it takes time. Such *weluri* is considered as LEK of *Using* people since it includes belief, knowledge, and practices that are in line with the principle of natural resources management or conservation. Local practices are the result of experiment and observation among generation (Turner et al., 2000). The experience in managing bamboo has created a specific interaction pattern between *Using* people and the environment. This experience is descended from generation to generation. There is a small difference in the practice of *angklung* manufacture by the ancestors. Local practices are considered as a dynamic creativity and innovation mechanism (Slathia et al., 2016). The *angklung* makers available today have the descendant of the *angklung* makers. They said that since they were a child they often see their grandfathers/grandmothers play and make *angklung* so that they like *angklung* and are interested in making it as well. Their ancestors give *weluri* to make *angklung* in the form of belief, advice, guidance, taboo, knowledge, and practice to make *angklung* through oral transmission. The makers then learn to their ancestors (*Fig. 5*).

Their beliefs on logging bamboo culm on *Pahing* day and between August up to October are guidance for *angklung* maker. The time of collecting or harvesting a certain plant has a conservation value (Agize et al., 2013). *Pahing* in Indonesian means bitter, so bamboo which is logged on *Pahing* day is believed to be durable because insects will not gnaw it due to its bitter taste. They also mean that it will be their prayers. The belief has been proven through experiences since bamboo culms which are logged in other than August up to October will be easily powdered (gnawed by insect). Borer is pests of harvested bamboo so they will harm the bamboo. The borer species are including *Dinoderus ocellaris, D. minutes,* and *D. brevis* (Dransfield and Widjaja, 1995; DIJPEN, 2011). The starch content in the bamboo culm is the lowest during the dry season, thus, logging a bamboo culm during dry season will make the bamboo durable since the borer will not gnaw it because of the low starch inside (Razal et al., 2013). *Using* people will not log bamboo culm in December up to February because there is a taboo to log bamboo tree during the rainy season. They believe it will distort the "breastfeeding time"

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(sprouting process). This belief helps preserve the bamboo regeneration. Local belief in something taboo plays a role in environmental preservation because it makes people beware in resources utilization (Tamalene et al., 2014), and has preserved various kinds of species (Gavali and Sharma, 2004). Bamboo logging during a shoot sprout will destroy the shoot itself and influence the bamboo regeneration (Razal et al., 2013).

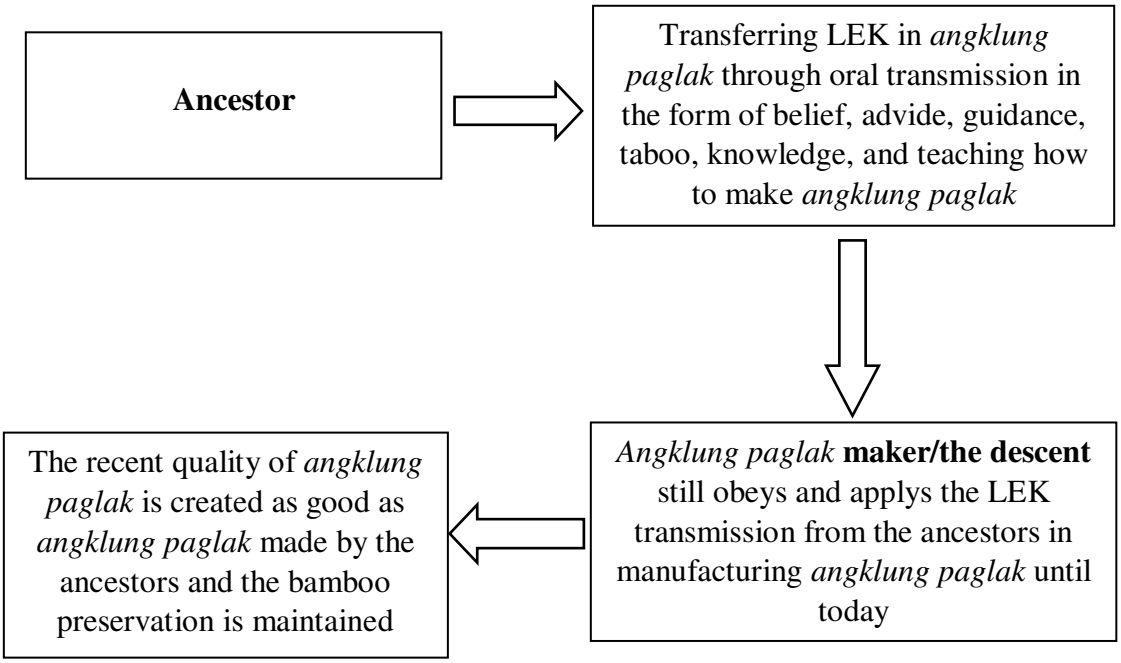


Figure 5. *Process of LEK transmission in angklung paglak making*

The knowledge of bamboo species (*benel* and *ori*) and criteria of suitable culm as the *angklung* material are precious knowledge, so as the process of its manufacture. Local knowledge about *benel* and *ori* as angklung materials will lead to the preservation of these bamboo varieties. The difference between the former and today's *angklung* manufacture becomes the reflection of LEK evolutionary in which new knowledge may exist replacing the previous knowledge based on experiences or trial of a generation (Sunaryo and Joshi, 2003). Such difference in knowledge includes the difference in selecting the bamboo age, drying process, and the variation of bamboo in one *ancak angklung*. The most important thing is the consistency in making melodious *angklung*, stable tones, and durable or unbreakable bamboo tubes.

The high demand of *angklung* makes the bamboo stock dropped. Therefore, the local practice and wisdom which is possible to be done by the makers is to log as many bamboo culms as possible in August up to October for stocks. Yet, selective logging is still practiced by *Using* people. They only log the potential bamboo, with appropriate age (more than three years old) and erected culm. The makers, who choose *glagaran* bamboo, will not make *angklung* of carelessly chosen bamboo to fulfill the demand. They sometimes stock the *angklung* which are ready to be sold. If there is a request and they run out of bamboo, they will ask the customer to wait. They do not want to disappoint their customers because of its quality.

The bamboo conservation practices can be seen during the whole process of *angklung* manufacturing, beginning from the bamboo selection, bamboo drying, and

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angklung harmonizing. The logging day and the selective felling which are not done when shoot bamboos are sprouting are the practice of bamboo management by considering the bamboo preservation. The selective felling can conserve the bamboo and its reproduction (Dransfield and Widjaja, 1995), while clear felling of bamboo clumps will affect the number of sprouts and the height of bamboo (Nath et al., 2009). The selective felling which is done by logging only a potential bamboo is a practice of showing respect to the nature, i.e. the right of bamboo to live and grow naturally. Meanwhile, drying the bamboo culm is done by for a particular period without additional substances. *Angklung* makers do not manipulate the bamboo culm and only manage it naturally. The culm is only cut into several tubes, which are harmonized regarding to the potential tones until get melodious tones. Briefly, the procedure to make *angklung* includes four stages (*Table 1*).

Table 1. *Stages of Angklung making*

No.	Stages of Angklung Making	Activities
1	Bamboo culm selection	• The type of bamboo used: (1) *Benel* (*Gigantochloa atter*): produced gentle sounds (2) *Ori* (*Bambusa arundinacea*): produced high tone sound • The type of *benel* is the most frequently used bamboo • The age of bamboo: more than three years old bamboos with the erect culm. Two methods used (1) alive bamboo: it needs a drying process firstly (2) dead (dry) bamboo: it does not need the drying process; it can be directly harmonized
2	Bamboo logging	• A belief that *Pahing* (one of the Javanese days) in August, September, and October is the best day to log bamboo for *angklung* • Do not log bamboo during sprouting time in rainy season (a taboo) • Dead bamboo (*glagaran*) can be logged since June to October
3	Bamboo drying	• Alive bamboo is air-dried at the upright position where the base part is in the bottom for about three months
4	*Angklung* harmonizing	• One of the bamboo tube sides is partly cut • That part is shaved with a knife (*ladeng lanang*) to get a right tone • Every tube is perforated for the rope hole • Arranging the the bamboo tube at the wood buttress is started from the low to the high tones using plastic rope

The *angklung* maker does not log wild bamboo at a riverside or forest. Whereas, logging the wild one will not be as costly as when logging the clumps from the bamboo farmers. One *ancak angklung* needs 6-8 bamboo culms since each culm might produce

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different potential tones. The *angklung* makers mostly buy bamboo from clumps at farmer's field or garden. Meanwhile, the bamboo management done by the bamboo farmers in Kemiren Village includes two things namely planting and preserving the bamboo (*Table 2*). Home gardens play an important role in conservation and management of the sustaining natural resources species (Agize, 2013). The decision to buy bamboo culm is considered as a conservation practice for it preserves the wild bamboo life.

Table 2. *Bamboo management*

No	Type of Management	Technique of Management
1.	Bamboo planting	• The 1-2 years old bamboo is cut with the height about 1 meter from the ground • The bamboo with its rhizome (*Using* people call it *bongkotan*) are taken; it is then planted in the place they want • The new sprouts (different from the shoot) will grow close to the stem base or rhizome while the stem is weathered over time
2.	Preservation	• People apply selective felling to preserve the bamboo to keep the productivity • The three years-bamboo will be cut/harvested • The harvesting is undertaken out of rainy season (out of sprouting period)

Unfortunately, there is a belief in *Using* community that *angklung* makers must be the descendant of the previous *angklung* makers. *Using* people believe that only those who descent the blood of *angklung* makers or those who have art talent are capable to make melodious *angklungs*. These people are considered to have diligence and capability in listening to tones, and they must inherit this ability from their ancestors. This ability causes other people who want to learn to make an *angklung* less confident. Besides, the instructor or the *angklung* maker will also doubt the students' perseverance in manufacturing *angklung*, since they have to be very patient in listening and finding the correct tone.

Angklung paglak is a symbol of cooperation in farming of *Using* community. This art is disappearing within the decreasing cooperation tradition (*ngrasah*) in field, even though it is not reducing the social interaction between *Using* people. The cooperation in harvesting time no longer exists since they harvest the rice by using a sickle and a rice mill. The rice is directly sold in field, so the process of harvesting runs quickly. It is different from the past, where people used traditional finger-bladed knife (*ani-ani/gampung*) therefore the harvest time took several days. *Angklung paglak* performance which was done formerly in harvest time is no longer practiced today. A local tradition has an important role in conserving biodiversity. A local tradition that is still done by a community may preserve the conservation practices related to that tradition (Tamalene and Al Muhdhar, 2017). The biodiversity conservation is appropriate when it applies cultural value and religion approach compared to constitution and law regulation (Hongmao et al., 2002).

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Conclusion

In brief, *angklung paglak* is one of the cultural products of *Using* community as the expression of farming culture. The process of *angklung* manufacturing consists of several stages: bamboo selection, logging, drying, and *angklung* harmonizing. Each process is considered as a system of local knowledge or LEK which includes belief, knowledge, and practices of managing bamboo. Local knowledge in *angklung* making including selecting *benel* (*Gigantochloa atter*) and *ori* (*Bambusa arundinacea*) as *angklung* materials, age of bamboo (more than three years old), best month for logging in August up to October, and air drying the bamboo without exposing it to the direct sunlight in upright position. The belief on a particular day that is on *Pahing* day and a taboo for logging in December up to February may affect to the time of bamboo logging. The selective felling practice by choosing only potential bamboo, buying it rather than logging the wild one also contribute to the bamboo preservation. The bamboo drying, which is done in order to prevent borer to gnaw the bamboo starch, is also done without any manipulation and chemical substances addition. This reflects ecological human characteristics. *Angklung* making teaches people to preserve and live in harmony with the nature. The perseverance in harmonizing *angklung* also contains value respect to the nature, for what it has given to humans.

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A STUDY OF THE ECOLOGICAL PATTERNS OF FARMING-PASTORAL ZONES IN SEMI-ARID REGIONS: USING THE WEST LIAOHE RIVER PLAIN OF CHINA AS AN EXAMPLE

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Abstract. The farming-pastoral zones in semi-arid regions of China are known to be typically ecologically vulnerable areas. It is required to research the agricultural and pastoral area ecological pattern that can guarantee ecological safety, and put forward the reasonable ratio for plough and grassland. This study selected the West Liaohe River Plain as the research area, and utilized a remote sensing interpretation and field visit method to analyze the land use situation during different periods of time. Also, the distribution scope of the natural grassland was identified, and the ecological security patterns of the agriculture and animal husbandry in the West Liaohe River Plain were adjusted based on the basic principle of natural grassland expansion. Then, the distribution of groundwater flow field in the research area was simulated, and the rationality of the ecological security pattern supported by the groundwater conditions was analyzed. The grassland areas after the adjustments measured 28,760.7 km^2, and accounted for 43.87% of the total area of the West Liaohe River Plain. The grassland area will be recovered to the level of 1990 following the adjustment of this ecological pattern.
Keywords: *land use, natural grassland, expansion, critical water levels, groundwater*

Introduction

The breadth and depth of the influences of human activities on global ecosystems have been expanding continuously with the rapid progress of urbanization. Therefore, the structures and functions of natural ecosystems have significantly changed. The ecological environmental problems caused by the degradation of the ecological systems have become a bottleneck, and have restricted social and economic development. In the early 1980s, ecological security had become a research hotspot in the field of nascent international ecological systems (Naveh, 1994; Yu, 1996; Steffen et al., 2015), and as a result the ecological security pattern theory came into being at the right moment. On the basis of the mutual feedback between patterns and processes, it achieved effective control of the ecological process through the construction of regional ecological security patterns, in order to give full play to the ecological function; realize the effective and reasonable allocations of regional natural resources and green infrastructures; guarantee the necessary ecological natural resources and material welfare; and ultimately achieve ecological security (Dabelko, 1995; Peng et al., 2017). Ecological security patterns have become one of the important spatial methods used to alleviate the contradictions between ecological protection and economic development (Yu, 1996; Meng et al., 2012; Ou et al., 2015; Zhao and Xu, 2015). The majority of the hot spots include the farming-pastoral zones (Li and Cao, 2014), arid/semi-arid areas (Meng et al., 2017), scenic areas (Zhang et al., 2008). These types of areas are potentially strongly disturbed by human activities, resulting in the ecological safety problems becoming outstanding in these

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areas. Previously, many researchers have identified ecological sources based on a "source-sink" theory (Chen et al., 2006) of landscape ecologies, and established the resistance surfaces of the expansions of the landscape elements using minimum cumulative resistance models. The security patterns were then determined with different elements and classes according to the expansion trends (Pan and Liu, 2015), in order to effectively control the landscape space structure. In addition, cellular automata models (Zhu and Liu, 2013), scenario analyses (Mao et al., 2013), GAP analyses (Liu et al., 2009) were used for the establishment of the land ecological security patterns.

However, at the present time, the majority of the research studies conducted on the construction of the ecological security patterns have been analyzed from the perspective of landscape ecologies, without identifying the driving factors which had led to the changes of the regional ecological patterns. Also, the previous related studies also lacked evaluations of the effectiveness or rationality of the construction of the ecological security patterns based on the driving factors (Xu et al., 2017; Zhang et al., 2018). The natural attributes of the regional hydrological cycles determine the basic patterns of an ecosystem (Chen, 2007). For example, for semi-arid areas, the natural ecosystems are grassland vegetation supported by groundwater. The spatial patterns and landscape changes of ecosystems reflect the pattern evolution of the groundwater spatial distribution, and the realization of the ecological safety patterns must be supported by the groundwater conditions. For a long time, the agricultural structure in the farming-pastoral zones in semi-arid regions is unbalanced, with over-developed water sources, and the unsustainable problem in development is more and more serious. In part of the region, the development intensity of water source has approached extremity, with increasingly serious land desertification, grassland degradation and weakening ecological functions. Unbalance even reversal in ratio of plough and grassland has been a comparatively common phenomenon in farming-pastoral zones in semi-arid regions, which obviously does not conform to the national policy for ecological civilization construction, and it is required to research the agricultural and pastoral area ecological pattern that can guarantee ecological safety, and put forward the reasonable ratio for plough and grassland, which is an important ecological safety problem with far-reaching influence. In this research study, by regarding the West Liaohe River Plain as the research area and based on a "source-sink" theory, a minimum cumulative resistance model was used to establish the landscape ecological security patterns. Also, the rationality of the ecological security pattern supported by the groundwater conditions was analyzed, in order to provide references for the ecological protection in semi-arid areas.

Materials and methods

In this research study, by taking the West Liaohe River Plain as an example, with the aim of protecting the natural attributes of the ecological system, a method was established for evaluating the ecological structure in the farming-pastoral zone, and a reasonable natural and human ecosystem was constructed. First, the remote sensing information and field visit data were used to analyze the changes in land usages in the West Liaohe River Plain, along with the distribution scope of the natural grassland. Second, the critical groundwater level of the vegetation community in the West Liaohe River Plain was calculated in combination with the data obtained from the field visit and historical investigations. Third, the existing natural grassland vegetation was found

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to have a good protection of biological diversity, and could be used as the gene pool for the ecological restoration of the grassland. Therefore, the outward expansion was based on the natural grassland in order to adjust and merge the grassland scope. Finally, the critical burying depth of the groundwater recharged vegetation was regarded as the constraint condition, in order to analyze the rationality of the ecological pattern supported by the groundwater subsurface flow field.

Study areas

In China, the semi-arid area is a strip zone between the arid area of the northwestern inland river, and the semi-humid area in the north and northeastern regions of China. This includes the west section of the Songliao River Basin; northwestern section of the Haihe-Luanhe River Basin; upper and middle reaches of the Yellow River Basin, and so on. Among these areas, the West Liaohe River was determined to be a relatively complete basin, and was therefore selected as the research area for this study (*Fig. 1*).

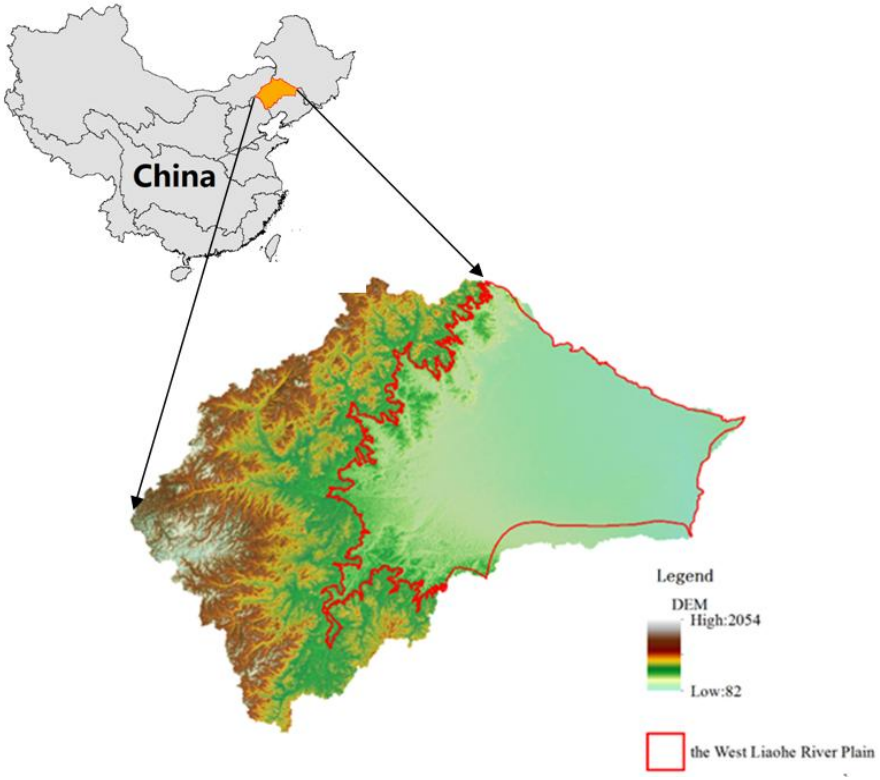


Figure 1. *Study areas*

The West Liaohe River Plain is located east of the West Liaohe River Basin. Its main section is located in Tongliao City, within 41°05′ to 45°13′N and 116°10′ to 123°35′E, with a land area of approximately 65,000 km^2. The West Liaohe River Plain is a typical farming-pastoral zone in the semi-arid region of China. The regional average annual precipitation is less than 380 mm, and the annual average water evaporation is more than 1,000 mm. Also, this area has obvious semi-arid characteristics. The rainfall in this area does not directly generate surface runoff, and the precipitation has a significant vertical infiltration. The groundwater is abundant, and the vertical recharge is stable.

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The vegetation in the pasturing area includes mainly typical herbaceous steppe vegetation communities, as well as arbor, shrub, and sub-shrub steppe vegetation and meadow vegetation communities. The natural ecology in a semi-arid area is a steppe vegetation supported by groundwater. The changes in the spatial patterns and landscapes of ecosystems are known to reflect the pattern evolution of the spatial distributions of the groundwater. The grassland ecologies represent the natural attributes of the ecosystems in semi-arid areas, and are the ecological foundations which support these areas. The grassland ecological security is not only related to the pasture itself, but also greatly affects the ecological quality, safety, and stability of the entire region.

Changes in the land usages

This study selected four periods for the interpretation of the land usage and analyzed the dynamic changes of the ecological patterns of the West Liaohe River Plain, in order to identify the distribution scope of the natural grassland in the West Liaohe River Plain, in combination with the field visit. The remote sensing interpretation results are shown in *Figure 2*.

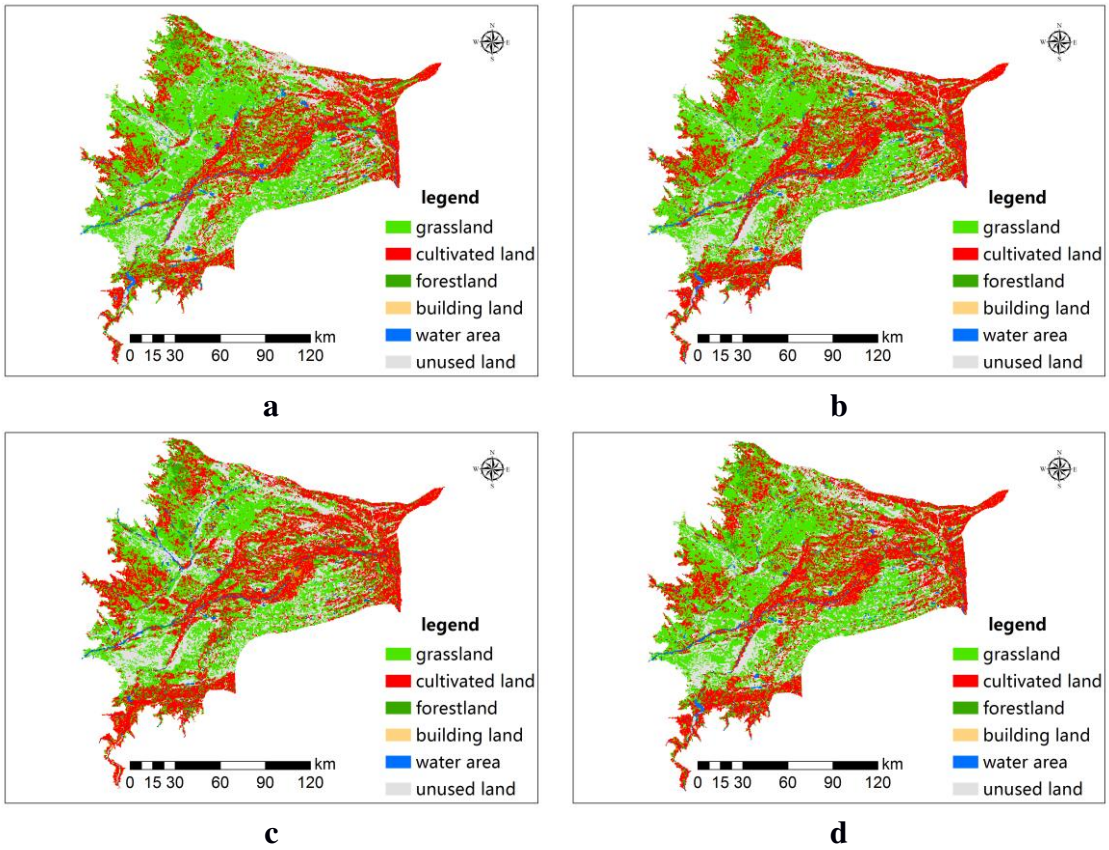


Figure 2. *Distribution of the land use types in the West Liaohe River Plain between 1990 and 2016:* ***a*** *1990,* ***b*** *2000,* ***c*** *2010 and* ***d*** *2016*

It was determined that, in the land use types of each region, the grassland and cultivated land areas occupied a larger proportion, while the water and construction land areas occupied a smaller proportion. The southwest, northeast, and middle areas were

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basically covered by cultivated land, while the grassland areas were mainly distributed in the Jarud Banner in the northwest, and Horqin Left Back Banner in the south. According to the comparison of the grassland and cultivated land areas during the different periods, the grassland area in the West Liaohe River Plain was found to have been sharply decreased, from 29,092 km^2 in 1990 to 21,428 km^2 in 2010. Also, the grassland areas were increased after 2010, with the grassland areas reaching 22,439 km^2 in 2016. The changes in the cultivated land areas showed an opposite trend. For example, the cultivated land areas increased from 18,251 km^2 in 1990 to 26511 km^2 in 2010. Moreover, the cultivated land areas had decreased after 2010, and were 23,372 km^2 in 2016. It was found that the proportion of the agriculture and animal husbandry land usages in the West Liaohe River Plain had already been reversed by 2010.

Grassland vegetation investigation

The author carried out grassland vegetation investigation in grassland of West Liaohe River Plain for three times in July to August, 2016, and July to August, 2017 (*Fig. 3*), with vegetation investigation area of 6,702.89 km^2, and 306 sampling points are selected in all. 94.1% of sampling points are concentrated in natural grassland, with 297 sampling points distributed in all; and 9 sampling points are spread in other regions. It can reflect the vegetation distribution condition of grassland in the West Liaohe River Plain basically, with good representativeness and comprehensiveness. Longitude and latitude positioning can be done for all the sampling points with handheld GPS, and the phytology professionals carry out statistics and record for the plant category and species name, perform photograph and description for the surrounding ecological environment of the sampling point, screen out the dominant species, and determine the community name and species composition of the sampling point.

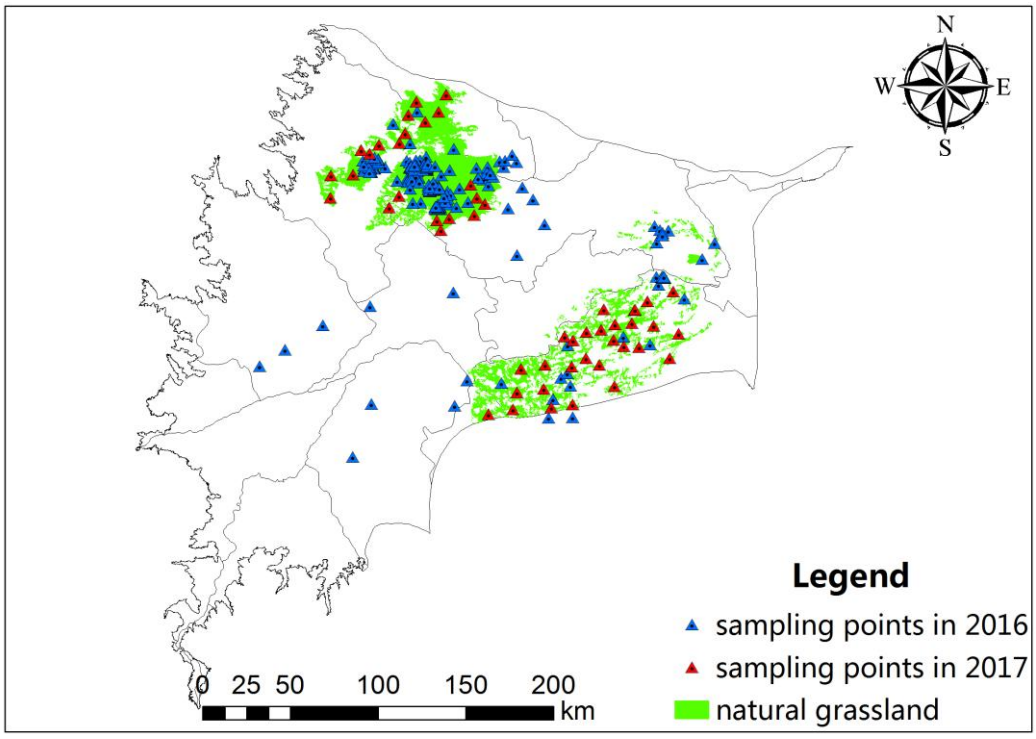


Figure 3. *Sampling points of grassland vegetation investigation in West Liaohe River Plain*

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Calculation method for critical buried depth of groundwater recharge vegetation

In regard to grassland plants in semi-arid areas, the ability of plants to absorb water is the most important condition for their competitive survival. Previously, many research studies have examined the adaptability rates of different plants under drought stress conditions (Zhou, 1999;Jin, 2010; Zhang, 2011). The author puts forward a kind of calculation method for critical buried depth of groundwater recharge vegetation (Chen et al., 2016). The analysis and calculation are composed of two parts: one is the vegetation root system active layer connected to ground, the other is the underwater diving active layer connected to groundwater surface. Under certain climate and soil condition, the root system layer thickness is related to vegetation type, which can be acquired according to the field investigation and the existing vegetation ecology research result. The forming of underwater diving active layer is the result of capillary force action, and the maximum lifting height of capillary water can be used as the thickness index of underwater diving active layer. Calculation formula for maximum lifting height H of capillary water is as follows:

$$H = 2\gamma / \rho gR$$

ρ is liquid density, g is gravity acceleration, R is effective aperture.

Natural grassland expansion based on a source-sink theory

In order to minimize the grassland degradation, the grain for green and closing measures have been widely applied in the farming-pastoral zones in recent years. The natural restorations of rehabilitated land areas are very slow processes. The formation of a stable vegetation community usually takes between 15 and 20 years. Moreover, the formations of such vegetation communities are relatively singular and do not contain rich species. Natural grasslands basically maintain the natural attributes of grassland areas due to good groundwater conditions, and less human interference. Natural grasslands have abundant plant species, and provide gene pools for grassland ecological restorations. Therefore, the adjustments in the ecological patterns in the farming-pastoral zone of the West Liaohe River Plain should include the expansion of the natural grassland as the basic principle, which will potentially increase the connectivity of the habitat patches and increase the mobility of the species among the patches. It will promote inter-plate gene exchanges and species mobility, as well as enhance the opportunities for species re-immigration. At the same time, the species in the isolated patches will be able to survive and continue, which will then provide a continuous network of habitats for the species which lack space diffusion capabilities. Therefore, the natural grassland areas will be continuously enlarged, and the natural nature of the natural grasslands can be maintained.

A “source-sink” theory is a basic theory which is used in the study of landscape ecology. The “source” landscape refers to the landscape type which can promote the development of the ecological processes in the study of the patterns and processes of an area. The “sink” landscape is the landscape type which can stop and delay the development of the ecological processes. The positive significance of the application of this theory was to couple the static landscape pattern with the dynamic ecological process. This assisted in the understanding of the relationship between the ecological pattern and the process, and provided a scientific basis for putting forward effective

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ecological spatial regulation and management methods. At the present time, this theory has been effectively applied on a large scale in the field of biodiversity conservation. Therefore, by taking the natural grassland expansion as the basic principle, this study used a "source-sink" theory method in the landscape ecology in order to adjust and optimize the ecological pattern of the agriculture and animal husbandry land usages in the West Liaohe River Plain. The natural grassland, as a gene pool for the grassland ecological restoration, played a key role in the safety of the entire plain ecosystem. Therefore, it was used as a "source" in this study, while the farmland and unused land were regarded as a "sink". In order to avoid direct conflicts among the different landscape functions (such as the grassland, farmland, and so on) from the aspect of a spatial pattern, a buffer zone was arranged in the contact zone between the grassland and large irrigation zone (including the closed grassland or artificial green belt) which eliminated the impact of the irrigation zone on the grassland, and facilitated the conservation and restoration of the natural attributes of the natural grassland areas.

In order to reflect the process in which the "source" landscape experienced different media in order to overcome resistance, it was necessary to evaluate the landscape resistance based on the dynamic characteristics of the "source" landscape in the study area. Therefore, with the help of GIS technology, this study used a minimum cumulative resistance model to express the influence of the landscape media on the spatial diffusion of the landscape "source", and thereby established a landscape resistance surface. The minimum cumulative resistance model considered the landscape "source", distance, and landscape interface characteristics, as shown in *Equation 1:*

$$MCR = f_{min} \sum (D_{ij} \times R_i)(i = 1,2,3,\ldots m;\ j = 1,2,3,\ldots,\ n) \quad \text{(Eq.1)}$$

where D_{ij} refers to the field distance from the landscape "source" j to a landscape unit i in the space; and R_i denotes to the resistance coefficient of a certain landscape unit i in the space, and represents the difficulty level of the movement passing. The minimum cumulative resistance model used in this study was essentially the measurement and evaluation on the accessibility of a path from the "source" landscape to a point in the space. The smaller the resistance value, the higher the accessibility to this point. Therefore, the resistance model was able to reflect the potential possibility and tendency of the landscape unit movement, and was a type of abstract expression of the landscape changes. This provided an analysis basis for the landscape function characteristics. As far as the protection and utilization of the municipal ecosystem were concerned, the areas with stable municipal ecosystem structures were favorable to the migration and diffusion of the landscape unit, and the resistance to migration and diffusion were smaller. In contrast, there was a higher resistance to the unit movement and diffusion.

Different landscapes suffer different resistances during the processes of movement and migration, which are dependent on the suitability degree of the landscape interface to the survival, reproduction, and migration of the elements. However, the resistance coefficient is also relative, and can be used to calculate the cost distance since it can relatively reflect the differences in the different resistance factors. This research study set 1 as the minimum resistance coefficient, which represented the fact that a certain landscape unit could move freely, and its area was the most suitable space. Also, this study set 100 as the maximum resistance coefficient, which assumed that the landscape unit could not move, and its area was the most unsuitable space. As far as the research area was concerned, the areas which could not be adjusted involved urban development

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planning (such as urban construction land, large irrigation areas, and so), which were determined to have the largest resistance value (100). The farmland areas had a smaller resistance value of 70; unused land had a resistance value of 50; and the deteriorated grassland areas were given a resistance value of 10.

The classified statistical measurements were made for the cumulative resistance values by using a 1/2 standard derivation in GIS. The critical threshold was determined to divide the corresponding resistance intervals according to the frequency discontinuity and spatial distribution characteristics of the resistance value. Therefore, the influence degree of the natural grassland on the entire expandable area was divided into the expansion core area, expansion connection area, and expansion buffer area.

Analysis of the rationality of the groundwater supported ecological pattern

The natural grassland expansion areas had been adjusted to the different types of landscapes only from the perspective of landscape ecology. However, whether or not the groundwater conditions supported these adjustments of the ecological pattern still required verification. This study used a distributed type water cycle simulation model MODCYCLE as the tool (Lu et al., 2012), and simulated the groundwater flow field in the West Liaohe River Plain through establishing a water cycle simulation model for the West Liaohe River Basin. The period ranging from 2001 to 2014 was used as the base year period for the study of the West Liaohe River Basin. The hydrometeorological and water consumption data during the corresponding period were used as the basis to conduct the water cycle simulation construction, parameter calibration, and model validation in the base year of the West Liaohe River Plain area. The input data which were involved in the MODCYCLE model included the fundamental spatial data and the water cycle driving data. The spatial data included the basic region/basin information, such as the elevation, soil distribution, and so on, while the water cycle data included the meteorological driving data, water for human activities, and so on. This research study collected the meteorological data from 22 National Meteorological Stations in the West Liaohe River Plain, along with the precipitation data from 89 local rainfall stations (*Fig. 4*).

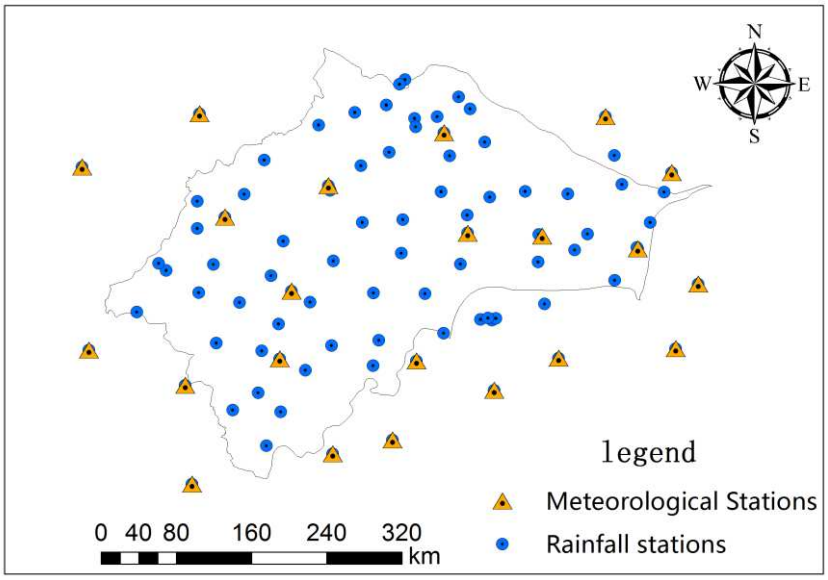


Figure 4. *Distribution of national meteorological stations and rainfall stations*

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The water intake and usage processes for human activities utilized the 2001 to 2014 statistical data from the water resources bulletin for the West Liaohe River Basin. Contour map for the groundwater depths in the West Liaohe River Plain in 2014 is shown in *Figure 5*.

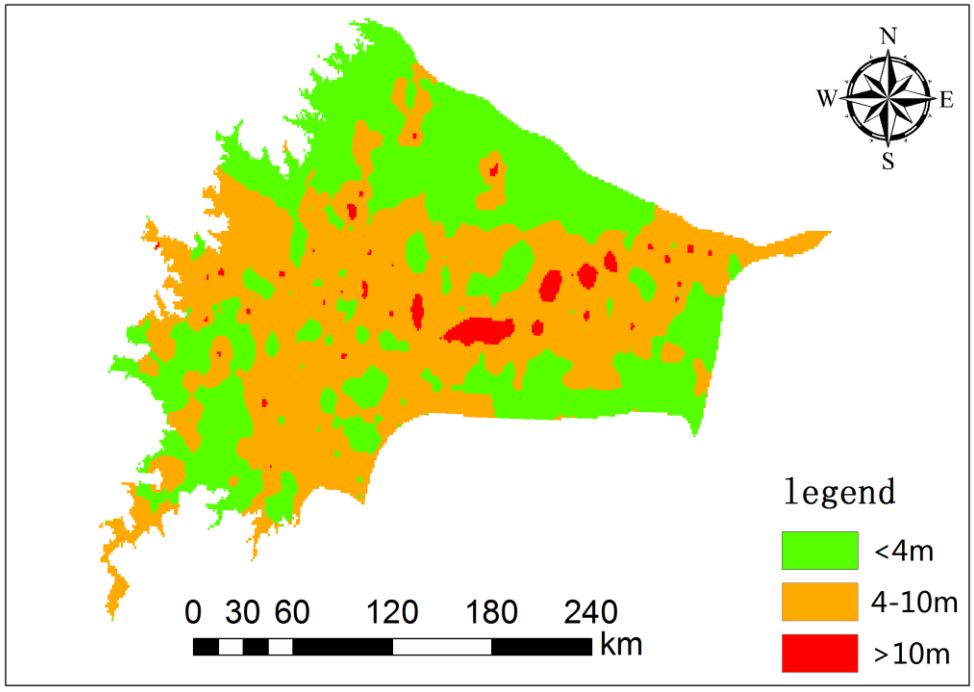


Figure 5. *Contour map for the groundwater depths in the West Liaohe River Plain in 2014*

The contour maps of the groundwater levels in the natural grassland expansion areas and the West Xiliao River Plain could be superposed. Then, the rationality of the ecological pattern of the farming and pastoral areas supported by the groundwater flow field were analyzed. The groundwater conditions in the expansion areas were divided into the supporting, basically supporting, and non-supporting areas according to the groundwater level values of ≤ 3 m, 3 to 4 m, and > 4 m.

Results and discussion

Distribution scope of natural grassland

Through carrying out vegetation investigation of the West Liaohe River Plain, 306 valid sampling points are obtained, with 258 species of plants found in total, divided into 52 families and 169 categories. Grassland of the West Liaohe River Plain includes three types: natural grassland, sown pasture and deteriorated grassland (*Fig. 6*). Wherein, the natural grassland is influenced slightly by man-made interference, with rich vegetation species diversity and high vegetation coverage, and the grassland with natural property is the gene pool for grassland ecology restoration. The sown pastures in the West Liaohe River Plain are mainly planted with the commercial crop alfalfa, with high vegetation coverage but single composition. The deteriorated grassland is influenced by man-made interference intensely, and the vegetation composition is mainly successive

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vegetation, with low vegetation coverage. The grassland degraded seriously may be degraded to mobile sandy land, with exposed sand head and scare vegetation, and only the pioneer plants such as Agriphyllum arenariun Bieb. can grow here, which has lost the natural property of grassland basically. This paper interpreted the distribution condition and field vegetation investigation result of grassland with different coverages by using remote sensing, and recognized the distribution scope of grassland influenced by man-made interference slightly with natural property. The area of natural grassland is 6,702.89 km^2, which is mainly distributed in Jarud Banner in northern part and Kezuohou Banner in southern part. Wherein, the natural grassland area of Jarud Banner is 3,932.43 km^2, and that of the Kezuohou Banner is 2,770.46 km^2.

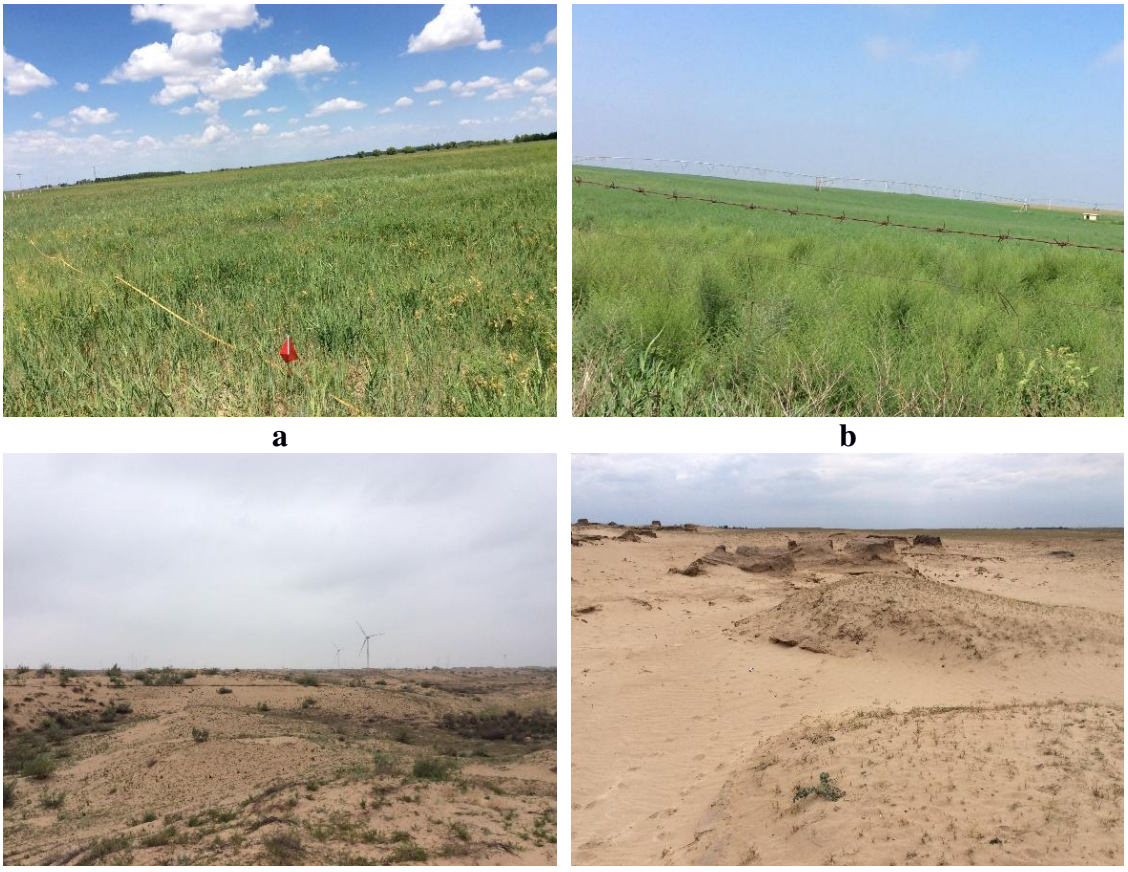


Figure 6. *Types of grassland of the West Liaohe River Plain:* ***a*** *natural grassland,* ***b*** *sown pasture,* ***c*** *deteriorated grassland and* ***d*** *mobile sand land*

Critical water levels of the groundwater recharged vegetation

Through the examination of the research data and vegetation investigation, and in accordance with the different water conditions, the steppe vegetation community in the West Liaohe River Plain was roughly divided into seven types and 22 communities. The critical burial depths of the groundwater of the different vegetation communities in the West Liaohe River Plain were obtained through calculation. The calculation results are shown in *Table 1* as follows: the critical burial depths of the majority of the vegetation communities were within 4 m. Meanwhile, the agriophyllum squarrosum community,

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which represented the pioneer community growing in the shifting sandy land areas, had root system lengths of up to 6 m, and maximum critical burial depths of up to 7 m.

Table 1. *Critical water levels for the growth of the vegetation communities in the West Liaohe River Plain grassland areas*

Vegetation community	Soil type	Capillary lift (m)	Root length (m)	Critical water level (m)
Typha orientalis Presl	Moisture soil	0.5-1.0	0.2	In water
Scirpus validus Vahl	Moisture soil	0.5-1.0	0.15	In water
Scirpus triqueter L.	Moisture soil	0.5-1.0	0.2	1.2
Phragmites communis	Moisture soil	0.5-1.0	0.3	1.3
Hemarthria altissima	Meadow soil	0.5-1.0	0.3	1.3
Carex spp.	Meadow soil	0.5-1.0	0.3	1.3
Agrostis alba L.	Meadow soil	0.5-1.0	0.3	1.3
Sanguisorba officinalis L.	Meadow soil	0.5-1.0	0.3	1.3
Potentilla anserine L.	Meadow soil	0.5-1.0	0.3	1.3
Cyperus rotundus L.	Meadow soil	0.5-1.0	0.3	1.3
Calamagrostis epigeios	Meadow soil	0.5-1.0	0.6	1.6
Iris lactea	Meadow soil	0.5-1.0	0.8	1.8
Suaeda glauca Bunge	Meadow soil	0.5-1.0	1.2	2.2
Achnatherum splendens	Meadow soil	0.5-1.0	1.5	2.5
Leymus chinensisTzvel.	Chestnut soil	1.5	0.3	1.8
Stipa grandis P. Smirn.	Chestnut soil	1.5	0.5	2
Cleistogenes squarrosa Keng	Chestnut soil	1.5	0.6	2.1
Artemisia frigida Willd.	Chestnut soil	1.5	1	2.5
Lespedeza bicolor Turcz	Chestnut soil	1.5	1	2.5
Artemisia halodendron	Sandy soil	0.5-1.0	1.5	2.5
Caragana microphyllaLam	Sandy soil	0.5-1.0	3	4
Agriophyllum squarrosum	Sandy soil	0.5-1.0	6	7

Distribution of the natural grassland expansion

As detailed in *Figure 7,* The expansion core area of the natural grassland was determined to be 7,922 km^2. It is distributed around the natural grassland, and suffered a lower level of resistance. Also, it played a key role in protecting the natural grassland from the interferences of human activities, thereby maintaining its natural attributes. It was considered to be the most direct area for the expansion of the natural grassland. The patches of farmland and unused land areas in this area were all completely adjusted to grassland. The farmland to grassland areas were implanted to the dominant and common associated species of the adjacent natural grassland plant communities through artificial cultivation or introduction processes.

The expansion connection areas of the natural grassland were determined to be 3,799.85 km^2. With the outward expansion of the natural grassland along the expansion core area, it had suffered from major influences of human activities. This area showed a landscape pattern in which large sheets of deteriorated grasslands were staggered with patches of farmland and unused land. The differences between this area and the expansion core area were that the dominant species from the vegetation communities in

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the adjacent expansion core area had difficulties adapting when only the attributes of the patches were changed to those of closed grassland or further artificial planting. Therefore, in this study it was necessary to adjust the vegetation communities in the connected areas in combination with the aforementioned factors.

The expansion buffer areas of the natural grassland were determined to be 1859.51 km^2. The larger the distance to the natural grassland resulted in higher resistance. The existing landscapes mainly included large irrigation areas, highly fragmented farmland areas near towns, unused land, and degraded grassland interlacing areas. These areas had a very low possibility of recovering the natural attributes of the natural grassland due to the limits imposed by urban development planning. This easily led to the loss of the natural attributes of the grassland under enhanced artificial interference conditions. Therefore, it was determined to be necessary to protect the patches in these areas and maintain stability by taking such measures as returning farmland to grassland, limiting land reclamation, closing degraded grassland, and so on. These measures provided security boundary conditions for the restoration of the grassland ecosystems within the core and connected regions of the study area.

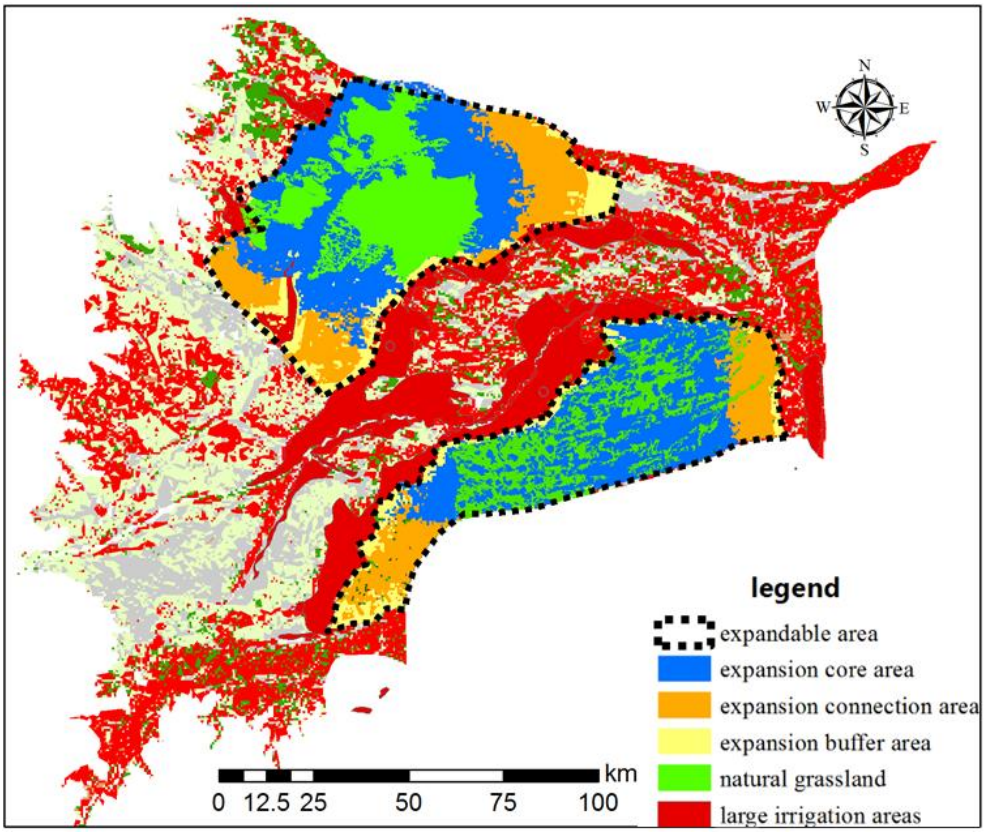


Figure 7. *Distribution of the natural grassland expansion in the West Xiliao River Plain*

Ecological pattern

As detailed in *Figure 8,* in the natural grassland expansion areas, the areas with groundwater levels within 3 m consisted of 5,258.96 km^2. The groundwater conditions in these areas met the requirements to recharge water for the critical burial depths of the vegetation. Also, the adjustments of the ecological patterns in this area could be

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supported. The areas with groundwater levels within 3 to 4 m measured 4,565.86 km^2, and the groundwater conditions in these areas had the ability to recharge water for the growth of fruticeta with long root systems, such as the dominant species Caragana microphylla Lam. The areas with ground water levels above 4 m accounted for 3,896.51 km^2, and the ground water conditions in these areas were determined not to meet the recharging requirements of the critical burial depths of the vegetation. It was therefore concluded that the expansions of the natural grassland in these areas would be meaningless if the conditions of the groundwater were not improved. Therefore, the expansions of the natural grassland should first be transformed in the ecological pattern adjustment areas which were supported by the groundwater conditions. Second, shrub communities with long root systems, such as Caragana microphylla Lam, could be planted in the ecological pattern adjustment areas which were basically supported by the groundwater conditions. Finally, the ecological pattern adjustment areas which were not supported by the relative groundwater conditions could be eliminated.

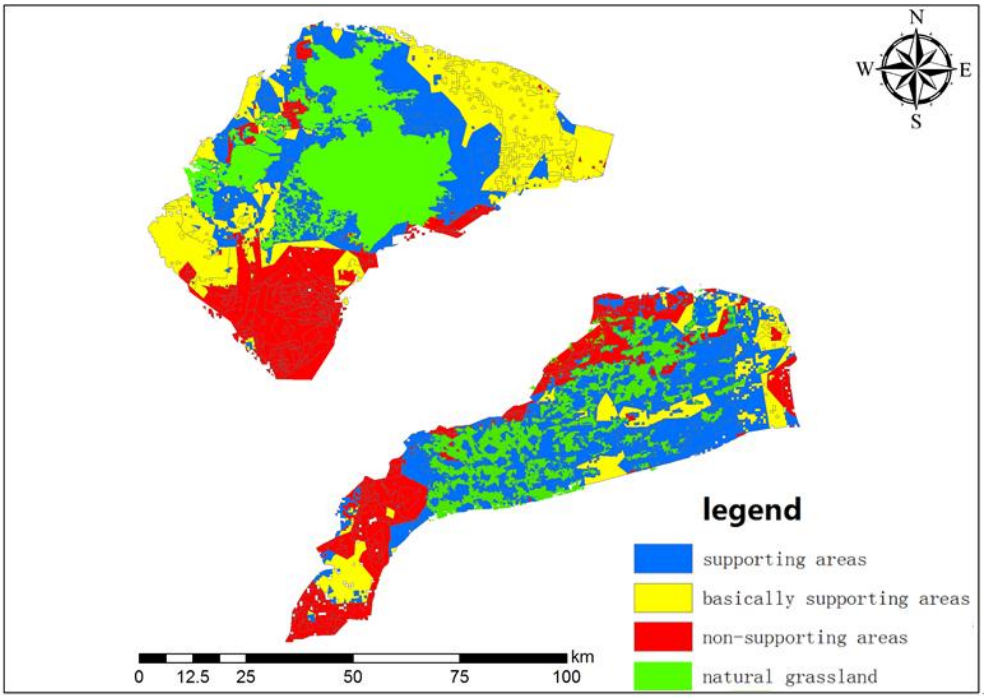


Figure 8. *Distribution of the rationality of the ecological patterns supported by the groundwater subsurface flow field*

The adjustments to the reasonable ecological patterns in the farming-pastoral zone of the West Liaohe River Plain were based on the basic principle of the natural grassland expansion. The optimization and adjustments of the ecological patterns were conducted using a landscape ecology method, and the demands of urban planning and social-economic development were also considered. Moreover, the groundwater conditions were used to analyze the rationality of the adjustments, and the results are shown in *Figure 9*. The grassland areas after the adjustments measured 28,760.7 km^2, and accounted for 43.87% of the total area of the West Liaohe River Plain. Among the grassland areas, the natural grassland areas had been expanded by 8,909.24 km^2, which increases from the original 6,702.89 km^2 to 15,612.13 km^2, which accounted for

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23.87% of the total area of the West Liaohe River Plain. The farmland areas were determined to be 20,932.4 km^2, which accounted for 31.93% of the total area of the West Liaohe River Plain. Among the farmland areas, the large irrigation areas were 7,729.58 km^2, and accounted for 11.81% of the total area of the West Liaohe River Plain.

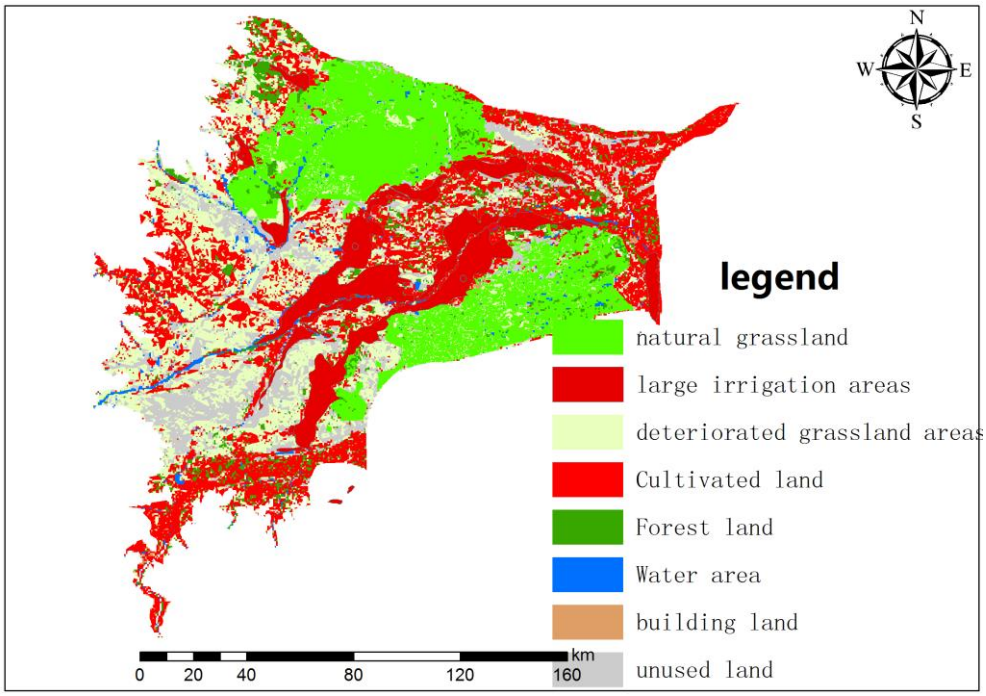


Figure 9. *Ecological patterns of the farming-pastoral zone in the West Liaohe River Plain*

Table 2 details the statistics of the land usage situations in the West Liaohe River Plain since 1990. Through this adjustment of the ecological pattern, the grassland areas have been basically recovered to the level of 1990, which has provided conditions for the ecological restoration of the grassland areas.

Table 2. *Land usage situations in the West Liaohe River Plain during the different periods*

Land usage	1990 (km^2)	2000 (km^2)	2010 (km^2)	2016 (km^2)	Ecological pattern (km^2)
Cultivated land	18251	21215	26511	23372	20932.4
Grassland	29086	26640	21428	22439	28760.7
Forest land	2475	2950	2917	4296	4296
Water area	1876	1697	1564	1492	1492
building land	1616	1566	1665	1712	1712
Unused land	12257	11493	11476	12250	8367.9
Proportion of cultivated land	27.84%	32.36%	40.44%	35.65%	31.93%
Proportion of grassland	44.36%	40.63%	32.68%	34.23%	43.87%

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Conclusion

The farming-pastoral zones in semi-arid regions are typically ecologically fragile zones, as well as important ecological barriers. The ecological environments are not only the basis and guarantee of regional economic development, they are also barriers relating to the ecological security of the Yellow River Basin, Haihe River Basin, and Beijing-Tianjin-Hebei Regions. In this research study, by taking the West Liaohe River Plain as the research area, and the natural grassland expansion as the basic principle, adjustments were made to the ecological security patterns of the agriculture and animal husbandry in the West Liaohe River Plain. Then, the rationality of the ecological security patterns supported by the groundwater conditions was analyzed. The grassland areas were expanded through the adjustments of the ecological patterns, which provided the conditions and foundation for the ecological restoration of grassland. The natural attributes of the regional hydrological cycles determine the basic patterns of an ecosystem, it was necessary to recognize the decisive influences of the regulation and management of the groundwater level on the grassland vegetation community pattern. In future research, we should focus on how to improve the biodiversity of grassland and how to control the water level of the groundwater.

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PONGAMIA PINNATA (L.) PANIGRAHI AQUEOUS EXTRACT ALLEVIATES MERCURIC CHLORIDE INDUCED STRESS ON SEEDLING GROWTH OF MAIZE

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Abstract. Aqueous extract of *Pongamia pinnata* (PALE) was accessed to alleviate mercuric chloride ($HgCl_2$) induced stress on seed germination and early seedling growth of *Zea mays* L. Maize seeds were soaked in 5 and 2.5% PALE prior to sowing in the pots and $HgCl_2$ was applied at rate of 1 and 0.5 mg kg^{-1} to the soil to induce an artificial stress for the germination and seedling growth. The results showed that seed germination (%), root dry weight and photosynthetic pigments were significantly reduced by $HgCl_2$ at 1 mg kg^{-1}, which resulted in accumulation of phenolic compounds in maize leaves. It was found that the total soluble phenolic content was negatively correlated with shoot dry weight ($r = -0.656$, $p = 0.001$). On the other hand, seed soaked with PALE significantly alleviated adverse effects of $HgCl_2$ stress on seed germination and growth attributes of maize, thus it was found to be effective to protect maize plants from adverse effects of mercury and can be recommended for application in mercury contaminated areas of farmer's field.

Keywords: *mercury stress, phenolic, Pongamia pinnata, phytoremidiation, PALE, Zea mays, $HgCl_2$*

Introduction

The term 'heavy metal' is often used to cover a diverse range of elements, which are considered to be toxic to human health as being an important class of pollutants. Although heavy metals are naturally present in the soil (Zakir et al., 2014), but the geologic and anthropogenic activities have caused them to increase to the toxic level that are harmful to both plants and animals (Alyemeni and Almohisen, 2014). Heavy metals adversely affect the development of both vegetative and generative parts of the plants (Jeliazkova and Craker, 2003) leading to losses in yield (Chatterjee and Chatterjee, 2000). Therefore, the remediation of heavy metal polluted soils cannot be over emphasized (Chibuike and Obiora, 2014).

Heavy metals block functional groups of metabolically important molecules, alter hormonal balance, reduce nutrient assimilation, protein synthesis, and DNA replication (Nagajyoti et al., 2010). They inhibit chlorophyll synthesis by substitution of magnesium in chlorophyll and also water uptake (Boening, 2000). Among the various types of heavy metals, mercury accumulates in all parts of the plant but mostly in roots indicating that roots serve as a barrier to mercury uptake (Bibi et al., 2016).

To reclaim the soils contaminated with heavy metals, various physical and chemical approaches have been adopted but these are expensive and make the soil unsuitable for plant growth (Marques et al., 2009). Biological approach (bioremediation) on other hand is the best for establishment / reestablishment of plants on polluted soils. Being a natural process, it is an environment friendly method. Phytoremediation is a part of bioremediation in which plants are used for the treatment of polluted soils (Cuypers et

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al., 2010). The different mechanisms used for phytoremediation are phytostabilization, phytovolatilization and phytoextraction (Chaney et al., 1997). The amount of phytochemicals such as plant phenolics are reported to increase under the stress of heavy metals. This increase of phenolic content is correlated with increased activity of enzymes involved in biosynthesis of phenols under heavy metal stress (Winkel-Shirley, 2002; Rizvi, 2006; Bibi et al., 2016).

Pongamia (*Millettia pinnata*), formerly known as *Pongamia pinnata*, is a tree/shrub widely cultivated in Pakistan. It can tolerate a wide range of soil types including saline, alkaline, sandy, heavy clay and rocky soils and waterlogged soils. Pongamia is used in land reclamation and as a soil stabilizer (Shirbhate and Malode, 2012). Keeping in view the harmful effects of heavy metals on plants and the role of phytochemicals in heavy metal stress tolerance of plants, the present investigation was carried out to alleviate mercury induced stress on maize plants by *Pongamia pinnata* leaf extract.

Material and methods

The healthy and fully expended leaves of *Pongamia pinnata* (200 g) were collected (20 gm per tree) from the campus of the University of Science and Technology, Bannu, mixed together to form a composite sample, dried under shade for 2 to 3 weeks and finely ground in an electric grinder. The elemental composition of *Pongamia Pinnata* leaf was analysed following the method described by Rashid (1986). The powdered leaf material was digested in a solution of nitric acid and perchloric acid (2:1) and heated at 150 °C. After cooling it to room temperature, the volume was raised to 50 ml using deionized water and filtered. Elemental analyses were carried out using an Atomic Absorption Spectrophotometer (Model: Perkin Elmer Analyst-200, USA).

Preparation of Pongamia pinnata leaf extract

Formal maceration techniques were used in the preparation of extracts. The *Pongamia pinnata* leaf powder (20 g) was soaked in 200 mL of distilled water and was kept there for 48 h. It was filtered first with fine porous cloth and then with a Whatman No. 1 filter paper. The aqueous extract obtained was designated as a 10% (w/v) stock solution. This stock solution was further diluted to 5 and 2.5% (w/v) in distilled water and were denoted as PALE.

Bioassay

The seeds of maize (*Zea mays* L., variety Azam) were obtained from the Agriculture Research Centre Sara-e- Nourang, Lakki Marwat, KP, Pakistan. Finely ground uniformed soil (sand and clay 1:1) was filled in plastic pots (8×12 cm^2) for sowing of maize seeds in order to facilitate easy penetration of roots and uprooting of plants at the time of harvest. The soil used as culture medium was analyzed for pH, content of available nitrogen and phosphorous using standard protocols. Determination of soil pH was carried out by the method of McLean (1982). For electrical conductivity (EC) determination, the saturated paste of soil was prepared by suspending soil into water and thoroughly mixed by using magnetic stirrer for 30 min. After settling, the EC was noted by using conductivity meter (Page et al., 1982). Phosphorous content of the soil was determined according to the method of Olsen and Sommers (1982). For determination of total nitrogen content 10 g of soil was digested in micro-Kjeldahl flask

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with 20 mL of concentrated H_2SO_4 and 10 g digestion mixture. After digestion, the flasks were allowed to cool. The contents were transferred to a 50 mL flask and distilled. The distillation was carried out in the presence of 20 mL of 40% NaOH and collected in 5 mL boric acid. The distillate was titrated against 0.1 N H_2SO_4 using $KMNO_4$ as indicator. The particle size of the soil was determined at Agriculture Research Station Sari Naurang Lakki Marwat, Pakistan. The physico-chemical properties of the soil are shown in *Table 1*.

Table 1. *Physico-chemical properties of the soil used in the experiment*

Parameters	Values
pH	7.5
E.C.	3.10 dS m^{-1}
Total nitrogen content	0.41% dry weight
Total phosphorous content	29 mg kg^{-1}
Clay (<0.002 mm)	34%
Fine silt (0.002–0.02 mm)	13%
Fine sand (0.05–0.2 mm)	23%
Coarse sand (0.2–2 mm)	30%

To impose artificial stress of mercury, $HgCl_2$ was added at 1 and 0.5 mg kg^{-1} soil. The experiment was carried out statistically in completely randomized design (CRD) with three replications for each treatment (various levels of mercury and PALE) in a glass house in the Department of Botany, University of Science and Technology, Bannu, KP Province, Pakistan. Maize seeds were soaked in various treatment levels of PALE for three hours before sowing them in pots. The following samples were examined: Control pots where seeds soaked in distilled water were sown, Pots with soil contaminated with $HgCl_2$ at 1 mg kg^{-1} soil, Pots with soil contaminated with $HgCl_2$ at 0.5 mg kg^{-1} soil, Pots with soil contaminated with $HgCl_2$ at 1 mg kg^{-1} soil where seeds soaked in 5% PALE were sown, Pots with soil contaminated with $HgCl_2$ at 1 mg kg^{-1} soil where seeds soaked in 2.5% PALE were sown, Pots with soil contaminated with $HgCl_2$ at 0.5 mg kg^{-1} soil, where seeds soaked in 5% PALE were sown, Pots with soil contaminated with $HgCl_2$ at 0.5 mg kg^{-1} soil, where seeds soaked in 2.5% PALE were sown.

When 90% of the seeds were germinated in control pots, the seeds germination (%) was calculated according to the following formula:

$$\text{Seed germination } (\%) = (\text{Seeds germination / Total number of seeds grown}) \times 100$$

The plants were harvested 28 days after sowing and were analyzed for the following morphological, physiological and biochemical growth attributes. The shoot and root weight were determined using electronic balance. Root length was determined using a measuring tape. The leaf photosynthetic pigments were analysed according to the method described by Arnon (1949). The total soluble phenolic content of the maize leaves were determined by the Folin-Ciocalteau method (Wolfe et al., 2003). The extract (200 µL) was mixed with the Folin-Ciocalteau reagent (Sigma-Aldritch, Cat # F9252-100ML) and incubated for five minutes at room temperature in dark. The

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mixture was added to a 1.5 mL sodium carbonate (7%, w/v) solution and incubated in dark for 90 min. The optical density of the mixture was determined at 760 nm using a spectrophotometer (Hitachi's U-5100 Tokyo, Japan). Standard curve made of various concentrations of gallic acid solutions was matched with the sample measurements. The concentration of total phenolic content was expressed as μg gallic acid equivalents g^{-1} sample.

Statistical analyses

Analysis of variance technique (ANOVA) was used for the analysis of data and the Least Significant Difference (LSD) test (Steel and Torrie, 1980) was used for the comparison of treatment means. Student Statistix (version 8.1 USA) software package was used for the determination of coefficient of correlation.

Results and discussion

The Pongamia leaf was analysed for elemental composition and total soluble phenolic content (*Table 2*). Among macronutrients, Na (204 μg g^{-1}), K (197 μg g^{-1}), Ca (67 μg g^{-1}) and Mg (89 μg g^{-1}) were determined. Among micronutrients Fe (42 μg/g), Mn (22 μg/g) and Zn (35.22 μg/g) were analysed. The total soluble phenolic content of Pongamia leaf was 139 mg gallic acid g^{-1} d.w. Plant extracts were characterized by the presence of natural phenolics and various types of nutrients having beneficial effect on plant growth (Ullah et al., 2014; Khattak et al., 2015).

Table 2. *Nutrient composition of Pongamia pinnata leaf*

Parameter studied	Value (μg g^{-1} d.w)
Na^+	204
K^+	197
Mg^{2+}	67
Ca^{2+}	80
Fe^{2+}	42
Mn^{2+}	22
Zn^{2+}	35
Total soluble phenolic content (μg gallic acid g^{-1} d.w)	139000

The data depicted that soil contamination with higher concentration of $HgCl_2$ (1 mg kg^{-1} soil) significantly reduced the seed germination compared to control (*Table 3*). On the other hand, seeds soaked with PALE significantly ameliorated adverse effects of $HgCl_2$ on seed germination (%). A negative impact of $HgCl_2$ stress on shoot fresh and dry weights was observed (*Table 3*). However, the exogenous application of PALE minimized adverse effects of $HgCl_2$ on shoot fresh and dry weights. Root length was also significantly reduced by $HgCl_2$ at 1 mg kg^{-1} soil as compared to untreated control (*Table 3*). The application of PALE (5%) exhibited stimulatory effects and minimized adverse effects of $HgCl_2$ on root length. The soil contamination with $HgCl_2$ significantly decreased root fresh weight as compared to control ($p < 0.05$). The PALE effectively protected fresh weight of roots from adverse effects of $HgCl_2$ induced stress. At higher concentration of $HgCl_2$ (1 mg kg^{-1} soil), the beneficial effect of PALE on root fresh

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weight was also found to be stronger. The $HgCl_2$ has no significant effect on root dry weight (*Table 3*).

Table 3. *Effect of PALE on morphological growth attributes of maize under mercuric chloride stress*

Treatment		Germination (%)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)
T0	**Control** (pots where seeds soaked in distilled water were sown)	100.0 a	1.286 a	0.214 a	0.780 a	0.077 ab	68.22 ab
T1	Pots with soil contaminated with **$HgCl_2$ at 1 mg kg^{-1} soil**	83.2 b	0.440 c	0.013 c	0.420 d	0.057 ab	53.42 d
T2	Pots with soil contaminated with **$HgCl_2$ 0.5 mg kg^{-1} soil**	99.4 a	0.905 ab	0.096 bc	0.440 cd	0.112 a	63.05 bc
T3	Pots with soil contaminated with **$HgCl_2$ 1 mg kg^{-1} soil and** seeds soaked in **5% PALE** were sown	100.0 a	0.873 b	0.137 ab	0.603 bc	0.064 ab	72.22 a
T4	Pots with soil contaminated with **$HgCl_2$ 1 mg kg^{-1} soil and** seeds soaked in **2.5% PALE** were sown	94.4 a	0.817 bc	0.141 ab	0.534 cd	0.062 ab	57.75 cd
T5	Pots with soil contaminated with **$HgCl_2$ 0.5 mg kg^{-1} soil and** seeds soaked in **5% PALE** were sown	94.4 a	0.910 ab	0.095 bc	0.743 ab	0.048 ab	65.49 abc
T6	Pots with soil contaminated with **$HgCl_2$ 0.5 mg kg^{-1} soil and** seeds soaked in **2.5% PALE** were sown	100.0 a	0.941 ab	0.140 b	0.730 ab	0.042 b	71.70 ab
LSD		**9.03**	**0.400**	**0.0875**	**0.1659**	**0.0697**	**8.86**

Means sharing common English letters are statistically similar ($p = 0.05$, n = 3)

Mercury stress results in abnormal seed germination with a significant reduction in germination indices (Patra and Sharma, 2000). Mercury reduces mitotic activity in plant organs, therefore results in lower biomass production (Patra et al., 2004). The beneficial effect of PALE on seed germination and morphological growth characters of maize under $HgCl_2$ could be attributed to its phytochemical composition. Previous studies have shown that the aqueous leaf extract of *Moringaoleifera* was highly effective and protected maize plants from adverse effect of $HgCl_2$ (Bibi et al., 2016).

Results showed that $HgCl_2$ at 1 mg kg^{-1} soil significantly reduced chlorophyll *a* content as compared to control (*Table 4*). The application of PALE at 5% concentration significantly ameliorated adverse effects of $HgCl_2$ stress on chlorophyll *a* content. All the treatments showed non-significant effect on chlorophyll *b* content (*Table 4*). The reduction of chlorophyll content under mercury stress has been reported in *Marrabiumvulgare* (Moreno-Jiménez et al., 2007). Mercury disrupts the photosynthetic machinery by inactivating the magnesium ion of the chlorophyll molecule (Patra et al., 2004).

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Table 4. *The effect of PALE on physiological attributes of maize under mercuric chloride stress*

Treatment		Chl *a* (mg/g f.w)	Chl *b* (mg/g f.w)	Total soluble phenolic content (mg gallic acid eq./g f.w)
T0	**Control** (pots where seeds soaked in distilled water were sown)	12.88 a	6.224 a	34.94 c
T1	Pots with soil contaminated with **$HgCl_2$ at 1 mg kg^{-1} soil**	4.98 c	3.665 b	64.52 b
T2	Pots with soil contaminated with **$HgCl_2$ 0.5 mg kg^{-1} soil**	11.66 ab	4.667 ab	45.46 c
T3	Pots with soil contaminated with **$HgCl_2$ 1 mg kg^{-1} soil and** seeds soaked in **5% PALE** were sown	12.54 a	7.120 a	106.88 a
T4	Pots with soil contaminated with **$HgCl_2$ 1 mg kg^{-1} soil and** seeds soaked in **2.5% PALE** were sown	10.37 b	6.443 a	105.15 a
T5	Pots with soil contaminated with **$HgCl_2$ 0.5 mg kg^{-1} soil and** seeds soaked in **5% PALE** were sown	13.19 a	6.637 a	67.10 b
T6	Pots with soil contaminated with **$HgCl_2$ 0.5 mg kg^{-1} soil and** seeds soaked in **2.5% PALE** were sown	10.24 b	5.063 ab	49.41 bc
LSD		**2.025**	**2.502**	**17.803**

Means sharing common English letters are statistically similar ($p = 0.05$, n = 3)

In the present investigation, it was found that $HgCl_2$ induced stress caused accumulation of phenolic compounds in the leaves of maize (*Table 4*). Total soluble phenolic content was found to be higher when $HgCl_2$ was applied at 1 mg kg^{-1} soil. However, soaking seeds in PALE resulted in further augmented accumulation of phenolics in maize leaves. The most effective concentration of PALE was 5%. Plants accumulate phenolic compounds when exposed to heavy metal stress (Klepacka et al., 2011). Accumulation of phenolic under heavy metal stress has also been reported in *Phaseolus vulgaris* (Hamid et al., 2010), *Boerhavia diffusa* (Abdussalam et al., 2015) and *Trigonellafoenum-graecum* (Askari and Azmat, 2013). Phenolic compounds protect cell biomolecules from oxidative damage by scavenging reactive oxygen species and prevent lipid peroxidation. This is achieved by the reduced forms of phenolic compounds whereas in oxidized state, these may function as prooxidants (Sakihama et al., 2000). Antioxidant activity of Pongamia extract was correlated with its flavonoid and phenol content (Behera et al., 2012). The higher increase in phenolic compounds by seed soaked with PALE under $HgCl_2$ stress indicates the possible role of bioactive compounds present in the extract which contributed to the mercury stress tolerance of maize.

It was observed that Hg stress resulted in the accumulation of phenolic compounds with a reduction in the content of photosynthetic pigments. On the other hand PALE not only caused accumulation of phenolic compounds but also improved the content of photosynthetic pigments. This suggested that exogenous application of PALE reduced chlorophyllase activity responsible for chlorophyll degradation. Phenolic compounds

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either stimulate or inhibit the content of photosynthetic pigments that is why phenolics are considered as compounds with bifunctional role, one of stimulation and another of inhibition (Yang et al., 2004). This may be attributed to the fact that phytochemicals present in PALE have stimulatory effect on photosynthetic pigments in maize.

The correlation of chlorophyll *a* was found to be positive with chlorophyll *b* ($r = 0.691$, $p = 0.000$), germination (%) ($r = 0.581$, $p = 0.005$), root fresh weight ($r = 0486$, $p = 0.025$) and shoot fresh weight ($r = 0.571$, $p = 0.006$). The total soluble phenolic content was negatively correlated with shoot dry weight ($r = -0.656$, $p = 0.001$). The correlation of root fresh weight was also positive with shoot fresh weight ($r = 0.491$, $p = 0.023$).

Conclusion

The growth of maize was inhibited when grown on $HgCl_2$ contaminated soil due to the toxicity of mercury. This mercury stress was associated with the accumulation of phenolic compounds. It was found in the present studies that exogenous application of PALE was effective in alleviating the adverse effects of $HgCl_2$ on maize plants grown under $HgCl_2$ contaminated areas, thus, PALE can be used in bioremediation of contaminated soils for better plant growth. *Pongamia pinnata* is a drought tolerant and nitrogen fixing plant species. Its oil has potential for use as a feedstock for biodiesel production and can survive under harsh environmental conditions. Therefore, it is a more suitable tree species for exploitation as a source of future bioregulators for improving growth and productivity of crop plants.

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VEGETATION ANALYSIS OF SOME WETLAND HABITATS IN CENTRAL REGION OF SAUDI ARABIA

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Abstract. In this study, the vegetation of 63 sabkha and sewage canals in central region of Saudi Arabia was analyzed. A total of 109 plant species belonging to 83 different genera and 34 different plant families were collected. The most common plant families were *Chenopodiaceae* (18 species), *Poaceae* (16 species) and *Asteraceae* (10 species). Vegetation in the study area was classified into 6 groups; two of them were found in sabkhas only, one was found in sewage canals only and the other three were found in both habitats. The main factors controlling the plant presence and vigour in the studied wetlands were soil salinity, pH, EC, soil texture and content of organic matter.
Keywords: *wetlands, vegetation, DECORANA, TWINSPAN*

Introduction

Wetlands are defined as the areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support a prevalence of vegetation typically adapted for life in saturated soil conditions (LePage, 2011). They include, for example, salt marshes (sabkhas), shallow ponds and sewage canals. Wetlands are identified by unique soils (hydric soils), plants adapted to life in wet environments (hydrophytes) and the presence of water (hydrology) during the growing season. From an ecological perspective, wetlands are simply a particular slice of the continuum between the wettest ecosystem types (rivers, lakes, estuaries, and the marine environment) and the driest environments within a physiographic (broad-scale subdivisions of the Earth's surface based on terrain texture, rock type, geologic structure, and history) region (Brinson, 2011).

Wetlands in the central region of Saudi Arabia are considered among the main diverse habitats. However, there have been no studies of their vegetation diversity until now. In Saudi Arabia, wetlands are located in a hot and dry region that is experiencing major climate changes. They are generally characterized by high biodiversity in terms of both animals and plants. With their hydrologic characteristics, wetlands have a great ecological importance. They have plants with high productivity and provide habitats for many wild fishes and birds (LePage, 2011). Saudi Arabia has several natural and industrial wetlands including lakes, sabkhas and wadis. Beside their importance to various animals especially amphibians, wetland habitats have a great potential to conserve biodiversity of plants (Flinn et al., 2008). Al-Obaid et al. (2017) stated that wetlands are also of great economic importance in agriculture and grazing. Nevertheless, the plant communities of different wetlands in Saudi Arabia has not been fully described. The aim of this study was to characterize the vegetation of different wetlands in central region of Saudi Arabia.

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Materials and methods

Study area

The central "Najd" region is the large central plateau in Saudi Arabia; it is divided into two parts: the higher Najd in the west (located on the Arabian Shield region) and the lower Najd in the east. The altitude in this area ranges from 500 - 1000 m above sea level. The higher Najd is characterized by an extensive system of large wadis. Wadis in general flow from west to east, following the slope of the land, from the higher mountains in the west to the lower plains of Najd.

Qassim is located in the northern central region of Saudi Arabia with an area of 80.000 km^2. It has two different geological regions; one is located on the Arabian Shield region and the other is located in the sedimentary sector (the Arabian Shelf). Qassim has many sabkhas and wadis in addition to several mountains. Riyadh is located at 24° 38' N lat. and 46° 43' E long. in the central region of Saudi Arabia occupying a large area of Najd plateau with an average altitude of 600 m above sea level. Riyadh is characterized by several mountains (e.g. the Tuwaiq Mountains) and sand dunes. Similar to Qassim, Riyadh is divided into two geological sectors; the western part is the Arabian Shield sector and the eastern is the sedimentary sector (*Fig. 1*).

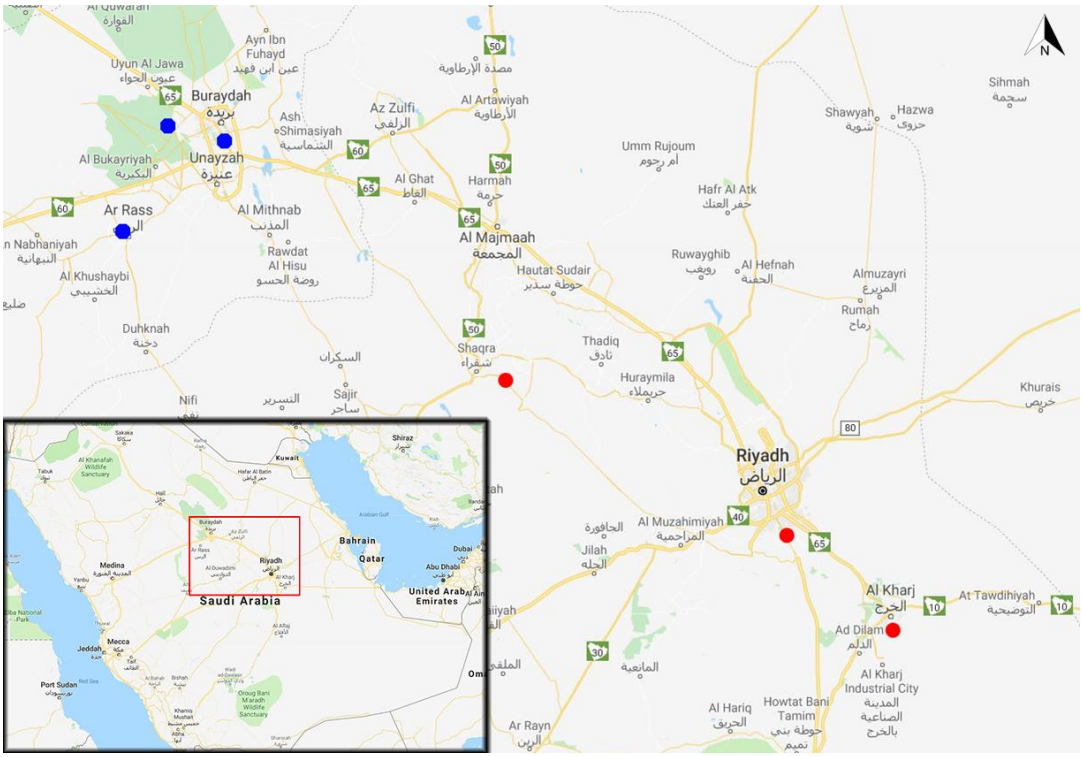


Figure 1. *A map showing the different studied sites*

The study area is characterized by high temperatures especially from June to August when the temperature can exceed 45 °C. The general climate in the study area is hot summer and mild to cold winter. The rainfall is very low with an average of less than 200 mm/ month, and most of the rain falls during spring with almost no rain in the summer. The wind is usually laden with dust from upwind desert areas. *Table 1* summarizes the climate characteristics of the two studied areas; Riyadh and Qassim.

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Table 1. *Average of climate records for 10 years (2005 – 2014) in Riyadh and Qassim areas (Source: Saudi General Authority of Meteorology and Environmental Protection)*

Climate variable	Riyadh		Qassim	
	Range	Mean	Range	Mean
Max air temperature (°C)	20.95 – 43.93	32.44	20.43 – 44.72	32.58
Min air temperature (°C)	7.48 – 27.66	17.57	6.74 – 27.33	17.00
Rainfall (mm/ month)	0.00 – 320.91	160.46	0.00 – 320.00	160.00
Relative humidity (%)	8.30 – 94.30	51.30	4.20 – 99.80	52.00
Wind speed (km/ h)	4.10 – 7.10	5.6	4.90 – 7.40	6.15

Sample stands

In the present study, 63 different stands were selected to represent the two main studied habitats (sabkhas and sewage canals) that exist in different wetlands in the central region of Saudi Arabia. GPS coordinates for all the studied stands were recorded (*Appendix 1*) and in every stand, an area of 50 m × 50 m was sampled. Sampling was carried out during the spring season 2016 in order to ascertain the maximum presence of the different plant species. Plant samples collected from each stand were identified according to Collenette (1999) and Chaudhary (2001) and presented at the herbarium of King Saud University, Riyadh, Saudi Arabia. Plants were classified into annual herbs, annual grasses, perennial herbs, perennial grasses and shrubs (*Appendix 2*). The pattern of geographical distribution (chorotype) of each species were studied following Chaudhary (2001). The total and mean cover and presence of each species were calculated for each stand following the method described by Kent (2012).

Soil analysis

Three soil samples were collected from each stand at a depth of 50 cm and mixed together to form a composite sample. Soil physical and chemical characteristics were assessed. The hydrometer method was used to determine the soil texture (Day, 1965). The "Loss on Ignition" method (combustion at 550 °C) was used to calculate the total organic matter present in the soil samples. Soil pH and electrical conductivity (EC) was calculated in soil suspension 1: 5 w: v. Nitrogen content in soil samples was determined using the micro Kjeldahl method (AOAC, 1990). Phosphorus content was determined following the method of APHA (2005). Concentrations of various nutrients (Ca, K, Mg, Fe, Na) were measured following the methods described by Allen (1989).

Water analysis

One water sample from each stand was collected (where applicable) and chemical analyses were performed. Samples were collected at a depth of 1 m above surface that is around the middle of each water body. Water pH and EC were examined according to Allen (1989). Phosphorus content was determined following the method of APHA (2005). In addition, levels of Ca, K, Mg, Fe and Na in the water samples were investigated (Allen, 1989).

Statistical analysis

Canonical correspondence analysis (CCA) and detrended correspondence analysis (DCA) using DECORANA software (Hill, 1979a) and multivariate analysis using

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TWINSPAN software (Hill, 1979b) were performed to calculate the estimates of cover for 109 plant species collected from the 63 stands. Species richness (α-diversity) was defined as the average number of species present in each individual stand. Gamma diversity (γ-diversity) was determined based on the total number of species for each stand. The relative cover (p_i) of each stand was used to calculate the relative evenness using the Shannon-Wiener index $\widehat{H} = -\sum_{i=1}^{s} p_i \log p_i$ and the relative dominance concentration using the Simpson index $C = \sum_{i=1}^{s} p_i^2$ where s is the total number of species collected (Pielou, 1975; Magurran, 1988).

Results

The total number of plants collected from the different wetlands studied in the Riyadh and Qassim areas was 109 plant species belonging to 83 different genera and 34 different plant families, with a species richness factor of 1.31 (109 species / 83 genera). The most abundant plant families were Chenopodiaceae with 18 different plant species (16.5%), Poaceae represented by 16 different plant species (14.7%), Asteraceae with 10 different plant species (9.2%) and Fabaceae with 7 different plant species (6.4%). The least abundant families were Aizoaceae, Amaranthaceae, Apocynaceae, Asphodelaceae, Cistaceae, Cucurbitaceae, Ephedraceae, Frankeniaceae, Juncaceae, Lamiaceae, Malvaceae, Molluginaceae, Neuradaceae, Orobanchaceae, Portulacaceae and Ruppiaceae, with each family being represented by only one plant species (*Fig. 2a*, bar chart). *Fig. 2b* (pie chart) shows that the predominant life forms were annual herbs with 40 different plant species (36.7%) and perennial herbs with 34 different plant species (31.2%). The total number of different annual species was 45 (41.3%), while the perennial species were represented by 64 different species (58.7%). In terms of the global geographical distribution of the plants collected, the most represented regions were the Saharo-Arabian region with 39 (35.10%) different plant species followed by the bi-regional Saharo Arabian-Somalia Masai with 10 (9.2) different plant species and the Tropical and Saharo Arabian-Irano Turanian regions with 8 (7.3%) different plant species for each. Generally, the most abundant species were those typically found in a single region (52 species, 46.9%) and the bi-regional species (30 species, 28.4%) while the multi-regional and cosmopolitan species were the least abundant with 12 species (11.0%) and 1 species (0.9%), respectively. The most abundant plant species in all the habitats studied were *Phragmites australis* (with a relative presence 'P' = 41.3%, a relative coverage 'C' = 15.4%), *Tamarix nilotica* (P = 39.4%, C = 5.71%), *Salosola imbricate* (P = 34.9%, C = 1.32%), *Zygophyllum propinquum* ssp. *migahidii* (P = 25.4%, C = 16.0%) and *Tamarix aucheriana* (P = 25.4%, C = 16.0%). In contrast, the rarest plants (≤ 1) in the habitats studied included (for example) *Aizoon canariense*, *Chenopodium album*, *Plantago amplexicaulis* and *Salvia spinosa*.

In the present study, two forms of wetlands in Saudi Arabia were studied, namely sabkhas (natural) and sewage canals (industrial). Seventy-nine different plant species belonging to 69 genera and 28 families were found in sabkhal habitats. Chenopodiaceae, Poaceae and Asteraceae were the most highly represented plant families with 14, 14 and 7 plant species, respectively. Most of the plant species in sabkhas were annual herbs (34 species, 43%) and perennial herbs (20 species, 25.3%). The most abundant plant species were *Tamarix aucheriana* (P = 39.47%, C = 13.16%) and *Zygophyllum propinquumm* ssp.*migahidii* (P = 34.21%, C=4.03%), while the rarest plant species were *Chenopodium album* and *Salvia spinosa*. In contrast, in sewage canal habitats, 58 different plant

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species belonging to 50 genera and 26 families were found. The most abundant families there were members of the Poaceae (9 species, 15.5%) and Chenopodiaceae (8 species, 13.8%). Perennial herbs twenty different species (34.5%) were the most represented group followed by annual herbs with 16 species (27.6%). *Phragmites australis* was the most abundant plant species (P=57.69%, C=24.04%) followed by *Tamarix nilotica* (P=50.00%, C=8.85%), *Calotropis procera* (P=38.46%, C=8.31%) and *Pulicaria undulata* (P=38.46%, C=6.92%). In contrast, *Aeluropus lagopoides* and *Amaranthus viridis* were the rarest plants (*Appendix 1*).

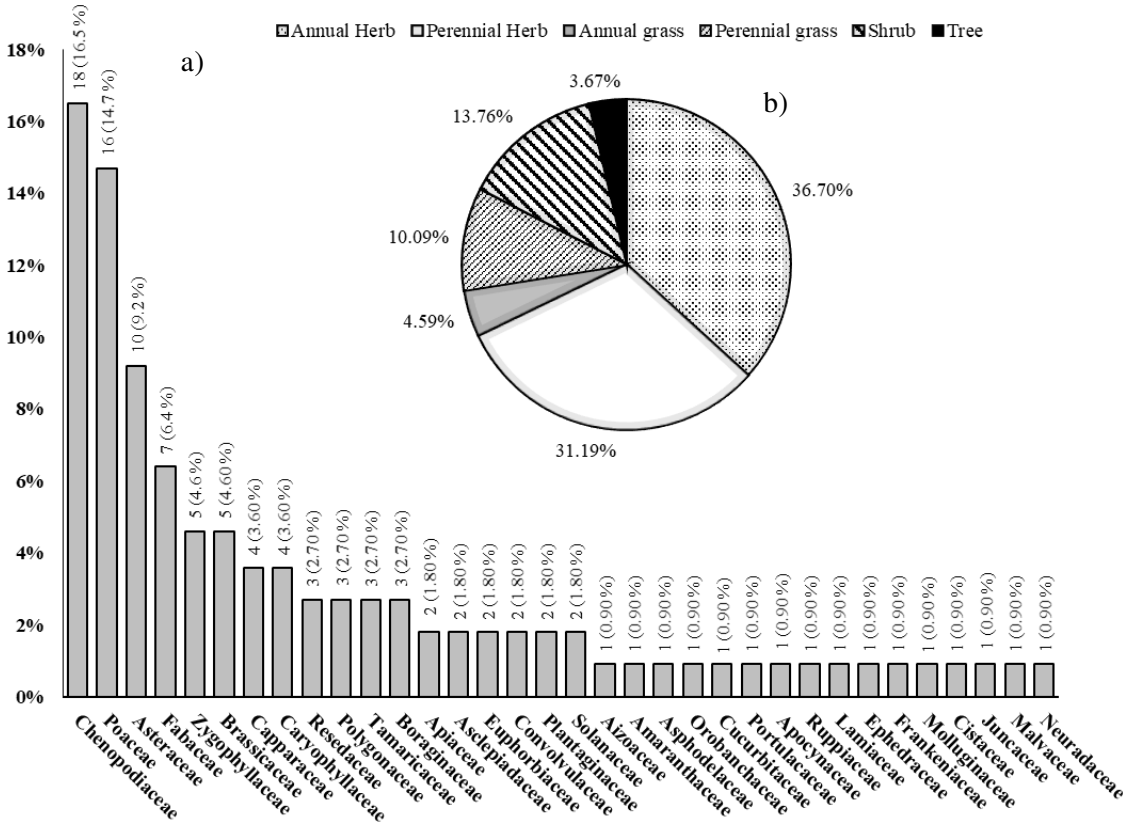


Figure 2. *(a) Number and percentages of different represented families (bar chart) and (b) lifeforms (pie chart) in the studied stands.*

Cover estimates for 109 plants collected from 63 different stands using TWINSPAN software generated 6 different vegetation groups at the third level of classification (*Fig. 3a, Table 2*). Of these groups, only two groups only occupied sabkhas habitats; a) that characterized by *Tamarix aucheriana – Phragmites australis* and b) that characterized by *Centropodium forsskalii – Emex spinosa*. One group occupied sewage canals only (*Calotropis procera – Citrullus colocynthis*), two groups mainly occupied sabkhas; a) *Suaeda vermiculata – Seidletzia rosmarinus – Zygophyllum propinquum* ssp. *Migahidii* and b) *Haloxylon salicornicum – Suaeda aegyptiaca*. One group mainly occupied sewage canals (*Tamarix nilotica – Salsola imbricata*). Further analysis of the same data set using DECORANA software showed a reasonable segregation between the groups that had been generated (*Fig. 3b*).

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Table 2. *Characteristics of different vegetation groups produced by TWINSPAN*

VG	Stands	Stands (%)	Species	Habitats		First dominant species	P%	C%	Second dominant species	P%	C%
				Sabkhas	Sewage						
1	7	11.00	35	71.4	28.6	*Haloxylon salicornicum*	57	18.86	*Suaeda aegyptiaca*	43	6.43
2	10	15.87	31	80	20	*Suaeda vermiculata*	43	10.9	*Seidletzia Rosmarinus* *Zygophyllum propinquum ssp. migahidii.*	50 50	10 8.2
3	16	25.40	70	100	0	*Tamarix aucheriana*	56	25	*Phragmites australis*	56	16.31
4	19	30.16	59	36.8	63.2	*Tamarix nilotica*	63	16.84	*Salsola imbricata*	47	3.58
5	9	14.29	41	0	100	*Calotropis procera*	44	15.78	*Citrullus colocynthis*	33	2.0
6	2	3.17	6	100	0	*Centropodium forsskalii*	50	3.89	*Emex spinosa.*	50	1.0

P: presence of species, C: relative cover of species

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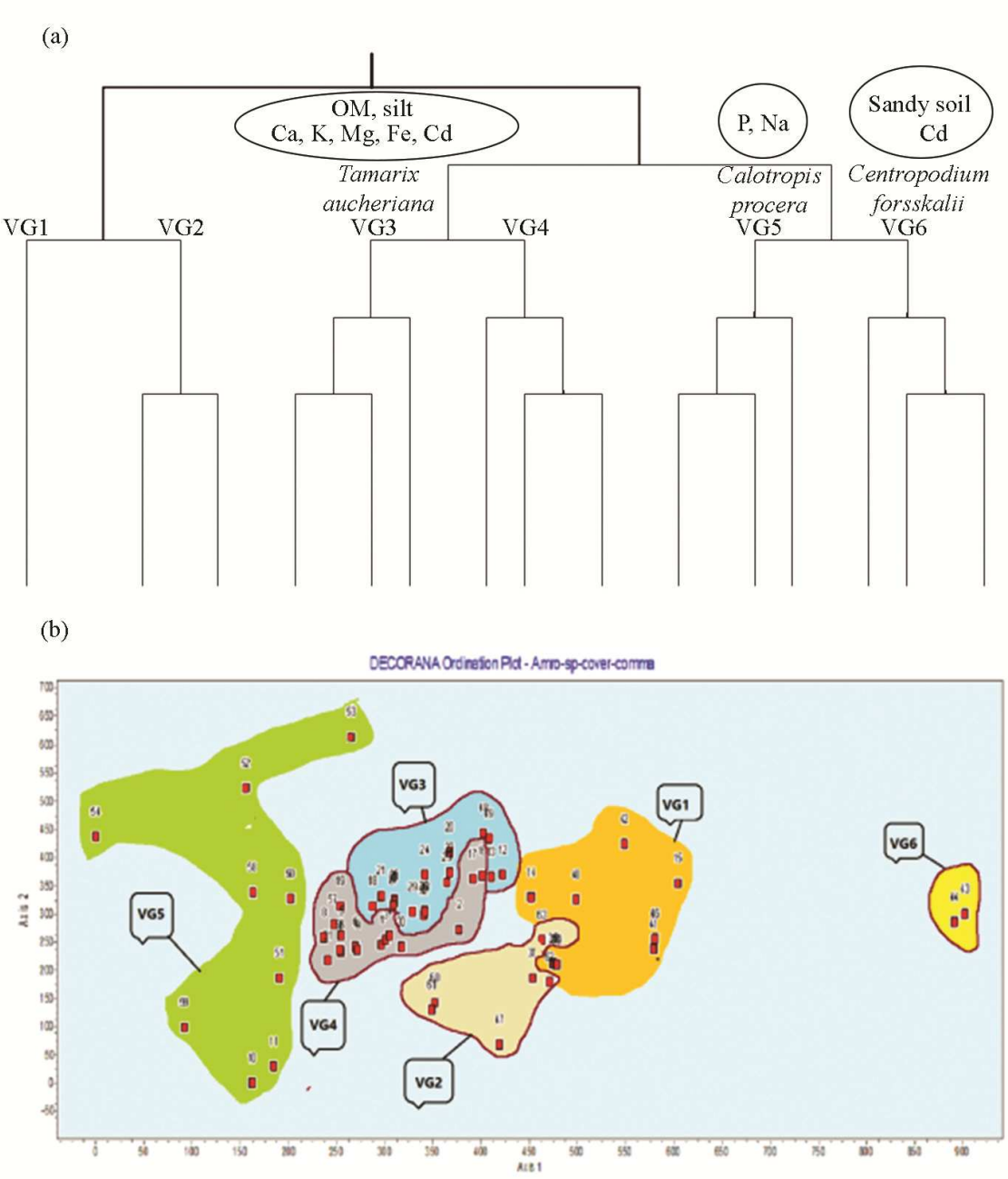


Figure 3. *(a) Dendogram showing the relationship between 6 different vegetation groups generated by TWINSPAN and (b) Segregation of different vegetation groups after applying DECORANA analysis.*

CCA analysis showed that different environmental factors related to soil affect the species distribution on the two axes. There was a positive correlation between the Simpson index value and the first axis and a negative correlation between the same axis and EC, Ca, Mg, Cr and Cd. Meantime, the second axis correlated negatively with silt percentage in soil, organic matter, Ca, K, Mg, and Cd (*Fig. 4a*). Plant species recognized as psammophytic (associated with sandy soils) occupied the positive upper left and right sides of the CCA plot (*Fig. 4b*) and showed a positive correlation with the

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Shannon-Wiener index, evenness, species richness, sand percentage in soil, Zn and Na. Psammophytic plants included, for example, *Bassia eriophora*, *Pennisetum divisum*, *Cynodon dactylon* and *Asphoedelus tenuifolius*. Shrubs such as *Calotropis procera*, *Tamarix aucheriana* and *Heliotropium digynum* were found on the lower side of the first axis and showed a strong correlation with the Simpson index and P content. Halophytes mainly occupied the negative lower side of the first axis and showed strong correlation with increasing pH, EC, Cr, Pb, Mg and organic matter. This group of plants included, for example, *Aeluropus lagopoides*, *Salicornia europaea* and *Juncus rigidus*.

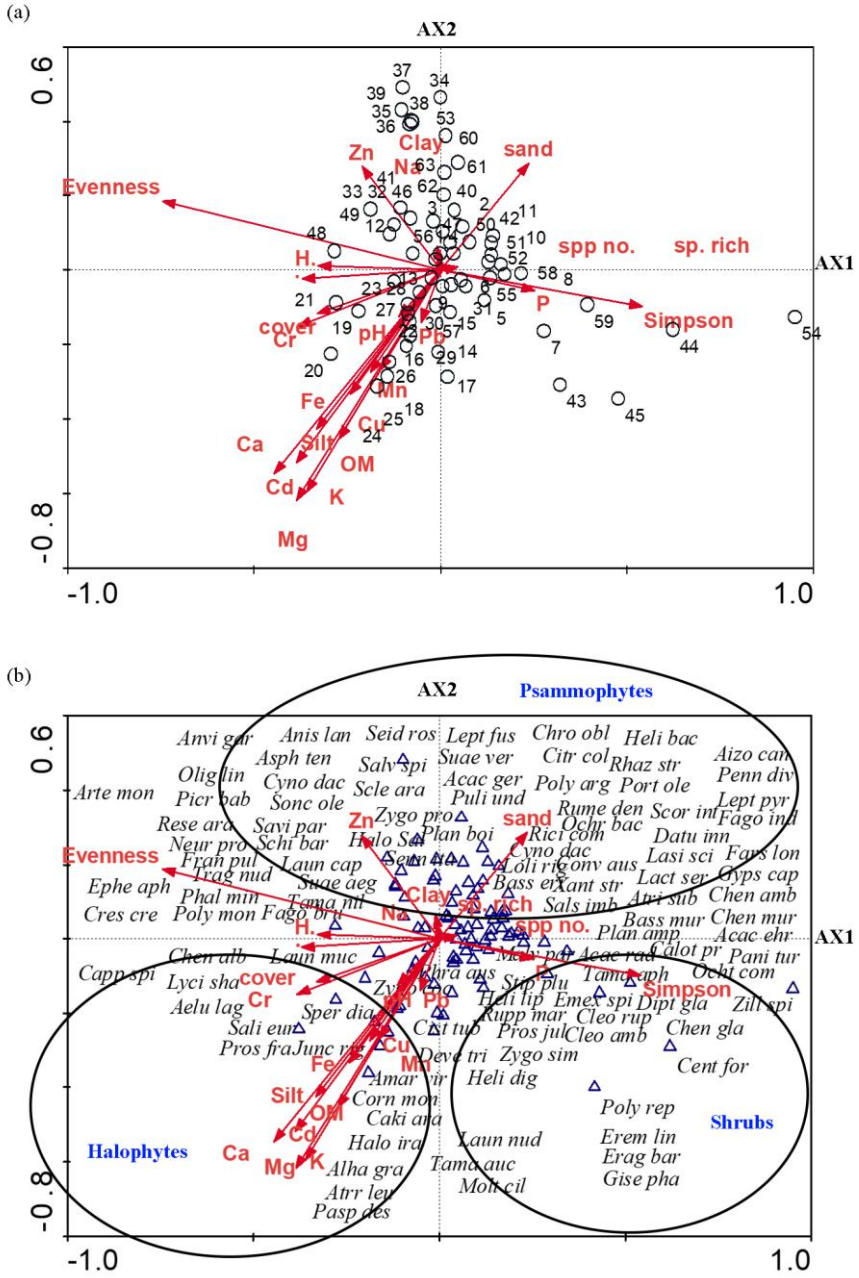


Figure 4. *(a) The CCA plot shows the relationships between the stands studied and the soil characteristics and biological indices (the Simpson index and measures of evenness). (b) The same plot showing the abundant species represented by the first four letters of genus and first three letters of species.*

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Discussion

Wetlands are considered a very significant habitat for diverse group of animals, plants and even microbes. In addition to their important role in the water cycle, and in removing excess nutrients and other contaminants from polluted water, they are one of the most biodiverse sites around the world. Wetlands contribute significantly in keeping the ecological balance between different plant species. In Saudi Arabia, wetlands are the richest habitats from the point of view of plant diversity because of the availability of moisture, in contrast to the many dry places around the kingdom. In this study of the vegetation of 63 different sites, it was found that perennial plants were the most abundant life forms. Our earlier (unpublished) studied on wetlands in Saudi Arabia have also shown that perennial plants are more abundant than annual plants in valley habitats in the Riyadh area. In contrast, we found that annual plants were more abundant in Sabkha habitat in the Qassim area (unpublished data). Levels of species richness (1.31) in this study indicated the high plant diversity at the region. Members of the Chenopodiaceae family were the most abundant family in the stands studied. This may be due to the high tolerance of drought and salinity typical of the members of this family. Stebbins (1974) pointed out that the Chenopodiaceae plants were typically able to grow in saline sandy soils such as sabkhas. It could, also, be due to the fact that members of the Chenopodiaceae disperse readily due to their lightweight seeds (El-Sheikh, 2013). In the present study, where the Chenopodiaceae were most abundant, they were followed by Poaceae and Asteraceae. In contrast, at the level of Saudi Arabia as a whole, Chaudhary (2001) found that the most abundant families were first, the Poaceae followed by the Chenopodiaceae and the Zygophyllaceae.

In the present study, perennial plants were more represented than annuals. Wetlands habitats such as sabkhas and sewage treatment areas are very different from the dryland areas, in that they provide the moisture required by plants, and do so year-round. In addition, soils in wetlands have higher concentrations of various nutrients that promote the growth of various perennial species. The dominant plants in wetlands are trees as they can tolerate drought stress for longer periods, and they can draw water from surface and deep soil via their extensive root systems (Al-Rowaily et al., 2012). Chorotype analysis showed that mono-regional plants were the most abundant and were represented by the Saharo Arabian, Tropical and Irano-Turanian groups, respectively. The study stands are located, mainly, in the Arab Sahara Region located between the Tropical and Irano-Turanian regions (Alatar et al., 2012). The central region of Saudi Arabia is a part of the Arab Sahara Region and their plants are mainly mono-regional belonging to the Saharo Arabian group (Al-Nafie, 2008). The presence of plants normally associated with Saharo Arabian-Somalia Masai, Saharo Arabian-Irano Turanian and Mediterranean-Irano Turanian floras indicate that the central region of Saudi Arabia is a transitional area with some of the characteristics of surrounding regions. This supports earlier vegetation studies of the central region (Alatar et al., 2012; Alatar et al., 2015) and studies of Kuwait (El-Sheikh et al., 2012).

TWINSPAN analysis showed that there were 6 different vegetation groups in the study sites. Shaltout et al. (1997) studied the vegetation of coastal lowland of eastern Saudi Arabia. In their analysis, they found 12 different vegetation groups include *Tamarix aucheriana* and *Phragmites australis* communities. In addition, *Phragmites australis* was found in the Hail region in northern Saudi Arabia (El-Ghanim et al., 2010). In the present study, it was found that salinity, soil texture and nutrient levels were the main environmental factors affecting the distribution of plants in each stand.

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Our results are supported by other studies of similar habitats (Alatar et al., 2012; El-Sheikh et al., 2012). The Shannon index showed a positive correlation with sand content in soil because soils with more sandy texture provide soil aeration in wetlands such as sabkhas and sewage canals. Furthermore, percentage of cover was positively correlated with organic matter content and potassium levels. Shrubs and trees benefit from the presence of high organic matter which as it decomposes provides nutrients for growth, allowing development of significant biomass (Alatar et al., 2015). Other macronutrients especially K, Mg and Ca also play a critical role in maintaining plant osmotic pressure in a saline environment, adjusting the pH of the plant cell, protecting cells from toxicity and serving as co-factors for several enzymes (Shiel, 2001).

Psammophytic plants were the most abundant lifeform in the stands studied followed by halophytes and shrubs. This pattern reflects characteristics of the whole flora of Saudi Arabia (Chaudhary, 2001). Psammophytic plants were correlated with Shannon index, species evenness, species richness and number of species in each stand. Increased plant cover may promote higher chlorophyll content in plants as a result of competition and shading. Abou-Sitta and Al-Taisan (1995) found that desert plant growing in shade had higher chlorophyll content than plants growing in sunny areas. Higher chlorophyll content enhancing plant's ability to photosynthesize and produce the carbohydrates essential for growth. There was a clear correlation between halophytes and the EC of the soil. Clearly, we could expect halophytic plants to be the most abundant group of plants under high salinity conditions (Alatar et al., 2012; El-Sheikh et al., 2012). In the present study, the third and sixth vegetation groups which dominated by *Tamarix aucheriana* and *Centropodium forsskalii*, respectively, were found only in sabkha habitats. This could be attributed to the high salinity levels in these habitats. Halophytes are, also, able to hyperaccumulate some of the heavy metals including Cr, Pb, Fe, Mn, Cu and Cd. Heavy metals hyperaccumulators can remediate contaminated soil by sorting such metals in inert forms in their vacuoles using a mechanism similar to the one that halophytes use to cope with salinity (Van Oosten and Maggio, 2015). In the present investigation, the fifth vegetation group dominated by *Calotropis procera* was found only in sewage canals habitats. These habitats were characterized by high levels of heavy metals. This could be an explanation for the high presence of *Calotropis procera* in these habitats because its high ability to accumulate heavy metals (Galal et al., 2016).

The vegetation of the sabkhas and sewage canals in this study fell into 6 different vegetation groups. The third vegetation group, dominated by *Tamarix aucheriana* and *Phragmites australis*, showed high values of percentage of cover and a high Shannon index associated with high soil organic matter, silt percentage in the soil and concentrations of Ca, K, Mg, Fe and Cd. In contrast, the fifth vegetation group, dominated by *Calotropis procera* and *Citrullus colocynthis*, was associated with high soil concentrations of Na and P. This could be due to the role of Na in maintaining pH and ionic balance in plant cells and tissues, and the essential role of P as a macronutrient required for the biosynthesis of amino acids and nuclear proteins (Shiel, 2001). The sixth vegetation group, dominated by *Centropodium forsskalii* and *Emex spinosa*, was associated with high values of species richness and a high Simpson index. This vegetation group was mainly found in sand habitats with good soil conditions. Sandy soils are particularly suitable for growth of various annual herbs and grasses. The distribution of such plants is related to salinity.

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APPENDIX

Appendix 1. *GPS coordinates of the studied stands in Riyadh and Qassim regions*

Stand No.	Habitat	Coordinates	Altitude (m)	Stand No.	Habitat	Coordinates	Altitude (m)
1	Sewage, Riyadh	N 24° 06' 05.5" E 47° 23' 21.4"	421	33	Sabkha, Qassim	N 26° 01' 20.57" E 44° 10' 33.07"	608
2	Sewage, Riyadh	N 24° 06' 01.9" E 47° 23' 14.9"	420	34	Sabkha, Qassim	N 26° 23' 09.1" E 43° 48' 14.5"	629
3	Sewage, Riyadh	N 24° 05' 58.7" E 47° 23' 08.5"	420	35	Sabkha, Qassim	N 26° 23' 23.3" E 43° 47' 42.5"	629
4	Sewage, Riyadh	N 24° 06' 07.9" E 47° 23' 32.3"	421	36	Sabkha, Qassim	N 26° 23' 39.13" E 43° 47' 21.48"	629
5	Sewage, Riyadh	N 24° 06' 15.7" E 47° 23' 22.4"	421	37	Sabkha, Qassim	N 26° 23' 44.8" E 43° 47' 54.3"	634
6	Sewage, Riyadh	N 24° 06' 38.7" E 47° 23' 34.4"	420	38	Sabkha, Qassim	N 26° 23' 48.5" E 43° 48' 39.7"	635
7	Sewage, Riyadh	N 24° 05' 50.9" E 47° 22' 42.7"	417	39	Sabkha, Qassim	N 26° 23' 26.26" E 43° 48' 58.21"	625
8	Sewage, Riyadh	N 24° 04' 47.2" E 47° 22' 14.2"	431	40	Sabkha, Riyadh	N 25° 14' 28.2" E 45° 32' 33.5"	645
9	Sewage, Riyadh	N 24° 04' 28.0" E 47° 22' 11.3"	434	41	Sabkha, Riyadh	N 25° 14' 00.7" E 45° 32' 59.8"	646
10	Sewage, Riyadh	N 24° 11' 10.0" E 47° 26' 03.3"	410	42	Sabkha, Riyadh	N 25° 13' 50.7" E 45° 33' 07.7"	648
11	Sewage, Riyadh	N 24° 11' 07.98" E 47° 25' 58.41"	410	43	Sabkha, Riyadh	N 25° 13' 03.1" E 45° 33' 13.6"	669
12	Sabkha, Qassim	N 26° 04' 04.6" E 44° 09' 58.5"	621	44	Sabkha, Riyadh	N 25° 21' 09.07" E 45° 19' 58.99"	671
13	Sabkha, Qassim	N 26° 04' 10.1" E 44° 09' 59.9"	621	45	Sabkha, Riyadh	N 25° 14' 02.5" E 45° 33' 30.0"	646
14	Sabkha, Qassim	N 26° 03' 51.9" E 44° 01' 41.2"	619	46	Sabkha, Riyadh	N 25° 14' 10.3" E 45° 31' 32.2"	651
15	Sabkha, Qassim	N 26° 03' 37.6" E 44° 09' 39.0"	613	47	Sabkha, Riyadh	N 25° 16' 07.6" E 45° 31' 54.0"	644
16	Sabkha, Qassim	N 26° 03' 16.9" E 44° 09' 54.3"	609	48	Sabkha, Riyadh	N 25° 20' 31.4" E 45° 22' 22.0"	655
17	Sabkha, Qassim	N 26° 02' 59.80" E 44° 10' 02.86"	610	49	Sabkha, Riyadh	N 25° 20' 13.5" E 45° 22' 13.0"	662

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18	Sabkha, Qassim	N 26° 03' 46.6" E 44° 08' 28.1"	604	50	Sewage, Riyadh	N 24° 20' 58.2" E 046° 55' 49.9"	499
19	Sabkha, Qassim	N 26° 03' 44.3" E 44° 08' 30.0"	605	51	Sewage, Riyadh	N 24° 22' 02.9" E 046° 54' 28.4"	505
20	Sabkha, Qassim	N 26° 03' 49.5" E 44° 08' 13.6"	601	52	Sewage, Riyadh	N 24° 22' 16.5" E 46° 54' 20.8"	506
21	Sabkha, Qassim	N 26° 03' 46.1" E 44° 08' 13.3"	602	53	Sewage, Riyadh	N 24° 21' 58.8" E 46° 54' 15.9"	506
22	Sabkha, Qassim	N 26° 03' 38.6" E 44° 08' 07.8"	604	54	Sewage, Riyadh	N 24° 21' 48.7" E 46° 54' 20.4"	506
23	Sabkha, Qassim	N 26° 03' 33.6" E 44° 08' 18.6"	603	55	Sewage, Riyadh	N 24° 21' 31.9" E 46° 54' 31.1"	502
24	Sabkha, Qassim	N 26° 03' 26.7" E 44° 08' 20.7"	604	56	Sewage, Riyadh	N 24° 18' 36.35" E 46° 58' 40.14"	501
25	Sabkha, Qassim	N 26° 03' 22.3" E 44° 08' 22.3"	604	57	Sewage, Riyadh	N 24° 23' 01.1" E 046° 49' 45.0"	564
26	Sabkha, Qassim	N 26° 02' 51.39" E 44° 08' 42.48"	605	58	Sewage, Riyadh	N 24° 22' 31.8" E 46° 49' 53.3"	566
27	Sabkha, Qassim	N 26° 02' 26.6" E 44° 08' 13.2"	610	59	Sewage, Riyadh	N 24° 22' 11.6" E 046° 50' 23.8"	559
28	Sabkha, Qassim	N 26° 02' 21.8" E 44° 08' 45.8"	599	60	Sewage, Qassim	N 25° 56' 04.4" E 43° 28' 22.3"	650
29	Sabkha, Qassim	N 26° 00' 27.81" E 44° 09' 16.58"	600	61	Sewage, Qassim	N 25° 56' 06.8" E 43° 28' 27.1"	650
30	Sabkha, Qassim	N 25° 58' 48.0" E 44° 10' 14.9"	602	62	Sewage, Qassim	N 25° 54' 33.3" E 43° 26' 26.9"	649
31	Sabkha, Qassim	N 25° 58' 00.7" E 44° 10' 47.7"	604	63	Sewage, Qassim	N 25° 54' 07.8" E 43° 27' 24.2"	647
32	Sabkha, Qassim	N 25° 58' 58.3" E 44° 11' 30.5"	607				

Appendix 2. *Collected plant species and their families, lifeforms and chorotype*

Species	Families	Lifeform	Chorotype*
Acacia ehrenbergiana	Leguminosae	Shrub	SH-SM
Acacia gerrardii	Leguminosae	Tree	SH-SM
Acacia raddiana	Leguminosae	Tree	SH-SM
Aeluropus lagopoides	Gramineae	Perennial grass	Eu. Sib-Med-IT
Aizoon canariense	Aizoaceae	Annual herb	SA-SM
Alhagi graecorum	Leguminosae	Perennial shrub	MED-IT
Amaranthus viridis	Amaranthaceae	Annual herb	TR
Anisosciadium lanatum	Umbelliferae	Annual herb	SA
Anvillea garcinii	Compositae	Perennial shrub	SA
Artemisia monosperma	Chenopodiaceae	Shrub	SA
Asphoedelus tenuifolius	Asphodelaceae	Annual herb	SA-SM
Atriplex suberecta	Chenopodiaceae	Perennial shrub	Am
Atrriplex leucoclada	Chenopodiaceae	Shrub	SA
Bassia eriophora	Chenopodiaceae	Annual herb	SA-IT
Bassia muricata	Chenopodiaceae	Annual herb	SA-IT
Cakile arabica	Cruciferae	Annual herb	SA
Calotropis procera	Asclepiadaceae	Shrub	SM
Capparis spinosa	Capparaceae	Shrub	Med-SA
Centropodium forsskallii	Gramineae	Perennial grass	SA-IT
Chenopodium album	Chenopodiaceae	Annual herb	PALEO
Chenopodium ambrosioides	Chenopodiaceae	Perennial shrub	TR
Chenopodium glaucum	Chenopodiaceae	Annual herb	EU-MED
Chenopodium murale	Chenopodiaceae	Annual herb	PALEO
Chrozophora oblongifolia	Euphorbiaceae	Perennial shrub	IT

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Species	Families	Lifeform	Chorotype*
Cistache tubulosa	Orobanchaceae	Annual herb	SA-IT
Citrullus colocynthis	Cucurbitaceae	Annual herb	SA
Cleome amblyocarpa	Capparaceae	Annual herb	SA-SM
Cleome rupicola	Capparaceae	Annual herb	SH-SM
Convolvulus austro-aegyptiacus	Convolvulaceae	Perennial shrub	SA
Cornulaca monacantha	Chenopodiaceae	Perennial shrub	SA
Cressa cretica	Convolvulaceae	Annual herb	MED-IT
Cynodon dactylon	Gramineae	Perennial grass	TR
Datura innoxia	Solanaceae	Shrub	AM
Devera triradiata	Umbelliferae	Shrub	SA
Dipterygium glaucum	Capparaceae	Perennial shrub	SA
Emex spinosa	Polygonaceae	Annual herb	MED-SA
Ephedra aphylla	Ephedraceae	Shrub	SA
Eragrostis barrelieri	Gramineae	Perennial grass	MED-SA
Eremobium lineare	Cruciferae	Annual herb	SA
Fagonia bruguieri	Zygophyllaceae	Perennial shrub	SA
Fagonia indica	Zygophyllaceae	Perennial shrub	SA
Farsetia longisiliqua	Cruciferae	Perennial shrub	SA
Frankenia pulverulenta	Frankeniaceae	Annual herb	Eu-Sib-Med-IT
Gisekia pharnaceoides	Molluginaceae	Annual herb	TR
Gypsophila capillaris	Caryophyllaceae	Annual herb	IT
Halothamnus iraqensis	Chenopodiaceae	Perennial shrub	SA-IT
Haloxylon salicornicum	Chenopodiaceae	Perennial shrub	SA
Helianthemum lippii	Cistaceae	Perennial shrub	SA-SM
Heliotropium bacciferum	Boraginaceae	Perennial shrub	SA-SH
Heliotropium digynum	Boraginaceae	Perennial shrub	SA
Juncus rigidus	Juncaceae	Perennial shrub	SA-IT
Lactuca serriola	Compositae	Perennial shrub	Med-Eu-Sib-IT
Lasiurus scindicus	Gramineae	Perennial grass	SA-SH-SM
Launaea capitata	Compositae	Annual herb	SA
Launaea mucronata	Compositae	Annual herb	SA
Launaea nudicaulis	Compositae	Annual herb	SA
Leptadenia pyrotechnica	Asclepiadaceae	Shrub	SA-SM
Leptochloa fusca	Poaceae	Perennial grass	TR
Lolium rigidum	Gramineae	Annual grass	MED-IT
Lycium shawii	Solanaceae	Shrub	SA
Malva parviflora	Malvaceae	Annual herb	MED-IT
Moltkiopsis ciliata	Boraginaceae	Perennial shrub	SA
Neurada procumbens	Neuradaceae	Annual herb	SA
Ochthochloa compressa	Gramineae	Perennial grass	SA-SM
Ochradenus baccatus	Resedaceae	Shrub	SH-SM-SA
Oligomeris linifolia	Resedaceae	Annual herb	SH-SM-SA
Panicum turgidum	Gramineae	Perennial grass	SH-SM-SA
Paspalidium desertorum	Gramineae	Annual grass	SH-SM
Pennisetum divisum	Gramineae	Perennial grass	SA
Picris babylonica	Compositae	Annual herb	SA
Phalaris minor	Gramineae	Annual grass	MED-IT
Phragmites australis	Gramineae	Perennial grass	MED-IT-SA
Plantago amplexicaulis	Plantaginaceae	Annual herb	SA
Plantago boissieri	Plantaginaceae	Annual herb	SA
Polycarpaea repens	Caryophyllaceae	Perennial shrub	SA-SM
Polygonum argyrocoleum	Polygonaceae	Annual herb	MED-IT
Polypogon monsepeliensis	Gramineae	Annual grass	Med-IT-SA
Portulaca oleracea	Portulacaceae	Annual herb	COSM
Prosopis fracta	Leguminosae	Perennial shrub	IT

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Species	Families	Lifeform	Chorotype*
Prosopis juliflora	Leguminosae	Tree	AM
Pulicaria undulata	Compositae	Perennial shrub	SA-SM
Reseda arabica	Resedaceae	Annual herb	SA
Rhazya stricta	Apocynaceae	Perennial shrub	SA
Ricinus communis	Euphorbiaceae	Shrub	TR
Rumex dentaus	Polygonaceae	Perennial shrub	Med-Eu-Sib-IT
Ruppia maritima	Ruppiaceae	Perennial shrub	TR
Salicornia europaea	Chenopodiaceae	Annual herb	Med-Eu.Sib
Salsola imbricata	Chenopodiaceae	Perennial shrub	SA-SH
Salvia spinosa	Labiatae	Annual herb	IT
Savignya parviflora	Cruciferae	Annual herb	SA
Schismus barbatus	Gramineae	Annual grass	SA-IT
Sclerocephalus arabicus	Caryophyllaceae	Perennial shrub	SA
Scorzonera intricata	Compositae	Perennial shrub	IT
Seidletzia rosmarinus	Chenopodiaceae	Shrub	SA
Senna italica	Leguminosae	Perennial shrub	SH-SM
Sonchus oleraceus	Compositae	Annual herb	Eu-Sib-Medi-IT
Spergularia diandra	Caryophyllaceae	Annual herb	Med-IT-Eu.sib
Stipagrostis plumosa	Gramineae	Perennial grass	SA-IT
Suaeda aegyptiaca	Chenopodiaceae	Annual herb	SA
Suaeda vermiculata	Chenopodiaceae	Perennial shrub	SA
Tamarix aphylla	Tamaricaceae	Tree	SA-SM
Tamarix aucheriana	Tamaricaceae	Shrub	SA-SM
Tamarix nilotica	Tamaricaceae	Shrub	SA
Traganum nudatum	Chenopodiaceae	Perennial shrub	SA
Xanthium strumarium	Compositae	Perennial shrub	TR
Zilla spinosa	Cruciferae	Annual herb	SA
Zygophyllum coccineum	Zygophyllaceae	Perennial shrub	SA
Zygophyllum propinquum ssp. migahidii	Zygophyllaceae	Perennial shrub	SA
Zygophyllum simplex	Zygophyllaceae	Annual herb	SH-SM

* TR: Tropical, SH-SM: Sahel-Somalia Masai, SA-SM: Saharo Arabian-Somalia Masai, SA-SH-SM: Saharo Arabian-Sahel-Somalia Masai, SA-IT: Saharo Arabian-Irano Turanian, SA: Saharo Arabian, Paleo: Paleotropic, Med-SA: Mediterranean-Saharo Arabian, MED-IT-SA: Mediterranean-Irano Turanian-Saharo Arabian, MED-IT: Mediterranean-Irano Turanian, IT: Irano Turanian, Eu-Sib-Med-IT: Euro Siberian-Mediterranean-Irano Turanian, COSM: Cosmopolitan, Am: American

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SHORT-TERM PATTERNS IN THE POST-FIRE DIVERSITY OF LIMESTONE GRASSLANDS AND ROCKY GROUND VEGETATION

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Abstract. In the summer of 2007 an enormous wild fire occurred on Vidlič Mt. (Southeast Serbia), which burned down more than 2500 ha of vegetation. Our aim was to study the short-term patterns of diversity changes and vegetation dynamics in the grasslands and rocky ground vegetation affected by the fire. Plots of vegetation were observed according to Braun-Blanquet phytosociological methods. Species richness and Simpson's diversity index were calculated per quadrant for each of three seasons. ANOVA, Unequal HSD *post hoc* test and Linear Regression were performed in order to follow the post-fire vegetation dynamics and diversity. The species richness and diversity of the fire-affected sites were highest in the zone of thermophilous oak forests, slightly lower in the transitional belt, and lowest in the belt of beech forests. With increasing altitude, the values of richness and diversity decreased. Considering life-forms composition, the most important influence of wildfire was the relationship between two most represented categories, hemicryptophytes and therophytes. It was concluded that the cover values of 13 species show comparatively high levels of statistically significant differences between the stages of vegetation formed in the post-fire period. Limestone grasslands and rocky ground vegetation showed quick recovery after the fire, which indicates that these habitats show comparatively high resilience to fire.
Keywords: *wildfire, fire-affected, community composition, floristic similarities, burned plots, species richness, life-forms changes, vegetation recovery*

Introduction

Over the last few years, an increasing number of fires have occurred in natural areas. Worldwide, more than 50,000 forest fires occur annually (Aleksić et al., 2009), on average destroying more than 40 million ha of forest (Lukić et al., 2017). There are "fire climate regions" in which fires have a greater frequency and intensity on substrate with good drainage, and this, combined with the flammability of the dead combustible materials results in any fire having a greater surface area (Dimitrov, 1999). Jurjević et al. (2007) point out that there are three areas that are seriously endangered by fires on Earth: the Mediterranean, the North American area of pine forests and the area of African savannas because of their climate, weather conditions and the character of the substrate. In Europe alone 30,000–40,000 forest fires a year take place and the fire-affected area amounts to over 500,000 ha annually (Jurjević et al., 2007). Damage in

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North America is significantly higher. In Europe, most fires take place in the in ecosystems of the Mediterranean: Italy, France, Greece, Spain, Portugal and Croatia (Margaletić and Margaletić, 2003; Türkmen and Düzenli, 2011). In humid regions there are not as many forest fires and the consequences are not nearly as disastrous (Vukelić and Rauš, 1998).

Between 2004 and 2013, 3800 ha (2250 ha categorized as forests) were destroyed by fire in Serbia (Nikolov, 2015). In just five years (2003-2007) 580 fires were counted and most of them (64%) were in 2007 (Tabaković-Tošić et al., 2009).

The comparative fire ecology of plant communities on serpentinite and sandstone substrates, especially the degree to which species diversity depends on fire, has been underlined by Safford and Harrison (2004). In addition, Pausas and Ribeiro (2017) provided insights into several positive effects of fire on grasslands formed on rocky areas, emphasizing its role in increasing the landscape variability, creating new ecological niches, thereby ensuring the improvement of general plant diversity.

Although several studies on the effects of fire on vegetation in Serbia and its surroundings have been reported (Čolić, 1960, 1966; Vukićević, 1965; Dinić et al., 2003; Janković, 2003; Marković et al., 2015a, 2016), only a few of them consider long-term post fire regeneration patterns in forests focusing on species richness and diversity (Vukićević, 1965; Čolić, 1966). The short-term patterns of dry grasslands and rocky outcrop vegetation over the limestone formations have not yet been investigated in the central Balkans. Furthermore, no studies have yet evaluated the effects of fire on grasslands growing over limestone derived soils, which is one of the most highly represented types of vegetation in the mountain regions of the northeastern, eastern and southeastern parts of Serbia. The carbonate substrate predominant on Vidlič Mountain contributes to the faster and greater warming and drying of terrestrial vegetation (Margaletić and Margaletić, 2003) and it is more sensitive to the occurrence of fire.

The wildfire on Vidlič Mountain happened in 2007 and burned over 2500 ha of grasslands, shrubbery and forest vegetation, considerably changing the landscape of the mountain (Ministry of the Environment and Spatial Planing, 2008), and a forest area of 1000 ha was impacted by uncontrolled wildfire (Panić, 2007). Subsequently, a large area of open grassy and dwarf shrubbery semi-opened vegetation of approximately 1000-1500 ha was also engulfed by fire. The wildfire crossed the skeletal slopes rapidly. The repercussions of the fire on these habitats were neither recorded nor repaired by any governmental organization, as they were in the case of forests, whereby the State Forestry Enterprise was fully engaged.

Several fundamental studies of xeric vegetation in Serbia have already been performed by Jovanović-Dunjić (1955, 1956), Danon (1960), Diklić (1962), Diklić and Nikolić (1964). This vegetation plays an important role in stopping further denudation in limestone areas. During the previous century, a well-developed area of forest on Vidlič Mountain was largely destroyed, remaining mainly on the northern and eastern slopes. Deciduous forest of Hungarian and Turkish oak (*Quercetum frainetto-cerris* Rudski 1949), one of the most widespread types of forest is considered to be climax in the region (Mišić et al., 1978). Different stages of degradation of this community are present on the mountain. The fragments with oriental hornbeam (*Carpinus orientalis*) are one of them. The upper region of the mountain is covered by moesian beech forests (*Fagetum montanum moesiacum*), considered to be a climate-regional community. After woodcutting, and the period of subsequent grazing, many types of meadows, xeric pastures and shrub-like vegetation native to rocky slopes were formed over the vast area

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of eroded slopes. Thus, xeric vegetation in the region is secondary by origin, representing the plagioclimax in the habitat of well-developed deciduous woodland. Its origin, floristic composition and structure are also determined by the warm climate, and particularly by edaphic factors (Diklić and Nikolić, 1964). Bearing steppe characteristics, which is indicated by presence of numerous sub-Mediterranean species, this vegetation is important for many reasons. It plays an important role in stopping further denudation not only regionally, but also in the wider limestone areas of the Peninsula. Different varieties of xeric pastures are traditionally used for grazing, which was particularly popular in previous centuries. This vegetation is also important as a source of medicinal (Marković et al., 2010) and especially aromatic plants (Marković et al., 2009). Finally, in spite of the barren and shallow soils, the xeric vegetation of this region is rich in plant species, especially endemic, endangered and steppe relict representatives (e.g. *Adonis vernalis*, *Paeonia tenuifolia, Ranunculus illyricus, Sternbergia colchiciflora, Hyacinthella leucophaea*).

Our general hypothesis was that the effects of wildfire on plant communities and their diversity would be weaker on more fertile limestone soils and soils with frequent rocky outcrops. We assumed that well represented rocky fraction, typical for the limestone areas, could play an important role in following phenomena:

1) Limestone soils have higher ratios of herbaceous to woody fuels, thus increasing fire spread rates, decreasing fire intensity, and reducing soil heating - fire move faster, and therefore, its impact could be less severe.

2) Soil layer could be only shallowly affected, so the seed bank in the ground would be better preserved.

3) Limestone soils have frequent rocky outcrops which can provide safe sites for plants to avoid burning, saving unburned “patches” of initial vegetation.

The short-term patterns of post-fire diversity for the open and semi-opened, herbaceous to shrubby vegetation on the Vidlič Mt. calcareous plateau in the central Balkan Peninsula were chosen as the object of the present study. To the best of our knowledge, this is the first significant contribution to the post-fire ecology of limestone areas across the Peninsula.

Material and methods

Study area

The study was conducted in fire-affected areas of Vidlič Mt., including surfaces covered by dry grasslands and rocky ground vegetation (*Fig. 1*). Vidlič Mt. is an extension of the Stara Planina (Balkan Mt.) mountain chain, which is a large calcareous plateau located in eastern Serbia (Vidanović, 1955). Its ridge stretches in a prevailing northwest-southeast direction between Serbia and Bulgaria (Petrović, 1999). In geological terms the mountain is mostly built of limestone and dolomite formations, with well-represented cretaceous, jurassic and triassic layers (Vidanović, 1960; Petrović, 1999). Smaller areas are covered with marlstone and sandstone (Andjelković, 1977). Dry skeletal and barren land covers the mighty rocky deposits over the whole area. The prevailing soil types are rendzina and calcomelanosol, which achieve different evolutionary forming phases (Antonović and Mrvić, 2008). Rendzina is nutrient-poor soil formed on soft limestone, as well as on carbonate and silicate substrates like marlstone and sandstone. Calcomelanosol is shallow, humus rich soil formed on limestone and dolomite containing a high percentage of calcium and magnesium

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carbonate (Miljković, 1996). The climate is temperate continental, with hot summers and prominent drought periods. Climate steppe and mediterranean influences are represented across the large base, while the upper part of the mountain has features of a mountain climate (Vidanović, 1960). The mountain is characterized by dynamic relief, inclined slopes, and vast deforested areas exposed to extensive erosion.

The main focus in our previous study (Marković et al., 2015b) was on grasslands belonging to the already familiar alliances *Festucion valesiacae* (Festuco-Brometea) and *Seslerion rigidae* (Festuco-Seslerietea). *Carici humilis-Stipetum pulcherrimae, Potentillo-Caricetum humilis* and *Sanguisorbo-Festucetum vallesiacae* are the most commonly represented associations in the investigated area. Great attention was also dedicated to the fire-affected vegetation of extremely eroded areas, in particular *Euphorbio myrsiniti-Bothriochloetum* (Chrysopogoni-Satureion), that represents final degradation stage of termophilous forests, as one of the most common associations on rocky ground.

In our previous study we investigated the vegetation of 10 unburned plots on Vidlič Mt. in detail (Marković et al., 2015b). The phytocoenoses investigated from unburned areas were found to be similar to those that cover other limestone areas in this region. In the present study, unburned areas were compared with burned ones.

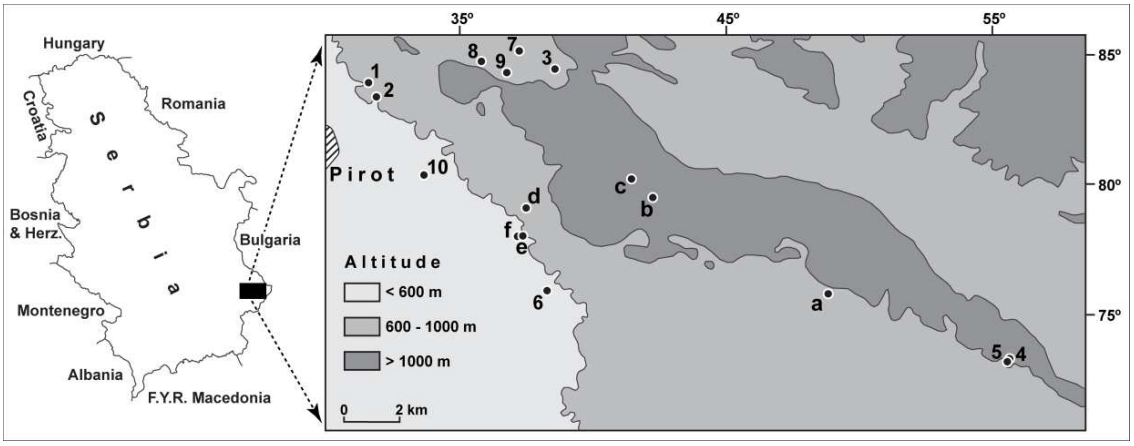


Figure 1. *The area of Vidlič Mt. investigated and location of the plots analyzed: the spatial position of the plots referring to fire non-affected vegetation (1-10) and plots referring to the vegetation of fire-affected localities (a-f). (1, 2 – Mali Vrh; 3 – Basara; 4, 5 – Vlkovija; 6 – Vučje; 7, 8, 9 – Crni vrh;10 – Izvor; a – Visočki Odorovci; b, c – Basarski kamik; d, e, f – Vučje)*

Data collection procedures

The vegetation dynamic of burned xeric grasslands in three consecutive seasons (2008-2010) was followed, and a comparison between the stages of vegetation established at the burned sites with the vegetation of similar, unburned habitats was carried out. In order to track changes in the vegetation at the burned and unburned plots, six phytocoenological plots (a-f) were made in the first, second and third years after the fire. Plots from unburned areas were chosen randomly, and plots from burned areas were then taken from the same vegetational belt. In the beech forests belt unburned plots for comparison were found at greater distances from the burned plots (*Fig. 1*). Plot data for the fire non-affected vegetation as well as for fire affected vegetation are

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presented in *Table 1*. The position of every plot was georeferenced using Ozi explorer and displayed on the map (*Fig. 1*) using DIVA-GIS (5.2) software (Hijmans et al., 2005).

Table 1. *Plot data for the fire non-affected (1-10) and fire-affected vegetation (a-f) at Vidlič Mt. Plots 1-10 represent unburned areas, which was the subject of the investigation in our previous study (Marković et al., 2015b)*

Plot	Locality	Latitude [N]	Longitude [E]	Altitude [m]	Vegetation belt	Exposure	Terrain inclination [°]
1	Mali vrh	43°10'53''	22°37'15''	635	Oak forests	W	20
2	Mali vrh	43°10'37''	22°37'26''	620	Oak forests	S	5
3	Basara	43°11'04''	22°42'03''	900	Transitional	SW	10
4	Vlkovija	43°05'24''	22°53'35''	1100	Beech forests	S	40
5	Vlkovija	43°05'23''	22°53'34''	1090	Beech forests	S	30
6	Vučje	43°06'54''	22°41'44''	490	Oak forests	W	15
7	Crni vrh	43°11'25''	22°41'08''	810	Transitional	S	5
8	Crni vrh	43°11'14''	22°40'10''	930	Transitional	S	35
9	Crni vrh	43°11'01''	22°40'48''	890	Transitional	S	15
10	Izvor	43°09'07''	22°38'37''	480	Oak forests	W	40
a	Visočki Odorovci	43°06'44''	22°48'58''	900	Transitional	S	35
b	Basarski kamik	43°08'36''	22°44'30''	1100	Beech forests	E	35
c	Basarski kamik	43°08'58''	22°43'58''	1150	Beech forests	E	10
d	Vučje	43°08'28''	22°41'14''	710	Transitional	S	10
e	Vučje	43°07'56''	22°41'08''	558	Oak forests	S	30
f	Vučje	43°07'55''	22°41'03''	524	Oak forests	SW	25

The Braun-Blanquet method (1964) was applied for phytocoenological analysis. The 10 plots per fire affected site (quadrants 100 m^2) were analysed. Phytocoenological analysis included data on the locality, altitude, exposure, terrain inclination, plot area, abundance, cover, plot code, floristic composition and degree of presence. The plant material collected was identified according to Josifović (1970-1977) and Velchev (1982-1989) and the nomenclature was adjusted according to Tutin et al. (1964-1980, 1993). The plant material was collected during the summers of 2008-2010 (June, July and August) and deposited in the "Herbarium Moesiacum Niš", University of Niš (HMN).

Data analysis

The combined values were transformed into a numerical scale as proposed by Westhoff and Van der Maarel (1973) to enable comparison of the floristic composition of the plots in different seasons. Simpson's diversity of species in the community, within the minimum range, was performed in the Flora software package (Karadžić et al., 1998). The species richness (the total number of species), and Simpson's diversity index (Whitaker, 1972) that includes species evenness, were calculated per quadrant (100 m^2) for each of three seasons. The mean values of richness and diversity for the plots were presented.

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Descriptive statistics (Means, Standard Deviations) of richness and diversity were calculated. The influence of fire damage and elevation on the values of richness and diversity were tested using one way and two way analysis of variance (ANOVA) and the 95% LSD test was used to analyze the differences between the fire-affected and non-affected plots and different altitudes. Linear regression was performed in order to estimate the correlations between the richness/diversity and altitude, exposure and slope. The analysis of variance (ANOVA) was also used to determine whether there were differences in the floristic composition of the main stages of vegetation in the post-fire period. The unequal HSD *post-hoc* procedure was used to identify the exact seasons (I-III) that show differences in the mean values of significant species. The univariate analysis of species cover values was performed on taxa with highest presence in the plots (60-80% and 80-100%) per year, resulting in the descriptive statistics presented in *Table 4*. All of the statistical analyses quoted here were performed using the STATGRAPHICS Plus 5.0 software.

A comparison of the floristic similarities between the burned and unburned plots in the second and third year were made using Sorensen's coefficient of similarity (Sc) based on presence vs. absence (Smith and Killeen, 1995).

The system proposed by Mueller-Dombois and Ellenberg (1974) modified by Stevanović (1992) is used for the identification of plant life-forms as follows: phanerophytes (P), chamaephytes (Ch), hemicryptophytes (H), geophytes (G) and therophytes (T). In order to find out how the life-form composition changed, the percent of the basic groups was observed during the first, second and third year of post-fire period and compared with the components of fire non-affected vegetation.

Results

Species richness and diversity

In the first year after the fire (*Fig. 2A, B*) the species richness and diversity of the fire-affected sites was the highest in plots e and f, located in the zone of degraded thermophilous oak forests (*Quercetum frainetto-cerris* subass. *carpinetosum orientalis*) formed at low altitudes (500-600 m) (*Fig. 3A, B*). Slightly lower richness and diversity were noted in localities (a and d) from the transitional belt between the oak (*Quercetum montanum*) and beech forests at upper altitudes (700-900 m). On the plots (b and c) located in the belt of beech forests (*Fagetum moesiacae montanum*) over 1000 m above sea level, the poorest values of richness and diversity were recorded. As expected, in the second and third year after the fire (2009 and 2010) an increasing trend of richness and diversity was registered in almost all plots (*Fig. 2A, B*).

By calculating the average values of diversity, it was noticed that the richness and diversity of the burned vegetation were generally reduced in comparison to the unburned areas (*Fig. 4A, B*). After an initial fall, richness and diversity generally had an increasing trend, but did not reach the values of unburned vegetation in the third year (*Table 2*). However, over time, richness (expressed as extreme range and standard deviation values) exerted an upward trend, while for diversity the opposite was true. Fire damage and elevation caused statistically significant differences in both richness and diversity but no interaction between them was found (Two-way ANOVA) (*Table 3*). Furthermore, differences between burned (a-f) and unburned areas (1-10) as well as differences between altitude belts (up to 600 m, 600-1000 m and over 1000 m)

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were also confirmed (homogenous groups - A and B, obtained by LSD tests, denoted differences in 95% of the cases) (*Table 2*).

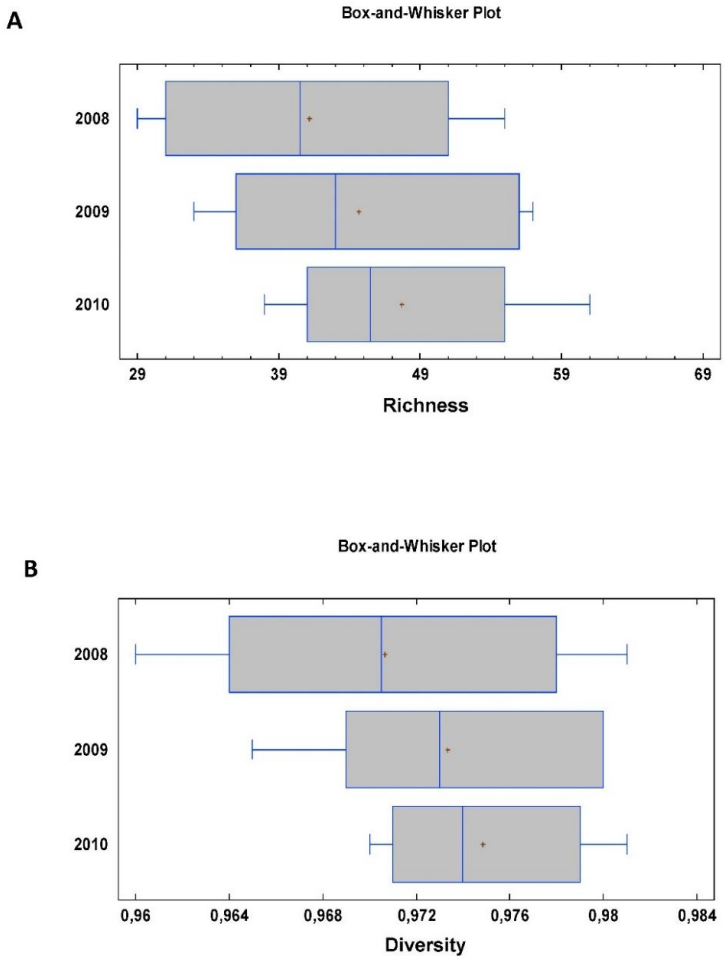


Figure 2. *Species richness (total number of species) (A) and diversity (Simpson's index) (B) of the plots in three consecutive years*

Table 2. *Differences between plots in terms of richness (total number of species) and diversity (Simpson's index). All F-ratios are based on the residual mean square error*

Source	Count	Richness			Diversity		
		Range	Mean value	SD	Range	Mean value	SD
FACTOR: FIRE DAMAGE							
a-f (2008)	6	29-55	40.33 ^{A}	11.18	0.960-0.981	0.970 ^{A}	0.01
a-f (2009)	6	33-57	44.67 ^{A}	10.46	0.965-0.980	0.973 ^{A}	0.01
a-f (2010)	6	38-61	47.67 ^{A}	8.76	0.970-0.981	0.975 ^{A}	0.00
1-10 (2008)	10	46-76	5768 ^{B}	9.54	0.976-0.985	0.980 ^{B}	0.00
FACTOR: ELEVATION (m)							
< 600 m	8	51-61	55.13 ^{B}	3.23	0.977-0.981	0.980 ^{B}	0.00
600-1000 m	12	29-62	43.14 ^{A}	8.40	0.966-0.982	0.973 ^{A}	0.00
> 1000 m	8	29-76	44.50 ^{A}	17.44	0.960-0.985	0.971 ^{A}	0.01

Different letters (A and B) indicate significant difference at $P \leq 0.001$ among the cases

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Table 3. *Two-way analysis for richness and diversity - Type III Sums of Squares. Factors: fire damage and elevation. All F-ratios are based on the residual mean square error*

Source	df	Richness		Diversity	
		F-ratio	P-value	F-ratio	P-value
MAIN EFFECTS					
Fire damage	1	13.78	0.0011	15.05	0.0007
Elevation (m)	2	4.67	0.0193	7.43	0.0031
RESIDUAL	24				
TOTAL (CORRECTED)	27				

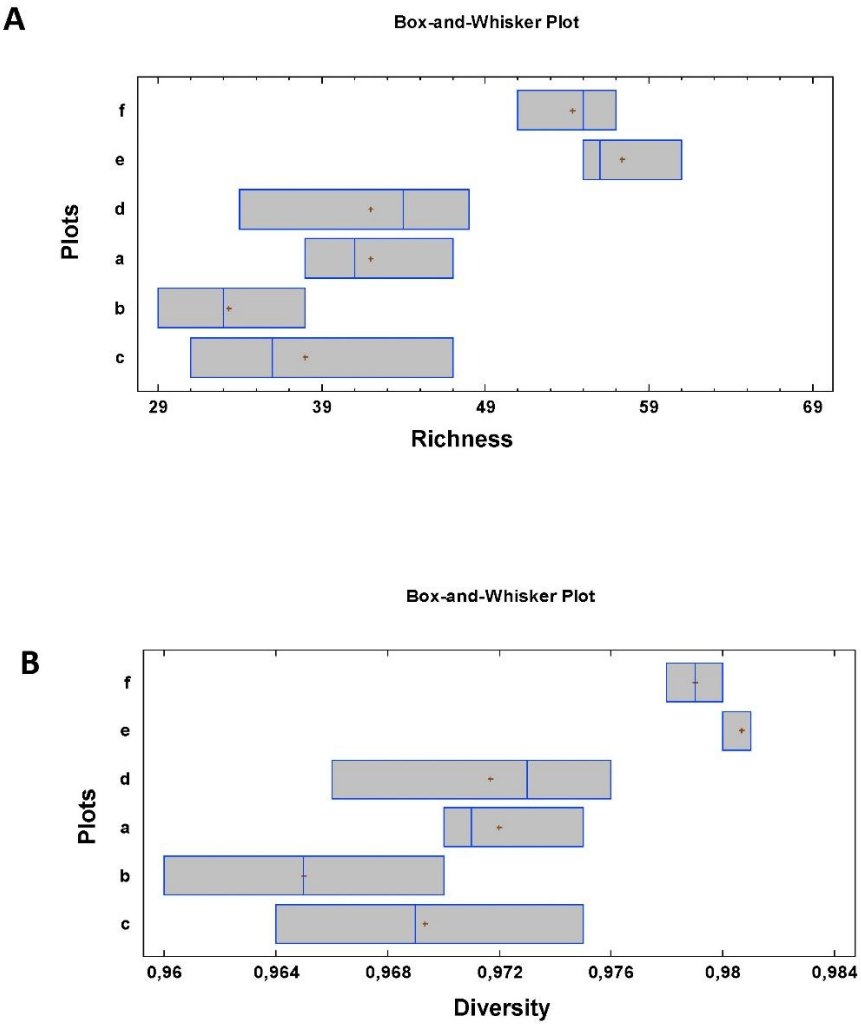


Figure 3. *Mean values of species richness (total number of species) (A) and diversity (Simpson's index) (B) per plots*

Correlations with environmental variables

Richness and diversity had a strong positive correlation (r = 0.98; ANOVA *p*-value = 0.0001) (see also *Fig. 3A, B*). With increasing altitude, the values of richness and diversity decreased (r = -0.80 and -0.78, resp.; *p*-values = 0.0001). But richness and

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diversity increased towards E-WS showing moderate positive correlations (r = 0.69 and 0.68, resp.; *p*-values = 0.0001). The correlation between diversity and richness and the slope of the terrain were weak and without statistically significant differences (r = 0.10 and 0.05, resp.; ANOVA *p*-values > 0.01). But richness and diversity have significant strong negative correlations with the vegetation belt (from oak, through transitional to beech forest) (r = -0.85 and -0.85, resp.; *p*-values = 0.0001).

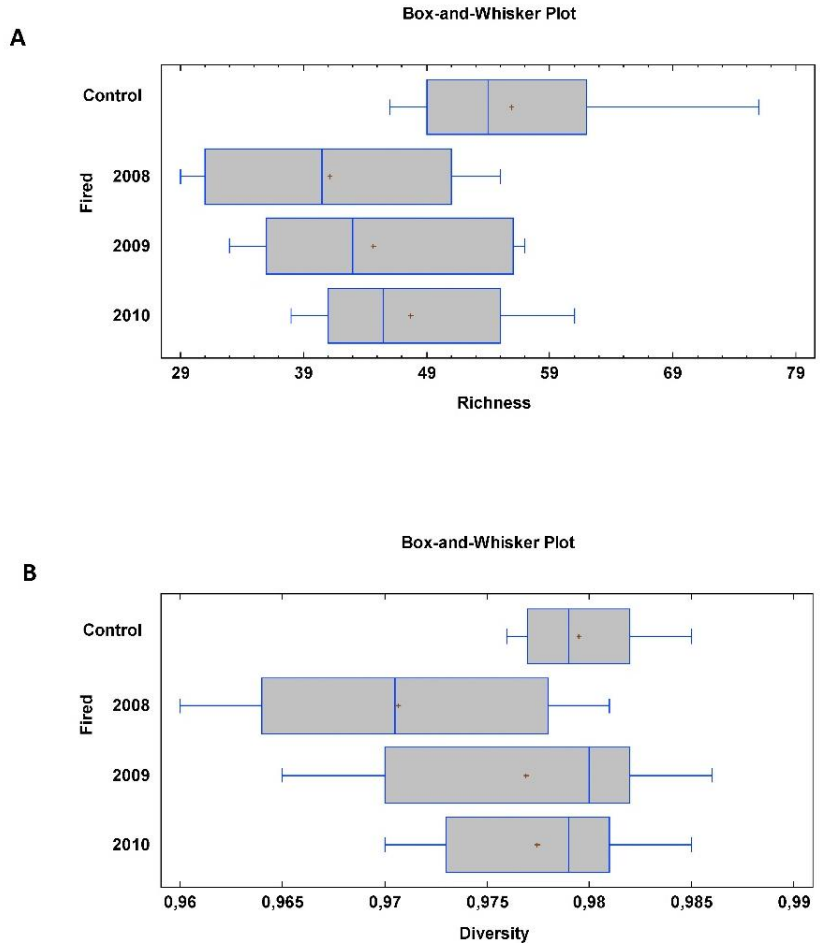


Figure 4. *Mean values of richness and diversity of fire non-affected plots (control) and fire-affected plots in three consecutive years*

Life-forms dynamics in post-fire period

The life-form composition of the vegetation of fire non-affected plots (1-10) could be described by the dominance of herbs (H), including forbs and grasses, represented with 54%, but also large percent of annual plants (T), represented with 32% of all registered species. The group of generally low plants, with semifruticose sprouts (Ch) was presented with 7% in this vegetation. The plants that survived thanks to solid subterranean organs (G) were represented with 5%, while the percent of shrub species (P) was approximately 2%. The composition of life-forms in the vegetation of fire-affected plots (a-i) is considerably different due to the fire influence and became unstable, especially in the first period of post fire recovery. The most important consequence of wildfire is the relationship between two best represented growth-form

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categories, hemicryptophytes and therophytes. The percent of annual representatives in the vegetation of the fire-affected plots (a-i) rapidly increased in the first stage of its revitalization. Under the new conditions, the presence of therophytes (44%) almost exceeds percentages of herbaceous perennial plants, represented with 46% in the first year. Other functional groups, chamaephytes (8%), geophytes (7%) and phanerophytes (1%) were lesser represented in that phase. In the second year of post-fire period, the proportion of therophytes gradually decreased compared to hemicryptophytes (36% *vs.* 47%) while percentage of chamaephytes (11%) began to be more significant. As expected, during the third year of observation, the percent of hemicryptophytes increased (49%) while of therophytes decreased to 32%. The contribution of other vegetation components in the final year of observation was as follows: chamaephytes 12%, geophytes 5% and phanerophytes 2%. Considering life-form of taxa with higher phytocoenological importance, a significant role could be assigned to perennial grass species *Festuca valesiaca* that was dominant in vegetation of all studied post-fire stages. However, significant abundance and coverage were also achieved by some annual species, e.g. *Bromus squarrosus, Filago montana* (firs year) and *Sideritis montana* (first and second year), which easily inhabited burned plots in the initial stages. During the middle post-fire period the vegetation was characterized by higher cover values of *F. valesiaca* and perennial species *Teucrium chamaedrys*, as well as comparatively low cover values of annual species. Increasing cover values of other herbaceous perennial plants beside *F. valesiaca* (e.g. *Euphorbia cyparissias, Medicago falcata* and *Galium purpureum*), along with almost insignificant coverage of therophytes, which was recorded in the final year of observation.

Univariate analysis of species cover values in relation to vegetation dynamics

According to the results of the ANOVA test it was concluded that the values of 13 out of the 21 species compared possess comparatively high levels of statistically significant differences between groups of taxa based on their phytocoenological importance in the post-fire period (*Table 4*). The following species stand out because of their high F values: 1) *Centaurea stoebe* and 2) *Sanguisorba minor*. These species can have an extremely significant role in floristic as well as phytocoenological differentiation between the stages in the early post-fire period of restoration. Among the other species, *Bromus squarrosus, Filago montana, Hieracium bauhinii, Rosa canina, Koeleria splendens, Eryngium campestre, Medicago falcata* and *Fragaria vesca* could also play an important role in the post-fire dynamics of the vegetation.

The unequal HSD *post hoc* test confirmed that all 13 species represent important indicators of post-fire dynamics in calcareous grassland and bedrock vegetation. There were especially significant differences in the cover values of *C. stoebe*, *S. minor* and *R. canina* in the third year, while *B. squarrosus* and *F. montana* were more represented during the first year after the fire (*Table 4*).

Floristic similarity between the unburned and burned vegetation

The floristic similarity between the unburned and burned vegetation in the first three years after the fire was lower than 50% according to Sorensen's coefficient of similarity. The unburned and burned plots in the oak forest belt at lower altitudes showed the highest values (*Table 5*). The lowest similarity was recorded between the unburned and burned plots in the beech forest belt at higher altitudes (20–27%).

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Table 4. *Results of the univariate analysis of species cover values during the post fire period*

Taxon	F[1]	P[2]	I[3]	II	III
Asperula cynanchica [H]	1.97		2.17±0.41	2.33±0.52	1.67±0.82
Bromus squarrosus [T]	10	**	1.33±1.03[a]	0.00[b]	0.00[b]
Centaurea stoebe [H]	25	***	0.00[a]	0.00[a]	1.67±0.82[b]
Crataegus monogyna [P]	0.28		1.33±1.03	1.67±0.82	1.67±0.82
Eryngium campestre [H]	8.08	**	0.00[a]	1.67±0.82[b]	1.33±1.03[b]
Euphorbia cyparissias [H]	1.65		1.50±1.22	1.83±0.98	2.67±1.21
Festuca valesiaca [H]	0.12		3.33±2.07	2.67±2.80	2.83±2.48
Filago montana [T]	10	**	1.33±1.03[a]	0.00[b]	0.00[b]
Fragaria vesca [H]	5	*	0.00[a]	1.33±1.03[b]	1.33±1.03[b]
Galium purpureum [H]	0.02		2.00±1.10	2.17±1.94	2.17±1.94
Hieracium bauhinii [H]	10	**	0.00[a]	0.00[a]	1.33±1.03[b]
Koeleria splendens [H]	9	**	0.00[a]	0.00[a]	1.50±1.22[b]
Medicago falcata [H]	6.94	**	0.00[a]	2.50±1.64[b]	2.50±1.64[b]
Medicago lupulina [H]	5.99	*	0.00[a]	0.00[a]	1.83±1.83[b]
Petrorhagia saxifraga [H]	0		1.67±0.82	1.67±0.82	1.67±0.82
Rosa canina [P]	10	**	0.00[a]	0.00[a]	1.33±1.03[b]
Sanguisorba minor [H]	25	***	0.00[a]	0.00[a]	1.67±0.82[b]
Satureja kitaibelii [Ch]	0.02		2.17±1.94	2.00±1.90	2.00±1.90
Sideritis montana [T]	3.87	*	3.17±3.19[a]	1.50±1.22[ab]	0.00[b]
Teucrium chamaedrys [H]	1.57		1.67±1.37	3.00±1.10	3.33±2.42
Teucrium montanum [H]	5	*	0.00[a]	1.33±1.03[b]	1.33±1.03[b]

[1]F: ANOVA F-test
[2]P: Level of significance (*: P < 0.05; ** P < 0.01; ***: P < 0.001)
[3]Cover values (mean ± standard deviation) according to Van der Maarel (1966); means with different superscript letters within the same row differ significantly (Unequal HSD post-hoc test)
Life-forms: [Ch]) chamaephytes, [H]) hemicryptophytes, [P]) phanerophytes, [T]) therophytes

Table 5. *Floristic similarity between fire non-affected and fire-affected vegetation in the first, second and third year after burning summed up to the vegetation belts. Total number of species found in fire non-affected (A) and fire-affected vegetation (B), number of species in common between non-affected and fire-affected vegetation (C), Sorensen's coefficient of similarity based on presence vs. absence - Sc (%)*

Vegetation belt	Total number of species		Floristic similarity	
	A	B	C	S_c (%)
	2008 (1st year)			
Oak forests < 600 m	120	80	45	45
Transitional belt 600 m-1000 m	138	65	41	40
Beech forests >1000 m	113	47	16	20
	2009 (2nd year)			
Oak forests < 600 m	120	91	52	49
Transitional belt 600 m-1000 m	138	71	45	43
Beech forests >1000 m	113	52	20	24
	2010 (3rd year)			
Oak forests < 600 m	120	90	46	44
Transitional belt 600 m-1000 m	138	72	46	44
Beech forests >1000 m	113	62	24	27

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Discussion

The specific patterns of succession of calcareous grasslands in different conditions and regions in Europe have been the subject of several recent studies (Alard et al., 2005; Schrautzer et al., 2009). Rutherford et al. (2011) have studied post-fire plant succession immediately after disturbance. The majority of the conclusions suggest that there is still much to be learned about how the conditions following a fire are reflected in the interactions between species in the early stages after a fire, and what impact they may have on later successional development.

Plants respond in two different ways to fire: some species survive fire and then resprout, while others are killed by fire, but germinate from fire-resistant seeds (Oliveira et al., 2015). The survivorship of plants after fire is dependent on their characteristics, the meteorological conditions and other disturbances such as grazing (Robberecht and Defossé, 1995; Peláez et al., 2009).

A previous study has shown that the highest values of diversity, exactly referring the most diverse vegetation in the highest zone, were followed by plots from the transitional zone at medium altitudes (Marković et al., 2015b). As the altitude increased, the diversity of fire non-affected vegetation increased. In the present study this trend was not noticed in the first year after the fire. In contrast, the greatest diversity was recorded at lower altitudes, while on plots at higher altitudes the diversity was lower. These findings are in accordance with the fact that more fertile soils have a stronger response to disturbance than other soils, which has been confirmed in previous investigations (Harrison et al., 2003; Grime, 2006). Limestone plots at lower altitudes have a deeper layer of soil than plots at higher altitudes. Short-term patterns of post-fire succession in the vegetation investigated were also dependent on exposure and slope, but to a much lesser extent in comparison to altitude.

The effects of the fire on plant communities were weaker in rockier habitats at higher altitudes where the fire moved faster and therefore lost its severity, giving a better chance to some of the plant forms to resist or to their seeds to survive in the sheltered places between the larger rocks of the fire impacted area.

In the study, the first post-fire year was characterized by moderately low diversity and more important role of annual species, in contrast to the wildfire non-affected vegetation. This was confirmed in a study of fire on limestone grasslands communities in Derbyshire (England) where the representation of short-lived species was higher in the burned plots than in those which were unburned (Lloyd, 1968). In the second year after the fire, a quantitative increase in the species richness and Simpson's index was recorded. The growth of species richness reflects a more intense process of species immigration from the surrounding preserved vegetation toward the burned plots. Hence, during the second year, the diversity of fire affected xeric vegetation on the mountain was improved, being more stable in most cases. The second post-fire year was characterized by an increase in the number of perennial plants. This trend continued in the third year after the fire when bunch grasses and other perennial species became dominant. According to Bennett et al. (2003) and Zedler (2007) grasses usually have capability to recover their full coenological and biological functionality, including reproduction, within one or few years after fire. As some recent studies have shown (Figueira et al., 2016; Wright and Fensham, 2018), the effects of fire on habitats can stimulates the production of inflorescences in some grass species, and hence support their faster spreading.

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The field surveys and observations show that the calcareous grassland vegetation in the study was very sensitive to the fire. Because of the removal of competition in burned areas, the importance of easy spreading plant species with short life cycles generally increased during the first period after the fire. Similar results were found by Ghermandi et al. (2004) and Papanastasis et al. (2002). According to our results the cover values of annual and biennial representatives increased due to the effects of the fire during the first two years. Consequently, the dominant species during the very first stage of vegetation formed after the fire were annual plants (*Bromus squarrosus, Filago montana*), and they were recorded in most of the plots, reaching high cover values in the overall vegetation. From the same group of plants, the annual (or sometimes biennial) species *Sideritis montana* was considerably important in the first, and especially second year after the fire, but was almost completely suppressed during the third year. This was plant especially abundant in waste, highly eroded and nutrient-poor, burned surfaces at higher altitudes. Since the cover value of the overall vegetation declines (caused by fire), annual species respond more rapidly and achieve greater cover values than perennial species in the first phase of its restoration. The same results were obtained in limestone Mediterranean mountain ecosystems in Greece (Papanastasis et al., 2002). At the same time, their further existence and cover values are strongly dependent on the degree of perennial species restoration. Namely, several perennial species were less affected by the fast-moving flame, and survived due to the presence of rhizomes, bulbs and similar subterranean organs or semifruticose aboveground sprouts. The production of a high number of new tillers in the early post-fire stages is one characteristic which increased the capability of restoring the perennial grass species (Ghermandi et al., 2004; Gittins et al., 2011; Gonzalez et al., 2015). As expected, sparse members of phanerophytes, as *Crataegus monogyna*, remained fire non-affected; while some of them (*Rosa canina*) additionally increased in the later period. Hence, perennial species like *Festuca valesiaca, Asperula cynanchica, Euphorbia cyparissias, Galium purpureum, Petrorhagia saxifraga, Teucrium chamaedrys* and *Satureja kitaibelii* were also important in the first stages, as they were minimally damaged by the fire. What's more, the cover values of dense caespitose grasses and semi-shrubs were high and stable all across the post-fire period. Their role in the stabilization of the floristic composition and restoration of the coenological relationships toward the initial communities in the period following the fire could be taken as important, as they were recorded in every season after the fire. Perennials rapidly achieved greater cover values, invading the destroyed habitats from the remnant patches of vegetation sheltered by the rocks, or by dispersion from the fire non-affected vegetation. During the final year of this study, they generally reached high phytocoenological importance, while annual species lost their role in the overall cover. A considerable number of perennials kept their cover values stable in the final two years, while some of them (e.g. *Festuca valesiaca, Euphorbia cyparissias, Teucrium chamaedrys, Centaurea stoebe, Sanguisorba minor*) increased their coverage. In that way the recovery stage of vegetation formed over the three years after the fire became more similar to the initial vegetation of calcareous grasslands on the mountain, which has been confirmed in other studies (Kucera and Enrenreich, 1962; Kucera and Koelling, 1964). This very quick floristic recovery after fire is also a characteristic of Mediterranean habitats (Caturla et al., 2000; Trabaud, 2003). In a wider sense, the limestone grasslands and rocky ground habitats on Vidlič Mountain have similar ecological characteristics to Mediterranean

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habitats as evidenced by the presence of large number of species with sub-Mediterranean, or even Mediterranean chorological character (Marković et al., 2015b).

In contrast, our results of Sorensen's coefficient of similarity indicate that the floristic composition of the unburned plots is not very similar to the floristic composition of the burned plots. The biggest differences were shown by plots at the highest altitudes, whereby the coefficient of floristic similarity was 20-27% in the first three years.

Such low floristic similarity can be explained by the geographic distance between the unburned plots and the burned ones. The species composition in grasslands depends much more on the surrounding vegetation than some other habitats such as scrubland (Chust et al., 2006). Community composition was enriched by new fugitive species which increased diversity in a quantitative sense but the floristic composition lost the quality it had before the fire. Similar results were obtained by Mitchell et al. (2009).

Conclusion

Having in mind species diversity, calcareous grasslands are of great importance for the conservation of biodiversity in Europe. The results for species richness and diversity after large fires in southeastern Serbia confirm that limestone grasses and rocky ground habitats have high resilience to fire. The trend was noticed that species richness and diversity are higher on lower altitudes. On the other hand, the result of Sorensen's coefficient of similarity indicates that the floristic composition of limestone grasses and rocky ground vegetation was changed and showed slow recovery in the stage after burning. The analyses of life form dynamics showed that the percent of annual plants rapidly increased in the first stage of revitalization. In the second and further stages the proportion of therophytes gradually decreased compared to hemicryptophytes. Additional studies in the future should be carried out on the changes in community composition.

Dry grasslands and other types of vegetation formed on limestone, rocky ground areas in Serbia and Balkan Peninsula are important habitats for endemic and some medicinal, especially aromatic plants. Therefore, results on the impact of uncontrolled wildfire on xeric vegetation on limestone terrains would have general application in wildfire management, as well as in nature conservation.

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POST-FIRE FUEL AND VEGETATION DYNAMICS IN AN UNGRAZED PHRYGANIC COMMUNITY OF CRETE, GREECE

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Abstract. Phrygana is a widespread vegetat type in the eastern Mediterranean, mainly represented by *Sarcopoteriwn spinosum* dominated formations in the Aegean islands and Crete. The effects of overgrazing and frequent burnings on phrygana are relatively known by scientists, but limited attention has been paid to the functioning of these communities when grazing and fires are suppressed. The aim of this study is to investigate post-fire vegetation and fuel dynamics in an ungrazed *S. spinosium* dominated phrygana in northwestern Crete. Fuel was first analyzed according to shrub, herb, litter, and total load; then live and dead materials; and finally, 0-0.5 cm and 0.6-2.5 cm of leaf diameter classes. Vegetation was analyzed based on cover and life form composition and dynamics. Also, species richness, diversity, and dynamics were investigated. One-way ANOVA was used to test for fuel and vegetation differences among sites, and Shannon-Wiener and Equitability indices were used to assess both diversity and evenness. Results showed a rapid reestablishment of both the vegetation (cover, life form, species richness and diversity) and fuel, as early as the 3rd and the 6th post-fire year, respectively. Fuel and vegetation were tremendously dominated by unpalatable and highly flammable dwarf shrubs, which smothered herbaceous plants. Species diversity was relatively low, indicating that few species crushingly structured the community by their high cover, while the remaining species were poorly abundant. The implications of the results in the context of a rational management planning of phryganic ecosystems are discussed.
Keywords: *biomass, Phrygana, post-fire dynamics, species diversity, species richness, vegetation Cover*

Introduction

Many, if not all, ecosystems of the world are known to undergo cyclic processes of disturbances and recovery, occurring at specific spatiotemporal scales. These natural phenomena allow ecosystems to maintain their ecological processes as well as their biological and ecological diversity in space and time (Naveh, 1994).

At least since the Jurassic geological time, fires had always been a natural selective force which affected plant community structure, productivity, and species composition (Le Houerou, 1974; Trabaud, 1994, 2000). In addition, fires have been associated with species diversity maintenance (Christensen, 1985), have contributed to stands rejuvenation (Trabaud, 2000), and have allowed species coexistence in space and time, by interrupting competitive advantage of dominant species (Malanson, 1987).

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Mediterranean forests and shrublands exemplify the abovementioned ecological systems that cope with natural disturbances such as natural fires' and grazing by wildlife.

As far as post-fire plant demography in phrygana is concerned, documented a great deal of dwarf shrubs' seed germination and seedling recruitment during the first post-fire year, contrasting with the unburned phrygana (Arianoutsou, 1998). By far, the dominant seedlings were made up of *Cistus* spp. and *Sarcopoterium spinosum* (with numbers multiplied by 10 and 20 times). On the contrary, a low seed germination level portrayed dwarf shrub in the 2nd post-fire year; 10 seeds per m^2 for *Cistus* spp. and 3 seeds per m^2 for *S. spinosum* were counted in winter. A similar demographic trend has also been reported for other phryganic communities by Naveh (1974), Papanastasis (1977), and Arianoutsou (1998), that is, a decrease in a number of dwarf shrubs' seedlings during the first post-fire year, and no conspicuous seed germination during the 2nd post-fire year. It is worthwhile referring that two main hypotheses have been suggested to explain the decrease in seedling density. The negative effects of the hot and dry Mediterranean summer on dwarf shrub seedlings' survival (Ferran et al., 1998) or the strong inhibiting competition exerted by the massive herbaceous plants on the young dwarf shrubs' recruits (Henkin et al., 1999; Seligman and Henkin, 2000).

Regarding to the post-fire fuel/biomass dynamics, there is insufficient research has been devoted to ecosystem structure and post-fire fuel dynamics in phryganic ecosystems. In Greece, the little literature available on the subject focused mainly on aboveground net primary production, whereas fuel and fire hazard-oriented studies are almost lacking. It is worthwhile mentioning that Christensen (1985) also highlighted the substantial contribution of herbaceous plants to shrubland production in immediate post-fire years.

To our knowledge, Papanastasis (1980) have provided the scanty, but useful, information available on fuel characteristics in Greek phrygana in relation to fire hazard. Thereby, the latter author mentioned that phryganic ecosystem are inclined to accumulate dead material as they age; thus, becoming decadent if unburned. This characteristic coupled with small sized material featuring phryganic dwarf shrubs contribute to render the older communities highly flammable (Papanastasis, 1977). However, as reported by Papanastasis (1980) in an experimental study, *Sarcopoterium spinosum* plants did not bum easily unless enough dead fuel had existed on them. This latter fuel required, at least, three post-fire years to be accumulated. Additionally, it has been indicated that herbaceous plants occurring in phryganic formations are also fire-adapted. Indeed, some species, such as *Andropogon hirtus*, a perennial grass that retains its old growth, contribute to the flammability of the phrygana (Papanastasis, 1977). Finally, fuel distribution has been documented to be uniform only on old and thick phrygana with no or few herbaceous plants. Most often, fuel is discontinuously distributed in grazed communities, made up of dwarf shrubs and herbaceous plants fairly intermingled (Papanastasis, 1977, 2000).

Studies have reported that both high ecosystem diversity and landscape maintenance in the Mediterranean have been promoted and ensured by the cyclic disturbances properly induced by humans and their livestock (Naveh, 1994; Naveh and Whittaker, 1980, 2007). However, improper land use such as wildfires and overgrazing for decades has significantly altered the ecosystems, and more particularly rangelands, such as phrygana in the eastern Mediterranean. Therefore, the objectives of the current research are to investigate post-fire fuel and vegetation dynamics in an ungrazed *Sarcopoterium*

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spinosum dominated phryganic community in northwestern Crete (Greece) for a relatively short period; that is, between 3 and 12 years.

Materials and Method

Study area general description

The study area is represented by the hills of Malaxa situated in northwestern Crete (*Figure 1*). This part of the island is administratively managed by the Prefecture of Chania, and more specifically by the municipality of Souda. The climate of Chania is typically the Mediterranean, characterized by a long dry summer season with a monotone wind regime, and precipitations ranging from September to May (Elhag and Bahrawi, 2016). The recent climatic data of Chania, for the last 20-year-period, indicated an annual precipitation averaging 690.4 mm. The Bagnouls-Gaussen ombrothermic diagram, drawn from the abovementioned data displays a longer dry summer period than mentioned by Elhag and Bahrawi (2016), beginning in April and ending in mid-September. The rainy period, ranging from October to March is characterized by an average precipitation of 615.65 mm, and the xerothermic period, extending from April to September, reaches 74.75 mm only. It is worth noting that January and February are the coldest months, with an average temperature of 10.6 °C, and July the hottest month with an average of 24.7 °C. Based on Emberger's pluviothermic quotient value (Q=86) calculated for Chania, Elhag and Bahrawi (2018) classified this region in the subhumid Mediterranean bioclimate. The differences among the designated study sites were evaluated based on its soil chemical properties (*Table 1*).



Figure 1. *Study area vegetation cover located at Malaxa, Crete*

Table 1. *Chemical properties of soil samples taken from the four sites*

	Soil Chemical Properties							
	pH	**Org. Matt.***	**N***	**P****	**Mg****	**Ca****	**K****	**Na****
Site 1 [3 years]	6.51	5.12	0.35	0.81	2.19	21.25	0.25	0.22
Site 2 [6 years]	6.36	5.87	0.38	0.82	2.65	19.85	0.27	0.21
Site 3 [9 years]	6.21	6.61	0.41	0.82	3.11	17.25	0.31	0.22
Site 4 [12 years]	6.16	6.96	0.44	0.81	3.64	15.74	0.33	0.23

* (%), **(mg/100 g soil)

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Sampling techniques and procedures

Fuel sampling and procedural steps

Sampling location was randomly selected in the central part of a physiognomically homogeneous vegetation, and line-transects were run in four directions from a central point. Each line-transect was 15 m long and one plot was taken at the end. The total number of plots per site was four. In each 1 m^2-plot, all the aboveground biomass was clipped and put in a plastic bag. As regards the laboratory work, the first step consisted in fuel separation to shrub, herb, and litter. Shrub was then divided to live and dead parts. It is noteworthy that live material included green leaves and fresh twigs, whereas dead material all the parts devoid of living tissues. Afterward, live and dead materials were separated into two leaf diameter classes: 0-0.5 cm and 0.6-2.5 cm. Finally, the fuel was oven dried at a temperature of 105° C for 24 hours, then weighted with a digital balance according to the categories.

Vegetation sampling and procedural steps

Vegetation sampling was carried out in the central part of a physiognomically homogeneous vegetation to ensure samples' representativeness and to avoid any edge effects. In each sampling location, four line-transects were run from a central point. Following the scheme used in fuel sampling, but transect length was 20 m. Cover values of plants, litter, and bare ground were then recorded using Line Transect and Point Intercept methods combined (Bonham, 1995); a 20 m measuring tape was stretched between two stakes along the line-transect, and a pin was dropped perpendicularly to the tape each 20 cm interval. Observations were recorded by reading the hits. The laboratory work was conducted in a herbarium: plant specimens were identified with the help of a stereoscope and the descriptive keys of Flora Europaea referential volumes (Tutin et al., 1980).

Analytical procedures and statistical treatments

Fuel analysis

Fuel was analyzed according to the several categorizations based on the fuel separation to shrub, herb, and litter, fuel separation to live, dead materials, and fuel separation to diameter classes. Post-fire changes in fuel composition and dynamics were investigated at the abovementioned three category levels. Analysis of variance (one-way ANOVA) was used to test for fuel load differences among sites, and Duncan multiple range tests for mean pair's comparisons.

Vegetation composition

Vegetation composition was analyzed based on shrub and herbaceous covers. In each site, both percent cover and percent cover composition were calculated according to the following two formulae of Bonham (1995):

1. Percent cover = (number of point intercepts / total points) x 100.
2. Percent cover composition = (number of point intercepts / total point intercepts of vegetation) x 100.

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Floristic composition

Floristic composition was analyzed based on life form richness. Following are the different life form groups (Kent, 2011):

- Phanerophytes: species with perennating buds emerging from aerial parts of the plant.
- Chamaephytes: species with perennating buds borne on aerial parts close to the ground (below 2 m).
- Geophytes: species with buds or shoot apices, which survive the unfavorable period below ground.
- Hemicryptophytes: all aboveground parts of the plant die back in unfavorable conditions, and buds are borne at ground level.
- Therophytes: plants that survive the unfavorable period as seeds. Species are annuals and complete their life cycle from seed to seed in the favorable summer months.

Species richness

Post-fire species richness dynamics was described using the following two ecological parameters (Blondel and Aronson, 1995; Magurran, 2013):

- A number of species per site, which combines the four lines-transects.
- Species richness per site, which is the sum of the total richness recorded in each line-transect of that site divided by the total number of line-transects (n = 4).

Species diversity

Post-fire species diversity dynamics was investigated using both heterogeneity and equitability (also referred as evenness) indices. Species diversity was calculated using the Shannon-Wiener heterogeneity index (H') which is based on the Information Theory (Naveh and Whittaker, 1980, 2007; Magurran, 2013). The formula is the following:

$$H' = -\sum_{i=1}^{s}(P_i)(log_e P_i) \quad \text{(Eq.1)}$$

where:
H' = the Information content of sample (nits/individual),
s = Number of species,
Pi = Abundance of the i^{th} species expressed as a proportion of total cover.

Equitability which refers to the pattern of distribution of individuals among species was calculated using the following index proposed by (Kent, 2011) based on Shannon-Wiener diversity index:

$$J = H'/H'_{max} \quad \text{(Eq.2)}$$

where:
J = the Equitability index,
H' = Shannon-Wiener diversity index,
$H'_{max} = \log_e (s)$ = Maximum possible value of H',
s = Number of species.

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The equitability index ranges from 0 to 1. It tends towards 0 when almost all abundances correspond to one species of the community, and towards 1 when each species is represented by the same abundance.

Species dynamics

Species dynamics was analyzed because of their cover changes throughout the different post-fire stages. The following community's characteristics were particularly brought out:

- The dominant species structuring the vegetation by its abundant cover.
- The diagnostic species of the community by their constant presence whatever fire interval considered.

Results

Post-fire fuel dynamics

In all sites, both shrub and litter accounted for about 97% of the total fuel composition. By far, shrub's contribution was the most important (about 80%). The herbaceous vegetation (about 3%) represented the remaining fuel. In addition, shrub, litter, and total fuel loads differed significantly (p=0.003, p=0.034, and p=0.002, respectively) among the sites, while herbaceous load did not (p=0.508) as it shown in *Table 2*.

Table 2. *Shrub, herb, litter, and total fuel loads (oven-dry weight) measured at four sites with different fire intervals (kg/ha)*

Site N [Fire interval]	Shrub			Herb	Litter	Total fuel
	Live	Dead	Total			
Site 1 [3 years]	3092.3^{a}	835.1^{a}	3927.4^{a} (84.0%)	209.2 (4.5%)	537.3^{a} (11.5%)	4673.9^{a} (100%)
Site 2 [6 years]	6097.5^{C}	6191.l^{b}	12288.6^{b} (83.7%)	459.7 (3.1%)	1928.7^{ab} (13.2%)	14677.0^{b} (100%)
Site 3 [9 years]	5044.3^{bc}	7284.9^{b}	12329.2^{b} (77.2%)	360.6 (2.2%)	3286.0^{b} (20.6%)	15975.8^{b} (100%)
Site 4 [12 years]	3986.4^{ab}	7380.6^{b}	11367.0^{b} (76.5%)	502.9 (3.4%)	2979.2^{b} (20.1%)	14849.l^{b} (100%)

Percentage of total fuel load between parentheses. Comparisons among sites performed by conducting one-way ANOVA and Duncan's Multiple Range Test, both at the p=0.05 level of significance. Values followed by the same letter are not statistically different

The results showed that shrub composed the bulk of the fuel between 3 and 12 years after fire. It is worth noting that a substantial amount of litter was accumulated as early as the 3rd post-fire year, whereas herbaceous biomass was lowly produced. Furthermore, a threefold amount of both shrubs and total fuel (about 12000 kg/ha and 15000 kg/ha, respectively) were noticed six years after fire, and a six-fold amount of litter (about 3000 kg/ha) nine years after fire, as compared to the amounts produced at the 3rd post-fire year. On the other hand, the statistical analyses suggested a stabilization of shrub, litter, and total fuel loads since the 6th post-fire year onwards.

Live and dead shrub fuel loads measured at the four sites with different fire intervals are presented in *Table 3*. The highest live material's contribution (about 80% of shrub

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fuel composition) was noted in the 3rd post-fire year. On the contrary, the dead material was found highly accumulated (about 65% of shrub fuel composition) in the 12nd post-fire year. One must notice the similar proportions of both live and dead materials in the 6th post-fire year. Additionally, both live and dead fuel loads were found differing significantly (p=0.038 and p=0.002, respectively) among the sites and the following differences were highlighted in the multiple comparison tests (Elhag et al., 2017):

- Site 1 had significantly lower live fuel load (3092.3 kg/ha) than site 2, characterized by almost double biomass (6097.5 kg/ha). Otherwise, similar loads characterized the three oldest sites.
- Site 1 had significantly lower dead fuel load (835.1 kg/ha) than site 2, site 3, and site 4, characterized by similar high loads (about 7000 kg/ha).

Table 3. *Live and dead shrub fuel loads (oven-dry weight) measured at the four sites with different fire intervals (kg/ha)*

Site N [Fire interval]	Live fuel	Dead fuel	Total fuel
Site 1 [3 years]	3092.3^{a} (78.7%)	835.1^{a} (21.3%)	3927.4^{a} (100%)
Site 2 [6 years]	6097.5^{c} (49.6%)	6191.1^{b} (50.4%)	12288.6^{b} (100%)
Site 3 [9 years]	5044.3b^{c} (40.9%)	7284.9^{b} (59.1%)	12329.2^{b} (100%)
Site 4 [12 years]	3986.4^{ab} (35.1%)	7380.6^{b} (64.9%)	11367.0^{b} (100%)

Percentage of live and dead fuel load between parentheses. Comparisons among sites performed by conducting one-way ANOVA and Duncan's Multiple Range Test, both at the p=0.05 level of significance. Values followed by the same letter are not statistically different

The results show that live material overwhelmingly dominated shrub fuel composition in the 3rd post-fire year, while the dead material was not substantially accumulated yet. On the other hand, a dead material composed the fuel bulk in the oldest post-fire stage (Elhag et al., 2017). Furthermore, the statistical analyses suggested that both live and dead fuel loads stabilized since the 6th post-fire year onwards, even though the following dynamic trends were noted:

- Live fuel load decrease since the 6th post-fire year onwards.
- Dead fuel load increase as early as the 3rd post-fire year onwards.

In all the sites, live fuel was almost entirely made up of material in the 0-0.5 cm diameter class. The latter size class material also dominated dead fuel composition (about 89%), while larger fuel in the 0.6-2.5 cm diameter class was poorly represented (about 11%). Additionally, live and dead fuel loads in the 0-0.5 cm diameter class differed significantly (p=0.046 and p=0.001, respectively) among the sites. On the contrary, dead fuel load in the 0.6-2.5 cm diameter class did not (p=0.143) as it shown in *Table 4*. Multiple comparison tests showed the following differences among the sites:

- Site 1 had significantly lower live fuel load in the 0-0.5 cm diameter class (3092.3 kg/ha) than site 2 characterized by a nearly twofold production (5832.5 kg/ha). Otherwise, similar loads characterized the three oldest sites.
- Site 1 had significantly lower dead fuel load in the 0-0.5 cm diameter class (796.9 kg/ha) than site 2, site 3, and site 4, characterized by similar high loads (about 6000 kg/ha).

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Table 4. *Live and dead shrub fuel loads (oven-dry weight) separated to diameter classes measured at the four sites with different fire intervals (kg/ha)*

Site N [Fire interval]	Live fuel			Dead fuel		
	D[0-0.5]	D[0.6-2.5]	Total	D[0-0.5]	D[0.6-2.5]	Total
Site 1 [3 years]	3092.3[a] (100%)	--	3092.3[a] (100%)	796.9[a] (95.4%)	38.2 (4:6%)	835.1[a] (100%)
Site 2 [6 years]	5832.5[b] (95.7%)	265.0 (4.3%)	6097.5[b] (100%)	5840.2[b] (94.3%)	350.9 (5.7%)	6191.1[b] (100%)
Site 3 [9 years]	5044.3[b] (100%)	--	5044.3" (100%)	6356.6[b] (87.3%)	928.3 (12.7%)	7284.9[b] (100%)
Site 4 [12 years]	3986.4[b] (100%)	--	3986.4[b] (100%)	5742.2[b] (77.8%)	1638.4 (22.2%)	7380.6[b] (100%)

D: diameter class (cm)
Percentage of fuel load between parentheses. Comparisons among sites performed by conducting one-way ANOVA and Duncan's Multiple Range Test, both at the p=0.05 level of significance. Values followed by the same letter are not statistically different

Post-fire vegetation dynamics

In all sites, shrubs composed about 73% of the vegetation cover, whereas herbs and grasses contributed to about 27% only. With respect to the herbaceous component, it is worth noting that the most contributing species to total vegetation was the perennial grass *Brachypodium retusum* with an average cover amounting to about 3%. Additionally, both shrub and herbaceous covers did not differ significantly (p=0.269 and p=0.087, respectively) among the sites. The above results show that shrubs overwhelmingly structured the vegetation, whereas herbaceous contribution was not substantial. Overall vegetation cover stabilization was also detected as early as the 3rd post-fire year onwards (Elhag et al., 2017).

114 species belonging to 23 families were recorded in the community using the line transect and point intercept methods combined. The floristic composition based on life form richness in the four sites with different fire intervals is summarized in *Table 5*.

Table 5. *Life form richness of the four sites with different fire intervals*

Life forms	Site 1 [3 years]	Site 2 [6 years]	Site 3 [9 years]	Site 4 [12 years]
Phanerophytes	4 (9.8%)	3 (5.4%)	4 (6.6%)	4 (5.9%)
Chamaephytes	5 (12.2%)	7 (12.5%)	8 (13.1%)	7 (10.3%)
Geophytes	3 (7.3%)	2 (3.6%)	3 (4.9%)	2 (2.9%)
Hemicryptophytes	11 (26.8%)	19 (33.9%)	14 (22.9%)	17 (25.0%)
Therophytes	18 (43.9%)	25 (44.6%)	32 (52.5%)	38 (55.9%)
Total	41 (100%)	56 (100%)	61 (100%)	68 (100%)

Percentage of total life form groups for each site is given between parentheses

In all the sites, therophytes and hemicryptophytes were the most dominant life forms, both contributing to about 76% of the floristic composition. It is worthwhile mentioning that therophytes were, by far, the most represented life form (about 49%), followed by hemicryptophytes (about 27%). The remaining forms, in decreasing richness, were the chamaephytes (about 12%), phanerophytes (about 7%), and geophytes (about 5%).

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The hemicryptophyte richness differed significantly (p=0.002) among the sites, whereas the other life forms did not (p=0.208, p=0.144, p=0.554, and p=0.255 for phanerophytes, chamaephytes, geophytes, and therophytes, respectively.

Multiple comparison tests showed the following differences among sites as regards hemicryptophyte richness:

- Site 2 had significantly higher mean richness than site 1, site 3, and site 4.
- Site 3 and site 4 were characterized by similar mean richness, lower than site 2.

In other respects, the statistical analyses revealed that species richness of phanerophytes, chamaephytes, geophytes, and therophytes did not differ significantly in all the studied post-fire stages, suggesting a stabilization process as early as the 3rd post-fire year onwards. On the other hand, it was noted a substantial increase of hemicryptophyte richness until the 6th post-fire year, and a decrease thereafter. The similar richness values obtained for the latter life form in the two oldest post-fire stages seemed to indicate a stabilization process since the 9th post-fire-year onwards. However, the study of a longer chronosequence is needed to confirm this latter suggestion (Elhag et al., 2017).

In *Table 6*, the number of species increased from site 1 (S=44) to site 4 (S=74). On the contrary, species richness was similar (s comprised between 16.75 and 22.75): no significant differences were detected among sites (p=0.075). Based on the latter ecological parameter which allows a subtle appreciation of the differences among sites, the results indicated that in all sites, an average of 21 species was collected in the covered transects. This average species number can be considered as representative of the community in each site (Blondel and Aronson, 1995; Magurran, 2013). Moreover, the species richness assigns to each species a weight that is proportional to its appearance probability along the sequence of transects; consequently, the less abundant species will be attributed a small weight (Blondel and Aronson, 1995; Magurran, 2013). On the other hand, it was noted that 70.4%, 70.2%, 85.1%, and 85.1% of the species occurring in site l, site 2, site 3, and site 4, respectively, were recorded in one transect only and with a minimal cover value.

Table 6. *A number of species and richness in the four sites with different fire intervals*

Site N [Fire interval]	**Site 1 [3 years]**	**Site 2 [6 years]**	**Site 3 [9 years]**	**Site 4 [12 years**
Number of species	44	57	67	74
Species richness	16.75 (±1.25)	21.50 (±0.29)	22.75 (±1.25)	22.50 (±2.72)

Standard error between parentheses

The values of heterogeneity index which were relatively low (less than 2.5) as compared to those usually obtained in more diversified communities (3.5). (Washington, 1984; Kent, 2011), suggested that in the four post-fire stages, the study phrygana was characterized by a relatively low diversity (*Table 7*). In other words, the environmental conditions prevailing in the community tended to be slightly unfavorable, allowing the settlement of a small number of species which were relatively abundant (Cowling et al., 1996). On the other hand, the values of mean equitability index (which did not exceed 0.7) suggested that in the covered transects, an appreciable number of species had a more or less similar importance, but few others were more abundant (Cowling et al., 1996).

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The description of plant species dynamics suggests that three main characteristics portray the ungrazed phryganic community between 3 and 12 years after fire:

- One overwhelmingly dominant dwarf shrub species, *Sarcopoterium spinosum*, chiefly structuring the vegetation by its abundant cover.
- Less represented species competing actively to occupy a position and coexisting with other species in the community. Their covers increase, decrease, or stabilize, depending on the ecological conditions prevailing in each post-fire dynamic stage.
- Several species present with a minimal cover, in all likelihood due to the strong competition pressures they undergo from the dominant taxa.

Table 7. *Shannon-Wiener heterogeneity and equitability indices in the four sites with different fire intervals*

Site N [Fire interval]	Site 1 [3 years]	Site 2 [6 years]	Site 3 [9 years]	Site 4 [12 years
Heterogeneity	1.867 (±0.21)	1.843 (±0.10)	2.177 (±0.06)	1.963 (±0.12)
Equitability	0.660 (±0.06)	0.601 (±0.03)	0.698 (±0.01)	0.634 (±0.02)

Standard error between parentheses

Within the frame of the studied chronosequence, *Table 8* outlines the plant species dynamics as follow:

Table 8. *Plant species dynamic in the four experimental sites*

	Site 1 (3 years)	Site 2 (6 years)	Site 3 (9 years)	Site 4 (12 years
Asparagus aphyllus	-	*	-	-
Asphodelus aestivus	**	**	-	-
Atractylis gummifera	**	*	-	*
Blackstonia perfoliata	*	*	-	-
Brachypodium retusum	**	**	*	*
Calicotome villosa	-	-	**	-
Calicotome villosa	-	-	-	**
Cistus creticus	***	***	***	***
Dactylis glomerata	-	*	*	**
Drimia maritime	-	*	-	*
Gastridium ventricosum	**	**	-	-
Hypericum empetrifolium	-	-	**	**
Knautia integrifolia	-	*	*	-
Knautia integrifolia	-	-	-	*
Linum corymbulosum,	*	*	*	*
Lotus angustissimus	**	-	-	-
Ononis spinosa	-	*	-	-
Origanum vulgare	-	-	-	*
Pidicaria dysenterica	*	*	-	-
Piptatherum miliaceum	-	-	-	**
Pulicaria dysenterica	-	-	*	*
Rhamnus lycioides	*	-	-	-
Sarcopoterium spinosum	***	***	***	***
Satureja thymbra	**	**	-	-
Tolpis virgata	-	*	-	-
Trifolium campestre	**	**	*	*
Trifolium physodes	-	*	-	-

*** is a dominant species, ** is a less abundant species, * is a poor abundant species

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Discussion

During the post-fire dynamic process, the fuel accumulated in the community was chiefly issued from shrubs, mainly made up of *Sarcopoterium spinosum* and *Cistus creticus*, and litter. Litter was enough accumulated as early as the 3rd post- fire year. In comparison with the results of Arianoutsou-Faraggitaki (1984) in a phrygana of Attica, there is an agreement about shrub dominance as soon as the 3rd post-fire year onwards. However, the community of Malaxa had almost double the shrub fuel load (3927.4 kg/ha vs. 1950.0 kg/ha) three years after the fire. Additionally, a total fuel load of the Malaxa phrygana in comparison with a *Sarcopoterium spinosum* dominated phrygana in Thessaloniki showed that the former had almost double fuel load (4673.9 kg/ha) than the latter (2800.0 kg/ha). Thus, the Malaxa phryganic community is characterized by a higher fuel production than the two other communities. These differences in biomass production may be attributed to differences in the prevailing environmental conditions or to the management practices (e.g., grazing), or even both.

It is important to highlight that the fuel characteristics favor particularly the high flammability of the vegetation. It seems clear that the high contributions of shrub and litter to the total fuel facilitate the spreading of any potential fire. The statement is based on (Specht et al., 2012) who reported that in shrub-dominated vegetation, the primary carrier of the fire is either shrubs or litter. Additionally, dead material accumulation with the age of the community is the expressed by an increase of the dead-to-live fuel ratio, leading inevitably to an increase of the likelihood of fire and its potential intensity (Henkin et al., 1999; Seligman and Henkin, 2000; Paula et al., 2009). On the other hand, small sized fuel featuring phryganic dwarf shrubs, like *Sarcopoterium spinosum* and *Cistus creticus*, and its substantial accumulation with the age since the last fire, increase the vegetation flammability. According to Specht et al. (2012), small diameter fuel dries out and ignites more rapidly than larger fuel because of its large surface area compared to its volume. Consequently, fire most often starts and spreads in fine fuel.

Regarding the vegetation composition, it was noted that in Malaxa community shrubs contributed the bulk of the vegetation cover, while herbaceous plants were not abundant. This confirms previous observations about the encroachment potential of shrubs after a transitional competition by herbaceous plants during the first two post-fire years (Le Houerou, 1974; Naveh, 1974; Papanastasis, 1977; Arianoutsou-Faraggitaki, 1984; Keeley and Babr-Keeley, 1999). Shrub cover dominance in other Mediterranean-type shrublands has been exemplified by Ferran et al. (1998) who reported 76% of the total cover chiefly dominated by *Quercus coccifera* in garrigues of Valencia, three-and-a-half years after fire passage.

As far as the floristic composition is concerned, it was remarked that therophytes and hemicryptophytes were the dominant life forms (in terms of species number) in the studied community, with a noticeable advantage of the therophytes. Within the mentioned life form groups, by far, herbs prevailed over grasses. The comparison between the biological spectra of the studied phrygana and the grazed *Sarcopoterium spinosum* dominated batha of Ariel investigated by Ish-Shalom-Gordon (1993) shows that therophytes are, by far, the most represented life form with 49% and 56%, respectively. However, the two communities differed regarding the second highest represented life form; hemicryptophytes (27%) in Malaxa as against chamaephytes (15%; referred as bush by Ish-Shalom-Gordon) in Ariel. Geophytes were better represented in Ariel (12%) than in Malaxa (5%).

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Furthermore, therophytes have been reported to be the most represented life form in other phryganic communities, but covering small areas (Bergmeier and Matthäs, 1996; Böhling and Gerold, 1995; Bergmeier, 1998; Ish-Shalom-Gordon, 1993) raised the possibility that the high annual therophytes richness portraying phryganic communities is favored by the occurrence of relatively predictable seasonal rainfall, but (Chilton and Turland, 2004) contended that the mentioned richness is the result of relatively rapid evolution under stress induced by drought, fire, and grazing. One has to note that due to their shallow-rooted system, therophytes depend on water supply in the upper soil layer to carry out their germination, growth, and reproduction (Bergmeier and Matthäs, 1996).

With respect to the floristic composition, the results obtained in Malaxa community are in agreement with those of Ish-Shalom-Gordon (1993) who recorded the same three dominant families in Ariel hatha: that is, Asteraceae, Fabaceae, and Poaceae. However, the two communities differed as regards the relative importance of the families: in Malaxa, the most represented family was the Fabaceae (22.8%), followed by the Poaceae (21.9%) and the Asteraceae (14.9%). On the contrary, in Ariel, the dominant family was the Asteraceae (16.6%), followed by the Fabaceae (14.6%) and the Poaceae (8.0%). The lower representation of Poaceae and Fabaceae in Ariel batha might be the fact of the higher grazing pressure (by goats and deers).

The richness values recorded in the studied phrygana cannot be fairly compared with the results obtained by other authors like Ish-Shalom-Gordon (1993) and (Bergmeier, 1997) who also investigated species richness in phrygana owing to the different methods used for data collection, and the management- types. For information, Ish-Shalom-Gordon (1993) found a total of 199 species in a grazed community of Ariel (Israel), using a 100 x 100 m plot; and Bergmeier (1997) counted a total of 91 and 86 species (and an average species number of 15.3 and 19.5) in grazed and non-grazed 20 x 30 microplots, respectively, in a phrygana of Sfakia (Crete). Nonetheless, the use of species richness as well as diversity indices provided a good insight into the organization of the community and the number of the more representative species at each post-fire stage.

The final interesting finding of the study is the similar stable species richness, heterogeneity, and equitability characterizing the community of Malaxa as early as the 3rd post-fire year onwards. These results are in line with other studies which indicated, that burned Mediterranean plant communities reestablish rapidly by auto succession process (Naveh, 1974; Trabaud and Lepart, 1980; Christensen, 1985; Trabaud, 1994; Arianoutsou, 1998; Henkin et al., 1999; Allen, 2014). Similar conclusions have also been drawn for Califomian chaparrals (Trabaud and Lepart, 1980). However, the relatively low diversity characterizing the community of Malaxa, as compared to other more diversified phrygana, is in all likelihood due to both fire suppression and non-grazing.

Conclusions

The unburned and ungrazed phrygana is characterized by a rapid reestablishment of both vegetation cover and overall aboveground biomass. These two latter's are overwhelmingly dominated by highly flammable dwarf shrubs, chiefly *Sarcopoterium spinosum*, in the case at hand. The cover tends to close and stabilize as early as the 3rd post-fire year with little or no risk of soil erosion, whereas the overall aboveground

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biomass needs a bit longer time to do so: that is, at the 6th post-fire year. In such a community, the herbaceous component is not substantially represented with respect to cover and aerial biomass (excepting during the first two post-fire years). This may be ascribed to the combination of both the high inhibiting competition pressure exerted by shrubs and the accumulation of litter, which restrict the spreading and the development of herbaceous plants to few openings which are temporarily free from competition.

Furthermore, the rapid reestablishment and stabilization of the representative species as well as the overall plant diversity as early as the 3rd post-fire year is in keeping with the general pattern of auto-succession and resilience characterizing Mediterranean-type communities which undergo cyclic fires. Phryganic communities recover faster than more advanced Mediterranean type shrublands. However, when the phrygana is ungrazed and unburned for a period, say 12 years as the community of Malaxa exemplifies it, the overall plant diversity becomes relatively low, probably after reaching its highest values during the first two post-fire years. Such a community may be characterized by a "dictatorial-type" organization (in which few species (chiefly dwarf shrubs in the present case), dominate by their abundances and broaden their niches to the detriment of the remaining species which are poorly represented.

In other respects, the conservation of the traditional practices, such as rotational burning and grazing, respectful of the diversity and the functioning of phryganic ecosystems may have positive socioeconomic repercussions. As previously, seen, a non-grazing situation associated with fire suppression for a period, both favor a rough encroachment of unpalatable dwarf shrubs such as *Sarcopoterium spinosum* and *Cistus creticus*, which heavily smother the herbaceous plants.

These are mainly annual legumes and grasses of high palatability and nutritive values. Consequently, the amount of pasture is reduced, and livestock production is lessened, affecting negatively the economy at both local and national levels. To optimize the benefits that can be gained from phryganic communities, both grazing and fire should be incorporated in the management plan, as pointed out by. To be beneficial, grazing management should observe a stocking rate equivalent to the grazing capacity of the rangeland, combine grazer and browser animals for a better control of plants, and respect the season and duration of grazing. Also, prescribed or controlled fires improve the grazing capacity of phryganic communities.

Tourism, as an additional source of income, can also be promoted by maintaining the biological and landscape diversity and attractiveness made up of mosaics of phrygana, maquis, and forests.

Post fire disturbances within the four study sites led to unpalatable and highly flammable dwarf shrubs, which smothered herbaceous plants. Species diversity was relatively low, indicating that few species crushingly structured the community by their high cover, while the remaining species were poorly abundant.

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DETERMINING OF PLASMA BIOCHEMICAL PARAMETERS ACCORDING TO DIFFERENT REPRODUCTIVE PERIODS IN THE POPULATION OF *Testudo graeca* (ÇANAKKALE, TURKEY)

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Abstract. In this study, some biochemical parameters (glucose, urea, calcium, triglyceride, cholesterol, creatinine, uric acid, total protein, iron, albumin, phosphorus, H.D.L., magnesium) hemoglobin and hematocrit depending on the pre-reproductive and reproductive periods and sex were determined in a total of 20 *Testudo graeca* specimens in the Çanakkale (Turkey). For the biochemical parameters, 0,5 ml of blood was collected from the scale in the lateral ligament of the neck with a 5-ml aspirating syringe. The blood was transferred into the biochemical tubes and centrifuged; the plasma part was separated and was measured by spectrophotometer. In conclusion, plasma biochemistry parameters were determined on sex-related pre-reproduction and reproduction periods in *T. graeca*. When comparing the pre-reproductive and the reproductive period in terms of glucose, uric acid and iron values, statistical differences were found in the females. It was determined that cholesterol values of males were significantly different between periods. In the females, glucose value was found high in the pre-reproductive period and uric acid and iron values were high in the reproductive period. Cholesterol values of males were found high in the pre-reproductive period. Iron levels in the pre-reproductive period were significantly different between males and females.

Keywords: *Testudo graeca, plasma biochemistry, hematology, reproductive period, Turkey*

Introduction

Reptiles have different adaptations to terrestrial life that are not usually found in other animal species. The turtle group, which includes species living on land, in freshwater, and in marine ecosystems and also has varying morphological and physiological characteristics is one of the important groups among reptiles (Godfrey and Campbell, 2002). Plasma biochemistry studies have been used to support and extend the results of studies in the field of hematology, as animal populations, physiology, guide the determination of the environmental status of the habitat in which they live (Jacobson et al., 1991; Raphael et al., 1994; Lopez-Olvera et al., 2003).

For all these reasons, biochemical analyses of blood plasma are an important method for finalizing the health of many animal species and in recent years, reptile-related hematology and plasma biochemistry studies have been increasing rapidly.

There are many studies on blood cells and clinical hematology (Marks and Citino, 1990; Dickinson et al., 2002; Lopez-Olvera et al., 2003; Tosunoğlu et al., 2005; Hamooda et al., 2014; Bielli et al., 2015) and plasma biochemistry associated with *Testudo* species (Gilles-Baillien, 1969, Lopez-Olvera et al., 2003, Dickinson et al., 2002, Marks and Citino, 1990, Scope et al., 2013, Hamooda et al., 2014; Özdamar, 2014, Hetenyi et al., 2016). Mediterranean Spurred Tortoise, *T. graeca* is classified in

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the vulnerable species category according to IUCN (Red list) criteria. It is spread in all suitable habitats (areas with less vegetation) in Çanakkale province (Tosunoğlu et al., 2017). Since *T. graeca* is an endothermic species, it may be affected by environmental conditions and external factors, as well as many internal and external factors affecting its blood parameters. Studies on hematology (Tosunoğlu et al., 2005), and plasma biochemistry (Hamooda et al., 2014) are very limited in *T. graeca* species. Tosunoğlu et al. (2005) have established reference intervals for blood cell measurements and clinical hematology parameters in *Testudo hermanni* and *T. graeca* species. Hamooda et al. (2014) conducted a study to determine sexually and seasonally related changes in plasma biochemical values of *T. graeca* species, which are spread in Libya.

There is no study about plasma biochemistry related to sex and different periods on *T. graeca* species which can be found around our country.

The purpose of this study is the determination of parameters of plasma biochemistry (glucose, urea, creatinine, calcium, albumin, cholesterol, total protein, uric acid, phosphorus triglycerides, magnesium, iron, HDL-cholesterol, hemoglobin and hematocrit) related to the sex (female-male) and different periods (pre-reproduction and reproduction period) of *T. graeca,* and the determination of reference intervals. It is also aimed to determine whether all parameters differ between pre-reproduction and reproduction period and between male and female individuals.

Materials and Methods

Çanakkale region is located in northwestern Turkey, and *T. graeca* samples used in this study were collected from mountainous areas around Bayramiç (39° 46' 33.2" K, 26° 46' 24.4" D, Altitude: 239 m), Lapseki (40° 14' 50.8" N, 26° 47' 13.9" E, Altitude: 315m), Ezine (39° 47' 15.30" N, 26° 14' 53.45" E, Altitude: 140m), Çan (40° 07' 31.8" N, 26° 31' 22.9" E, Altitude: 60m) and city center (40° 06' 55.0" N, 26° 25' 28.4" E, Altitude: 117m) (*Fig. 1*). In all of these localities, species were observed in open areas with low vegetation.

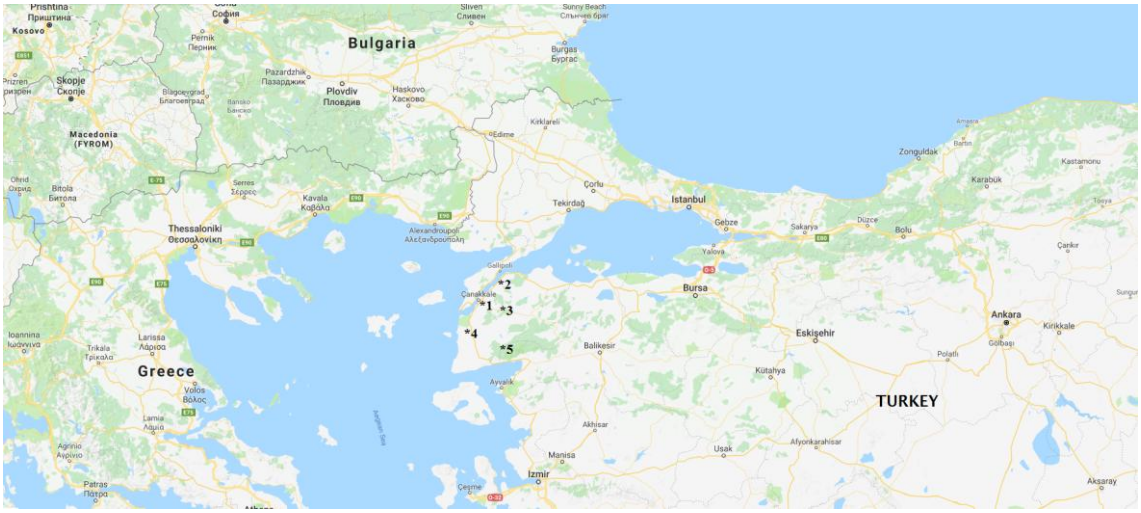


Figure 1. *Map of study area. 1- City Center, 2- Lapseki, 3- Çan, 4- Ezine, 5-Bayramiç (source: https://maps.google.com)*

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A total of 20 specimens were collected manually in two different periods; 11 (7 ♂♂, 4 ♀♀) in the pre-reproduction period (March and April 2016) and 9 (5 ♂♂, 4 ♀♀) in reproduction period (June and July 2016) in forested areas, through the bushes and the open areas. All the procedures on the samples were made with the permission of Çanakkale Onsekiz Mart University, Animal Experiments Ethics Committee with the decision numbered 2015/108. Morphological measurements were made with digital caliper and tortometer. After the samples were brought to the laboratory and the necessary blood samples were taken, the tortoises were left behind in the habitats from which they were collected.

For plasma biochemistry analysis, 0.5 ml blood was taken with a 5 ml scaled syringe on the right or left side of the ligament in the jugular vein on the neck (Wright, 2008). Blood samples were transferred to biochemical tubes containing lithium heparin (BD Vacutainer, Becton Dickinson, Plymouth, UK). The blood was centrifuged at 4000 rpm for 10 minutes in a centrifuge, and the plasma part was separated. Biochemical parameters (glucose, urea, calcium, cholesterol, triglyceride, creatinine, uric acid, total protein, iron, albumin, phosphorus, H.D.L. and magnesium) to be studied were obtained by measuring with ELIMAT brand auto analyzer using commercial kit in the obtained plasma. For hematocrit analysis, blood was taken into micro hematocrit tubes and centrifuged at 1000 rpm for 5 minutes in a micro hematocrit centrifuge and micro hematocrit tubes were calculated with hematocrit paper and the result was found as %. Total hemoglobin concentration was measured colorimetrically with a sahli hemoglobinometer (Tanyer, 1985).

Descriptive statistics of plasma biochemistry parameters were evaluated using SPSS (20.0 for Windows Student Version) statistical program. Statistical comparison was made using the Mann Whitney U test in the SPSS program.

Results

In this study, morphological measurements were taken from the samples and similar sized samples were used. The carapace lengths of specimens were measured as about 16.14 cm in male individuals and about 20.37 cm in female individuals. In this study, plasma biochemical values were examined separately in female and male individuals in pre-reproduction and reproduction period, and the data pertaining to a total of 15 parameters are given in *Table 1*.

Table 1. *Plasma biochemical values of T. graeca species pre-reproduction and reproduction periods. N: sample number, Min-Max: minimum-maximum values, SD: standard deviation*

		PRE-REPRODUCTION PERIOD				REPRODUCTION PERIOD			
PARAMETERS	Sex	N	Min-Max	Mean	SD	N	Min-Max	Mean	SD
Glucose * mg/dL	♂♂	7	24.87-198	134.02	55.90	5	61.7-143.3	82.63	34.34
	♀♀	3	121.3-166.7	144.86	22.74	3	71.39-86.01	79.35	6.05
	♂♂+♀♀	10	24.87-198	137.27	47.18	8	61.70-143.3	81.17	24.62
Urea mg/dL	♂♂	7	9.7-46.12	21.36	11.93	5	11.5-81.40	32.40	28.49
	♀♀	4	15.00-85.6	50.57	30.61	4	12.9-86.70	32.92	35.90
	♂♂+♀♀	11	9.70-85.60	31.98	24.16	9	11.5-86.7	32.63	29.82
Cholesterol * mg/dL	♂♂	6	166.3-386	266.13	83.03	5	22.35-130.1	76.69	45.37
	♀♀	4	94.2-623.7	247.6	252.57	4	79.49-208.6	136.75	64.77
	♂♂+♀♀	10	94.2-623.7	258.72	158.7	9	22.35-208.6	103.38	60.03

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Creatinine mg/dL	♂♂	7	0.1-0.6	0.43	0.16	5	0.24-0.49	0.34	0.098
	♀♀	4	0.2-0.73	0.45	0.24	4	0.1-0.38	0.23	0.12
	♂♂+♀♀	11	0.1-0.73	0.44	0.18	9	0.1-0.49	0.29	0.11
Uric Acid * mg/dL	♂♂	5	0.35-1.07	0.68	0.35	5	0.25-6.31	3.29	2.59
	♀♀	4	0.55-1.30	0.83	0.12	4	1.28-7.82	4.38	3.30
	♂♂+♀♀	9	0.35-1.07	0.68	0.27	9	0.25-7.82	3.78	2.79
Albumin g/dL	♂♂	7	1.25-2.8	1.59	0.56	4	0.85-1.59	1.22	0.33
	♀♀	4	0.45-2.14	1.31	0.83	4	0.4-2.06	1.33	0.69
	♂♂+♀♀	11	0.45-2.8	1.49	0.65	8	0.4-2.06	1.27	0.50
Calcium mg/dL	♂♂	6	3.6-9.91	7.02	2.45	5	6.96-30.20	15.16	10.38
	♀♀	4	2.63-38.61	15	16.26	4	1.07-25.13	16.29	10.87
	♂♂+♀♀	10	2.63-38.61	10.21	10.41	9	1.07-30.20	15.66	9.93
Triglyceride mg/dL	♂♂	6	111-673.5	366.55	203.33	4	121.30-743.7	384.75	278.14
	♀♀	4	105.6-276.7	185.47	70.24	4	84.10-309.3	202.70	92.20
	♂♂+♀♀	10	105.6-673.5	294.12	182.64	8	84.10-743.7	293.72	215.10
T.Protein g/dL	♂♂	6	2.27-8.64	6.21	2.39	5	0.22-12.31	6.29	3.83
	♀♀	4	1.02-7.76	4.68	3.45	4	4.82-10.80	6.75	2.77
	♂♂+♀♀	10	1.02-8.64	5.75	2.65	9	0.22-12.31	6.49	3.21
Phosphorus mg/dL	♂♂	7	0.37-10.33	2.80	3.46	5	0.19-2.19	1.40	0.88
	♀♀	4	0.3-4.88	2.44	1.94	4	0.17-4.20	2.05	1.65
	♂♂+♀♀	11	0.3-10.33	2.67	2.89	9	0.17-14.20	1.69	1.23
Magnesium mg/dL	♂♂	6	0.07-5.27	1.30	1.98	5	0.2-0.83	0.45	0.27
	♀♀	4	0.91-1.13	0.98	0.1	4	0.1-1.19	0.68	0.50
	♂♂+♀♀	10	0.07-5.27	1.20	1.57	9	0.1-1.19	0.55	0.38
H.D.L mg/dL	♂♂	7	31.52-207	86.94	59.71	5	5.03-52.17	32.59	20.49
	♀♀	4	39.75-148	88.24	51.37	4	24.31-106.2	64.73	43.59
	♂♂+♀♀	11	31.52-207	87.41	54.14	9	5.03-106.2	46.88	34.78
Iron * g/dL	♂♂	6	69.8-346.7	179.53	106.46	5	87.6-244.8	176.28	60.06
	♀♀	4	15.2-89.7	45.92	33.16	4	94.9-288.9	150.75	92.58
	♂♂+♀♀	10	15.2-346.7	126.9	106.88	9	87.6-288.9	164.93	72.10
Hemoglobin g/dL	♂♂	6	5-11	7.36	2.20	5	5-8.4	7.24	1.39
	♀♀	4	6-6.8	6.47	0.35	4	6-8	7.1	0.84
	♂♂+♀♀	10	5-11	7.01	1.72	9	5-8.4	7.17	1.11
Hematocrit (PCV) %	♂♂	7	0.15-0.35	0.22	0.08	5	0.13-0.3	0.24	0.069
	♀♀	4	0.16-0.33	0.21	0.076	4	0.18-0.25	0.23	0.035
	♂♂+♀♀	11	0.15-0.35	0.22	0.07	9	0.13-0.3	0.24	0.054

* P value of less than 0.05 indicates significant difference between the compared means.

When comparing the pre-reproductive period and the reproductive period in terms of glucose, uric acid and iron values, statistical differences were found in the females. It was determined that cholesterol values of males were significantly different between periods. In the females, glucose value was found high in the pre-reproductive period and uric acid and iron values were high in the reproductive period. Cholesterol values of males were found high in the pre-reproductive period. In addition, there was statistical difference between iron values of males and females in the pre-reproductive period.

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Discussion

Glucose

In our glucose values, when females were compared, it was found that there was a statistically significant difference in glucose between pre-reproduction period and reproduction period (P: 0.034; U: 0.000; Z: -2.121). When male and female individuals were evaluated together, there were significant differences between the two periods (P: 0.007; U: 12.00; Z: -2.694). In both sexes, glucose levels were found to be higher in the pre-reproduction period than in the reproduction period. It is thought that there is an increase after hibernation because the animal that is wakened from winter sleep fed too much to be prepared for the reproduction period. Hamooda et al. (2014) found that glucose values in the *T. graeca* specimens were close to each other in females and males.

Urea

In our urea values, no statistically significant difference was found between the sexes in the periods. Gilles-Baillien (1969) determined the urea value of *T. hermanni* as 2.46 mg/dL and found that the urea concentration increased when preparing for winter sleep.

Cholesterol

In our cholesterol values for males, when pre-reproduction and reproduction period were compared, it was determined that there is a significant difference (P: 0.006; U: 0.000; Z: -2.739). When male and female individuals are evaluated together, there are significant differences between the two periods (P: 0.007; U: 12.00; Z: -2.694). The cholesterol values obtained in both genders in the pre-reproduction period are higher than in the reproduction period. Hamooda et al. (2014) reported that cholesterol in *T. graeca* was significantly higher in females during the season in which they were active, and varied by season. These observations and analyzes have shown that there is a close relationship between egg development and vitellogenesis (Christopher et al., 1999, Hidalgo-Vila et al., 2007). And they have shown that testosterone hormone has a negative correlation with cholesterol.

Albumin

In our albumin values, there were no significant differences in pre-reproduction period and reproduction period, and similar values were determined. Dickinson et al. (2002) found that sex, location, and period cause significant differences in hematology and blood biochemistry in the desert turtle *Gopherus agassizii*, they found that there was no difference between the male and female individuals in albumin values. In our values, no statistically significant difference was found between the sexes in both periods.

Calcium

There was no statistic difference between the sex and periods in terms of the calcium parameter. Marks and Citino (1990) found that plasma vitelline causes an increase in the amount of calcium present during the reproduction period in the *Testudo radiata* specimens. And they found that calcium levels were not significantly different between males and females.

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Creatinine

In our creatinine values, there were no significant differences in pre-reproduction period and reproduction period, and close values were determined. There was no difference between the sexes in both periods. Hamooda et al. (2014) reported that creatinine values of *T. graeca* were found close to each other in males and females. There was no statistically significant difference in the creatinine values of *T. hermanni*, periodically in both sexes (Özdamar, 2014).

Total protein

There was no significant difference in pre-reproduction period and reproduction period, and close values were determined. There was no statistic difference between the sexes in both periods. In *G. polyphemus*, serum total protein levels were found to be higher in females compared to males (Taylor and Jacobson, 1982). Özdamar (2014) reported that the pre-reproduction period in *T. hermanni* species was shorter in females than in males.

Uric acid

There was no statistic difference between the sexes in both periods. It was determined that uric acid value in females was statistically different when pre-reproduction period and reproduction period were compared (P: 0.034; U: 0.000; Z: -2.121). When male and female individuals were evaluated together, there were significant differences between the two periods (P: 0.007; U: 8.000; Z: -2.694). It was determined that the uric acid levels in both sexes were lower in the pre-reproduction period than in the reproductive period. Scope et al. (2013) found that the concentration of uric acid in the *T. hermanni* specimens was the highest after winter hibernation. The lowest values were found to be 0.9 mg / dL in October and the highest value was found to be 5.4 mg / dL after winter sleep.

Phosphorus

In our phosphorus values, there were no significant statistical differences in the pre-reproduction period and reproduction period, but they were observed to be lower in reproduction period. Phosphorus was found to have no effect on sex. By Scope et al. (2013), phosphorus values of *T. hermanni* were found to be higher in females than in males and the maximum value for phosphorus was seen at the earliest in June.

Magnesium

There were no statistical differences between the sexes and the periods. Hamooda et al. (2014) found magnesium in *T. graeca* as 5.33 mg/dL in females and 5.64 mg/dL in males.

Triglycerides

In our triglyceride values, there was no statistical difference. Hamooda et al. (2014) observed that the concentration of triglycerides in female *T. graeca* showed a significant increase when compared to males.

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Iron

In our iron values, a significant difference was found between males and females in pre-reproduction period (P: 0.019; U: 1.000; Z: -2.345). When the female individuals were compared between the pre-reproduction and the reproduction period, the statistical difference was observed (P: 0.021; U: 0.000; Z: -2.309). A detailed study on the reference intervals of the iron values of tortoises has not been found.

HDL cholesterol

There was no statistical difference between the sexes and the periods. Sex and size were found to influence the biochemical parameters of HDL-cholesterol (Whiting et al., 2007; Anderson et al., 2011). Mader and Divers (2013) reported that in reptiles; females had high cholesterol during active laying period.

Hematocrit

In our hematocrit values, there was no statistical difference between the two periods and sexes. Hamooda et al. (2014) suggested that low levels of hematocrit in the *T. graeca* species are related to sexual affect. Tosunoğlu et al. (2005) found hematocrit values between 21-34% for *T. hermanni* and 24-36% for *T. graeca* species. They noted that hematocrit value is potentially informative about the state and physiology of the tortoises.

Hemoglobin

In our hemoglobin parameters, there was no statistical difference between the two periods and sexes. Tosunoğlu et al. (2005) reported hemoglobin levels of 4.9-7.4 g/dL for *T. hermanni* and 5.2-9.7 g/dL for *T. graeca*. Hamooda et al. (2014) found that the concentration of hemoglobin in the *T. graeca* species was characterized by low average values in both males and females, which have a significant effect in favor of male tortoises.

Conclusion

Biochemical analyses of blood plasma are an important method for assessing the health of many animal species. For this reason, comparative hematological results of *T. graeca*, which is on the Red List of the IUCN in the Vulnerable category, are given in this study. The data of this study will be useful for evaluating future health problems and for species conservation studies.

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METEOROLOGICAL DROUGHT ANALYSIS USING ARTIFICIAL NEURAL NETWORKS FOR BURSA CITY, TURKEY

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Abstract. Climate change is one of the most important environmental events of recent years. Floods and droughts may occur more frequently with climate change. Droughts could be classified as meteorological, hydrological or agricultural. When meteorological drought appears in a region, agricultural and hydrological droughts follow. In this study, the standardized precipitation index (SPI) was applied for meteorological drought analysis in Bursa City-Turkey. Analyses were performed on 3-, 6-, 9- and 12-month-long data sets. According to the analyses most of the percentages (67-72%) for all SPI values (3, 6, 9, 12 months) was in the near normal class close to Marmara Region-Turkey. Also meteorological variables and SPI-12 values were simulated with ANN models and had different structures. It was found that R and MSE values calculated were in the acceptable ranges and rising of hidden layers and input numbers in the model structures was ensured for more efficient model run. Modelling the precipitation from the meteorological parameters (Model 2) was possible with some error. Therefore simulations of SPI-12 and soil temperatures were indicative to meteorological and agricultural droughts. Comparison of the observed values and the modelling results showed a better agreement with SPI-12 and soil temperature parameters. Drought predictions made by ANN models would be useful for local administrations and water resources planners and would be extremely important for drought risk management.
Keywords: *aridness, standardized precipitation index (spi), modelling*

Introduction

In an increasing and demanding society with the development of industrial technology, mineral and energy resources are needed for fossil fuels. Besides the obvious benefits (rising of the living standards for most people) the exploitation of the natural resources of the Earth has not been without a cost. This cost is climate change. Climate change refers to both the rise in global temperatures because of human activities and the many consequential impacts such as more intense and frequent droughts and storms, melting glaciers and ice sheets, rising sea levels, warming seas (NOAA, 2017).

Droughts naturally occur in almost all climatic zones, and are a result of the reduction of precipitation from normal amounts for an extended period of time (Belayneh et al., 2016). Precipitation is not the only factor determining the development of drought. Hot weather, low soil moisture, wind, increasing demand of water due to population growth, irregular usage of water resources and human activities in the regions influence the drought events. Extended periods of drought can lead to several adverse consequences, which include a disruption of the water supply, low agricultural yields, and reduced flows for ecosystems (Belayneh et al., 2016). As the result of the long meteorological drought, agricultural and hydrological drought occurs. Drought may cause famine, hunger, and death and so it affects a community socially, economically and psychologically. Water shortages will become one of the most important environmental and social problems in some countries around the world in the near future (IPCC, 2014).

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Annual water amount per capita in Turkey is expected to decline (700-1910 m^3). Increasing evaporation because of global climate change will cause occurrence of more severe and prolonged droughts in Turkey (Kurnaz, 2014). Variation in the hydrological cycle will conduce to irrigation and water supply problems as well as increase in sudden flood events (Wen et al., 2013). Therefore, drought analysis is important in water resource management and planning, and for long-term economic and social planning (Buttafuoco and Caloiero, 2014; Ibrahimi and Baali, 2017).

Forecasting and analyzing meteorological drought characteristics specifically their initiation, time, frequency, severity and involved area are important. Because time, duration and severity of drought are not known it is analyzed with probability and statistical methods in any region (Belayneh et al., 2016; Deo et al., 2015; Ibrahimi and Baali, 2017; Buttafuoco and Caloiero, 2014). Precautions could be taken to minimize the drought with drought analysis and measures. Drought can be examined spatially and temporally. When the temporal analyses were made, duration, amplitude and intensity of drought might be calculated.

A drought period starts when the Precipitation Index falls below zero. When the drought period finishes the Precipitation Index rises above this level. Number of drought periods are denoted as "m"; duration of drought periods are showed as L_1, L_2, …….L_m; total of lacks lower from each drought level-magnitude demonstrated as "M_1 , M_2 , …….M_m".

The ratio of magnitude to duration of drought periods is identified as "drought intensity" (McKee, et al., 1993). It is formulated as *Equation 1:*

$$I_n = \frac{M_n}{L_n} \qquad \text{(Eq.1)}$$

The sum of the lack of n^{th} along a series M_n (Magnitude) is calculated as *Equation 2:*

$$M_n = \sum_{i=1}^{m} \left| X_0 - X_i \right| \qquad \text{(Eq.2)}$$

In this equation:
X_0: SPI cutoff level is zero for each drought,
X_i: It is a standardized series,
m: Number of drought periods for the cutoff level (X_0).

Maximum and minimum values of drought features (L_{max}, L_{min} ,M_{max} , M_{min} , I_{max} and I_{min}) are calculated for each cutoff level. Also, some basic statistical parameters as mean, standard deviation, median, skewness, kurtosis values are determined. These values inform about the statistical properties of the M, L and I values of data set (McKee et al., 1993).

A common tool utilized to monitor current drought conditions is a drought index. Drought indices are expressed by a numeric number, which is believed to be far more functional than raw data during decision-making (Pedro-Monzonís et al., 2015). Several drought indices have been developed around the world in the past based on rainfall as the single variable, including the widely used Deciles. There are some indices as Standardized Precipitation Index (SPI) (McKee et al., 1993) and Effective Drought Index (EDI) (Deo et al., 2015) and also the well-known Palmer Drought Severity Index

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(PDSI) which considers temperature along with rainfall (Belayneh et al., 2016). In temporal and spatial drought analysis, standard precipitation index and kriging methods are generally used respectively. SPI (*Eq. 3*) obtained by precipitation (mm) at a given time is subtracted from the mean precipitation and in the sequel divided by standard deviation value (Anonymous, 2014a, 2016a).

$$SPI = \frac{X_i - X_i^{mean}}{\sigma} \quad \text{(Eq.3)}$$

In this equation:
SPI: Standard Precipitation Index
X_i: precipitation (mm)
X_i^{mean}: mean Precipitation (mm)
σ: Standard deviation

The SPI drought index was chosen to forecast drought in this study due to its simplicity, its ability to represent droughts on multiple time scales, and because it is a probabilistic drought index. In addition, the study by Gürler (2017) determined that the SPI is the most appropriate index for monitoring the variability of droughts in Turkey because it is easily adapted to local climate, has modest data requirements, and can be computed at almost any time scale.

Application of statistical models has a long history in drought forecasting. Development of ANN (Artifical Neural Network) approaches with high precision of estimation was considered to be effective in eliminating the arduous task of field works and development of complex models that involve partial differential equations.

An application of ANN to solve civil and environmental engineering problems began in the late 1980s (Ibrahimi and Baali, 2017). ANN has recently been applied in hydrological forecasting to examine the rainfall-runoff relationship in watershed, to characterize daily stream flow and monthly reservoir inflow, to evaluate rainfall-runoff models, to forecast river flow, to forecast future precipitation values and for the purposes of drought forecasting (Daliakopoulos and Tsanis, 2016; Lima, et al., 2016; Pedro-Monzonís, et al., 2015; Belayneh et al., 2016). Also it could be used easily in climate models of catchment area (Salami and Ehteshami, 2016), forecasting for ecology, water quality and quantity (Sinha and Das, 2015; Lallahem and Hani, 2017; Chen et al., 2017)

In this paper, Standardized Precipitation Index-SPI (3, 6, 9, 12 months) were calculated based on monthly mean precipitation values, to determine drought categories and then total of 6 model structures were tested and validated with ANN model structures. The first aim of this paper was to develop suitable ANN models by considering the feed-forward back-propagation learning algorithm in the estimation of total monthly precipitation (mm), the average monthly minimum and surface soil temperatures (10 cm, °C) and SPI-12 values. The second aim was providing information from modeled parameters to meteorological and agricultural drought. Also the suitability of soil temperatures modelling for drought forecasting was investigated when the precipitation data was absent. For this purposes meteorological drought analysis was performed for the Bursa Centrum Station. In the drought analysis, it was determined that whether precipitation values were fitting gamma distribution and SPI method was used. Drought was investigated in SPI-12 and the obtained drought categories given by

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McKee et al. (1993) were modeled by ANN. Importance of this study lies in the risk management in countries around the world fighting with drought.

Materials and methods

Study area and data

Bursa is a large city in Turkey, located in Northwestern Anatolia, within the Marmara Region. It is the fourth most populous city in Turkey and one of the most industrialized metropolitan centers in the country. According to the 2013 general population determination results Bursa Province had 2,787,539 inhabitants (Anonymous, 2014b). Bursa between 28-30 degrees latitude and 40 degrees longitude circle is located in the southeast of the Marmara Sea (*Fig. 1*).



Figure 1. *Location of Bursa City and Meteorology Station*

It is 155 m above sea level and its total area is 10 819 km^2. The climate type of Marmara is dominant in Bursa. Marmara climate is known as a Terrestrial to Mediterranean transitional climate. Characteristics of the Marmara climate are as follows: annual precipitation is between 500-1000 mm, annual average temperature values are 14-16 °C, warmest month average: 23-25 °C, coldest month average: 5-6 °C. The most rain falls in winter, December, January, February. The arid months are June, July and August. Percent of precipitations are 38.3% of winter, 9.4% summer, 26.8% autumn and 25.5% in spring (Anonymous, 2014c). According to the long-term (1984-2013) averages, highest monthly average temperature was in July with 25.1 °C, the lowest monthly average temperature was in January with 5.6 °C and monthly average of

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the most rainfall was in December with 93.8 l/m² (Boz, 2015). There are 16 meteorological stations in districts of Bursa City. There are no stations in Yıldırım and Gürsu districts but two stations in the Osmangazi District. In this study, meteorological data (years of 1984–2013) from the 16 stations in Bursa was taken from Ministry of Forestry and Water Affairs, Meteorological Department Station 17116 (Bursa-Center) and meteorological drought was investigated. Between 12 and 40 station numbers were used for meteorological and agricultural drought analyses in many studies (Milanović et al., 2014; Sun et al., 2017; Łabędzki and Bak, 2017; Pablos et al., 2017). The stations are not located in district centers but in areas close to agricultural land. Therefore urban heat island could not cause significant difference between surface temperature in stations and in arable lands (Yang et al., 2016). The data collection was synoptic (3 hourly) and daily (Anonymous, 2014c). Reporting periods for long-term averages according to climatological standard were either 1971-2000, or 1981-2010. But the climatological values between different date ranges in the international reports were presented and evaluated (NOAA, 2017). Regular data recording for Bursa stations had started in 1984. Thus, the values between 1984 and 2013 were evaluated in this study. The parameters for statistical calculations were monthly total precipitation (mm), average air and soil temperature, pressure, vapor pressure, insolation intensity, wind speed, relative humidity were given in *Table 1*. Also long time averages of air temperature and monthly total precipitation are shown in *Figures 2* and *3,* respectively.

Table 1. *Statistical values of used parameters (1984-2013)*

Parameters	Max	Min	Average	Standard deviation	Total*	%*
Monthly average air temperature (°C)	27.90	1.10	14.70	7.27	3	0.80
Monthly average maximum air temperature (°C)	31.90	6.20	21.58	5.79	2	0.55
Monthly average minimum air temperature (°C)	24.50	-8.80	8.81	8.08	3	0.83
Monthly average minimum surface soil temperature (°C)	20.80	-5.50	7.47	5.89	17	4.72
Monthly average 10 cm soil temperature (°C)	33.30	2.20	16.56	8.93	9	2.50
Monthly total precipitation (mm)	396.60	0.00	58.26	48.26	3	0.83
Monthly average pressure (hPa)	1017.00	997.50	1004.31	3.72	5	1.39
Monthly average vapor pressure (hPa)	51.80	4.60	11.64	4.87	19	5.28
Monthly average-daily total global insolation intensity (cal/cm²)	644.70	86.20	312.67	149.29	9	2.50
Monthly total global insolution intensity (cal/cm²)	19341.60	2585.25	9484.77	4578.44	9	2.50
Monthly average wind speed (m/s)	3.50	0.80	1.95	0.44	11	3.06
Monthly average relative humidity (%)	84.50	46.30	66.68	7.64	4	1.11

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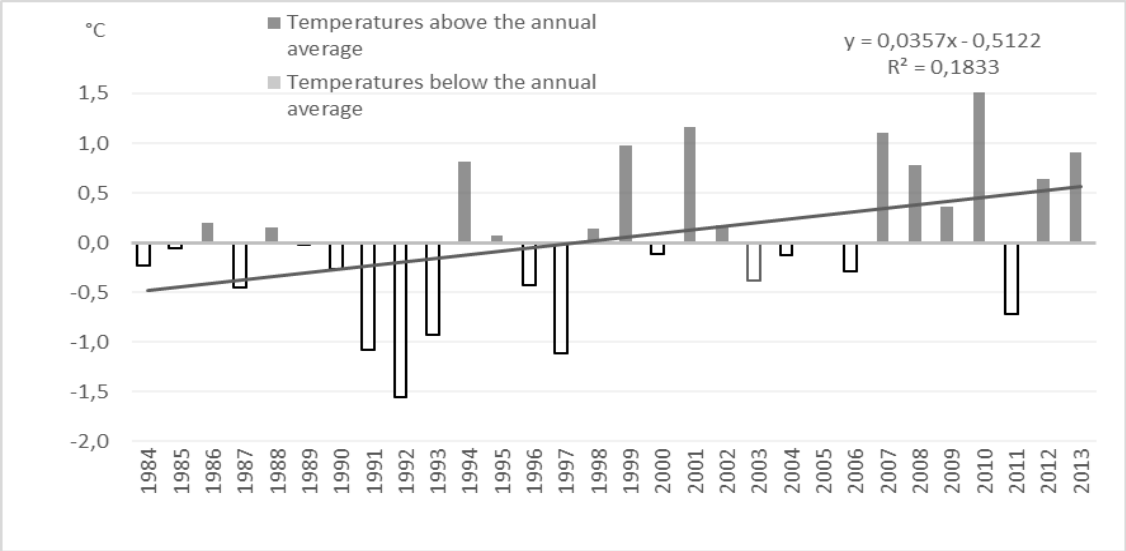


Figure 2. *Long-time averages of air temperature*

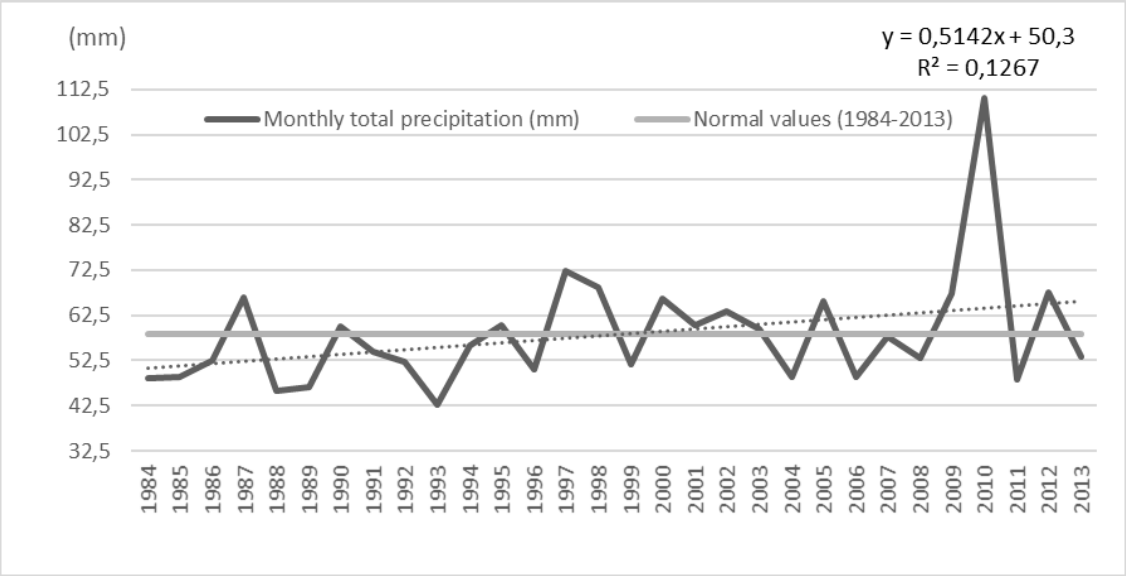


Figure 3. *Long-time averages of monthly total precipitation*

SPI calculation

The SPI is based on the probability of precipitation at any time scale (at least 30 years). The probability of observed precipitation is then transformed into an index. This long-term precipitation time series is then fitted to a gamma distribution, which is then transformed through an equal probability transformation into a normal distribution. Positive SPI values indicate wet conditions with greater than median precipitation and negative SPI values indicate dry conditions with lower than median precipitation. McKee and others (1993) originally calculated the SPI for 3-, 6-,12-, 24- and 48-month timescales (Buttafuoco and Caloiero, 2014; Belayneh et al., 2016; Anonymous, 2016a). *Table 2* presents SPI drought classes.

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Table 2. *SPI drought classification (Anonymous, 2016a)*

SPI Values	Class
>2	Extremely wet
1.5–1.99	Very wet
1.0–1.49	Moderately wet
−0.99 to 0.99	Near normal
−1 to −1.49	Moderately dry
−1.5 to −1.99	Very dry
<−2	Extremely dry

In order to calculate the SPI, a probability density function that adequately describes the precipitation data must be determined. The gamma distribution function was selected to fit the raw rainfall data in this study. The SPI is a *z*-score and represents an event departure from the mean, expressed in standard deviation units. The SPI is a normalized index in time. SPI values can be categorized according to the classes.

In this study, the SPI_SL_6.exe (Standardized Precipitation Index Program) was compiled in C++ for PC developed by the National Drought Mitigation Centre, University of Nebraska-Lincoln, and it was used to compute time series of drought indices (SPI) for the station in the Bursa and for each month of the year at different time scales (Anonymous, 2016a). The input file was set up to analyse the 1-, 3-, 6-, 9- and 12-month SPIs and output file of the months was constituted.

In this study, input and output data for the simulations were normalized SPI values depending on precipitation. Before the training SPI values were demeaned 0 to 1. So variables were made dimensionless and similarity selected at random has been removed.

The formulation for dimensionless (normalization) values was determined with *Equation 4:*

$$F = (F_i - F_{min}) / (F_{max} - F_{min}) \quad \text{(Eq.4)}$$

In this equation, F was any dimensionless value; F_i was i^{th} value in measurements; F_{max} and F_{min} were maximum and minimum measurements (Ibrahimi and Baali, 2017; Deo et al., 2015).

ANN model and training algorithm

Artificial neural networks are formed of a set of simple elements, the alleged artificial neurons. These elements are inspired by biological nervous systems. Models of neural networks are separated into two categories: feed forward neural networks and recurrent neural networks. Feed forward neural networks deliver data linearly from input to output and they are the most popular and most widely used models in many practical applications (Daliakopoulos and Tsanis, 2016). The feed forward neural network (FNN) with as few as a single hidden layer and arbitrary bounded and smooth activation functions can approximate a continuous nonlinear function. The model of a neuron and multi-layer FNN is represented in *Figure 4* (Chen et al., 2014; Yonar and Yalılı, 2014).

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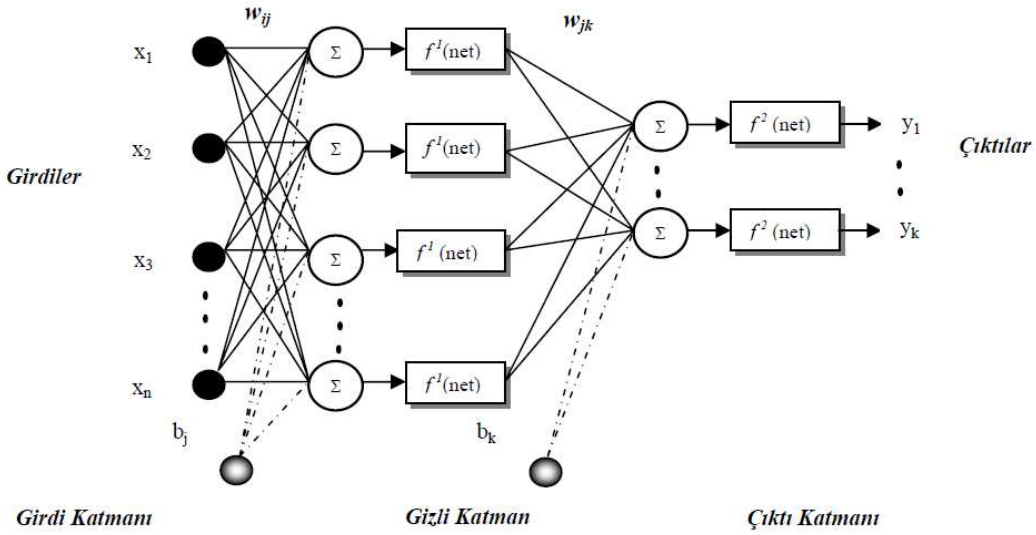


Figure 4. *Multi-layer feed forward neural network*

As shown in *Figure 4,* the elements constituting ANN are input layers, hidden layers (number of hidden layers are one or might be more}, output layer linkages between the layers and linkage weights.

The data of the problem is in the input and output layers. Productivity and importance of information in the input layer are provided with the weights. Net (basis) function expresses that effectiveness of input data to neuron. For an analytical study, the connection networks are mathematically represented by a basis function u (w, x). The net value is a linear combination of the inputs and the output of a neuron can be expressed as *Equation 5:*

$$out = f(n)$$

where

$$n = \sum_{j=1}^{R} w_j x_J + b \qquad \text{(Eq.5)}$$

x_1, x_2, … ,x_R are the input signals; ω_1,ω_2, . . .,ω_R are the weights of neuron; b is bias value; and f(·) is the activation function.

Activation function is a function determining the neuron output with processing the net inputs obtained from net function. The linear and sigmoid are common used activation functions in the construction of artificial neural networks.

An example of the sigmoid is the logistic function, defined by *Equation 6:*

$$f(n) = \frac{1}{1+e^{-n}} \qquad \text{(Eq.6)}$$

As sigmoid function (*Eq. 7*) can also be used as hyperbolic tangent function and it gives the outputs range between [0-1] (Cömert and Kocamaz, 2017):

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$$f(n) = \frac{1-e^{-n}}{1+e^{-n}} \quad \text{(Eq.7)}$$

The inputs x_1, x_2, . . ., x_R are multiplied by weights $\omega_i,j_{(1)}$ and summed at each hidden neuron i.
Then the summed signal would be calculated as *Equation 8*:

$$n_{i(1)} = \sum_{j=1}^{R} w_{i,j(1)} x_J + b_{i(1)} \quad \text{(Eq.8)}$$

The output y at a linear output node can be calculated as *Equation 9:*

$$y = \sum_{i=1}^{z} w_{1,i(2)} \frac{1-e^{-(\sum_{i=1}^{R} x_j w_{i,j(1)} + b_{i(1)})}}{1-e^{-(\sum_{i=1}^{R} x_j w_{i,j(1)} + b_{i(1)})}} + b_{1(2)} \quad \text{(Eq.9)}$$

where R is the number of inputs, z is the number of hidden neurons, ωi.j(1) is the first layer weight between the input j and the i^{th} hidden neuron, ω1,i(2) is the second layer weight between the i^{th} hidden neuron and output neuron, bi(1) is a biased weight for the ith hidden neuron and b1(2) is a biased weight for the output neuron (Chen et al., 2014)

The learning algorithms using in ANN are heuristics, partial Newton methods, matched gradient methods and Levenberg Marquardt methods. In this study feed forward neural network structure was used and Levenberg Marquardt training algorithm supervised training algorithms was preferred for training. Levenberg Marquardt algorithm is the least squares calculation method based on maximum neighborly idea (Chen et al., 2017). The sum of squared errors of any one of the element in the training set (N number of elements) could be calculated as *Equation 10:*

$$E(w) = \frac{1}{2}\sum_{i=1}^{N} (y_{mi} - y_i)^2 = \frac{1}{2}(y_m - y)^T (y_m - y) \quad \text{(Eq.10)}$$

Symbol *(w)* in *Equation 10* would be defined as *Equation 11:*

$$w = [w_{1,1(1)}, w_{1,2(1)}, \ldots, w_{z,R(1)}, b_{1(1)}, b_{2(1)}, \ldots, b_{z(1)}, w_{1,1(2)}, w_{1,2(2)}, \ldots, w_{1,z(2)}, b_{1(2} \quad \text{(Eq.11)}$$

Two number of different hidden layer neuron for each input neurons was tested in this study. This was because; in the recent study were two different approaches used in determining the number of hidden layer neurons. The first was (N+1)/2 and the second one was (2*N+1) (Chen et al., 2017). “N” was the number of neuron in the input layer (Chen et al., 2017).

Performance and sensitivity analysis of ANN

The performance of the forecasts from the data-driven models was evaluated. The Pearson correlation coefficient is one of the most commonly used performance in selecting proper inputs for the ANN (Chen et al., 2014). Correlation coefficient is

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described as the degree of correlation between the empirical and modeled values (*Eq. 12*):

$$r = \frac{\sum_{i=1}^{N_o}(y_i - \bar{y})(y_{mi} - \bar{y}_m)}{\sqrt{\sum_{i=1}^{N_o}(y_i - \bar{y})^2 \sum_{i=1}^{N_o}(y_{mi} - \bar{y}_r}} \quad \text{(Eq.12)}$$

where y_i and y_{mi} enounce the network output and measured value from the i^{th} element; $\bar{y}$ and $\bar{y}_m$ conceive their average respectively, and N_o describes the number of measurements.

Another performance computation is mean absolute error (MAE). The MAE is used to measure how close forecasted values are to the observed values. It is the average of the absolute errors. The smaller values of MAE and MSE (mean square error) provide the better performance (Belayneh et al., 2016). MAE and MSE are estimated with *Equations 13* and *14:*

$$MAE = \frac{1}{N_0}\sum_{i=1}^{N_0}|y_{mi} - y_i|^2 \quad \text{(Eq.13)}$$

$$MSE = \frac{1}{N_0}\sum_{i=1}^{N_0}(y_{mi} - y_i)^2 \quad \text{(Eq.14)}$$

ANN application

In this study, all the ANN models were created with the MATLAB ANN toolbox (Matlab, 2015). The tangent sigmoid transfer function was the activation function for the hidden layer, while the activation function for the output layer was a linear function. All the ANN models in this study were trained using the Levenberg Marquardt (LM) back propagation algorithm. The LM back propagation algorithm was chosen because of its efficiency (Nawi et al., 2016; Cömert and Kocamaz, 2017).

All the ANN models the cross validation technique was used to partition the data sets; 80% of the data was used to train the models, while the remaining 20% of the data was used to test and validate the models, with 10% used for testing and 10% used for validation (Berhan et al., 2015; Belayneh et al., 2016). The training set was used to compute the error gradient and to update the network weights and biases. The error from the validation set was used to monitor the training process. If the network over fits the data the error in the validation set will begin rising. The testing data set is an independent data set and is used to verify the performance of the model.

Total number of 359 data based on monthly average values was used in this study and 6 model structures were tried. In the first model 6 parameters associated with air temperature, global insolation and precipitation parameters were used as inputs, the parameters of monthly average of 10 cm soil depth temperature and monthly average of surface soil minimum temperature were outputs. In the second model, 3 numbers of air temperature, 2 of soil temperature, 2 number of global insolation and monthly average of humidity parameters were used as inputs (total of the 8 parameters) and monthly total precipitation parameter was identified as output. 3 and 9 month SPI values in 3rd and 4th models and 3, 9 and 12 month SPI values in 5th and 6th models were used as input

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parameters. 12 month SPI was defined as output in3rd, 4th, 5th and 6th models. In the 1st and 2nd models, only meteorological parameters were modeled. SPI parameters were modeled in the other 4 models. The results related to ANN modelling experiments with a different number of hidden layers were defined in a comparative way.

Results and discussion

Assessment of SPI Values

3, 6, 9 and 12 month SPIs were calculated with SPI_SL_6.exe program. If the Standard Precipitation Index falls below zero a drought period start, number of drought periods "m", duration of drought periods L_1, L_2, …….L_m and total of lack from each drought level-Magnitude "M_1, M_2, …….M_m" and the ratio of magnitude to duration of drought periods identified as "drought intensity" parameters were calculated with Microsoft Office Excel (Windows 7 Home Premium) computer program. In addition to this cutting levels were decided as class intervals for SPI. According to the cutting levels M, L and I values were obtained for mild, moderate, severe and extreme drought conditions. The calculated statistical parameters of drought were given in *Table 3*.

Table 3. *The statistical parameters of drought*

Drought parameters		Number of events	Max	Min	Median	Most of repetition	Std	Skewness	Kurtosis	Average
M	3 Month	50	13	1	3	1.00	2.64	1.46	2.40	3.460
	6 Month	33	21	1	4.00	1.00	5.23	1.63	2.57	5.485
	9 Month	27	24	1	4.00	1.00	6.42	1.35	1.47	6.481
	12 Month	20	33.0	1	4.5	1.00	10.32	1.34	0.60	8.850
L	3 Month	50	13.41	0.02	2.065	0.05	2.80	1.54	3.30	2.861
	6 Month	33	24.59	0.01	2.07	-	5.73	2.05	4.41	4.395
	9 Month	27	25.86	0.07	2.04	1.42	6.90	1.63	2.15	5.322
	12 Month	20	30.87	0.09	1.7	-	10.35	1.59	1.27	7.065
I	3 Month	50	2.094	0.02	0.68	0.05	0.50	0.84	0.49	0.741
	6 Month	33	1.17	0.01	0.58	-	0.36	0.02	-0.97	0.567
	9 Month	27	1.21	0.07	0.53	-	0.35	0.19	-1.12	0.565
	12 Month	20	1.08	0.09	0.46	0.12	0.33	0.30	-1.45	0.507

As shown in *Table 3,* when the duration of drought becomes longer (3 Month to Month 12), number of events and median values of drought intensity were reduced but averages of drought intensities were found in same drought class "near normal". The calculated percentages of event number and drought classes (McKee et al., 1995) were given in *Table 4*.

Regarding the percent of event number it was seen that the most of the percentages (67%-72%) for all SPI values were in the near normal class. In the previous studies drought intensity of Marmara Region was found as maximum "1" (Sırdaş, 2002). Drought intensities of Bursa were determined close to Marmara Region in this study.

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Table 4. *The calculated percentages of event number and drought classes*

SPI Values	Class	3 Month	6 Month	9 Month	12 Month
		(%)			
>2	Extremely wet	4.857	3.077	2.250	5.176
1.5–1.99	Very wet	6.071	5.385	2.750	3.765
1.0–1.49	Moderately wet	21.250	13.846	8.750	11.765
−0.99 to 0.99	**Near normal**	**70.137**	**67.680**	**67.409**	**71.910**
−1 to −1.49	Moderately dry	7.671	10.773	12.256	9.551
−1.5 to −1.99	Severely dry	4.658	4.144	3.064	4.494
<−2	Extremely dry	3.014	1.381	1.950	1.685

The effect of neural network structure on performance function (MSE) and model estimation power (R)

The effect of neural network structure by changing of inputs, outputs and number of hidden neurons (layer) on model performance was investigated. Mean Square Error (MSE) has been adopted as a measure of performance (as performance function) for comparing the effectiveness of tested structures (Belayneh et al., 2016; Lalahem and Hani, 2017). The performance function (MSE) values and correlation coefficients (R) obtained for training, validation, testing and whole data sets. The model structures and their performance functions (MSE) values and correlation coefficients (R) of the data sets are presented in *Table 5*.

In the first model, air temperatures (3 parameters), global insolutions (2 parameters) and total precipitation were chosen as inputs also twin soil temperature parameters were chosen as outputs. This model was run quite efficiently.

In the second model air temperatures (3 parameters), soil temperatures (2 parameters), global insolations (2 parameters) and monthly average relative humidity (%) were inputs and monthly total precipitation (mm) was the output parameter. The calculation of MSE values of model 2 resulted in large numbers while correlation coefficients (R) were found little higher than in the other models. Therefore, modelling the precipitation from the meteorological parameters was possible with some error and this model run was not quite efficient.

In model 3 and model 4, modelling the SPI 12 from SPI 3 and SPI 9 was very efficient and also with increasing of hidden layer number in model 4 the model worked better than model 3. In model 4 MSE values of training and validation stages were decreased and correlation coefficient (R) of whole data was increased.

Increasing of input numbers in model 5 and model 6 (3, 6 and 9 months SPI) led to more efficient modelling than model 3 and model 4. R values were increased and MSE values were decreased in model 5 and 6. When model 5 compared to model 6, rising of hidden layer in model 6 was ensured more efficiently than in model 5.

As a result, it was found that all of the 5 models were successfully except model 2. R and MSE values calculated were in the acceptable range (Sinha and Das, 2015; Yonar and Yalili Kilic, 2014). MSE values in this study were found smaller than some water quality studies (Chen et al., 2014). This showed that hydrological and meteorological parameters were modeled well with ANN (Daliakopoulos and Tsanis, 2016; Sušanj et al., 2016; Sayagavi and Charhate, 2017). But in some water pollution and quality studies when number of data and tests were increased MSE values were calculated

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smaller (Sinha and Das, 2015; Yonar and Yalili Kilic, 2014). It was revealed in this study that precipitations in connection with meteorological drought were modeled better with SPI values. In the other studies SPI values were modeled well with neural network and Neural-based Fuzzy Inference System too (Ibrahimi and Baali, 2017; Belayneh et al., 2016). Also some modelling studies about the other drought indices (e.g. Effective Drought Index -EDI) gave good results (Pedro-Monzonís et al., 2015). *Figures 5* and *6* display the observed time series and forecasted values with the model structures. In addition, the corresponding scatter plots were also presented.

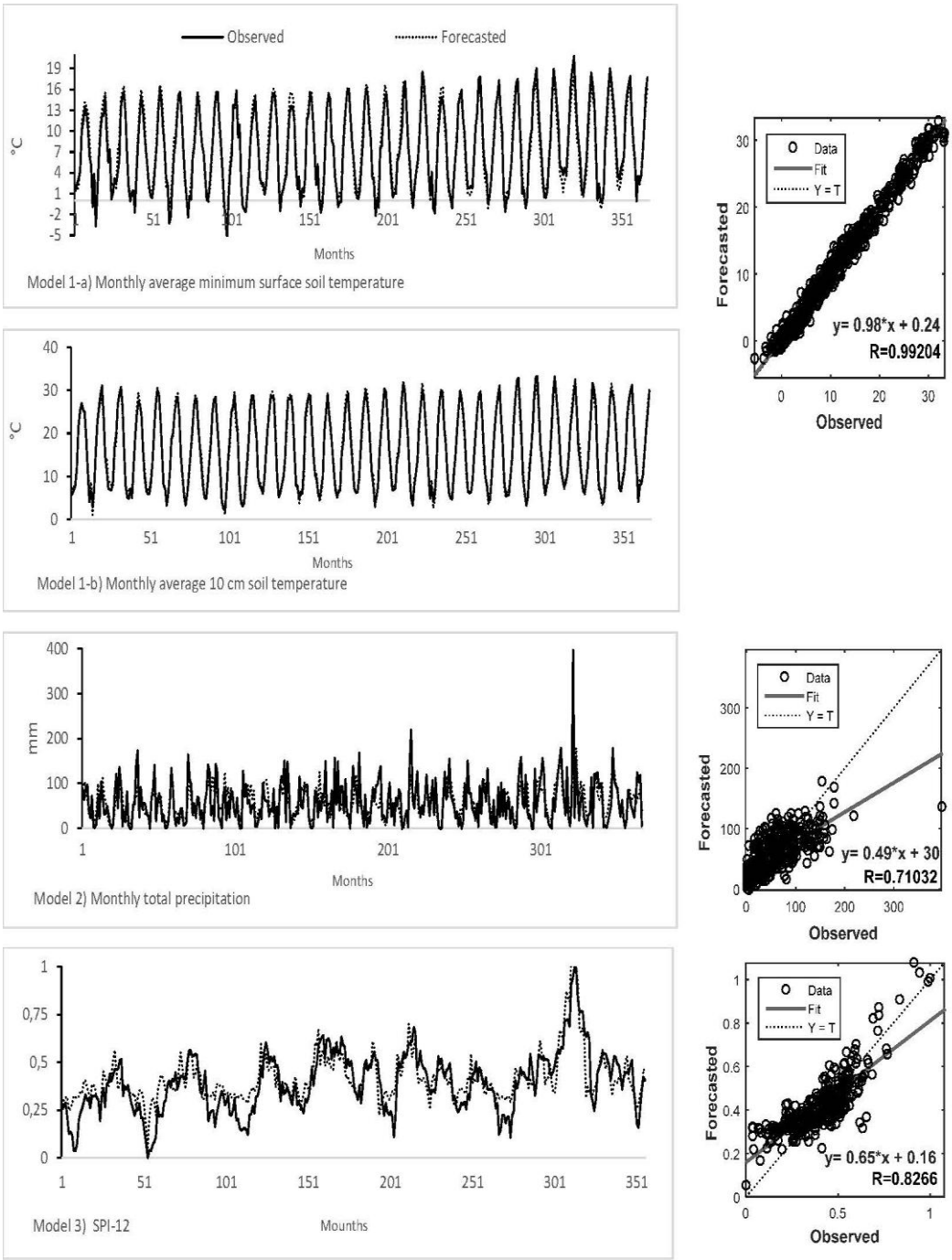


Figure 5. *Time series of observed and forecasted values with the model structures and the corresponding scatter plots*

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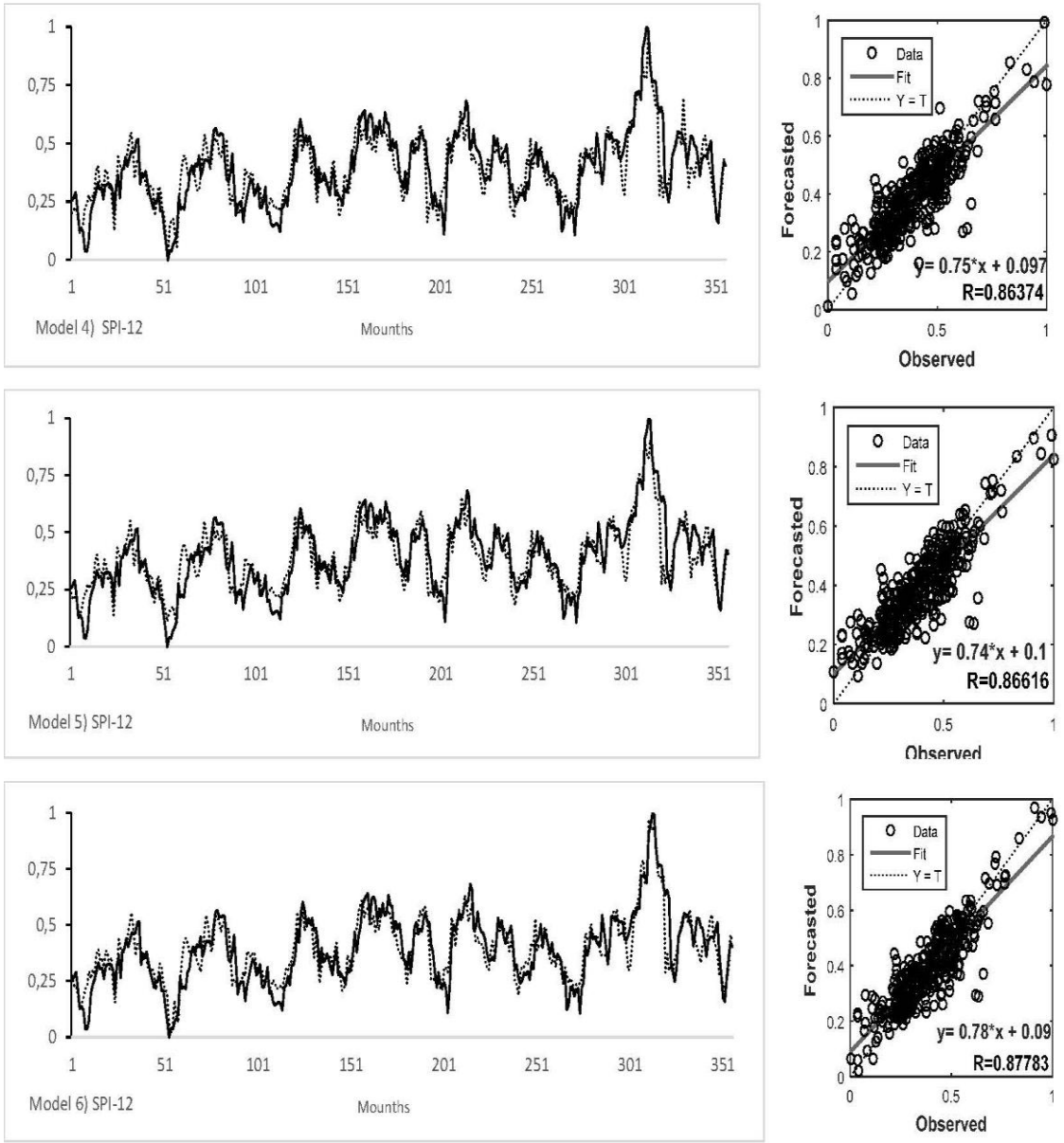


Figure 6. *Time series of observed and forecasted values with the model structures and the corresponding scatter plots (continued)*

Conclusion

Drought is a climate element that has devastating consequences on national economy and public life and Turkey is located in the semi-arid region. Therefore, monitoring and precautions for drought species are crucial.

In this study, time series of the Standardized Precipitation Index (SPI) are constructed for different periods based on monthly mean precipitation values, to determine drought categories. It was determined that the most of the percentages (67-72%) for all SPI values (3, 6, 9, 12 months) was in the near normal class close to Marmara Region-Turkey.

Not only benefiting from the precipitation series, other meteorological variables were also used for examination of drought. If precipitation data is absent, it is not possible to

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account for the drought index. Therefore, soil temperatures in Bursa City-Turkey as an indicator of agricultural and meteorological droughts were simulated with ANN model in this study. In addition, total precipitation and 12 month Standard Precipitation Index (SPI-12) parameters were also simulated with Artificial Neural Network Model had different structures.

Table 5. *The model structures and their performance functions (MSE) values and correlation coefficients (R) of the data sets*

Model No	Inputs	Outputs	ANN	MSE			R			
				Training	Validation	Testing	Training	Validation	Testing	Whole data set
Model 1	Monthly average air temperature (°C) Monthly average maximum air temperature (°C) Monthly average minimum air temperature (°C) Monthly total global insolation intensity (cal/cm²) Monthly average-Daily total global insolation intensity (cal/cm²) Monthly total precipitation (mm)	a) Monthly average minimum surface soil temperature (°C) b) Monthly average 10 cm soil temperature (°C)	6-1-2	1.3091	1.0263	0.8606	0.9917	0.9928	0.9945	0.99204
Model 2	Monthly average air temperature (°C) Monthly average maximum air temperature (°C) Monthly average minimum air temperature (°C) Monthly average minimum surface soil temperature (°C) Monthly average 10 cm soil temperature (°C) Monthly total global insolation intensity (cal/cm²) Monthly average-Daily total global insolation intensity (cal/cm²) Monthly average relative humidity (%)	Monthly total precipitation (mm)	8-1-1	1192.0973	741.9998	1142.2332	0.6761	0.8199	0.7924	0.71032
Model 3	SPI-3 Month SPI-9 Month	SPI-12 Month	2-2-1	0.0087	0.0068	0.0092	0.8262	0.7104	0.8832	0.8266
Model 4	SPI-3 Month SPI-9 Month	SPI-12 Month	2-5-1	0.0062	0.0050	0.0095	0.8657	0.8989	0.8488	0.86374
Model 5	SPI-3 Month SPI-6 Month SPI-9 Month	SPI-12 Month	3-2-1	0.0062	0.0084	0.0051	0.8644	0.7991	0.9192	0.86616
Model 6	SPI-3 Month SPI-6 Month SPI-9 Month	SPI-12 Month	3-7-1	0.0059	0.0076	0.0036	0.8841	0.8334	0.8714	0.87783

It was confirmed that 5 models of the 6 models were successful except for model 2. It was found that R and MSE values calculated were acceptable and rising of hidden

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layers and input numbers in the model structures were ensured efficiently. Modelling the precipitation from the meteorological parameters (Model 2) was possible with some error. For this reason, it was found that simulations of SPI-12 and soil temperatures were indicative to meteorological and agricultural droughts. Also this study showed that ANN was effective in forecasting the SPI and was effective tool for drought forecasting.

Finally, the developed ANN models were found to be suitable to make effective extreme point predictions. Drought predictions made by ANN models would be useful for local administrations and water resources planners. Hence, this paper presented an applicable approximation to this planning stage by means of the ANN modelling approach.

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GROWING SPINACH (*Spinacia oleracea* L.) IN A FLOATING SYSTEM WITH DIFFERENT CONCENTRATIONS OF NUTRIENT SOLUTION

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Abstract. This study was conducted in a greenhouse in Izmir/Turkey during the winter-early spring of 2016-2017, on spinach grown in the floating water culture system. Cultivation was carried out in aerated nutrient solutions on seedling trays, and peat was used as germination and rooting medium. Each seed was inserted to each hole (17 cc) in trays with 210 cells (957 plant m^{-2}). Following germination in the germination chamber, trays were moved to climate controlled greenhouse. After emergence, the seedlings were transferred to water culture on 07.12.2016 and 10.03.2017 for the 1st and 2nd period, respectively. The nutrient solution was applied as "full dose" (mg L^{-1}: N 150, P 50, K 150, Ca 150, Mg 50, Fe 5.0, Mn 0.50, Zn 0.05, B 0.50, Cu 0.03, Mo 0.02), "half dose" (with macro elements reduced by 50%) and "without nutrients" (water). Plants as baby leaves were harvested 3 times in both periods. Results showed that plant growth, yield, quality, and water consumption vary depending on the concentration of nutrient solution as well as the temperature during the growing season. Especially in the 2nd period when the temperatures were higher, plant water consumption and yield increase and earliness were achieved. It was observed that as the concentration of nutrient solution decreased, vitamin C, leaf Ca content and pH value of the nutrient solution increased while leaf N, P, K and Fe contents, EC value of the nutrient solution, leaf nitrate content, yield and plant biomass decreased. There was no statistical difference between full and half dose applications in many measured parameters. It is concluded that spinach can be grown as a baby leaf in a floating water culture and that in addition to reducing nitrate content, half-dose application may be preferred in terms of yield and water consumption, and production should be done during early spring season in greenhouse conditions for high yield and quality.
Keywords: *water culture, growing period, dose, yield, nitrate, vitamin C*

Introduction

In world markets, the demand and interest in clean and minimal processed food stuffs is continuously increasing with each passing day. Life styles of people on the other hand have evolved into a practical and a swift style. Employed or working women are able to spend less time in kitchens; therefore, they mostly prefer freshly consumed, instant, practical and healthy foods. Edible leaf vegetables are quite rich in vitamins and antioxidants (Yadav et al., 2013) and they are commonly used in salads and several other dishes. Thus, they are quite preferred by the consumers. These vegetables should be cleaned right after the harvest and such a cleaning process results in serious waste of time and water. In recent years, soilless culture, especially hydroponic culture, have become popular to facilitate the cleaning of edible leaf vegetables and to serve them for consumption with a minimum processing (Nicola et al., 2007).

Among the hydroponic culture systems, static solution culture is the oldest and easiest soilless culture technique. In today's modified version of the technique, plants

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are placed over styrofoam platforms freely floating over the nutrient solution and thus the method is called as "floating water culture" (Morgan, 1999; Gül, 2008). The system is composed of a basin containing nutrient solution, a light-weight material able to float over the nutrient solution to hold the seeds or seedlings and an air pump for aeration of the system.

Floating water culture was designed and developed by Arizona University in 1980; couple years later, was used in vegetable culture commercially in Florida (Resh, 1998). The method is commonly used in seedling production (Carrasco et al., 2003; Bilalis et al., 2010) and regular or miniature vegetable production (Fontana et al., 2010; Carrasco et al., 2011). The system today is widely used in unheated greenhouses of Mediterranean countries with minimum control of climate parameters for the same purposes (Duyar and Kılıç, 2016).

The advantages of floating hydroponic systems are specified as: easy system construction, economic production, low labor costs because of automation systems, rapid plant growth, homogeneous products, high and quality yields, clean products, greater number of plants per unit area, easy harvest, optimized fertilizer and water use, minimum evaporative losses, environment-friendly production and easy adaptation to small production areas (Fernandez et al., 2008; Franco et al., 2011; Tomasi et al., 2015). On the other hand, aeration demands of nutrient solution and resultant electricity requirement, changes (increase or decrease) in temperature of nutrient solution based on seasons are considered as potential disadvantages of the floating systems (Nicola et al., 2005; Rodriguez Hidalgo et al., 2010). Despite all these disadvantages, floating systems are largely preferred for production of various vegetable species since in soil plants are exposed to several soil-borne diseases, leaves are contaminated with soils resulting in leaf losses. The vegetables produced in soil cultures should also be cleaned and washed through before to serve them to the markets. When the leaves are washed and served to markets, leaf heat ups and resultant losses are experienced. On the other hand in floating hydroponic cultures, contamination with soil is totally eliminated, thus significant savings achieved in labor and water since post-harvest washing is not required.

Besides clean production, low nitrate contents are desired in directly consumed edible leaf vegetables. Excessive nitrogen uptake (excessive fertilization) or inhibition of nitrogen transformation into protein by some environmental factors result in nitrogen accumulation throughout the plant growth stages (Kara, 1993). A small part of nitrogen taken through nutrition is reduced to nitrite by the mouth bacteria and conveyed to the stomach. When the pH level of the stomach got above 5.5, nitrate is converted into hazardous nitrite ions through bacterial nitrate reductase activity. Nitrite then interacts with hemoglobin and result is methemoglobin formation; hemoglobin Fe^{+2} then oxidized to Fe^{+3} and ultimately blood oxygen carrying function is reduced or inhibited. Then the case is evolved into a hazardous disease for kids (methemoglobinaemia, blue-baby syndrome, cyanotic, cyanosis). Nitrite has various other negative impacts on human health, for instance it reacts with secondary amines and result in formation of carcinogen, mutagen and teratogen nitrosamines (Tannenbaum and Correa, 1985; Kara, 1993; Özdestan and Üren, 2010).

Existence of nitrate problems in leaf edible vegetables let the researchers mostly focus on nitrate in these species. Hydroponic researches revealed that micro element concentrations of the nutrient solution could be reduced (Cocetta et al., 2007; Duyar and Kılıç, 2016), nutrient solution could be diluted (Vernieri et al., 2005), plants could be stayed in water for couple days before the harvest (Gonnella et al., 2004; Jakse et al.,

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2013), NO_3-N could be withdrawn/replaced with Cl, SO_4 and NH_4 (Van Der Boon et al., 1990) all to reduce nitrate contents of the vegetables. These researches also revealed that floating culture could be used as an economic and environment-friendly culture technique.

Hoagland nutrient solution (Hogland and Arnon, 1950) is commonly used in floating culture researches undertaken for leaf edible vegetables (Vernieri et al., 2005; Cocetta et al., 2007; Alberici et al., 2008; Duyar and Kılıç, 2016). Although Hoagland nutrient solution supply quite quality nutrition for leaf edible vegetables harvested in short periods, it is known that this nutrient solution was primarily developed for tomato to get high yield in long growing periods. Thus in this case, potential use and efficiency of Hoagland nutrient solution in leaf edible vegetables are arguable issues. It was also considered that element contents of Hoagland prescription was higher than the prescriptions adapted for leaf edible vegetables and had quite much nutrient contents in post-harvest residues with potential environmental pollution (Alberici et al., 2008).

Spinach could be an alternative crop in greenhouses since it allows for multiple short-duration production cycles and a much faster economic return (Brandenberger et al., 2007). Besides, spinach is a vegetable with a high nutritional value (Ko et al., 2014) The present study was conducted to investigate the potential use of floating hydroponic culture for spinach production in unheated greenhouses in winter-early spring periods (1). Since there are not any species-specific nutrient solution prescription, availability of a nutrient solution other than Hoagland nutrient solution (winter nutrient solution prepared for lettuce) for spinach culture was also investigated in this study (2). Effects of dilution of macro element concentrations of the nutrient solution on plant growth and development, yield, quality and water consumption were also investigated in this study (3). In this way, high yield and quality spinach culture with floating hydroponic culture and proper nutrient prescription in proper periods in unheated greenhouses under Mediterranean climate conditions was assessed.

Materials and Methods

Experiments were conducted in vegetable greenhouse of Horticulture Department of Ege University Agricultural Faculty (Bornova-İzmir/Turkey, 38°27'17.03''N, 27°14'17.71''E) in winter-early spring growing periods of 2016-2017.

'Aletta F_1' (Küçükçiftlik Seed Co., Balıkesir/Turkey) spinach (*Spinacia oleracea* L.*)* cultivar was used as the plant material of the experiments. Styrofoam seedling trays (66.5 x 33.5 x 4.9 cm, with 17 cc, 210 cells) were used as the growing place. Peat (Klassman TS1) was used as the substrate for seed germination.

Seeds were sown in two successive periods on 24.11.2016 in the 1st period (winter) and on 21.02.2017 in the 2nd period (early spring) into the peat-filled seedling trays as to have one seed per cell (1.5 g seed per m^2). Excess plantlets were thinned after emergence. There were 957 plants per m^2. Following the seed sowing, growing medium were moistened, covered with stretch films and placed into germination chambers. Seedling trays were kept in germination chamber (dark, day/night 18-20°C, 80% humidity) for 4 days. They were then placed into seedling adaptation greenhouse and kept there until they had 1-2 true leaves. During this period, seedlings were irrigated with boom irrigation system of the greenhouse.

Hydroponic cultures were formed in 80×44×19 cm (52 liters) plastic basins (*Fig. 1*). PE-transparent air hoses were used to aerate nutrient solution. One end of the hose was

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connected to air compressor and the end was closed. The hose passed through culture basins and outlets with air stones were placed for each basin. Mini valves were used to adjust aeration rates (8.0-8.5 mg L^{-1}). Compressor electricity was supplied with a timer; the timer was set as 30:30 minutes start:stop mode for 24 hours.



Figure 1. *View of floating cultures in plastic basins*

The plant nutrient solution proposed by Gül (2008) for winter lettuce (mg L^{-1}: N 150, P 50, K 150, Ca 150, Mg 50, Fe 5.0, Mn 0.50, Zn 0.05, B 0.50, Cu 0.03, Mo 0.02) was used in this study. The recommended prescription was accepted as "**full dose**" (complete nutrient solution) and this full dose was compared with **"half dose"** with 50% reduced macro nutrients. Electrical conductivity (EC) of prepared nutrient solution was 1.6-1.8 dS m^{-1} in full dose; 1.2-1.3 dS m^{-1} in half dose while pH was 5.5-6.0 and 6.3-6.5, respectively. **"Water"** without any fertilizers was another treatment in order to test if irrigation water alone could be used due to short crop cycle. The content of irrigation water was as follows: N 7, P 1.10, K 1.19, Ca 127.4, Mg 45.8, Na 29.8, Cl 52.0, Fe 1.43, Mn 0.88, Zn 0.38, Cu 0.11 mg L^{-1}; EC 0.93 dS m^{-1}; pH 7.35. Each basin was filled with nutrient solution treatments or water (40 L). Seedlings were transplanted to water culture on 07.12.2016 for the 1st period and on 10.03.2017 for the 2nd period when they had 2 true leaves. Plants were kept in hydroponic culture for 59 days in the 1st period and for 38 days in the 2nd period. Throughout these periods, depleted nutrient solution or water was resupplied weekly and EC and pH adjustments were made in each time. Productions were terminated on 07.02.2017 for the 1st period and on 17.04.2017 for the 2nd period.

Greenhouse indoor temperature, relative humidity and solar radiation values were measured with sensors (Delta-T Devices) and recorded with data loggers (Delta-T Devices; GP1 and DL3000). Manual thermometers were used to measure the temperature of nutrient solution. Temperatures (°C) of the nutrient solutions and water treatments were measured in the morning, noon and evening of each day.

Harvests were performed when the outer, older leaves reached to a length of 10-15 cm. When the plants reached to harvest maturity, 10 homogeneous plants were selected from each replication and plant growth parameters were measured. Root lengths (from top to bottom) were measured with a tape meter and average root length was expressed in cm. The distance between the starting place of the roots and the tip of plant leaves was measured again with a tape meter (cm) and the values were used as plant height. Leaf fresh weight of the plants was measured with a precise balance. Leaves were dried

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at 65 ºC until a constant weight and weighted again to get leaf dry weights. The values were used as fresh (FW) and dry (DW) weights (g) and dry matter (%).

The first harvest was performed 33 days after transplantation to hydroponic culture in the 1st period and 20 days after transplantation in the 2nd period. A total of three harvests were performed on 10.01.2017, 27.01.2017 and 07.02.2017 in the 1st period and on 30.03.2017, 07.04.2017 and 17.04.2017 in the 2nd period. In each harvest, plants were cut above the growing tip to allow plants to regrow new leaves. Harvests were terminated after the 3rd harvest because of the losses in leaf quality. From the first to last harvest, plants harvested from each replication were weighted separately and yields per harvest (g) and total yields (g m^{-2}) were calculated.

From each replicates, 10 plants were randomly selected to determine leaf total chlorophyll content (mg g^{-1}) (Arnon, 1949), nitrate content (mg kg^{-1}) (Cataldo et al., 1975) and vitamin C content (mg 100 g^{-1} FW) (Pearson, 1970) spectrophotometrically.

Plant samples taken from the first harvest were separated as roots and upper sections and dried at 65°C. Dried samples were than ground and passed to sieves and subjected to N, P, K, Ca, Mg and Fe element analyses. P content of wet digested samples was determined colorimetrically with Vanadomolibdophosphoric yellow color method; K and Ca contents were determined with a flame photometer; Mg and Fe readings were performed in an atomic absorption spectrophotometer. Total N was determined with modified Kjeldahl method. Results were expressed as % dry matter and mg kg^{-1} DW (Kacar and İnal, 2008).

Plant water consumption (L $plant^{-1}$) was calculated using the following *Equation 1:*

$$Plant\ water\ consumption = \frac{applied\ nutrient\ solution - drainage\ solution}{number\ of\ plant} \quad \text{(Eq.1)}$$

Nutrient solution and water samples were taken from the basins twice a week and EC and pH of the samples were measured with a portable EC and pH meter.

In the present research, 105 plants were used per replicate. Experiments were conducted in randomized plots experimental design with 4 replications and two factors (periods - 1st and 2nd period; nutrient solution concentrations - full dose, half dose and water without fertilizer). Resultant data were subjected to variance analysis with JMP (version 5.0.1) statistical software. Tukey's test was used to compare treatment means. F test was used to specify significance levels (ns: nonsignificant, *: significant at $0.01<P\leq 0.05$ and **: significant at $P\leq 0.01$).

Results

Climate data

Average greenhouse indoor temperatures and relative humidity values were 11.3°C and 72.5% in the 1st period, 19.8°C and 71.1% in the 2nd period, respectively. Solar radiation value was 5.55 MJ m^{-2} day^{-1} in the 1st period and 10.64 MJ m^{-2} day^{-1} in the 2nd period.

Nutrient solution temperatures varied between 3.5 and 32.0ºC (average 10.7 ºC) in the 1st period and between 9.2 and 37.5 ºC (average 20.0 ºC) in the 2nd period.

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Plant growth and biomass values

The effects of growing periods on plant height, root length, leaf fresh and dry weights and dry matter contents, root dry weight and dry matter contents were found significant. All these parameters had greater values in the 2nd period than in the 1st period. In the 2nd period, plant heights increased by 27.1%, root lengths increased by 17.5%. As compared to the 1st period, plant fresh and dry weights increased respectively by 1.9 and 2.6 folds in the 2nd period (*Table 1*).

Nutrient solution concentration had significant effects on plant heights, root lengths, leaf fresh and dry weights and leaf dry matter. There were not significant differences in these measured parameters of full and half doses but water-treated plants had the lowest values. However, the greatest root length was obtained from water treatments (*Table 1*).

Period x nutrient solution concentration interaction had significant effects on plant height, root length, leaf biomass and root dry matter. The highest plant heights, leaf fresh and dry weights and leaf and root dry matters were higher in the 2nd period (early spring) in full and half dose treatments. The lowest measurements were obtained from water treatment excluding root lengths (*Table 1*).

Table 1. *Effects of different season and nutrient solution concentration on plant growth and biomass*

Period	Nutrient solution concentration	Plant height (cm)	Root length (cm)	Leaf			Root		
				Fresh weight (g)	Dry weight (g)	Dry matter (%)	Fresh weight (g)	Dry weight (g)	Dry matter (%)
1st		10.7 b	27.5 b	2.67 b	0.23 b	8.8 b	0.54	0.035 b	5.8 b
2nd		13.6 a	32.3 a	5.66 a	0.64 a	12.8 a	0.57	0.049 a	8.5 a
	Full dose	13.8 a	26.5 b	4.96 a	0.52 a	10.1 b	0.60	0.046	7.5
	Half dose	13.1 a	29.0 b	4.85 a	0.48 a	9.6 b	0.55	0.042	6.9
	Water	6.6 b	34.2 a	1.20 b	0.17 b	14.6 a	0.51	0.038	7.2
1st	Full dose	11.5 ab	24.3 c	3.05 b	0.26 b	8.4 b	0.62	0.037	5.6 cd
	Half dose	11.2 ab	25.3 bc	2.89 b	0.24 b	8.2 b	0.51	0.035	5.3 d
	Water	7.9 b	33.1 ab	1.47 b	0.16 b	10.8 b	0.47	0.032	6.7 bcd
2nd	Full dose	16.2 a	28.7 abc	6.87 a	0.78 a	11.9 b	0.57	0.054	9.3 a
	Half dose	15.1 a	32.8 ab	6.82 a	0.72 a	10.9 b	0.58	0.049	8.4 ab
	Water	5.3 b	35.4 a	0.94 b	0.17 b	18.4 a	0.55	0.044	7.6 abc
Analysis of variance	*Period*	*	*	**	**	**	*ns*	*	**
	Concentration	**	*	**	**	**	*ns*	*ns*	*ns*
	Period x Concentration	*	*	*	**	*	*ns*	*ns*	*

Means within each column followed by the same letters are not significantly different according to Tukey test.
ns, * and **: nonsignificant, significant at $0.01 < P \leq 0.05$ and $P \leq 0.01$, respectively.

Yield

Effects of the period, nutrient solution concentration and period x concentration interaction on yields values were found to be significant (*Table 2*, *Fig. 2*). The highest yield values were obtained from the 1st harvest. As compared to the 1st harvest, yield values in the 2nd and 3rd harvests respectively decreased by 41.5% and 50.3% in the 1st period and by 72.1% and 52.3% in the 2nd period.

In all three harvests, full dose-treated plants had the highest yield values, but they were placed in the same statistical group with the half dose-treated plants. As compared to the full dose, yields in half dose and water treatments increase respectively by 18.7% and 71.8% in the 1st harvest, by 10.0% and 70.8% in the 2nd harvest and by 18.8% and

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84.0% in the 3rd harvest. With regard to yield values under interactions, the greatest yields in the 1st and 3rd harvests were obtained from 2nd period x full dose treatments and the greatest yields in the 2nd harvests were obtained from 2nd period x full dose treatments. Yields decreased in water treatments and the lowest yield values were obtained from water treatments in both periods (*Table 2*).

Table 2. *Effects of different growing periods and nutrient solution concentrations on yield of harvests*

Period	Nutrient solution concentration	Yield of 1st harvest (g m^{-2})	Yield of 2nd harvest (g m^{-2})	Yield of 3rd harvest (g m^{-2})
1st		1256.2 b	733.9 a	623.3 b
2nd		1998.7 a	556.0 b	953.3 a
	Full dose	2083.1 a	788.2 a	1041.6 a
	Half dose	1692.7 a	709.1 a	845.9 a
	Water	585.8 b	229.9 b	166.6 b
1st	Full dose	1461.9 bc	911.1 a	820.9 bc
	Half dose	1303.7 cd	787.2 ab	655.0 c
	Water	749.6 cd	272.9 c	164.5 d
2nd	Full dose	2704.3 a	665.3 ab	1262.3 a
	Half dose	2081.6 ab	631.1 b	1036.7 ab
	Water	422.0 d	186.8 c	168.6 d
Analysis of variance	*Period*	*	*	*
	Concentration	**	**	**
	Period x Concentration	*	*	*

Means within each column followed by the same letters are not significantly different according to Tukey test.
* and **: significant at $0.01<P\leq 0.05$ and $P\leq 0.01$, respectively.

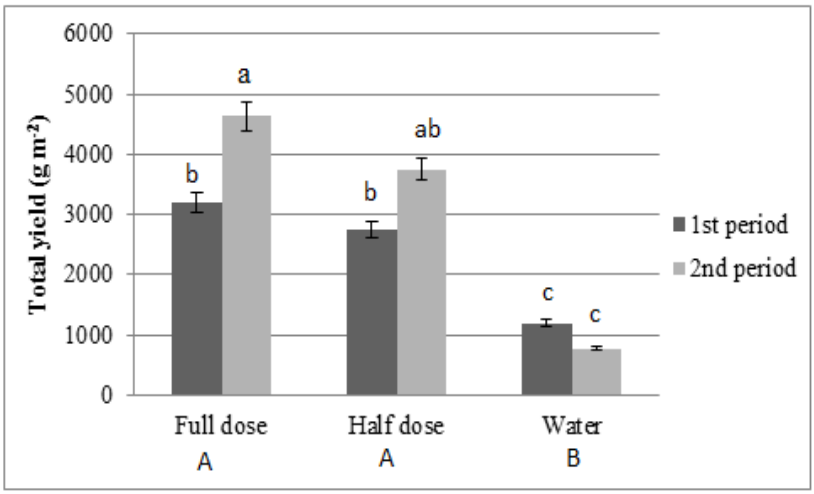


Figure 2. *Total yields of treatment in both growing periods*

Period: 0.01<P≤0.05, Concentration: P≤0.01, Period x Concentration: 0.01<P≤0.05
Capital letters indicate main effects of nutrient solution concentration; small letters show interaction effects of period and concentration.

The total yield was 2613.3 g m^{-2} in the 1st period and the value reached to 3508.0 g m^{-2} with 34.2% increase in the 2nd period. Total yield values of full dose, half dose and water treatments were respectively measured as 3912.8, 3247.6 and 982.2 g m^{-2}. As

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compared to full dose, 17.0% decrease was observed in half dose and 74.8% decrease was observed in water treatments. The greatest total yield was obtained from the 2nd period x full dose interaction and it was followed by the 2nd period x half dose which was placed in the same statistical group (*Fig. 2*). Of the total yield, 53.2% was obtained from the 1st harvest, 21.1% from the 2nd harvest and 25.7% from the 3rd harvest.

Quality parameters

Total chlorophyll contents were significantly influenced by the period, nutrient solution concentration and period x concentration interaction. While the total chlorophyll content was 1.18 mg g^{-1} in the 1st period, the value reached to 1.64 mg g^{-1} with 38.9% increase in the 2nd period. Total chlorophyll contents did not significantly change in full dose and half dose treatments, but decreased by 12.5% in water treatments. The greatest chlorophyll content was obtained from the 2nd period x full dose interaction and it was followed by the 2nd period x half dose interaction which was also placed in the same statistical group (*Fig. 3*).

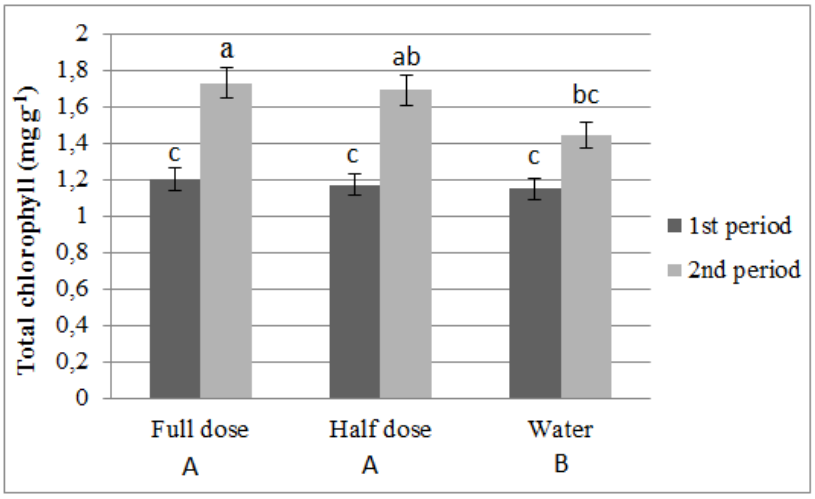


Figure 3. *Effects of treatments on total chlorophyll content*
Period: P≤0.01, Concentration: 0.01<P≤0.05, Period x Concentration: 0.01<P≤0.05
Capital letters indicate main effects of nutrient solution concentration; small letters show interaction effects of period and concentration.

While periods did not have significant effects on leaf nitrate content, nutrient solution concentration had significant effects on nitrate contents. Leaf nitrate contents decreased with reduced nutrient solution concentration, but the differences between full dose and half dose treatments were not significant. As compared to half dose, leaf nutrient contents decreased by 9.1% in half dose treatments. The lowest leaf nitrate content was obtained from water treatments. Considering the interactions, the lowest leaf nitrate contents were obtained from water-treated plants of both periods. Significant differences were not observed in leaf nitrate contents of the other treatments (*Fig. 4*).

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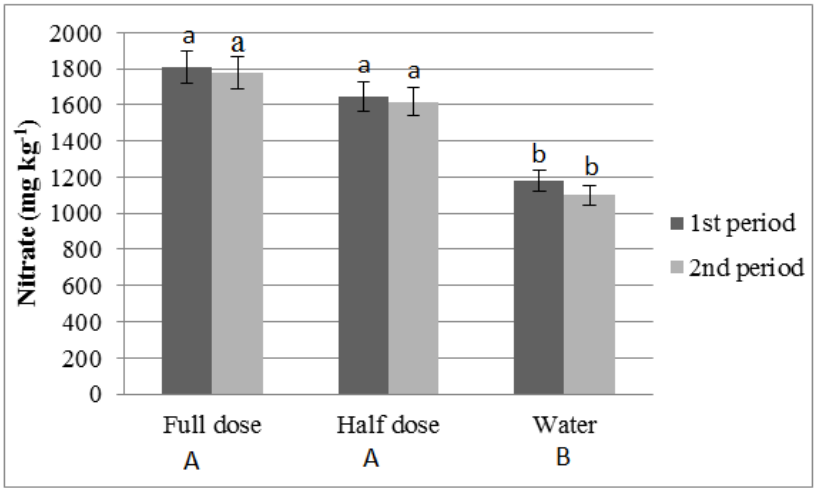


Figure 4. *Effects of treatments on leaf nitrate content*
Period: nonsignificant, Concentration: 0.01<P≤0.05, Period x Concentration: P≤0.01
Capital letters indicate main effects of nutrient solution concentration; small letters show interaction effects of period and concentration.

Vitamin C contents were also significantly influenced by the period, nutrient solution concentration and period x concentration interaction. As compared to the 1st period, vitamin C content reached to 47.5 mg 100 g^{-1} with 43.5% increase. Leaf vitamin C contents increased with decreasing nutrient solution concentration. Leaf vitamin C contents of full dose, half dose and water treatments were respectively measured as 29.8, 41.8 and 58.2 mg 100 g^{-1}. Under interaction, the greatest vitamin C content was obtained from the 1st period x water and the 2nd period x water treatments (*Fig. 5*).

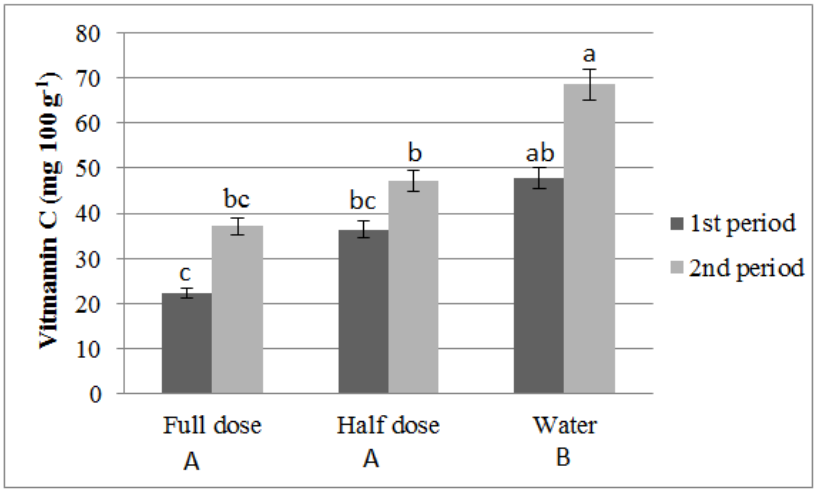


Figure 5. *Effects of treatments on vitamin C content*
Period: P≤0.01, Concentration: P≤0.01, Period x Concentration: P≤0.01
Capital letters indicate main effects of nutrient solution concentration; small letters show interaction effects of period and concentration.

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Leaf nutrient contents

Growing period had significant effects on P and Fe contents and the values were greater in the 1st period than in the 2nd period. The effects of growing period on N, K, Ca and Mg contents were not found to be significant (*Table 3*).

Table 3. *Effects of different growing periods and nutrient solution concentrations on leaf nutrient content*

Period	Nutrient solution concentration	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (mg kg^{-1})
1st		4.70	0.72 a	5.27	1.44	1.08	71.03 a
2nd		4.61	0.60 b	5.27	1.40	1.00	60.35 b
	Full dose	5.26 a	0.87 a	7.98 a	0.63 b	1.02	80.45 a
	Half dose	4.87 b	0.88 a	6.16 b	0.69 b	1.01	76.45 a
	Water	3.84 c	0.23 b	1.68 c	2.93 a	1.09	40.18 b
1st	Full dose	5.27 a	0.90 ab	7.84 ab	0.36 c	1.05	84.00 a
	Half dose	4.90 b	0.99 a	6.28 bc	0.39 c	1.07	82.40 a
	Water	3.93 c	0.28 c	1.70 d	3.55 a	1.12	46.70 c
2nd	Full dose	5.25 a	0.84 b	8.13 a	0.89 c	0.99	76.90 ab
	Half dose	4.83 b	0.77 b	6.04 c	0.99 c	0.95	70.50 b
	Water	3.76 d	0.19 c	1.66 d	2.32 b	1.06	33.65 d
Analysis of variance	*Period*	*ns*	*	*ns*	*ns*	*ns*	**
	Concentration	**	**	**	**	*ns*	**
	Period x Concentration	*	*	*	**	*ns*	*

Means within each column followed by the same letters are not significantly different according to Tukey test.
ns, * and **: nonsignificant, significant at $0.01 < P \leq 0.05$ and $P \leq 0.01$, respectively.

Except for Mg, nutrient solution concentration and period x concentration interaction had significant effects on all nutrients. P, Ca and Fe contents of full and half dose treatments were not significantly different, but water treatments had significantly different values. N, P, K and Fe contents decreased, Ca contents increased with decreasing nutrient solution concentration. A similar case was also observed under interaction (*Table 3*).

Plant water consumption

Depending on seasonal temperature differences and length of growth periods, amount of water applied to treatments varied between 0.316-0.361 L plant^{-1} in the 1st period and between 0.387-0.434 L plant^{-1} in the 2nd period. About 23.7% more water was applied to plants in the 2nd period. Removed water quantity was greater in this period and plant water consumption was also 52.7% more as compared to the 1st period. Effects of period and period x concentration interaction on plant water consumptions were significant, but nutrient solution concentrations did not have significant effects on water consumptions. Plant water consumption was 0.163 L plant^{-1} in the 1st period and 0.249 L plant^{-1} in the 2nd period. Plant water consumptions of full dose, half dose and water treatments were respectively measured as 0.205, 0.215 and 0.197 L plant^{-1}. Considering the interactions, the greatest plant water consumption was obtained from the 2nd period x half and full dose treatments (*Fig. 6*).

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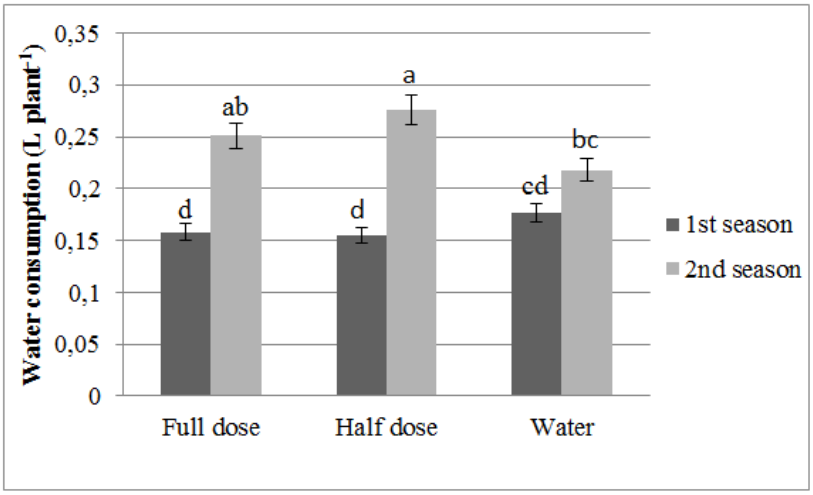


Figure 6. *Plant water consumption under interaction effects of treatments*
Period: P≤0.01, Concentration: nonsignificant, Period x Concentration: P≤0.01
Small letters show interaction effects of period and concentration.

Nutrient solution EC and pH values

Nutrient solution EC values of the 1st period varied between 0.75-0.90 dS m^{-1} in water treatments, between 1.16-1.40 dS m^{-1} in half dose treatments and between 1.56-1.75 dS m^{-1} in full dose treatments. The values were 0.8-1.0 unit higher in the 2nd period. Average EC of nutrient solution in full and half doses were 1.65 and 1.28 dS m^{-1} in the 1st period and 2.51 and 2.0 dS m^{-1} in the 2nd period, respectively.

Nutrient solution pH values were tried to be kept between 5.5 and 6.5 however, pH values of the 1st period varied between 6.62-7.89 in water treatments, between 6.38-7.35 in half dose treatments and between 5.59-7.23 in full dose treatments. In the 2nd period, pH values increased by 0.8-1.05.

Discussion

Plant development and biomass values of both periods revealed that plant development decreased with decreasing nutrient solution concentration. Similar findings were also reported in previous studies carried out on spinach (Cocetta et al., 2007) and rocket (D'Anna et al., 2003). In present study, there were not significant differences between the full and half dose treatments, but full dose treatments had better plant growth and development because of higher nutrient content of solution provided to the plants however water-treated plants had the least development due to lack of nutrients sufficient for plant growth.

Plant development data also revealed that besides nutrient solution concentrations, indoor temperatures of the growing periods influenced plant growth and development (Alberici et al., 2008). High temperatures of the 2nd period (average 19.8°C) stimulated plant development (Alberici et al., 2008; Eşiyok, 2012).

In present study, root lengths exhibited a different trend from the other plant development parameters. Water-treated plants had longer root lengths and this may be result from plant instincts to release their roots to deeper portions of the growth medium to get plant nutrients under nutritional stress conditions (Taiz and Zeiger, 2008).

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However root dry matter was the highest in full dose in the 2nd period which could be attributed either the nutrient concentration of solution or higher temperatures.

Hydroponic culture of miniature-leaf vegetables largely depends on environmental factors and species, but 3-6 weeks earliness is usually achieved with hydroponic culture compared with open field culture (Gonella et al., 2003 & 2004; Cocetta et al., 2007; Conte et al., 2008). Harvest was performed in present study 4 weeks after seeding and 20 days after transplantation to water culture in the 2nd period. In other word, an earliness was achieved with hydroponic culture (Eşiyok, 2012; Iannotti, 2017) due to higher temperatures, however in the 1st period lower temperatures delayed harvests up to 8 weeks after the seeding (Alberici et al., 2008; Eşiyok, 2012).

In hydroponic cultures yield varies according to plant species and cultivars, growth period temperatures, number of plants per unit area (plant m^{-2}), plant harvest lengths, growing periods, nutrient solution concentrations and number of harvests (D'Anna et al., 2003; Alberici et al., 2008; Jakse et al., 2013). For instance spinach yields for 1150 plant m^{-2} plant density and 10-15 cm harvest lengths was reported between 1130-1500 g m^{-2} (Cocetta et al., 2007) or between 832-1748 g m^{-2} with 12 cm harvest lengths (Alberici et al., 2008). In present study, plant density was 957 plant m^{-2} and harvests were performed at plant heights of 10-15 cm and 3 harvests were performed in each treatment. As the average of two periods, the 1st harvest yields at full dose, half dose and water treatments were respectively measured as 2083.1, 1692.7 and 585.8 g m^{-2}. Total yields were respectively calculated as 3912.9, 3247.7 and 982.2 g m^{-2} which were greater than the earlier results reported for spinach in hydroponic culture (D'Anna et al., 2003; Vernieri et al., 2005; Cocetta et al., 2007; Alberici et al., 2008; Rodriguez-Hidalgo et al., 2010; Duyar and Kılıç, 2016). Present findings revealed that spinach yields of hydroponic culture were also greater than the yield of soil-culture (Eşiyok, 2012). Water-treated plants had lower yield values than nutrient solution-treated (fertilized) plants. Such a finding indicated that very low amount of nutrients in the tap water and nitrogen coming from the nitric acid added to adjust element content and pH was not sufficient for plant growth and development particularly if the harvest period is extended.

In present study, yields decreased with decreasing nutrient solution concentrations. In previous studies, 20-50% decrease were reported with decreasing nutrient solution concentrations in rocket (Vernieri et al., 2005; Alberici et al., 2008; Duyar and Kılıç, 2016), spinach (Cocetta et al., 2007; Alberici et al., 2008) and lettuce (Alberici et al., 2008). Alberici et al. (2008) experimented 50% and 25% reduced macro elements as compared to control (100%) and reported varying decreases in yields of spinach, rocket and lettuce and recommended 25% reduction in micro nutrients. However, in our study full dose and half dose treatments were placed in the same statistical group. Thus, it was concluded that 50% reduction in macro element contents did not resulted in significant decreases in yields, thus could reliably be used in hydroponic spinach culture and provide significant savings in fertilizers and system costs.

Total chlorophyll contents varied between 1.15-1.73 mg g^{-1} and present values comply with the earlier findings (Cocetta et al., 2007; Franco et al., 2011). Total chlorophyll contents were not influenced by nutrient solution concentrations (Cocetta et al., 2007; Alberici et al., 2008), but influenced by growth periods (Fallovo et al., 2009). Present chlorophyll contents were higher in the 2nd period which could be attributed to higher temperatures (Popescu and Buruleanu, 2017).

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Leaf nitrate contents decreased with decreasing nutrient solution concentrations (Cocetta et al., 2007; Alberici et al., 2008; Fallovo et al., 2009; Duyar and Kılıç, 2016). Such a case can be explained with the decreased nitrate concentration of the plant tissues through using the nitrate already stored in vacuole in case of lower nitrate concentrations than the plant requirement (Okamoto et al., 2006; Tsay et al., 2006; Antonacci et al., 2007; Alberici et al., 2008). Besides genetic characteristics, growth period, light, temperature, growing conditions, fertilization and storage have also significant effects on plant nitrate accumulations (Premuzic et al., 2001; Frezza et al., 2005; Magnani et al., 2007; Kim and Ishii, 2007). In present study, leaf nitrate contents (1177.2-1811.9 mg kg^{-1}) also varied with the periods and nutrient solution concentrations which were in a harmony with previous research results (Cocetta et al., 2007; Alberici et al., 2008). European Commission Regulation No: 1258/2001 specify the maximum threshold value as 3500 mg kg^{-1} for spinach and lettuce (EUR-LEX, 2017). Present values were below this threshold value, thus did not exert any risks on human health.

Majority of leaf edible vegetables is a great source of vitamin C (Burrell and Ebright, 1940) and plant vitamin C contents may vary with the growing period and cultural conditions (Eşiyok et al., 2006). Spinach vitamin C contents were reported as 19.4 mg 100 g^{-1} by Cocetta et al. (2014), as between 25.0-71.0 mg 100 g^{-1} by Bergquist et al. (2007) and Çıtak et al. (2010), as 48 mg 100 g^{-1} by Bangash et al. (2011) and as 120.0 mg 100 g^{-1} by Fujiwara et al. (2005). Present values (22.42-68.60 mg 100 g^{-1}) comply with those earlier ones. Nutrient solution concentration influenced vitamin C contents and increasing vitamin C contents were observed with decreasing nutrient solution concentrations (Duyar and Kılıç, 2016). Vitamin C content of full and half dose treatments were not significantly different. Higher vitamin C contents of water-treated plants were resulted from nutrient stress conditions since plants increase vitamin C contents under stress conditions (Munzuroğlu et al., 2000). Zhang et al. (2016) related such a case with increasing mioinocytol oxygenize enzyme activity in ascorbic acid biosynthesis and recycle and decreasing ascorbate oxidase and glutathione reductase enzyme activities. Ascorbic acid accumulation increases with decreasing N concentrations.

Leaf nutrient contents vary based on plant species and cultivars, growing period, production system, fertilization program, plant tissue and sampling time (Kacar and Inal, 2008). In present study leaf N contents varied between 3.76-5.27%, P contents between 0.19-0.99%, K contents between 1.66-8.13%, Ca contents between 0.36-3.55%, Mg contents between 0.95-1.125 and Fe contents between 33.7-84.0 mg kg^{-1}. In both periods, leaf N, P, K and Fe contents decreased, but Ca contents increased with decreasing nutrient solution concentration. In IFA (1992) records, N, P, K, Ca, Mg and Fe contents of young spinach leaves harvested 40 days after plantation were respectively specified as 2.6%, 0.3%, 4.7%, 1.0%, 1.0% and 189 mg kg^{-1}. Barlas et al. (2011) reported N contents of rocket leaves as between 2.94-5.23% (average 4.32%), P contents as between 0.12-0.27% (average 0.25%), K contents between 3.99-5.98% (average 5.13%), Ca contents between 2.20-3.55% (average 2.95%), Mg contents between 0.33-0.79% (average 0.58%) and Fe contents between 183.3-776.9 mg kg^{-1} (average 350 mg kg^{-1}). Present N, Ca and Fe values of floating culture system were lower, but P, K and Mg contents were higher than those earlier findings. High nutrient contents may be related to sink effect of young leaves under existence of high nutrient concentrations (Gonnella et al., 2004).

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The amount of water applied to plants was greater in the 2nd growing period because of higher temperatures of the period. Since the system is a closed system, only the nutrient solution left at the end of the period was removed. Nutrient solution concentrations did not influence amount of water applied. Plant water consumption was the greatest in half dose treatments and the least in water treatments. However, the differences in water consumptions were not significant. Similar with the present findings, Duyar and Kılıç (2016) also reported higher water consumptions for half dose treatments in rocket and parsley grown in floating water culture in autumn and spring seasons and researchers indicated higher water consumption values in spring season.

Since plants are cultured in closed soilless systems, pH, EC and dissolved oxygen levels of the system should be carefully adjusted (Gonella et al., 2003). Potential EC and pH problems can be eliminated with a proper nutrient solution and dissolved oxygen problems can be eliminated through proper aeration of the system (Tesi et al., 2003; Lenzi et al., 2011).

Conclusion

Recent researches about floating hydroponic cultures mostly focused on practices to reduce system costs (seed bed ambient, ambient quantity). It is quite significant to find cheap and easily supplied seed germination media. In present study, peat (Klassman TS1) with quite high germination performance and can be easily supplied from the markets was used (Rodriguez-Hidalgo et al., 2010; Jakse et al., 2013) and a successful culture was achieved. Cocetta et al. (2007) used perlite in hydroponic study and reported yields as between 1.1-1.5 kg m^{-2}. Such values were lower than the value obtained from peat. Researches are still going on about different substrate materials. Amount of growing medium per seedling tray is also an important factor in reducing system costs. Jakse et al. (2013) achieved better outcomes from rocket hydroponic study with small-size cells, thus with greater plant density (160 cell seedling trays, 2000 seed m^{-2}). Further research is needed about optimum cell sizes for different species. In present study, 17 cc seedling trays with 210 cells (957 plant m^{-2}) were used and a successful culture was achieved. However, there is still a need for further research on seedling tray shapes (lined-trays), trays cell sizes and plant densities.

Considering entire results of the present study, it was concluded that (1) winter lettuce prescription (mg L^{-1}: N 150, P 50, K 150, Ca 150, Mg 50, Fe 5.0, Mn 0.50, Zn 0.05, B 0.50, Cu 0.03, Mo 0.02) could reliably be used for hydroponic spinach culture without any losses in yield and quality; (2) 50% macro nutrients reduced half dose (mg L^{-1}: N 75, P 25, K 75, Ca 75, Mg 25, Fe 5.0, Mn 0.50, Zn 0.05, B 0.50, Cu 0.03, Mo 0.02) could be used successfully in hydroponic spinach culture since the yields of half dose treatments were not significant different from the full dose treatments and half dose reduced leaf nitrate contents. In this way, significant savings could be achieved in fertilizer costs; (3) spinach culture should be performed under optimum climate conditions for high yield, quality and earliness since temperature is a limiting factor for growth and development of vegetables, yields are significantly influenced by low temperatures. However further researches are needed optimization for fast and continuous production at larger scale.

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HOW WELL DOES BORDERING FOREST COMPOSITION PREDICT TREE ESTABLISHMENT IN UTILITY CORRIDORS?

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Abstract. The relation between bordering tree forest composition, seed rain and tree seedling establishment was estimated in a powerline corridor of southern Quebec (Canada). Red maple was the most important tree species in the bordering forests, accounting for nearly half of the 18 tree species found, in terms of basal area. Seeds from 10 tree species were found in the seed traps, the large majority of which being from gray birch, followed by red maple, paper birch, and white ash. A good proportion of seeds from gray birch and eastern hemlock were dispersed in winter. Seedlings from 14 tree species were sampled in the right-of-way, the large majority being from red maple and gray birch. The relation between nearby bordering tree, seed and seedling abundances varied among tree species. Seed abundance collected within a single year appeared to be poorly related to tree representation and seedling abundance, suggesting high interannual variation in seed production. Distance to seed source was weakly related to seedling establishment for some species such as gray birch. However, for many species, tree composition in the neighboring forest was a good predictor of nearby seedling establishment, implying that dispersal limitation may be an important factor in determining tree composition.
Keywords: *seed dispersal, seedling establishment, tree composition, Acer rubrum, Betula populifolia*

Introduction

Tree establishment in open sites may be viewed as beneficial or detrimental depending on management purposes. On sites assigned to forest production, natural establishment contributes to reforestation, and cutting practices frequently include protection of seeding trees or forest patches in order to insure sufficient seed sources (Solarik et al., 2010). Lack of natural tree establishment may necessitate direct seeding (St-Denis et al., 2013) or planting (Corbin and Holl, 2012). In powerline corridors, on the other hand, tree establishment is often a nuisance and costly cutting operations are needed to keep a low vegetation cover that does not interfere with operation and safety (de Blois et al., 2004), Understanding the factors that influence seedling establishment in open sites can therefore contribute to better planning and vegetation management for a variety of purposes.

Because they act as seed sources, trees bordering an opening should be the best predictor of recolonization pressure (Martínez-Orea et al., 2014). However, bordering forest composition and seedling composition in the opening may not always be closely related. First, annual seed production, germination rate, and interannual variation vary depending on species and tree size (Hill et al., 1995). Also, seed rain at a particular location further depends on proximity to seeding trees and seed dispersion. Finally,

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seedling establishment varies between species depending on biotic and abiotic factors such as seed size, seed predation, soil requirement, herbaceous cover, predation, or climatic condition (Gill and Marks, 1991). All these factors interact to determine recolonization or tree invasion pressure.

How accurately can we therefore predict tree establishment potential from bordering tree composition alone? This question is important especially in the context of integrated vegetation management (IVM), where the goal is to minimize environmental damages and costs. IVM practices require adapting management strategies to the local ecological context (Nowak and Ballard, 2005). If the goal is to prevent or reduce tree invasion as in many utility rights-of-way, a sound assessment of tree invasion pressure is crucial to design efficient interventions. A situation where there is high invasion potential from a fast growing but little competitive tree species, for example, may require active prevention with the sowing of a competitive herbaceous cover to control invasion (de Blois et al., 2004), while sites with low invasion pressure may be best treated by targeting specific invaders as needed.

The objective of this study is to assess the relation between bordering tree forest composition, seed rain, and seedling establishment in a powerline right-of-way of southern Quebec, Canada. The work presented here is part of an ongoing long-term project that aims to design and implement efficient integrated vegetation management practices in powerline rights-of-way corridors.

Study site

The study site is located in Roxton County (45° 54'N, 73° 49'W), in the Montérégie Region of southern Quebec, Canada (*Fig. 1*).

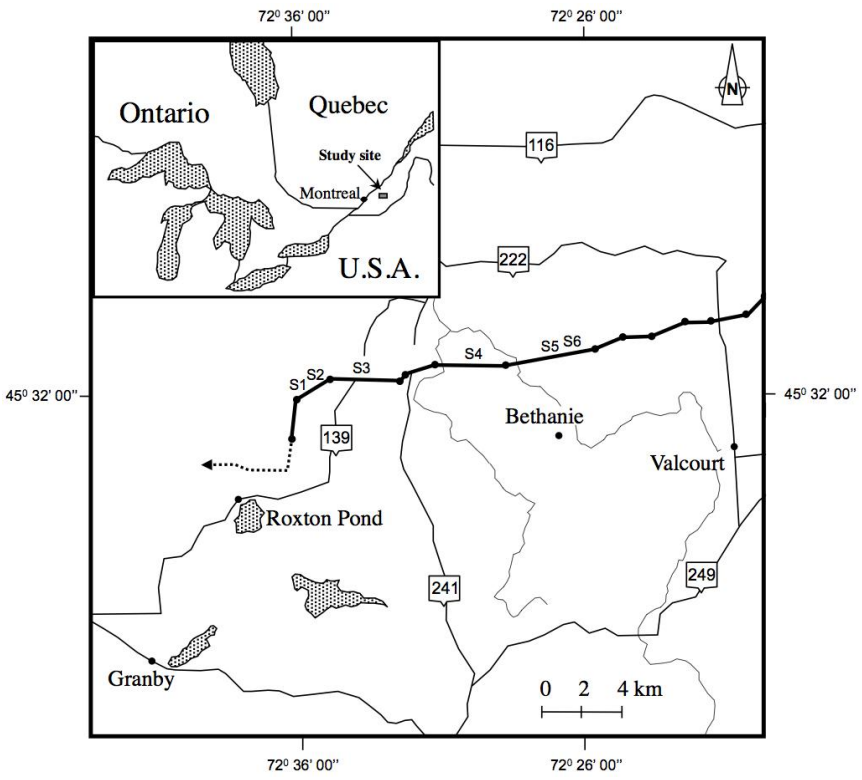


Figure 1. *Study site*

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This region has a humid continental climate with hot summers and cold winters. The mean annual temperature and total precipitation are, respectively, 6.3 °C and 1215 mm for the years 1981 to 2010 (Granby meteorological station). Mean maximal temperature is 25.2°C in July and –14.2°C in January (Environment Canada, 2015).

The sampling sites are distributed along a 14.6 km portion of a powerline right-of-way (*Fig. 1*). In this portion, the topography is slightly rugged, with a 79 m altitude difference between the first and last sampling site. A surficial deposit of sandy to gravel till lies over the bedrock made of calcareous shale and sandstone of the Sillery Formation (Prichonnet, 1984).

The Montérégie region is part of the Maple – Basswood Bioclimatic Domain (Ministère des Ressources naturelles du Québec, 2003) and the St. Lawrence Lowlands Ecoregion (Ecoregions Working Group, 1989). The studied powerline portion is mostly bordered by forests interspersed with cultivated fields and pastures. Mesic sites are mostly occupied by submature woodlands dominated by sugar maple (*Acer saccharum*) accompanied by red maple (*Acer rubrum*), yellow birch (*Betula alleghaniensis*), American beech (*Fagus grandifolia*), Ironwood (*Ostrya virginiana*) and eastern hemlock (*Tsuga canadensis*). These woodlands are often managed for maple sugar production. In the moderately drained sites, most of which are partially to severely disturbed by partial cutting, trembling aspen (*Populus tremuloides*), large-toothed aspen (*Populus grandidentata*), pin cherry (*Prunus pensylvanica*), paper birch (*Betula papyrifera*), white ash (*Fraxinus americana*) or gray birch (*Betula populifolia*) accompany red maple. Poorly drained sites are colonized by gray birch, balsam fir (*Abies balsamea*), willows (*Salix* sp), black ash (*Fraxinus nigra*) and white elm (*Ulmus americana*). Other common tree species found in the area include black cherry (*Prunus serotina*), eastern white cedar (*Thuya occidentalis*), tamarack (Larix laricina), balsam poplar (*Populus balsamifera*), red elm (*Ulmus rubra*) and eastern cottonwood (*Populus deltoides*) (Boivin et al., 2004).

The right-of-way is an 80m-wide corridor that has been deforested during a period going from September 1998 to February 1999, followed by the construction of the powerline. A portion of the right-of-way was grubbed and sown with an herbaceous mix while the rest was simply left as is.

Methods

Six sampling locations were selected in the right-of-way based on the following criteria: there had to be a forest border on each side of the right-of-way; the types of forest bordering the sampling sites had to cover a wide range of composition (*Table 1*). Data were collected in three phases over a period of 16 months (from April 2003 to August 2004) in order to characterize 1) forest border composition, 2) seed rain in the right-of-way, and 3) seedling establishment.

Bordering forest composition was sampled on July 2004 in two 80m x 20m plots adjacent to the right-of-way, one on each side, with the long axis perpendicular to the right-of-way (*Fig. 2*). We identified and measured the diameter at breast height (DBH) of every tree (DBH > 10 cm) in each plot. For gray birch, for which significant seed production starts when the trees are still small, we also counted the number of individuals in each of two small size-classes (sapling 1: 1-5 cm DBH; and sapling 2: 5-10 cm DBH). Because tree size for a particular species is generally correlated to seed production, we measured tree importance in terms of basal area.

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Table 1. *Characteristics of the sampled sites S1 to S6*

Name	Bordering vegetation: dominant [2] / co-dominant (forest age) [3]		Distance from S1 (km)
	North border	**South border**	
S1	Rma / Sma (young)	Rma / Las (young)	0
S2	Rma / Sma (young)	Rma / Tas (young)	0.4
S3	Sma / Rma (mature)	Rma / Sma (sub-mature)	3.8
S4	Rma / Gbi (sub-mature)	Rma / Tas (sub-mature)	8.6
S5	Rma / Ael (young)	Bfi / Tas (sub-mature)	13.7
S6	Gbi / Rma (young)	Rma / Wil (young)	14.0

1: Sowing was done with an herbaceous seeding mix (*Phleum pratense; Trifolium reprens; Trifolium pratense*) for revegetation purposes
2: Ael = American elm; Bfi = Balsam fir; Gbi = Gray birch; Las = Large-toothed aspen; Rma = Red maple; Sma = Sugar maple; Tas = Trembling aspen; Wil = willows
3: young (20-40 years); sub-mature (41-60 years) and mature (61-80 years). Estimations based on photointerpretation and field validation, reported on ecoforest maps of the Ministère des forêts (Québec, 1994).

Because certain types of seeds – notably birch seeds – are known to disperse on snow in winter, we used two types of seed traps to fully evaluate tree seed rain in the right-of-way. For summer seed rain, we used seed traps made of funnels of 30 cm of diameter (opening surface: 0.09 m^2), with a nylon mesh fastened at the small end to receive and store the seeds. The traps were fixed on a metal pole so that the opening end of the funnel was at 1 m above ground level in order to avoid the effect of the dense surrounding herbaceous vegetation. In April 2004, before seed fall of the earliest seed producer (red maple, starting seed fall in mid-May), 23 traps were installed in each sampling site, within a 10m-wide transect crossing the right-of-way (*Fig. 2*). Every month until late November, the nylon mesh were collected and replaced with new ones.

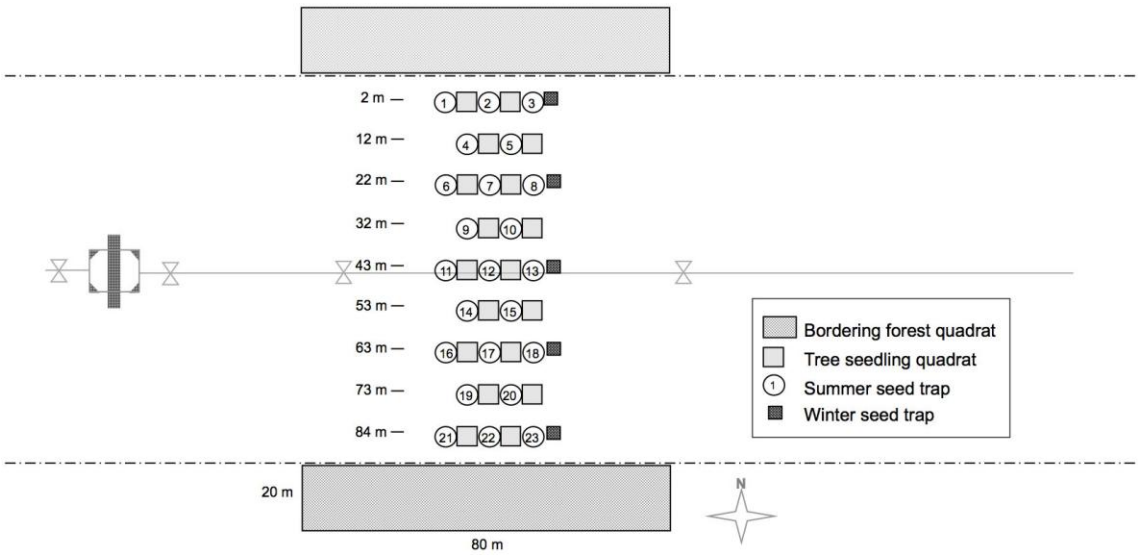


Figure 2. *Sampling design in a sampling site of the powerline right-of-way*

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Winter seed dispersion was evaluated using 1 m^2 nylon cloth pinned to the ground and covered with a screen of the same size to assure an adhesive surface for seed collection. Five of these winter seed traps were uniformly located along a transect crossing the right-of-way in each of the six sampling sites in fall 2013, before the first snowfall (*Fig. 2*). The traps were collected in the following spring, during snowmelt, in March 2004, in order to collect all seeds deposited over the $1m^2$ surface during winter. The snow remaining on the traps was melted and the organic material was filtered and added to those accumulated on the traps, and all tree seeds were sorted, dried, identified and counted. Birch seeds were identified using Kenneth and Brinkman (1974).

Tree seedlings were identified and counted in 18 $1m^2$ plots uniformly located in a transect crossing the right-of-way in each sampling sites of the same year. Information about the height and age were noted (*Fig. 2*). The number of seeds and seedlings counted in the plots along a transect were pooled in order to examine the relation between bordering tree, seed and seedling abundances between sites. For red maple and gray birch, the large number of seeds and seedlings allowed the study of their distribution along each transect, thus further allowing to study their spatial relation within sites.

Correlations using non-parametric Spearman tests were determined between 1) the distance to the nearest border and the abundances of seeds or seedlings (for red maple and gray birch only) and 2) seed abundance in summer and winter (for gray birch). Statistical tests were done using JMP statistical software version 6.

Results and discussion

All 6 sampling locations combined, 18 tree species were present in the forest plots bordering the rights-of-way (*Table 2*). Red maple was by far the most important tree species, accounting for nearly half of the total basal area for all trees, followed by sugar maple, trembling aspen, balsam fir, large-toothed poplar and gray birch. All the other tree species had a relative basal area lower than 3%. We found seeds from 10 tree species in the winter and summer seed traps, the large majority of which being from gray birch (80.7%), followed by red maple, paper birch, white ash, and yellow birch (*Table 2*). Note that there were no paper birch trees in the bordering forest plots despite the fact that it represented more than 5% of the seeds in the seed traps, suggesting long-distance dispersal. A good proportion of seeds from gray birch, eastern hemlock, and yellow birch were dispersed in winter (*Table 2*). This was particularly notable for yellow birch, which had almost half of its seeds dispersed in winter, confirming the necessity of sampling winter-dispersed seeds for a complete assessment of seed rain at a particular location (Matlack, 1989).

We found no seeds for several tree species that are otherwise represented in the adjacent bordering forest plots, including species known to produce large number of wind-dispersed seeds such as trembling aspen and large-toothed poplar. This absence may be due to a large interannual difference in seed production, with low production the year of our sampling.

Seedlings from 14 tree species were sampled in the right-of-way, the large majority being from red maple and gray birch (*Table 2*). There is generally little correspondence for species relative abundance between the tree – seed – seedling datasets, but there is a close relation for the relative rank of a species between the tree and seedling datasets: of the 6 most abundant trees in the bordering forests, 5 are also the most represented in the

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seedling sets, with both red maple and trembling aspen keeping the same rank (*Table 2*). There is little relation, both in terms of relative abundance or rank, between seed abundance and either tree abundance in the bordering forest or seedling abundance in the right-of-way. Again, this may be due to large interannual difference in seed production (Houle 1999).

Table 2. *Abundance and ranking of bordering trees, seeds and seedlings of the tree species in the right-of-way*

Species	**Tree basal area**			**Seed density (% winter)**			**Seedlings density**		
	(m^2/ha)	%	rank	Seeds/m^2	%	rank	Seedl./m^2	%	rank
Red maple	6.86	46.8	1	33.6	10.7	2	3.44	51.0	1
Sugar maple	2.10	14.3	2	0.4	0.1	8	0.16	2.4	5
Trembling aspen	1.28	8.7	3	-	-	-	0.25	3.7	3
Balsam fir	1.19	8.1	4	1.3	0.4	6	0.17	2.5	4
Large-toothed aspen	1.18	8.0	5	-	-	-	0.02	0.3	10
Gray birch	0.70	4.8	6	254 (17)	80.7	1	2.42	35.9	2
American elm	0.32	2.2	7	-	-	-	-	-	-
Black cherry	0.31	2.1	8	-	-	-	0.03	0.4	9
Yellow birch	0.15	1.0	9	3.6 (47)	1.1	5	0.06	0.9	7
White ash	0.15	1.0	10	4.0	1.3	4	0.05	0.7	8
Eastern white cedar	0.11	0.8	11	0.2	0.1	10	< 0.01	0.1	13
Tamarack	0.08	0.5	12	-	-	-	0.02	0.3	10
American beech	0.03	0.2	13	-	-	-	-	-	-
Ironwood	0.03	0.2	14	-	-	-	-	-	-
Eastern hemlock	0.03	0.2	15	0.4 (21)	0.1	9	-	-	-
Balsam poplar	0.02	0.1	16	-	-	-	-	-	-
Red elm	0.1	0.7	17	-	-	-	-	-	-
Pin cherry	0.02	0.1	18	0.4	0.1	7	0.08	1.2	6
Paper birch	-	-	-	16.9 (0.2)	5.4	3	-	-	-
Black ash	-	-	-	-	-	-	< 0.01	0.1	13
Eastern cottonwood	-	-	-	-	-	-	0.02	0.3	10

If we exclude species with little representation in the 3 data sets, we find that the relation between tree, seed, and seedling abundance in a particular sampling site varies largely among species (*Fig. 3*). The combined tree abundance of both borders is a good indicator of seedling presence within the right-of-way for red maple, sugar maple, balsam fir, white ash, and northern white cedar. Gardescu and Marks (2004) also found a close relationship between seedling emergence and seed source in red maple and white ash. The primary reason for this spatial relation is more likely attributed to a short distance dispersal than to local suitable habitat conditions since the absence of trees in a particular sampling site often results in a low number or in the absence of seeds (see sugar maple, balsam fir, white ash and eastern white cedar). For red maple, with trees

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present in all borders, there was a close relation between tree, seed and seedling abundances. For these species, seed dispersal is thus an important factor in explaining seedling establishment. In general, these species are also those that have the largest seeds, representing another example of the relation between dispersal limitation and seed size (Battaglia et al., 2008).

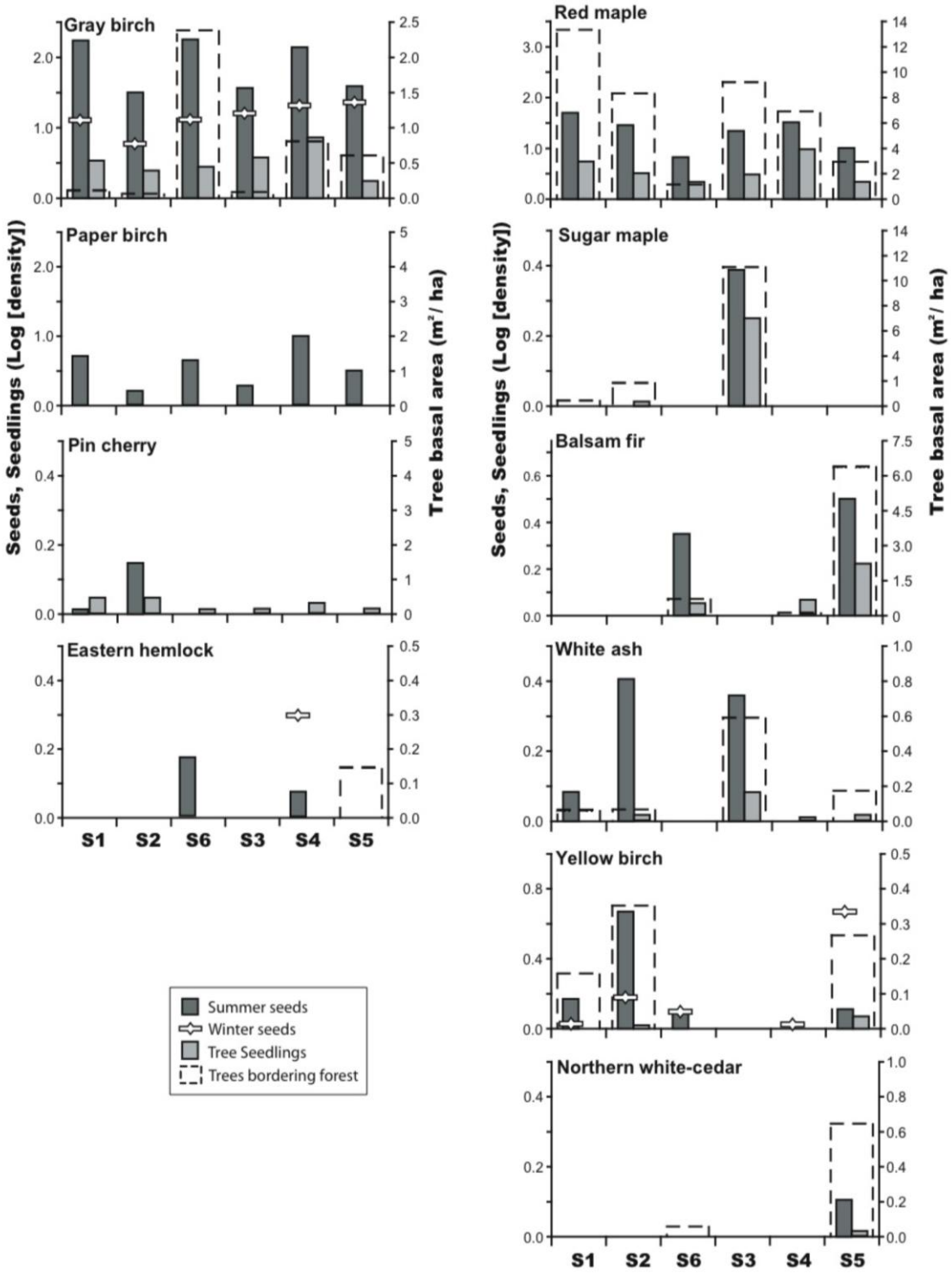


Figure 3. *Abundance of trees (in the bordering forest), seeds, and seedlings in each sampling site of the powerline right-of-way for the most common tree species*

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Other tree species showed little relation between tree and seed abundance within a sampling site. Gray birch seeds, both summer and winter dispersed, were present in great numbers in all sampling sites, irrespective of the abundance of seeding trees in the nearby border, suggesting a predominance of long-distance dispersal processes (Caughlin et al., 2014). Dispersal mechanisms in winter and summer did not create changes in dispersal patterns since the relative abundance in winter and summer appears to vary similarly. Paper birch seeds, present in all sites, also originated from more distant trees since they were absent in all borders. The large number of widely dispersed seeds combined with the total absence of seedlings suggest that paper birch does not find suitable conditions for seedling establishment in the right-of-way.

Dispersal of pin cherry seeds is known to be predominantly achieved by birds (Marks, 1974). While pin cherry trees are almost absent from the borders, seeds were found in all sites, although most often in small numbers. The two sites with the most pin cherry seeds were also the ones with the presence of seedlings. There was apparently no relation between the presence of eastern hemlock trees and the abundance of seeds (no seedlings were found), but the overall low tree and seed numbers in our sampling forces caution in interpreting this pattern.

Red maple and gray birch were sufficiently abundant in all sampling sites, both in terms of seeds and seedlings, to allow the study of their spatial patterns at the scale of a single sampling site. These two species are known to be aggressive colonizers (Lavoie, 1999, Abrams, 1998), and they both represent management problems in powerline rights-of-way (Boivin et al., 2004, Mercier et al., 2001). For red maple, there were often a decreasing number of seeds from the edge to the center of the right-of-way in several sampling sites (particularly apparent in S1, S2, S4, and S5) (*Fig. 4*). The same pattern occurred for seedlings in S5.

Overall, there was a significant negative correlation between the abundance of seeds and seedlings at a particular location in the rights-of-way and the distance from the nearest border ($r=-0.65$ and -0.42 respectively, $p<0.001$ in both cases). Both of these patterns indicate that the lower establishment in the center of the right-of-way is at least partly due to low seed dispersal distance at the scale of the transect. A greater tree seedling establishment at the edge of another powerline right-of-way was also related to the proximity of seeding trees and low seed dispersal distance by Brisson et al. (1997).

For gray birch, there was no relation between seed and seedling abundances and the distance to the nearest border ($r=-0.16$ and -0.02 respectively). Although spatial patterns of seed distribution for gray birch results from long-distance seed dispersion, the pattern along transects crossing the right-of-way implies smaller-scale dispersion processes, as shown by the apparent spatial autocorrelation in seed distribution within most sampling locations (*Fig. 4*). This may be due to local wind patterns within the rights-of-way.

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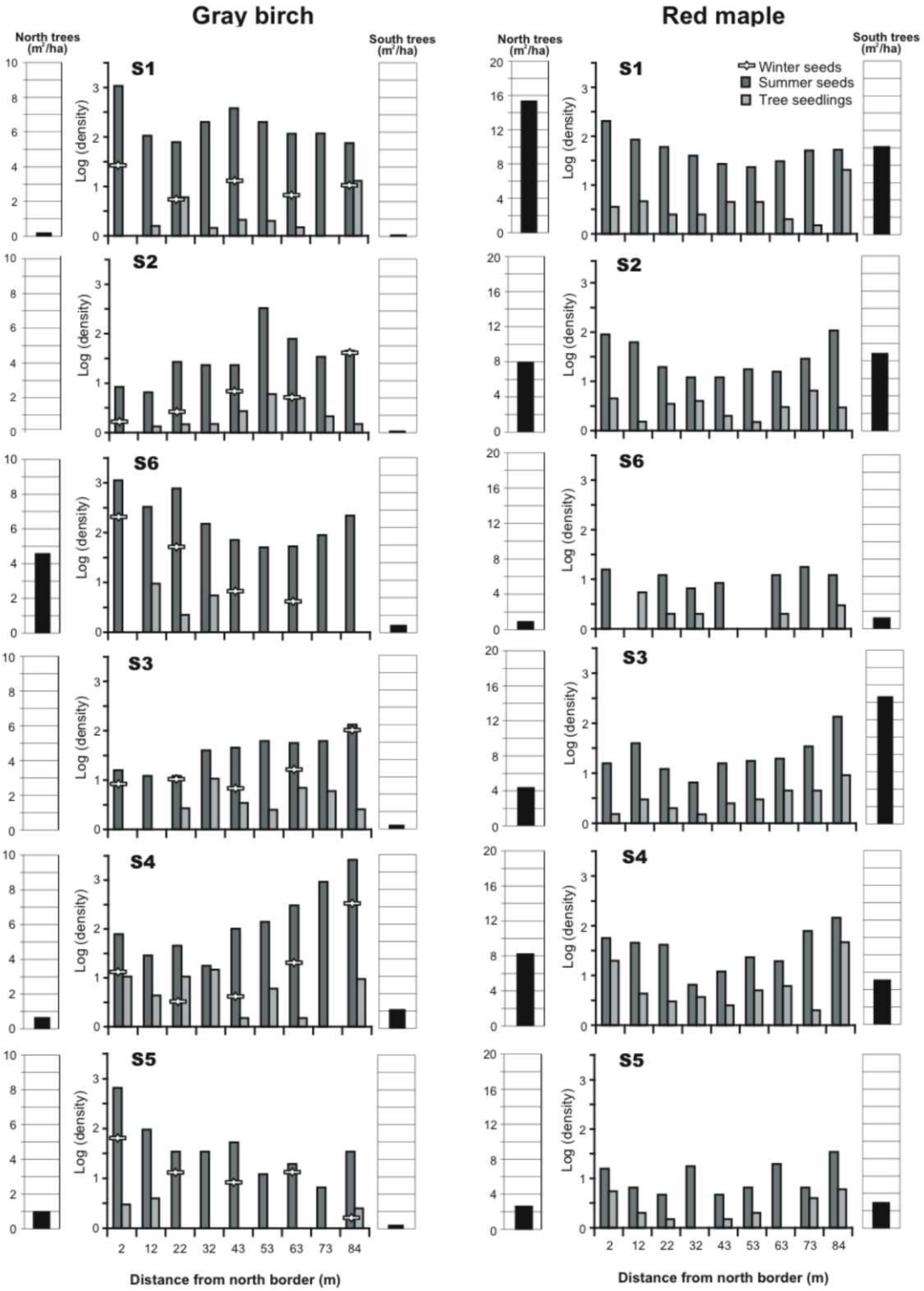


Figure 4. *Tree abundance for each forest border and spatial distribution of seeds and seedlings across the right-of-way for gray birch and red maple. The x-axis represents the distance (m), from the northern edge of the right-of-way to the center of the seed trap or seedling plot. At S3, the right-of-way was narrower, so that the transect had one plot less than for the other sites*

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Conclusion

Seed abundance collected within a single year appeared to be poorly related to tree representation in the bordering forest and seedling abundance in the right-of-way, most likely due to interannual variation in seed production (Wesołowski et al. 2015). We found that the relation between nearby bordering tree, seed and seedling abundances varies among tree species. Distance to seed source was not so important for natural seedling establishment for some species such as gray birch. Winter seed dispersal may contribute to its long-distance dispersal. However, for many species, tree composition was an overall good predictor of nearby seedling establishment in terms of ranking. While this may be partially related to local suitable conditions, it also suggests that dispersal limitation may be an important factor in determining tree composition (Abiyu et al. 2016). These results may better inform powerline rights-of-way managers about corridor portions that may need greater plant control operations, but they are also relevant to other applications such as forest restoration and forestry.

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Data accessibility. The field data used for this study are available at the Figshare online digital repository (https://figshare.com/); entry DOI: 10.6084/m9.figshare.6207431.

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DEGRADATION OF PEAT SURFACE ON AN ABANDONED POST-EXTRACTED BOG AND IMPLICATIONS FOR RE-VEGETATION

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Abstract. After peat extraction cut-over surface usually consists of moderately to highly humified peat which undergoes secondary transformation due to severely disturbed water conditions. The study was carried out within a mountain bog (Polish Carpathian) located in Orava-Nowy Targ Basin, southern Poland. The objectives of the study was to determine physical, hydrophysical and chemical properties of the upper layer of soil on two post-extracted areas of different age and to identify correlations between hydrophysical parameters and re-vegetation pattern. Vegetation was analysed in two groups: bog forming (*Sphagnum*, others) and non-bog forming (true mosses, trees, others). Secondary transformation of peat was quantitatively described by water-holding capacity index W_1. It correlated with thickness of residual peat and a range of properties of cut-over peat (e.g. porosity, bulk density, soil moisture content, ash content). The Principal Component Analysis (PCA) demonstrated the key importance of water table depth, residual peat thickness and hydrophysical conditions of the cut-over peat, especially water-holding capacity index W_1, soil moisture content and macropore volume on re-vegetation by typical bog species. Correlation of W_1 index with soil properties and *Sphagnum* occurrence indicates that it can be a useful indicator in evaluation of secondary transformation of cut-over bogs and therefore the potential for spontaneous regeneration of typical bog vegetation.
Keywords: *secondary transformation of peat, peat soil properties, water retention, cut-over peatland, peat quality*

Introduction

The European continent experienced the greatest loss of global resources of mires and peatlands, which is estimated at about 52% of their area (Joosten and Clarke, 2002). Management of peatland has a long tradition in Europe. Peatlands were drained and used mainly as farmlands and forests. Peat has also been extracted for fuel for centuries. In Western Europe its exploitation was the most intense in the 19th century, when mechanized peat extraction was introduced. Since the second half of the 20th century, peat has become an increasingly popular growing media in horticulture, and the most suitable substrate is poorly decomposed raised peat (Ilnicki, 2002). Rapid rate of degradation and disappearance of peatland ecosystems, particularly in Western Europe, encouraged development of a preservation and protection strategy for peatlands of Central Europe where losses have been less severe (Bragg and Lindsey, 2003).

In 2016 in Poland there were 290 peat deposits documented as suitable for peat extraction, of which 83 were still actively extracted and 78 were post-extracted sites (Szuflicki et al., 2017).

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Peat extraction requires drainage and removal of acrotelm, which severely alters natural functions of peatlands e.g. maintaining stable water level (Van Seters and Price, 2001). Peat soil after drainage experience transformation in aerobic conditions which is termed as secondary transformation (e.g. Kalisz et al., 2015). To quantitatively evaluate its state a water-holding capacity index W_1 may be used (e.g. Gawlik, 1992). This index expresses as a ratio of the lowest and the highest water-holding capacity of the soil and reflects changes in the physical, hydrophysical and chemical properties of peat soil subject to drying (Gawlik, 2000; Sokołowska et al., 2005).

When the cut-over sites are abandoned without any further treatment, the peat mass undergoes shrinkage, compression, and oxidation collectively known as peat subsidence (e.g. Eggelsmann, 1986). Those processes alter morphology of the soil profile as well as physical, hydrophysical and chemical properties of peat (Ilnicki and Zeit, 2003). As shown in other research (e.g. Schlotzhauer and Price, 1999; Price et al., 2003) increase in soil density after drainage results in pore size reduction, decrease of hydraulic conductivity and specific yield (the capacity of saturated soil to release water from storage by gravity drainage). This, in turn, further causes greater fluctuation of water table (e.g. Schouwenaars, 1993; Whittington and Price, 2006). Changes in pore size distribution trigger alterations in water storage that depends on the size of pores in the zone of water table fluctuation and the corresponding specific yield (Verry et al., 2011). Chemical transformation, mainly mineralization of nitrogen and carbon containing compounds, and composition of organic and inorganic material may change nutrient status of the soil as well as ground water quality (e.g. Wind-Mulder and Vitt, 2000; Laine et al., 2013). Peat oxidation leads to relative enrichment of ash content and to lowering of C:N ratio due to a preferential loss of C over N resulting from microbial decomposition (Krüger et al., 2015). It also causes substantial emissions of greenhouse gases (CO_2, N_2O), which is a considerable challenge in the context of climatic changes (e.g. Couwenberg, 2011; Hufnagel and Garamvölgyi, 2014).

Considering irreversible changes in peat structure and properties as well as greenhouse gases emission, restoration of post-extracted peatlands should begin right after the cutting is finished (Waddington et al., 2001). The only way to delay these processes is by complete inundation or by re-establishment of peat forming vegetation (Joosten, 1992). Regeneration of typical bog vegetation with *Sphagum* as a dominant component depends primarily on the hydrophysical conditions of the uppermost peat layer, as they are considered the main factors affecting water table fluctuations and the occurrence of water stress (Schouwenaars and Gosen, 2007).

Due to the history of peatland management in Europe and Poland, many studies on degradation of organic soils concern drained and agriculturally used (grasslands, arable lands) peatlands, especially fens (see Parent and Ilnicki, 2003). There have not been many research on the secondary transformation of soils of post-extracted bogs in Poland. This topic should be definitely focused on, especially in the context of numerous activities aimed at the conservation and restoration of peatlands (e.g. Herbichowa et al., 2007; Okruszko et al., 2011; Glińska-Lewczuk et al., 2014). Currently, degraded peatlands are often near or within various nature protection areas as in the case of the cut-over bog investigated in this study. That is why the assessment of regeneration potential of such sites is particularly important.

The objectives of the presented study were as follows: a) to determine physical, hydrophysical and chemical properties of the upper layer of peat soil on post-extracted areas of different age, b) to identify correlations between hydrophysical parameters of

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cut-over peat surface and re-vegetation pattern. This will expand our knowledge on changes in bog peat undergoing degradation in post-extraction areas. It would be also helpful in selecting soil parameters most strongly affecting the potential of cut-over bogs for restoration of typical bog peat-forming vegetation.

Materials and methods

Study area

The study was carried out within a raised bog Bór za Lasem which is located in Orava-Nowy Targ Basin, southern Poland (*Fig. 1*). It is one of 27 sites that form a complex of mountain peatlands belonging to the European Ecological Network Natura 2000 (PLH120016, PLB120007). Northern and southern borders of the Orava-Nowy Targ Basin are flanked by mountain ranges. It is located within moderately warm climate zone with local peculiarities (Kondracki, 2011). Mean annual air temperature is 5.5 °C (max: July 16 °C; min: February -6 °C), and annual total precipitation ranges from 750 mm to 825 mm (Olszewski, 1988). Basic weather characteristics were also recorded in 2016. Between June and August mean air temperature was 15.4 °C, with a maximum of 37.0 °C and minimum of -2.9 °C. Mean precipitation for that period was 375 mm.

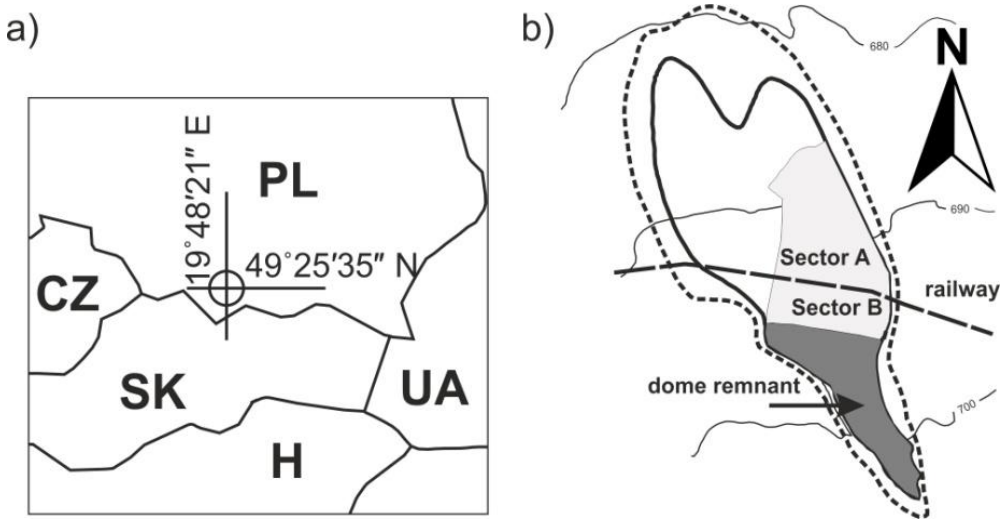


Figure 1. *Location of Bór za Lasem peat bog (a) and investigated post-extraction sectors (adapted from Łajczak, 2006) (b)*

Bór za Lasem bog was formed as a result of paludification of poorly permeable clay substrate. It is a raised bog but the supply of shallow groundwater was important at the initial stage of its formation. The site is dominated by ombrogenic peats, cotton-grass-*Sphagnum* (*Eriophoro-Sphagneti*) and hummock *Sphagnum* (*Eusphagneti*), and locally with poor-fen *Sphagnum*-sedge (*Sphagno-Cariceti*) peat and sapric peat at the base. The bog area is 55 ha, and mean peat thickness is ca. 1.80 m (max 3.65 m). Mean degree of peat decomposition (determined with microscopic method) in the remaining bog dome is 30%, and mean ash content is 2.2% (Lipka and Zając, 2014).

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After the Second World War, the bog was extracted within two sectors (*Fig. 1*), with block-cutting method using heavy machinery. Sector A (ca. 16 ha) was cut-over from the beginning of 1960s until the beginning of 1980s. Then the extraction continued on Sector B (ca. 8 ha) until the beginning of 1990s (personal communication). When the extraction was finished the sites were abandoned (*Fig. 2*). Both sectors are crossed with a network of secondary drainage ditches spaced at about 20 m that remove water to main ditches. Most of the secondary ditches are currently filled in and overgrown with vegetation and primary ditches remove water mainly during major floods after precipitation and spring snowmelt.



a



b

Figure 2. *General view of the investigated post-extraction Sectors A (a) and B (b) of Bór za Lasem peat bog*

Measurements of water level carried out from June to August 2016 in both post-extracted sectors in piezometers showed that if the residual peat was ca. 40 cm and

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less thick the water table dropped 13–22 cm below the peat layer (recorded for 58% and 17% of the plots on Sectors A and B respectively). If peat layer was thicker, mean water level was 25.4 cm for Sector A and 16.1 cm for Sector B. Control measurements of groundwater quality were performed twice in the summer in two piezometers from each sector. Mean water pH were 2.86 and 3.07 and electric conductivity 41 μS cm^{-1} and 50 μS cm^{-1} for Sectors A and B respectively.

Soil analysis

The study on post-extracted area of Bór za Lasem bog was conducted in the years 2015–2016. Twenty study plots (5 m × 5 m each) were laid out on each of the two sectors and they were arranged in W-shaped transects. At each study plot peat thickness was measured with a soil probe and samples for laboratory analysis were taken from two layers: 0–10 cm (after removing of litter) and 10–20 cm. Soil samples were collected in metal rings (160 rings, 250 cm^3) and into plastic bags (80 samples). Additionally, to determine peat type exposed after extraction, peat matrix samples were collected from eight randomly selected plots from different areas on both sectors (four locations from each sector). All the analyses were performed in duplicate.

The degree of peat decomposition was determined according to Von Post (1924), and peat type with microscopic method based on plant macrofossils composition (Kac et al., 1977; Tobolski, 2000).

The state of secondary transformation of peat was assessed based on physical, hydrophysical and chemical properties comprising: water-holding capacity index (W_1), dry bulk density (ρ_b), specific density (ρ), total porosity (n), volumetric moisture content (θ_v), saturated moisture content (θ_s), volumetric shrinkage (S_v), maximum hygroscopic moisture content (MH), water retention curve (pF), ash content (A), pH, electric conductivity (EC), total carbon (C), total nitrogen (N), mineral nitrogen forms (NO_3^-, NH_4^+), and available phosphorus (P).

Bulk density, soil moisture content and volumetric shrinkage were measured in samples of undisturbed structure following sample drying at 105 °C for to a constant weight. To determine saturated moisture content, soil samples were soaked with water for three weeks before drying. Bulk density and soil moisture content were then calculated with routinely used formulas. Volumetric shrinkage was estimated as a difference in sample volume in saturated and dry state (105 °C) to its volume in saturated state. Specific density was calculated with the formula by Okruszko (1971) based on ash content (see *Eq. 1*), and total porosity was worked out using specific density and bulk density.

$$\rho = 0.011 \cdot A + 1.451 \quad \text{(Eq.1)}$$

Water-holding capacity index was determined according to the method provided by Gawlik (1992). Each soil sample was divided into two parts. One part at field-moisture state was saturated in distilled water for 7 days. The second part was first dried to an absolutely dry weight (105 °C), and then saturated in distilled water (7 days). Water content was determined by a gravimetric method after centrifuging the samples at 1000 *g* for 1 h at 10 °C in a laboratory centrifuge with a cooling unit. The results were recalculated to 100 g of absolutely dry weight. Water holding capacity index was calculated as a ratio of water content in a sample dried up to 105 °C and in a sample of field-moisture. The state of secondary transformation of peat was

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evaluated according to the classification by Gawlik (2000): I – initially secondary transformed (W_1: 0.36–0.45); II – weakly secondary transformed (W_1: 0.46–0.60); III – moderately secondary transformed (W_1: 0.61–0.75); IV – strongly secondary transformed (W_1: 0.76–0.90); V – completely degraded ($W_1 > 0.90$).

Water retention characteristics of the soil were determined using the equilibrium centrifuging method (Smagin and Prusak, 2008). Then, laboratory data were fitted with Van Genuchten model using the RETC computer program (Van Genuchten et al., 1991). These data were used to find out water retention capacity and differential porosity of the peat soil assuming that field water capacity (FWC) corresponded to θ_v at pF 2.0, available water retention (AWR) to θ_v between pF 2.0 and pF 4.2, easily available water retention (EAWR) to θ_v between pF 2.0 and pF 2.7 and small pore retention (SPR) to θ_v between pF 2.7 and pF 4.2. Macropore volume was calculated as a difference between total porosity and volumetric moisture content at pF 2.0, mesopore volume was defined in the same way as AWR and micropore as volumetric moisture content at pF 4.2. Maximum hygroscopic moisture content was determined in a vacuum drying oven in the presence of saturated solution of K_2SO_4 (Mocek and Drzymała, 2010).

Soil ash content and pH analysis was performed in air dry samples as described by Sapek and Sapek (1997). Ash content was determined by a loss on ignition method where a peat sample (2 g) was incinerated in a muffle furnace at 550 °C, and then calculated as difference between weight of the sample dried up at 105 °C and its weight after incineration. Soil pH and electric conductivity were determined in 1:10 (soil:solution) ratio. Soil pH was measured by a potentiometer in distilled water and in 1 M KCl. The resulting values of electric conductivity were corrected for H^+ ions (Sjörs, 1950). Total carbon and nitrogen were determined on a CNS elemental analyzer. The samples were devoid of carbonates (inorganic carbon), and thus total carbon content corresponded to organic carbon content. These data were used to work out C:N ratio. The content of ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) was measured with a flow injection analysis method, and available phosphorus (P) was determined by Egner-Riehm method based on soil extraction with calcium lactate solution acidified with hydrochloric acid (Lityński et al., 1976).

Vegetation

At each study plot the percentage of cover by vascular plants and mosses was assessed. The bryophytes were determined to the genus level. Statistical analysis was based either on individual taxa or groups of species with similar habitat preferences. An analysis of effects of environmental factors on plant species occurrence (paper under review) demonstrated key importance of water level. Therefore, this paper analysed the vegetation pattern with regards only to chosen parameters affecting hydrological conditions of the soil surface. For the analyses vegetation was subdivided into groups: a) bog forming vegetation: *Sphagnum*, other bog species, b) non-bog forming vegetation: true mosses, trees (higher than 1.5 m), other non-bog species.

Statistical analysis

Distribution of the determined soil parameters was verified with Shapiro-Wilk test ($p > 0.05$). As some variables did not follow a normal distribution, and the data

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transformation was ineffective, the non-parametric Spearman rank correlation coefficient was used to assess the interdependencies between them. Significance of differences in the analysed parameters of the two soil layers between both sectors was assessed using a parametric t test for normal distribution variables, and nonparametric U-Mann Whitney test for non-normal distribution variables. The statistical analysis was performed in Statistica 12.

To compare the soil samples in terms of all investigated parameters and to estimate their relation to vegetation and environmental factors a multivariate statistical analysis method, i.e. Principal Component Analysis (PCA) was employed. It is a useful technique for identifying common patterns in data distribution that reduces the initial dimension of data sets and facilitates data interpretation. Ordination of all samples from 0–10 cm and 10–20 cm layers was based on eleven soil parameters (samples with negligible effect because of intercorrelations were excluded). The impact on vegetation was assessed with selected soil parameters (water-holding capacity index, volumetric moisture content, saturated moisture content, maximum hygroscopic moisture content, differential porosity) affecting hydrological conditions of the uppermost soil layer (0–10 cm). Since the soil parameters (variables) had different units, the data were centred and standardized to zero mean and unit variance before subjecting the data set to PCA. Residual peat thickness, water level and cover of vegetation were treated as passive variables not affecting the results of the ordination. Multivariate analysis was performed with Canoco software (ter Braak and Smilauer, 2002).

Results

When exploitation ceased on the younger Sector B, the site was covered with bog peat of cotton grass-*Sphagnum* (*Eripohorum-Sphagnum*) type with decomposition degree H4-6 as per Von Post scale. On Sector A harvesting exposed pine-*Sphagnum* peat (*Pinus-Sphagnum*) and sapric peat in which remains of pine wood were identified. One third of the study plots on this sector had a ca. 5 cm thick layer of compressed moss of decomposition degree H4 at a depth of approximately 5–6 cm. On other study plots peat decomposition ranged from H5 to H8.

Sectors statistically differed mainly in physical and hydrophysical properties and some chemical properties. On Sector B there were more significant differences in parameters between the two investigated layers than on Sector A. The largest with-in site variability was noticed for chemical properties, such as ash content (especially in 10–20 cm layer), mineral nitrogen forms and corrected EC. The most variable physical and hydrophysical parameters included thickness of residual peat (Sector B) and volumetric water content (Sector A, 0–10 cm). Low to moderate variability with a coefficient of variation usually below 30% was noticed for the other variables (*Tables 1* and *2*).

The soil water potential (pF)-moisture contents characteristic (*Fig. 3*) featured higher moisture content at FWC on older Sector A. Also, on this sector the volume of macropores was significantly lower (nearly two times) and that of micropores clearly higher, especially in 10–20 cm layer than on younger Sector B. In consequence, EAWR was higher on Sector B, while on Sector A more water was retained in smaller pores (SPR) (*Table 3*). In the layer 0–10 cm SPR represented 83% and 68% of AWR on Sectors A and B, respectively.

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Table 1. *Statistical measures of the analyzed physical and hydrophysical parameters in the upper soil layer on Sectors A and B*

Parameter	Unit	Layer [cm]	Min		Max		Mean		SD		V [%]	
			A	B	A	B	A	B	A	B	A	B
Water-holding capacity index W_1[1,2,3,4]	-	0–10	0.53	0.41	0.76	0.75	0.61	0.58	0.07	0.08	10.85	13.33
		10–20	0.41	0.27	0.65	0.58	0.53	0.45	0.07	0.08	13.31	17.41
Maximum hygroscopic moisture content[2,4]	vol. %	0–10	10.18	11.30	26.30	28.86	16.41	16.72	5.21	4.30	31.76	25.71
		10–20	10.80	8.85	22.80	14.51	15.45	11.58	3.81	1.55	24.52	13.37
Volumetric moisture content[1,2,3,4]	vol. %	0–10	12.48	58.95	67.66	88.59	34.65	76.05	17.87	8.75	51.59	11.50
		10–20	61.92	57.39	89.37	90.43	76.94	83.45	7.48	7.85	9.72	9.41
Saturated moisture content[1,2,3,4]	vol. %	0–10	61.13	75.87	90.85	85.69	73.00	81.86	7.14	3.06	9.79	3.73
		10–20	76.41	76.80	91.37	92.58	83.06	87.18	4.49	3.99	5.41	4.58
Bulk density[2, 4]	g cm^{-3}	0–10	0.16	0.14	0.33	0.35	0.25	0.22	0.05	0.06	20.10	25.07
		10–20	0.20	0.10	0.35	0.31	0.21	0.16	0.07	0.05	33.06	29.27
Specific density[3,4]	g cm^{-3}	0–10	1.50	1.47	1.73	1.78	1.59	1.57	0.06	0.10	4.02	6.59
		10–20	1.46	1.47	2.20	1.93	1.61	1.52	0.23	0.10	14.57	6.88
Total porosity[1,2,3,4]	%	0–10	78.21	77.02	88.57	90.71	83.58	85.90	3.16	3.18	3.78	3.70
		10–20	76.51	83.78	92.32	93.22	86.42	89.70	4.14	2.27	4.79	2.52
Volumetric shrinkage[1,2,3,4]	%	0–10	15.75	19.14	53.12	59.93	31.68	45.07	9.07	10.40	28.63	23.08
		10–20	30.61	36.31	66.11	69.74	47.86	54.77	9.53	10.93	19.91	19.95
Peat thickness[5]	cm	-	18.67	32.00	98.00	113.00	39.32	64.60	21.83	23.93	55.52	37.04

SD – standard deviation; V- variability coefficient
[1]Statistically significant difference ($p < 0.05$) in the layer 0–10 cm between Sectors A and B
[2]Statistically significant difference ($p < 0.05$) in the layer 10–20 cm between Sectors A and B
[3]Statistically significant difference ($p < 0.05$) between the layers 0–10 cm and 10–20 cm on Sector A
[4]Statistically significant difference ($p < 0.05$) between the layers 0–10 cm and 10–20 cm on Sector B
[5]Statistically significant difference ($p < 0.001$) between Sectors A and B

Table 2. *Statistical measures of the analyzed chemical parameters in the upper soil layer on Sectors A and B*

Parameter	Unit	Layer [cm]	Min		Max		Mean		SD		V [%]	
			A	B	A	B	A	B	A	B	A	B
Ash content[3,4]	%	0–10	4.24	2.00	25.35	30.34	12.69	10.94	5.81	9.42	45.76	86.05
		10–20	1.21	1.46	68.08	43.78	14.25	6.28	21.30	9.50	149.67	151.16
Total N	%	0–10	1.38	1.19	2.02	2.02	1.70	1.65	0.17	0.21	10.09	12.79
		10–20	0.81	1.27	1.89	1.99	1.56	1.58	0.30	0.20	19.34	12.69
Total C	%	0–10	47.45	33.84	58.14	63.07	53.79	55.57	2.81	6.71	5.22	12.08
		10–20	26.83	53.36	63.10	61.07	52.87	56.60	10.67	2.39	20.19	4.23
C:N ratio[2,3,4]	-	0–10	24.36	26.75	39.37	48.34	31.85	33.96	3.60	4.55	11.29	13.40
		10–20	29.87	29.05	37.84	42.24	33.97	36.38	2.09	4.28	6.15	11.78
NO_3^-	mg kg^{-1}	0–10	0.83	0.44	4.44	6.24	1.54	1.51	0.97	1.26	62.95	83.44
NH_4^{+1}	mg kg^{-1}	0–10	7.68	35.84	44.58	172.46	16.19	77.54	9.03	33.79	55.74	43.58
Available P	mg kg^{-1}	0–10	10.90	2.62	48.77	41.94	23.30	17.92	9.98	9.70	0.43	0.54
pH_{H2O}[1,2,4]	-	0–10	3.56	2.96	3.96	3.60	3.72	3.29	0.12	0.21	3.26	6.40
		10–20	3.28	2.92	4.05	3.85	3.75	3.61	0.20	0.20	5.32	5.40
pH_{KCl}[1,4]	-	0–10	2.35	2.52	3.24	2.90	2.82	2.68	0.23	0.13	8.12	4.86
		10–20	2.72	2.73	3.15	3.18	2.90	2.90	0.13	0.09	4.48	3.25
Corrected electric conductivity[1, 2, 3]	µS cm^{-1}	0–10	119.00	60.62	252.08	264.00	177.77	134.58	38.34	50.35	21.57	37.41
		10–20	115.08	61.73	381.36	220.36	245.30	113.47	61.08	44.93	24.90	39.60

SD – standard deviation; V- variability coefficient
[1]Statistically significant difference ($p < 0.05$) in the layer 0–10 cm between Sectors A and B
[2]Statistically significant difference ($p < 0.05$) in the layer 10–20 cm between Sectors A and B
[3]Statistically significant difference ($p < 0.05$) between the layers 0–10 cm and 10–20 cm on Sector A
[4]Statistically significant difference ($p < 0.05$) between the layers 0–10 cm and 10–20 cm on Sector B
[5]Statistically significant difference ($p < 0.001$) between Sectors A and B

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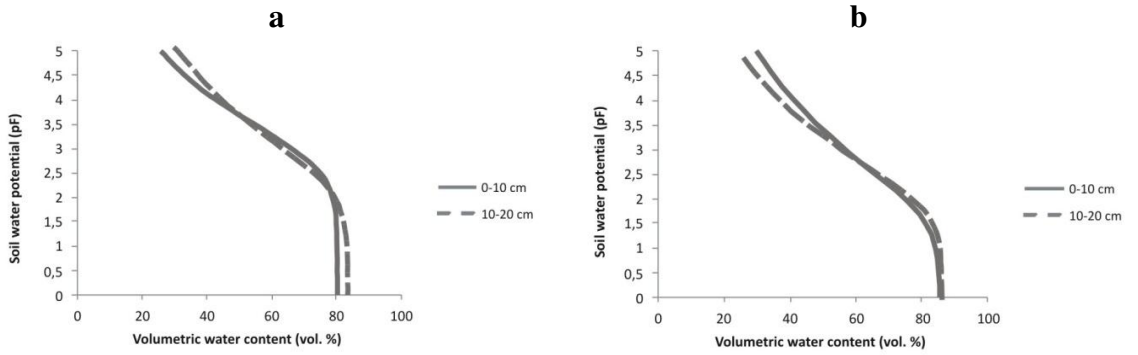


Figure 3. *Water retention (pF curve) of the upper soil layer for Sectors A (a) and B (b)*

Table 3. *Differential porosity and water retention characteristics in the upper soil layer on Sectors A and B*

Parameter	Unit	Layer [cm]	A			B		
			Mean	SD	V [%]	Mean	SD	V [%]
FWC (pF 2.0)	vol. %	0–10	78.42	6.01	13.05	74.60	8.22	9.08
		10–20	78.08	7.33	10.66	74.91	9.60	7.81
AWR[3] (pF 2.0–4.2)	vol. %	0–10	39.71	5.44	7.30	36.56	7.50	4.74
		10–20	37.71	10.46	3.61	44.88	10.23	4.39
EAWR[1] (pF 2.0–2.7)	vol. %	0–10	6.77	3.39	1.20	11.35	3.43	3.31
		10–20	7.39	3.95	1.87	9.33	4.87	1.91
SPR[1,3] (pF 2.7–4.2)	vol. %	0–10	32.94	5.88	5.60	24.21	6.25	3.88
		10–20	30.32	12.24	2.48	35.55	14.36	2.48
Micropores[2,3] (pF 4.2)	%	0–10	38.71	9.05	4.28	39.03	6.87	7.56
		10–20	40.37	7.41	5.45	30.04	3.71	8.10
Macropores[1]	%	0–10	5.16	7.41	0.70	11.30	8.33	1.36
		10–20	9.11	7.18	1.27	14.79	9.70	1.53

Explanations: FWC – field water capacity; AWR – available water retention = mesopores; EAWR – easily available water retention; SPR – smaller pores retention; SD – standard deviation; V – variability coefficient
[1]Statistically significant difference ($p < 0.05$) in the layer 0–10 cm between Sectors A and B
[2]Statistically significant difference ($p < 0.05$) in the layer 10–20 cm between Sectors A and B
[3]Statistically significant difference ($p < 0.05$) between the layers 0–10 cm and 10–20 cm on Sector B

Water-holding capacity index W_1 for the majority, i.e. 57% of analysed samples ranged between 0.46 and 0.60 (weakly secondary transformed), for 22% of samples it was 0.61–0.75 (moderately secondary transformed), and for 17% of samples between 0.36 and 0.45 (initially secondary transformed). Mean values of W_1 in the layers 0–10 cm and 10–20 cm (*Table 1*) indicated moderate to weak secondary transformation of peat mass on Sector A, while on Sector B it was weak to initial. There was a strong positive correlation of the W_1 index with bulk density and ash content (consequently with specific density), and a negative correlation with total porosity, volumetric shrinkage and soil moisture content. Moderate correlation was observed with thickness

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of residual peat, total C, C:N ratio, and pH. The W_1 index least strongly correlated with the content of micro- and mesopores and corrected EC (*Table 4*).

Table 4. *Spearman's rank correlation between water-holding capacity index W_1 and analyzed soil parameters. The statistically significant ($p < 0.05$) values of correlation coefficient (Rho) are marked in bold*

Parameter	Rho
Total porosity	**-0.653**
Bulk density	**0.637**
Volumetric moisture content	**-0.631**
Saturated moisture content	**-0.597**
Ash content	**0.586**
Volumetric shrinkage	**-0.541**
Peat thickness	**-0.474**
Total C	**-0.423**
C:N ratio	**-0.394**
pH	**0.348**
Corrected electric conductivity	**0.295**
Micropores	**0.284**
NH_4^+ [1]	-0.285
Mesopores (AWR)	**-0.249**
Maximum hygroscopic moisture content	**0.232**
Macropores	-0.224
NO_3^- [1]	0.140
Available P [1]	-0.006

[1]0–10 cm layer only

In PCA of all soil samples (both sectors and layers) (*Fig. 4*), the first PCA axis explained 45.3% and the second 12.4% of total variance. Water level and residual peat thickness most strongly correlated with the first PCA axis. Dispersion of soil samples was higher on Sector A than on Sector B. The samples were arranged from those from the older Sector A, layer 0–10 cm (left-hand part of the diagram), to those from the younger Sector B, layer 10–20 cm (right-hand part of the diagram). The samples from Sector A, layer 10–20 cm and Sector B layer 0–10 cm occupied a common central area of the diagram. Single samples deviated from this trend. There was a significant ($p < 0.05$) negative correlation ($r=-0.771$) between residual peat thickness and water level. In general, an increase of peat thickness and water table level was associated with a decrease in bulk density, water-holding capacity index W_1, maximum hygroscopic moisture content and total N and an increase in total porosity, macropore volume, soil moisture content, volumetric shrinkage, pH, total C and C:N ratio. Contrary to that, micropore volume, ash content and corrected EC were increasing with depth on the older Sector A but decreasing on the younger one.

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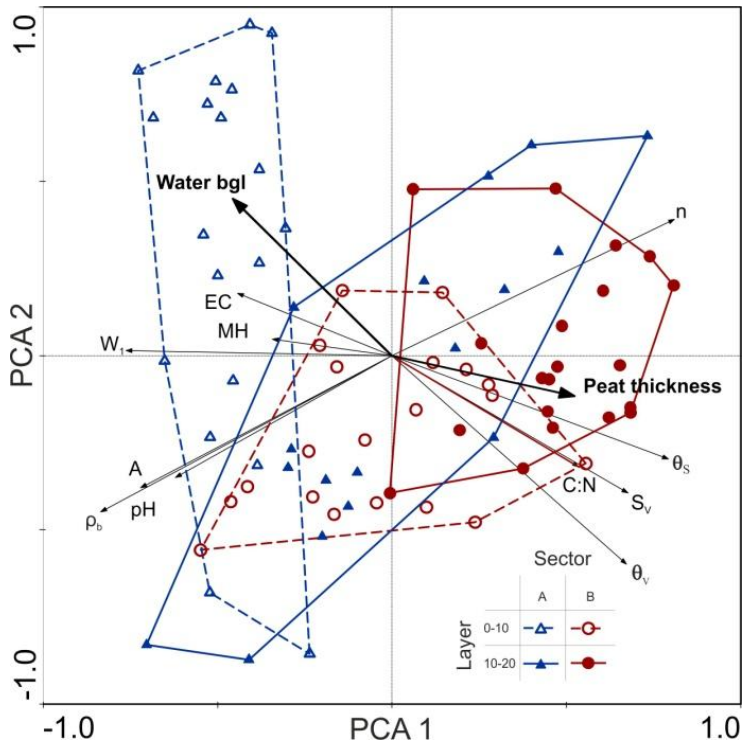


Figure 4. *Result of PCA ordination conducted on soil parameters of all samples. Abbreviations: W_1 – water-holding capacity index, MH – maximum hygroscopic moisture content, θ_v – volumetric moisture content, θ_s – saturated moisture content, ρ_b – bulk density, n – total porosity, S_v – volumetric shrinkage, A – ash content, C:N – total nitrogen and total carbon ratio, EC – corrected electric conductivity, Water bgl – mean water level below ground. Environmental factors (Peat thickness, Water bgl) are displayed as passive variables*

Typical bog-forming species occurred on all plots (*Table 5*). Mosses of the *Sphagnum* genus, which are the main bog forming plants, were found only on 44% of the plots and did not achieve a large coverage. Among the bog forming vascular plants the most frequent (97%) and of the largest cover (on average 36%) was *Eriophorum vaginatum*. Trees exceeding 1.5 m were found on the majority of the plots (82%), sometimes reaching high cover (up to 60%), especially on the older Sector A (*Fig. 5*). These were mainly *Betula pendula* and *Pinus sylvestris*. Small trees (up to 1.5 m) appeared with a relatively high frequency. However their share in the area cover was small. *Calluna vulgaris*, *Vaccinium myrtillus* and *V. vitis-idea* were among the most common other non-bog forming species. Very few minerotrophic species were recorded more than once (*Empetrum nigrum*, *Juncus effusus*, *Sorbus aucuparia*, *Carex nigra*, *Eriophorum angustifolium*, *Dryopteris carthusiana*). The most frequent was *Dryopteris carthusiana* with frequency 11%.

In PCA ordination based on the parameters affecting hydrological conditions (*Fig. 5*) the first axis explained 34.7%, and the second 30.1% of total variance. Water level, residual peat thickness and vegetation most strongly correlated with the second axis. Typical bog forming vegetation was clearly concentrated in the lower part of the diagram, opposite to the non-bog forming vegetation. Especially cover of *Sphagnum* was associated with studied plots showing lower W_1 index, higher water level, peat thickness, soil moisture content and macropores volume.

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Table 5. *Frequency of occurrence [%] and cover [%] of plant groups (in bold) and species. Mean cover is calculated only for plots on which the species was present. In the group of other non-bog species only the species with frequency > 30% are presented*

	Cover			Frequency
	Min	**Max**	**Mean**	
Bog forming vegetation				
Sphagnum	1	30	9	44
Other bog species	**5**	**95**	**21**	**100**
Eriophorum vaginatum	0	95	36	97
Vaccinium uliginosum	0	50	15	82
Ledum palustre	0	25	6	67
Oxycoccus palustris	0	20	4	18
Non-bog forming vegetation				
True mosses	**0**	**57**	**22**	**95**
Trees (higher than 1.5 m)	**3**	**60**	**20**	**82**
Other non-bog species	**0**	**30**	**10**	**92**
Calluna vulgaris	0	60	20	82
Vaccinium vitis-idaea	0	45	11	41
Vaccinium myrtillus	0	25	6	44
Betula pendula	0	5	2	44
Pinus sylvestris	0	5	1	59

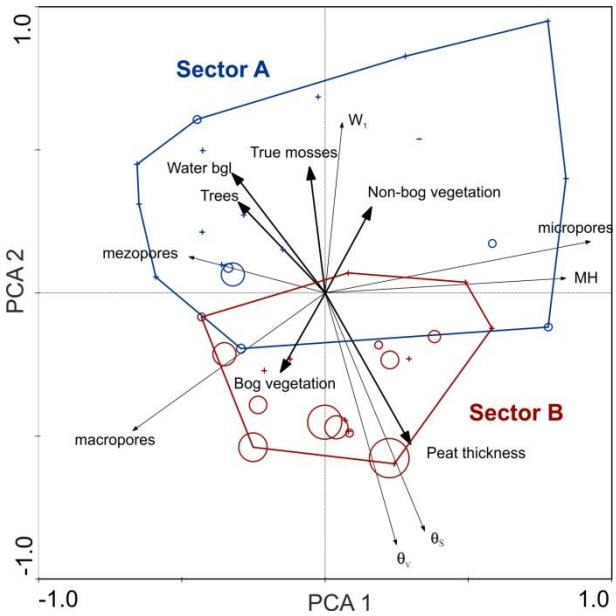


Figure 5. *Result of PCA ordination conducted on chosen parameters affecting hydrological conditions of surface peat layer (0–10 cm). Abbreviations: W_1 – water-holding capacity index, MH – maximum hygroscopic moisture content, θ_v – volumetric moisture content, θ_s – saturated moisture content, Water bgl – mean water level below ground. Environmental factors (Peat thickness, Water bgl) and vegetation cover are displayed as passive variables. Circle diameter is proportional to Sphagnum cover of plot, + depict plots with no Sphagnum*

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Discussion

Post-extracted areas of peatlands are divers mainly in terms of characteristics of the exposed peat (type, degree of decomposition, thickness), drainage intensity, climatic conditions, as well as the re-vegetation pattern. That is why making direct comparisons of hydrological conditions and soil properties of cut-over peat with other sites is difficult. However, there are some distinct trends in secondary transformations of analysed peat soil. The study indicated that the changes in a bog peat were similar to that observed in minerotrophic fens (e.g. Ilnicki and Zeitz, 2003).

The longer time after abandonment of post-extracted areas without any treatments e.g. drainage blocking, the more advanced secondary transformation of peat, reaching deeper layers. Differences in physical and hydrophysical soil properties of examined sites were essential as compared with fibric material (e.g. Boelter, 1969) of the uppermost (acrotelm) layer of a natural bog. An increase in pH, electric conductivity and mineral forms of nitrogen (NO_3^-, NH_4^+) at peat surface was recorded, as on other post-extracted areas (Wind-Mulder et al., 1996), but overall it remained acidic and poor in nutrients. We used water holding capacity index W_1 as a quantitative indicator of secondary transformation of cut-over bog. The correlations stated between W_1 index and the range of analysed soil properties were similar to those reported in other studies, mainly for fens (Gawlik, 1993, 1996; Szajdak et al., 2000; Boguta and Sokołowska, 2014; Glina et al., 2016). This suggests that water-holding capacity index W_1 can be a valuable measure reflecting the degree of peat soil degradation on post-extracted areas, which is manifested primarily in changes of soil hydrophysical conditions.

We observed strong correlation between parameters affecting water conditions of surface peat, i.e. water level, residual peat thickness, soil moisture, pore sizes and water-holding capacity index W_1 and establishment of different vegetation groups (bog forming and non-bog forming). In time, peat substrate quality created more unfavourable hydrophysical conditions for re-vegetation by typical bog species, especially *Sphagnum* what was evidenced in multivariate analysis (older vs. younger sector). However, to be more specific the state of secondary transformation of peat depends more on soil water conditions after drainage than the time that elapsed since drainage (Gawlik, 1996).

Hydrophysical conditions of the peatland surface are considered the main variable affecting *Sphagnum* growth (e.g. Price and Whitehead, 2001). Peat (catotelm) that was exposed after ceasing peat extraction on investigated site maintained high water retention capacity but water was retained with high pressure. Similar results were reported for other post-extracted sites (Schlotzhauer and Price, 1999; Mustamo et al., 2016). High water retention capacity results from the moderate to high decomposition degree of the peat matrix, hence high fraction of small pores (e.g. Boelter, 1969). Increase in bulk density due to peat consolidation and further decay in drainage conditions subsequently alter size of pore spaces (e.g. Whittington and Price, 2006). Hence increased peat decomposition and soil compaction result in a decrease in easily available water retention and a rise in smaller pores retention (Gawlik, 1992).

Macropore volume in analysed soil was comparable with specific yield reported by Price (1996) or Mustamo et al. (2016) on post-extracted sites, as well as with the water yield coefficient (which represents the same area on pF curve as macropores and is an equivalent of specific yield) given by Boelter (1969) for sapric peat. Their percentage was very low compared to slightly decomposed *Sphagnum* peat (Verry et al., 2011). Low macropores volume and specific yield may seriously inhibit *Sphagnum*

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development as unlike vascular plants it is unable to uptake water from the substrate when pore-water pressure is lower than –100 mb (Hayward and Clymo, 1982 after Price, 1997). According to Price and Whitehead (2001), hydrological conditions suitable for *Sphagnum* recovery require high water table level (24.9 ± 14.3 cm), volumetric moisture content above 50%, and pore-water pressure above –100 mb. However, if volume of small pores is high, water is held at such low pressure that it cannot be drawn by *Sphagnum* through capillary suction (Price et al., 2003). Furthermore, Dixon et al. (2017), who studied moss water availability demonstrated that both factors, deeper profile and shallower starting water table, delayed the beginning of very high surface tension and that peat deposits shallower than 0.5 m showed the lowest capability of equalizing prolonged periods of evaporative stress.

The cited studies are consistent with our results that indicated better conditions for establishment of bog forming vegetation, especially *Sphagnum* on areas with thicker residual peat layer, shallower water table, higher soil moisture content and macropore volume. On such areas surface peat layer was less secondary transformed. This was expressed in lower W_1 index, which indirectly denoted lower density of peat, hence higher porosity. Hydrophysical properties of the uppermost layer of cut-over peat are particularly important to *Sphagnum* as they lack roots and vascular channels (Schouwenaars and Gosen, 2007) and unlike vascular plants they cannot take up water from deeper layers of soil. On the contrary, non-bog forming vegetation (mainly true mosses and trees) developed better on drier conditions, when peat layer was thin and secondary transformation of peat expressed as W_1 index, more advanced. Increased share of true mosses, like *Polytrichum* associated with lowered water level was described by Potvin et al. (2015). Dry conditions also facilitate tree seedling establishment, as *Sphagnum* growth is then impeded (Limpens et al., 2014). In humid climatic conditions of Central Europe woodland is a typical potential vegetation (Ellenberg and Leuschner, 2010), so trees occupy abandoned post-extracted peatlands over time. Trees strongly affect peatland ecosystems through changes in the structure and species composition, availability of light and soil physical and chemical properties (Mitchell et al., 2007). Trees (Van Seters and Price, 2001) and also ericaceaous shrubs (Farrick and Price, 2009) increase peat drying by interception of precipitation water and transpiration, which especially in birch is high (Fay and Lavoie, 2009). Therefore, they may further exacerbate secondary transformation of peat.

It is important that restoration should begin directly after extracted area is abandoned (Price, 1997). Only then it can be possible to mitigate effects of drainage and consequently changes in peat structure and properties as well as to inhibit establishment of trees.

Conclusions

The properties of the peat surface deteriorate on abandoned cut-over bogs, where peat dries up over time. The process can be also exacerbated by vascular vegetation, especially trees. Secondary transformation of peat may be comprehensively described by water-holding capacity index W_1. It correlates with thickness of residual peat and a range of properties of cut-over peat (e.g. porosity, bulk density, soil moisture content, ash content), that change as the degradation advances. Identification of these correlations means that W_1 index may be used in evaluation of secondary transformation of peat mass on cut-over bogs.

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The study demonstrated the key importance of water table depth, residual peat thickness and hydrophysical conditions of the cut-over peat surface, especially water-holding capacity index W_1, soil moisture content and macropore volume on re-vegetation of *Sphagnum.* Negative correlation of W_1 index with *Sphagnum* occurrence indicates that it may also be a useful measure in evaluation of the potential of cut-over bogs for spontaneous regeneration of typical bog vegetation, mainly *Sphagnum* species. Implementation of such approach into practice requires further research on other degraded bogs for verification.

Our study suggests that prospective restoration strategy of post-extracted areas should consider implications of unfavourable physical properties of the cut-over peat surface on hydrological conditions. However, any solutions aimed at providing appropriate hydrological conditions must be site-specific due to huge diversity of local conditions (e.g. Gorham and Rochefort, 2003; Price et al., 2003; Shantz and Price, 2006).

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EXAMINATION OF ANATOMICAL FEATURES OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) VARIETIES CULTIVATED UNDER HYDROPONIC SYSTEM

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Abstract. In the study it was aimed to examine and determine some anatomical features of *Lycopersicon esculentum* Mill. cv. Ceren and *Lycopersicon esculentum* Mill. cv. Panda varieties cultivated under hydroponic condition in greenhouse in Şanlıurfa, Turkey. Cross sections were cut from the root, stem and leaf by microtome and examined by light microscopy. The results were photographed. Biometric measurements of some tissues were performed. The roots of the plants are surrounded by 1 layered epidermis formed by big and small cells. Vascular bundle gathered in the centre. Stems are formed 1 layered epidermis, 1-2 layered chlorenchyma, 3-4, 4-5 layered collenchyma and 3-4, 4-5 layered parenchymatic tissues. Vascular bundles are bicollateral. Pith is parenchymatic. Stomate, epidermal hair and glandular hair are observed. Leaves are bifacial and amphistomatic. Mesophyll is formed by 1 layered palisade parenchyma and 2-3 layered spongy parenchyma. Big vascular bundle is bicollateral and small vascular bundle is collateral. They have epidermal and glandular hairs. As a result, some basic anatomical features examined showed that cv. Ceren and cv. Panda are convenient for cultivation under hydroponic system.The data obtained will contribute to soilless agricultural cultivation in the province and will pave the way for similar scientific studies.
Keywords: *light microscopy, plant histology, soilless agriculture, water stress*

Introduction

Greenhouse cultivation has developed related to negative developments such as climate changes and agricultural fields' abuse. In greenhouse soil and soilless agriculture can be applied depending on available irrigation and cultivation technologies. In soilless agriculture aggregate and nonaggregate (hydroponic) cultivation conditions can be used (Alan, 1990). Soilless agriculture is more productive than soil agriculture and its production cost is half of soil agriculture (Özkan, 2014).

Plant cultivation in soilless conditions is particularly developed in horticulture. It is mostly used for the cultivation of vegetable and ornamental plants. Yield can differ according to the variety of plants in cultivation using soilless agriculture techniques. For instance, while 1.25-2.5 ton da^{-1} tomato is cultivated in soil agriculture, 15-75 ton da^{-1} tomato is cultivated in soilless agriculture. 1.625 ton da^{-1} cabbage is cultivated in soil agriculture while 2.25 ton da^{-1} cabbage is cultivated in soilless agriculture (Alan, 2010).

Plants grow in biotic and abiotic conditions depending on their needs. These conditions may cause stress in plants. As stated in Korkmaz and Durmaz (2017) plants undergo changes in terms of quality of stress factor and genetical and ontogenic features of plants. Thus, plants adapt to factors of their environment by developing stress reactions. Stress reactions of plants exposed to stress effects can be observed when the general features of plants which are original to species are compared.

In this case not only morphological reactions of plants but also their physiological and anatomical reactions and etc. should be considered as a whole. When all these features are considered together, it can be seen if plants complete their developments and adapt to the conditions of their habitat.

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Tomato has been conventionally cultivated by using soil culture techniques. In the study, it was aimed to anatomically examine root, stem and leaf of the two varieties of tomato cultivated by using hydroponic system and to histologically specify their adaptations to the condition. Two different varieties of tomato (*Lycopersicon esculentum* Mill.) were randomly chosen from among varieties which are preferred cultivating in greenhouse by farmers in Turkey and were examined.

Materials and methods

The research was carried out in Şanlıurfa in Southeastern Anatolia. The study area is 465 m above sea level and located between 37°08'N latitude and 38°46'E longitude (Demirok and Tuylu, 2017). *Lycopersicon esculentum* Mill. cv. Ceren (offspring F1) and cv. Panda (offspring F1) which are hybrid varieties of tomato were cultivated by using aerated deep water culture in polycarbonate covered greenhouse in Harran University, Şanlıurfa, Turkey in spring in 2016 *(Fig. 1).* Anatomical studies were carried out in the laboratories in Ankara University, Ankara, Turkey. The data of climate belonging to 2016 and 1985-2016 long years (LY) in the province of cultivation (Anonymous, 2016) is given in *Table 1,* some climate values measured in the greenhouse in 2016 when the research was carried out are shown in *Table 2*.

Table 1. *Average climate values of 2016 and 1985-2016 long years (LY) in research area (Anonymous, 2016)*

Climate parameters		Max. temperature (°C)	Min. temperature (°C)	Ort. Temperature (°C)	Relative humidity (%)	Wind speed (2 m s^{-1})	Hours of sunshine (h)
Months	Year						
April	2016	32.7	7.4	20.6	36.1	1.4	8.90
	LY	22.5	10.8	16.2	53.4	1.5	7.55
May	2016	35.0	10.7	23.2	38.3	1.9	10.20
	LY	29.7	19.7	21.9	39.4	1.7	9.70
June	2016	42.0	18.9	29.8	28.0	1.9	11.90
	LY	34.9	21.0	28.3	35.2	2.0	11.95
July	2016	43.0	20.9	33.0	25.4	1.9	12.40
	LY	37.4	25.7	33.4	29.1	1.9	12.00

Table 2. *Some climate parameters measured in greenhouse in 2016*

Months	Decades	Temperature* (°C)	Relative humidity* (%)
April	2	23.4	30.6
	3	24.3	28.0
May	1	23.8	33.1
	2	27.9	30.0
	3	26.1	31.6
June	1	29.9	28.7
	2	31.0	26.8
	3	35.3	28.3
July	1	35.4	27.1
	2	35.8	28.7
	3	35.4	28.3

*Measured at 14:00 pm

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Figure 1. *An appearance of hydroponic culture in greenhouse*

The treatment was created as with 3 replications according to the random parcels plant pattern. Seedlings were transplanted to the hydroponic system at primary stage. 8 plants were cultivated in each basin. 2 of 8 plants cultivated were separated as border effect. Samples were taken at random from the remaining 6 plants and were observed. The basins which were made of galvanized sheet in the size of 50x200x30 cm were used for hydroponics. Nutrient solution was modified by using Arnon and Hoagland (Tuylu et al., 2018). Stock A and Stock B solutions were separately prepared (*Table 3*). Electrical conductivity (EC) of nutrient solution was measured as 2.5 dS m^{-1} by EC meter and pH of nutrient solution was set to 5.8-6.5 by using nitric acid. In soilless agriculture EC of nutrient solution has to be kept at optimum level. While high electrical conductivity (EC) reduces the yield affecting number of fruit, diameter of fruit and thickness of pericarp negatively, it causes increase of parameters of quality such as titratable acidity, amount of dry substance which is easily soluble in water, reducing glucose and lycopene (Söylemez ve Pakyürek, 2017). Air compressors were used to aerate nutrient solution used in hydroponics and nutrient solution was changed once a week. The plants were cultivated as a single stem by pruning. They were applied pesticide against diseases and pests if necessary.

Table 3. *The content of element in nutrient solution (mg l^{-1})*

Stock A										Stock B	
N	P	K	S	Mg	Mn	B	Cu	Zn	Mo	Ca	Fe
210	31	234	64	48	0.5	0.5	0.02	0.05	0.01	200	2.8

The samples of plants vegetatively and generatively grown were kept in alcohol 70% for anatomical studies. The samples of 3rd harvest root, stem and leaf were anatomically examined from period of production including 5 harvests. Small pieces taken from lower, middle and upper parts of root, stem and leaf were dehydrated by putting them in

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different concentration series of ethanol. Then, they were saturated by paraffin. The samples were embedded in paraffin blocks to take cross sections. 8-10 µm thick cross sections from root and leaf and 20-30 µm thick cross sections from stem were taken by microtom (Leica SM 2000 R). The cross sections were stained by safranin-fast green (Algan, 1981; Tuylu et al., 2017). They were examined by light microscope (Leica 1000) and the results were photographed by digital camera (Leica EC3). The photos are completely original. Biometric measurements were performed in some tissues by using Las v4.3 program.

In the anatomical study 3 groups in itself for each replication were created. The total number of cross sections taken from the plants and examined is 90 on 9 preparats for each replication and total 270 values of measurement were obtained by taking 3 from each section. The arithmetic mean of the values of measurements and mean standard deviation were calculated by using Excel computer software and the results were commented.

Results

Some anatomical features of root, stem and leaf of cv. Ceren and cv. Panda varieties

Roots

It is observed that the general shape of cv. Ceren's root is curved. It is surrounded by one sequenced epidermis formed by large and small cells. Parenchymatic cells (cortex) between epidermis and vascular bundles have such a very thin wall that the area between epidermis and bundles looks like a large air space. It is observed that xylem vessels are big and clear and phloems are generally smaller and less than xylem vessels (*Fig. 2a, b* and *c*).

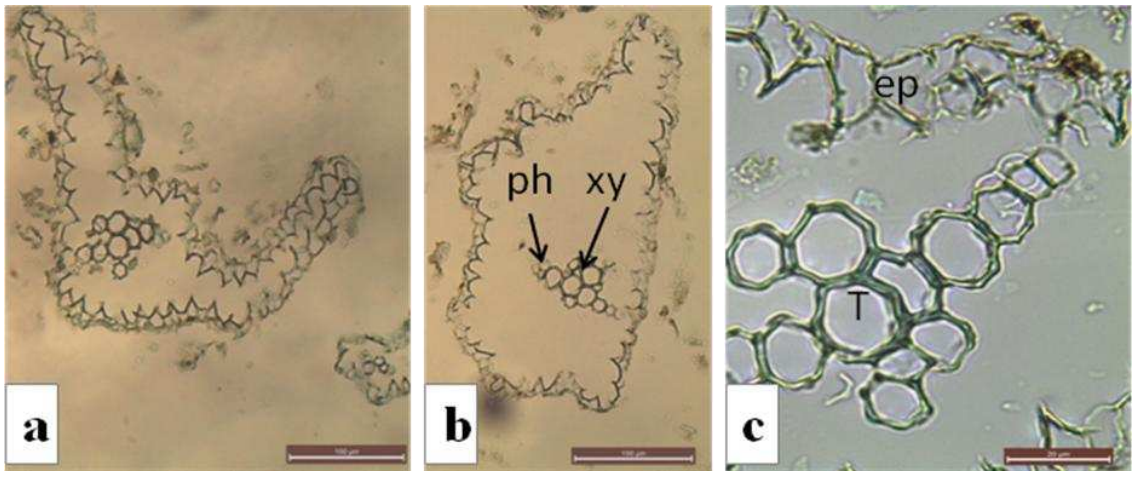


Figure 2. *Root cross section in cv. Ceren. a. general shape of root, bar = 100 µm, b. xylem (xy), phloem (ph), bar = 100 µm, c. epidermis (ep), trachea (T), air space (as), bar = 20 µm*

cv. Panda's root is surrounded by one layered epidermis formed by large and small cells. Endodermis and pericycle are not clearly observed. Vascular bundle is gathered in the centre (*Fig. 3a* and *b*). Parenchymatic cells (cortex) are observed between the epidermis and the vascular bundles (*Fig. 3c*).

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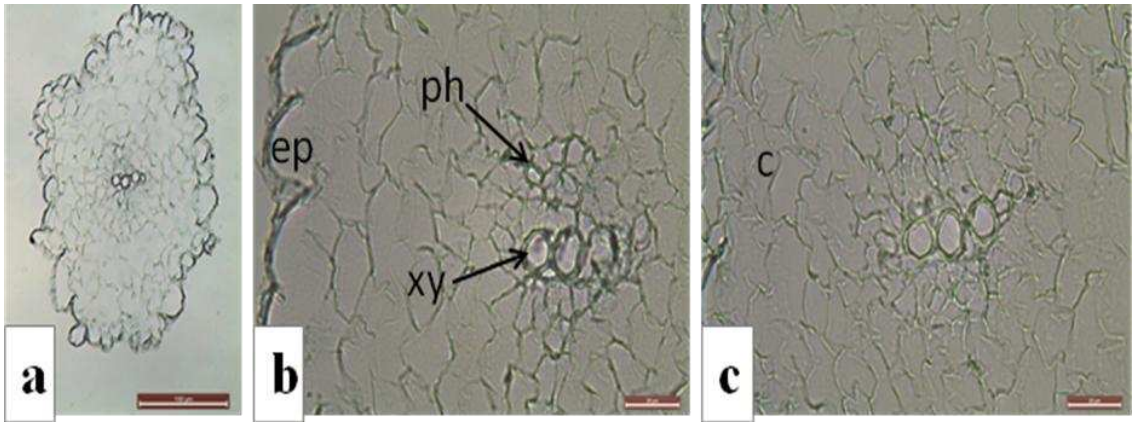


Figure 3. *Root cross section in cv. Panda a. general shape of root, bar = 100 µm, b. epidermis (ep), xylem (xy), phloem (ph), bar = 20µm, c. cortex (c), bar = 20 µm*

Biometric measurements were performed in xylem vessels in roots of two varieties. For these measurements, it was statistically determined that cv. Ceren's xylem vessels are slightly bigger than cv. Panda's xylem vessels (*Table 4).*

Table 4. *Results of xylem vessels diameter in roots (µm) ($p < 0.05$)*

Plants	Mean diameter of xylem vessels ± std deviation
cv. Ceren	9.982 ± 3.980
cv. Panda	8.059 ± 1.721

Stems

Stomate, epidermal hair and glandular hair are observed in stem of cv. Ceren which is surrounded by one layered epidermis (*Fig. 4a, b, g, h* and *i*). There are 1-2 layered chlorenchyma, 4-5 layered collenchyma and 4-5 layered parenchymatic tissue (*Fig. 4a* and *b*). Bicollateral vascular bundles surround the stem like a ring (*Fig. 4c* and *d*). 1-2 layered sclerenchyma around pholem facing in the direction of epidermis and 1-2, sometimes 3 sclerenchyma cells around phloem facing in the direction of pith are observed (*Fig. 4a, c* and *e*). Pith is parenchymatic. Crystal-sands are observed in parenchymatic cells (*Fig. 4f*).

One layered epidermis, stomate, epidermal hair and glandular hair are observed in stem of cv. Panda (*Fig. 5b, g, h* and *i*). There are 1-2 layered chlorenchyma, 3-4 layered collenchyma and 3-4 layered parenchymatic cortex (*Fig. 5a*). Vascular bundles surround the stem like a ring and they are bicollateral (*Fig. 5a* and *c*). 1-2 layered sclerenchyma around phloem facing in the direction of epidermis and 1-2 sclerenchyma cells around phloem facing in the direction of pith are observed (*Fig. 5a, d* and *f)*. Pith is parenchymatic (*Fig. 5e*). Crystal-sands are rarely observed in parenchymatic cells.

Biometric measurements were performed in some tissues in stems of two varieties. For these measurements, there is no clear difference between thickness of cuticle and diameter of xylem vessels of two varieties statistically. It was determined that the

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epidermis, the cortex and the collenchyma in cv. Ceren are thicker than those in cv. Panda (*Table 5*).

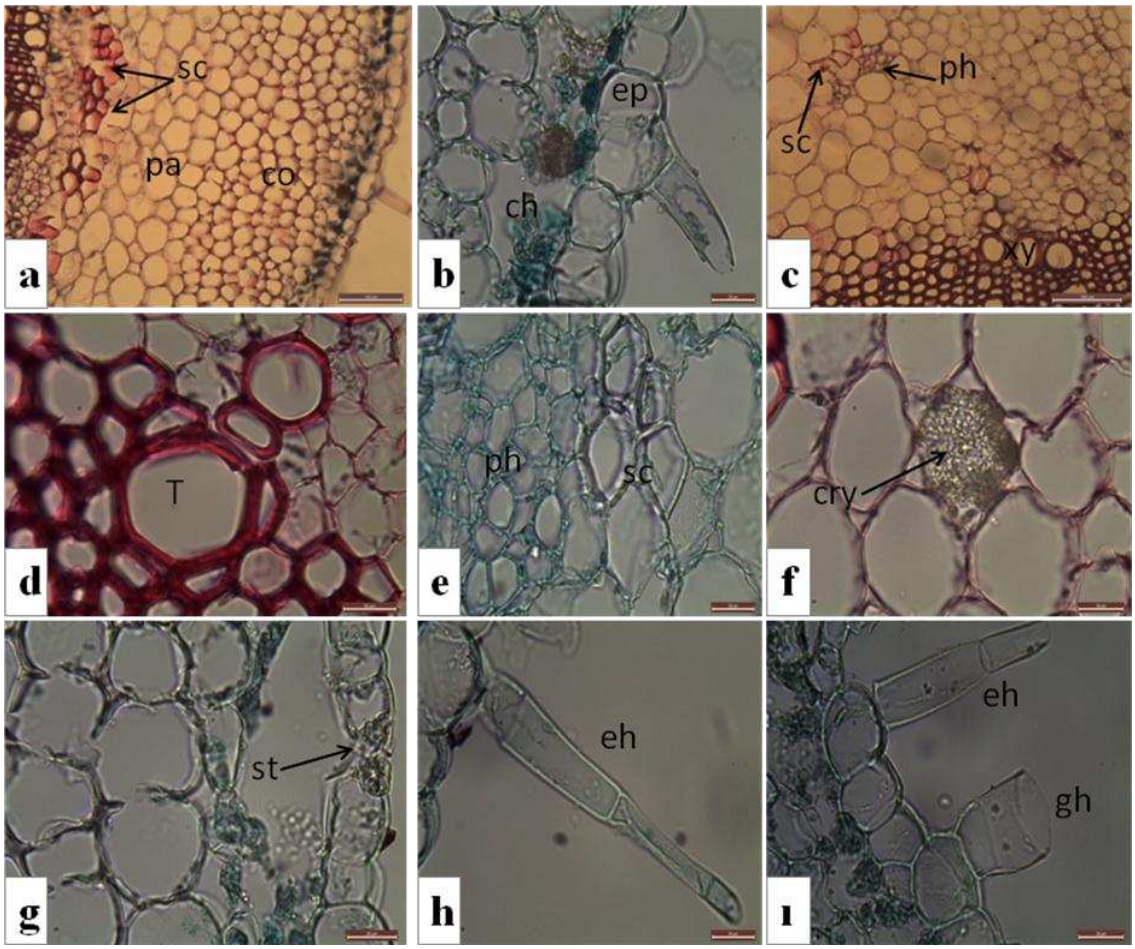


Figure 4. *Stem cross section in cv. Ceren. a. collenchyma (co), parenchymous (pa), sclerenchyma (sc), bar = 100 µm, b. epidermis (ep), chlorenchyma (ch), bar = 20 µm, c. sclerenchyma cells (sc), phloem (ph), xylem (xy), bar = 100 µm, d. trachea (T), bar = 20 µm, e. sclerenchyma layer (sc), phloem (ph), bar = 20 µm, f. crystal-sand (cry), bar = 20 µm, g. stomate (st), bar = 20 µm, h. epidermal hair (eh), bar = 20 µm, i. epidermal hair (eh), glandular hair (gh), bar = 20 µm*

Table 5. *Results of biometric measurements performed in some tissues of stems (µm)*

Plants	**Mean biometric measurements ± std deviation in some tissues of stems**			
	Thickness of cuticle**	**Thickness of epidermis***	**Thickness of cortex + collenchyma***	**Diameter of xylem vessels****
cv. Ceren	1.659 ± 0.292	25.681 ± 4.730	254.789 ± 23.388	31.144 ± 11.305
cv. Panda	1.864 ± 0.497	22.909 ± 9.014	236.890 ± 53.503	32.780 ± 13.570

*$p < 0.05$, **ns > 0.05

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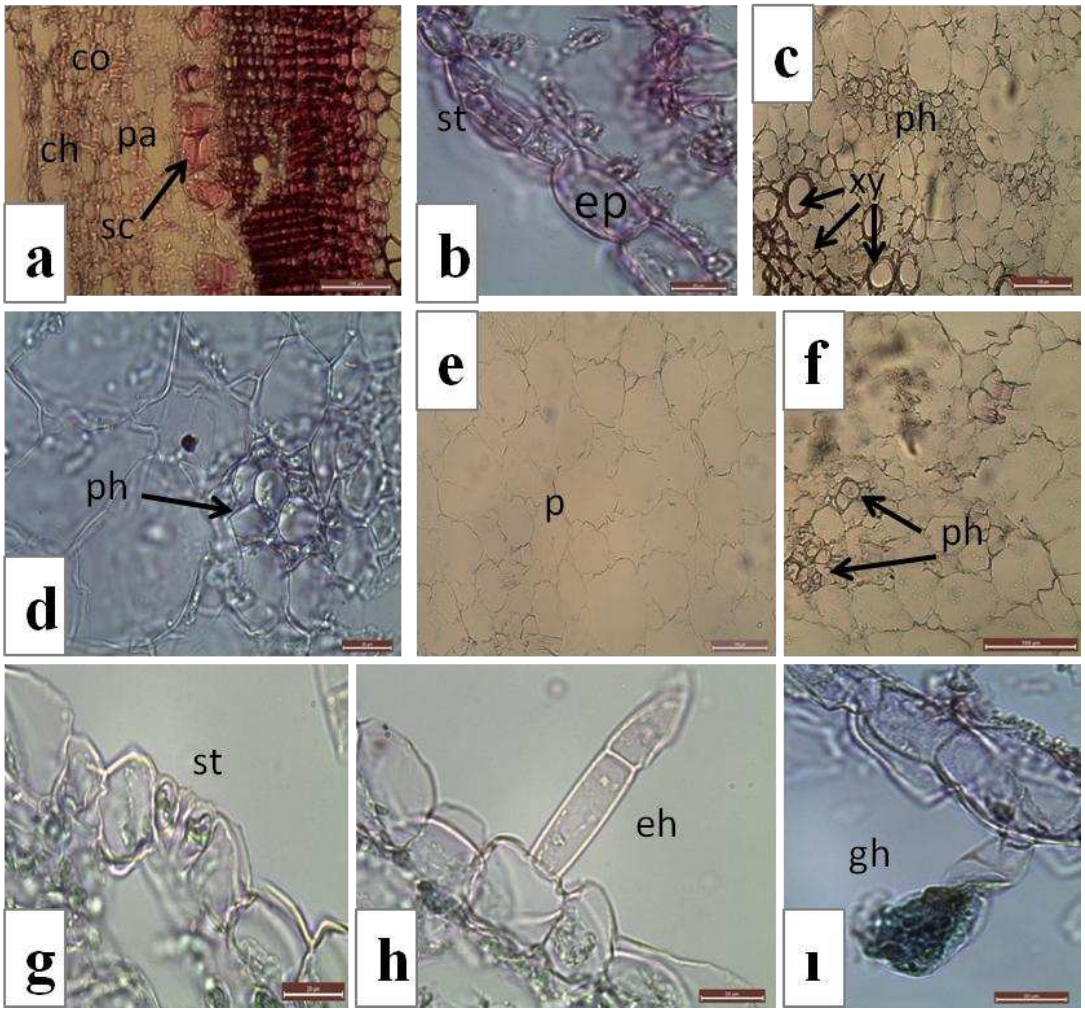


Figure 5. *Stem cross section in cv. Panda a. chlorenchyma (ch), collenchymas (co), parenchymous (pa), sclerenchyma layer (sc), bar = 100 µm, b. stomate (st), epidermis (ep), bar = 20 µm, c. xylem (xy), phloem (ph), bar =100 µm, d. phloem (ph), bar = 20 µm, e. pith (p), bar = 100 µm, f. phloem (ph), bar = 100 µm, g. stomate (st), bar = 20 µm, h. epidermal hair (ep), bar = 20 µm, i. glandular hair (gh), bar = 20 µm*

Leaves

cv. Ceren' s leaf is bifacial (*Fig. 6a*). Mesophyll is formed by one layered palisade parenchyma with spaces between the cells and 2-3 layered spongy parenchyma with spaces between the cells (*Fig. 6b*). Big vascular bundle is bicollateral (*Fig. 6c*). Collateral small vascular bundles are embedded into mesophyll and surrounded by bundle sheath (*Fig. 6d* and *e*). It is amphistomatic. They have glandular and epidermal hairs (*Fig. 6f* and *g*). Crystal-sands are observed (*Figure 6e*).

cv. Panda's leaf is bifacial. Mesophyll is formed by one layered generally short-celled palisade parenchyma with spaces between the cells and 2-3 layered spongy parenchyma with spaces between the cells (*Fig. 7a*). Big vascular bundle is bicollateral (*Fig. 7d*). Collateral small vascular bundles are embedded into mesophyll and surrounded by bundle sheath (*Fig. 7b*). It is amphistomatic (*Fig. 7a*). They have glandular and epidermal hairs (*Fig. 7e* and *f*). Crystal-sands are observed (*Fig. 7c*).

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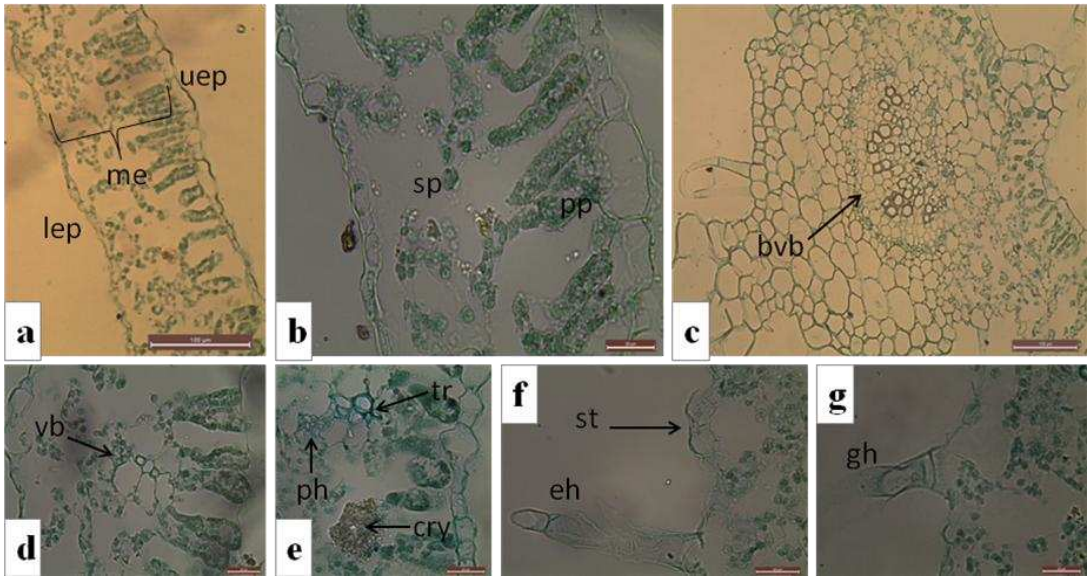


Figure 6. *Leaf cross section in cv. Ceren. a. upper epidermis (uep), mesophyll (me), lower epidermis (lep), bar = 100 µm, b. palisade parenchyma (pp), spongy parenchyma (sp), bar = 20 µm, c. big vascular bundle (bvb), bar = 100 µm, d. vascular bundle (vb), bar = 20 µm, e. phloem (ph), tracheid (tr), crystal-sand (cry), bar = 20 µm, f. stomate (st), epidermal hair (ep), bar = 20 µm, g. glandular hair (gh), bar = 20 µm*

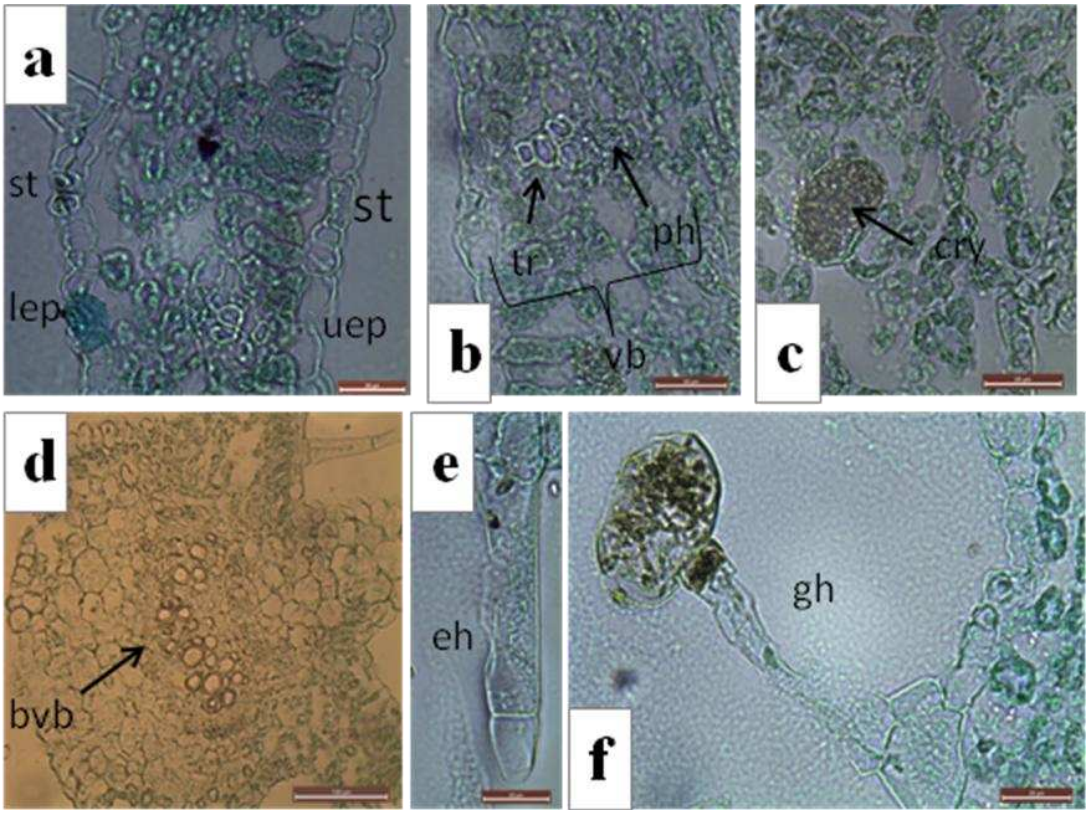


Figure 7. *Leaf cross section in cv. Panda a. stomate (st), upper epidermis (uep), lower epidermis (lep), bar = 20 µm, b. phloem (ph), tracheid (tr), vascular bundle (vb), bar = 20 µm, c. crystal-sand (cry), bar = 20 µm, d. big vascular bundle (bvb), bar = 100 µm, e. epidermal hair (ep), bar = 20 µm, f. glandular hair (gh), bar =20 µm*

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Biometric measurements were performed in some tissues in leaves of two varieties. These measurements showed no clear difference between mean thickness of cuticle, lower epidermis and diameter of xylem vessel of two varieties statistically.

Upper epidermis is thicker in cv. Ceren than in cv. Panda. It is determined that cv. Ceren's mesophyll is wider, and its big vascular bundle is longer than those in cv. Panda. Width of big vascular bundle in cv. Ceren is smaller than the one in cv. Panda (*Table 6*).

Table 6. *Results of biometric measurements performed in some tissues of leaves (µm)*

Mean biometric measurements ± std deviation in some tissues of leaves	**Plants**	
	cv. Ceren	**cv. Panda**
Thickness of cuticle***	1.048 ± 0.103	1.727 ± 0.159
Thickness of upper epidermis*	19.460 ± 4.292	13.642 ± 3.065
Thickness of lower epidermis***	6.692 ± 1.723	8.488 ± 2.451
Width of mesophyll*	118.373 ± 7.485	70.830 ± 17.463
Diameter of xylem vessels***	9.066 ± 4.742	7.674 ± 5.562
Lenght of big vascular bundle between parenchymatic tissues**	159.990 ± 16.062	147.356 ± 11.308
Width of big vascular bundle between parenchymatic tissues**	263.381 ± 7.527	270.616 ± 28.103

*$p < 0.01$, **$p < 0.05$, ***ns > 0.05

Discussion

The root of cv. Ceren is formed by very thin-walled parenchymatic cells between epidermis and vascular bundles and these cells could not be clearly observed. Parenchymatic cells in cv. Panda are more thick-walled than in cv. Ceren. Endodermis and pericycle could not be observed in cv. Panda either. According to Sam et al. (2000) structure and functions of all plant organs are affected by water stress. The diameters of root xylem vessels are different from each other in cv. Ceren and cv. Panda. In Tuylu et al., (2018), Malatya Kurucaova variety of tomato was examined and stated that cortex is thicker in the root cultivated in hydroponic culture and the diameters of root xylem vessels are narrower than those in the root cultivated in perlit. Regarding the two studies it is clear that cortex and xylem vessels in the root were affected by water stress.

The anatomical features in the stems and leaves examined are similar to the general anatomical features of Solanaceae family and *Lycopersicon esculentum* Mill. mentioned in Metcalfe and Chalk (1950) and Rost (1996). The fact that both plants have some features such as palisade parenchyma cells with spaces is the result of adaptation which they developed under water stress conditions. According to the results of mean biometric measurements in stems and leaves of cv. Ceren and cv. Panda, they adapted to the condition with histologically different sizes. The mean thickness of epidermis and mean thickness of cortex and collenchyma in stem of cv. Ceren are more than the ones in cv. Panda. Significant differences between thickness of cuticle and diameter of xylem vessel of the two varieties were not observed. In Tuylu et al. (2018) it was determined that cuticle and epidermis are thicker and cortex and xylem vessel are narrower as a

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result of water stress in the stem cultivated under hydroponic system than those cultivated in perlit condition.

According to the results of mean biometric measurements in leaves, significant differences between thickness of cuticle, lower epidermis and diameter of xylem vessels of the two varieties were not observed. The fact that in cv. Ceren upper epidermis is thicker than in cv. Panda is the result of different adaptation of the two varieties to water stress. Sam et al. (2000) studied the effects of water deficit on epidermis of leaf at preflowering stage in *Lycopersicon esculentum* Mill. cv. INCA9 and stated that water stress have slight effects on width and lenght of epidermis cells. Thus, it is understood that the plant also shows epidermal change under water stress as it shows under water deficit. The width of mesophyll in cv. Ceren increases more clearly than the one in cv. Panda. In parallel with this, the length of big vascular bundle in midrib region increases and its width reduces. Tuylu et al. (2018) figured out that midrib region is wider due to water stress in leaf of tomato cultivated in hydroponic system and big vascular bundles are larger than the ones cultivated in perlit condition. They stated that no clear change was measured in other tissues of leaf.

Conclusion

Considering the histological changes and biometric measurements it is understood that adaptation of cv. Ceren and cv. Panda have some differences from each other under the same cultivation conditions. According to the results it is figured out that some anatomical features such as parenchymatic cells, endodermis, pericycle, xylem vessel in root, epidermis, cortex and collenchyma in stem, upper epidermis, mesophyll, big vascular bundle in midrib region in leaf can vary from each other in terms of histologically thickness, width, length, arrangement of cells and etc. in the two different varieties belonging to the same genus cultivated at the same time and under the same condition.

In the study, the two varieties could grow in alternative condition which is out of their natural cultivation condition and it was observed that they could complete their developments in some basic tissues. In this cultivation the roots of plants were exposed to excess water, but both plants adapted to water stress condition and developed by means of some anatomical changes. In both plants no clear anomaly was observed. cv. Ceren and cv. Panda are convenient for cultivation under hydroponic culture mentioned in terms of some basic anatomical features examined. However; features of fruit of the plants should be evaluated in terms of parameters of yield and quality before recommending the producers to cultivate the two varieties under hydroponics. In addition, in the study the anatomical features of the two varieties determined will contribute to other studies.

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ASSESSING THE TEMPORAL AND SPATIAL DYNAMICS OF THE FOREST FIRES IN SOUTHEASTERN CHINA

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Abstract. This study presented the distribution of the largest forest fires in the area of Sanming, Fujian province of China from 2000 to 2009 and focused on the spatial and temporal dynamics of forest fire occurrences. The fire location distribution, occurrence causes, and daily, monthly and annual distribution of fires influenced by weather were examined. The weather data was analyzed using the software FWI Calc. v.10.3.1.106. There were a total of 818 forest fires occurred in the period 2000-2009 that burned 87 million m^2 of forests; the fires have been detected during the fieldwork. The time distribution of forest fire occurrence had a regular pattern daily, and most forest fires occurred between 9 a.m. and 5 p.m., accounting for 92.42% of total fires. It was found that drier and warmer weathers provided favorable conditions for forest fire occurrences, and the majority of serious fires were occurred between 2008 and 2009, which was a period with relatively dry and warm weather. Significant relationships between forest fire occurrence and weather were determined in the study. Because of human activities and more combustible species in Youxi and Datian counties in the Sanming area, the two counties had the highest fire risks.

Keywords: *fire dynamics, forest meteorology, weather parameters, Fire Weather Index (FWI), Canadian Forest Fire Danger Rating System (CFFDRS), Sanming*

Introduction

Over the last decades, scientists and forest managers have begun to explore the emulation of forest fire disturbance in managing forest landscape (Seymour et al., 2002; Cleland et al., 2004; Nitschke, 2005; Ekoungoulou et al., 2017) because forest fire is a key process in regulating vegetation succession, plant regeneration, and maintaining biodiversity in many forest ecosystems (Rollins et al., 2004; Ekoungoulou et al., 2018b). Trees, the major components of forests (Ekoungoulou et al., 2014a and 2014b), absorb large amounts of atmospheric carbon dioxide by photosynthesis (Ekoungoulou et al., 2015), and forests return an almost equal amount to the atmosphere (Ekoungoulou et al., 2018c) by auto-and heterotrophic respiration (Ekoungoulou et al., 2014b and 2014c). Understanding the spatial and temporal variation of forest fire provides a long-term perspective of dynamics of ecosystem processes and vegetation patterns (Gonzalez, 2005; Nonaka and Spies, 2005). Use of forest fire statistics, including records of ignition sources, number of forest fire occurrences, and burnt area, is an effectual method to understand the spatial and temporal characteristics of forest fire (Kasischke et al., 2002; Liu et al., 2015). Variability of climate and fire weather also influences forest fire behaviors and the fire severity at various time scales (Liu et al., 2010).

Different geographical, topographical, and climatic characteristics exist across the vast landmass of China; as a result, forest fire regimes would vary spatially in the

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country. Forest fire has been a serious issue in China's forest management; in the period 1950-2000, there were 13,600 fire occurrences per year in China. The yearly average of burned area in the period was approximately 758,000 ha, according to the official report from the China's forestry bureau (Zhang and Qingwen, 2008). Because of climatic differences across China, fire season varies in major forest regions. In the Northeastern China and Inner Mongolia, fire season lasts from spring to autumn. Fire season is from winter to spring in the southern and southwestern China and from summer to autumn in the northwestern China. In China, wildland fires are not distributed uniformly over the country; most of the fires occurred in approximately 100 key counties from 16 main forestry regions (Guo and Fu, 2007). Also, the number of forest fires in China and the burnt area fluctuated largely annually, and the burnt area was over 1 million ha in years 1951, 1955, 1956, 1961, 1962, 1972, 1977, 1987 and 1996 (Zhong et al., 2003).

The increase of human population and human activities has resulted in an increase in forest fire frequency (Guo and Fu, 2007). As modern society has encroached on forests and thereby forest fires threaten the regional economy and the safety of residents, forest fires have become an important issue socially as well as scientifically (Kalabokidis et al., 2002). From the statistical data reported by the Ministry of Forestry, China in 1999, most wildland fires are caused by non-productive fire utilizations such as smoking (accounting for 40.6%). The rest were caused by productive fire utilizations such as prescribed fires for land reclamation (30.9%), lightning and other natural disasters (0.38%), arson (1.52%), and others (21.18%) (Anon, 1999). There is no doubt that much progress has been achieved in forest fire prevention and protection in China since the foundation of the new China (Lifu et al., 2003). However, with the fast growing economy in China, the present situation of forest fire protection is becoming even more urgent. Therefore, the forest fire study in Sanming becomes important. The study focused on spatial and temporal fire distribution, ignition causes, and the influence of temperature, humidity, precipitation and wind speed on forest fire occurrence. The study can help develop local fire management and forest fire prevention projects.

Materials and Methods

Study site

Sanming is located in the northwest of Fujian Province, between 116.22 to 118.39 °E and 25.30 to 27.07 °N, with ~230 km from east to west and ~180 km from south to north (*Fig. 1*). The total area of Sanming is approximately 22,900 km^2, including the urban area of 1,178 km^2. Mountainous area farm land, river and others account for 82%, 7.3%, 8.7%, and 2% of Sanming city, excluding the urban area. Sanming belongs to a sub-tropical climate of monsoon with considerable precipitation and clear presence of four seasons. Annual mean temperature is between 17-19.4°C; average annual precipitation is from 1,565 to 1,795 mm with the maximum annual precipitation 2,255 mm and the minimum annual precipitation 1,131 mm, and average relative humidity is between 79 to 94%.

The forest cover of the city is around 28.26 million hm^2, and by the year of 2000, the forested area in the city had already reached its upper limit with 71% of available lands being forested (Guo and Fu, 2007). Chinese fire (*Cunninghamia lanceolata* (Lamb.) Hook) and Masson pine (*Pinus massoniana* D. Don) account for 76% of the forested area. The dominance of the two species resulted in an overall lack of bio-diversity and

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an increasing challenge to the ecological health of the forest in the city (Huang, 2009; Song et al., 2004).

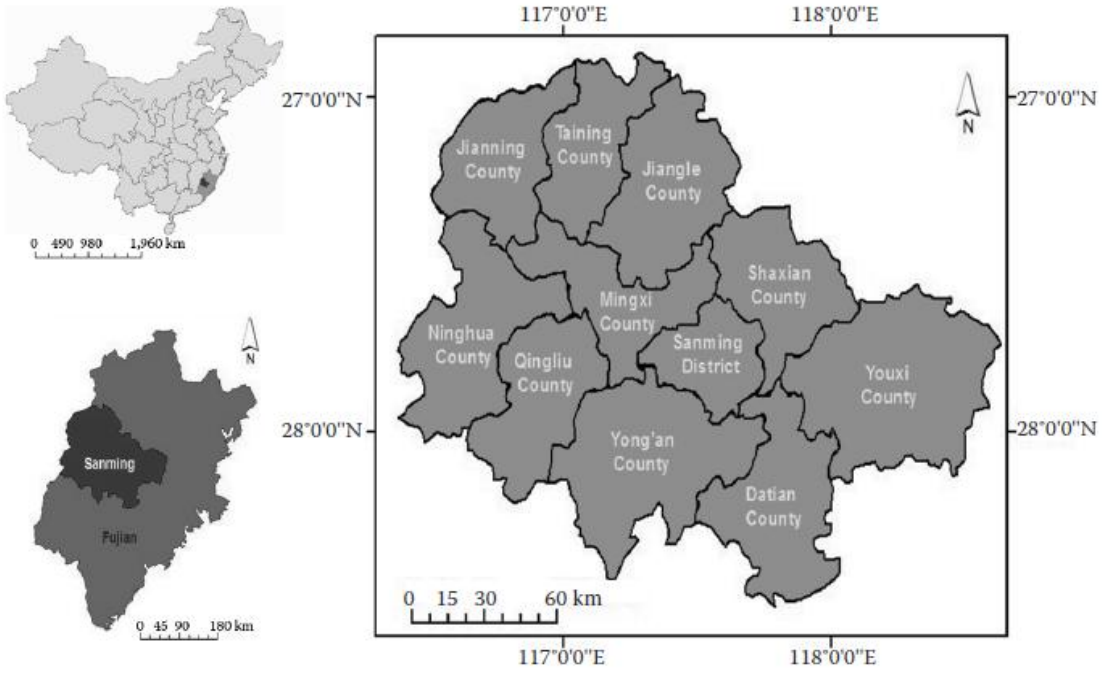


Figure 1. *Location of Sanming city and Fujian Province in Southeast China*

Data collection

In this study we used fire occurrence records provided by the Administrative Divisions of Fujian Province from 2000 - 2009. Each record consists of fire information such as fire locations, fire severity classes, ignition causes and the date of fires. Weather data of temperature, relative humidity, precipitation and wind speed were collected from the Yong'an Meteorological Station, which is located at 117.21 °E longitude and 25.58 °N latitude with the elevation of 206 m. Both fire dandy weather data were analyzed using statistical software Minitab 2002 to find out relationship between forest fire occurrence and weather conditions. The spatial and temporal analyses were conducted using the software FWI Calculator v.10.3.1.106 and the graphic results were prepared using the software SigmaPlot v.10.0.

Data analysis

The Fire Weather Index (FWI) system is the first part of Canadian Forest Fire Danger Rating System (CFFDRS) introduced into New Zealand in 1980. It has proved to be a suitable fire danger rating system for the country. The FWI was evaluated for several seasons before it was introduced for the 1980-81 fire season. FWI is based on weather readings taken at noon standard time and it rates fire danger at the midafternoon peak from 2:00 - 4:00pm. Weather readings required include the air temperature in the shade, relative humidity in the shade, wind speed at 10 meters aboveground level for a mean over 10 minutes; rainfall for the previous 24 hours. FWI calculator v.10.3.1.106 can read the weather input file every 15 minutes after the initial midday reading. If the

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weather station software keep updating this file (e.g., every 10-15 min), FWI Calculator will record the peak temperature and other relevant weather values. The FWI System outputs three moisture codes: the Fine Fuel Moisture Code (FFMC), the Duff Moisture Code (DMC), and the Drought Code (DC), with higher values indicating drier conditions and greater fire danger. The FWI System also generates four fire behaviour outputs based on the above indices: the Initial Spread Index (ISI), the Build-Up Index (BUI), the Fire Weather Index (FWI), and the Daily Severity Rating (DSR), with higher values indicating elevated fire danger. A fire danger rating system should act as a tool on tracking the day-to-day susceptibility of forests to fire, or the system should enable fire managers to properly assess the levels of preparedness and the suppression resources needed to keep fire losses to a minimum. The system should be capable of measuring weather variations that cause the day to day changes in fire danger. The fire danger information provided through the system can be used to define the fire season (Liu et al., 2015), determine appropriate fire prevention measures, assess the likelihood of fire occurrence, determine fire suppression response and resources deployment, inform public, make decisions to close areas at high risk, issue or cancel burn permits, plan and conduct controlled burns.

The graphic results have been prepared using the SigmaPlot v.10.0 (Ekoungoulou et al., 2018a) software. Analysis of Variance (ANOVA) was used (Ekoungoulou, 2014) to test possible differences between fire occurrence among different months and years. Fire ignition causes and their spatial distribution were showed as topographic maps using the version 9.3 of ArcGIS software.

Results and Discussion

Spatial distribution of three classes of forest fire

Spatial distribution of forest fires in Sanming was showed in *Table 1*, and the fires were categorized into three classes. Class 1 means fire warning, Class 2 means ordinary fire, and Class 3 indicates serious fire. According to this classification scheme, the fires that burn less than 1 hm^2 are classified as Class 1 fire; the fires that burn between 1 hm^2 and 100 hm^2 are Class 2 fires, and Class 3 fires are the ones burn more than 100 hm^2. There were 133 Class 1 fires, 674 Class 2 fires and 11 Class 3 fires occurred in Sanming between 2000 and 2009, respectively, and total fire area and total burnt areas in this period were 11,730.27 hm^2 and 8,721.16 hm^2, respectively. In the southeastern Youxi and Datian counties, Class 3 fires occurs more frequently than in other counties, and burnt areas in the two counties were relatively large compared to other counties (*Fig. 2*). There were 172 and 146 fires, and burnt areas were 1,703.70 and 2,350.50 hm^2, respectively in Youxi and Datian counties from 2000 to 2009. So, Datian counties was considered the highest vulnerable to forest fire in Sanming city during the study period. The results revealed that Classes 1 and 2fires distributed in all countries, but only Class 1 fires occurred in the Sanming district from 2000 - 2009.

Ignition causes

According to the statistical data of forest fire occurrences in the last 10 years in Sanming, a substantial number of forest fires were caused by human. *Fig. 3* indicates that 64.97% of fires were caused by waste-land-burning, followed by 9.41 of careless smoking, 4.48% of fire from outside, 3.47% of children-playing-fire, and lightning of

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0.82%. Clearly, most forest fires in Sanming are caused by human activities, whereas lightning only cause a small number of forest fires. Waste land burning is the major causes of forest fire in Sanming, and such caused fires were unevenly distributed in the study area. A total of 462 fires were caused by waste-land-burning; among these fires, 60 were Class 1 fires; 398 were Class 2 fires, and only four Class 3 fires, respectively. *Fig. 4* indicates that most of waste-land-burning fires were n Youxi (172 cases) and Ninghua (95 cases) counties, and in the two counties human disturbance was mainly cause for forest fires. The spatial and temporal changes of forest fires in the study are studies in the following aspects: daily changes, monthly and seasonal variations, and annual changes.

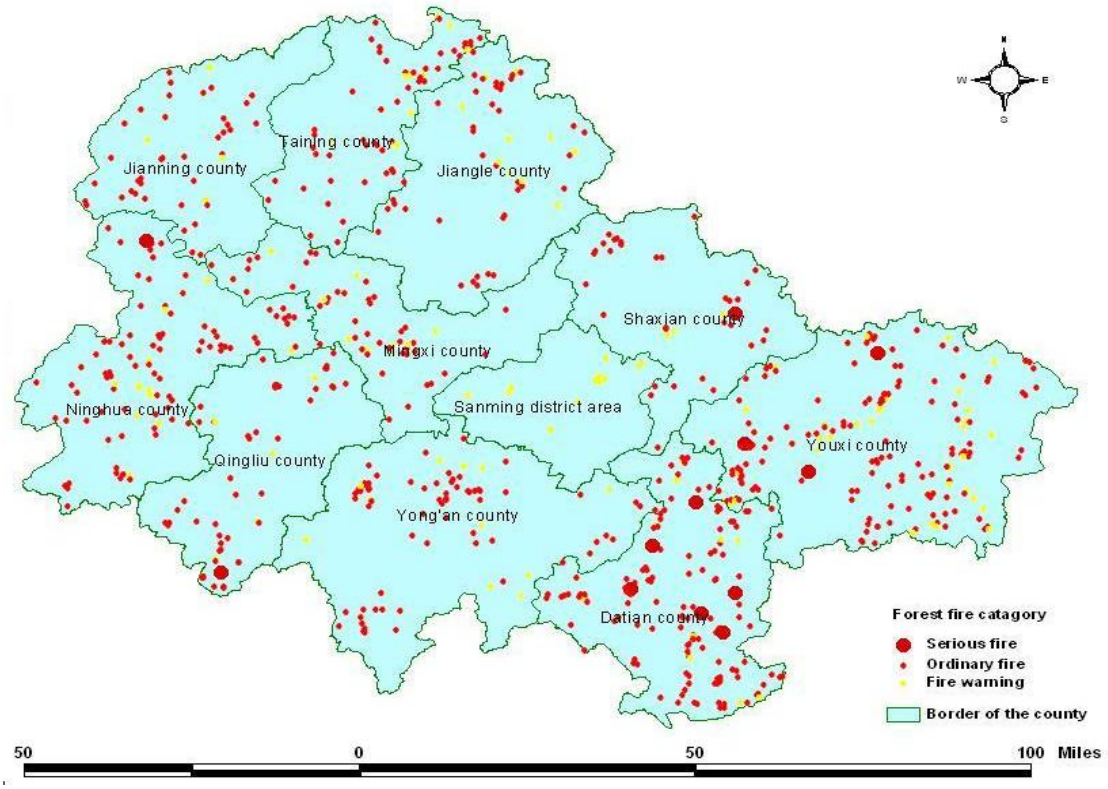


Figure 2. *Spatial distribution of three classes of forest fire the study area*

The daily variation of forest fires

In the *Fig. 5a*, it was found that most of the forest fires occurred in Sanming between 9 a.m. to 5 p.m., and during this time period 92.42 % of the total fires, or 161 fires, occurred at 2 p.m with a burnt forest area 2,271.10 hm^2 (*Fig. 5b*). No fire has ever occurred from 1:00 am to 5:00 am, and only nine fires occurred between 5:00am to 6:00am. This is because there were almost no human activities in such time period daily and weather at such times were relatively cool.

Fig. 5c and *Fig. 5d* showed the temporal distributions of fire frequency and burnt area along the day time from 1:00am to 11:00pm. The fires caused by waste land burning peaked mainly from noon to 3 p.m., with a total of 308 fires burnt 5,213.90 hm^2 areas. The fires with unknown causes occurred from 9 a.m. to 9 p.m. with a total of 108 fires and a burnt area of 1,207.70 hm^2, which were comparatively lower than those of

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the waste land burning fires. Lightning-caused fires mainly occurred from noon to 5 p.m., and the total burnt area was 120.10 hm^2.

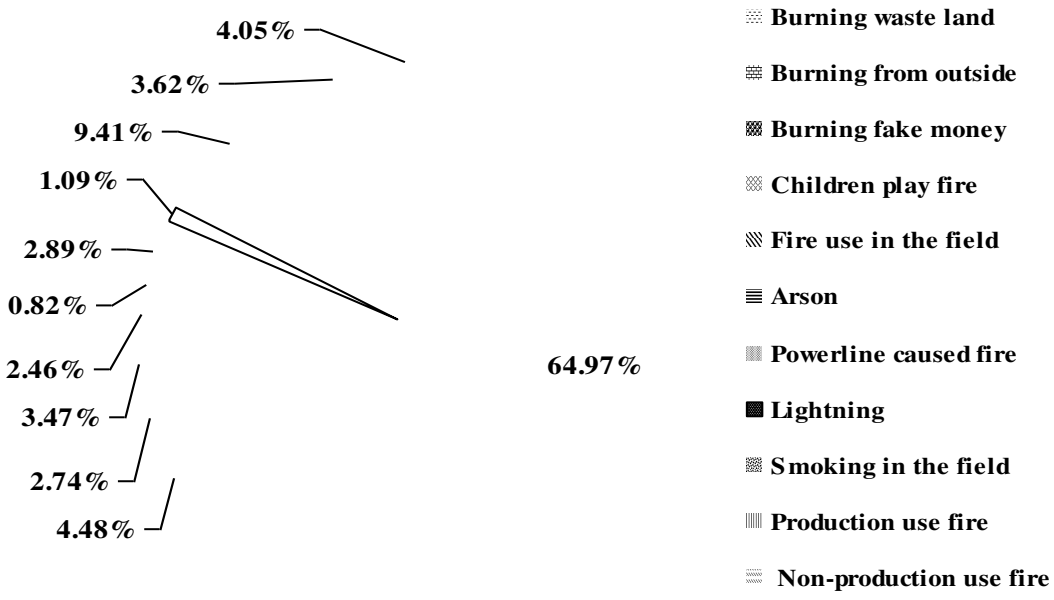


Figure 3. *Causes of forest fires in Sanming during the years 2000 – 2009*

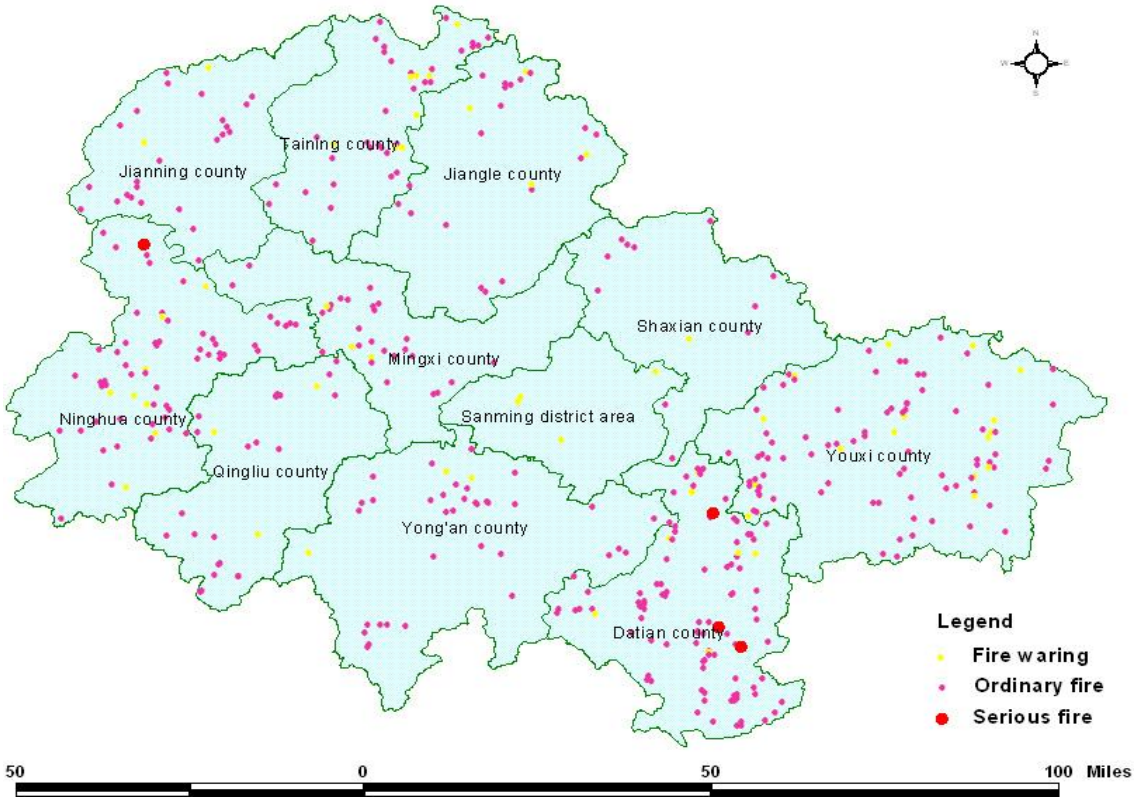


Figure 4. *Forest fire occurrences in Sanming due to burning waste land*

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Table 1. *Forest fire statistics in Sanming between 2000 and 2009*

Study Area	Number of fire	Forest fire classes			Fire area (hm^2)	Burnt area (hm^2)
		Class 1 Fire warning	Class 2 Ordinary fire	Class 3 Serious Fire		
Datian	146	13	127	6	3528.00	2350.50
Youxi	172	34	136	2	2442.10	1703.70
Yong'an	95	12	82	1	1315.50	1139.10
Qingliu	46	11	34	1	1027.60	832.20
Shaxian	58	5	52	1	946.00	779.70
Mingxi	85	13	72	0	774.90	524.32
Jiangle	42	4	38	0	512.80	438.40
Jianning	51	10	41	0	480.10	374.90
Taining	52	9	43	0	384.30	305.80
Ninghua	59	10	49	0	311.27	269.84
Sanming	12	12	0	0	7.70	2.70
Total	**818**	**133**	**674**	**11**	**11730.27**	**8721.16**

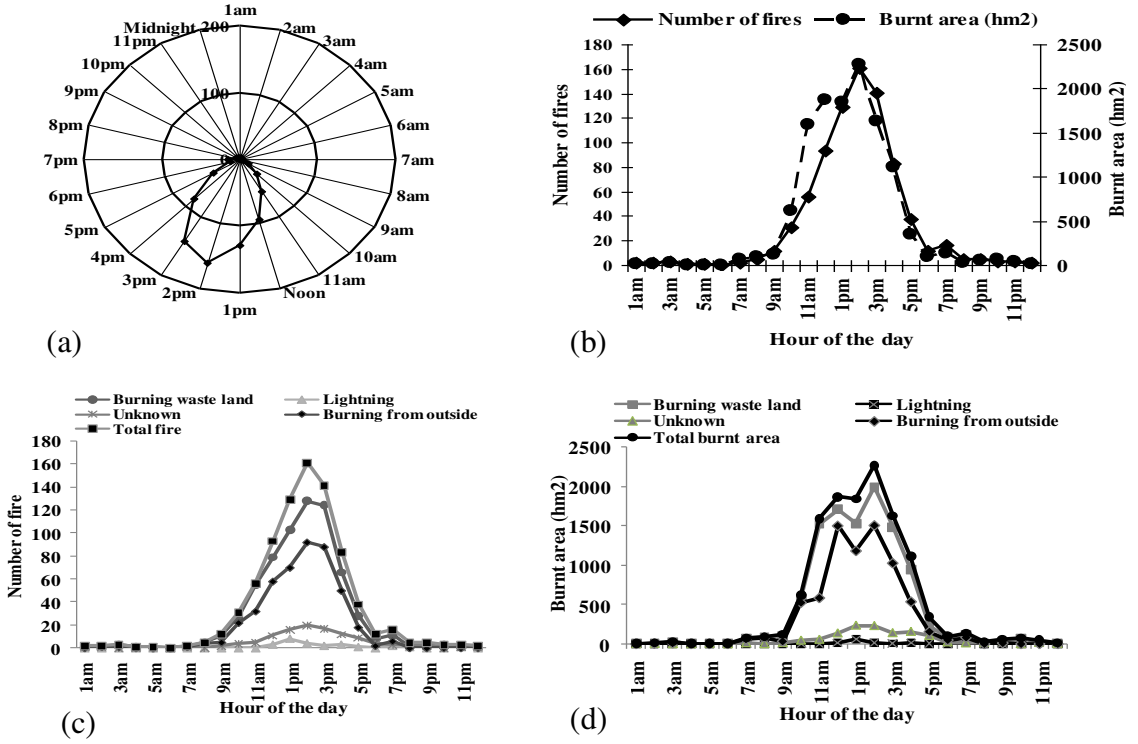


Figure 5. *Forest fire and burnt area distributions in a day time*

Monthly change of forest fire

As *Fig. 6a* indicates, over the 10-year period 2000- 2009, fire frequency was higher in spring (January-March) than in other seasons., because during the period of January-March weather are relatively dry and relative humidity are low (*Fig. 6b*). March is the month with most forest fires occurred and large areas burned, followed by February and

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January. During the period 2000-2009, there were 269 fires occurred in March that burned 4,976.40 hm^2, which accounted for 33% of fire occurrences and 41% of total area burnt, respectively. February and January were ranked as the second and third in terms of fire frequency and areas burned; there were 179 fires in February with an area burned of 3,730.50 hm2, which accounted for 22% of total fire occurrences and 31% of areas burned. The same figures for January were 87 and 1,170 hm^2, which were 11% of total occurrences and 10% of areas burned.

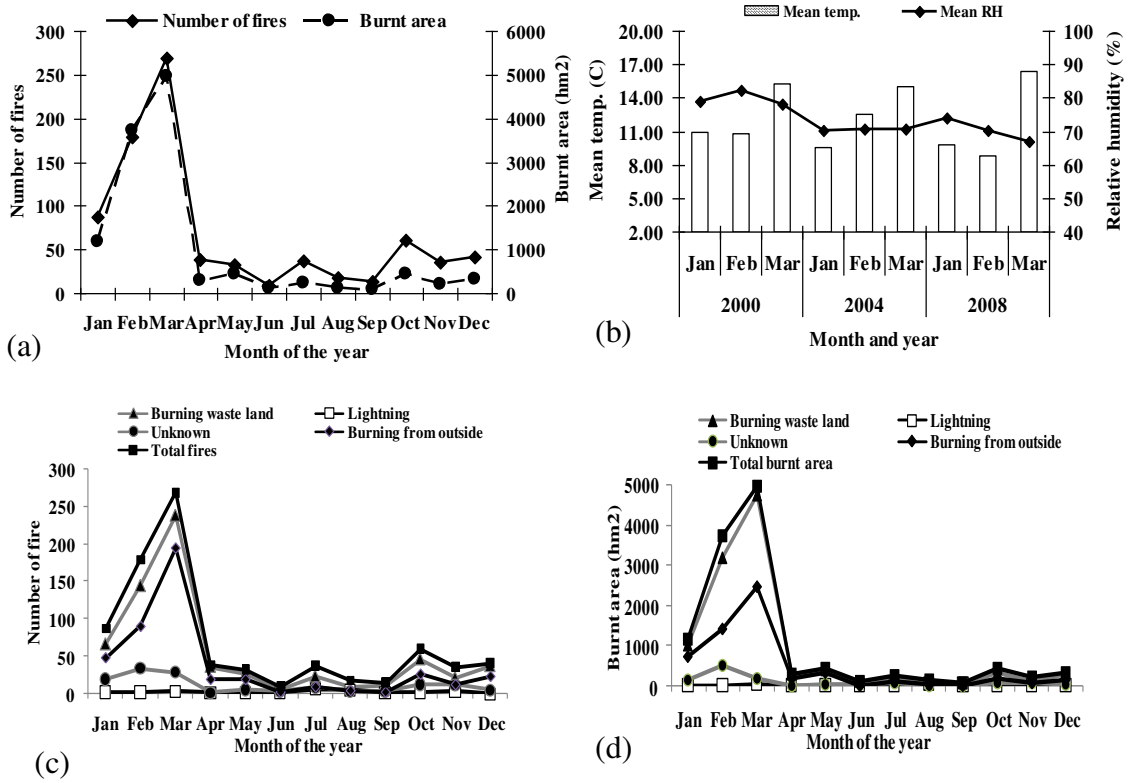


Figure 6. *Forest fire and burnt area distribution in the month of the year*

Fig. 6c and *Fig. 6d* contain the monthly distributions of fire frequency and areas burned by different ignition causes. Waste land burning for crop production and burning from outside sources were major ignition causes that accounted for about 70% of total fire occurrences 84% of all area burnt in three months from January to March. The period of May to December appeared to be the time least prone to forest fires. The fires of unknown causes were most likely to occur in the beginning months of a year and the frequency of such fires was highest in February. Lightning-caused fires were the least serious in the study area.

Forest fire annual distributions

Fire situations varied from year to year in the period 2000-2009, and the statistics showed in *Fig. 7a* and *Fig. 7b* suggested that the years with more than 100 forest fires and an area more than 1,500 hm^2 were 2000, 2004, 2008 and 2009. Other years suffered less forest fires. Waste land burning, burning from outside sources, and unknown causes were the three major ignition causes. The severity of such fires was raising from 2007,

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and in 2009 the total forest occurrences and areas burned by such fires were 165 and 3,861.30 hm^2, respectively.

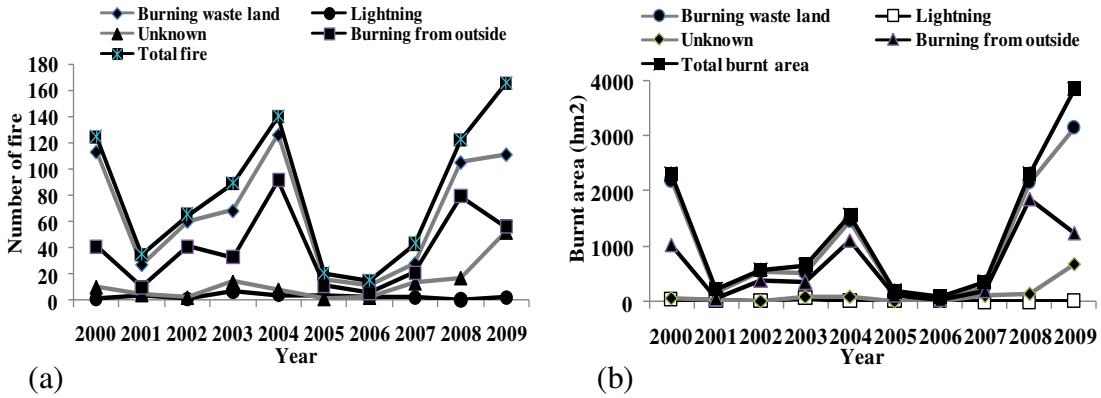


Figure 7. *Forest fire annual distributions in Sanming*

Influence of forest fire by weather parameters

Forest fires are more likely to occur when it is dry and less likely to occur when it is wet and with low temperature. *Fig. 8a* and *Fig. 8b* depicted decreasing trends of rainfall and relative humidity caused by increasing trends of temperature and wind speed. Such weather change patterns lead to a dry weather condition that favors more forest fire occurrences. All serious fires occurred in the day time between 10:30 and 16:15 hours because of dry and warm weather during this time period of the day. In Sanming, most of serious fire occurred in 2008 and 2009 in the early noon when temperature is maximum (> 29 °C), wind speed reaches > 3 m/s, and relative humidity is low with no precipitation (*Table 2*).

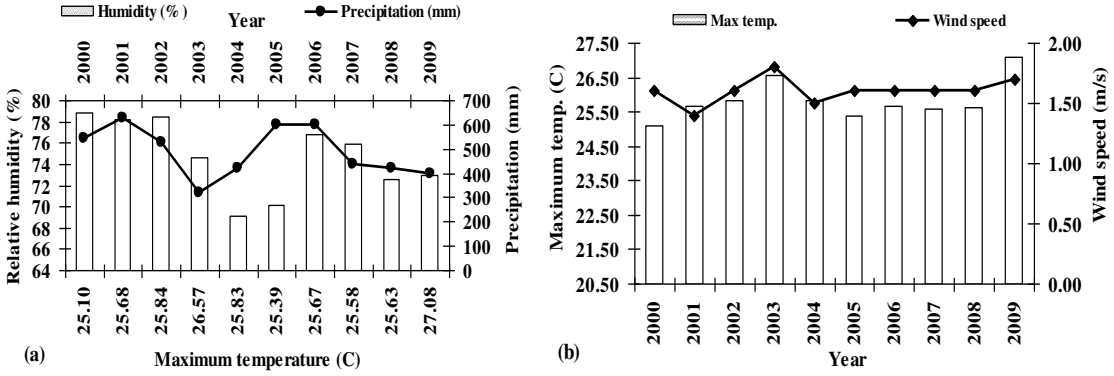


Figure 8. *Weather parameters change trend in Sanming between 2000 - 2009*

Table 3 consisted of the F-test results on annual and monthly forest fire distributions. Have significant relationship with changes of temperature, precipitation, relative humidity and wind speed, and such atmospheric movement prone to highest forest fires in Sanming.

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Table 2. *Serious forest fire occurrences in Sanming during the study period*

Sl. No.	Date (Year/month/day)	Time (24 hours)	Area burnt (hm^2)	Maximum Temperature (°C)	Relative humidity (%)	Precipitation (mm)	Wind speed (m/s)
1	2000-03-28	11:53	179.50	30.6	50	000	1.5
2	2000-03-28	13:30	236.10	30.6	50	000	1.5
3	2008-03-02	12:40	254.00	23.7	55	000	1.4
4	2008-03-03	12:10	265.50	23.6	48	000	1.3
5	2008-03-04	10:30	170.00	22.3	48	000	1.6
6	2008-03-04	12:50	113.90	22.3	48	000	1.6
7	2009-01-18	14:30	185.80	23.7	65	000	0.9
8	2009-02-12	13:40	176.10	29.2	50	000	3.5
9	2009-02-12	16:02	294.60	29.2	50	000	3.5
10	2009-02-12	11:30	287.00	29.2	50	000	3.5
11	2009-02-12	11:20	498.70	29.2	50	000	3.5

Table 3. *Statistical analysis showed relationship between forest fire and weather parameters*

Analysis (95% confidence interval)			
	F	P	Remark
Forest fire annual distributions	1.64	0.078	Significant
Forest fire monthly distributions	1.18	0.002	Significant
Effect of temperature on forest fire occurrences	1.28	0.032	Significant
Effect of relative humidity on forest fire occurrences	1.39	0.067	Significant
Effect of precipitation on forest fire occurrences	1.45	0.042	Significant
Effect of wind speed on forest fire occurrences	2.20	0.012	Significant

Sanming zoning map based on fire proneness

Sanming can be divided into three zones based on fire proneness, i.e., the high risk zone, the reasonable risk zone, and the low risk zone (*Fig. 9*). The southeasttern Youxi and Datian counties are delineated as the high fire risk zone (Class I fire danger area) because of high human disturbance and concentrated distribution of more combustible Masson pine forests. The reasonable risk zone (Class II fire danger area) includes the northwestern Jianning, Mingxi, Ninghua, Qingliu, and South Yong'an counties; the human disturbance in these counties is minimal and the area has been covered by less flammable Chinese fire forest. Taining, Jiangle, Shaxian counties and Sanming district belong to the low risk zone (Class III fire danger area) where only few fires occurred in small. The vegetation cover in the zone is less combustible species of eucalyptus and bamboo. The map provides a useful tool for local forest managers for planning and executing their fire prevention and suppression exercise.

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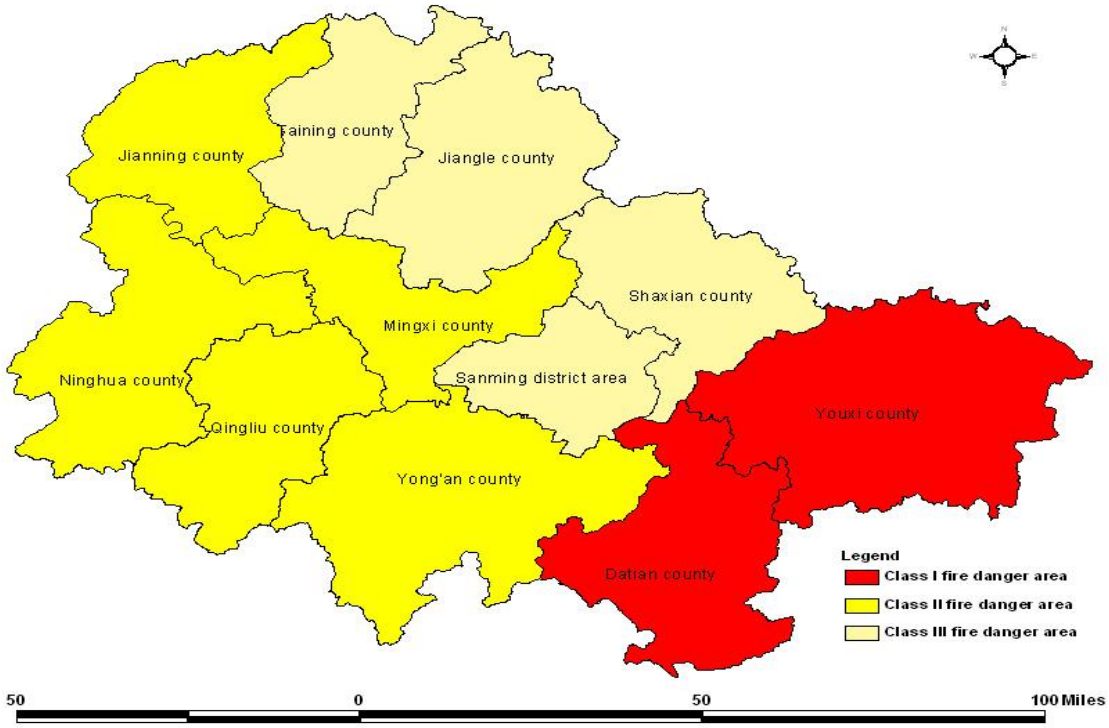


Figure 9. *Sanming zoning map based on forest fire proneness*

In Sanming city, most forest fires are caused by human-being, and lightning and arson fires only make up a small portion of all fires in the period 2000-2009. Our results are similar to the finding by (Zhong et al., 2003) that most forest fires in China are human-caused. Temporal and spatial analyses of forest fires are important for successful forest fire management (Keane et al., 2003). In the current study, we analyzed the temporal and spatial patterns forest fires based on forest fire statistics between 2000 and 2009 in the city. The highest numbers of fires occurred in Sanming around 2 p.m., and there are similar studies in other localities in China that forest fires are more likely to occur at 2:00 p.m in Beijing, Wuyishan Mountain and Hangzhou city (Huang, 2009; Liu et al., 2009). A study conducted Toronto, Canada illustrated that the fires incident density changes over time (in hour), the period between after-lunch time and the beginning of evening time seems to have the densest patterns of forest fire appearance (Asgary et al., 2010). The study also found the important number of occurs of forest fire in the spring and the feeble number of fire damage occurs in the autumn. The time-series processing of monthly forest fire occurrences and zone burned did show a strong seasonal pattern. Spring season was highly incident of fire of forest occurrences in Sanming and feeble fire of forest distributed in other seasons of year. Dry weather, heavy sunshine, low humidity and high wind speed from January to March explains this district difference among seasonal and monthly forest fire characteristics.

Forest fire occurrences goes upward over time especially the ascending trend after the year 2007 and in 2009 the highest number of fire (165 cases) and areas burned (3,861.30 hm^2) was found there. Weather and climate influence forest fire comportment and account for the variability in fire harshness at different time scales. Valdiya (2006) asserted that the accretion in temperature increment the chance of fire appearance

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whereas precipitation and humidity have the different relation with fire. The data of weathers processed regarding this study have also strong relationship with forest fire occurrence. The counties throughout all of Sanming were classified into three group based on fires of forest proneness to examine the spatial assessment of fires of forest. In Sanming, many fires of forest were occurred in spring from January to March and a little forest fires can be registered in others seasons of the year. Dynamic of the wildland fires were clearly and detected in Youxi County and Sanming District, respectively. Southeast areas had larger forest fire appearances compare to other areas because of heavy forest use by human and more combustible species, a well-developed firefighting system with rapid suppression prevented large forest fires about the fire risk zone.

Conclusion

Fujian is a green province in China with forest coverage of 65.95%, and Sanming is one of the green cities in the provinces with forest coverage of 71%. Forest fires are recurrent disturbance events in Sanming because the city is dominated by over-matured, more combustible and flammable species. In this study, we found that the temporal and spatial patterns of forest fires exhibited considerable variations along time and by changing weather characteristics. The new insights into the temporal and spatial dynamics of forest fires in Daming could be applied to help allocate firefighting resources in fire season for immediate fire suppression in the southeastern China. Also, our results can be used to verify and readjust the fire precautionary period. During the 1960s, a wide range of people migrated to Sanming from other parts of Fujian and the increased urbanization and human activities increased opportunities for fire ignition. The results from this study can be used to help closely monitor fire danger conditions in the city. For example, population can be notified that January-March is the period most venerable to fire through some education programs, such that people would be more careful in their life and production activities in the period. Also, our results can be incorporated into legislation such that some practices in forest areas that are conducible to fire could be banned legally. According to this research's results, it's recommended to set and slopes, and to collect weather and forest fire data daily in Fujian area. These findings indicate that either the frequency or the mean length of prolonged weather, alone, cannot fully describe the short-term climate influence of an area.

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Conflict of interests. The authors have declared that no competing interests exist.

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ASSESSMENT OF PHYTOPLANKTON DIVERSITY IN TWO LAKES FROM THE NORTHEASTERN ALGERIAN SAHARA

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Abstract. Plankton is the basis of many food webs and is the main food of aquatic trophic chains. The primary productivity achieved by phytoplankton depends on the availability of nutriments. Phytoplankton biodiversity of two natural connecting lakes (Lella Fatma and Zerzaim) in the region of Megarine in South East of Algeria was studied during the period from January 2016 to December 2016. The relationships between the biomass of phytoplankton and the physicochemical properties of water were assessed in 28 genera of phytoplankton belonging to five classes (Mediophyceae, Coscinodiscophyceae, Bacillariophyceae, Euglenophyceae and Cyanophyceae). The statistical analysis performed during this study clearly shows that there is a strong correlation between differing physicochemical water parameters of the studied lakes and the influence on the phytoplankton population diversity.
Keywords: *phytoplankton, Megarine, lakes, diversity, nutriments*

Introduction

Natural lakes in arid areas constitute an important source to both ecological equilibrium and economical uses and in this ecosystem, biotic and abiotic components interact with them and determine the ecological statue of ecosystem (Awah, 2008). Population growth and the economic development are responsible for major modifications of water and the aquatic cycles are often perturbed by the excessive contributions in nourishing elements (Flandroy et al., 2018).

In addition to enriched nutrient content, these inputs induce an enhanced vegetation growth leading to an anarchic algal development, which propagate the unsightly appearance of water plans, emanation of unpleasant odors, death of aquatic species (Benredjeb and Romdhane, 2002; Brient et al., 2001). This has a detrimental effect on valuable ecosystem services, such as water supply, production, recreation, aesthetics, and more importantly the availability in sufficient quantity and quality that contributes to the maintenance of health. Enhanced anthropogenic activities deteriorate water quality (Chukwu et al., 2008) and impair their basic use through the process of pollution (Hur and Jung., 2009; Wu et al., 2005; Zhang et al., 2009).

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Algae are widely present in environments, such as streams, lakes and rivers and although relatively inconspicuous, they have a major importance, in terms of ecology and in relation to human use of natural resources. The maximum production of phytoplankton is obtained when the physicochemical factors are at optimum level (Muhammad et al., 2005).

The relation between the biodiversity and the functioning of the ecosystems is a fundamental ecological question and it is essential to know the potential effect of various abiotic and biotic elements (Zhang et al., 2018).

Phytoplanktons are highly diverse group of photoautotrophic organisms with unicellular reproductive structures, which are important for aquatic habitats (Ariyadej et al., 2004; Welker, 2008). They are important primary producers in the base of the food chain, constitute a vital link and an important biological indicator of the water quality (Laskar and Gupta, 2009). Maintenance of a healthy aquatic ecosystem depends on the biotic properties of water and the biological diversity of the ecosystem (Hillebrand et al., 2017).

In order to visualize the state of lakes in the Saharan region of Algeria, we have studied the spatiotemporal distribution of phytoplankton of two Saharan lakes during the year 2016, to determine the phytoplankton diversity and the abiotic factors responsible for their distribution.

The main objectives of the present study were (i) to measure the physicochemical factors of both lakes, (ii) to establish the inventories of phytoplanktons and (iii) to determine the spatiotemporal variations.

Material and methods

Study site

Megarine (Arabic: المقارين) is a town and commune in Ouargla province Algeria, it is located just North of Touggourt city. The region is made up of a large area of palm plantations surrounding an oasis network that extends from Sidi Slimane beyond Touggourt through to Balidat Ameur. Beyond the oases is the arid and barren landscape of the Sahara, featuring areas of sand dunes (ergs) and flat rocky plains (regs). The region of Megarine is approximately 285 km² (Dubost, 2002) in area and is located in the center of Touggourt region which is limited to the North by Sidi Slimane, to the South by Meggar and on the West by el Alia. The approximate meteorological conditions of the area are a mean annual precipitation 35.05 mm, temperature 23.3 °C, relative humidity 42.8%, visibility 12 km and wind speed is 12.4 km/h (ONMT, 2016) and the climate is hyper-arid, with a long dry season.

The lake of Megarine is divided into two small lakes namely Lella Fatma and Zerzaim (*Fig. 1*). Lella Fatma is located at latitude 33°12′21″ North and longitude 06°05′54″ East and Zerzaim lake is located at latitude 33°12′12″ North and longitude 06°05′50″ East. The two lakes are connected to each other by a natural trench but still distinct during the rainy period. The lakes are covered with *Juncus* and other macrophytes like *Phragmites*, *Tamaricaceae*, etc…

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Figure 1. *Photo shows the two lakes of the study Lella Fatma and Zerzaim*

Sampling sites and physicochemical analysis

Sample capturing used 0.5 l PVC bottles and were rinsed with distilled water and rinsed with sample water pre-sample collection. Samples were taken from a depth of 20 cm each month for one year (2016), at the five previously mentioned points of each lake (*Fig*. 2, *Table 1*).

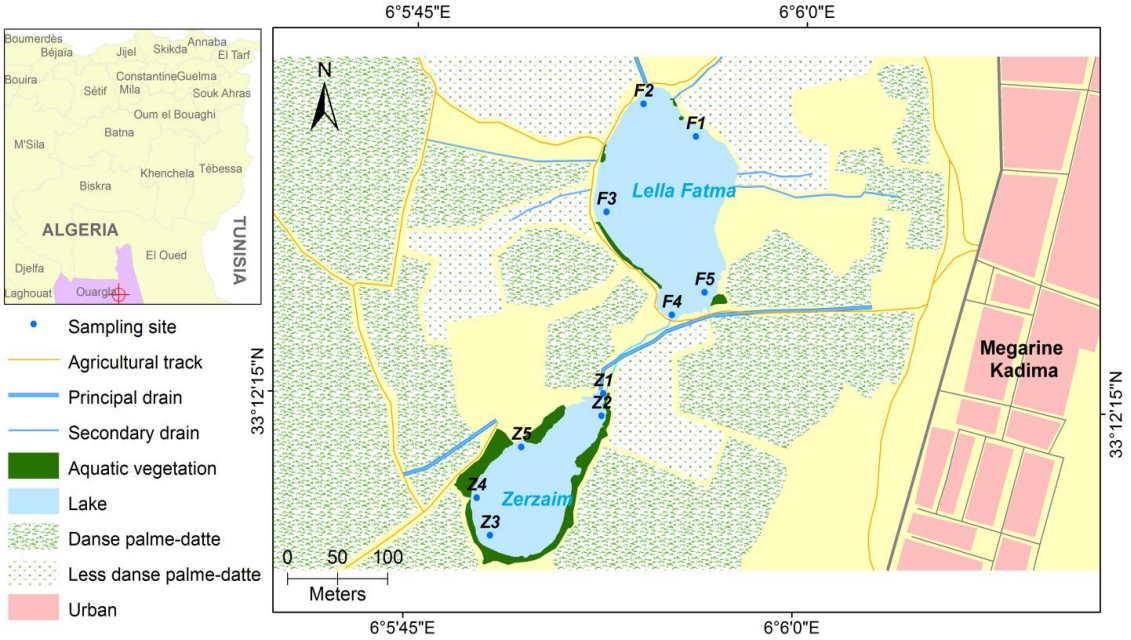


Figure 2. *The Location of Megarine lakes (Lella Fatma and Zerzaim) and the sampling points*

Table 1. *Characteristics of the sampling points of the two studied lakes*

Characteristics of LellaFatma lake sampling points		Characteristics of Zerzaim lake sampling points	
F1	Near a secondary drain	**Z1**	Connection point with LellaFatma Lake
F2	Evacuation point of a primary drain	**Z2**	Near a palm grove
F3	Near a palm grove	**Z3**	Near a palm grove
F4	Connection point with Zerzaim Lake	**Z4**	Presence of emerged plants
F5	Wastewater drainage point	**Z5**	Presence of salt crusts

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Some parameters are measured in situ, such as: temperature, electrical conductivity, pH and dissolved oxygen, using a multi-field parameter (Multi 350; SET5, Germany), while other parameters such as: nutrients (nitrogen compounds (NO_2^-, NO_3^-, NH_4^+) and PO_4^{3-} phosphates), were determined by using the standardized methods of Rodier (1996) using a spectrophotometer (60310) at the laboratory of the Scientific and Technical Research Centre for Arid Areas (CRSTRA), Biophysical Station, Touggourt, Algeria.

Simultaneously, to the abiotic data, phytoplankton samples were taken at the same points using a conical net ending with a collector and the collected phytoplankton samples were preserved in small plastic containers in 4% formalin (Anderson et al., 2002; Cemagref, 2007). Water samples containing the algae were collected from between 20 and 40 cm depth, away from the shore in order to have all the ecological types of algae and several techniques have been used, pressing plants and other submerged substrates and scraping from submerged or simply wet stones, branches or debris (Bourrely, 1966; Iltis, 1980). The harvested samples were placed in opaque glass jars previously washed and labeled (date of harvest, name of the study station and sampling point).

The determination of the different taxa that we concentrated by a net, was made in the laboratory under a microscope; for the systematic study, a series of specialized books of determination were consulted (Germain, 1981; Komàrek and Anagnostidis, 1994, 1997; Krammer and Lange-Bertalot, 1991, 1986).

Statistical analyses

Relative abundance and frequency were realized in order to evaluate the importance, the place and influence of species within a stand. Statistical analysis (correlation and correspondence analysis) was performed using Statistica 10 software, to remove the effects between the abiotic and biotic components.

Results

Physicochemical characteristics

During the study period, the region was characterized by: an air temperature average of 23.3°C (max = 33.5 °C in July and min = 12.7 °C in January), the annual rainfall of 49.6 mm with highest value in January (20.55 mm) and lowest in July (0 mm), and the average annual wind speed of 12.4 km/h (O.N.M.T., 2016).

Physicochemical characteristics of our lakes showed that: water temperature average ranged from 22.86 °C in Lella Fatma lake and 22.37 °C in Zerzaim lake. The temperature of water trace a seasonal evolution (*Fig. 3a*) relatively related to air temperature, which increases in summer (El Haouati et al., 2015). The minimum values are found in the winter with typical 16 °C in January for two lakes, and the maximum during August with 30 °C in Lella Fatma Lake and 29 °C to the Zerzaim Lake. The temperature augmentation is due to the absorption of the Sun's rays (Zheng et al., 2015).

In general the change in pH in the two lakes has a trend that varies between 7.46 and 8.6. For both lakes, these values are close to neutral (over 7). This deviation in the alkalinity was due to a good photosynthetic activity and also to the nature of the ground (Parinet et al., 2004). Aquatic plants consume dissolved CO_2 (by respiration) and

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displace the calcium-carbon balance, this tends to decrease the concentration of H^+ ions caused by the increase in pH, which is a good indicator of photosynthesis (Nagaraju et al., 2018). The pH value indicates the trophic state of a lake: an acidic water surface is oligotrophic, a neutral water surface is mesotrophic and an alkaline water surface is eutrophic (Stager et al., 2018).

The electric conductivity average value was 43.8 mS/cm in Lella Fatma Lake and 38.86 mS/cm in Zerzaim Lake, these results obtained showed a fluctuation in the interval of 11.4 and 76.8 mS/cm, which is higher in summer (*Fig. 3b*). The electrical conductivity is proportional to the amount of dissolved ionisable salts, it is a good indicator of the degree of mineralization of water, in our case the mineralization was high (S.E.E.E., 1996).

Dissolved oxygen variation is related to several factors, mainly temperature and salinity (Franco, 2017). The mean values of dissolved oxygen during the study period were 2.56% (min: 0.13%, max: 13.84%) for Lella Fatma Lake and 2.63% (min: 0.12%, max: 11.07 mg/l) for Lake Zerzaim. The high values of dissolved oxygen were found in the month of May and the autumn season; this elevation was due to the air movement (wind) (*Fig. 3c*).

Phosphorus and nitrogen are the two main nutrients essential for plant growth and are naturally present in soil, rock and vegetation. An accelerated load of phosphorus and nitrogen from anthropogenic sources can cause harmful algal blooms and is one of the biggest problems facing many countries around the world.

Nutrients observed during the study had an average of nitrogen components of 7.97 mg/l in total within Lella Fatma lake and 9.85 mg/l in Zerzaim lake (*Fig. 3d*).

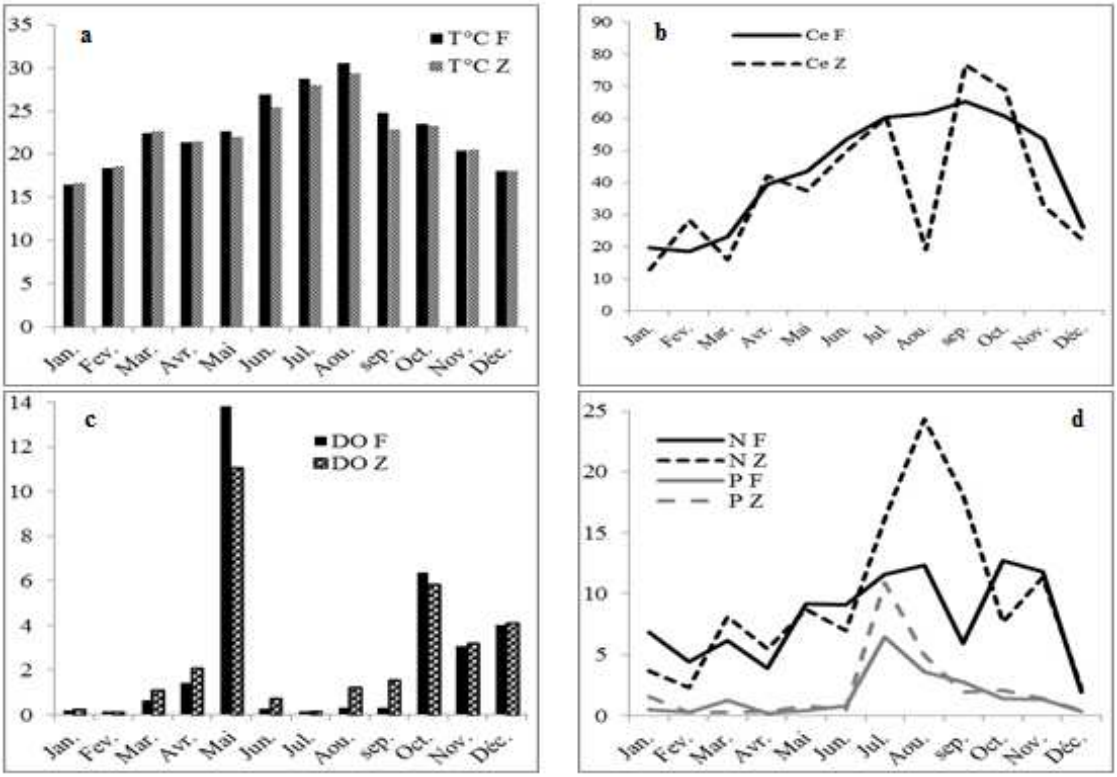


Figure 3. *Annual variation of the physicochemical parameters of the two lakes.* ***a.*** *Water temperature,* ***b****. electrical behavior,* ***c.*** *dissolved oxygen,* ***d.*** *nutriments N & P*

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The N/P ratio (Redfield, 1963) of ambient concentrations may indicate the limiting nutrient. N/P ratio < 16/1 indicates nitrogen limitation, while phosphorus limitation occurs when N/P 16/1 (Goman et al., 2017).

The graphs in *Figure 3d* demonstrate that the values of nitrogen in totality are higher than phosphorus' values.

To determine whether it is phosphorus or nitrogen that limits the development of algal biomass, the most frequently used method is to compare the value of the total nitrogen/total phosphorus (N/P) ratio measured in water with that measured in a non-deficient algal population. If results show that an N/P ratio is above 7.2 it indicates a phosphorus limitation and an N/P ratio of less than 7.2 is due to nitrogen limitation (Goman et al., 2017). The application of this criterion indicates that phosphorus is the limiting factor in the lakes, where it was found that the N/P ratio averaged at 4.98 for Lella Fatma lake and 4.58 for Zerzaim lake.

Phytoplankton biodiversity

Diversity, abundance and dominance of phytoplankton community of lakes were studied during January 2015 to December 2015 with a total of 58 spices belonging to 20 family, 14 orders, 5 classes and to 3 phylums (Bcillariophyta, Euglenophyta and Cyanobacteria) being observed. A total of 23 species of Cyanophyceae, 21 species of Bacillariophyceae, 12 species of Mediophyceae, 1 species of Coscinodiscophyceae, and 1 species of Euglenophyceae (*Table 2*).

Table 2. *Various species found during the period of study*

Phylum	Class	Order	Family	Species	Lella Fatma	Zerzaim
Bcillariophyta	Mediophyceae	Thalassiophysales	Catenulaceae	*Amphora coffeaformis* Kützing	75.0	75.0
				Amphora ovalis Kützing	33.3	41.7
				Amphora lineolata Ehrenberg	50.0	41.7
		Naviculales	Amphipleuraceae	*Amphiprora sp* Ehrenberg	0.0	25.0
				Frustulia rhomboides Ehrenberg	8.3	8.3
			Brachysiraceae	*Brachysira aponina* Kützing	16.7	33.3
			Naviculaceae	*Navicula halophila* Grunow	25.0	41.7
				Navicula *margalithie* Lange-Bertalot	16.7	41.7
			Pleurosigmataceae	*Pleurosigma angulatum* W.Smith	25.0	41.7
		Cocconeidales	Cocconeidaceae	*Cocconeis placentula* Ehrenberg	83.3	100.0
		Stephanodiscales	Stephanodiscaceae	*Cyclotella menighiniana* Kützing	66.7	66.7
				Cyclotella striata Kützing	41.7	50.0
	Coscinodiscophyceae	Melosirales	Melosiraceae	*Melosira dickiei* Kützing	0.0	33.3
	Bacillariophyceae	Cymbellales	Cymbellaceae	*Cymbella pusilla* Grunow	50.0	66.7
			Gomphonemataceae	*Gomphonema angustatum* Kützing	25.0	41.7
		Fragilariales	Fragilariaceae	*Fragilaria tenera* W.Smith	50.0	66.7
				Fragilaria fasciculta C.Agardh	8.3	16.7
		Mastogloiales	Achnanthaceae	*Achnanthes minutissima* Kützing	75.0	83.3
			Mastogloiaceae	*Mastogloia braunii* Grunow	58.3	83.3
				Mastogloia elliptica C.Agardh	58.3	66.7
		Bacillariales	Bacillariaceae	*Denticula kuetzingii* Grunow	58.3	66.7

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				Hantzschia elogantula Grunow	8.3	16.7
				Nitzschia constricta Kützing	41.7	41.7
				Nitzschia fonticola romana Grunow	58.3	58.3
				Nitzschia vitrea G.Norman	16.7	33.3
				Nitzschia geitleri Hustedt C	8.3	16.7
				Nitzschia tubicola Grunow	8.3	16.7
				Nitzschia palea Kützing	33.3	50.0
				Nitzschia obtusa W.Smith	25.0	41.7
				Nitzschia recta Grunow	41.7	66.7
				Nitzschia sigmoidea W.Smith	25.0	25.0
		Surirellales	Surirellaceae	*Campylodiscus clypeus* Kützing	8.3	16.7
				Surirella striatula Turpin	33.3	41.7
				Surirella ovata var. *pinnata* W.Smith	8.3	25.0
Euglenophyta	Euglenophyceae	Euglenales	Phacaceae	*Phacus orbicularis* K.Hübner	8.3	25.0
Cyanobacteria	Cyanophyceae	Chroococcales	Chroococcaceae	*Chroococcus minutus* Kützing	66.7	66.7
				Chroococcus turgidus Kützing	33.3	41.7
				Chroococcus lemnticus Lemmermann	41.7	50.0
			Cyanobacteriaceae	*Cyanothece major* Komárek	8.3	0.0
			Microcystaceae	*Gloeocapsa sp* Kützing	16.7	25.0
				Microcystis sp Kützing	8.3	0.0
			Gomphosphaeriaceae	*Gomphosphaeria salina* Komárek	25.0	33.3
		Oscillatoriales	Oscillatoriaceae	*Oscillatoria chalybea* Mertens	33.3	58.3
			Phormidiaceae	*Phormidium chalybeum* Anag. & Komá.	16.7	25.0
			Coelosphaeriaceae	*Coelomoron pusillum* Komárek	33.3	50
				Coelosphareium sp Nägeli	16.7	8.3
				Woronichinia karelica Komá. & Legn	41.7	75
				Woronichinian aegeliana Elenkin	0.0	16.7
			Merismopediaceae	*Merismopedia warmingiana* Lagerheim	33.3	16.7
			Leptolyngbyaceae	*Leptolyngbya granulifera* Anagnostidis	33.3	58.3
				Planktolyngbya sp Anag. & Komá.	25.0	33.3
			Romeriaceae	*Romeria sp* Koczwara	25.0	333
			Pseudanabaenaceae	*Jaaginema subtilissimum* Anag. & Komá.	8.3	16.7
				Pseudanabaena recta Komá. & Cronb.	25.0	33.3
				Pseudanabaena galeata Böcher	8.3	8.3
				Pseudanabaen amucicola Naum. & Hube.	33.3	58.3
				Pseudanabaena papillaterminata Kisselev	16.7	8.3
		Spirulinales	Spirulinaceae	*Spirulina tenuior* Lagerheim	8.3	33.3

F = 100%: ubiquitous species
F = 100-75: constant species
F = 75-50: frequent species
F = 50-25: common species
F = 25-5: accessory species
F ≤ 5: rare species

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Frequency and spatiotemporal distribution of phytoplankton species

According to the calculation of the relative abundance that is based on the number of individuals observed under the microscope, phytoplankton populations exhibit fluctuations in abundance as a function of time and space. This spatiotemporal variation (*Fig. 4*) differs from one species to another, and in this study was as follows:

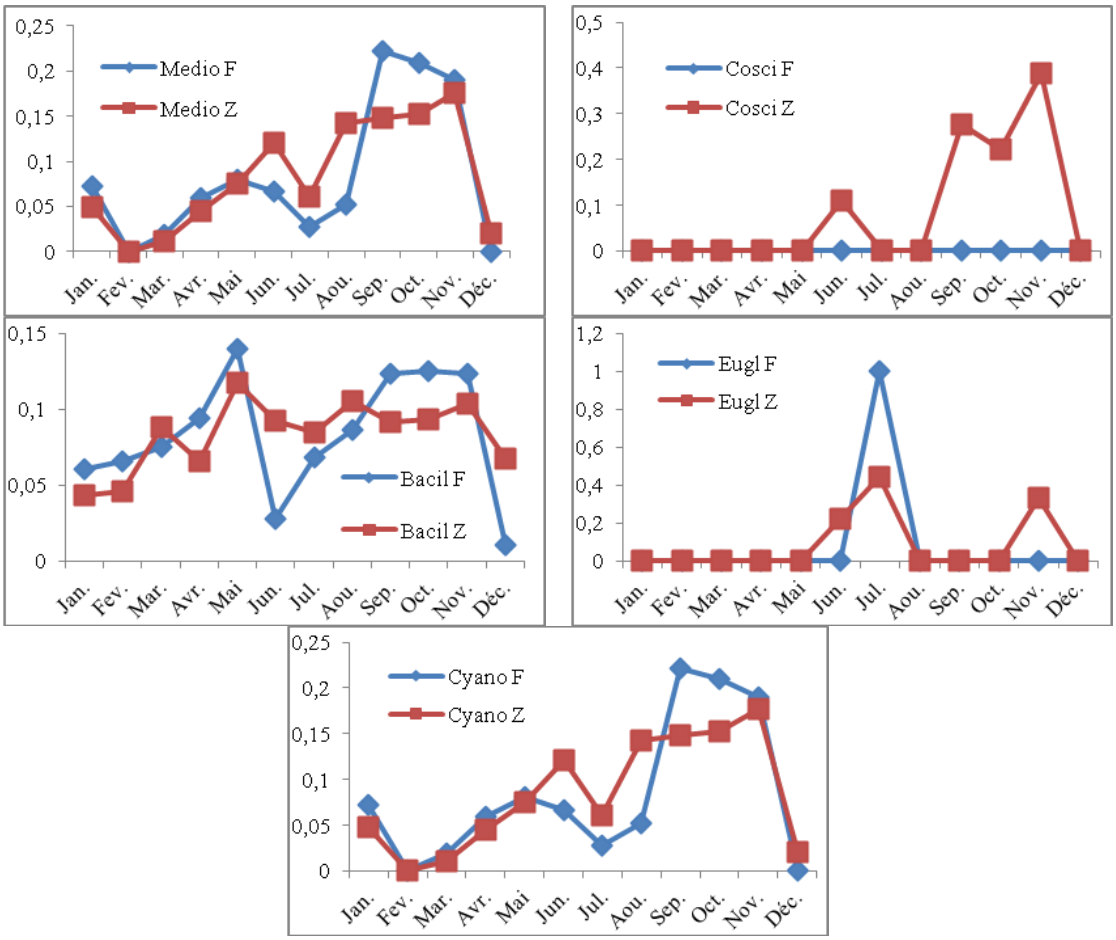


Figure 4. *Spatiotemporal variation in relative abundance of phytoplankton in lakes (Lella Fatma & Zerzaim) (2016). (Cyano: Cyanophyceae, Bacil: Bacillariophyceae, Medio: Mediophyceae, Cosci: Coscinodiscophyceae, Eugl: Euglenophyceae)*

Cyanophyceae forms the most dominant group with 23 species, or 39.7% of the total phytoplankton community andare represented in the majority by the genus Chroococcus.

Bacillariophyceae forms the most diverse group, with 21 taxa, or 36.2% of the entire population, the representative species of the group are: *Achnanthes minutissima*, *Fragilaria, Mastogloia and Nitzschia.*

Mediophyceae, this class contains 12 species, with a proportion of 20.7%, dominated by genre *Amphora* and species *Cocconeis placentula*.

The two final groups **Coscinodiscophyceae** and **Euglenophyceae** are represented by only one species respectively: *Melosira dickiei* in Zerzaim Lake only and *Phacus orbicularis*in both lakes.

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The influence of environmental variables on the distribution of phytoplankton groups in the Megarine lakes' was assessed using PCA analysis (*Fig. 5*).

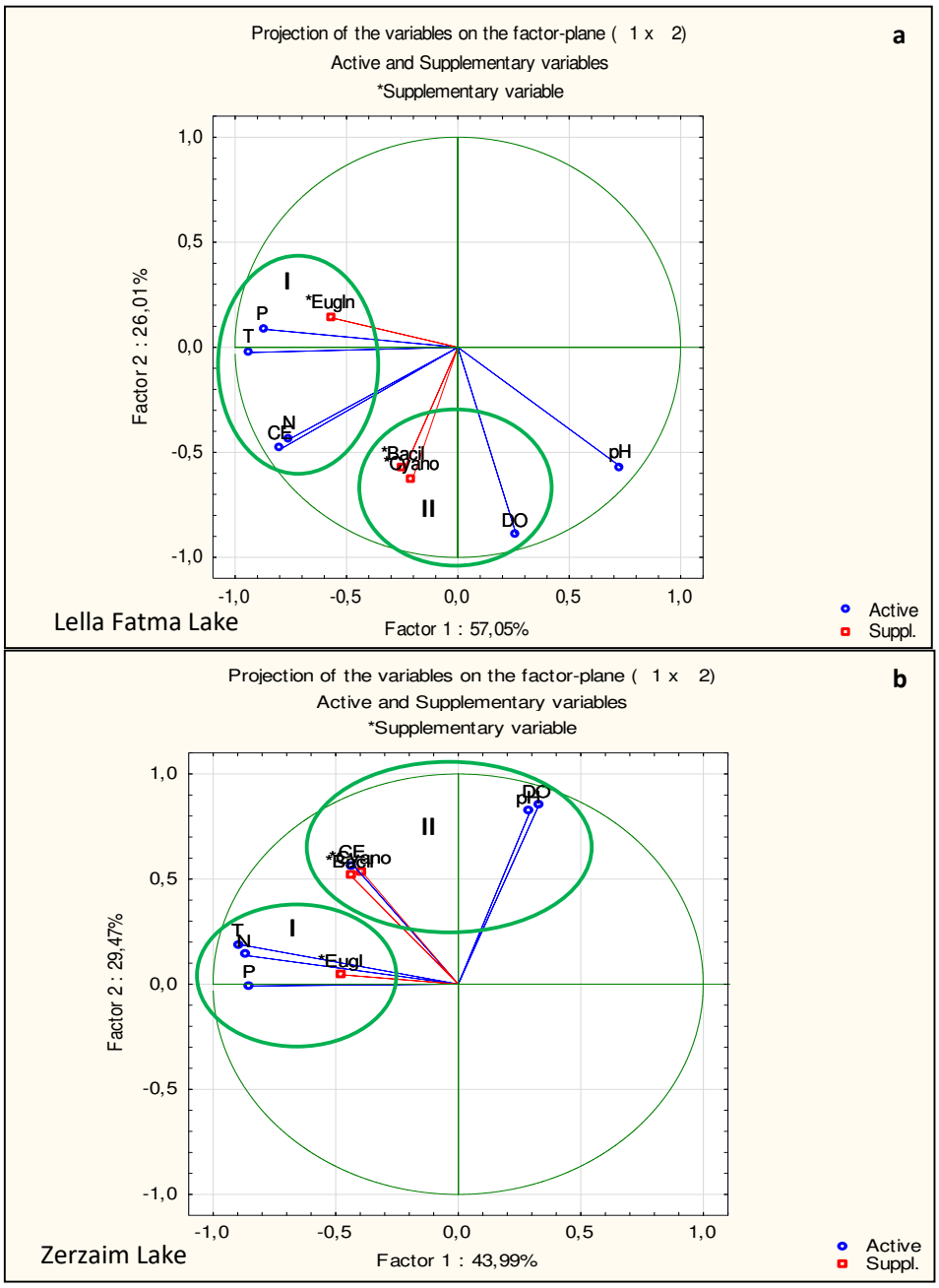


Figure 5. *Principal components and Classification Analysis diagram of environmental variables and phytoplankton groups in lakes (Lella Fatma & Zezeim) during period study (2016). (T: water temperature, pH: hydrogen potential, CE: Electric conductivity, DO: Dissolved Oxygen, P: Phosphate, N: nitrogen elements, Bacil: Class Bacillariophyta, Eugle: Euglenophyta, Cyano: Cyanobacteria)*

The PCA of the biotic and abiotic parameters, in the two studied lakes, showed that the first two axes F1 and F2 contain most of the data since they present 83.06% of the total inertia for the Lake Lella Fatma and 73.46% for Lake Zerzaim. Examining the

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correlations between the axes and the different components makes it possible to explain the meaning of each axis in the structured distribution of the variables clouds.

"Lella Fatma" Lake: The F1 axis (57.05% of total inertia) is mainly correlated with temperature, electrical conductivity and nutrients (nitrogen and phosphorus), as long as the F2 axis (26.01% total inertia) is mainly bound with dissolved oxygen (*Fig. 5a*). The PCA allows for discrimination of the two groups:

Group I contains the Euglenophyta, directly proportional to the presence of nutrients, water temperature and electrical conductivity and from this a conclusion can be made that the axis represents organic matter mineralization phenomenon.

Group II, the Bacillariophyta and Cyanobacteria prefer well-oxygenated waters, therefore this axis relates to eutrophication.

"Zerzaim" Lake: The F1 axis (43.99% of total inertia) is correlated in particular with temperature and nutrients (nitrogen and phosphorus), as long as the axis F2 (29.47% of total inertia) is bound with dissolved oxygen (*Fig. 5b*).

Group I contains the Euglenophyta, always remains directly proportional to the presence of nutrients and the temperature of the water, it is therefore the mineralization of organic matter that takes place at this level.

Group II, Bacillariophyta and Cyanobacteria have a relationship proportional to the oxygen level, the potential of hydrogen and the electrical conductivity, so the presence of the phenomenon of eutrophication is noted

Discussion

An annual evolution in phytoplankton community with regard to physicochemical properties was evident in this study, and monitoring of the phytoplankton compartment in both lakes highlights that it is directly influenced by the fluctuation of abiotic parameters (Wetzel, 2001).

The phytoplankton populations in Lake Zerzaim were denser and more diversified in relation to the environmental parameters and more favorable than in Lake Lella Fatma, especially with the contribution of nitrogen and phosphorus.

Cyanobacteria perform photosynthesis with release of oxygen, and it is beneficial to find them in abundance within the lakes.

Cyanobacteria were also known to produce a variety of bioactive compounds of which some have toxic effects and are called cyanotoxins**.** The effects of some harmful algal blooms were not related to toxin production, but they were involved in depletion of dissolved oxygen concentration caused by algal proliferation, death and decay or night respiration (Shaista, 2008)**.**

Several freshwater, brackish water and marine cyanobacterial produce hepatotoxins (Welker, 2008) and in this study, it highlights: the genera of Microcystis that may produce linear and cyclic peptide (Ploutno et al., 2005), Oscillatoriamay produce toxins, aplysiatoxins which may cause skin problems in swimmers (Shaista, 2008).

Phytoplankton require nitrogen as a basic element in protein anabolism (Forsberg, 1977), and the nutritional role of phosphorus is assumed to be responsible for the rapid eutrophication of water bodies (Griffith et al., 2009), which was the case for the study.

Finally in the Megarine region lakes the development of phytoplankton populations was obviously caused by nutrients (nitrogen and phosphorus); and has caused a migration of micro-algae towards the deeper depths of lakes (Serizawa H et al., 2008).

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The remarkable difference between the two lakes in terms of phytoplankton density was due to human activity, because the road to the Lake Lella Fatma is accessible and easy for people and even vehicles. For this reason, the lake is a place of tourist events in each summer. Unlike the Zerzaim lake, which characterized by the absence of clear pathways and surrounded only by palm trees and other plants on each side.

Physicochemical and phytoplankton data obtained in this study could be used as a baseline and reference point when assessing further changes caused by nature or man, since there has not been published information or data on these lakes.

Subsequent studies should allow a more precise approach to the interactions existing between the phytoplankton compartment and the adjacent levels of the food web and the impact of the abiotic parameters of the lakes. The rapid evolution of phytoplankton communities in response to environmental changes reinforces the importance of their ecological monitoring because of the importance of these lakes.

Conclusion

The study of some physicochemical parameters of Megarine lakes made it possible to characterize the general state of water during the year 2016. The results obtained showed that the lakes suffer from an agricultural pollution and shows that these lakes were favorable ecosystems to microalgae proliferation. The succession of phytoplankton populations was therefore conditioned by abiotic parameters. Phytoplankton represents a remarkable compartment by the role it plays, not only in the aquatic environment but also in all areas of our life: environmental quality (oxygen), food (fish and seafood), well-being (health, beauty), industrial resource, therefore it is imperative to monitor them in the global ecosystems.

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SILVER NITRATE ($AgNO_3$) BOOSTED HIGH-FREQUENCY MULTIPLE SHOOT REGENERATION FROM COTYLEDONARY NODE EXPLANTS OF OKRA (*ABELMOSCHUS ESCULENTUS* L.)

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Abstract. Okra (*Abelmoschus esculentus* L.) regeneration frequency to a large extent depends on its genetic structure and its recalcitricity. In present study, the influence of silver nitrate ($AgNO_3$) in combination with cytokinins such as 6-benzyl amino purine (BAP) and kinetin (KIN) to regenerate multiple shoots from cotyledonary node explants of *A. esculentus* were optimized. Cotyledonary node explants were excited from 10-days old okra *in vitro* seedlings and incubated on Murishage and Skoog (MS) medium supplemented with different concentration and the combination of BAP alone and in combination with KIN. The highest percentage of shoot bud regeneration (75%) was achieved on MS medium supplemented with 2.0 mgL^{-1} BAP. Highest percentage of shoot multiplication and proliferation (85%) with 6.75 shoots per explant having 3.75 cm length was obtained on MS medium containing 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} KIN + 3.0 mgL^{-1} $AgNO_3$. On the other hand, non-containing $AgNO_3$, the combination of 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} KIN, a maximum of 53% shoot multiplication with 2.3 shoots per explant having 3.75 cm length was recorded. Individual shoots were elongated on MS medium supplemented with 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} GA_3 (Gibberellic acid) (shoot length 5.60 ± 0.70^a cm) and rooted on half strength MS medium + 1.0 mgL^{-1} IBA (Indole 3-butiric acid) + 200 mgL^{-1} AC (Activated charcoal), with highest root formation 83% with 5.20 roots per shoot having 4.8 cm length was achieved. The current protocol will be useful for the development of okra transgenic plant as a result of genetic transformation.

Keywords: *growth regulators, shoot multiplication, organogenesis, cytokinins and auxins*

Abbreviations: $AgNO_3$ – silver nitrate, MS – Murishage and Skoog, ddH_2O – double distilled water, $HgCl_2$ – mercuric chloride, NaOH – sodium hydroxide, HCL – hydrochloric acid, PGRs – plant growth regulators, AC – activated charcoal, 2,4-D- 2,4 – dicholorophenoxyacetic acid, BAP – 6-benzyl aminopurine, KIN – kinetin, GA_3 – gibberellic acid, IBA – indole-3-butyric acid, NAA – α-naphthyl acetic acid, μmol m^{-2} s^{-1}– micromoles per meter square per second

Introduction

Okra (*Abelmoschus esculentus* L.) is a member of Malvaceae family, which is mostly known as lady's finger. It is an important vegetable crop owing to its palatability for human consumption. It is cultivated in sub-tropical and tropical areas throughout the world (Dhande et al., 2012). India is the leading country followed by Nigeria, Sudan, Mali and Pakistan with the production of 5.5 million tons and covers an areas of 0.48 million hectares in the world (FAOSTAT, 2016). It is tremendous source of balanced human food containing protein, vitamins, and minerals which provides important compounds for medical (Gemede et al., 2015; Düzyaman and Vural, 2001). Moreover, its regular consumption could prevents hepatitis, cancer, ulcers and diabetes (Kumar et al., 2009a). Plants from the Malvaceae family such as okra are particularly susceptible to various

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environmental stresses such as abiotic and biotic stresses which reduce crop growth, yield and cause economic loss (Venkataravanappa et al., 2015).

Development of plant transformation and genetic engineering made it capable to isolate different disease and pest's resistant genes from bacteria and plants. Then transfer these resistant genes into economically valuable crops which can be grown under stressed conditions (Manickavasagam et al., 2015). Effective regeneration system is the pre-requirement for transgenic plants (Michel et al., 2008). In addition, *in vitro* reproduction technique offers a powerful tool to protect genetic resources and mass-multiplication of endangered plant species (Choffe et al., 2000). However, okra is one of the most recalcitrant crops for genetic modification with the lack of an efficient *in vitro* regeneration system amongst the leading reasons. It is widely reported that okra regeneration protocol using different explants such as hypocotyl, cotyledon, shoot tips, cotyledonary nodes, cotyledonary axis and plumule from zygotic embryo (Mangat and Roy, 1986; Roy and Mangat, 1989; Irshad et al., 2017; Narendran et al., 2013; Dhande et al., 2012; Manickavasagam et al., 2015; Daniel et al., 2018). However, available okra regeneration protocols are not well-organized for the development of transgenic plants. The main reasons for poor regeneration protocols of okra are due to its recalcitrant nature which takes longer time and requires skills (Manickavasagam et al., 2015).

Shoot multiplication through direct organogenesis is a good alternative way of regeneration, which takes less time, has lower soma clonal variability and higher efficiency index than indirect regeneration system (Juturu et al., 2015). Additionally, it has the ability to overcome the limitation of genotype requirement in transformation system. Several efforts have been made for getting an efficient regeneration system of okra by using different protocols (Anisuzzaman et al., 2008; Dhande et al., 2012; Kumar et al., 2009a; Mangat and Roy, 1986; Narendran et al., 2013; Irshad et al., 2017; Kumar et al., 2015). Various factors including media composition, explants, culture conditions, carbon sources, browning of callus, phenolic secretion, culture vessels and slow *in vitro* response have been shown to influence *in vitro* regeneration of okra (Irshad et al., 2017; Kumar et al., 2015). Media composition plays an important role in the regeneration efficiency. Kumar et al. (1998) reported that the development and growth of cells cultured *in vitro* rely on existence of ethylene and phytohormones in culture environment. Different evidence's showed that the morphogenic response of adventitious bud induction can be improved by inhibition of ethylene biosynthesis and gaseous environment in the culture medium (Pua, 1999). Inhibition of ethylene biosynthesis is a good alternative to improve the adventitious bud induction.

Silver Nitrate ($AgNO_3$) has been reported a potential ethylene inhibitor in various economically important plant species (Zhang et al., 2001; Jakubowicz et al., 2010; Kumar et al., 2016; Mookkan and Andy, 2014; Venkatachalam et al., 2017; Aylin Ozudogru et al., 2005). Addition of regeneration enhancer such as silver nitrate ($AgNO_3$) in growth medium plays a vital role in shoot multiplication and stated in various economically important plants such as *Vigna mungo* (Mookkan and Andy, 2014), *Gossypium hirsutum* (Kumar et al., 2016), *Arachis hypogaea* (Aylin Ozudogru et al., 2005), *Cucumis sativus* (Venkatachalam et al., 2018), *Catharanthus roseus* (Panigrahi et al., 2017) and *Prosopis cineraria* (Venkatachalam et al., 2017).

In this study, we reported an effective *in vitro* regeneration system to induce multiple shoots through cotyledonary node explants of *A. esculentus* by using various plant growth regulators with different combination, concentration and growth promoter e.g. silver nitrate ($AgNO_3$). The basic objectives of this study were (a) to improve direct regeneration

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protocol for okra by using cotyledonary node as explant (b) to examine the impact of cytokinins and auxins by using alone or in combination with $AgNO_3$ on shoot multiplication through *in vitro* seedling derived cotyledonary node explants. This alternative method will be beneficial for attaining transgenic plants of commercial okra genotypes.

Materials and methods

Silver nitrate ($AgNO_3$) stock solution preparation

For the preparation of stock solutions, chemicals were purchased from Solarbio® (China). A Stock solution of $AgNO_3$ (0.5 mgL^{-1}) was prepared with sterilized distilled water and stored in screw-capped bottles. The stock solution was kept at 4 °C until further use.

Plant material and seed germination

Abelmoschus esculentus "Xiang Fu" cultivar was used in the experiment. Seeds purchased from vegetable seed market (P. R. China, Fuzhou), were surface sterilized by washing with sterilized double distilled (ddH_2O) water 3-5 times and then treated by immersion in 0.1% weight per volume (w/v) $HgCl_2$ (mercuric chloride) for 3 minutes, following the 5 rinses with ddH_2O to remove the residual of $HgCl_2$, then treated with 70% ethanol for 30 seconds and washed 3-5 times with ddH_2O and immersed overnight in ddH_2O. The sterilized seeds were inoculated in half-strength Murishage and Skoog (1962) (MS) basal solid medium without addition of plant growth regulators in sterilized baby food jars (5×9 cm), 5-8 seeds per jar and was kept in dark condition.

Culture medium and conditions

The cotyledonary node explants were cultured on MS medium fortified with different concentration and combination of BAP, KIN, thidiazuron (TDZ), silver nitrate ($AgNO_3$), gibberellic acid (GA_3), indole 3-butiric acid (IBA), α-naphthalene acetic acid (NAA) and activated charcoal (AC). For the whole experiment, MS basal medium containing 2.5% sucrose (w/v), 0.8% agar (w/v) and pH was adjusted to 5.8 by the use of 0.5 N NaOH or HCl prior to autoclaving at 121 °C for 20 min. The culture conditions were maintained at 25 $\pm$ 2 °C with 70% relative humidity and 16:8 h light/dark photoperiod supplied by cool white fluorescent lamps (Philips, Beijing, China) at an intensity of 60 $\mu mol\ m^{-2}\ s^{-1}$.

Explant preparation and shoot induction

Cotyledonary node explants were excised aseptically from 2-week old *in vitro* raised seedlings (*Fig. 1A*) and cultured on MS medium augmented with different concentration of BAP (0.5, 1.0, 2.0, 2.5 mgL^{-1}) alone or in combination with KIN (0.5, 1.0, 2.0, 2.5 mgL^{-1}) for shoot bud induction. Consequently, the shoot buds were sub-cultured into same fresh MS medium for two weeks intervals to enhance the shoot bud development. Data on shoot proliferation percentage (%) and number of shoots per explant were measured after two weeks intervals.

Multiple shoots induction

To investigate the effect of $AgNO_3$ on shoot multiplication, two experiments were carried out containing $AgNO_3$ and non-containing $AgNO_3$. In the first experiment (containing $AgNO_3$), *in vitro* derived shoot buds were cultured on MS medium containing

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various concentration of BAP (0.5, 1.0, 2.0, 2.5 mgL^{-1}), KIN (0.5, 1.0, 2.0, 2.5 mgL^{-1}) and TDZ (0.5, 1.0, 2.0, 2.5 mgL^{-1}) in combination with $AgNO_3$ (0.5, 1.0, 2.0, 2.5 mgl^{-1}), as growth enhancer. In second experiment (non-containing $AgNO_3$), shoot buds were cultured on MS medium supplemented with BAP, KIN and TDZ at equimolar concentration. The shoot bunches were sub-cultured on same fresh shoot multiplication medium at every two weeks intervals for fast shoot growth with more number of shoot buds. Shoot initiation and growth characteristics were noticed per week, while data for shoot multiplication responses, mean number of shoot per explant and shoot length was noted after four weeks of inoculation.

Shoot elongation

In order to obtained healthy shoots with appropriate length for rooting, well developed shoots (1-1.5 cm) in length were isolated from shoot bunches and cultured on MS medium containing different combination and concentration of BAP (1.0, 1.5, 2.0 mgL^{-1}) and GA_3 (1.0, 1.5, 2.0 mgL^{-1}) alone or in combination. The shoot length (cm) and shoot elongation frequency (%) were noticed after three weeks of culture.

Rooting of regenerated shoots and acclimatization

Elongated shoots (2.0-5.0 cm in length) were excised aseptically and cultured on half strength MS medium containing (100, 200 mgL^{-1}) AC with various concentration (0.5, 1.0, 1.5 mgL^{-1}) of IBA and NAA. Root formation frequency, root length and number of roots per shoot were recorded after four weeks of cultured. The plantlets with roots were cautiously detached from the culture medium and distilled water was used to remove the agar traces and rooting medium. Plantlets were shifted into plastic cups comprising of vermiculite and garden soil (1:1) and watered every second day. The plastic cups were covered with polyethylene bags for 5 days and kept in the growth chamber at 25 ± 2 °C with relative humidity (70%) and 16/8-h of light/dark photoperiod having light intensity of 80 µmol m^{-2} s^{-1} supplied by cool white fluorescent tubes. These plantlets were then transferred to greenhouse under natural conditions for further growth and development.

Statistical analysis

The experiments were carried out based on a CRD (complete randomized design) with n = 10, and each experiment was performed three times. MS medium without plant growth regulators was used as a control treatment at every step of experiment. One-way analysis of variance (ANOVA) was performed using SPSS version 17.0 version (SPSS Inc., Chicago, IL, USA) for all statistical analysis and difference among mean values were analyzed by using Tukey's test, p-value ≤ 0.05.

Results

Effects of plant growth regulators on shoot bud induction

Morphogenetic responses of cotyledonary node explants (*Fig.1B*) with various concentrations of BAP and KIN were given in *Table 1*. Cotyledonary nodal explants cultured on MS medium without PGRs (control) gave no response. However the response was quicker in the presence of cytokinins including BAP and KIN (*Fig.1C*). Various results were observed on medium supplemented with different type and concentration of

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cytokinins. Among the tested concentration of BAP and KIN, shoot induction percentage (75%), with 4.5 numbers of shoots, was observed on MS medium supplemented with 2.0 mgL^{-1} BAP alone was higher as compare to the combination of 2.0 mgL^{-1} BAP + 2.0 mgL^{-1} KIN, (4.15 number of shoots) with 70% response.

Table 1. *Effects of different concentration of plant growth regulators (PGRs) on shoot bud differentiation from cotyledonary node explants of okra.*

Culture media	Plant growth regulator (mgL^{-1})		Shoot proliferation %	No. of shoots/explant
	BAP	KIN		
M1	0	0	0.00 ± 0.00^{j}	0.00 ± 0.00^{d}
M2	0.5	-	43.00 ± 0.58^{g}	1.33 ± 0.17^{cd}
M3	1.0	-	52.00 ± 0.58^{e}	2.75 ± 0.13^{abc}
M4	2.0	-	**75.00 ± 0.76^{a}**	**4.50 ± 0.29^{a}**
M5	2.5	-	60.00 ± 0.58^{d}	3.80 ± 0.44^{a}
M6	-	0.5	58.00 ± 0.50^{d}	1.68 ± 0.23^{bcd}
M7	-	1.0	48.00 ± 0.29^{f}	2.55 ± 0.26^{abc}
M8	-	2.0	65.00 ± 0.76^{c}	3.50 ± 0.29^{ab}
M9	-	2.5	38.00 ± 0.87^{h}	2.90 ± 0.67^{abc}
M10	0.5	0.5	31.00 ± 0.58^{i}	1.40 ± 0.70^{cd}
M11	1.0	1.0	40.00 ± 0.76^{gh}	3.75 ± 0.25^{a}
M12	2.0	2.0	70.00 ± 0.64^{b}	4.15 ± 0.45^{a}
M13	2.5	2.5	28.00 ± 0.58^{i}	2.90 ± 0.59^{abc}

The labels M1-M13 (M1- Controlled) presents PGRs combination, values represented mean ± standard error; data were recorded after four weeks of culture on MS medium supplemented with BAP and KIN. Best results are indicated in bold, mean followed by the same letter within a column are not significantly different according to Tukey's test with p-value ≤ 0.05

Shoot multiplication and proliferation

Multiple shoot induction was not observed in medium without PGRs (control). Among the tested combinations medium containing $AgNO_3$, maximum shoot multiplication percentage (85%) was noticed on a medium fortified with 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} KIN + 3.0 mgL^{-1} $AgNO_3$ with maximum number of shoots (6.75 shoots/ explants, 3.90 cm in length) followed by the other combination (1.75 shoots/ explants, 1.0 cm in length), with lowest multiple shoot percentage (37%), on a medium containing 2.0 mgL^{-1} TDZ + 4.5 mgL^{-1} $AgNO_3$ was recorded (*Table 2A*). Whereas highest percentage of shoot multiplication (51%) with maximum shoot numbers (3.80 shoots/explants, 2.30 cm in length) was observed on MS medium having the combination of 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} KIN with non-containing $AgNO_3$ (*Table 2B*) (*Fig. 1D, 1E*).

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Table 2A. *Effects of different concentration and combination of plant growth regulators containing $AgNO_3$ on shoot multiplication from cotyledonary node explant of okra*

Culture media	Plant growth regulator (mgL^{-1})				Shoot proliferation %	No. of shoots/explant	Shoot length (cm)
	BAP	KIN	TDZ	$AgNO_3$			
M1	0	0	0	0	0.00 ± 0.00^{i}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}
M2	-	-	-	1.5	38.00 ± 0.58^{h}	3.80 ± 0.47^{bcd}	$1.750.38^{abc}$
M3	-	-	-	3.0	75.00 ± 0.29^{b}	4.00 ± 0.58^{bcd}	2.60 ± 0.31^{abc}
M4	-	-	-	4.5	50.00 ± 1.15^{fg}	3.33 ± 0.44^{bcd}	2.50 ± 0.29^{abc}
M5	1.0	-	-	1.5	45.00 ± 1.73^{g}	3.80 ± 0.44^{bcd}	1.75 ± 0.18^{abc}
M6	1.5	-	-	3.0	77.00 ± 1.15^{b}	5.50 ± 0.29^{ab}	3.40 ± 0.38^{ab}
M7	2.0	-	-	4.5	68.00 ± 0.58^{cd}	4.75 ± 0.14^{abc}	2.35 ± 0.62^{abc}
M8	-	1.0	-	1.5	50.00 ± 1.00^{fg}	2.90 ± 0.67^{cd}	1.50 ± 0.87^{abc}
M9	-	1.5	-	3.0	63.00 ± 0.58^{de}	3.83 ± 0.17^{bcd}	2.00 ± 0.29^{abc}
M10	-	2.0	-	4.5	67.00 ± 1.04^{cd}	3.60 ± 0.31^{bcd}	2.80 ± 0.42^{ab}
M11	-	-	1.0	1.5	60.00 ± 2.89^{e}	2.75 ± 0.38^{cd}	2.50 ± 0.50^{abc}
M12	-	-	1.5	3.0	49.00 ± 0.58^{fg}	3.00 ± 0.58^{cd}	1.80 ± 0.61^{abc}
M13	-	-	2.0	4.5	37.00 ± 0.76^{h}	1.75 ± 0.38^{de}	1.00 ± 0.50^{bc}
M14	1.0	1.0	-	1.5	53.00 ± 1.53^{f}	5.00 ± 0.58^{abc}	2.75 ± 0.38^{ab}
M15	1.5	1.5	-	3.0	**85.00 ± 0.58^{a}**	**6.75 ± 0.63^{a}**	**3.90 ± 0.49^{a}**
M16	2.0	2.0	-	4.5	49.00 ± 0.76^{fg}	4.33 ± 0.33^{bc}	2.55 ± 0.29^{abc}
M17	1.0	-	1.0	1.5	53.00 ± 0.58^{f}	3.10 ± 0.49^{bcd}	1.90 ± 0.95^{abc}
M18	1.5	-	1.5	3.0	48.00 ± 1.15^{fg}	2.90 ± 0.38^{cd}	1.75 ± 0.52^{abc}
M19	2.0	-	2.0	4.5	69.00 ± 1.00^{c}	3.75 ± 0.66^{bcd}	2.90 ± 0.38^{ab}

The labels M1-M19 represent PGRs combination, values represented mean ± standard error; data were recorded after four weeks of culture on MS medium supplemented with $AgNO_3$ in combination with BAP, KIN and TDZ. Best results are indicated in bold, mean followed by the same letter within a column are not significantly different according to Tukey's test with p-value ≤ 0.05

Table 2B. *Effects of different concentration and combination of plant growth regulators on shoot multiplication from cotyledonary node explant of okra with non-containing $AgNO_3$*

Culture media	Plant growth regulator (mgL^{-1})			Shoot proliferation %	No. of shoots/explant	Shoot length (cm)
	BAP	KIN	TDZ			
M1	0	0	0	0.00 ± 0.00^{i}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
M2	1.0	-	-	26.00 ± 0.58^{h}	2.10 ± 0.38^{ab}	1.00 ± 0.29^{ab}
M3	1.5	-	-	43.00 ± 1.53^{bcd}	2.75 ± 0.52^{a}	2.18 ± 0.43^{ab}
M4	2.0	-	-	33.00 ± 0.87^{fg}	2.38 ± 0.62^{ab}	1.80 ± 0.42^{ab}
M5	-	1.0	-	28.00 ± 1.15^{gh}	1.90 ± 0.49^{ab}	1.38 ± 0.31^{ab}
M6	-	1.5	-	36.00 ± 0.58^{ef}	2.00 ± 0.58^{ab}	1.85 ± 0.45^{ab}
M7	-	2.0	-	40.00 ± 1.53^{cde}	2.60 ± 0.38^{ab}	2.15 ± 0.45^{ab}
M8	-	-	1.0	35.00 ± 1.26^{ef}	1.75 ± 0.25^{ab}	1.50 ± 0.87^{ab}
M9	-	-	1.5	45.00 ± 0.29^{abc}	2.80 ± 1.17^{a}	2.00 ± 0.29^{ab}
M10	-	-	2.0	38.00 ± 1.53^{def}	1.70 ± 0.30^{ab}	1.00 ± 0.29^{ab}
M11	1.0	1.0	-	34.00 ± 2.08^{efg}	1.80 ± 0.42^{ab}	1.38 ± 0.19^{ab}
M12	1.5	1.5	-	**51.00 ± 1.15^{a}**	**3.80 ± 0.12^{a}**	**2.30 ± 0.35^{a}**
M13	2.0	2.0	-	47.00 ± 0.29^{ab}	2.60 ± 0.70^{ab}	1.00 ± 0.50^{ab}
M14	1.0	-	1.0	38.00 ± 2.52^{def}	2.15 ± 0.34^{ab}	1.60 ± 0.31^{ab}
M15	1.5	-	1.5	40.00 ± 1.73^{cde}	3.00 ± 0.58^{a}	1.92 ± 0.58^{ab}
M16	2.0	-	2.0	48.00 ± 0.50^{ab}	2.90 ± 0.38^{a}	1.40 ± 0.31^{ab}

The labels M1-M16 represent PGRs combination, values represented mean ± standard error; data were recorded after four weeks of culture on MS medium supplemented with BAP, KIN and TDZ (Thidiazuron). Best results are indicated in bold, mean followed by the same letters within a column are not significantly different according to Tukey's test with p-value ≤ 0.05

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Shoot elongation

Well-proliferated shoots were isolated from the shoot bunches and transferred to the MS medium supplemented with different concentration of BAP and GA_3 for shoot elongation. Media without PGRs showed a negligible result of shoot length (0.5 cm) with 10% shoot elongation. On the other hand, Media containing GA_3 and BAP showed significantly effects on plant height with maximum shoot length (5.6 cm) and highest shoot elongation percentage (80%) in medium supplemented with 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} GA_3 following the lowest shoot length (2.50 cm) was measured on MS medium comprising 2.0 mgL^{-1} GA_3 alone. Among the tested PGRs, medium supplemented with GA3 alone was not effective for shoot elongation (*Table 3, Fig. 1F& 1G*).

Table 3. *Effects of MS medium supplemented with GA_3 and BAP on elongation of isolated shoots of okra*

Culture media	Plant growth regulator (mgL^{-1})		Shoot length (cm)	Shoot elongation %
	BAP	GA_3		
M1	0	0	0.50 ± 0.29^{c}	10.00 ± 1.00^{e}
M2	1.0	-	2.85 ± 0.45^{b}	34.00 ± 0.58^{d}
M3	1.5	-	3.57 ± 0.54^{ab}	40.00 ± 1.53^{d}
M4	2.0	-	4.20 ± 0.42^{ab}	63.00 ± 0.76^{bc}
M5	1.0	1.0	3.75 ± 0.43^{ab}	65.00 ± 1.53^{b}
M6	1.5	1.5	**5.60 ± 0.70^{a}**	**80.00 ± 1.32^{a}**
M7	2.0	2.0	4.50 ± 0.29^{ab}	57.00 ± 0.76^{c}
M8	-	1.0	2.60 ± 0.31^{b}	40.00 ± 1.04^{d}
M9	-	1.5	3.88 ± 0.19^{ab}	58.00 ± 1.53^{c}
M10	-	2.0	2.50 ± 0.29^{bc}	60.00 ± 2.31^{bc}

The labels M1-M10 represent PGRs combination, values represented mean ± standard error; data were noted after four weeks of inoculation on MS medium containing GA_3 and BAP. Best results are indicated in bold, mean followed by the same letters within a column are not significantly different according to Tukey's test with p-value ≤ 0.05

In vitro rooting and acclimatization

Well-developed, elongated shoots were excised from the *in vitro* regenerated shoots and cultured on half strength MS medium supplemented with different concentration of IBA, NAA along with AC for root induction. Highest rooting frequency (83%) as well as normal roots production (5.20 roots/shoot, 4.80 cm in length) was observed on MS medium supplemented with 1.0 mgL^{-1} IBA + 200 mgL^{-1} AC combination. While lowest rooting response (38%) was observed on medium fortified with 0.5 mgL^{-1} IBA alone (*Table 4, Fig. 1H*). Before transferring to the green house, plantlets with well-developed root system were transferred to the plastic cups having vermiculite and garden soil (1:1) and they were raised in the growth chamber for two weeks at 25 ± 2 °C with 70% relative humidity and consequently grown in the field with an of 87% survival rate (*Fig. 1I*).

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Table 4. *Effects of half strength MS medium supplement with different concentration of activated charcoal and auxins on rooting of okra*

Culture media	Plant growth regulator (mgL^{-1})			Root proliferation %	No. of root/explant	Root length (cm)
	IBA	NAA	AC			
M1	0	0	0	36.00±1.53^{k}	1.20±0.42^{e}	0.80±0.44^{c}
M2	0.5	-	-	38.00±1.00^{jk}	1.90±0.26^{de}	2.50±0.29^{abc}
M3	1.0	-	-	43.00±0.29^{ijk}	2.70±0.35^{cde}	3.10±0.67^{abc}
M4	1.5	-	-	39.50±0.29^{jk}	4.00±0.50^{abc}	2.80±0.42^{abc}
M5	0.5	-	100	45.00±0.87^{ij}	3.75±0.43^{abcd}	2.50±0.29^{abc}
M6	1.0	-	100	54.00±2.65^{fgh}	3.20±0.61^{abcde}	2.70±0.35^{abc}
M7	1.5	-	100	68.50±0.76^{b}	4.80±0.20^{ab}	3.50±0.29^{ab}
M8	0.5	-	200	63.00±1.53^{bcde}	3.10±0.40^{bcde}	2.50±0.29^{abc}
M9	1.0	-	200	**83.00±1.76^{a}**	**5.20±0.20^{a}**	**4.80±0.35^{a}**
M10	1.5	-	200	60.00±1.26^{cdef}	4.00±0.29^{abc}	2.80±0.20^{abc}
M11	-	0.5	-	50.00±0.58^{ghi}	4.10±0.10^{abc}	3.10±0.46^{abc}
M12	-	1.0	-	48.00±0.50^{hi}	2.50±0.29^{cde}	1.80±0.42^{bc}
M13	-	1.5	-	57.00±0.76^{efg}	3.10±0.49^{bcde}	2.50±0.29^{abc}
M14	-	0.5	100	65.00±2.89^{bcd}	2.80±0.57^{bcde}	3.00±0.58^{abc}
M15	-	1.0	100	58.00±1.15^{def}	3.33±0.33^{abcd}	2.10±0.67^{bc}
M16	-	1.5	100	67.00±1.00^{bc}	4.00±0.58^{abc}	3.00±1.15^{abc}
M17	-	0.5	200	54.00±1.53^{fgh}	3.80±0.15^{abcd}	2.80±0.20^{abc}
M18	-	1.0	200	70.00±1.04^{b}	4.88±0.40^{ab}	3.70±0.15^{ab}
M19	-	1.5	200	68.00±1.44^{b}	3.90±0.38 ^{abcd}	3.00±0.58^{abc}

The labels M1-M19 represent PGRs combination, values represented mean ± standard error; data were recorded afterward the culture of four weeks on half strength MS medium composition with auxins IBA and NAA in combination with AC. Best results are indicated in bold, mean followed by the same letter within a column are not significantly different according to Tukey's test with p-value ≤ 0.05

Discussion

Direct *in vitro* regeneration system has the potential to improve the phyto-pharming genetics under the influence of internal and external factors, used for genetic transformation. In present study, our main purpose was to establish, an efficient regeneration system in okra through shoot multiplication by cotyledonary node as explant. Shoot multiplication was enhanced from cotyledonary node explants which varied with the type of growth regulator, concentration, and combination used. In general, cotyledonary nodes was found more effective for shoot initiation and multiplication explants source. Till date, regeneration through cotyledonary node explants have been found in various plant species including *Abelmoschus esculentus* (Irshad et al., 2018), *Pisum sativum* (Jackson and Hobbs, 1990), *Punica granatum* (Naik et al., 2000), *Gossypium hirsutum* ((Kumar et al., 2016), *Vigna mungo* (Mookkan and

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Andy, 2014), *Lathyrus sativus* (Tesfaye et al., 2017), *Prosopis cineraria* (Venkatachalam et al., 2017) and *Cucumis sativus* (Venkatachalam et al., 2018).

Cytokinins play a key role in shoot organogenesis. BAP and KIN used alone or in combination showed different responses. When BAP was used alone, at low level of 0.5 mgL^{-1} BAP showed only (43%) shoot induction with an average of (1.33) number of shoots, but highest shoot induction (75%), with (4.5) number of shoots was observed at 2.0 mgL^{-1} BAP. Similar observation was reported previously in *Prosopis cineraria* (Venkatachalam et al., 2017; Sangeetha and Venkatachalam, 2014), *Dendrocalamus strictus* (Goyal et al., 2015) and *Tinospora cordifolia* (Panwar et al., 2018). Whereas shoot induction slightly decreased (60%, 3.80 number of shoots) with increasing of BAP concentration up to 2.5 mgL^{-1} (*Table 1*). Increased concentration of cytokinins suppressed the initiation of shoot buds. Similar results have been reported in various plant species *Enicostema axillare* (Sasidharan and Jayachitra, 2017) and *Prosopis cineraria* (Venkatachalam et al., 2017).

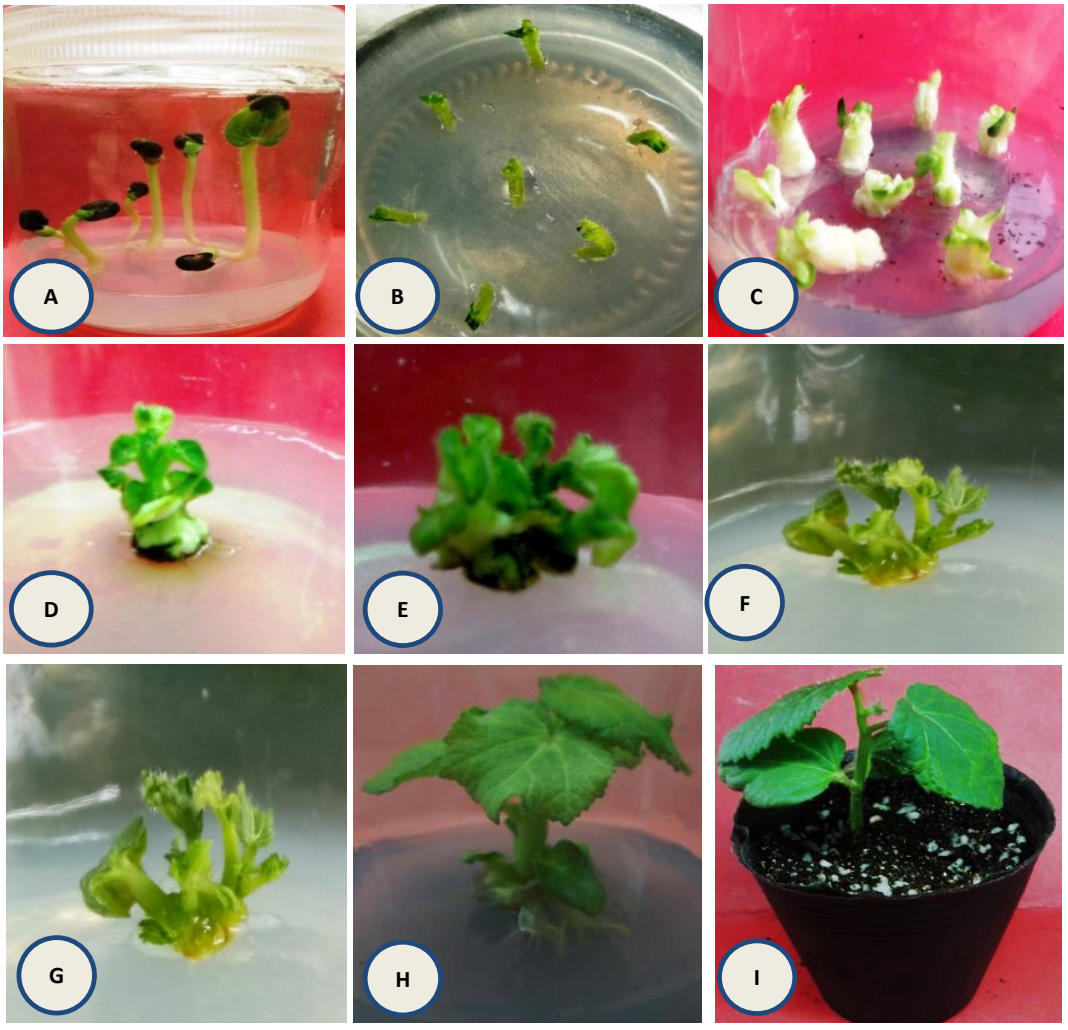


Figure 1. *Direct in vitro regeneration of okra (Abelmoschus esculentus L.) through cotyledonary node explant and the effect of $AgNO_3$ on multiple shoot formation. (A) 10-days old okra seedling. (B) Cotyledonary node explants. (C) Vigorous cultures after two weeks in culture media. (D) Multiple shoots inductions from nodal regions. (E) Multiple shoots formation and development. (F, G) Proliferation and elongation of multiple shoot. (H) Rooting of elongated shoots. (I) Regenerated plant*

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KIN showed less potential to shoot initiation then BAP. At lowest concentration of 0.5mgL^{-1}, it showed 58% with 1.68 shoots/explant and 65% with 3.50 shoots/explant at 2.0 mgL^{-1} BAP respectively. But the number of shoots per explant were significantly decreased at 2.5 mgL^{-1} BAP producing only 2.90 number of shoots with 38% response. Our results are in contrast with (Sivakumar et al., 2014). Rathore et al. (2016) also reported that KIN showed fewer shoot induction results in *Withania coagulans*. Fewer responses were observed at the lowest and highest plant growth regulator concentrations then intermediate level. Bakrudeen et al. (2011) in *Catharanthus roseus* preferred the use of BAP and KIN alone but not in combination. Our finding matches with the earlier reports of *Colocynthis citrullus* (Ntui et al., 2009), *Catharanthus roseus* (Bakrudeen et al., 2011) and *Prosopis cineraria* (Venkatachalam et al., 2017) in which BAP demonstrated positive influences on regeneration of shoot bud than to KIN.

Ethylene produced in culture medium, is known as an inhibitor of plant development and morphogenesis relying on the species and culture stage (Mohiuddin et al., 1997). $AgNO_3$ can induce variable responses *in vitro* by the inhibition of ethylene action. Kumar et al. (2009b) and Zhang et al. (2001) reported that the shoot multiplication is owing to the action of silver ions as a competitive ethylene inhibitor level by the crosstalk of ethylene and hormones rather than the ethylene biosynthesis inhibition. Use of regeneration enhancer in culture medium has been reported to be useful for shoot multiplication. Among the various compounds that have been widely used, $AgNO_3$ showed a positive effect on plant tissue cultures (Zhang et al., 2001). At 1.0 mgL^{-1} and 4.5 mgL^{-1} $AgNO_3$ induced 3.8 and 3.33 shoots/explant with 38 and 50% responses. But highest number of shoots/explant (4.0) was recorded at 3.0 mgL^{-1} $AgNO_3$ with 75% response. At 1.5 mgL^{-1} BAP + 3.0 mgL^{-1} $AgNO_3$ induced highest number of shoots 5.5 shoots/explant with 77% response but response decreased (68%) with increasing the concentration and produced 4.75 number of shoots/explant at 2.0 mgL^{-1} BAP + 4.5 mgL^{-1} $AgNO_3$, our results are similar with the finding of (Mookkan and Andy, 2014; Balkhande et al., 2013).

In case of 1.0 mgL^{-1} KIN + 1.5 mgL^{-1} $AgNO_3$ showed 50% response with 2.90 number of shoots per explant, which slightly increase with increasing the concentrations of 1.5 mgL^{-1} KIN + 3.0 mgL^{-1} $AgNO_3$ 50% response, 3.83 shoots/explants and at 2.0 mgL^{-1} KIN + 4.5 mgL^{-1} $AgNO_3$ highest response 67% with decreasing number of shoots (3.60 shoots/explant) was recorded. Our findings are in line with (Mookkan and Andy, 2014). Whereas in comparison, at lowest concentration of 1.0 mgL^{-1} TDZ + 1.5 mgL^{-1} $AgNO_3$ showed higher response 60% with 2.75 shoots/explants, but at higher dose of 2.0 mgL^{-1} TDZ + 4.5 mgL^{-1} $AgNO_3$ presented lowest response 37% with 1.75 shoots /explants (*Table 2A*). Results clearly indicate that TDZ and $AgNO_3$ at low concentration favored multiple shoot induction while higher dose shows negative results. Same findings were observed by Ouma et al. (2004) in cotton. Among the combinations evaluated, maximum number of shoot percentage (85%) was observed at a medium 3.0 mgL^{-1} $AgNO_3$ + 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} KIN by inducing (6.75 shoots/explant) which was comparatively higher than earlier reports but at higher dose of 4.5 mgL^{-1} $AgNO_3$ + 2.0 mgL^{-1} BAP + 2.0 mgL^{-1} TDZ, consequently decreased the number of shoots (4.33 shoots/explant), with (49%) shoot induction response (*Table 2A*). Whereas, lower number of shoots (2.90 shoots/explant) were observed at a medium containing 3.0 mgL^{-1} $AgNO_3$ + 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} TDZ having 48% response with (2.90 shoots/explant) followed by the lowest response 37% with 1.75 shoots/explant was observed at 4.5 mgL^{-1} $AgNO_3$ + 2.0 mgL^{-1} BAP (*Table 2A, Fig. 1E*). In general BAP

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was found to be most efficient cytokinins in comparison to TDZ and KIN, similar phenomenon was reported by (Venkatachalam et al., 2018) and the addition of $AgNO_3$ along with other cytokinins enhanced the multiple shoot induction was also previously reported by (Venkatachalam et al., 2017; Mookkan and Andy, 2014; Naik et al., 2000).

The results achieved from our study suggests that $AgNO_3$ could be used as a worthy improver or regeneration enhancer for *in vitro* regeneration of okra through shoots multiplication, meanwhile the responses may vary with the plant species. Fasolo et al. (1989) reported that cytokinins have the feature to stimulate the shoot proliferation but inhibit the elongation. Whereas, shoot elongation is important in plant tissue culture, which varies with the medium type, concentration and combination of PGRs used in different plants. Highest response of shoot elongation (80%) was achieved with 5.60 cm shoot length on optimal concentration of 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} GA_3 (*Fig. 1F, 1G*) followed by the lowest shoot length (2.50 cm) which was recorded at a medium containing 2.0 mgL^{-1} GA_3 (*Table 3*). Our results indicated that use of BAP and GA_3 enhance the shoot elongation. Similar results of BAP and GA_3 combinations were reported by (Selvaraj et al., 2006) in *Cucumis sativus,* (Gonbad et al., 2014) in *Camellia sinensis* and (Kumar et al., 2016) in *Gossypium hirsutum*.

Well-developed rooting is essential for successful *in vitro* regeneration in many plants and the use of half strength MS medium promotes the rooting (Rahman et al., 1992; Oakes et al., 2016). The addition of AC enhances the root formation due to the large surface area which can adsorb many types of substances leadings to improve the cell growth and development (Ghazzawy et al., 2017). IBA at 1.0 mgL^{-1} produced the 2.70 roots/shoot with 43% response, which varies on higher and lower concentration. While in combination with 100.0 mgL^{-1} AC + 1.5 mgL^{-1} IBA produced 4.80 roots/shoot with 68.50% response, which increases with increasing the concentration of AC and showed the highest response 83% with 5.20 roots/shoot on medium containing 200.0 mgL^{-1} AC + 1.0 mgL^{-1} IBA. In case of NAA, the highest responses 70% with 4.88 roots/shoots on a medium containing 200.0 mgL^{-1} AC + 1.0 mgL^{-1} NAA were observed. Our results are in agreement with previous reports of (Şan et al., 2015; Irshad et al., 2017; Kumar et al., 2016). The rooted plantlets were hardened by transferring into plastic cups containing vermiculite and garden soil (1:1) mixture. They were then kept in a growth chamber for two weeks at 25 ± 2 °C with 70% relative humidity and consequently established in the field condition with 87% survival rate. This protocol will be useful for multiple shoot induction, direct plant regeneration, shoot elongation, rooting and hardening of other relevant plant species.

Conclusion

In this study, an efficient *in vitro* plant regeneration protocol was established using cotyledonary node explants of *A. esculents* "Xiang Fu" cultivar. BAP at 2.0 mgL^{-1} was found to be most effective for shoot bud initiation from cotyledonary nodal explants. Shoot multiplication frequency was enhanced by the addition of silver nitrate ($AgNO_3$) in the medium and the highest shoot multiplication frequency was achieved on MS medium fortified with 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} KIN + 3.0 mgL^{-1} $AgNO_3$ with maximum number of shoots (6.75 shoots/explants, 3.90 cm in length). Highest shoot elongation (80%) was observed in a medium containing 1.5 mgL^{-1} BAP + 1.5 mgL^{-1} GA_3 with maximum shoot length 5.6 cm. Highest rooting frequency (83%) with (5.20 roots/shoot, 4.80 cm in length) was observed in MS medium supplemented with 1.0

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mgL^{-1} IBA + 200 mgL^{-1} AC. Furthermore, the whole protocol from seedling establishing to regenerated plants was completed in only 3.5 to 4.0 months. This efficient protocol for high-frequency plant regeneration through shoot multiplication in okra (*Abelmoschus esculentus* L.) will be a valuable tool for further genetic transformation.

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DETERMINATION OF OPTIMUM MANAGEMENT STRATEGY FOR HONEY PRODUCTION FOREST LANDS USING A'WOT AND CONJOINT ANALYSIS: A CASE STUDY IN TURKEY

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Abstract. In this study, it was aimed to develop alternative management strategies for Honey Production Forest (HPF) lands, which are planned to be set up in Bartin, and to select the optimum one. For this, a model (A'WOT-CJA), in which A'WOT (SWOT-Analytical Hierarchy Process) and Conjoint Analysis (CJA) are used together, was developed within the framework of ecological, social and economic criteria based on the participation principle. Within the scope of A'WOT-CJA, the honey status of Bartin was determined by SWOT in the first step and it was prioritized using AHP by digitizing in the second step. The highest SWOT values found as a result of AHP are "honey type" with 0.245 in terms of strength, "honey production season length" with 0.202 in terms of weakness, "bee product type" with 0.224 in terms of opportunity and "beekeeping type" with 0.147 in terms of threat. However, in order to improve the effectiveness and comprehensiveness of the strategies, 81 strategies with four factors, each consisting of three levels, were developed and evaluated by CJA in the third step. However, since it was difficult and time consuming to prioritise these 81 strategies, these strategies were reduced to 9 through orthogonal rotation and were arranged to be 110 participants among those related to the subject (50 people from the local population, 10 people from public institutions, and 50 people from NGOs). According to the A'WOT-C results, HPF optimum management strategy of Bartin Province was formed as follows; *"In the establishment of honey forests, giving priority to the chestnut species with high economic return, putting the lands, where the honey production season will last at least six months, forward in land selection, preparing the lands especially to increase the honey and royal jelly yield and the lands being in such form to support migratory beekeeping."*
Keywords: *forest management, beekeeping, participation, multi-criteria decision-makings, rural development*

Introduction

The necessity of using the natural resources effectively and efficiently in consideration of increasing population, needs and expectations refers to a precise equation in today's world and human being is the basis of this equation. Planners and implementers endeavour to meet the ever-changing and evolving needs. This process also increases the expectations for natural resources.

Although the fuel wood and timber are the most known among the resources obtained from the forests, the non-wood forest products (NWFP) are the most common products obtained from forest resources. Within the scope of plant-derived non-wood forest products in Turkey, apicultural products and honey have important roles as well as wild food plants, medical and aromatic plants, bulbous plants, dye plants and mushrooms (HFAP, 2013). Forest resources also provide an important input to apicultural products with their rich biodiversity and gene resources.

Forests are important resources for the production of quality honey (HFAP, 2013). Furthermore, while forests provide a low investment environment for beekeeping activities, they create high income opportunity with the labour force input and constitute an important source of income for forest villagers (Yilmaz, 2006; Gungor and Ayhan,

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2016). In addition, they make significant contributions to reduce the rural-urban migration and ensure community stability with the influence of income (Yilmaz, 2006). When viewed from this aspect, it can be stated that beekeeping is one of the most important rural development instruments (Korkmaz et al., 2015). On the other hand, while forests contribute to honey production, they also help pollination and soil conservation and they fulfill an important function at the point of conservation and development of biodiversity (HFAP, 2013).

Honey and apicultural products produced together with honey such as propolis, royal jelly, pollen and beeswax have an important place in world trade. Today, there are around 59 million bee hives in the world and around 1 million 250 thousand tonnes of honey is produced from them. India is the country with the highest number of hive assets in the world (12 million hives). Nevertheless, China, which has 9 million hives, is the country that produces the biggest amount of honey (466.6 thousand tonnes) in the world. After China, Turkey (94.7 thousand tonnes), Iran (74.6 thousand tonnes), Ukraine (73.7 thousand tonnes) and Russian Federation (68.4 thousand tonnes) come respectively in terms of honey production (*Figure 1*) (FAO, 2018). As can be understood from these statistics, Turkey is the second most important honey producer of the world.

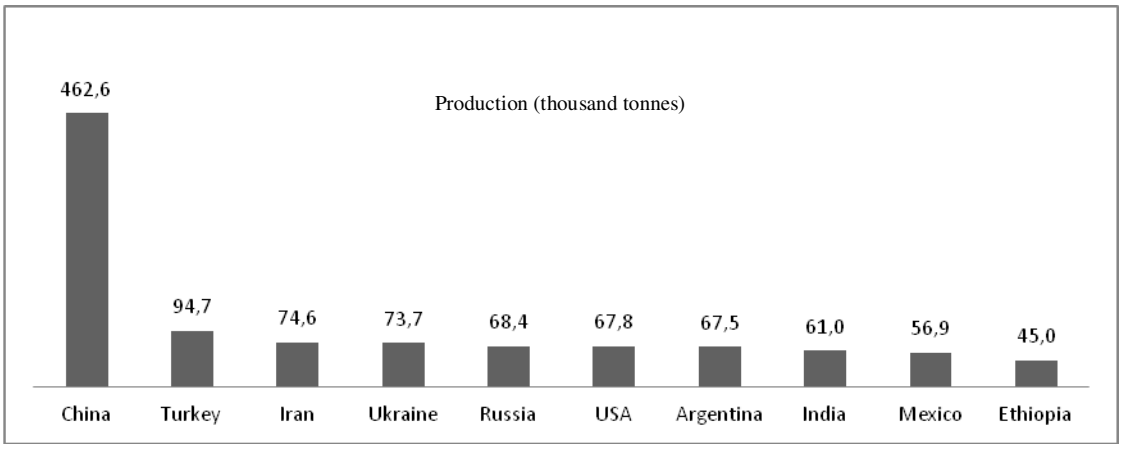


Figure 1.*World's 10 largest honey producers (FAO, 2003)*

The average honey production per hive is around 22 kg in the world. Canada (56 kg/hive), China (52 kg/hive), Mexico (39 kg/hive), Argentina (27 kg/hive) and the USA (26 kg/hive) are the countries that carry out honey production above the world average. Turkey is considerably lower than the world average with the production value of 15 kg/hive (ranks number 12) (FAO, 2009, 2018). Although 75% of honey plant species and varieties, which are available in the world, are available in Turkey (HFAP, 2013), Turkey does not yet have a sufficient role in the world honey trade, as the honey yield per hive is below the world average (OCEP, 2013).

On the other hand, forest areas in Turkey cover the 27.6% (21.678.134 ha) of the area of the country (*Table 1*). The areas, where there are forest trees and shrubs such as chestnut, tilia, acacia and rhododendron, which can be used as honey forests, form about 4% of the forest areas (GDF, 2015). When forest gap inside the forests are added to these areas, it is understood that Turkish forest areas have sufficient potential for Honey Production Forests (HPF).

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In order to increase honey production yield and to establish sustainable honey forests, the areas, where forests are to be established, should be determined with a participatory approach by taking the social demands and resource constraints into consideration. For this reason, interest groups should be included in the planning process during the process of determining the optimal management strategy of HPF. Through the HPFs to be planned in this way, the forests will be capable of providing many ecological-environmental, economic and socio-cultural benefits as well as the yield increase.

Effective strategies should be developed and implemented at the determination, management and planning stages of HPF lands in forests due to the contributions of beekeeping and apicultural products. There are number of examples of forest products and services in the world that are integrated into management strategies (Leuschner, 1992; Clutter et al., 1992; Duerr, 1993; Klempeer, 1996; Martell et al., 1998; Carsjeans and Vanderknaap, 2002; Eastman et al., 1998; Yilmaz, 2004; Gungor, 2005; Sen and Gungor, 2018).

Food safety, which has become even more important with the increase in world population, and the production of honey and other apicultural products within this context (FAO, 2009), have become one of the ultimate goals that should be achieved for Turkish forestry (GDF, 2015). However, the studies conducted to increase honey production are not carried out by scientific methods but by the initiatives of decision makers. Within this scope, it was aimed to develop alternative management strategies for the planning of HPF sites using multi-criteria decision making methods and to select the most suitable HPF site by prioritising the alternatives.

Study area

Bartin Province, which is the study area, is located in the Western Black Sea region of Turkey between 41°53' northern latitude and 32°45' eastern longitude (*Figure 2*). Its surface area is 2,143 km^2. In Bartin, mild marine climate (Black Sea Climate) with warm (26 °C and over) summers and cooler winters (-2 °C and below) prevail (BMST, 2017). The fact that it is close to the sea and that the mountain ranges, which are not very high, are parallel to the shore causes the temperature differences on the coastline to decrease and humidity to increase (BG, 2017). For this reason, Bartin Province has an important potential of honey production in terms of its geographic location and climate. The general area of Bartin Province is 228,576 ha. The forest area is 135,437 ha and the forest area ratio of the province is 59.3%. This value is above the average of Western Black Sea (50%) and the country (28%) (GDF, 2015). Bartin Province has an important potential of honey production in terms of its forest area values.

Bartin is one of the significant regions in terms of potential honey production areas due to its rich biodiversity in the Black Sea region, its edaphic and climatic conditions, for having tree species with high nectar yield such as chestnut, tilia and false locust etc. (GDF, 2011; WWF, 2003, 2010; Gungor and Ayhan, 2016).

On the other hand, in terms of honey production, forest area, general area, number of forest villages, population, general population and number of hives of Bartin Province and region and country values are given in *Table 1* (Gungor and Ayhan, 2016).

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Figure 2. *Geographical location of Bartin Province*

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Bartin Province, which has a high portion of forest assets, is also well above the country average in terms of forest villager density. Therefore, Bartin Province, which has an important potential for honey production, is well below the country and the region average in terms of honey yield level (*Table 1*). As can be understood from these statistics, Bartin Province could not tip the scales in its own favour in honey production.

Table 1. *Comparison of forest area, general area, number of forest villages, population, general population and number of hives of Bartin Province with region and country values*

Region Provinces		Area Information[1]			Population Information[2]			Honey Production Values		
		Forest Area (Ha)	General Area (Ha)	Forest Area/ General Area (%)	Forest Villagers (Person)	Total Population (Person)	Forest Villagers/ General Population (%)	Number of Hives[3] (Piece)	Honey Production[4] (Tonnes)	Honey Yield (Kg/Hive)
Western Black Sea	Bolu	514295	806204	64	78849	271208	29	22645	260	11
	Duzce	119420	243411	49	85728	338188	25	36164	518	14
	Zonguldak	156586	385604	41	199013	619703	32	38892	124	3
	Bartin	**110227**	**199491**	**55**	**72260**	**187758**	**38**	**25587**	**352**	**14**
	Karabuk	116804	129980	90	43466	227610	19	19222	248	13
	Kastamonu	477316	1250775	38	160724	361222	44	59419	418	7
Western Black Sea Sum		1494648	3015465		640040	2005689		201929	1920	
Western Black Sea Mean		249108	502578	50	106673	334282	32	33655	320	10
Black Sea General Sum		6727514	11483861		1830241	7539694		1318876	22313	
Black Sea General Mean		373751	637992	59	101680	418872	24	73271	1240	17
TURKEY Sum		21678134	78534470		7073020	76667864		6348009	94694	
TURKEY Mean		267631	969561	28	87321	946517	9	78370	1169	15

(1) GDF, 2014, (2) TBMM, 2010, (3) OCEP, 2013, (4) TUIK, 2017.

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Materials

Data necessary to realize the study aim is obtained from various public institutions, non-profit organizations and literature. Within this scope, Management Plans by the Forestry Operation Directorate of Bartin (FODB) (FODB, 2005), Honey Forest Action Plan (HFAP) by the General Directorate of Forestry (GDF) (HFAP, 2013) and Bartin Honey Forest Project (BHFP) (BHFP, 2013) were utilized. Moreover, the statistical data of the state institutions and organizations (TBMM, 2010; BAKKA, 2011, FAO, 2018, TUIK, 2017) and the Honey Forest Projects (GDF, 2012a; 2012b; 2014) conducted in various provinces and scientific research studies conducted in the region (Genc, 2003; Tutkun, and Bosgelmez, 2003; Misir, 2011; Dogaroglu, 2017; Bekar and Acar, 2017; Gungor and Sen, 2018; Sen and Gungor, 2018) were also utilized within the scope of this study. In addition, the records of Turkish Association of Beekeepers (TAB) (TAB, 2017) and Beekeepers Association of Bartin (BAB) (BAB, 2017), which is connected to this centre, were also used within the scope of the study together with the employers of association and the local public talks.

Methods

In the development of alternative management strategies for HPF sites and in the selection of the optimum one, the evaluation of the ecological, social and economic criteria together and the opinion of the interest groups (local community, public institution representatives, NGO representatives) will also increase the likelihood of making the right selection. There are many pure and hybrid Multi-Criteria Decision-Making (MCDM) techniques that can be used for this purpose in the literature such as AHP (Analytic Hierarchy Process), ANP (Analytic Network Process), A'WOT (SWOT-AHP), CJA (Conjoint Analysis), TOPSIS (Technique for Order of Preference by Similarity to Ideal Solution), TOPSIS-G (Technique for Order of Preference by Similarity to Ideal Solution with grey numbers), VIKOR (Multi-Criteria Optimization and Compromise Solution), ELECTRE (Elimination and Choice Expressing Reality), PROMETHEE (Preference Ranking Organization Method for Enrichment of Evaluations), SAW (Simple Additive Weighting), DEMATEL (Decision-Making Trial and Evaluation Laboratory) and DANP (DEMATEL-based ANP).

Considering the aim of the research, the most appropriate MCDM hybridization was decided to be A'WOT (SWOT-Analytic Hierarchy Process) and Conjoint Analysis (CJA) combination (A'WOT-CJA). Because within the scope of A'WOT-CJA, the honey status of Bartin Province was determined by SWOT and the obtained SWOT criteria were prioritized by digitizing by AHP. Thus, the factors standing out in each SWOT group formed the basis of HPF management strategies. Sub-levels should also be defined for the factors developed for more comprehensive strategies. In this way, the participants can better interpret and analyse the strategies. In this direction, a total of 81 strategies were developed by adding sub-levels to each SWOT factor. However, the strategies were reduced to 9 through orthogonal rotation in CJA, since it was difficult and time consuming to prioritise these 81 strategies. By this means, more consistent results were obtained. These 9 strategies were ranked by the 110 participants in the direction of CJA, and thus HPF optimum management strategy for Bartin Province was determined. The MCDM techniques used within the scope of the study are explained below.

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A'WOT technique

In the study, a hybrid technique A'WOT (SWOT Analysis and Analytical Hierarchy Process (AHP) combination) was used. A'WOT is a hybrid method developed by Kurttila et al. (2000) and Pesonen et al. (2001a). This method provides a structure combining the AHP method of Saaty (1980) with SWOT. The purpose of utilizing AHP in SWOT analysis is to evaluate the SWOT factors systematically. While SWOT analysis provides a frame for the decision status analysis, AHP supports more analytical application of SWOT (Kurttila et al., 2000; Kangas et al., 2001; Kajanus et al., 2004).

SWOT (Strengths, Weaknesses, Opportunities and Threats) is a commonly applied tool in the analysis of internal and external environment in order to provide a systematic approach and support to the cases of strategic decision making (Wheelen and Hunger, 1995, Hill and Westbrook, 1997). The purpose of SWOT implementation in the process of strategic planning is to develop and adopt a strategy that is generally adaptable between internal and external factors. SWOT can also be used, when alternative strategies emerge (McDonald, 1993). SWOT analysis is not capable of assessing the case of strategic decision making in a comprehensive way. It remains at the level of detecting the factors only. In addition to this, the expression of individual factors is often very general and short. Moreover, SWOT does not include tools for determining the significance of factors analytically or evaluating decision alternatives according to the factors. Therefore, the more use of SWOT is mainly based on the qualitative analysis carried out during the planning process and the skills and expertise of the people involved in the process. In summary, SWOT analysis is a list of internal and external factors or an incomplete qualitative examination. For this reason, it is sometimes called as "SoWOT" (Hill and Westbrook, 1997). General structure of SWOT Analysis realized within the scope of A'WOT is given in *Table 2*.

Table 2. *Structure of SWOT analysis*

SWOT ANALYSIS		
	POSITIVE FACTORS	NEGATIVE FACTORS
Internal factors	STRENGTH (How can we utilize the Strength)	WEAKNESS How can we convert these to Strength
External factors	OPPORTUNITY (How can we convert these to Strength)	THREAT (How can we convert these to Opportunity)

When the operation strategy is required to be determined by using SWOT analysis only, the internal and external factors, which are effective on the strategy, are listed roughly, indefinitely and superficially and evaluated as qualitatively insufficient (Hill and Westbrook, 1997). That is to say, in the traditional SWOT analysis, effect of each factor on the proposed plan or strategy is not determined. This problem is overcome by using AHP. In the AHP, each factor is assigned relative priorities in the direction of binary comparisons (Masozera et al., 2006). Therefore, the missing aspects of the SWOT analysis in terms of measurement and evaluation are eliminated by AHP. Thus, SWOT groups and their factors become measurable and their priorities are quantified.

The idea of using AHP within the framework of a SWOT is to assess the SWOT factors systematically and to balance them according to their intensity (Saaty, 1980;

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Kurttila et al., 2000). When AHP is included in the SWOT analysis, it analyses the factors of SWOT analysis by comparing them with each other. AHP helps to conduct SWOT in a more analytical way and the AHP – SWOT combination is a hybrid model that is referred to as A'WOT. The range of application of A'WOT technique is quite extensive and there are many studies available since it is mostly used in the field of forestry (Leskinen and Kangas, 1998, Kurttilla et al., 2000, Leskinen 2000, Pesonen et al., 2000, Pesonen et al., 2001b, Kajanus et al., 2004; Yilmaz, 2007; Leskinen et al., 2008; Gungor, 2011; Gungor and Sen, 2018). A'WOT decision hierarchy benefiting from the AHP is given in *Figure 3*.

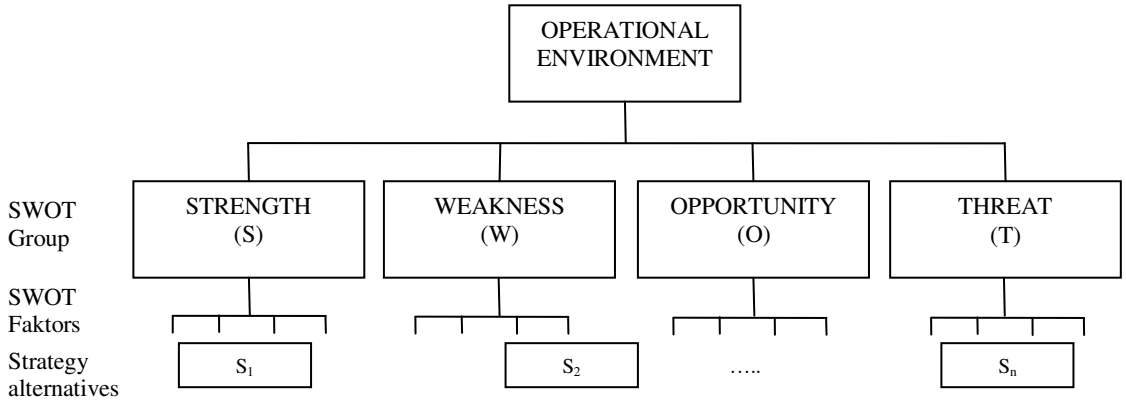


Figure 3. *Hierarchical representation of A'WOT analysis (Kangas et al., 2001)*

In the first step of the A'WOT technique, SWOT analyses are carried out. For this, firstly SWOT groups consisting of Strengths, Weaknesses, Opportunities and Threats are formed (*Figure 3*). Subsequently, the SWOT factors for each SWOT group are ranked as neutrally as possible. Thus, the internal and external equity factors obtained are included in the SWOT analysis. However, considering the fact that the human brain can compare up to 7 ± 2 elements at the same time (Schomoldt et al., 1995), the number of factors in each SWOT group should not be more than nine.

Later in the A'WOT technique, binary comparison operations are performed between the SWOT factors in each SWOT group. While these binary comparisons are being made, the one, who is performing the comparison is asked to state ordinance by asking questions such as "which factor is more preferred (important) when factor 1 is compared to factor 2?" and "how much more the factor, which is preferred more after that, is preferred than the other factor?" According to the information obtained from these comparisons, the relative significance (priorities) of the SWOT factors are calculated using the eigenvalue approach within the AHP technique. Then, the binary comparisons are made between the four SWOT groups. For this, a SWOT factor with the highest priority value is selected from each group to represent the group. Thus, by using these four SWOT factors selected from each group, the priority values of the SWOT groups are calculated using binary comparisons and eigenvalue approach, as in the previous step. In the next stage, the relative priority value of each SWOT group and the relative priority value of each of the SWOT factors in this group are multiplied separately. Thus, the overall priority value of each SWOT factor included in the relevant SWOT group is obtained. This operation is carried out separately for each of the four SWOT groups. As

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a result, the overall priority values of all SWOT factors, of which the total value is equal to one, are obtained (Kurttila et al., 2000; Pesonen et al., 2001a).

After making the comparisons in A'WOT, useful quantitative information about decision-making can be obtained (Kurttila et al., 2000). In addition, the use of A'WOT ensures the selection of alternatives according to each SWOT factor and each SWOT group (Pesonen et al., 2000). When the significance of different SWOT groups are identified, the selection alternatives can be prioritized as a whole in terms of strategic selection.

By including the AHP technique in SWOT analyses, SWOT groups and their factors become measurable and their priorities are set forth numerically. The weights of each calculated SWOT group and factors constitutes the basis of the strategies to be developed in the next step.

After the hierarchical structure model is created, matrices are created by making binary comparisons. Binary comparison is the comparison of two factors or criteria and is based on the judgment of the decision maker. By binary comparison, it is aimed to determine the relative significance of the elements in the hierarchy comparing to the element at the top level (Kurttila et al., 2000). In order to determine the significance levels of SWOT groups and factors among themselves in the AHS, (n×n) binary comparison matrices, which are shown in the formula given in equation 1, are created (Saaty, 1995) and the criteria or alternatives are compared binarily.

$$A=\begin{bmatrix} 1 & a_{21} & a_{31} & \cdots & a_{n1} \\ 1/a_{21} & 1 & a_{32} & \cdots & a_{n2} \\ 1/a_{31} & 1/a_{32} & 1 & \cdots & a_{n3} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 1/a_{n1} & 1/a_{n2} & 1/a_{n3} & \cdots & 1 \end{bmatrix}_{nxn} \quad \text{(Eq. 1)}$$

The relative significance of contribution to the purpose of each criteria given in Equation 1 and the superiority of each target are determined in a binary comparison of judgments (Saaty, 1980). The 1-9 significance scale developed by Saaty (1980) is used in order to assign value in the comparisons. The meanings expressed by the significance levels are shown in *Table 3*.

Table 3. *Scale of relative significance (according to Saaty, 1980)*

Intensity of importance	Definition	Explanation
1	Equal importance	Two factors contribute equally to the objective.
3	Weak importance of one over another	Experience and judgement slightly favor one over the other.
5	Essential or strong importance	Experience and judgement strongly favor one over the another
7	Very strong or demonstrated importance	Experience and judgement very strongly favor one over the other.
9	Absolute importance	The evidence favoring one over the other is of the highest possible validity
2,4,6,8	Intermediate values	When compromise is needed.

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Following the formation of the binary comparison matrices the eigenvector of each item is calculated showing that an item is more important than the other items (Kurttila et al., 2000). The eigenvector of the matrix in dimension of n×1 is determined as follows (equation 2):

$$i = 1,2,3, \ldots., n \; ve \; j = 1,2,3, \ldots, n$$

$$b_{ij} = \frac{a_{ij}}{\sum_{i=1}^{n} a_{ij}} \quad \text{(Eq. 2)}$$

In order to determine the significance distributions of the criteria, it is necessary to calculate the W = [w_i] nx1 column vectors. W column vector is obtained from the arithmetic mean of the row elements of the matrix, which is the value of the b_y values given in the formula given in Equation 2.

The consistency ratio (CR) for each of the binary comparison matrices is calculated (equation 3). For this ratio, an upper limit of 0.10 is required. The ratio being over 0.10, means that there is inconsistency in decision making. In this case, improvement is necessary in judgement. The largest eigenvector of the matrix A (λ_max) is calculated and the CR value is obtained (equation 4).

$$i = 1,2,3, \ldots., n \; ve \; j = 1,2,3, \ldots, n$$

$$D = [a_{ij}]_{nxn} \times [W_i]_{nx1} = [d_i]_{nx1} \quad \text{(Eq. 3)}$$

$$\lambda_{max} = \frac{\sum_{i=1}^{n} \frac{d_i}{w_i}}{n} \quad \text{(Eq. 4)}$$

The randomness index (RI) is another value needed for calculating the consistency ratio. *Table 4* contains the RI values which are determined from constant numbers and determined according to n value (Kurttila et al., 2000). The CR value is calculated according to the formula given in Equation 5.

$$\boldsymbol{CR} = \frac{\boldsymbol{\lambda - n}}{\boldsymbol{(n-1).RI}} \quad \text{(Eq. 5)}$$

Table 4. *Randomness index*

n	1	2	3	4	5	6	7	8	9	10
RI	0.00	0.00	0.58	0.90	1.12	1.24	1.32	1.41	1.45	1.49

Later, the factors taking the highest values as a result of A'WOT calculations are detailed to make the strategies more effective. In this stage, Conjoint Analyses were utilized and more detailed strategies were developed in this way by adding criteria to the factors found in A'WOT results in the Conjoint Analysis.

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Conjoint analysis

The word Conjoint is a combination of the words CONside and JOINT. Conjoint Analysis (CJA) was developed in the 1970s (Orme, 2009). CJA is a market research method determining the consumer preferences of multi-featured goods or services that are effective in demand determination. By using this analysis, it is assumed that the value given to any product by a person corresponds to the total utility deriving from all of the determined qualities of that product and then, the likelihood of purchasing that product is in proportion to the benefit they obtain from that product. CJA determines the most customer satisfactory combination of the factors related to the product or service that they consider during the purchasing stage and at the same time ensures the determination of the most preferred factor levels for that product or service.

The measurement of tools is one of the most basic concepts in CJA which is designed as factors and sub-levels. A utility gives a subjective decision of preference of each participant. It also forms the conceptual basis for the measurement value which is a very important concept for this research (Anderson et al., 2010).

In case the number of factors and their levels are high in CJA, an extra burden will be added on the participants and the time speared for the research by participants will increase. Many participants may not want to participate in the survey since it will take too much time or even they do participate, they may give up they may quibble in order to save time (Louviere, 1988; Marshall and Bradlow, 2002). For this reason, there is no need to include a factor, which does not make any difference in the study to be carried out (Hair et. al, 1995). The number of factors to be used in CJA is usually between 4 and 7 and this number can increase up to 10 to 15. The number of levels is determined to be between 2 and 5 (Scholl et. al, 2005).

A simple model of CJA, which is generally used to understand consumers' reactions or preferences to a product or service, is given in equation 6 (Malhotra, 1996; Raghavarao et. al, 2010):

$$y_k = \sum_{j=1}^{J} \sum_{m=1}^{M_j} \beta_{jm} x_{jm} \qquad \text{(Eq. 6)}$$

Here:

y_k : Total utility of an alternative,

β_{jm} : Utility value of factor j at level m,

x_{jm} : The dummy variable, which takes the value of 1 for level m of the factor j and 0 for the other cases, 1 if incentive k has value m of factor j 0 else.

CJA, which includes statistical analysis such as correlation and regression and uses qualitative and quantitative data, analyses the effects of many independent variables by using the (utility) function as a dependent variable. In this way, the effect of each variable on consumer preference is determined.

CJA was used in many researches such as Irish consumer preferences for honey (Murphy et al., 2000), in the preference of local food producers (Darby et. al, 2008): in the preference stage of honey consumers in Czech Republic (Sanova et. al, 2017).

In this research, the strategies found as a result of A'WOT were ranked by CJA. For this, the significance of each factor and sub-level influencing the preferences were

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determined by CJA (utility, weight) and their combinations were presented to the participants. By this means, the optimum management strategy of HPF was determined.

Preparation and implementation of survey forms

SPSS (Statistical Package for Social Science) 21.0 package program was used for A'WOT and CJA. Surveys conducted under the context of A'WOT and CJA were implemented through face-to-face interviewing method in place. The surveys were made in the Central District, Amasra District and Kozcagiz District of Bartin.

The number of participants was determined according to the simple random sampling method. In this method, the sub-groups/layers are represented in the sample at the proportion of their weights in an environment of which the boundaries are set. In order to be able to make the sampling, the number of units to be selected from each sub-population is determined by the share of that sub-population within the entire population (Kurant et al., 2011). Thus, the sample to be formed is guaranteed to represent the population with all its sub-units (Fuller, 2009). Participants in the study were assessed in three interest groups (in the sub-layer) as local residents, public institutions and NGOs in order to increase the precision of A'WOT and CJA analyses. Later, the number of participants, who were subject to the survey in each interest group, was calculated using the formula given in equation 7 (Daniel, 1999):

$$n = \frac{NZ^2pq}{d^2(N-1)Z^2pq} \quad \text{(Eq. 7)}$$

Where:

N : Population size (102 people from the local population, 20 people from public institutions, and 98 people from NGOs),

n : Sample size (50 people from the local population, 10 people from public institutions, and 50 people from NGOs),

Z : Z statistic for a level of confidence. Confidence level 95% (1.96),

p : Probability of the presence of the aspect aimed to be measured within the main group (taken 50% due to the multi-purpose nature of this study),

q : 1-p,

d : Sampling error considered 10% (0.1).

As a result of the calculations made, surveys were made within the context of both A'WOT and CJA with a total of 110 people, 50 from local residents, 10 from public institutions and 50 from NGOs.

Results and Discussion

SWOT findings and A'WOT calculations

Evaluation of Bartin Province in terms of honey production is given in *Table 5*. SWOT outputs were evaluated by AHP under the scope of A'WOT and the values of SWOT groups and factors, which were considered to be effective in the development of HPF management strategies in Bartin, were obtained.

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Table 5. *Consistency rates[1] and priorities of SWOT groups and factors according to A'WOT results*

SWOT group	Group priority	SWOT factors	Consist-ratio	Priority of factor within group	Overall priority of factor
Strengths	0.352	S1. Natural forests increasing the honey yield	0.104	0.109	0.038
		S2. Old growth with organic honey production capability		0.171	0.060
		S3. Nectar plants contributing in pollen quality		0.178	0.063
		S4. Potential of producing chestnut, tilia, false locust and rhododendron honey that are sold in high prices		**0.245**	**0.086**
		S5. Proximity to major markets such as Istanbul and Ankara		0.094	0.033
		S6. Scientists with experience in beekeeping		0.050	0.018
		S7. Bee races resistant to disease		0.032	0.011
		S8. Climatic and ecological structure that is suitable for beekeeping		0.120	0.042
Weaknesses	0.172	W1. Honey yield below the average of Turkey,	0.101	0.178	0.031
		W2. Low-yield stationary beekeeping activities instead of high-yield migratory beekeeping,		**0.202**	**0.035**
		W3. The understanding of seeing beekeeping as a hobby,		0.170	0.029
		W4. Restricted capital transfers to beekeeping,		0.161	0.028
		W5. Lack of information on food safety,		0.080	0.014
		W6. Lack of registered beekeeper and uncontrolled beekeeping,		0.042	0.007
		W7. Inadequate and untended beekeeping education,		0.103	0.018
		W8. High migration rate.		0.063	0.011
Opportunities	0.324	O1. High rate of increase in demand for apicultural products,	0.109	0.141	0.046
		O2. The rural population capable of beekeeping,		0.039	0.013
		O3. Flora's potential to produce high-yield apicultural products (such as royal jelly, propolis, beeswax)		**0.224**	**0.073**
		O4. Ever-growing market for organic products,		0.221	0.072
		O5. National and international supports in financing beekeeping,		0.060	0.019
		O6. The key role of beekeeping in reducing migration,		0.088	0.029
		O7. New international markets with export potential,		0.153	0.050
		O8. Positive developments in OGM policies.		0.073	0.024
Threats	0.151	T1. Social insecurity due to fake honey production,	0.079	0.121	0.018
		T2. Bottlenecks in the marketing channels for the apicultural products in Turkey,		0.112	0.017
		T3. Lack of corporate company in honey production,		0.123	0.019
		T4. Lack of understanding the importance of beekeeping in rural development,		0.118	0.018
		T5. In spite of favourable season for long-term honey production, the short-term honey production season in practice,		**0.147**	**0.022**
		T6. Financial bottlenecks experienced in beekeeping,		0.098	0.015
		T7. Shadow economy in honey production and sales,		0.110	0.017
		T8. Lack of scientific knowledge on bee breeding and honey production.		0.170	0.026

[1]The consistency ratio of the comparisons between four SWOT groups was 0.093

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When *Table 5* is examined, Strengths group has the highest score in the SWOT group with 0.352. This is followed by Opportunities with 0.324. Weaknesses with 0.172 ranks third and Threats with 0.151 is the last. According to these results, it is understood that in the development of the HPF management strategies for Bartin Province, a structure, which emphasizes Strengths and Opportunities and dismisses Weaknesses and Threats, should be adopted. Moreover, "S4" in the Strengths group has the highest score among the SWOT factors with 0.245 (0.086 in the total average). "O3" with 0.224 (0.,073 in the total average) from Opportunities group ranks second. "W2" with 0.202 (0.035 in the total average) from the Weaknesses group ranks third and "T5" with 0.147 (0.022 in the total average) from the Threats group is the last one. In addition, showing the values obtained on the figure is given in *Figure 4*.

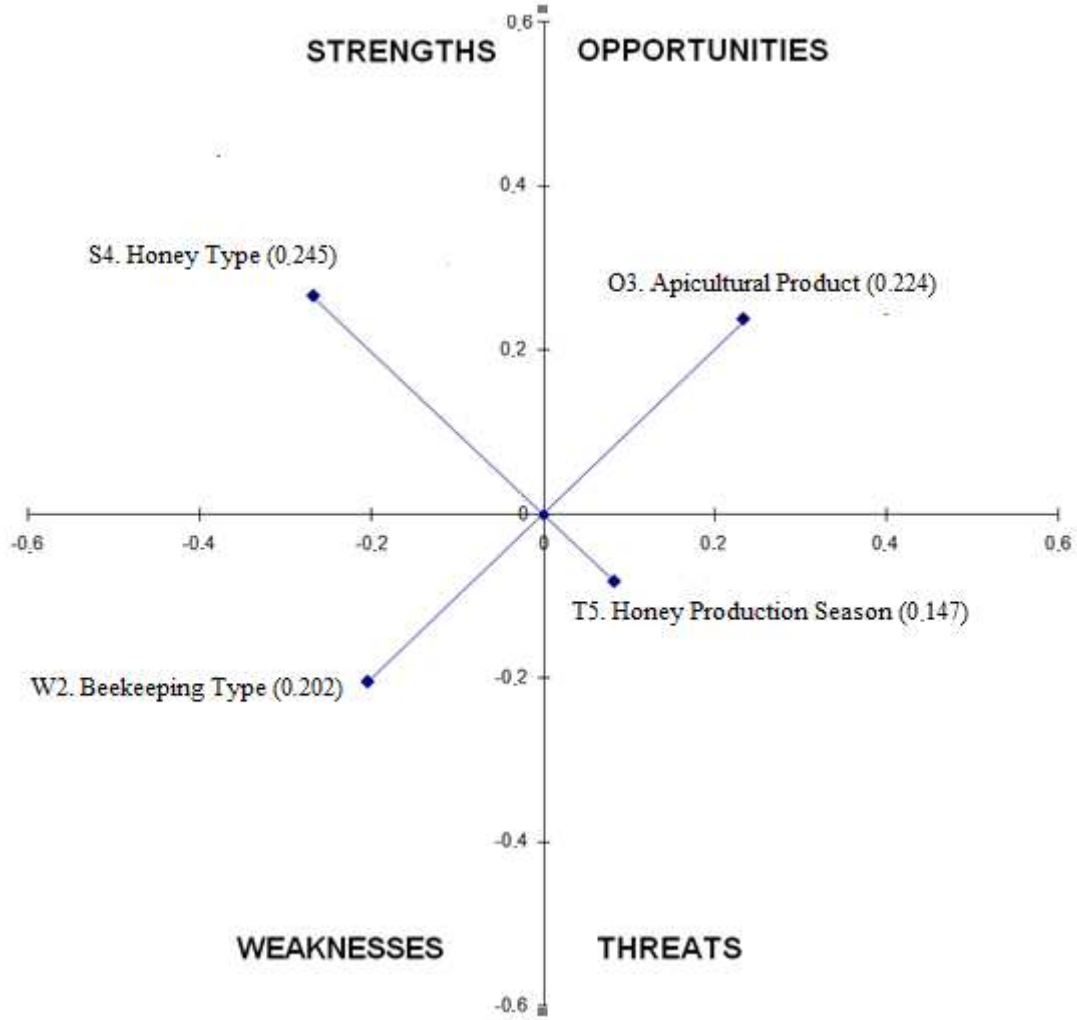


Figure 4. *An illustration of the relative significance of SWOT factors in an A'WOT application to HPF optimum management strategy*

CJA calculations

CJA was used in determining the sub-criteria of the factors determined by A'WOT. In the CJA calculations made according to the formula given in Equation 1, "*preference score and order of priority for alternative HPF management strategies"* was taken as the

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dependent variable. Dependent variable was measured by ordering and 9 strategies were used in ordering. In the development of the strategies, there were four independent factors (variables) determined by A'WOT and three levels developed under CJA (*Table 6*).

Table 6. *A'WOT factors and CJA levels*

1st Step: Factors determined under A'WOT			2nd Step: Levels developed under CJA
SWOT groups	**SWOT factors**	**Factor Name**	**Factor Level**
Strengths	S4	Honey Type	1. Tilia + False Locust Honey 2. Chestnut Honey 3. Rhododendron Honey
Weaknesses	W2	Beekeeping Type	1. Stationary beekeeping on the same land 2. Migratory beekeeping on the same land 3. Migratory beekeeping on different lands
Opportunities	O3	Apicultural Product	1. Honey + Royal Jelly 2. Propolis + Bee Venom 3. Pollen + Beeswax
Threats	T5	Honey Production Season	1. Maximum 3 months 2. Approximately 6 months 3. 9 months and longer

By taking the factors and sub-levels in *Table 4*, it is possible to develop a total of 3^4=81 combinations (strategies) on the basis of full design (full profile) (Green and Sirinavasan, 1978; Malhotra, 1996, Gungor, 2005). However, in this case it is difficult and time consuming for participants to prioritise 81 strategies. For this reason, management strategies have been developed in which each factor and sub-levels are independent (as an orthogonal design) (Hair et al., 1995; Smith, 1999). In the development of these strategies, in accordance with the CJA and for the purpose, the most negative level of each factor was taken as a reference (*rhododendron honey* for the factor of honey type, *pollen+beeswax* for apicultural product factor, *maximum 3 months* for honey production season, for the type of beekeeping *stationary on the same land*). For the remaining levels, 8 dummy variables with the values of 0 or 1 were defined. Based on this dummy variable defined, 9 alternative management strategies were developed based on orthogonal design (*Table 7*).

Table 7. *HPF management strategies formed on the basis of orthogonal design in CJA*

Strategy No	Factors and Levels			
	Honey Type	**Apicultural Product**	**Honey Production Season**	**Beekeeping Type**
1	Tilia + False Locust Honey	Pollen + Beeswax	Approximately 6 months	Stationary beekeeping on the same land
2	Tilia + False Locust Honey	Propolis + Bee Venom	Maximum 3 months	Migratory beekeeping on the same land
3	Chestnut Honey	Honey + Royal Jelly	Approximately 6 months	Migratory beekeeping on the same land
4	Tilia + False Locust Honey	Honey + Royal Jelly	9 months and longer	Migratory beekeeping on different lands

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5	Rhododendron Honey	Pollen + Beeswax	9 months and longer	Migratory beekeeping on the same land
6	Rhododendron Honey	Propolis + Bee Venom	Approximately 6 months	Migratory beekeeping on different lands
7	Chestnut Honey	Propolis + Bee Venom	9 months and longer	Stationary beekeeping on the same land
8	Chestnut Honey	Pollen + Beeswax	Maximum 3 months	Migratory beekeeping on different lands
9	Rhododendron Honey	Honey + Royal Jelly	Maximum 3 months	Stationary beekeeping on the same land

Later on, special cards were prepared for the strategies in *Table 7* and presented through personal interviews. The interviews were held in Bartin in 2016 with representatives of local residents and state institutions and non-governmental organizations. The number of persons interviewed was found using the formula (Equation 7) that gives sample size in limited communities.

Determination of optimum management strategy

As required by CJA, the participants were asked to rank the strategies, which are formed according to *Table 7*, from the best to the worst. The optimum management strategy for the HPF in Bartin was determined as the result of the analysis of the linear regression model, in which the average of the priorities given by 110 participant to each strategy is considered the dependent variable and the dummy variable levels of the factors as independent variable, using CJA.

According to the CJA results, in the strategies developed during the establishment process of honey forest, "*Honey Production Season* "with 34.44% ranks first, "*Beekeeping Type*" with 28.15% ranks second, "*Honey Season*" with 22.66% ranks third and "*Apicultural Product*" factor with 14.75% ranks last (*Table 8*).

Table 8. *CJA results*

Factor Name	**Factor Levels**	**Utility Coefficient**	**Significance Level (%)**
1. Honey Type	1.1. Tilia + False Locust Honey	-0.151	22.66
	1.2. Chestnut Honey	0.572	
	1.3. Rhododendron Honey	-0.421	
2. Apicultural Product	2.1. Honey + Royal Jelly	0.507	14.75
	2.2. Propolis + Bee Venom	-0.363	
	2.3. Pollen + Beeswax	-0.144	
3. Honey Production Season	3.1. Maximum 3 months	-0.748	34.44
	3.2. Approximately 6 months	0.421	
	3.3. 9 months and longer	0.327	
4. Beekeeping Type	4.1. Stationary beekeeping on the same land	-0.993	28.15
	4.2. Migratory beekeeping on the same land	0.565	
	4.3. Migratory beekeeping on different lands	0.428	

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For the most preferred and most important "Honey Production Season" factor, the "*Honey production season being approximately 6 months*" level with a utility coefficient of 0.421 ranked first, the "*Honey production season being 9 months and longer*" level with the utility coefficient of 0.327 ranked second, the "*Honey production season being maximum of 3 months*" level with the utility coefficient of -0.748 ranked third.

According to these results, participants want the honey production season to be approximately 6 months. Bartin Province (770 mm), which in fact has a rainfall average above the Turkey's rainfall average (573 mm), (BMST, 2017) is also above the country average (17%) in terms of honey plant rate (the average of Bartin Province is 23%) (GDF, 2011). This situation provides an advantage to Bartin Province in terms of honey production. However, when the yield level statistics per hive are examined (*Table 1*), it is understood that the hive yield is below the country average.

The honey production season shorter than three months means a low income for the beekeeper and it causes them to spend the rest of the year actively and makes it necessary for them to deal with other works. This prevents the desired specialization in beekeeping and causes people to consider beekeeping as a hobby (Korkmaz at all, 2015). It is possible to find the same result in other countries as well. For example, according to data of 2010, around 76% of beekeepers in Europe see this work as a hobby. However, around 10% does beekeeping professionally. However, in Kosovo and Hungary, 20% or less of the beekeepers were hobbyists. In Romania, about half of all beekeepers were hobbyists. Except for Kosovo, Greece, Romania and Spain, professional beekeepers represented less than 10% of the total population of beekeepers (Chauzat et al., 2013).

Although the beekeepers react positively to the fact that the honey production season is 9 months or longer, the latter statements indicates that this will not be possible in Bartin and its surrounding regions. Likewise, experienced beekeepers and institutional officials stated that honey yield has decreased after the extension of season (6 months and more), especially in the production of chestnut honey (Gungor and Ayhan, 2016).

For the "*Beekeeping Type*" factor, which has the second important place in the strategy, the "*Migratory beekeeping on the same land*" level is the most preferred one with a utility coefficient of 0.565. This was followed by "*Migratory beekeeping on different lands*" with a coefficient of 0.428. The least preferred is the "*Stationary beekeeping on the same land*" with the coefficient of -0.993. According to these results, it is understood that the participants are more interested in migratory beekeeping than stationary beekeeping. When the literature related to the subject is examined (GDF, 2011, 2012a, 2012b; BAKKA, 2011; BHFP, 2013; HFAP, 2013; Gungor and Ayhan, 2016; Gungor and Sen, 2018) it is understood that for the success of beekeeping, it is a must to shift from stationary beekeeping to migratory beekeeping.

The point to be considered here is the performance of "migratory beekeeping on the same land" activity instead of "migratory beekeeping on different lands", which is a more comprehensive beekeeping activity, due to limited capital and labour in shifting from stationary beekeeping to migratory beekeeping. Likewise, when considering the rough structure of the Bartin Province and its surrounding topography (BHFP, 2013), and the honey plant richness (Genc, 2003; Misir, 2011), different vegetation periods can be observed even in the case of a single honey forest in the same year. For this reason, a certain amount of accumulated capital and labour should be ensured in beekeeping first

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and in the next stage, migratory beekeeping, which is the way of professional beekeeping that should be adopted.

For the "Honey Type" factor, which has the third important place, the level of "Chestnut Honey" is the most preferred one with a utility coefficient of 0.572, while the other levels are less preferred. When looking at the market sales prices of the region for honey, it is understood that the highest return belongs to chestnut honey (BAKKA, 2011; FAO, 2009, 2018; Gungor and Ayhan, 2016; TUIK, 2017; BAB, 2017; Gungor and Sen, 2018). This, naturally draws the attention of the beekeepers and the community to chestnut honey. Just like the honeydew honey in Mugla and Anzer honey in Rize, the chestnut honey draws the attention of honey consumers and producers in Bartin. The obtained results of the survey are also corroborative regarding the interest in chestnut honey in Bartin. The interest for the chestnut honey in Bartin Province was also observed in another province of Turkey. In other words, it was determined that the consumers from Izmir knew the chestnut honey with 33.3%, citrus honey with 9.4%, thyme honey with 21.6%, hayit honey with 11.8% and French Lavender honey with 3.9% (Baki et al., 2017). However, due to climatic conditions, the production values of chestnut honey in the region change from year to year (TUIK, 2017). Likewise, the average rainfall of the last 5 years (690 mm) is lower than the average of the last 50 years (770 mm) (BMST, 2017). For this reason, during the last 5 years, there have been significant decreases in the production of chestnut honey in Bartin Province (TUIK, 2017) and many beekeepers have suffered from this (BAB, 2017). In order to avoid experiencing this suffering, beekeepers need to enrich the honey variety taking into account the climatic conditions and to take preventive and protective measures against fluctuations in honey prices such as the diversity of the portfolio in the economy. Based on what has been told, it will be appropriate to take other types of honey (linden honey, false locust honey and rhododendron honey) into consideration in addition to chestnut honey.

For the “Apicultural Product” factor, which ranks the last meaning the least important one, the "Honey + Royal Jelly" level was the most preferred one and the "Propolis + Bee Venom" level was least preferred one with a utility coefficient of 0.507. The obtained results show that level of interest and knowledge of beekeepers and people of the region on apicultural products is inadequate.

In another study conducted in this subject (Baki et al., 2017), it was understood that 60% of the consumers consumed pollen, 36% consumed propolis and 4% consumed the royal jelly. However, it was determined that they did not use cream containing bee venom for therapeutic purposes. While the consumption of cream honey, organic honey and royal jelly was not encountered in studies conducted in previous years (Saner et al., 2011), in this study the consumer, who consumed these products, were encountered despite the low rate and number. Based on the formation of required consumer awareness, increased consumption of such apicultural products depends on ensuring healthy production and accessibility to these products in Turkey (Baki et al., 2017).

When it comes to beekeeping, the most common product coming to mind is honey. In fact, since honey is the most profitable product among the apicultural products, the result of this survey can be considered as natural. However, both for the sustainability of production and in order to increase the income generated from apicultural products and to reduce the risks, other apicultural products such as propolis and royal jelly should also be produced and consumed in the region (Baki et al., 2017).

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In order to ensure diversity in apicultural products, some progress has to be made in beekeeping as well. For example, the approach of regarding beekeeping as a second occupation or even as a hobby should be put up, specialized and qualified beekeeping activities should be performed and the capital allocated by the beekeepers for this work in this context should be increased (Park ve Youn, 2012). In addition, it is a must to have extensive knowledge on beekeeping in order to obtain all of the apicultural products. In a study conducted in Japan and South Korea (Kohsaka et al., 2017) on this subject, it was emphasized that the combination of traditional methods of beekeeping with modern methods would be important for beekeepers.

When the honey yield values of the World, Turkey and Bartin Province are compared, the average annual honey yield of our country (15 kg) is considerably lower than the world average (22 kg) (FAO, 2018). Bartin's honey yield value (14 kg), which is already below the world average, is also lower than the country's average (TUIK, 2017). Hive presence of Bartin and Turkey, which is in good state when compared in terms of the hive presence of the world, does not comply with the values obtained in honey production and honey yield (Gungor and Ayhan, 2016). In a comparison made for European countries, it was reported that honey yield levels and hive numbers of EU countries are also below the world average (Chauzat et al., 2013).

On the other hand, it should also be known that the increase in crop yields of plants provided by bees through pollination is 10-12 times (Yakovleva, 1975), even 20 times (Crane, 1972; 1975) of the monetary value gained from apicultural products. Also, there are many studies regarding the benefits of bees on pollination (EFSA, 2013; Goulson, 2013; IUCN, 2013, etc.). When including the economic values to be obtained indirectly through positive externalities in the light of this information, the honey production quantities and economic return of the HPF in Bartin Province will rise to higher levels.

Conclusions

In the research, a structure, which takes the ecological, social and economic values into consideration and acts according to the participation principle, was taken into consideration and A'WOT-CJA calculations were made within this scope. In the establishment phase of HPF, the current situation is set forth by SWOT analysis, prioritized with AHP (according to A'WOT technique) and arranged by CJA. For this reason, the research is the first forestry study in Turkey where A'WOT-CJA are used together.

According to the A'WOT findings obtained, Strengths (0.352) ranks first, Opportunities (0.324) ranks second, Weaknesses (0.172) ranks third, and Threats (0.151) ranks fourth in SWOT groups. In the development of management strategies in the direction of A'WOT, a structure, which emphasizes Strengths and Opportunities and dismisses Weaknesses and Threats, was adopted. According to this, the presence of natural forests and honey plants increasing the honey yield in A'WOT, the fact that the chestnut honey, which has a high market price and is in demand, is produced in the region, the long honey season and the existence of production potential of apicultural products such as pollen, beeswax and propolis including honey are the factors standing out.

In the research, sub-levels were added to the factors found in the A'WOT result *(honey type, apicultural product, honey production season, beekeeping type)* in CJA and optimum management strategy was determined to be *"In the province of Bartin,*

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chestnut type with high yield of honey should be given priority, the lands, where the flora is rich in terms of honey plants, should be selected as honey forest areas, these lands should be prepared especially in the way of increasing honey and royal jelly yield, the lands, where the honey production lasts approximately six months, should be highlighted in land selection and the honey forest lands should be constructed in a way to support migratory beekeeping." as a result of CJA calculations.

A'WOT-CJ model used under the scope of the research can be used in the prioritization stage of HPF lands, which are planned to be set up in Turkey, and in the decision making stage. By using A'WOT-CJA, which has an analytical structure, including the HPF management plans will have a more dynamic structure and potential conflicts between beneficiaries, local residents and management can be avoided as the opinions of the interest groups, meaning participants, were taken into consideration at the planning stage. Besides, the HPFs to be established in line with the developed strategy will contribute to the formation of a sustainable structure, to local beekeeping and to rural development. The HPFs, which have an important position in terms of food security, should be one of the priority issues in the strategies to be developed in order to support the rural population in Turkey.

In the study, a hybrid MCDM method was applied in determining the HPF management strategies. In this method, A'WOT and CJA techniques are used together. From this aspect, it is possible to state that it is a case study in the field of prioritizing natural sciences, forestry and management strategies. In addition, A'WOT-CJA followed in this study will be a role model for future research projects.

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THE EFFECT OF GABIONS ON THE QUALITY OF A STREAM HABITAT

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Abstract. Research on the effect of gabions (wire mattresses filled by stones) on stream biota was conducted on the regulated and natural reference reaches of the Oščadnica stream in northern Slovakia. The fish community reacts sensitively not only to overall sets of changes but also to the shape and material of a riverbed. Therefore, the assessment of the impact of the gabions on the stream biota was realized by the bioindication of the ichthyofauna. Based on a comparison of the quantitative indicators in the regulated and natural reference reaches of the Oščadnica stream, we can say that gabions do not have any negative effects on the ichthyofauna, which is mainly represented by brown trout. Brown trout is the species that reacts most sensitively to morphological stream changes. The results of the research show that gabions do not have any negative effects on the stream biota.
Keywords: *bioindication, ichthyofauna, river regulation, armouring of the river channel, abundance, ichthyomass*

Introduction

River regulation changes a river´s basic morphological and flow characteristics, for example, by alternating riffle zones with calm pool areas and introducing variable riparian borders with a wide range of cover places for fish. This type of activity has a negative impact on river biota and results in decreased biodiversity (Cianfrani et al., 2009; Palmer et al., 2010). Modifications resulting from regulations are very noticeable in assessments of the availability of specific habitats that are necessary for a river´s restoration (Mažeika et al., 2006). It has been shown that the negative impacts of river regulation are mainly restricted to a river´s upper reaches or rhitral streams (Macura, 2012). The variability of a riverbed decreases in the upper part of a stream, which has a significant impact on the ichthyofauna. Therefore, any morphological changes are reflected by the fish (Macura et al., 2012).

The research shows that freshwater fish are good indicators of morphological changes in the river channel and biotic integrity in freshwater ecosystems (Pont et al., 2006, Cheek et al., 2016, Roni et al., 2014, Schmutz and Jungwirth, 1999). They also respond sensitively to changes in temperature and flow (Avery-Gomm et al., 2014, Schlosser, 1987, 1990; Cowx and Welcomme, 1998). Due to their longevity, mobility and sensitivity to biotope changes, they are suitable for assessing the ecological river

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integrity (Keeley et al., 2015, Aarts and Nienhuis, 2003, Chovanec et al., 2003, Welcomme et al., 2006).

The Water Framework Directive (2000/60/EU) requires information about morphological changes and other characteristics of the quality of a stream that affect the presence of macrophytes and macroinvertebrates (Artemiadou et al., 2005; Friberg et al., 2005).

A river restoration scheme that preserves the high variability of a riverbed does not have any negative impact on an overall ecosystem. The design parameters of covers, pools and other structures that create a variable morphology in a regulated channel are therefore important components of restoration proposals of rivers (Koščo et al., 2010) and water reservoirs (Augustinková et al., 2017). Biological and hydrological relations have been studied in many streams, and authors have drawn attention to specific flow patterns that are specific to individual regions. However, these relations are better described by biological responses to alterations in flow regimes (Snelder and Lamouroux, 2010).

Therefore, it is necessary to focus on the factors that affect the living conditions of aquatic organisms and their life processes. Therefore, fish are suitable bioindicators for a water environment because their lives are closely connected to their environment. Individual species always occupy a certain kind of habitat, so that each change in the environment can cause changes in its fish community. Species that are able to adapt can survive; others recede into more suitable conditions or are completely eliminated. Other species that can survive in these conditions come to that location, which changes not only the whole original fish community but also the ecological nature of the flow. The fish community reacts sensitively not only to a whole set of changes but also to the shape of and the material constituting the riverbed.

Intensive discussions are ongoing among water managers, ichthyologists and hydrobiologists. Nowadays, these specialists believe that the success or failure of any restoration project depends on understanding of river biotopes (Zhang et al., 2016) and continuous stream monitoring. The aim of current research is to quantify the design characteristics of appropriate habitats for river restorations (Döll et al., 2010; Pastuchová et al., 2008). Taking this trend into account, the results in this article are focused on the impact of the objectification of gabions on the aquatic habitats of streams.

The characteristics of gabions

The basic philosophy of a gabion´s structure is its fusion with a natural environment. After decades, the whole gabion´s construction is overgrown by vegetation and is thereby perfectly stabilized. The system is ecological; the materials used have a natural character, except for the use of wires that are covered by a PVC protective layer. This type of gabion is only used when the wiring construction is expected to have a long life and is located in an aggressive environment. Based on the type of construction for which the structure is designed, several basic types of products, such as baskets, mattresses and sacks, are available. These elements can be combined. To stabilize a slope, a combination of a basket with a rod that forms a mesh of different lengths is also often used.

An important area in gabion constructions is their use in water management structures. It is even possible to say that we find gabion constructions the most

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important constructions in the field of water management structures. As in other types of constructions, different systems and materials can be used. In water management structures, wire mattresses are the most suitable construction elements due to their elasticity. Because a flexible construction can copy a terrain and carry large deformations without any destruction, wire mattresses can be recommended for fragmented riverbanks in river restorations.

Materials and methods

The Oščadnica stream in northern Slovakia (*Fig. 1*), where the two reference reaches were chosen, was selected to determine the impact of wire mattresses in a regulated stream channel. The natural reference reach (49.4193° N 18.8417° E) is 150m above the regulated stream channel (49.4198° N 18.8388° E). The stream channel is structured, and the riverbanks are stabilized by the root system of the riverbank vegetation (*Fig. 2*).

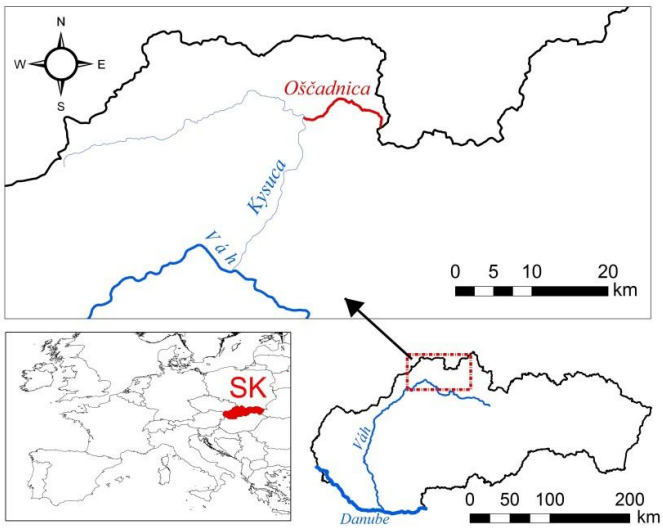


Figure 1. *Location of the Oščadnica stream within Europe*



Figure 2. *Natural reference reach of the Oščadnica stream*

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In the regulated reference reach, the stream channel has a simple trapeziodal profile with slopes on a gradient of 1:1.5. The slopes and the base are stabilised by wire mattresses. The reference reach lacks riverbank vegetation (*Fig. 3*).



Figure 3. *Regulated reference reach of the Oščadnica stream; the riverbanks are stabilized by wire mattresses*

The water quality

The data about the water quality in the Oščadnica stream were taken from the hydroecological projects of the Váh catchment. The oxygen content was determined by direct measurements by an oxygen searcher (Hana Instruments) during the ichthyological research. The saturation of the water by oxygen ranged from 95.2% to 98.8%.

Topographic measurements in the reference reaches

The topography of the reference reaches is represented by cross sections that are related to the polygonal traction. The polygonal traction is created by points located between 20 - 25 m apart. The cross section was measured by nivelation from each point. The polygonal traction points were fixed with wooden pins. Properly measured polygonal traction allows for the changing of any fixed points in case of their damage. Longitudinal and cross sections were implemented for the hydraulical model.

Ichthyological research

The research on the Oščadnica stream was realised five times between 2015 and 2016. The selected reference reaches of the stream were covered by 10 x 10 mm mesh barriers at both ends after hydrometric measurements. The fish were pursued by an electrical aggregate. After the fish were caught, they were placed in a relay located below the lower barrier in the stream and outside the electric field. In each reference reach obstructed by mesh, three hunts were undertaken at an average interval of 45 minutes. After each hunt, the fish were counted, measured, weighed and released back to the stream below the mesh.

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The abundance was evaluated from the measured data, according to the Leslie and Davis method (1939).These values, in combination with the average weight of each species, were used to calculate the biomass of each species and the total ichthyomass. „Abundance“ refers to the frequency rate of each species, whereas the biomass is its total weight. The total abundance means the entire frequency rate of all the individual species in the community in the selected stream, and the ichthyomass means the total weight of the community. The abundance, biomass and ichthyomass values are presented per hectare of the water surface.

“Occurrence constancy” is evaluated according to the presence of individual species in a given reference reach depending on the time. This “occurrence constancy” expresses the persistence of the generic composition of the ichtyocenosis.

The relevant calculations are:

$$K = \frac{n_i}{s} \quad \text{(Eq.1)}$$

where K is the “Occurrence constancy”, n_i is the amount of samplings of the occurrence of the species i, and s is the number of all the samplings. The occurrence constancy is expressed in the following categories:

I uncommon species: 0-20%
II rarely occurring species: 20-40%
III species often occurring: 40-60%
IV species predominantly occurring: 60-80%
V species almost always occurring: 80-100%

Other characteristics of the ichthyofauna are shown by the species diversity index and equitability index. The diversity index represents the ratio of the number of species and the number of individuals in the population. The diversity index is a structurally quantitative expression of the characteristics of each community, in this case, ichthyocenoses. The relevant calculation is:

$$H' = \sum_{i=1}^{s} \left(\frac{n_i}{N}\right) \cdot \ln\left(\frac{n_i}{N}\right) \quad \text{(Eq.2)}$$

where S = total number of species, n_i = number of individuals for “i” species, and N = total number of individuals of all the species.

Equitability (equanimity) relates to diversity and expresses the relative distribution of all the individuals in the community of the species present. The relevant calculations are:

$$K = \frac{H'}{\ln S} \quad \text{(Eq.3)}$$

where H'= diversity index, and S = the total number of species detected.

Schedule of the field measurements

From the perspective of the determining the impact of the stream regulation, the impact of climate change or the conservation of fishery resources, it is important to focus on the summer period (Carlson et al., 2015, Murchie et al., 2008). Summer period at the minimum discharge can be considered as a design condition, because it creates

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the highest load on the biota of the stream. High discharges do not have any significant negative impact on the biota of the stream. Hence, the dates of ichthyological, topographic and hydrological measurements were scheduled during the summer period at low water levels. To accentuate the impact of higher temperatures, ichthyological measurements have started at 11 am and ended after 4 pm. After all, the discharge was determined by hydrometry and the water level in the individual cross sections was marked. The topography of the riverbed was always measured the following day, after ichthyological and hydrometric measurements. Specific dates for ichthyological and hydrometric surveys were: 4.6.2015, 27.10.2015, 12.6.2016, 24.7.2016 and 2.9.2016.

Results

In the natural reference reach of the Oščadnica stream, there are demonstrably higher abundance values but small differences in the biomass (*Fig. 4* and *Table 1*). From the data, it is clear that fewer fish were present in the regulated stream reference reach but that their weight was higher. The hydrobiological research also confirmed that the wire mattresses did not have any negative impact on the macrozoobenthos. An evaluation of the macrozoobenthos on both reference reaches indicated that no significant difference was found in the structure of the species and in the amount of the bottom fauna in the regulated and natural reference reaches. The amount of organisms was only lower in the sampling in 2015 in the regulated reference reach, but the species diversity was higher in the regulated reference reach. There were no differences in the number of taxa found (the measurements were taken twice, i.e., in May and September).

The results obtained indicate that even in a prismatic stream channel that is not suitable for fish, there are the same ichtyomass and macrozoobenthos. This confirms the assumption that wire mattresses have a similar influence on the biota as rockfill or as a natural riverbank.

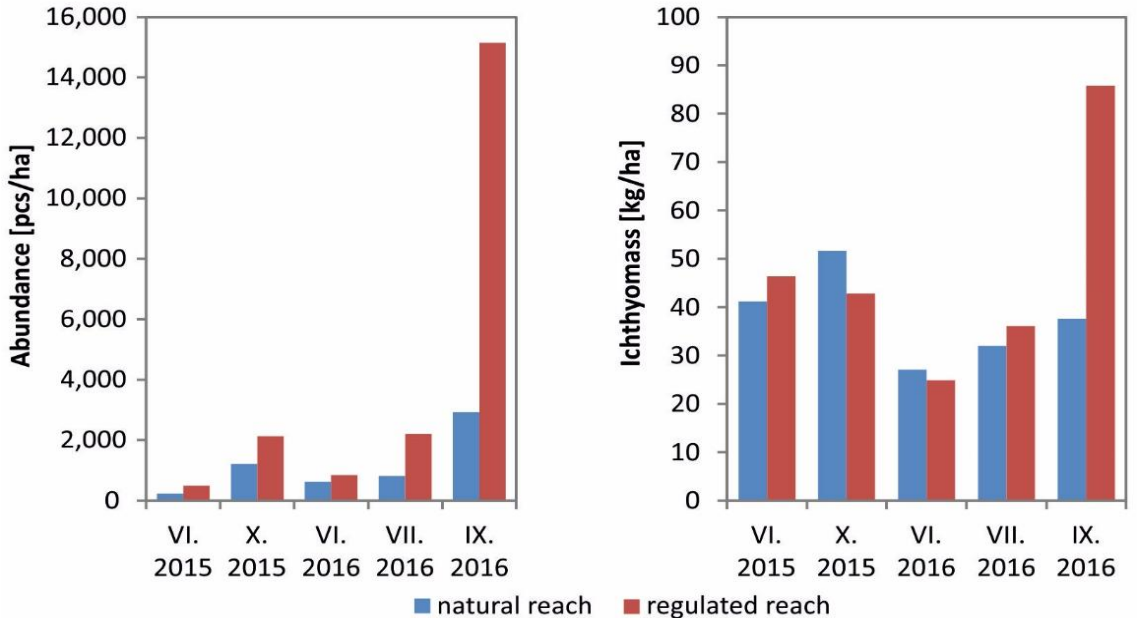


Figure 4. *Abundance (pcs per ha^{-1}), ichtyomass (kg per ha^{-1}) in the regulated and natural parts of the Oščadnica stream*

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Table 1. *The abundance (pieces per ha^{-1}), biomass (kg per ha^{-1}), and the average individual weight (g.ind^{-1}) of the fish species detected in the natural and regulated reference reaches of the Oščadnica stream between 2015 and 2016*

Species	Date	Natural stream (49.4193° N 18.8417° E)			Regulated stream (49.4198° N 18.8388° E)		
		pcs.ha^{-1}	kg.ha^{-1}	g.ind^{-1}	pcs.ha^{-1}	kg.ha^{-1}	g.ind^{-1}
BT	4.6.2015	117	34.6	55.4	164	35.4	46.8
	27.10.2015	860	44.5	52.3	35	0.7	39
	12.6.2016	366	20.4	58.4	283	18.5	61.9
	24.7.2016	699	28.2	37.6	407	21	55.2
	2.9.2016	1665	31.4	27.1	814	22.8	26.3
EM	4.6.2015	1	0.04	8	186	3.7	4.3
	27.10.2015				952	11.5	14.2
	12.6.2016				308	3	9.1
	24.7.2016				1245	5.7	4.8
	2.9.2016				11788	43.7	3.5
AB	4.6.2015	92	6.5	13.4	18	0.6	7.6
	27.10.2015	356	7.2	20.5	35	0.7	10.2
	12.6.2016	250	6.5	27.7	60	1	15
	24.7.2016	114	3.8	30.7	37	0.9	26.7
	2.9.2016	1262	6.2	7.1	1159	5.2	4.3
SL	4.6.2015	3	0.1	8.5	123	5.1	9
	27.10.2015				335	3.6	12.8
	12.6.2016	8	0.1	13	197	2.4	11
	24.7.2016				469	5.1	11.7
	2.9.2016				1381	14.1	9.6
CH	27.10.2015				18	1.1	70
G	27.10.2015				9	0.2	25
	24.7.2016				49	3.4	74.7
Σ	4.6.2015	213	41.24	85.3	491	44.8	67.7
	27.10.2015	1216	51.7	72.8	1384	17.8	171.2
	12.6.2016	624	27	99.1	897	28.3	171.7
	24.7.2016	813	32	68.3	2158	32.7	98.4
	2.9.2016	2927	37.6	34.2	15142	85.8	43.7

Explanations: BT = brown trout, EM = Eurasian minnow, AB = Alpine bullhead, SL = stone loach, CH = chub, G = grayling

There are six species of fish (*Table 2*) in the monitored reference reaches of the Oščadnica stream. While all six species were present in the regulated reference reach, only four species were detected in the natural reference reach. Grayling and chub are not found in this reference reach. The "constantly occurring" species in both reference reaches are Eurasian minnow, brown trout and Alpine bullhead; the regulated reference reach also contains stone loach. The grayling and chub in this reference reach belong to category I, uncommon species. The stone loach in the regulated reference reach belongs to category II, species that rarely occur.

The state of these indexes of the diversity and equitability of the species in the regulated reference reach is statistically higher than in the natural reference reach (*Table* 3).

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Table 2. *Composition of the species of ichthyofauna in the monitored reference reaches of the Oščadnica stream between 2015 and 2016*

Family / Species	Natural stream	Regulated stream
Cyprinidae		
Eurasian minnow - *Phoxinus phoxinus*	+	+
Chub - *Leuciscus cephalus*	-	+
Balitoridae		
Stone loach - *Barbatula barbatula*	+	+
Salmonidae		
brown trout - *Salmo trutta m. fario*	+	+
Thymallidae		
Grayling - *Thymallus thymallus*	-	+
Cottidae		
Alpine bullhead - *Cottus poecilopus*	+	+
Total frequency rate number of species	4	6

Table 3. *The summarized statistical data on the diversity index (H′) and equitability (E′) in the monitored reference reaches of the Oščadnica stream between 2015 and 2016*

	Natural ref. reach (1)		**Regulated ref. reach (2)**	
Parameter	*H′*			
	Spring (1a)	Autumn (1b)	Spring (2a)	Autumn (2b)
Θ	0.92	0.9	1.74	1.38
s	0.29	0.12	0.12	0.39
v	31.43	13.28	6.65	28.08
n	3	2	3	2
Θ	0.92		1.6	
s	0.21		0.29	
v	23.37		18.01	
n	5		5	
t (1a, 1b)	-			
t (1a, 2a)	4.54**			
t (2a, 2b)	-			
t (1b, 2b)	-			
t (1, 2)	4.23**			
	E′			
	Spring (1a)	Autumn (1b)	Spring (2a)	Autumn (2b)
Θ	2.01	3	2.63	1.98
s	0.19	0.4	0.36	0.2
v	9.58	13.2	13.68	10.33
n	3	2	3	2
Θ	2.41		2.37	
s	0.59		0.45	
v	24.56		18.89	
n	5		5	
t (1a, 1b)	-			
t (1a, 2a)	2.63*			
t (2a, 2b)	-			
t (1b, 2b)	-			
t (1, 2)	0.15			

Explanations: Θ = average, s = standard deviation, v = variance, n = number of cases, t = t-test for equal means, * = P < 0,05; ** = P<0,01

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The abundance and biomass of brown trout in the natural part of the Oščadnica stream is significantly higher than in the regulated part, with the exception of the regulated part, where the autumn biomass is lower. The average weight does not show any significant differences between the natural and regulated streams; however, the spring values are higher than the autumn values.

Discussion

There is enough information about the impact of the stream restoration on ichthyofauna (Bovee, 1982, 1986, Booker and Dunbar, 2004, Parasiewicz and Walker, 2007). Most fish species prefer certain combinations of water depths, velocity rates, bedding material and hiding possibilities. Information about the influence of velocity rate and depth of the water on habitat preferences is also sufficient, for example in Macura et al. (2016). However, there is a lack of information on the impact of the environment material, especially on the impact of the stabilisation material. Therefore, the basic aim was to compare the impact of the gabions armouring of the riverbed on the quality of the aquatic habitat. It would be ideal, if the gabion armouring was used for a natural flow for a sufficient length. Such a regulation does not exist in Slovakia, neither in the Czech Republic. Therefore, the Oščadnica stream, where the river channel is armoured by gabions on both sides and the regulation is long enough to reflect the fish habitat preferences, was selected for this study. This regulation is directly linked to the natural stream.

If there would be a significant negative effect of the regulated stream on the ichthyofauna, the research would continue by modelling the quality of the aquatic habitat by the IFIM methodology, specifically the SEFA model. This would allow a partial selection of the impact on the morphological changes in the regulated stream and the impact of the armouring material. Therefore, the reference reach was topographically measured in detail and a hydraulic model for the SEFA program was prepared. The measured results of the abundance and ichthyomass did not confirm any negative state of the restored stream compared to the natural one.

Several authors report that a suitable bioindicator of the environment is brown trout, because it responds sensitively to the changes in flow velocity and depths of the stream (Hooper, 1973; Shirvell and Dungey, 1983; Reiser and Wechse, 1976; Macura, 2016). Alpine bullhead and the stone loach are less sensitive to morphological changes. If we evaluate the trout independently, the abundance and the ichthyomass is higher in the natural stream; except the measurements in June 2015, where the parameters of regulated flow are slightly predominated. If we consider the trout together with minnow, which also sensitively responds to morphological changes, then the parameters will be again higher for the regulated stream, except for the ichthyomass in the measurements from October 2015. For the brown trout, the Wilcoxon signed-rank test has been performed, resulting in 6% significance of the abundance only. This value indicates the difference between the regulated and the natural stream, but it is not statistically significant. However, it is important to note that the number of measurements is relatively small for a good statistical evaluation. Therefore, the statistical data provided in the article is necessary to comprehend as additional data only. Based on the obtained data it cannot be clearly stated that there is a negative impact of the regulation of the Rajčianka stream as well as influence of the riverbank armouring by gabions is not pronounced.

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Conclusion

Ambiguous differences in the abundance and ichthyomass values between the regulated and the natural reach confirm that the regulated reach with gabion armouring has no proven (negative or positive) effect on the ichthyofauna, that indicates the biota quality in the stream. Similarly, small differences in zoobenthos were observed. Regarding to the mentioned facts it can be said that there is no significant impact of the gabions on biota in the stream. The aim of the article was to highlight the results of the research and the description of those properties that may affect the use of gabions. The specific properties of this armouring material allow a wide range of uses in the stream regulations and restorations.

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CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF HUNGARIAN SANDY SOILS UNDER DIFFERENT MANAGEMENT PRACTICES

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Abstract. The type and the management have strong effect on the composition and activity of microbial community and therefore on fertility of the soil. In this study ecological and conventional managements were compared in a sandy area of the Nyírség (North-Eastern) region, Hungary. Soil samples were collected twice from the upper and lower part of the sloping study sites, in autumn 2012 and in spring 2013, from the 0-30 cm and 30-60 cm depth intervals. Beside the main physical and chemical soil properties, the community structure (phospholipid fatty acid characterization) and activity (invertase and catalase enzymes) of microbes were investigated. The recycled plant residues can buffering the negative environmental/human effects, decreases the differences between the chemical and microbiological results of two elevation positions, and results favourable conditions to the soil microbes. Higher pH, total and organic C content, total and nitrite-nitrate-N content, furthermore higher enzymatic activities were measured in the ecological than in conventional site. Our results showed that ecological management had positive effect on the quality and fertility of the sandy soils in the Nyírség region by improving the chemical soil parameters and increasing the community size and the activity of microbes. Most of the measured parameters were more favourable in the 0-30 cm than the lower sampled layer.
Keywords: *sandy soils, ecological management, conventional management, soil microbiological properties*

Introduction

The global human population is 7.5 billion and it is expected to reach 9.7 billion by 2050 (UN, 2015). Thus, applying the rules of sustainable management is essential to meet the growing demand for food. Organic production is a management system based on best environmental practices, a high level of biodiversity, the preservation of natural resources and the application of high animal welfare standards. In contrast, conventional management system focuses firstly on high crop yield, however the demand for healthy foods has been started to emerge.

In ecological management mineral fertilizers could be replaced with green manure, manure, recycled crop residues. In Hungary, the ecological management is carried out in accordance with the 834/2007 and 889/2008 EU Commission Regulations. Pesticides and mineral fertilizers are allowed only in conventional management based on different regulations of the countries. The European Union is divided into three zones concerning

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the authorisation of a Plant Protection Products and Hungary belongs to the zone "B", with Belgium, Czech Republic, Germany, Ireland, Luxembourg, Netherlands, Austria, Poland, Romania, Slovenia, Slovakia and the United Kingdom. Furthermore in Hungary a list of the currently authorized plant protection products and enhancing substances is published yearly. The dose of applied nitrogen fertilizers is regulated by the Nitrates Directive (1991).

Nowadays, the negative effects of used chemicals on the biodiversity and human health are known (EASAC, 2015; Kim et al., 2017; Kidd et al., 2017), moreover there are several reports on qualitative and quantitative differences of soil microbial community in ecological and conventional managements (Romaniuk et al., 2011; Ge et al., 2013). Beside sustainability, there is growing demand for healthy food and plant production and ecological management opens this door.

The soil microbial community has important role in the soil fertility, because of its influence on the dynamics of organic matter and nutrient cycles (Bowles et al., 2014).

Phospholipid fatty acids (PLFAs) are the main component of the membrane of all living microbial cells, where special PLFA patterns characterise different microbial groups (Gude et al., 2012; Zogg et al., 1997; Zelles et al., 1992; Bossio et al., 1998). Thus, the application of PLFA techniques results in direct information for the group-level identification, classification and quantification of microbial community composition (Wu et al., 2009). Properly selected and applied soil cultivation method results in higher soil organic carbon content, hereby provides available nutrients for the soil microbes (Prasad et al., 2016). The microbial activity and biodegradation as well as the organic carbon and total nitrogen content also are more pronounced in the ecological management than in conventional management system (Ge et al., 2013).

The objective of the present study was to give a complex characterization of the studied ecological and conventional sites in a topographically heterogenous area. We hypothesized, that the ecological management had favourable influence on the soil physical and chemical properties as well as on the community structure and microbial activity of soil, hereby contributing to sustainable plant production.

Materials and methods

Sampling sites and management

The studied fields belong to the Research Institute of Nyíregyháza, University of Debrecen, in the North-Eastern part of Hungary. The region has a moderately cold-dry continental climate with 10.5 °C annual mean temperature and 500-750 mm annual mean precipitation. The soil type is acidic sandy soil (Dystric Arenosols) with diverse forms of dunes (Michéli et al., 2006). The selected areas were close to each other (about 400 m, *Fig. 1*) however, soils varied according to the topographical position (*Fig. 2a,b*). Soil samples were collected from two representative upper and lower parts of the slope of both ecological and conventional management sites, so we distinguished four sample types: EU: ecological upper part of slope (475849°17'N to 475849°72'N, and 214036°82'E to 214037°35'E, 156 m above the sea level); EL: ecological lower part of slope (475848°81'N to 475849°57'N and 214031°48'E to 214031°97'E, 151 m above the sea level); CU: conventional upper part of slope (475841°35'N to 475841°91'N and 214051°10'E to 214051°55'E, 158 m above the sea level); CL: conventional lower part of slope (475842° 43'N to 475842°99'N and 214054°64'E to 214054°96'E, 153 m

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above the sea level). The difference in elevation between the upper and lower part of the slope is 5 m in both site. We used for the measure of GPS coordinates an Trimble NOMAD 900 G device.

Ecological crop production has been maintained since 1997. In the studied sampling period, cereal crops (rye with hairy vetch and oat) were cultivated in the ecological and conventional fields, respectively. In both managements soils were ploughed upto 30 cm depth every year. Furthermore, in the conventional management fertilizers and pesticides were applied according to the needs of the plants. In the ecological management the loss of organic and mineral matters were compensated with farmyard and green manure (at least once in 5 years, respectively) and regular incorporation of the crop residues.

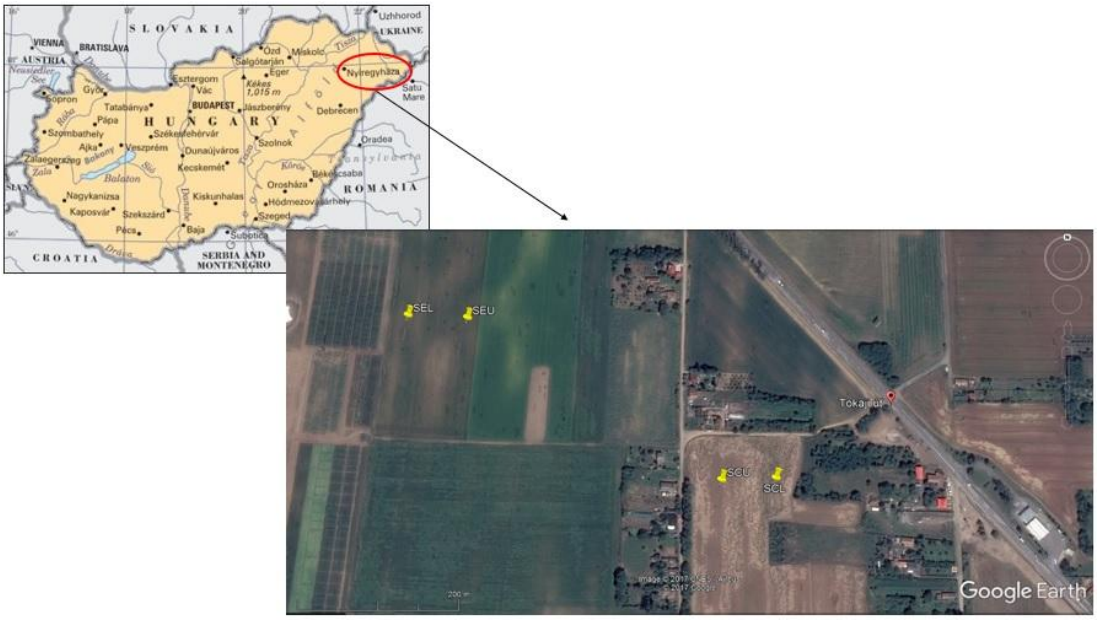


Figure 1. *Location of the investigated areas*

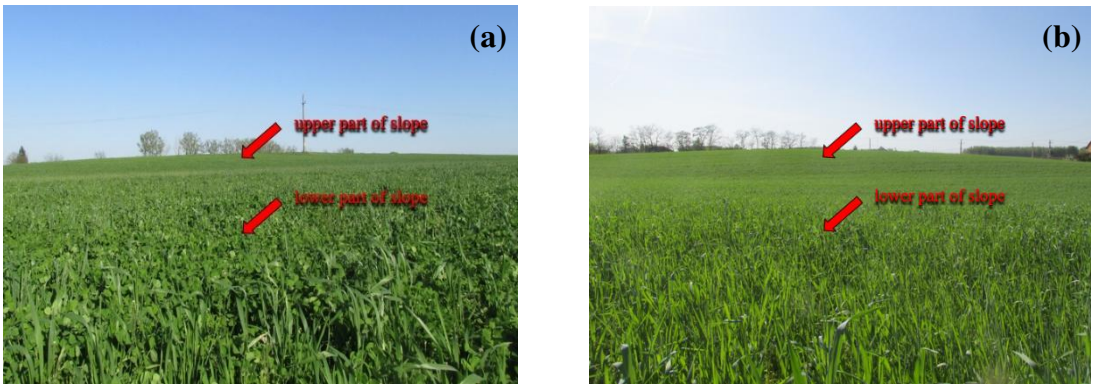


Figure 2. *Topographical differencies in ecological (a) and conventional (b) sites*

The physical properties indicated the heterogeneity of sandy soils in Nyírség region (*Table 1*). These parameters were determined with dry sieving and sedimentary method

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from the upper 30 cm depth interval in autumn 2012 (Buzás, 1993), from the same samples than chemical properties. Although the two investigated sites were located within a few hundred meters, the textures of these soils were different. The soil moisture was significantly higher in the ecological site.

The cultivated plants in ecological field were rye with hairy vetch (the previous crop was buckwheat), and in the conventional site oat was cultivated and rye with hairy vetch was cultivated in 2012 year.

Table 1. *Physical soil properties of the investigated soils (0-30 cm, 2012)*

Sampling site	gravel	sand	fine sand, silt, clay	soil moisture
	$^{m}/_{m}$ % dry matter		%	
EU	30.28 ± 1.33[c]	15.35 ± 0.52[a]	54.37 ± 1.05[b]	5.77 ± 0.08[b]
EL	34.65 ± 0.54[d]	24.07 ± 0.81[b]	41.28 ± 1.06[a]	6.90 ± 0.29[c]
CU	2.53 ± 0.35[a]	15.51 ± 0.52[a]	81.97 ± 0.69[d]	3.17 ± 0.22[a]
CL	9.39 ± 1.63[b]	17.91 ± 0.95[a]	72.71 ± 2.44[c]	2.58 ± 0.16[a]

[1]Data are expressed as mean ± standard error (n=12)
[2]Within a column the letters following the numbers represent the differences by one-way ANOVA followed by Tukey's-b test ($P < 0.05$)
[3]EU, ecological upper part of the slope; EL, ecological lower part of the slope; CU, conventional upper part of the slope; CL, conventional lower part of the slope

Sampling time and method

Samples were collected in two different seasons. The first sampling was completed in autumn 2012 (25-26 October), and the second sampling was done on 25 April 2013. The observed anomalies of weather are shown in *Fig. 3*, based on the data of the meteorological station of the Research Institute of Nyíregyháza.

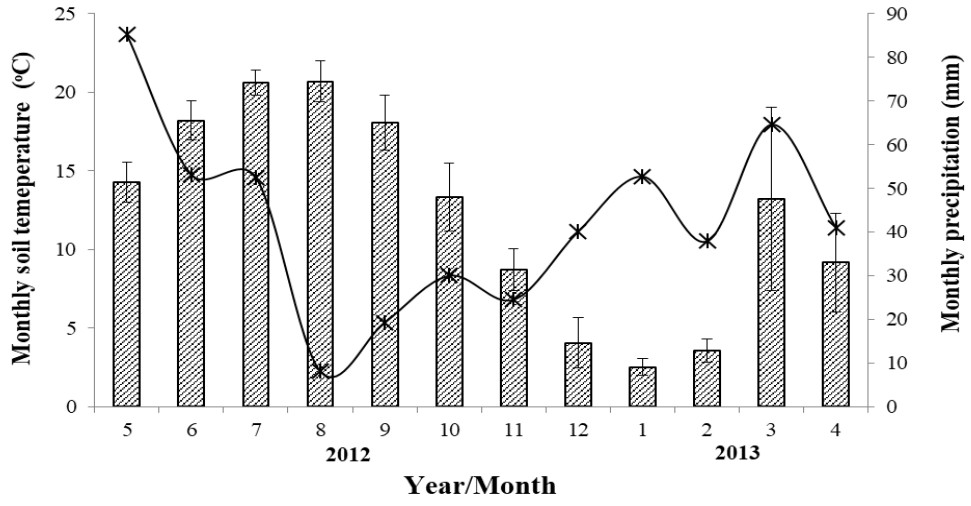


Figure 3. *Soil temperature and precipitation of the investigated areas measured by the Research Institute of Nyíregyháza, from May 2012 until April 2013. Data were provided by a μ-Metos meteorological station (Pessl Instruments GmbH.,Weiz, Austria), which recorded the data in every 2 minutes in the area of the Research Intstitute*

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Soil samples were collected from two depth intervals, 0-30 cm (ploughed) and 30-60 cm, in four repetitions. One repetition is a composite of four drilled subsamples. Samples for microbial analysis were put into a cooler bag in the field, and were frozen immediately after being carried to the laboratory. The samples for chemical analysis were stored air-dried until the analysis.

Determination of chemical soil properties

These properties were measured in the samples collected from the 0-30 cm soil depth. The larger plant roots were removed and the air-dried samples were sieved (2 mm mesh size) before the chemical analyses. Soil pH was measured with a Hach-Lange, HQ411D type digital pH meter (Hach-Lange, Loveland, Colorado, USA) in a 1:2.5 soil:KCl suspension (MSZ-08-0206-2 Hungarian Standard Method, 1978). Total carbon (C) and nitrogen (N) content were measured with varioMax CNS elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The organic carbon content was measured spectrophotometrically (UNICAM UV2 spectrophotometer, Thermo Scientific, Waltham, Massachusetts, USA) after acidic digestion (potassium dichromate and cc. sulphuric acid; MSZ-08-0452 Hungarian Standard Method, 1980). The KCl extracted nitrite-nitrate-N concentration was measured with an autoanalyzer FIA Star 5000 (Foss, Hilleroed, Denmark; MSZ 20135 Hungarian Standard Method, 1999).

Phospholipid fatty acid (PLFA) analysis

For PLFA analysis the samples were chosen based on the results of enzyme activities measurements. PLFAs were extracted, fractionated and methylated as described by White et al. (1979) with minor modifications. Methyl nonadecanoate was used as internal standard after the methylation step. The prepared samples were stored at -20 °C until the analysis. The PLFAs were separated and identified using a gas chromatograph-mass spectrometer system (GC 6890N with MS 5975, Agilent, Santa Clara, CA, USA) with a 100 m Supelco SP-2560 column, in selected ion mode and scan mode as well (50-350 amu).

Detected PLFAs were expressed in nmol PLFA g^{-1} dry soil unit (the results were corrected with the soil moisture content), and these data were used to represent the different microbial groups of soil. The unbranched, saturated PLFAs 14:0, 15:0, 16:0 and 18:0 represented the general bacterial biomass (Gude et al., 2012). The branched, saturated PLFAs iC15:0, aC15:0, iC16:0, iC17:0, aC17:0 indicated the G-positive (G^+) (Zogg et al., 1997), as well as the monoenoic and cyclopropane unsaturated C16:1n7c, C16:1n5c, C18:1n9c, cyC19:0 indicated the G-negative (G^-) bacteria (Zelles et al., 1992). The 10MeC16:0 and 10MeC17:0 represented the Actinobacteria (Bossio et al., 1998), and C18:2n6 was used as a fungi marker (White et al., 1979). Sum of these measured PLFAs were used to calculate the total PLFA concentration of the soil microbial community.

Soil enzyme activities

Samples were stored at -20 °C and were incubated for one day at 4 °C before the analysis. Before sieving (Ø = 2 mm), larger plant roots were removed in the laboratory. Invertase activity was measured photometrically at 508 nm, with 3,5-dinitrosalicylic acid (Mikanová et al., 2001). Catalase activity was determined with potassium

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permanganate titration from air-dried soil samples (MSZ-08-1721/4-86 Hungarian Standard Method, 1986).

Statistical analysis

All measurements were reported as mean values with standard error (SE). Statistical analysis was done using three internal repetitions respectively (n = 12) for the more accurate statistical result except in case of PLFA analyses, where internal repetitions were not measured (n = 4). All statistical analyses were carried out with the IBM SPSS Statistics 22.0 software package (IBM Inc., USA), applied a significance level of $P < 0.05$ was applied. One-way analysis of variance (ANOVA) followed by Tukey's-b and Games-Howell tests (depending on to results of the test of homogeneity of variences) were used to compare the means of different sampling sites. Correlations between abiotic and biotic soil parameters were determined using Pearson's correlation. Principal Component Analyis (PCA) was used to compare PLFA profiles between two management systems and soil layers. For PCA the two enzyme activities and the PLFA results, furthermore the total C and N content were used as variables, because the total C, N content significantly influenced these parameters.

Results

Chemical soil properties

The soils in the Nyírség region generally have acidic pH in accordance with the measured low pH_{KCl} (3.89-5.98) of the sampling sites (*Table 2*). The pH_{KCl} was more favourable in ecologically managed sites than in conventional sites. Chemical parameters were better on the lower part of slopes, but topography had reverse effect on pH_{KCl} because it was higher in the samples originated from the upper part of slopes (EU: 5.98; CU: 3.94) than samples from the lower part of slopes (EL: 5.02; CL: 3.89).

Table 2. *The main chemical parameters of investigated soils*

Sampling site	pH_{KCl}	total C *	organic C *	total N *	NO_2^- NO_3^- - N *	C:N ratio
EU	5.98±0.13^{c}	0.573±0.005^{b}	0.462±0.008^{b}	0.063±0.000^{b}	0.012±0.003^{ab}	9.13±0.05^{c}
EL	5.02±0.04^{b}	0.727±0.033^{d}	0.711±0.013^{d}	0.085±0.004^{d}	0.020±0.001^{b}	8.52±0.09^{b}
CU	3.94±0.02^{a}	0.313±0.003^{a}	0.236±0.002^{a}	0.040±0.001^{a}	0.007±0.000^{a}	7.91±0.11^{a}
CL	3.89±0.03^{a}	0.645±0.016^{c}	0.556±0.005^{c}	0.073±0.002^{c}	0.010±0.000^{a}	8.88±0.03^{c}

* in $^{m}/_{m}$ % dry matter unit

[1]Data are expressed as mean ± standard error (n=12)

[2]Within a column the letters following the numbers represent the differences by one-way ANOVA followed by Games-Howell test ($P < 0.05$)

[3]EU, ecological upper part of the slope; EL, ecological lower part of the slope; CU, conventional upper part of the slope; CL, conventional lower part of the slope

Generally, comparing the two managements, favourable values were found in ecological management, where all the measured chemical parameters were better. The most significant differences were in cases of available-N and organic-C content. The values measured in the conventional management on the upper part of slopes are only 58.3% and 51.1% of values measured in ecological site, respectively, while on the lower

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part of slopes these portions are 50.0% and 78.2%, respectively. However the differences in total-N and total-C content between the managements were less, 63.5% and 54.6% on the upper part of slopes, respectively, while 85.9% and 88.7% on the lower part of slopes, respectively. According to the elevation positions, the C:N ratio was higher on the upper part of the slope in ecological site, while in the conventional management higher ratio was measured on the lower part of the slope.

Microbial community structure

The actual structure of microbial community was identified by PLFA markers. Lower concentrations of the individual PLFA markers were measured in spring 2013 (*Table 4*) than in the previous sampling time (*Table 3*). Microbial biomass, estimated as total PLFA showed significant differences ($P < 0.05$) between the management systems, reliefs and depths. The highest value was measured in EU upper soil depth interval (23.736±1.05 nmol PLFA g^{-1}) in autumn 2012. In the first sampling time the highest G^+:G^- ratio was measured in samples from EU 0-30 cm depth interval (1.623±0.07). Fungi:general bacteria ratio was the highest in CU in upper soil depth interval (0.186±0.01). In contrast to the other data, actinobacteria:general bacteria ratio was higher in the 30-60 cm soil depth interval (except in CU), and was significantly higher in ecological fields (0.291±0.01 in EU; 0.162±0.00 in EL). There is a large difference in this ratio between the two soil depth intervals in the ecological field while it is similar in both studied depth intervals of conventional field.

Table 3. *The PLFAs indicated structure of microbial community in autumn 2012*

Biomarker PLFA	EU		EL		CU		CL	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
General bacteria[1]	7.998±0.32^{d}	1.445±0.07^{A}	2.861±0.13^{b}	2.193±0.07^{D}	3.447±0.15^{c}	1.883±0.01^{B}	2.150±0.05^{a}	1.048±0.02^{A}
$Gram^+$ bacteria[1]	8.495±0.40^{d}	1.872±0.09^{A}	3.347±0.15^{b}	2.071±0.06^{C}	3.685±0.16^{c}	1.890±0.07^{B}	2.247±0.06^{a}	0.766±0.17^{A}
$Gram^-$ bacteria[1]	5.236±0.22^{d}	1.169±0.06^{A}	2.480±0.11^{b}	1.439±0.04^{B}	3.344±0.14^{c}	1.452±0.05^{C}	2.097±0.05^{a}	0.583±0.12^{A}
Actinobacteria[1]	0.832±0.06^{d}	0.421±0.02^{D}	0.275±0.01^{a}	0.355±0.01^{C}	0.430±0.02^{c}	0.201±0.01^{A}	0.292±0.01^{b}	0.131±0.00^{A}
Fungi (C18:2n6)[1]	1.175±0.04^{d}	0.134±0.01^{B}	0.328±0.02^{b}	0.133±0.00^{A}	0.641±0.02^{c}	0.279±0.01^{D}	0.257±0.01^{a}	0.115±0.00^{A}
Total PLFA[1]	23.736±1.05^{d}	5.041±0.25^{A}	9.291±0.39^{b}	6.203±0.21^{C}	11.546±0.51^{c}	5.691±0.18^{B}	7.043±0.17^{a}	2.643±0.05^{A}
$Gram^+$:$Gram^-$ bacteria	1.623±0.07^{d}	1.602±0.08^{D}	1.350±0.05^{c}	1.426±0.05^{C}	1.102±0.04^{b}	1.314±0.04^{B}	1.072±0.02^{a}	1.313±0.03^{B}
Fungi:general bacteria	0.147±0.01^{c}	0.093±0.00^{B}	0.115±0.00^{a}	0.061±0.00^{A}	0.186±0.01^{d}	0.148±0.00^{D}	0.119±0.00^{b}	0.109±0.00^{A}
Actinobacteria: general bacteria	0.104±0.05^{b}	0.291±0.01^{D}	0.096±0.00^{a}	0.162±0.00^{C}	0.125±0.01^{c}	0.107±0.00^{A}	0.136±0.00^{d}	0.125±0.00^{D}

[1]Data are expressed as mean ± standard error (nmol PLFA g^{-1} dry soil), (n=4)
[2]Within a row the letters following the numbers represent the differences (small letters in 0-30 cm soil depth and capital letters in 30-60 soil depth) by one-way ANOVA followed by Tukey's-b test ($P < 0.05$)
[3]EU, ecological upper part of the slope; EL, ecological lower part of the slope; CU, conventional upper part of the slope; CL, conventional lower part of the slope

In spring 2013 (*Table 4*) the G^+:G^- bacteria ratio was also higher in the ecological samples on the upper part of the slope (1.386±0.05 in EU), but this ratio was close to each other on the top and the bottom of the hill. Fungi:general bacteria ratio was higher on the upper part of the slope in the upper soil depth interval (0.160±0.01 in EU, 0.223±0.01 in CU). In contrast to the other PLFAs, the actinobacteria:general bacteria ratio was higher in the 30-60 cm depth interval in both managements(except of CL).

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Comparing the managements, significantly higher ratios were found in ecological fields (0.202±0.01 in EU, 0.209±0.01 in EL). The calculated ratios show similar trend to the previous sampling time.

Table 4. *The PLFAs indicated structure of microbial community in spring 2013*

Biomarker PLFA	EU		EL		CU		CL	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
General bacteria[1]	3.047±0.13^{d}	0.508±0.01^{A}	2.218±0.11^{b}	0.728±0.03^{B}	1.730±0.29a	0.895±0.02^{C}	2.816±0.09^{c}	1.018±0.03^{D}
Gram^{+} bacteria[1]	3.396±0.14^{c}	0.871±0.03^{C}	2.402±0.11^{b}	0.603±0.03^{B}	1.822±0.07^{a}	0.431±0.02^{A}	3.479±0.12^{d}	1.310±0.04^{D}
Gram^{-} bacteria[1]	2.451±0.10^{c}	0.673±0.02^{C}	1.863±0.09^{b}	0.467±0.02^{B}	1.366±0.05^{a}	0.349±0.01^{A}	2.747±0.11^{d}	0.999±0.04^{D}
Actinobacteria[1]	0.244±0.01^{c}	0.181±0.01^{C}	0.178±0.01^{a}	0.152±0.01^{B}	0.186±0.01^{b}	0.075±0.00^{A}	0.343±0.02^{d}	0.199±0.01^{D}
Fungi (C18:2n6)[1]	0.487±0.02^{d}	0.060±0.00^{D}	0.347±0.02^{a}	0.041±0.00^{A}	0.385±0.01^{c}	0.043±0.00^{B}	0.378±0.01^{b}	0.059±0.00^{C}
Total PLFA[1]	9.625±0.39^{c}	2.679±0.07^{C}	7.009±0.06^{b}	1.993±0.08^{B}	5.489±0.22^{a}	1.405±0.04^{A}	9.763±0.41^{d}	3.585±0.15^{D}
Gram^{+}:Gram^{-} bacteria	1.386±0.05^{d}	1.295±0.03^{C}	1.289±0.01^{b}	1.291±0.05^{B}	1.333±0.05^{c}	1.234±0.04^{A}	1.266±0.05^{a}	1.311±0.05^{D}
Fungi:general bacteria	0.160±0.01^{c}	0.067±0.00^{C}	0.157±0.01^{b}	0.057±0.00^{A}	0.223±0.01^{d}	0.085±0.00^{D}	0.134±0.01^{a}	0.058±0.00^{B}
Actinobacteria: general bacteria	0.080±0.00^{a}	0.202±0.01^{C}	0.080±0.00^{a}	0.209±0.01^{D}	0.107±0.00^{b}	0.149±0.00^{A}	0.122±0.01^{c}	0.195±0.01^{B}

[1]Data are expressed as mean ± standard error (nmol PLFA g^{-1} dry soil), (n=4)
[2]Within a row the letters following the numbers represent the differences (small letters in 0-30 cm soil depth and capital letters in 30-60 soil depth) by one-way ANOVA followed by Tukey's-b test ($P < 0.05$)
[3]EU, ecological upper part of the slope; EL, ecological lower part of the slope; CU, conventional upper part of the slope; CL, conventional lower part of the slope

Soil enzyme activities

Both measured enzyme activities showed similar trends to the main chemical parameters with higher values in the ecological field and in the upper depth interval. In case of invertase enzyme (*Fig. 4a,b*), a large decrease in activity was observed in spring 2013 in both sampling sites and depth intervals, with most pronounced changes in the upper 30 cm of EU site (-13.92 mg glucose * g^{-1} dry soil * 4 h^{-1}). However this decrease was more significant, than in the case of PLFAs. In both managements and depth intervals the intensity of decrease was similar, about hundredth.

The invertase activity was effected in different ways by the elevation position. Generally, higher activity was measured in the upper depth interval on the upper part of slopes, while in the 30-60 cm depth interval higher activity was found on the lower part of slopes. The only exception was in the upper depth interval in autumn under conventional management, where the activity was higher on the lower part of the slope.

On the contrary, a slight increase (0.09-0.83 mg O_2 * g^{-1} * h^{-1}) of catalase activity was observed in spring 2013 (*Table 5*), but the ratios of ecological:conventional sites in different elevation positions were similar to the results of autumn 2012. In most cases, the activity was higher on ecological sites and on the lower part of slopes. Our results suggest the higher stability and independence of catalase enzyme from the environmental factors comparing to the invertase activity.

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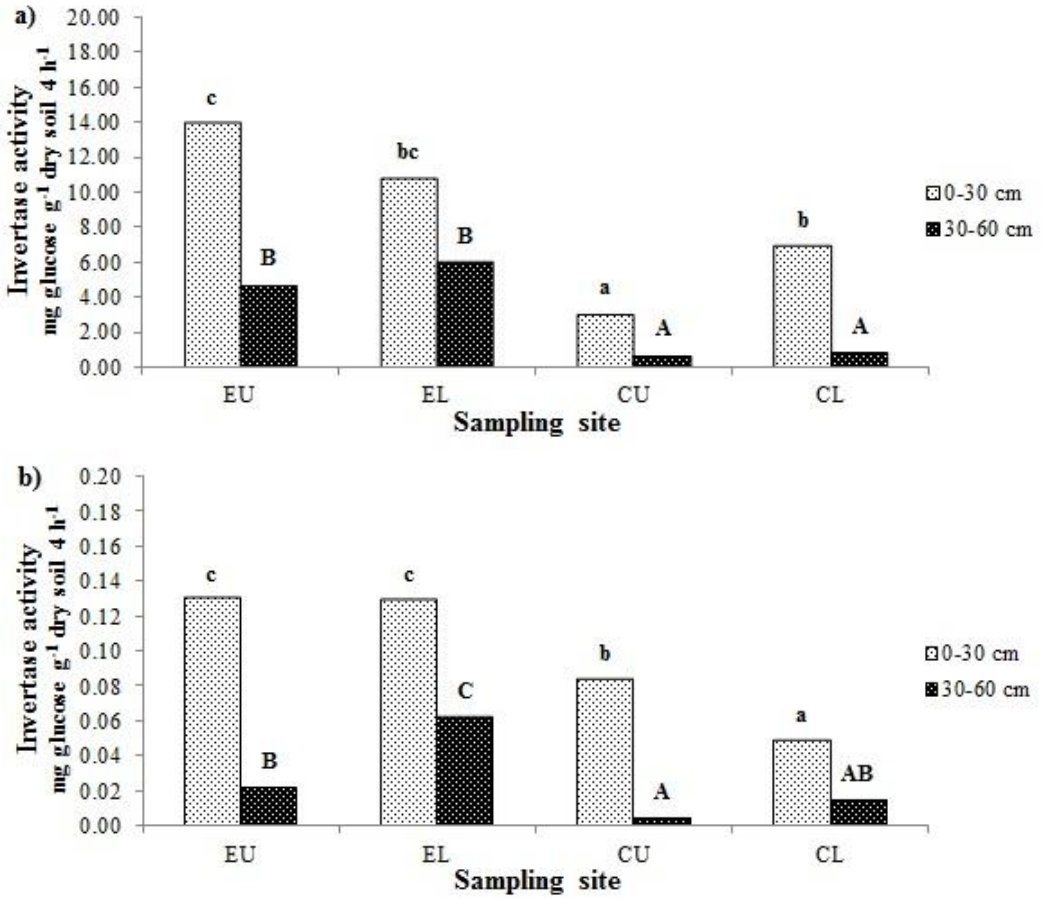


Figure 4. *Invertase activity in autumn 2012 (a) and in spring 2013 (b). Above the columns the letters represent the differences (small letters in 0-30 cm soil depth and capital letters in 30-60 soil depth) by one-way ANOVA followed by Games-Howell test (n=12; P < 0.05). EU, ecological upper part of the slope; EL, ecological lower part of the slope; CU, conventional upper part of the slope; CL, conventional lower part of the slope*

Table 5. *Catalase activity of investigated samples*

Sampling site	Catalase activity (mg O_2 g^{-1} dry soil h^{-1})			
	Autumn 2012		Spring 2013	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm
EU	1.548±0.061[b]	1.296±0.084[B]	1.927±0.073[b]	1.210±0.066[A]
EL	2.231±0.091[c]	1.845±0.053[C]	2.592±0.146[c]	2.208±0.155[C]
CU	0.823±0.026[a]	0.516±0.023[A]	1.388±0.086[a]	1.006±0.044[A]
CL	1.500±0.044[b]	0.793±0.085[A]	2.072±0.162[bc]	1.618±0.089[B]

[1]Data are expressed as mean ± standard error
[2]Within a column the letters following the numbers represent the differences (small letters in 0-30 cm soil depth and capital letters in 30-60 soil depth) by one-way ANOVA followed by Games-Howell test (n=12; P < 0.05)
[3]EU, ecological upper part of the slope; EL, ecological lower part of the slope; CU, conventional upper part of the slope; CL, conventional lower part of the slope

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Principal component analysis

Results of principal component analysis (PCA) indicated that the sampling sites under different managements, elevation positions and depth intervals were separated (*Figs. 5-6*). In autumn 2012 the first component contained the bacterial PLFA groups and total PLFAs while fungal PLFA belonged to this component just in the upper depth interval. The second component represented C and N content, invertase and catalase enzymes in every cases while fungi and actinobacteria PLFAs in the below depth interval. Ratios of individual microbial groups usually were weak indicators of soil properties. In spring 2013 similar trend was observed.

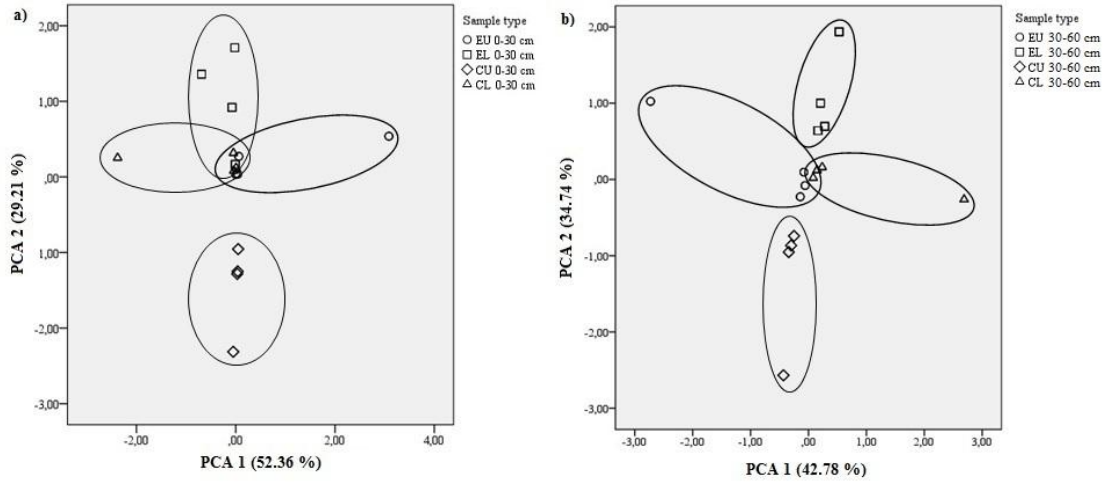


Figure 5. *Results of principal component analysis in autumn 2012 from the 0-30 cm (a) and the 30-60 cm soil layer (b). EU, ecological upper part of the slope; EL, ecological lower part of the slope; CU, conventional upper part of the slope; CL, conventional lower part of the slope*

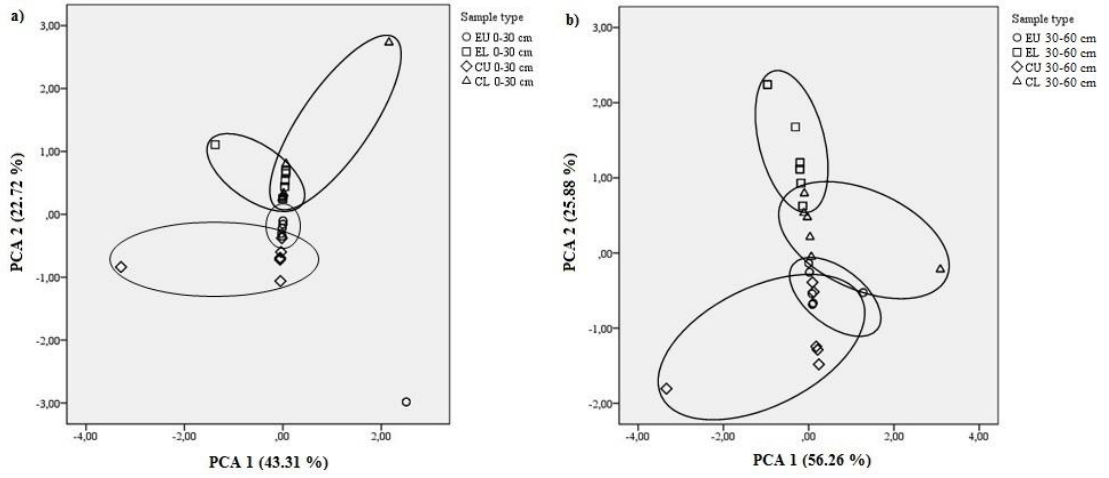


Figure 6. *Results of principal component analysis in spring 2013 from the 0-30 cm (a) and the 30-60 cm soil layer (b). EU, ecological upper part of the slope; EL, ecological lower part of the slope; CU, conventional upper part of the slope; CL, conventional lower part of the slope*

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Discussion

Chemical soil properties

Our results excellently presented the heterogeneity of sandy soils in Nyírség region. Earlier results (Yan-Jun et al., 2014; Wang et al., 2013) showed that the nutrient content was more favourable in those sites, where organic manure/crop residues were used as fertiliser, and the higher proportion of larger aggregates (> 2 mm) were observed. This probably resulted in increased microbial biomass and activity with increased extracellular polysaccharides providing good cementing agent to soil aggregation process (Liu, 2007). These are in accordance with our measured enzyme activities and PLFA results. The favourable effect of ecological management is indicated by the smaller difference between the two topographical sites than in conventional field. Furthermore the lower soil moisture in the conventional sites also may be responsible in the lower enzymatic activities (Stark and Firestone, 1995).

The different microorganisms are located in different parts of soil. While bacteria live in the smaller pores, fungi are located in the larger pores and on the surface of aggregates therefore fungi are more sensitive to different environmental effects (Gordon et al., 2008). Nevertheless our sampling sites on hills were not affected by topographical exposure which is proved by the higher microbial activity and PLFA values on the upper part of slopes.

In this study higher soil pH on ecological site was observed which suggests that organic manure apply and/or lack of fertilizer could, to some extent, alleviate soil acidification. Furthermore, the measured lower values of pH_{KCl} in conventional site probable were caused by the acidifying effect of the applied fertilizers (Pais and Horváth, 1990). The pH seems to be a chemical soil factor with high importance on microbial properties (Bååth and Anderson, 2003; Wang et al., 2013), which is also confirmed the results of our correlation studies (*Table 6*).

Table 6. *Relationships of biotic and abiotic variable pairs in autumn 2012*

Variables	pH_{KCl}	Total C	Organic C	Total N	NO_2^-, NO_3^- - N	C:N ratio	gravel	sand	fine sand, silt, clay	soil moisture
Invertase activity	0.620**	0.561**	0.495**	0.504**	0.204	0.535**	0.617**	0.375**	-0.621**	0.619**
Catalase activity	0.452**	0.838**	0.920**	0.843**	0.626**	0.386**	0.761**	0.738**	-0.836**	0.713**
General bacteria	0.906**	-0.081	-0.258	-0.201	0.779**	0.458	0.390	-0.188	-0.277	0.444
$Gram^+$ bacteria	0.913**	-0.079	-0.243	-0.182	0.778**	0.401	0.438	-0.118	-0.331	0.499
$Gram^-$ bacteria	0.802**	-0.291	-0.423	-0.358	0.612*	0.192	0.334	-0.120	-0.246	0.439
Actinobacteria	0.843**	-0.202	-0.379	-0.325	0.696*	0.421	0.275	-0.282	-0.162	0.343
Fungi (C18:2n6)	0.798**	-0.282	-0.453	-0.399	0.635*	0.369	0.215	-0.313	-0.106	0.296
Total PLFA	0.884**	-0.141	-0.305	-0.244	0.738**	0.376	0.385	-0.159	-0.279	0.455
$Gram^+$:$Gram^-$ bacteria	0.899**	0.629*	0.448	0.480	0.980**	0.812**	0.654*	0.039	-0.544	0.565
Fungi:general bacteria	-0.021	-0.949**	-0.971**	-0.948**	-0.276	-0.344	-0.468	-0.464	0.487	-0.297
Actinobacteria: general bacteria	-0.733**	-0.561	-0.554	-0.620*	-0.730**	-0.058	-0.990**	-0.762**	0.980**	-0.989**

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

Among others pH has strong effect on substrate availability of enzymes (Voroney, 2007) which play the basic role in energy and nutrient supply of microbial communities. Usually the PLFA content increases with increasing pH (Bååth and Anderson, 2003)

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which was proved by the 0.884 correlation coefficient between total PLFA and pH_{KCl}. That means 2-6 times higher concentrations of different PLFAs in the samples with higher pH.

The organic matter and nutrient pool is more stable where organic nutrient inputs are applied, rather than in case of other nutrient supplying techniques (Nardi et al., 2004). The quality and quantity of plant residues input into the soil can affect the organic matter content of soils (Chen et al., 2000; Edmeades, 2003). Greater than 100 kDa SOM fraction, which improves the soil structure and increases the plant and microbial nutrient uptake, was observed at the highest percentage in case of organic manure application (Dell'Angola et al., 1964). Furthermore, application of animal manure in organic management could increase the microelement content of soil (Bíró et al., 2005). The increasing organic C and PLFA content of ecological field indicated the possible presence of this SOM fraction. The increase of total and organic carbon, total nitrogen and the carbon:nitrogen ratio in our ecologically managed sampling sites resulted in higher available N content for plants. Based our results acidic pH in conventional sites could inhibit the availability of these nutrients.

Soil community structure and microbial activity

The different organic matter content and the value of available substrates resulted in the structural changes of microbes, because the nutrient supply method had strong effect on the community structure (Hartman et al., 2006). When the easily available, unstable carbon forms are present in the soil, the increase of G^- bacteria markers can be observed (Peacock et al., 2001), but less easily available carbon source increases the value of G^+ bacteria in the soil (Keynan and Sandler, 1983). The higher G^+:G^- bacteria ratio in an ecological site indicates the presence of more stable carbon forms.

Higher rate of fungi in the ecological management than in the conventional one has already observed (Esperschütz et al., 2007; Marschner et al., 2004). The increasing organic matter input resulted in an increase of fungi, thereby the increase of fungi:bacteria ratio (Frostegård and Bååth, 1996). However, our results showed that fungi:general bacteria ratio was depended on the climate conditions and can varied seasonally. In case of easily available fertilizer application the ratio of bacteria increased, because the fungi are primarily responsible for breakdown of the persistent materials (Grayston et al., 2004). This was confirmed by our results, where the values of fungi markers were higher in the ecological samples from the upper 30 cm soil layer because of the presence of recyclable but not easily available plant residues.

The main difference of the two management systems was found in the upper soil layer. While in this layer the conditions were more favourable for the microbes especially in case of substrates sources (Prasad et al., 2016), the number and activity of microbes were higher and the differences between the two cultivated systems were stronger.

When the values of easily available nutrients decreased, the decomposition process of actinobacteria became dominant (Bastida et al., 2013). The found negative correlation between actinobacteria markers and organic C ($r = -0.379$) indicated that the degradation processes started in autumn after the harvest and recycling of plant residues, but this relationship was not statistically significant.

Seasonal variation had strong effect on the microbial status of soils in both management systems (*Fig. 4* and *Table 5*). In spring 2013 the results were not significantly different probably due to the reduced microbial activity and the inactive

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microorganisms because of the combined effect of autumn soil cultivation and the extremely cold spring weather.

Our study revealed that the land use impact on soil enzyme activities vary under different management and environmental conditions. The recycled plant residues could increase the essential substrates' concentrations of enzymes (e.g. cellulose, hemicellulose) (Wick et al., 1998) which could result in higher enzyme activities. The organic matter, increased by the recycling of plant residues, can protect the extracellular enzymes against destroying them (Martens et al., 1992) and provide their substrates which could increase the enzymes activities in ecological field. However, the acidic pH usually inhibits the microbial activity (Sahoo et al., 2010). Differences are significant also in the lower soil layer which indicate the positive effect of long-term ecological management on the whole soil profile.

Invertase enzyme can break down some carbohydrate polymers at the end of the C cycle therefore its activity depends on other enzymes using higher organic matter as a substrate. Catalase protected cells against the oxidative damage. Invertase activity usually positively correlated with soil organic carbon and total nitrogen content (Frankenberger and Johanson, 1983). Catalase activity usually was stable in the soil, and showed significant correlation with organic carbon content and reduced with depth (Alef and Nannipieri, 1995). Close vertical connection of different soil layers is represented by the results of invertase activity. The unfavourable climate in spring 2013 decreased the enzyme activity not only in the upper, but also in the lower soil depth interval. Although both measured enzymes are extracellular but their climate-sensitivity seems to be rather different. Similar decreasing of invertase activity and PLFA values suggested the closer connection of invertase activity with the living microbial cells. However, the differences between the PLFA values in two sampling time were not so high than in case of invertase activity. This fact indicated a metabolically inactive microbial biomass (Šnajdr et al., 2008).

Locations of enzymes and PLFAs (living cells) in soil are different: our results suggest that living microbial cells are connected rather to the higher soil particles while enzymes, especially the catalase, are connected rather to the medium sized particles. The soil temperature and moisture, the available substrates are the most dominant factors having combined effects on the seasonal dynamic of microbial activity (Bing-Cheng and Dong-Xia, 2012).

Conclusions

Beside altered microbial activity, structural changes in microbial biomass were observed between the ecological and conventional managements but the differences were less in unfavourable climate conditions.

We confirmed that as low as 5 m differences in the topography can result in different physical, chemical and microbiological properties in different soil depth of the upper and lower parts of the slope. These differences due to the elevation positions are less in the ecological field, indicating stronger soil protecting management.

The relationships between living cells and enzyme activities were different at the four studied sites. Our results proved the strong correlation between invertase activity and microorganisms while catalase activity was found more stable and independent from living cell number. The bacterial and fungal microbe groups showed different

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sensitivity to the environmental factors (temperature, moisture, pH), and their seasonal variability was also observed.

Ecological management enhanced the microbial activity of acidic sandy soil compared to the conventional one. Positive effects of recycled crop residues were observed in ecological site, but more investigations are required on the relationships between the quality and quantity of recycled crop residues and the different chemical and microbial properties on the upper and lower part of slopes.

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[1]H NMR-BASED METABOLIC PROFILE OF *LACTOBACILLUS SALIVARIUS* FDB89 UNDER OSMOTIC STRESS

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Abstract. In their natural habitats and application environments, lactic acid bacteria are often exposed to osmotic changes. At metabolic level, bacteria are able to *de novo* synthesize or uptake specific solutes from environment to maintain constant turgor pressure. In the work, the [1]H nuclear magnetic resonance (NMR) spectroscopy based on intracellular metabolites profiles of *Lactobacillus salivarius* under hyper-osmotic growth conditions was characterized. New characteristic peaks of compatible compounds, such as betaine, carnitine, choline and/or their derivatives were detected. Especially, the cytoplasmic concentrations of proline increased dramatically when the osmolarity was raised from about 0.40 to 1.84 osmol/kg by adding 0.8 mol/l NaCl to the medium. Comparative analysis of the metabolites of *L. salivarius* FDB89 grown under aerobic and anaerobic conditions with NaCl stress revealed an overlap of up-regulated compounds of more than 50%. 2-aminobutyrate, pyruvate, glycine, trimethylamine-*N*-oxide, and acetylcholine were detected only under anaerobic condition, and 2-oxoglutarate, tyrosine, glutamine and glutamate were detected only under aerobic condition, which suggested oxygen also has a non-negligible effect on osmotic response in *L. salivarius* FDB89.
Keywords: *Lactobacillus salivarius*, metabolomics, hyper-osmotic stress, quantitative [1]H NMR spectroscopy, principal component analysis

Introduction

Lactobacillus is a genus of gram-positive facultative anaerobic or micro-aerophilic bacteria that produce lactic acid as the predominant end-product during the sugar fermentation. *Lactobacillus* is normally isolated from oral cavity, intestinal tract, vagina and fermented products, and generally recognized as safe (GRAS). As a major part of lactic acid bacteria (LAB) group, they especially have an important role in the development of the organoleptic, nutritional and/or hygienic quality of fermented foods, and are extensively exploited for the health-promoting properties (Machado et al., 2004; Shellhammer et al., 2017). *Lactobacillus salivarius* FDB89 used in this study, was isolated from the healthy centenarian's fecal samples from the volunteers inhabited in Bama longevity villages in China (the fifth longevity area in the world), and was

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confirmed to be able to convert the carcinogen 4-nitroquinoline-*N*-oxide to a less toxic compound and its geno-toxicity is significantly inhibited in previous study (Zhao et al., 2013), which highlights its potential probiotic use.

As we know, microorganisms are often exposed to hyper-osmotic environments generally produced by high concentrations of common salt or sugar used in food processing, which has been accepted as a desired and effective preservation method to suppress spoilage of microbes (Tsakalidou et al., 2011). However, high-osmolarity might initially result in plasmolysis, loss of turgor, shrinkage of the cell, and further elevate reactive oxygen species (ROS), cause cytoskeleton rearrangement, which increases DNA strand breaks and oxidation of proteins and DNA bases. It is interesting that cells could accommodate by accumulating osmoprotectants or increasing the expression of relative shock proteins, and the failure to adapt usually results in cell apoptosis (Burg et al., 2007). Osmotic stress response of some microorganisms (e.g., *Escherichia coli*, *Salmonella typhimurium*, *Lactococcus lactis*, and *Lactobacillus plantarum*) have been studied intensively by genetic, proteinic, physiological, and biochemical methods (Glaasker et al., 1996; Hoffmann et al., 2013; Meadows and Wargo, 2015; Métris et al., 2016; Rep et al., 1999; Teo et al., 2009). But the osmotic response studies at metabolic level are limited. And it was found that different microorganisms may have different response mechanisms. Bacteria reduced the deleterious osmotic effects by uptake or synthesis of some compatible solutes including amino acids (glutamine, glutamate and proline) and their derivatives (peptides, glycine-betaines and *N*-acetylated amino acids), polyols (glycerol) and/or sugars (trehalose and sucrose), which are not inhibitory to most cellular processes, but rather stabilize the biological structure and function of macromolecules (Hoffmann et al., 2013; León et al., 2018).

Nuclear magnetic resonance (NMR), as an important non-targeted analytical techniques, enables to rapidly produce reliable, reproducible, and unbiased metabolite profiles of each sample, and has been widely applied in analyzing metabolic networks and distinguishing biopolymers (Pinto et al., 2014; Tomita et al., 2018).

In this work, we aimed at employing ^{1}H NMR technique combined with principal component analysis (PCA) approach to evaluate the metabolic response of *L. salivarius* to osmotic stress (under aerobic and anaerobic condition) and indicate potential biomarkers that may provide comprehensive information of osmo-adaptation on the microbial fermentative behavior.

Materils and methods

Bacterial strain and growth conditions

L. salivarius FDB89 (*L. salivarius* subsp. *salivarius*) was provided by culture collection of MOE Key Laboratory of Functional Dairy (China Agriculture University, China). Two kinds of culture mediums incluing control (MRS medium) and hyper-osmotic medium obtained by adding NaCl (final concentration 0.8 mol/l) to the basal MRS were used. The changes of physiochemical characteristics in aerobic and anaerobic condition for hyper-osmotic medium was compared with control (only aerobic condition). The strain was sub-cultured twice in deMan-Rogosa-Sharpe (MRS) medium for 12 h at 37°C prior to experimental use, 20 ml of cell suspensions in 100 ml flasks were incubated at 120 rpm under aerobic conditions as control. For typical anaerobic growth condition in hyper-osmotic medium, cells were cultivated in Hungate

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tube previously excluding oxygen with high purity nitrogen at 37°C without shaking. For each treatment, the assay was repeated at least three times.

Growth and pH assays

Samples were taken at two-hourly intervals for 24 h. The absorbance at 600 nm (OD_{600}) was determined for growth curve of *L. salivarius* FDB89 (Michael et al., 2010). And the pH was measured after calibration of the pH meter (Sartorius, Goettingen, Germany) with standardized pH buffer solutions 4.01 and 7.00 (Hamilton, Switzerland) prior to the analysis.

Preparation of intracellular extract

Stationary phase cells were carefully micro-filtrated to avoid lysis and washed for twice with physiological saline solution to remove any remaining nutrient solution. The sediment was quickly transferred to a clean Eppendorf tube and quenched immediately into liquid nitrogen. The whole process should be accomplished within 2 min (De Angelis and Gobbetti, 2011).

A biphasic system was performed to extract the intracellular metabolites with some modifications (Aliferis and Jabaji, 2010; Tomita et al., 2017). Briefly, 2.0 ml of cold methanol/chloroform (1:1, v/v) was added to the tube containing the cells and fully vortexed for 60 s, then 1.0 ml of Milli-Q water (0°C) was added, sonicated for 2 min and centrifuged at 8944 × *g* for 5 min at -20ºC. And 1.5 ml of the aqueous layer was carefully pipetted into a new tube and immediately frozen. The extraction was lyophilized and stored at -80ºC for analysis.

^{1}H NMR data acquisition and processing

Lyophilized extraction were dissolved in 650 µl of 0.1 mol/l phosphate buffer (pH 7.0), in deuterium oxide (D_2O, Norell Inc., USA), containing 0.5 mmol sodium 3-(trimethylsilyl) propionate-2,2,3,3-d_4 (TSP, Sigma-Aldrich, UK)(chemical shift and reference indicator). Additionally, sodium azide (0.2% w/v) was added to preserve the samples for longer storage. Insoluble material was removed by centrifugation at 8944 × *g* for 2 min at 4°C, 600 µl of the supernatant was transferred into a 5-mm diameter NMR tube for determination (Galafassi et al., 2013; Graham et al., 2010).

NMR spectra were acquired at 300 K using a DRX-600 NMR spectrometer (Bruker, Germany, proton frequency 600.13 MHz, 14.1 T) equipped with a 5-mm PATXI probe. One-dimensional solution ^{1}H NMR experiments were done with the following parameters (Galafassi et al., 2013; Li et al., 2018; Nieva-Echevarría et al., 2017): pulse program for water suppression; relaxation delay, 4 s; mixing time (for noesy), 1 s; acquisition time, 2.28 s; number of steady states transients (dummy scans) is 4; number of transients, 256; solvent suppression, pre-saturation with spoil gradient; spectral width is 12 ppm; time domain size of 32 k. Peak-width at half height of TSP was less than 1.50 ± 0.15 Hz (mean ± SD).

All NMR spectra were zero filled to 65, 536 points and applied with an exponential line broadening factor of 0.3 before Fourier transformation, manually phased, baseline corrected and calibrated by the TSP signal at 0 ppm using TOPSPIN 2.1 (Bruker Topspin GMBH, Rheinstetten, Germany). Selected peaks were assigned and quantified using the 600 MHz library from Chenomx NMR suite 6.0 (Chenomx Inc., Edmonton, Canada).

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Principal component analysis

The spectral region between 0.20 and 10.00 ppm of each spectrum was divided into equally sized buckets with a width of 0.04 ppm. The regions of water (between 4.75 and 5.00 ppm), methanol (between 3.35 and 3.37 ppm) and chloroform (between 7.67 and 7.70 ppm) were excluded. Scaled to the total spectrum area of the residual regions, each bucket area was integrated and each spectrum was transferred into a 1 × 238 vector. Principal component analysis (PCA) was performed on processed ^{1}H NMR data with AMIX 3.7.10 (Bruker BioSpin, Rheinstetten, Germany).

Results

Growth of L. salivarius FDB89 under different conditions

The growth rate and final pH were similar for *L. salivarius* FDB89 at NaCl stress with or without oxygen (*Figs. 1* and *2*). The absence of NaCl stress in the growth media resulted in about 1.4-fold increased maximum absorbance of *L. salivarius* FDB89. The result was in agreement with previous study (data not shown) and supported by other reports (Arsenijevic et al., 2013; Varelas et al., 2017). Based on the results, the cell culture in stationary phase was sampled at 12 h for NMR measurement.

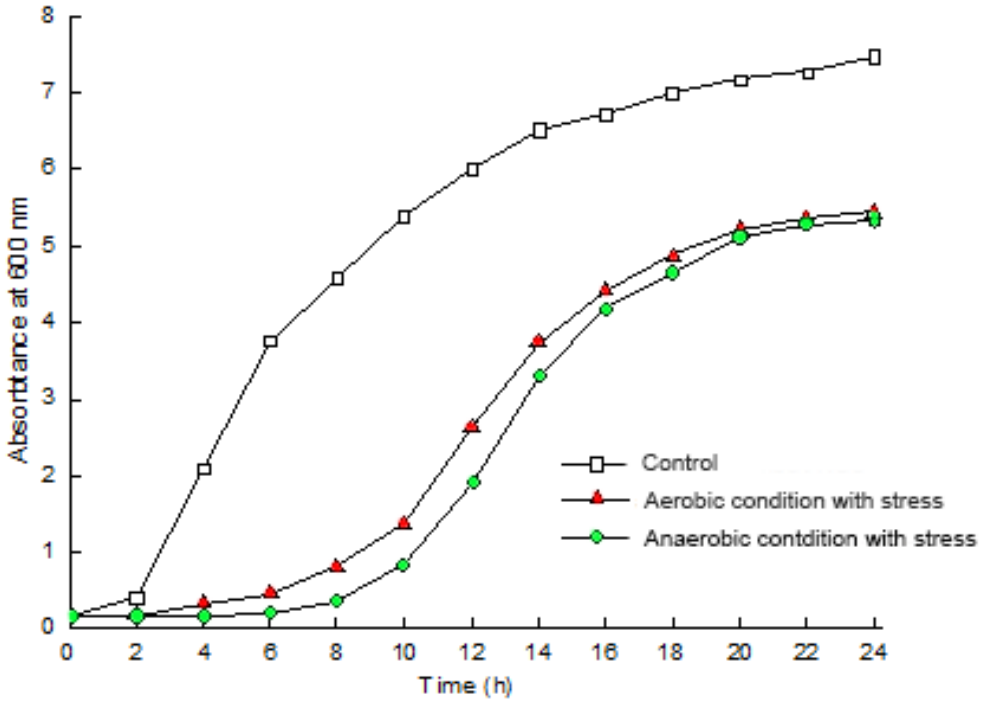


Figure 1. *Growth curves of L. salivarius FDB89 cultivated under different growth conditions*

Visual inspection of ^{1}H NMR spectra and assignment of compounds

Representative ^{1}H NMR spectra are presented in *Fig. 3*, which shows the metabolic profiles of *L. salivarius* FDB89 stationary phase cultivated under normal and NaCl (aerobic and anaerobic) stress conditions. About 44 metabolites were presumptively identified by Chenomx software (*Table 1* in Supplemental Data).

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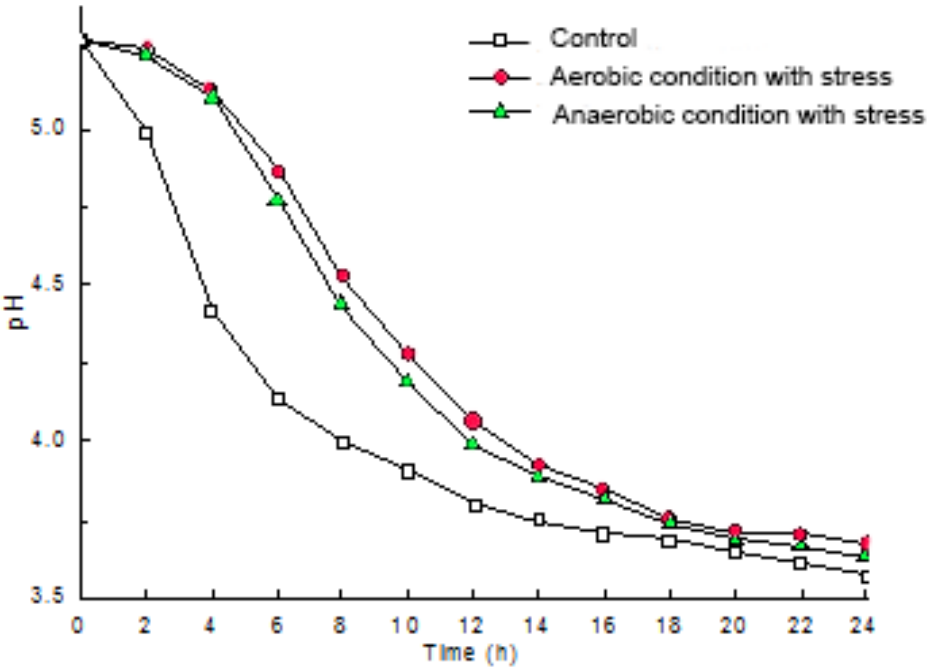


Figure 2. *Changes in pH when L. salivarius FDB89 grown under different culture conditions*

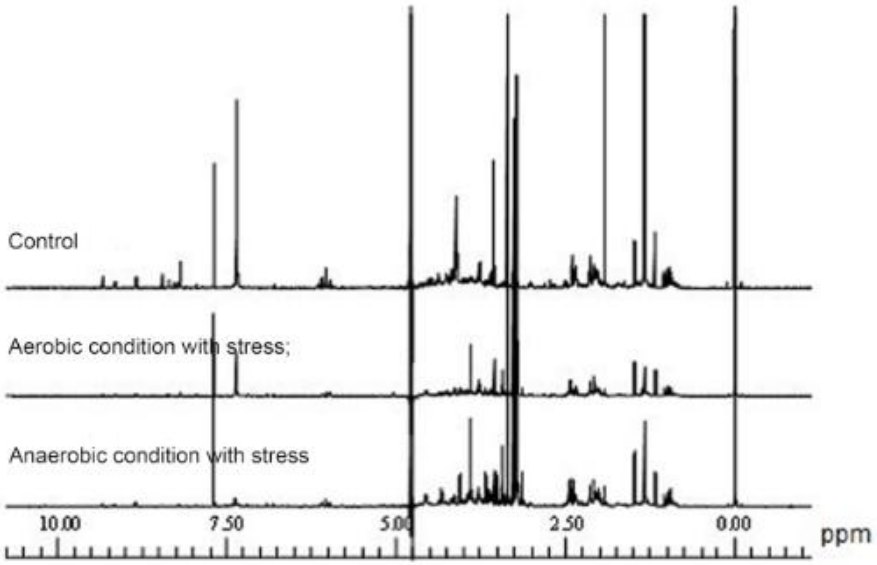


Figure 3. *Representative 600 MHz [1]H NMR spectra of intracellular extracts of L. salivarius FDB89 under different growth conditions*

It was difficult to discern the individual peaks with certainty in the [1]H NMR spectra between approximately δ 1.9-2.5 and δ 3.0-4.5 ppm due to overlapping amino acid and sugar resonances. However, conspicuous differences were observed among the three spectra patterns and peak intensities. In the osmotic spectrum, new peaks of betaine, carnitine, choline and its derivatives (e.g., sn-glycero-3-phosphocholine, phosphocholine, and acetylcholine), and isoleucine, phenylalanine, pyrimidine and

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cyclopentanecarboxylic acid were also observed. Higher peaks of methionine, proline, malonate, and aspartate have been discerned compared to control one. In the spectrum without NaCl stress, the characteristic peaks of isocitrate, arginine, kynurenine, propionate, UDP-glucose and succinate were identified, and the peak intensity of 2-hydroxybutyrate, alanine, glycine, valine, glutamine, acetate, lactate, pyroglutamate, threonine and NAD^+ increased.

2-Aminobutyrate, pyruvate, acetylcholine and trimethylamine oxidexide were only observed in hyper-osmotic and anaerobic condition. In contrast, tyrosine, glutamine and glutamate were the particular compounds in aerobic condition regardless of NaCl stress. The peak intensity of methanol at δ 3.30 in anaerobic condition is 1.5-fold greater than those of aerobic ones. The results showed different metabolic pathways of *L. salivarius* FDB89 under anaerobic and aerobic conditions. Being a major part in one carbon metabolism, more methanol was observed in anaerobic condition, it might be used as energy and carbon skeleton in this condition.

As a rapid and non-targeted method, NMR technique provides information about a very wide range of different compounds, in spite of its deficiency in the database system. In this work, many unknown peaks in NMR spectra could not be assigned.

Principal component analysis

When visual inspections of ^{1}H NMR spectra of different growth conditions are compared, only marginal variations at metabolite levels are obvious. Both the peak overlapping and low intensities between the signals also affect the full interpretation, and it is difficult to accurately assess statistical differences between datasets and comprehend the individual variability in each sample. So PCA was performed to reduce and analyze the acquired NMR data for discrimination. The corresponding points from one sample group occur close together on score plot, but far from other ones (*Fig. 4*). The values of PC1 and PC2 cumulatively accounted for 99.59% of the total variability (*Fig. 4A*), so the preparation of intracellular extraction in this study was reproducible and reliable.

Score plots well define the notable separations between PCA comparisons with the PC1 of 99.50% (*Fig. 4B*) and 90.68% (*Fig. 4C*), respectively, illustrating significant differences in the metabolic profiles of *L. salivarius* FDB89 cultured under different growth conditions. The loading plots indicate the metabolites that are contributed significantly to the individual variables in terms of magnitude and direction to PCs, and permit the potential biomarkers to be identified.

Fig. 4B shows oxygen also has an important influence on the stress response of *L. salivarius* FDB89. For aerobic condition, higher levels of glycylproline, oxoglutarate, glutamine, tyrosine and lysine were characterized. Whereas trimethylamine-*N*-oxide, pyruvate, 2-aminobutyrate and glycine were the major metabolites accumulated in anaerobic condition. With betaine, carnitine, choline, alanine, leucine, proline, phenylalanine, valine, actate, lactate, malonate, pyroglutamate, serine, and isopropanol were all identified in the both salt stress conditions. But their contents in anaerobic condition are much lower than those in aerobic one except acetate and pyroglutamate. Those metabolites were regarded as the representative metabolites or biomarkers, respectively, for aerobic and anaerobic condition with osmotic stress.

Fig. 4C shows higher levels of betaine, carnitine, choline and its derivatives, sn-glycero-3-phosphocholine, proline, glycylproline, isoleucine, phyenylalanine, malonate, isopropanol, cyclopentanecarboxylic acid and taurine, which were identified as

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important metabolites for *L. salivarius* FDB89 under osmotic stress. And arginine, propionate, lactate, acetate, proglutamate, and succinate were shown to be important contributors in control condition.

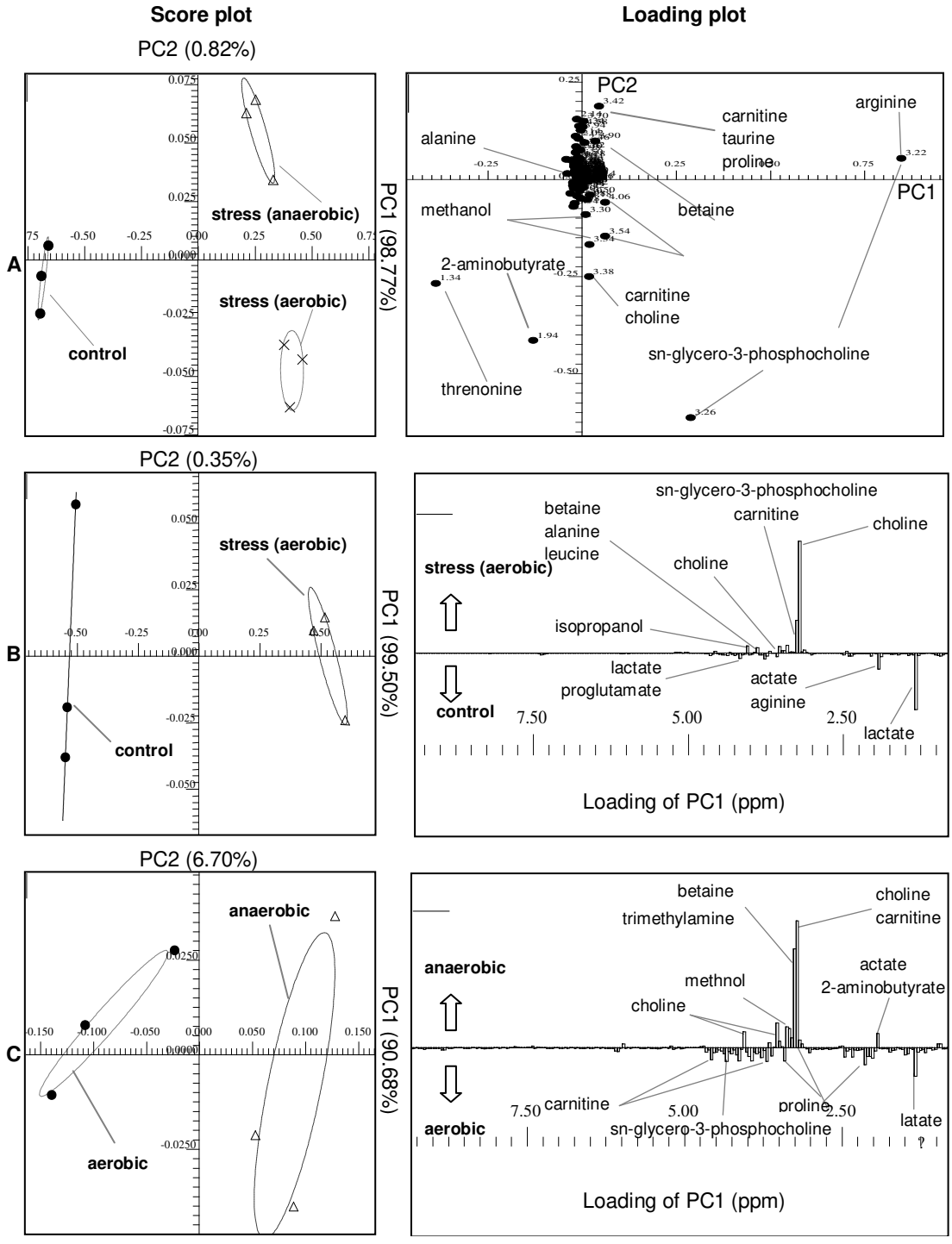


Figure 4. *PCA of intracellular extracts of L. salivarius FDB89 under different growth conditions: (A) PCA of control and aerobic and anaerobic conditions with stress; (B) control versus anaerobic condition with stress; (C) aerobic condition with stress versus anaerobic condition with stress*

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Biomarker identification by direct comparison of their peak intensities

The biomarkers identified by PCA could be identified directly by their increased metabolite resonances among the three types of growth models. In this study, PCA of ^{1}H NMR spectra indicated that betaine, carnitine, choline and its derivatives (mainly including sn-glycero-3-phosphocholine, phosphocholine, and acetylcholine), proline, malonate, aspartate, cyclopentanecarboxylic acid, and to a much lesser extent isoleucine, and phenylalanine were potential biomarkers of *L. salivarius* FDB89 when exposed to osmotic stress.

Discussion

Cellular responses induced by stress are essential for the survival of cells under adverse conditions. These responses, resulting in cell adaptation to the stress, are accomplished by a variety of processes at the molecular or metabolic level. Initial adaptive responses to high-osmolarity is a large increase in the uptake rate and the amount of cytosolic potassium, which normalizes cell turgor, and followed by a slower accumulation of different intracellular organic osmolytes at metabolic level, mainly including polyhydric alcohols, free amino acids and their derivatives. They are able to additionally normalize intracellular ionic strength, and enhance their osmotic tolerance and survival. These responses have presumably occurred in cells chronically adapted to osmotic stress (Hoffmann et al., 2017). In this study, the intracellular metabolic profiles of *L. salivarius* FDB89 cells under NaCl stress has been investigated using ^{1}H NMR method, and the effect of oxygen on osmotic response was evaluated (*Fig. 4*). Our results indicated that *L. salivarius* FDB89 grown under osmotic stress, compared to control condition, consistently accumulated abundant betaine, carnitine, choline and its derivatives (e.g., sn-glycero-3-phosphocholine, acetylcholine and phosphocholine), regardless of the presence of oxygen, while none of them are observed in the cells without stress (*Table 1* in Supplemental Data). These compounds are presumed to play a dominating adaptive role in mediating osmotic adjustment and protecting macromolecular structures and function at high and variable concentrations in osmotic stressed cells, which was also in accordance with previous studies (Hoffmann et al., 2017; Meadows and Wargo, 2015; Sheehan et al., 2006; Li et al., 2013).

Proline, and to a lesser extent, isoleucine, phenylalanine and methionine as the most important amino acids increased in *L. salivarius* FDB89 when exposed to NaCl stress. However, it could not be considered that all these amino acids except proline are biomarkers due to an opposite result obtained using gas chromatography mass spectrometry (GC/MS) (data not shown). For example, aspartic acid was identified as an observably increased amino acid to defend against NaCl stress in GC/MS analysis. Moreover, cells grown in anaerobic condition with osmotic stress clearly face energy limitation, resulting in the differences of their osmotic response ways. In this work, more than 50% of the overlapping peaks down-regulated except methanol and pyroglutamate. 2-aminobutyrate, pyruvate, glycine, trimethylamine-*N*-oxide and acetylcholine were detected only under anaerobic high-osmolarity condition, which suggested oxygen also has a non-negligible effect on the selection of osmolytes for *L. salivarius* FDB89 under osmotic stress. In contrast to conventional methods, which most often focus on the analysis of few specific metabolites as markers, NMR technique combined with PCA approach enables to rapidly screen large quantities of metabolites and identify potential biomarkers easily using pattern recognition techniques in a single

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experiment (Li et al., 2013; Miller et al., 2007). But there are some unknown peaks with higher abundance and much lower abundance in the complex matrices have not distinguished, additional verification using other methods is often necessary to confirm that the biomarkers identified by NMR-based screening are reproducible and reliable (McKelvie et al., 2009).

Conclusion

In their natural habitats and application environments, microorganism is usually exposed to osmotic changes. At metabolic level, bacteria are able to de *novo* synthesize or uptake specific solutes from environment to maintain constant turgor pressure. In the present study, the ^{1}H NMR and GC/MS metabolomics of *L. salivarius* FDB89 subjected to hyper-osmotic culture conditions was analyzed. Results indicate that nitrogen compounds (mainly amino acids and amines) including proline, isoleucine, phenylalanine and methionine may serve as potential biomarkers. However, not all organisms accumulate the same organic osmolytes, and the selection of osmolytes depends on the duration of the osmotic stress and the availability of substrates and osmolytes in the surroundings. The analysis of the metabolic profiles exposed to iso-osmotic concentrations of ionic and/or non-ionic (e.g., sucrose, lactose, trehalose, and sorbitol) compounds is also needed in furtuher metabolomics studies.

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SUPPLEMENTAL DATA

Table 1. *Presumptive metabolites and their contents in intracellular extracts of L. salivarius FDB89 under different growth conditions by [1]NMR method*

Metabolite	External concentration (mmol/l)	Intracellular concentration (millimoles per gram of dry weight)		
		Control	Aerobic growth (0.8 M NaCl)	Anaerobic growth (0.8 M NaCl)
2-hydroxybutyrate	0.16	1.42	0.82	0.84
2-aminobutyrate	nd[a]	nd	nd	1.36
Pyruvate	0.14	nd	nd	1.55
2-oxoglutarate	nd	nd	0.92	nd
Isocitrate	nd	1.10	nd	nd
Betaine	0.13	nd	8.66	6.91
Carnitine	nd	nd	4.46	2.05
Acetylcarnitine	nd	0.17	0.11	0.12
Choline	0.07	nd	13.95	13.74
Phosphocholine	nd	nd	0.59	0.93
Acetylcholine	nd	nd	nd	1.21
Sn-glycero-3-phosphocholine	nd	0.00	3.05	1.09
Alanine	1.42	6.32	4.10	3.11
Isoleucine	0.51	nd	1.61	0.97
Leucine	1.03	2.28	2.22	1.86
Lysine	0.59	nd	0.76	nd
Methionine	0.10	nd	0.27	0.08
Proline	nd	1.13	5.82	3.40
Glycyl proline	nd	nd	1.32	nd
Glycine	1.81	3.12	nd	0.65
Phenylalanine	nd	nd	0.77	0.93
Tyrosine	0.14	0.75	0.80	nd
Arginine	0.99	0.32	nd	nd
Threonine	0.74	8.16	6.85	5.29
Aspartic acid	0.15[b]	1.51[b]	3.36[b]	3.60[b]
Valine	0.61	2.50	2.09	1.28
Glutamine	nd	2.50	0.79	nd
Trimethylamine-*N*-oxide	nd	nd	nd	3.21
Kynurenine	nd	0.45	nd	nd
Acetate	1.44	16.54	1.51	2.77
Lactate	0.23	4.12	2.35	1.63
Malonate	nd	nd	4.33	3.37
Propionate	nd	1.01	nd	nd
Glutamate	0.83	3.40	3.62	nd
Pyroglutamate	0.77	8.87	3.15	4.78
Methanol	1.32	77.33	93.69	233.26
Isopropanol	nd	1.64	2.29	1.70
NAD^+	nd	0.31	0.09	0.05
Pyrimidine	nd	nd	0.50	0.16
UDP-glucose	nd	0.45	nd	nd
d TTP	nd	nd	0.37	nd
Cyclopentanecarboxylic acid	nd	nd	3.04	1.85
Succinate	0.04	0.38	nd	nd
Taurine	nd	0.79	3.10	0.71

[a] nd, not detected.
[b] based on GC/MS analysis (data not shown).

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DEGRADATION KINETICS AND ORGANIC MATTER REMOVAL CHARACTERISTICS OF ANAEROBIC TREATMENT OF PICKLE CLEANING WASTEWATER

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Abstract. The method of asynchronous cultivation was used to cultivate surplus sludge in sewage treatment plant under anaerobic environment. The best anaerobic treatment scheme was determined by the single factor and orthogonal experiments, and the degradation kinetics of organic matter in pickle washing wastewater was studied. The characteristics of organic matter removal were studied by Excitation-Emission-Matrix Spectra (3D-EEM) and parallel factor analysis (PARAFAC). The experimental results show that when the influent salinity is 11.98 g/L, chemical oxygen demand (COD) is 3500 mg/L and pH is 7, the removal rate of COD can reach more than 80% after anaerobic static 24 h. The order of importance of each factor is: substrate concentration > salinity > pH > residence time. The kinetics of anaerobic degradation of pickle washing wastewater shows that when the salt content in water samples is 0-30.22 g/L, the degradation reaction type of inhibitor salt is anti-competitive inhibition. When salt content was 36 g/L, the inhibition type is a mixed type. 3D-EEM shows that after anaerobic treatment, tryptophan substances in water decreased, humus increased, anaerobic biodegradation transformed most of the macromolecular proteins into humus substances, and the anaerobic ammonification reaction has occurred, which made COD lower while NH_3-N increased.
Keywords: *orthogonal experiment, salinity, kinetic study, inhibition type, three-dimensional fluorescence spectroscopy (3D-EEM), parallel factor analysis (PARAFAC), tryptophan, humus*

Introduction

Kimchi wastewater is a kind of typical high salt wastewater, including salt water and cleaning wastewater. It has the characteristics of weak acid and high organic content (Sichuan, 2012). It is difficult to treat, and the quality of water varies greatly with season and the type of pickled vegetables (Li et al., 2012). Kimchi wastewater has great harm. Its rich organic matter, ammonia nitrogen, and phosphorus nutrients can lead to eutrophication of the water body. High salt content will also cause serious salinization of the soil (Sichuan, 2010; Chang et al., 2014). At the same time, because of its weak acidity and high organic content, it can cause odor and breed mosquitoes and flies, Therefore, in order to reduce its impact on the water environment, the pickle wastewater must be concentrated and timely treated (Yang et al., 2017). Therefore, reducing the content of organic pollutants in kimchi wastewater is not only the necessary work to respond to the environmental protection policy but also the necessary work to reduce or even eliminate the threat to the environment and ecological balance (Zhao et al., 2011).

The treatment methods of high salt wastewater mainly include physicochemical method (Wu, 2017; Zhen et al., 2017; Von, 2016), biological method (Li et al., 2013; Du et al., 2014) and biological physicochemical combined technology. The biological

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method has always been a common and efficient treatment method (Sima, 2013) in wastewater treatment. At present, from the domestic and foreign research status of pickle wastewater, there are many studies on pickle salinized wastewater, and most of them are concentrated on the optimization of the bioreactor and the culture of salt - eating bacteria. For example, Li et al. (2013) have studied the treatment effect of ASBBR process to the wastewater of pickled vegetable. The results show that the effluent COD can be reduced to 375 mg/L when the system runs to 79 d, the removal rate is 92.86%. Du et al. (2014) studied the effect of MBR technology on the treatment of salty pickled mustard wastewater. The results showed that the ammonia nitrogen, SS, COD, and phosphate were greatly removed under the intermittent aeration condition. Ali et al. (2011) studied the effect of COD/N ratio and salinity on the performance of the batch reactor. The experiment showed that high salinity inhibited nitrification, and high COD/N ratio was beneficial to nitrogen removal (> 90%).

However, little research has been done on pickle cleaning wastewater, and little research has been done on organic composition in pickle wastewater. Compared with saline wastewater, the washing wastewater also has high salinity, and the amount of wastewater is larger, and the water quality fluctuates more. Therefore, this paper takes the pickle cleaning wastewater as the research object, uses the method of domestication of sludge to reduce the inhibitory effect of high salinity on the microorganism, and establishes a stable and efficient anaerobic biological treatment system (Yu, 2009). While seeking the best anaerobic treatment conditions and exploring the inhibition mechanism, 3D-EEM and PARAFAC analysis methods are used to further understand the bubble. The feasibility of anaerobic degradation of organic compounds in vegetable cleaning wastewater was verified and provided a theoretical basis for the practical treatment of pickle wastewater. This study not only improved the stability and degradation efficiency of the anaerobic treatment system of pickle wastewater by using the domesticated sludge, but also deeply explored the inhibition mechanism of the salinity on the anaerobic treatment, undertook the task of reducing most of the organic matter, and filled the blank of the organic matter analysis of the pickle wastewater by using 3D-EEM technology.

Experimental materials and methods

Experimental materials, reagents and instruments

Wastewater: the pickled vegetable wastewater is taken from pickle plant in Xin Fan Town and be treated by flocculation. The wastewater is light yellow, low turbidity and good transparency. The main index of wastewater is shown in *Table 1*.

Table 1. *Quality indexes of pickle wastewater*

Project	Content
COD/(mg/L)	2000~3500
pH	6.5~7.5
Turbidity	10~30
Salt content (calculate by NaCl)/(g/L)	10~15
SS/(mg/L)	50~100
NH_3-N/(mg/L)	30~80
TP/(mg/L)	10~15

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Anaerobic initial culture sludge: The surplus sludge comes from the sewage treatment plant of hi-tech west district. The concentration of mixed liquor suspended solids (MLSS) is 10.42 g/L, and the concentration of mixed liquor volatile suspended solids (MLVSS) is 6.42 g/L.

Experimental reagents: glucose (water), urea, potassium dihydrogen phosphate, two sodium hydroxide, sulfuric acid and silver sulfate, potassium dichromate, mercury sulfate, ammonium ferrous sulfate, potassium sodium tartrate, potassium iodide, mercuric iodide, sodium hydroxide, ammonium chloride, potassium sulfate, ammonium molybdate, potassium antimony tartrate acid, ascorbic acid, potassium dihydrogen phosphate, chloride sodium, silver nitrate, potassium chromate etc..

Experimental instruments: DZKW type electric thermostatic water bath, BS-124-S electronic balance, vacuum suction device, WXJ-III type microwave digestion device, 722s spectrophotometer, JSM280G-18 type portable pressure steam sterilizer.

Experimental device: The anaerobic reactor is a self-made reaction device. The anaerobic bottle adopts a 1.5-L filter bottle as an anaerobic reactor, and the gas is collected by drainage method. The experimental device diagram is shown in *Figure 1*.



Figure 1. *Solid diagram of anaerobic experimental device*

Method of experiment and analysis

Acclimatization of anaerobic activated sludge

Rinsing the excess sludge with water, and then settle for half an hour later and discard the upper layer of cloudy liquid for so many times. An appropriate amount of sludge was placed in a 1.5-L closed glass reactor, which accounted for about 1/3 of the total volume of the container. Then the culture medium with a nutrient ratio of CODcr:N:P = 200:5:1 (CODcr at about 4000 mg/L, composed of glucose, urea, and two

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potassium hydrogen phosphate) was added for continuous culture. Changing the water every two days, and stirring properly, and controlling the temperature at 37 °C. After a period of time, it was observed that the sludge became darker and darker, and the acidity of the effluent weakened. A large number of bubbles appeared on the surface, indicating that sludge could start domestication. A certain concentration of NaOH solution was used to adjust the influent pH to keep it between 6.8 and 7.5. The adaptability of sludge to wastewater water quality is improved by continuous cultivation and acclimatization with flocculating effluent and nutrient solution, and the proportion of flocculated effluent in the influent is gradually increased. COD was measured every 4 days in the domestication period, and COD was measured every 2 days once the acclimation became stable.

Anaerobic experimental process

After domestication for a month or so, the influent is completely replaced by flocculation effluent, and the anaerobic effluent is basically stable. The best experimental scheme was determined by studying the effects of different influent pH, salinity, substrate concentration, residence time and temperature on COD removal. By changing the salinity of influent, the Monod fitting equation of anaerobic biodegradation kinetics under different salinity conditions was investigated, and the kinetic equation of anti-competitive inhibition was deduced by the steady-state method, which has a further understanding of biodegradation process.

Analysis method of 3D-EEM

Preparation of water sample: In order to analyze the composition and change characteristics of dissolved organic matter in water samples. First, the original water, anaerobic stable effluent sample and the same batch of water samples were collected in 50 ml plastic sample tube; Then, the water system membrane with a diameter of 50 mm and 0.45 m pores was installed in the sand core filter device (Yang et al., 2014). The water samples taken from the previous step were poured into the filter device, the vacuum pump was opened and filtered, and the 50-ml filtrate was collected in the Tetrafluoroethylene bottle, and the water samples were frozen in the icebox at 4 °C to slow down the metamorphic (Zhu et al., 2014); Finally, the water samples after treatment are sent to the fluorescence spectrophotometer for testing.

The measurement method of water sample: Using deionized water as the blank water sample, the three-dimensional fluorescence characteristics of water samples were scanned by Hitachi F-7000 fluorescence spectrophotometer (Produced by Hitachi high-tech company), its parameters were shown in *Table 2*.

Table 2. *Parameter meter of the fluorescence spectrophotometer*

Project	Parameter setting
Excitation wavelength range/nm	200-600
Emission wavelength range/nm	200-600
Excitation bandwidth/nm	5
Emission bandwidth/nm	5
Sampling interval/nm	5
Scanning speed/nm/min	30000
PTM Voltage/V	400

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Analysis method of 3D-EEM: The FL Solutions software is used to extract the fluorescence intensity data matrix within the wavelength range, and the matrix is introduced into the drawing software Origin 8.5 to get the three-dimensional contour map of the water sample, at the same time the data is introduced into the Matlab R2014b data processing software platform which is pre-installed with the N-way320 and parafacmodeling VER2.0 Toolbox toolkit. After deducting the Raman peak value of the blank water sample, the parallel factor analysis method is used to analyze the parallel factor. Finally, the three-dimensional fluorescence spectrum and the number of parallel factors, the fluorescence peak position and the fluorescence intensity of each parallel component are obtained, and the weakening amplitude is calculated.

Experimental results and discussion

The degradation of COD during domestication

As can be seen from *Figure 2,* 0-30 d is a period of domestication. During this period, with the increasing of raw water in the entering water, the concentration of chlorine ion is also increasing, and the removal rate of COD is decreasing. The removal rate of COD is reduced to 27.9% when the entering water is all the raw water; 32-50 d is a continuous domestication period, and the water entry conditions are consistent. With the prolongation of the culture period, the removal rate of COD increased gradually and reached more than 60% in the 44 d. At the end of continuous domestication, the removal rate of COD reached more than 65%; 50-68 d is the stable period of effluent. It can be seen that under the influent water quality, the water quality of the effluent can reach COD below 700 mg/L, and the removal rate of COD is above 70%. Thus, it can be seen that anaerobic sludge acclimation is completed.

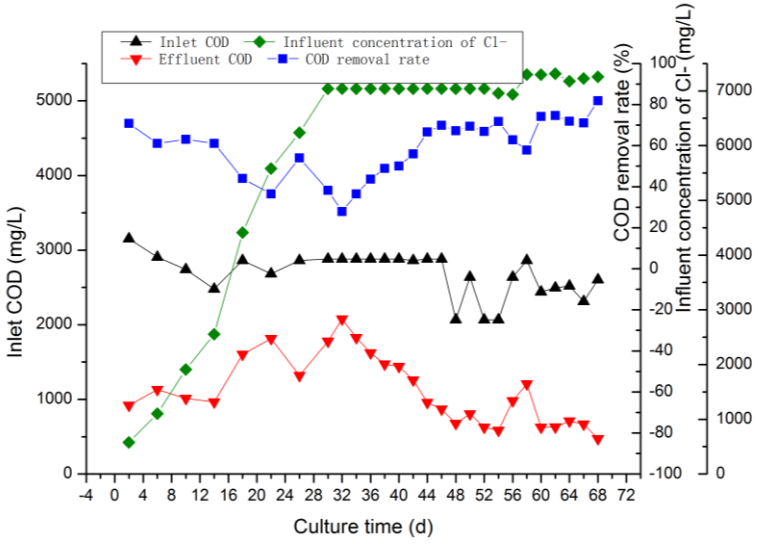


Figure 2. *Changes in each index during acclimatization*

The effect of pH on the removal of COD

The reaction temperature was 38 °C, the coagulation effluent was added to the anaerobic device to 1.5 L, and the water was changed every 24 h. The COD degradation

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of the residence time HRT = 24h under different pH influent conditions was investigated, such as *Figure 3*. The pH range is 5-9, shaking the reactor manually several times a day, and ensuring that every pH influent condition stays 4 d in the anaerobic plant.

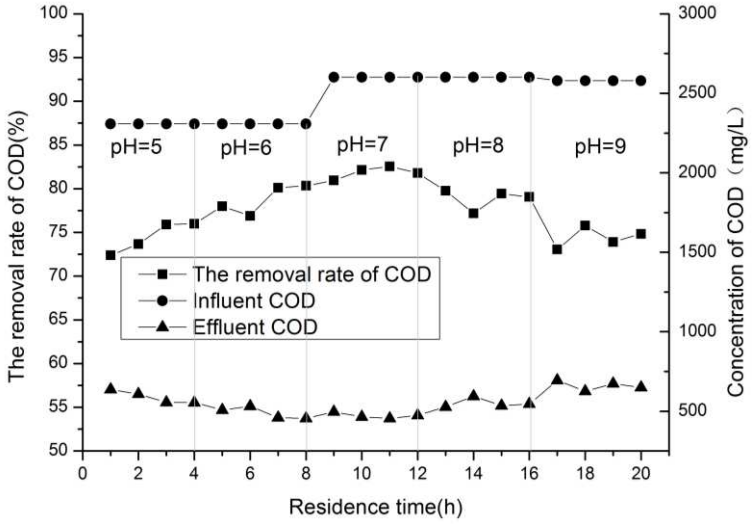


Figure 3. *pH effect on COD removal*

As can be seen from *Figure 3,* in the 5-7 range, with the increase of pH value, the COD removal rate gradually increased, while the pH value continued to rise, the removal rate began to decline. The best effect was achieved at pH = 7, the COD removal rate reached 81.8%, and the COD effluent concentration was 474 mg/L. The degradation effect of COD in the acid environment is better than that of COD in the alkaline environment. This is due to the hydrolysis acidification reaction under acidic conditions, which makes a large number of macromolecule carbon chains in the wastewater break into small molecular carbon chains and decompose organic matter into small molecular substances such as CO_2, CH_4 and H_2O under anaerobic digestion, causing the whole environment to be under the weak acid condition (Hu and Yan, 2009; Liu, 2011), so the best pH is 6-7.

Effect of substrate concentration on the removal of COD

The reaction temperature was 38 °C, the coagulation effluent was added to the anaerobic device to 1.5 L, and the water was changed every 24 h. The removal of COD under different COD concentration of influent was investigated, as shown in *Figure 4*. COD changes in the range of 1000-5500 mg/L, gently swaying several times a day and ensuring a stable operation of each concentration for 4 days.

As can be seen from *Figure 4,* when HRT = 24 h, in the range of 1000-3500 mg/L COD increased with the concentration of substrate removal rate increased gradually, but when the concentration continues to rise, the removal rate began to decline, so the best effect in COD is 3500 mg/L, the removal rate of COD reached 85.6%, the effluent COD concentration is 466 mg/L. The low substrate concentration will cause the low organic load of activated sludge and increase the operating cost, while too high substrate concentration will cause the excessive organic load to cause the excessive acid

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production of sludge to make the reactor acidosis and affect the effect of effluent. Therefore, it is necessary to control the COD concentration at the appropriate level (Liu, 2011).

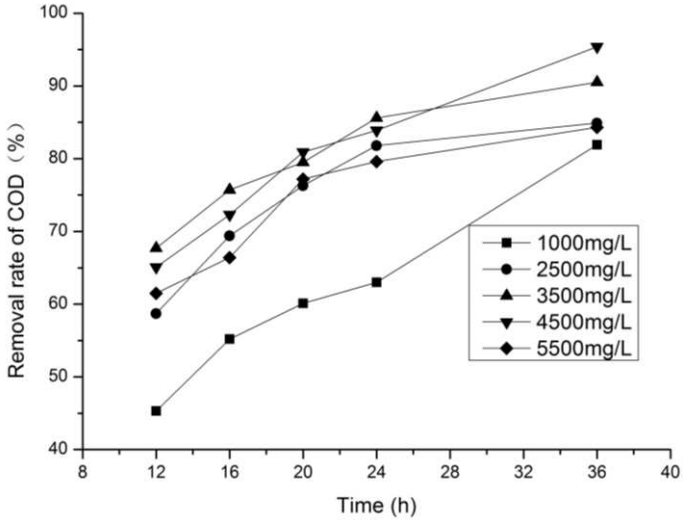


Figure 4. *The effect of substrate concentration on the removal of COD*

Influence of residence time on the removal efficiency of COD

The reaction temperature was 38 °C, the coagulation effluent was added to the anaerobic device to 1.5 L, and the water was changed every 24 h. The influent COD concentration is 2602 mg/L, pH = 7 and salinity is 11.98 g/L, The removal of COD under different residence time was investigated, as shown in *Figure 5.*

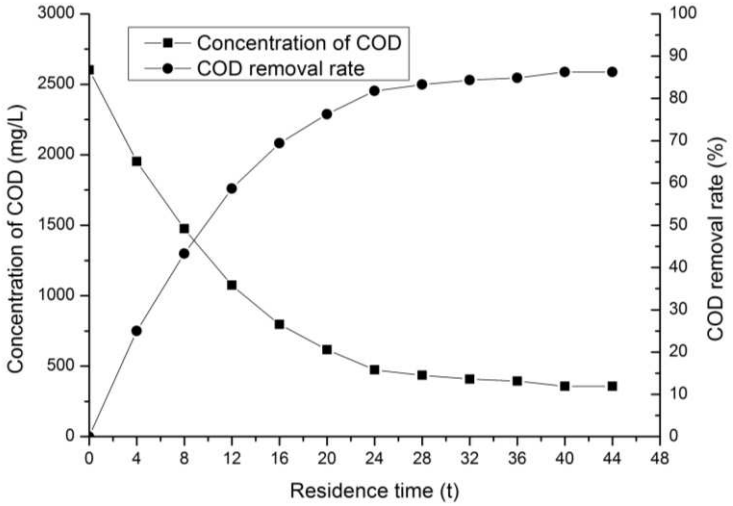


Figure 5. *The effect of residence time on the removal of COD*

As can be seen from *Figure 5,* with the prolongation of residence time, the effluent concentration of COD decreases gradually, and the COD removal rate increases gradually, and tends to balance at HRT = 24h. Therefore, the best residence time is

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24 h, the COD removal rate reaches 81.8%, and COD effluent concentration is 474 mg/L.

Study on the effect of salinity on the anaerobic degradation of pickle cleaning wastewater and its kinetics

The reaction temperature was 38 °C, the coagulation effluent was added to the anaerobic device to 1.5 L, and the water was changed every 24 h. The removal of COD under the different salt concentration of influent was investigated, as shown in *Figure 6.* Salt changes in the range of 0-36 g/L, gently swaying several times a day and ensuring a stable operation of each concentration for 4 days.

From *Figure 6* we can see that the removal rate of pickle cleaning wastewater increases with the increase of HRT, and it can be seen that at the same HRT, the removal rate of COD decreases with the increase of salt concentration. When the salinity was maintained at 11.98 g/L, the removal rate is 81.8%; when the salinity was reduced to 0 g/L and 6.5 g/L, the removal rate of COD increased to 90.3%, 82.6%; when the salinity was increased to 17.93 g/L, 24.16 g/L, 30.22 g/L, 36 g/L, the removal rate decreased to 79.3%, 68.9%, 67.4%, 64.1% respectively. It can be seen that the acclimated anaerobic sludge can achieve good results in the treatment of pickles cleaning wastewater in the 0-24 g/L salinity range.

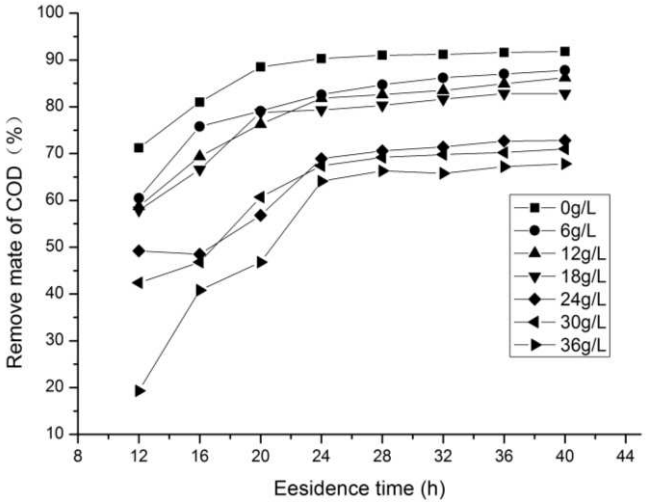


Figure 6. *Effect of different salinity at different residence time on COD removal*

As can be seen from *Figure 6,* different amount of salt in the anaerobic digestion process has an inhibition on microbial metabolism at different levels, the relationship between the specific growth rate and the matrix concentration is no longer consistent with the Monod relationship. Therefore, it is necessary to modify the kinetic formula of Monod specific growth rate according to the characteristics of wastewater and inhibitor.

The anaerobic biological removal rate of the usual matrix is described by the Monod equation (*Eq. 1;* Liu, 2011):

$$V = \frac{V \max S}{Ks + S} \quad \text{(Eq.1)}$$

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The Monod fitting equation for the kinetics of anaerobic biodegradation of organic matter under the conditions of salinity is shown in *Figure 7a-g*. The Monod fitting equation for the kinetics of anaerobic biodegradation of organic compounds under different salinity conditions in *Figure 7* is shown in *Table 3*.

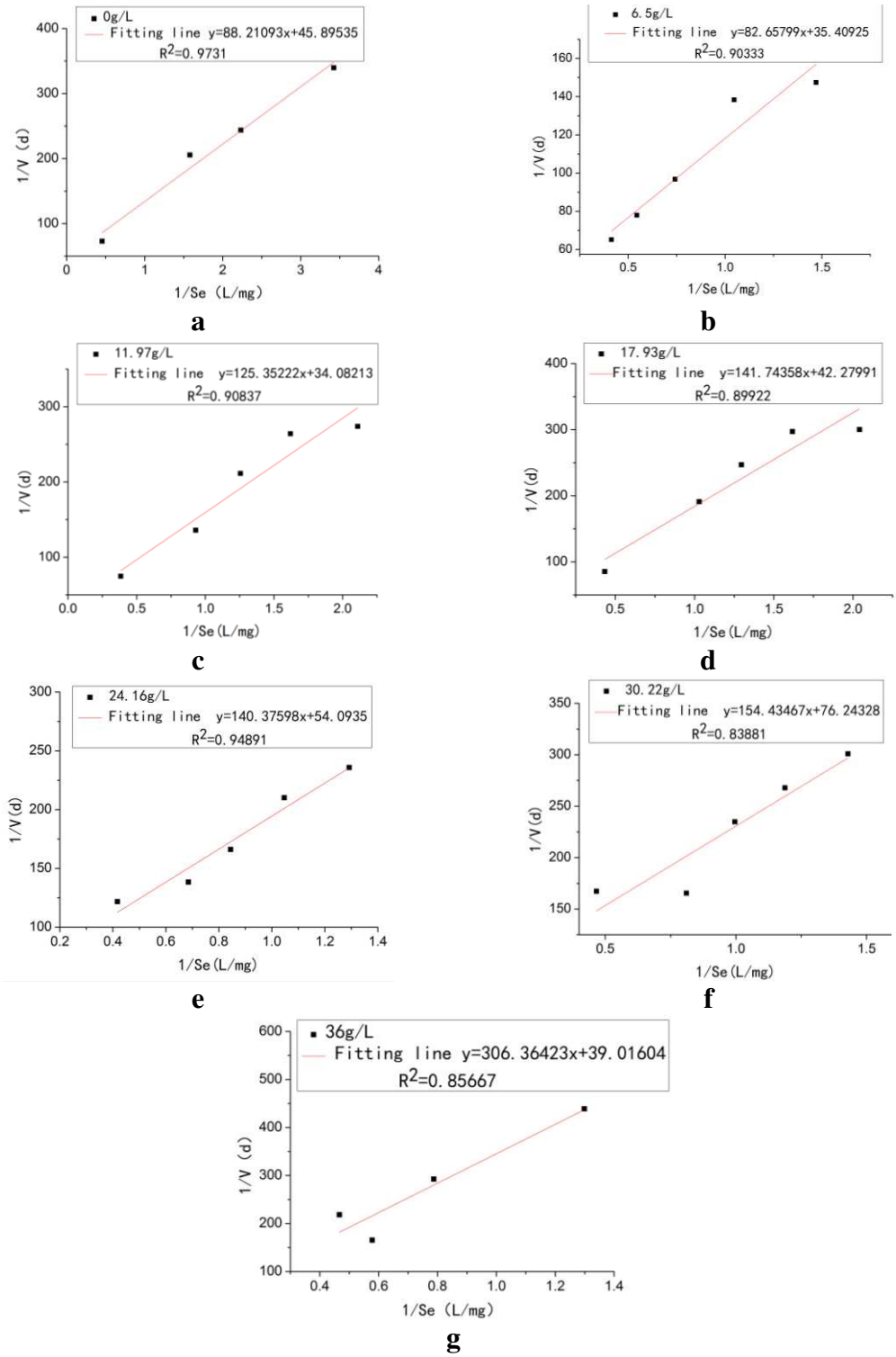


Figure 7. a. *0 g/L fitting line;* ***b.*** *6.5 g/L fitting line;* ***c.*** *11.97 g/L fitting line;* ***d.****17.93 g/L fitting line;* ***e.*** *24.16 g/L fitting line;* ***f.*** *30.22 g/L fitting line;* ***g.*** *36 g/L fitting line*

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Table 3. *Experimental results of anaerobic biodegradation reaction of acclimated sludge from 11.98 g/L wastewater*

Serial number	Salinity (g/L)	Fitting equation	R^2	MLSS (mg/L)	K or V_{max} (d^{-1})	K^*	K_S (mg/L)	K_S^*
1	0	y=88.21093x+45.89535	0.9731	9510	0.0218	0.743	1.923	0.52
2	6.5	y=82.65799x+35.40925	0.90333	9510	0.0282	0.96	2.33	0.63
3	11.98	y=125.35222x+34.08213	0.90837	9510	0.02934		3.68	
4	17.93	y=141.74358x+42.27991	0.89922	9510	0.0237	0.81	3.359	0.91
5	24.16	y=140.37598x+54.0935	0.94891	9510	0.01849	0.63	2.596	0.705
6	30.22	y=154.43467x+76.24328	0.83881	9510	0.01312	0.447	2.026	0.55
7	36	y=306.36423x+39.01604	0.85667	9510	0.0256	0.087	7.85	2.13

Comparing the value of K, K_0, Ks, and K_{S0}, according to the following formula A-D in *Table 4* can infer the type of inhibition.

Table 4. *Inhibition type table*

Formula number	Inhibition type	Inhibition condition
A	Competitive	$K = K_0, K_s \rangle K_{S0}$ and $K^* = 1.0, K_s^* \rangle 1.0$
B	Non-competitive	$K \langle K_0, K_s = K_{S0}$ and $K^* \langle 1.0, K_s^* = 1.0$
C	Anti-competitive	$K \langle K_0, K_s \langle K_{S0}$ and $K^* \langle 1.0, K_s^* \langle 1.0$
D	Mixed	$K \langle K_0, K_s \rangle K_{S0}$ and $K^* \langle 1.0, K_s^* \rangle 1.0$

Note: V, V_{max} – to the removal rate and the maximum ratio of removal (d^{-1}); S_0, S, S_e – influent, reactor and water matrix concentration (mg/L); X – microbial concentration (mg/L); t – eaction time (h); K^* and K_s^* – inhibition constants; K – The maximum ratio of substrate removal in the absence of inhibitor (d^{-1}); K_{S0} – The saturation constant in the absence of a suppressing substance; K_I – The dissociation equilibrium constant of an inhibitor and an enzyme (microorganism) complex, also known as the inhibitor constant

According to the inhibition types determined by the above methods, the inhibition mechanism can be analyzed. Further, the inhibition kinetic constant K_I of substrate anaerobic biodegradation can be obtained by dynamic steady-state treatment.

It can be seen from *Table 3* and *Figure 7a-g* that the substrate V_{max} of the organic compounds in the anaerobic degradation wastewater is lower than the acclimated salt concentration when the content of salt is below 11.98 g/L and higher than 11.98 g/L. This shows that the change of salt content in the wastewater has a certain inhibitory effect on the anaerobic microorganism which has been adapted to the high salt concentration. At the same time, it can be seen from *Table 4* that when the concentration of salt is 0-30.22 g/L, $K^* < 1$ and $Ks^* < 1$, it is known that the type of degradation reaction is anti- competitive inhibition, that is, the sodium chloride in wastewater cannot combine with the enzyme directly, NaCl can only combine with

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enzyme-substrate complex to form an intermediate complex, enzyme-substrate-inhibitor.

$1/V_{max}$ is used to make a straight line of salt concentration I, its longitudinal intercept is $1/V_{max}$, the slope is $1/V_{max}K_I$, the fitting line equation is y = 1.10392x + 24.75291, and KI = 22423 mg/L can be calculated. The greater the K_I value, the less inhibition of anaerobic degradation of organic matter. Anti-competitive inhibition kinetic fitting curve sees *Figure 8*.

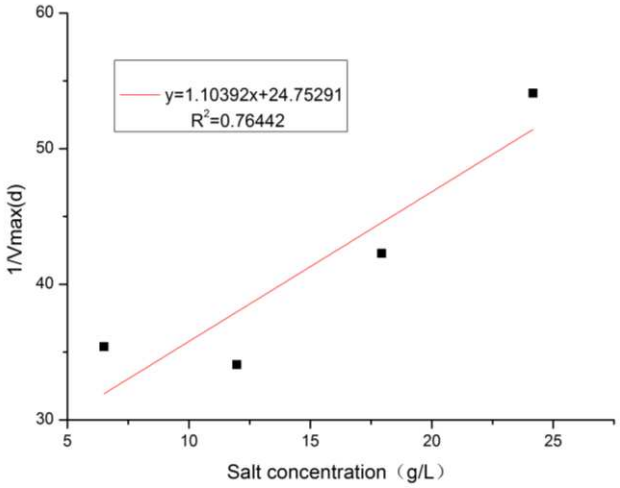


Figure 8. *Anti-competitive inhibition kinetic curve fitting curve*

Therefore, when the salt content in the water sample is ≦ 30.22 g/L, the anti-competitive inhibition kinetic equation of the salt concentration to the anaerobic COD degradation reaction is as follows (*Eq. 2*):

$$V = \frac{V_{max}S/\left(1+\frac{i}{K_I}\right)}{K_s/\left(1+\frac{i}{K_I}\right)+S} = \frac{V_{max}S}{K_s+S/\left(1+\frac{i}{K_I}\right)} = \frac{0.02934S}{3.68\left[1+\frac{i}{22423}\right]} \quad \text{(Eq.2)}$$

When the salt content is 36 g/L, K^* < 1 and Ks^* > 1, the type of NaCl's inhibition of wastewater is mixed type, the reaction process is relatively complex.

Optimization of anaerobic activated sludge for COD degradation

Formulation of the orthogonal experiment

Through the above single factor experiments, 4 factors were determined in this experiment, the concentration of A: the substrate, B: salinity, C: pH, and D: residence time. Each factor was selected in three levels. The level table of the experimental factors was shown in the lower *Table 5,* and the orthogonal experiment was carried out by the L9 (3^4) orthogonal table.

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The result and analysis of the orthogonal experiment

The results of the orthogonal experiment are shown in *Table 6*. From the result of *Table 6,* the order of importance of each factor is B > C > A > D. The main influence factor in the experimental range is B substrate concentration, followed by C salinity, and then CpH, factor D residence time is the smallest. It is determined that the best scheme of anaerobic biodegradation to the pickle cleaning wastewater is A2B2C1D1, that is, when COD concentration is 2500 mg/L, the salt content is 6 g/L, pH is 7, residence time is 20 h, the effluent is the best.

Table 5. *Experimental factor level table for anaerobic treatment*

Level	**Factor**			
K	**pH**	**Substrate concentration (mg/L)**	**Salinity (g/L)**	**Residence time (h)**
1	6	1500	6	20
2	7	2500	12	24
3	8	3500	18	28

Table 6. *Orthogonal experimental results of anaerobic treatment*

Serial number	**A**	**B**	**C**	**D**	**COD removal rate /%**
1	1	1	1	1	73
2	1	2	2	2	70.15
3	1	3	3	3	66.84
4	2	1	2	3	56
5	2	2	3	1	78.48
6	2	3	1	2	78.57
7	3	1	3	2	49.87
8	3	2	1	3	77.92
9	3	3	2	1	62.19
K1j/3	70	57.34	76.5	71.22	
K2j/3	71.02	75.52	62.78	66.2	
K3j/3	63.33	69.2	65.06	66.92	A2B2C1D1
Rj	6.67	18.18	13.72	4.3	
Optimal scheme	A2	B2	C1	D1	
The importance of each factor	B>C>A>D				

Note: Kij – the index value of the test results at the level I of the j factor; the difference in the Rj – the j factor

Removal of fluorescent organic matter

Measurement results of 3D-EEM

The three-dimensional fluorescence spectrum and contour map of water intake and anaerobic effluent were shown in *Figures 9* and *10*. Before and after treatment, COD decreased from 2068 mg/L to 584 mg/L, and the removal rate was 71.8%. NH_3-N increased from 52.8 mg/L to 135.3 mg/L and increased by 156.3%. The spectrogram of the contour clearly expresses the excitation of the peak intensity of the spectral peak

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intensity of the influent and the effluent, and the position and relative intensity of the emission wavelength. You can see from *Figure 9,* the water has a strong peak excitation/emission band at 270 ~ 295 nm/340 ~ 360 nm; can be seen from *Figure 10,* the anaerobic effluent has 4 fluorescence peak excitation / emission bands are located at 270 ~ 295 nm/340 ~ 360 nm, 245 ~ 285 nm/420 ~ 450 nm, 295 ~ 310 nm/420 ~ 450 nm. 345 ~ 370 nm/420 ~ 450 nm. Because of the large changes in the front and back-fluorescence peaks, it can be found that the species of soluble organic matter in water has changed greatly before and after the anaerobic treatment. At the same time, it can be seen from the contour map that the spectral map overlaps to a certain extent.

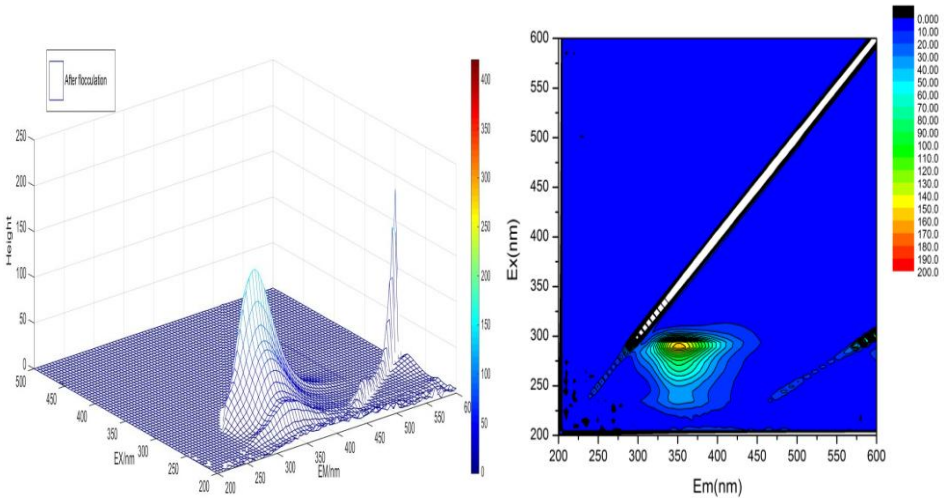


Figure 9. *Influent fluorescence spectrum and contour map*

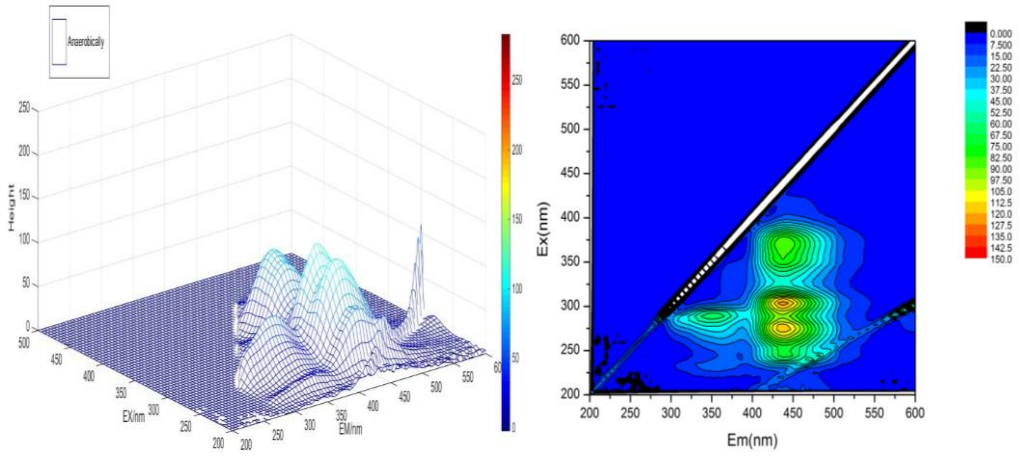


Figure 10. *Fluorescence spectra and contour maps of anaerobic effluent*

Results of parallel factor analysis

For the same batch of 5 samples, the original data matrix (5 * 82 * 82) was measured by PARAFAC parallel factor analysis. 3 parallel factors in the water sample were obtained by the parallel factor analysis model, and the core consistency map is shown in *Figure 11*. Parallel factor decision rule: when the kernel consistency is greater than or

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equal to 80% or in the vicinity, show that this factor is reliable and that 50-80% is not stable, then, < 50% for the problem. The choice of factor number is to select the maximum number of factors before the kernel conformance is rapidly falling (Ma, 2014).

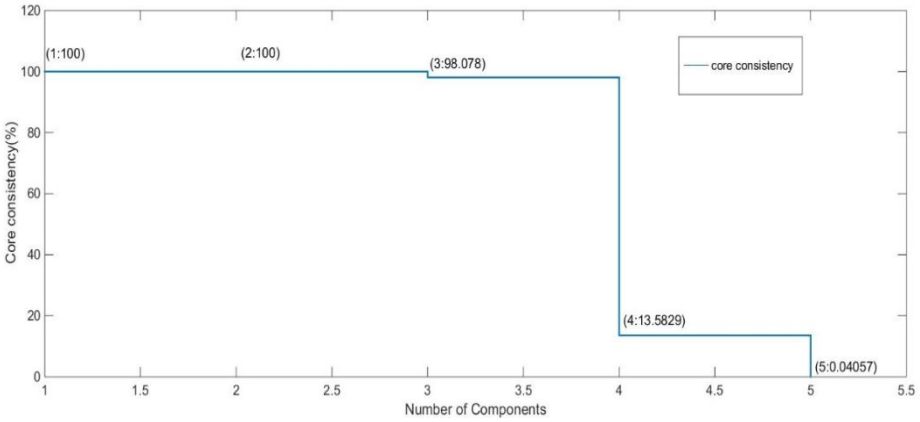


Figure 11. *Core consistency diagram*

The 3-factor excitation and emission spectral profiles obtained by using the parallel factor model are shown in *Figure 12*.

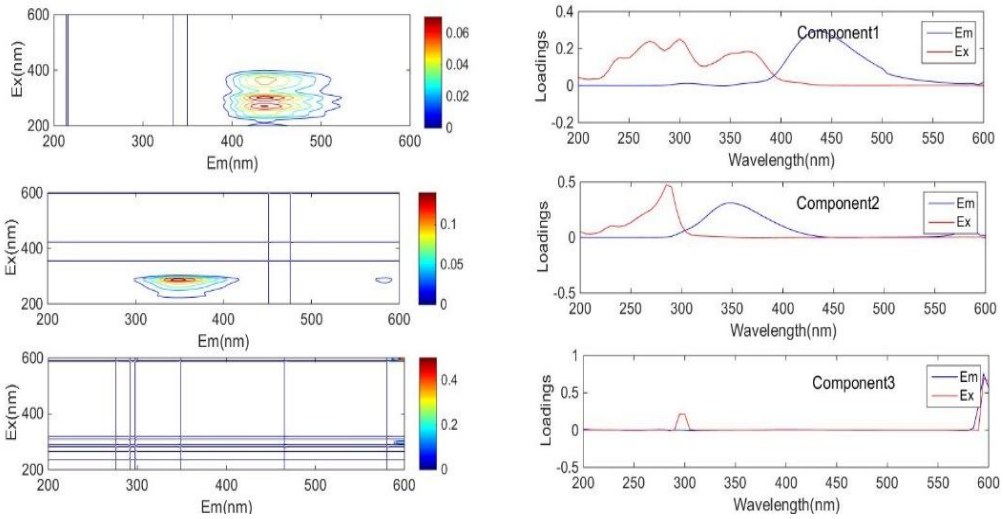


Figure 12. *Parallel factor excitation and emission spectrogram*

As can be seen from *Figure 12,* factor 1 has 4 optimal excitation wavelength, were 250 nm, 270 nm, 300 nm, 360 nm, optimal emission wavelength is 435 nm, respectively characterizing that UVA humus produced by terrestrial plants or soil organic matter, the unknown humus produced by microbiological processes, UVA humic substances produced by terrestrial plants, endogenous microorganism processes, and UVC humic substances produced by terrestrial plants or soil organic matter, belonging to visible fulvic acid. Therefore, factor 1 indicates a kind of humus-like substance (Zhu et al., 2014; Ma, 2014; Hao et al., 2007; Liu et al., 2012). The optimal excitation and emission wavelength of factor 2 is 285/350 nm, characterizing that highly excited luminescent

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tryptophan produced by terrestrial plants, endogenous generation and microbial processes, which is amino acids that are free or bound to proteins and the fluorescence characteristics are similar to those of free tryptophan, indicating a complete protein or less degraded peptide (Hao et al., 2007; Liu et al., 2012). While factor 3 did not find the full fluorescence peak and the best emission wavelength. The inference is that it should be caused by scattering and cannot indicate organic matter in water. From *Figure 9* and *10* can be seen that after anaerobic treatment factor 2 fluorescence peaks decreased, that is, the fluorescence peak intensity of tryptophan is reduced by 58.7%. While factor 1 fluorescence peaks increased, the fluorescence peak intensity of the humus is increased by 11.5 times. Protein reduced and humic substances in water increased after anaerobic treatment, we can indicate that anaerobic biodegradation has converted most of the macromolecular proteins into humus, and the anaerobic ammonification reaction has been taken, which made COD lower while NH_3-N increased.

Conclusion and prospect

(1) The concentration and impact of salinity will inhibit the anaerobic microorganism's degradation ability. In this study, the adaptability of anaerobic microorganisms to high salt wastewater was improved by acclimation of anaerobic sludge. After domestication, the removal rate of COD increased from 27.9% to over 70%. The single factor optimization results showed that the removal rate of COD could be raised to more than 80% again under the condition of pH = 7 and the residence time for 24 h. It is indicated that the water quality is the most significant factor affecting the anaerobic effect. Try to ensure that the influent water quality is stable at COD concentration of 2500 mg/L, the salinity of 6 g/L and pH of 7, so as to achieve the best effect of effluent. Therefore, the anaerobic treatment system in this study is feasible for treating pickle cleaning wastewater.

(2) According to the results of 3D-EEM research, there are a lot of tryptophan (protein) in the pickle cleaning wastewater. The anaerobic biodegradation can convert most of the large molecular protein into humus, and the anaerobic ammonification reaction has occurred, which made COD lower while NH_3-N increased. Therefore, anaerobic treatment in this study has a significant contribution to the removal of organic matter from Pickles wastewater.

(3) Follow up studies can focus on the following aspects: First of all, biological or physicochemical methods should be combined for further treatment of anaerobic effluent to ensure effluent quality. Secondly, in the future research, we need to further optimize the methods of cultivation and domestication of anaerobic sludge, further shorten the cycle of acclimation and put into operation at an early date. Finally, it is necessary to expand the scale of the anaerobic reactor and investigate the treatment effect in the actual engineering application.

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EXCLUSION NETS INFLUENCE ON THE ABUNDANCE OF GROUND BEETLES (COLEOPTERA: CARABIDAE) IN APPLE ORCHARDS

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Abstract. Ground beetles (fam. Carabidae) as beneficial insects are valuable organisms to study whether any of the new implemented agricultural measures in fruit production is beneficial. The aim of our study was to analyze impact of different types of exclusion nets on ground beetle species composition and abundance in an apple orchard. The study was conducted in an IPM apple orchard, situated in the north-west part of Croatia. The treatments for the study were four types of exclusion (colored) nets (Agritenax white, Agritenax yellow and Agritenax red all with photo selective properties and Stop Drosophila Normal net - pearl color) and non-covered trees acted as a control. Ground beetles were collected during the summer and autumn (from July 1st until October 15th in 2015) with pitfall traps. During the study, a total of 1559 specimens of ground beetles were identified and sorted into 28 species. Significant differences in the captures under nets were observed for species: *Anchomenus (Anchomenus) dorsalis* (Pontoppidan 1763), *Microlestes minutulus* (Goeze 1777), and *Pterostichus (Feronidius) melas melas* (Creutzer 1799). The most of the ground beetles (413) were recorded under the Agritenax yellow nets and the least (184) in the control. Our study showed the positive effect of exclusion nets on ground beetle species composition and diversity in IPM apple orchard thus confirming their value in assessing the introduction of new agricultural measures into practice.
Keywords: *Agritenax and Stop Drosophila Normal net, Croatia, IPM fruit production, pitfall traps, soil dwelling fauna*

Introduction

Ground beetles (Coleoptera: Carabidae) are beneficial organisms as predators of various pests in different developmental stages in agricultural production (Lövei and Sunderland, 1996; Kromp, 1999). Their communities are not bound to a certain crop type, but shift in dominance according to the crop-specific rhythm of cultivation and changes in crop phenology and microclimate (Kromp, 1999). Any change in agricultural practices, especially novel practices, can directly affect ground beetles. Because they are highly sensitive to anthropogenic impacts, ground beetles can be considered as bioindicators of environmental impacts from cultivation practices (Kromp, 1999). The composition and richness of Croatian ground beetle fauna have been studied in a variety of arable crops represented in different agricultural practices (e.g. alfalfa and red clover, winter wheat and barley, maize, sugar beet and vegetable gardens) (detail described in: Kovačević and Balarin, 1960; Sekulić et al., 1973; Bažok et al., 2007; Stančić, et al., 2010; Kos et al., 201 201 2013; Lemić et al., 201 2017; Drmić et al., 2016; Virić et al.,

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2017). Clearly there have been few studies of changes in ground beetle communities in response to orchard production, and particularly to those agricultural and crop protection practices that are now standard in apple production.

The standard method for crop protection in Croatian apple orchards is Integrated Pest Management (IPM), which is a suite of environmentally and toxicologically acceptable procedures to control pest populations. Those measures could affect activity, density and species richness of apple orchard ground beetle communities. A new approach of applying colored photo selective shade nets (exclusion nets; anti-hail nets) for improving the utilization of solar radiation by fruit trees was introduced in practice recently (Shahak et al., 2004). The photo selective netting in apple orchard has a positive effect on flowering, fruit-set, fruit size, color and internal quality, in addition to non-specific reduction of water stress, superficial damage and sunburn (Shahak et al., 2004). The main reason to set up the exclusion nets during this study in IPM Croatian orchards was to test their effectiveness in preventing codling moth to apple fruits (Pajač Živković et al., 2016). This experiment resulted in a highly significant reduction of codling moth in IPM orchards (Pajač Živković et al., 2016). The main consequence of applying exclusion nets is the change in the microclimate beneath the trees, accordingly any change of this kind could lead to a disturbance and towards affect orchard ground beetle communities. Therefore, we hypothesized that exclusion nets could have a negative impact on ground beetles in IPM orchards.

The main objective of this study was to define and analyze what type of exclusion net has the least negative impact on ground beetle abundance and diversity in an IPM Croatian apple orchard.

Materials and methods

Study area

The study was conducted in an IPM apple orchard (46°9’47”N, 15°52’52”E), situated in north-west Croatia (Krapina County; *Fig. 1*).



Figure 1. *Experimental site in Croatia (Krapina County)*

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Beetle collection was carried out during the growing season in 2015. The study area is characterized by a continental-humid climate of warm, rainy summers and cold winters (Penzar and Penzar, 2000). The average annual precipitation in this region is 850 mm and the average annual temperature is 11 °C (MHS, 2017). The soil in the study area is typical pseudogley (IUSS Working Group WRB, 2015). The orchard elevation is 282 a.s.l. Apple cultivars of 'Braeburn', 'Idared', 'Golden Delicious', 'Granny Smith' and 'Jonagold' are grown on a surface of 600 m^2. During the study period, insecticide treatments were not applied on the experimental site in order to exclude their impact on orchard entomofauna. Fungicides were applied by standard IPM principles in order to maximize apple health and minimize the risk of economic and ecological threats for the environment. Weeds are managed by mulching treatments between rows several times in the season, and vegetation in the row was not treated with herbicides. The orchard is surrounded by agricultural fields (arable crops and grasslands).

Experimental design

Experimental design was a random block arrangement with four treatments in three repetitions. The treatments were four types of exclusion nets (Agritenax white, Agritenax yellow, Agritenax red – photo selective ones and Stop Drosophila Normal net - pearl color) and non-covered trees as a control. Agritenax nets produced by Tenax S.r.l., Italy were of the same density (2.4 × 4.8 mm) while Stop Drosophila Normal net, produced by Artes Politecnica, Italy was of 0.9 × 1 mm in density. Each treatment was placed on three neighboring trees, while in three cases neighboring trees were uncovered with exclusion nets what represented a control plot (*Fig. 2*).

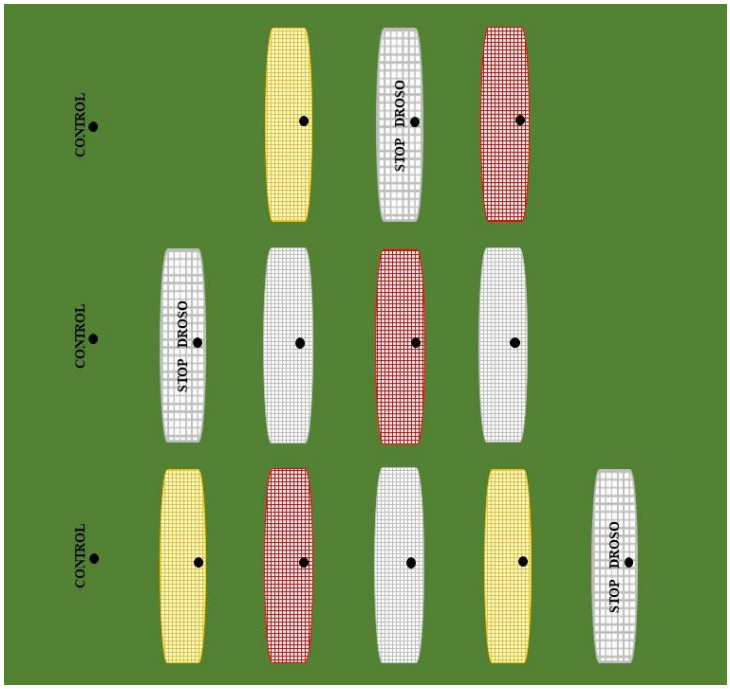


Figure 2. *Schematic representation of experimental design*

The study occurred in the center of the IPM apple orchard planted with the variety 'Braeburn'. Trees were completely covered with nets and fixed to the ground with metal

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pegs at the petal fall and the nets were removed after the harvest of apple fruits. Before setting up nets, existing arthropod fauna on apple trees were eliminated by applying neonicotinoid insecticide thiacloprid (Calypso® 480 SC, Bayer CropScience). One pitfall trap was placed in each treatment in the soil beneath the trees.

Beetle sampling and data analysis

Ground beetles were collected during summer and autumn (from July 1st until October 15th in 2015) (week 28 till week 42 of the year) with pitfall traps. Traps were a metal cylinder covered with a metal lid (Ø = 10 cm wide and h = 20 cm). The cylinder was buried in the soil and a round edge plastic southpaw was fitted above. A polythene pot was placed at the bottom of the cylinder. The plastic pot was half filled with saline water (20% solution) for preservation. For transportation purposes the pots had lids. Traps were inspected on a weekly basis and beetles were preserved in 70% EtOH until identification. The identification of the collected ground beetles was based on the works of: Auber (1965), Bechyne (1974), Harde and Severa (1984) and Freude et al. (2006).

The abundance of captured beetles on a weekly base was used as the response variable, and both week and net type, were used as fixed predictor variables in a two-factor analysis of variance, performed separately for each identified beetle taxon. The Tukey honestly significant difference test was used for post-hoc comparisons among means within levels of the two fixed factors (R Core Team, 2017).

Results

During the study, a total of 1559 specimens of ground beetles were identified and sorted into 28 species represented in *Table 1*.

The maximum number of specimens, 98 belonged to *Pterostichus (Feronidius) melas melas* (Creutzer 1799), which is five times the number of specimens of *Carabus (Procrustes) coriaceus* (Linne 1758), with a total of 196 individuals. In addition to these two species, among the most abundant was *Microlestes minutulus* (Goeze 1777) with a total of 81 specimens (*Table 1*).

Table 1. *Identified species, captures of species under different net treatments and total capture of species during collection in IPM apple orchard, Krapina, Croatia, 2015 (*p < 0.05; **p < 0.001)*

Species	A.R.	A.W.	A.Y.	S.D.N	CONT.	Σ	Date	Net°°	Date ×Net
Nebria (Nebria) brevicollis (Fabricius 1792)	0	0	1	0	0	1	0.463	0.413	0.499
Carabus (Procrustes) coriaceus Linne 1758	30	50	33	50	33	196	1.39e-14***	0.204	0.891
Brachinus (Brachinus) crepitans (Linne 1758)	7	3	9	4	0	23	0.0109*	0.3006	0.8817
Trechus (Trechus) quadristriatus (Schrank 1781)	0	0	0	0	1	1	0.456	0.410	0.487
Dinodes (Dinodes) decipiens (L. Dufour, 1820)	0	0	1	0	2	3	0.0934	0.2602	0.8331
Anisodactylus (Anisodactylus) binotatus (Fabricius 1787)	6	6	9	5	3	29	0.0166*	0.7018	0.2131
Harpalus (Harpalus) affinis (Schrank 1781)	0	1	1	0	0	2	0.0212*	0.5595	0.8911
Harpalus (Harpalus) atratus Latreille 1804	3	7	2	2	6	20	0.0966	0.3998	0.3861

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Harpalus (Harpalus) dimidiatus (P. Rossi 1790)	7	11	9	11	7	45	2.09e-07***	0.726	0.180
Harpalus (Harpalus) rubripes (Duftschmid 1812)	1	0	0	0	3	4	0.049371*	0.026589*	8.1e-05***
Harpalus (Harpalus) tardus (Panzer 1796)	1	1	1	2	0	5	0.0212*	0.7358	0.9252
Pseudoophonus (Pseudoophonus) griseus (Panzer 1796)	2	3	1	1	0	7	9.4e-04***	0.366895	0.345923
Pseudoophonus (Pseudoophonus) rufipes (De Geer 1774)	1	5	0	12	2	20	0.496	0.237	0.644
Ophonus (Hesperophonus) azureus (Fabricius 1775)	1	3	1	2	4	11	0.0315*	0.6250	0.1279
Ophonus (Metophonus) gammeli (Schauberger 1932)	0	1	0	0	1	2	0.530	0.559	0.455
Ophonus (Metophonus) parallelus (Dejean 1829)	1	1	1	2	2	7	0.121	0.930	0.791
Ophonus (Ophonus) sabulicola (Panzer 1796)	3	2	1	1	4	11	9.1 e-04***	0.544563	0.999960
Parophonus (Parophonus) maculicornis (Duftschmid, 1812)	0	0	0	1	0	1	0.456	0.410	0.487
Microlestes minutulus (Goeze 1777)	13^{bc}	40^{a}	10^{bc}	16^{bc}	2^{c}	81	0.00746**	0.02031*	0.44138
Badister (Badister) bullatus (Schrank 1798)	2	0	2	0	0	4	0.0864	0.4096	0.7991
Anchomenus (Anchomenus) dorsalis (Pontoppidan 1763)	4^{bc}	10^{bc}	6^{bc}	21^{a}	0^{c}	41	1.39e-05***	0.00115**	0.44221
Abax (Abax) exaratus (Dejean 1828)	0	0	0	2	0	2	0.456	0.410	0.487
Pterostichus (Calopterus) selmanni (Duftschmid 1812)	0	0	2	0	3	5	0.651	0.227	0.542
Pterostichus (Feronidius) melas melas (Creutzer 1799)	156^{c}	209^{bc}	299^{a}	219^{bc}	101^{c}	984	< 2e-16***	4.3e-05***	0.742
Stomis (Stomis) pumicatus pumicatus (Panzer 1796)	0	1	0	0	0	1	0.456	0.410	0.487
Calathus (Calathus) fuscipes subsp. graecus Dejean 1831	5	8	17	4	9	43	0.0218*	0.0686	0.5972
Calathus (Neocalathus) melanocephalus (Linne 1758)	1	0	6	1	1	9	0.0296*	0.2284	0.6879
Amara (Amara) ovata (Fabricius 1792)	0	0	1	0	0	1	0.463	0.413	0.499
Σ (total)	244	362	413	356	184	1559			

Abbreviations: A.R. – Agritenax red; A.W. – Agritenax white; A.Y. – Agritenax yellow; S.D.N. – Stop Drosophila Normal; CONT. – No net, control
Different letters indicate significantly different treatment levels

The population dynamics of the most abundant species under different exclusion nets and on the control is shown in *Figures 3*, *4* and *5*. Significant captures of species *P. melas melas* started on all treatments from August 13th and lasted till the end of monitoring period. During September two stresses for the population were detected, the first lasted from August 27th to September 3rd while the second lasted from 17th to 24th September. The trend of captures under all nets as well as on the control during the study period was more or less uniform (*Fig. 3*).

The first significant increase in captures of *C. coriaceus* started on all treatments in the first half of September and the most ground beetles were collected at the beginning of October. A significant number of adults have been captured till the end of monitoring period (*Fig. 4*).

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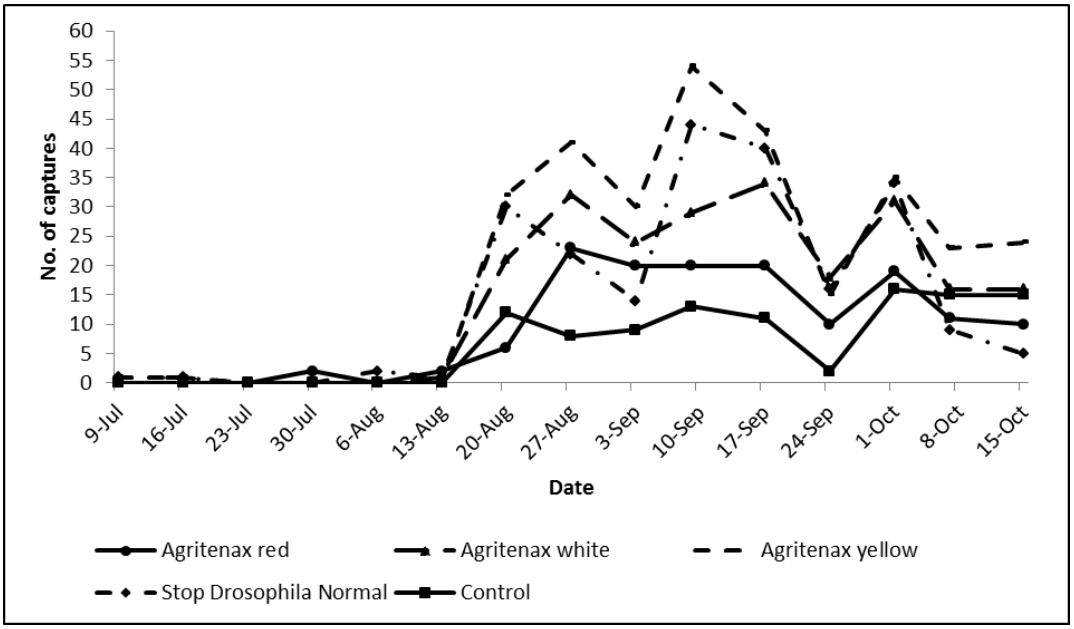


Figure 3. *Population dynamic of species Pterostichus melas melas in IPM apple orchard under different exclusion nets and on the control*

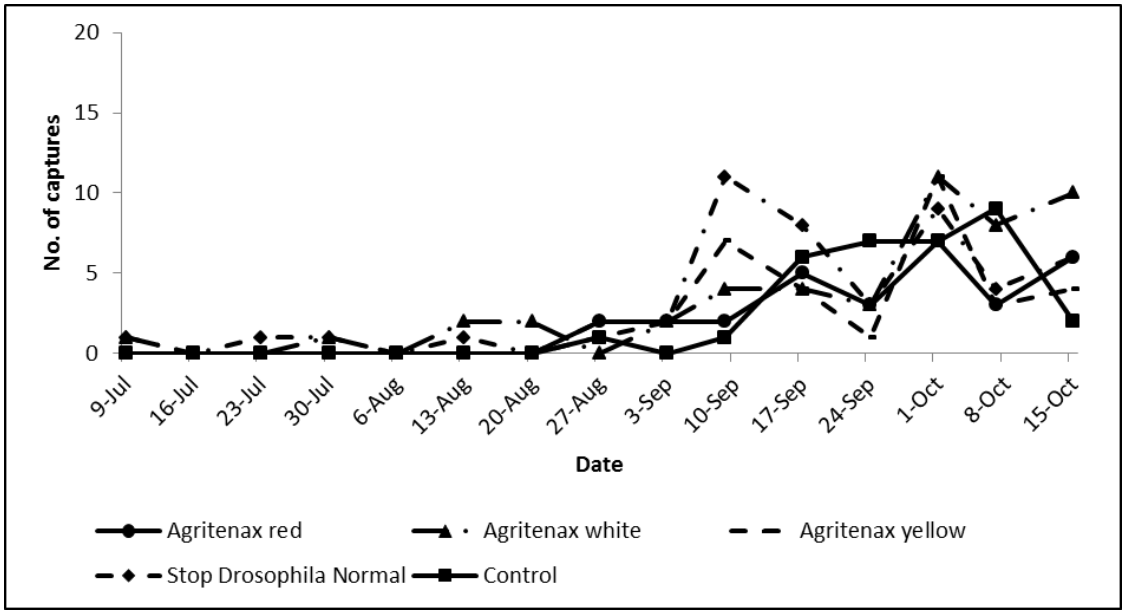


Figure 4. *Population dynamic of species Carabus coriaceus in IPM apple orchard under different exclusion nets and on the control*

The captures of species *M. minutullus* started on all treatments at the end of July and lasted until the beginning of October. Greater stress for the population of this species occurred from 3rd to 10th of September when the capture fell suddenly on all traps. Under Agritenax white nets captures were higher in September than in August, while the captures under the Agritenax red nets as well as on the control were higher at the beginning of August compared to September (*Fig. 5*).

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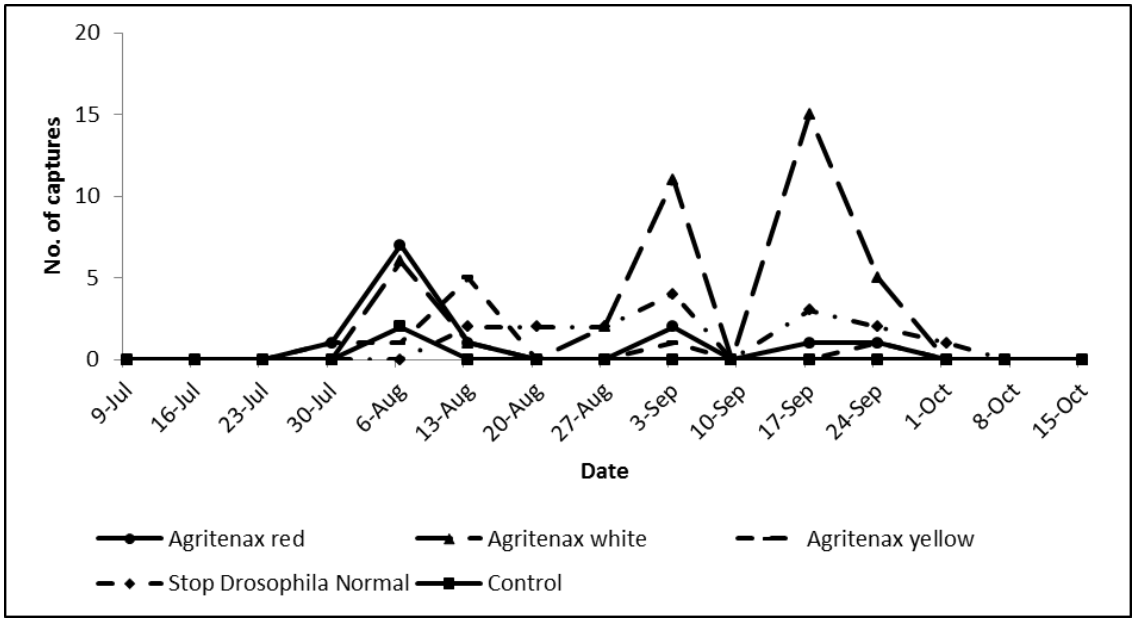


Figure 5. *Population dynamic of species Microlestes minutulus in IPM apple orchard under different exclusion nets and on the control*

The biggest catch of one species of ground beetles under one type of exclusion nets was 299 and belonged to *P. melas melas* under Agritenax yellow nets. During the study just one individual of *Anchomenus (Anchomenus) dorsalis* (Pontoppidan 1763) and *Nebria (Nebria) brevicollis* (Fabricius 1792) were captured.

The most ground beetles (413) were recorded under the Agritenax yellow nets and the least (184) on the control (*Table 1*). Significant effects of time of capture were observed generally for more abundant species. Extremely high ($p < 0.001$) and high ($p < 0.01$) differences between the week of the capture were established on seven species (*Table 1*). The effect of net type was observed for only four species. A significant interaction between net and time of sampling was observed only for *Harpalus (Harpalus) rubripes* (Duftschmid 1812). This species was caught in low numbers; therefore because of its rarity further conclusions about it will not be made.

Significant differences in the captures under nets were observed for species *A. dorsalis*, *Microlestes minutulus* (Goeze 1777), and *P. melas melas* (*Table 2*). This study shows that there are significantly more captures of the species *A. dorsalis* under the Stop Normal Drosophila nets in relation to Agritenax red, white and yellow nets. On the other hand, for the same species, there is a lack of captures on the control. For *M. minutullus* the highest capture was established under Agritenax white nets in relation to Agritenax red and yellow where the captures were significantly lower (*Table 2*). For this species, captures on the control were the lowest. Captures of adult specimens of the species *P. melas melas* were the highest under the Agritenax yellow nets (*Table 2*). While captures under Agritenax red and white nets, also as for the Stop Normal Drosophila nets, did not defer statistically. Captures for *P. m. melas*, were similar to those of the other two species, and were the lowest on the control (*Table 2*).

There was no difference in captures between closely observed species beneath Agritenax red nets and control. *P. melas melas* had higher rates of captures under the Agritenax yellow nets than other two species in relation to the control. Species of *P.*

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melas melas and *M. minutulus* were captured more beneath the Agritenax white nets, and *P. melas melas* and *A. dorsalis* beneath the Stop Normal Drosophila nets in relation to the control.

Table 2. *Tukey adjusted p-values for pairwise differences among all net treatments for species: Anchomenus dorsalis (A.d.), Microlestes minutulus (M.m.) and Pterostichus melas melas (P.m.m.), Krapina, Croatia, 2015*

Treatments	Agritenax red			Agritenax yellow			Agritenax white			Stop Drosophila Normal		
	A.d.	***M.m.***	***P.m.m.***	***A.d.***	***M.m.***	***P.m.m.***	***A.d.***	***M.m.***	***P.m.m.***	***A.d.***	***M.m.***	***P.m.m.***
Agritenax red	-	-	-	-	-	-	-	-	-	-	-	-
Agritenax yellow	0.99	0.99	0.0**	-	-	-	-	-	-	-	-	-
Agritenax white	0.77	0.14	0.67	0.93	0.08	0.16	-	-	-	-	-	-
Stop Drosophila Normal	0.01**	0.99	0.51	0.03*	0.98	0.27	0.21	0.24	0.24	-	-	-
Control	0.93	0.87	0.64	0.77	0.95	0.0**	0.30	0.01**	0.01**	0.0**	0.75	0.03*

**$0.001 \leq P \leq 0.0$ *$0.01 \leq P \leq 0.05$

Discussion

Various authors have developed different kinds of experiments or have gathered knowledge to prove that the presence of ground beetle, as wide spread beneficial organism, could be an indicator for habitat quality (Bourassa et al., 2008; Avgın and Luff, 2010; Koivula, 2011). Our investigation showed a similar importance for the bio-indicative value of this family and their presence in our apple orchard habitat with 28 species found. These results are in a similar range as that of studies in apple orchards from Hungary (Kutasi et al., 2004), Spain (Miñarro et al., 2008), Italy (Paoletti et al., 1995), Canada (Smith et al., 2004) and Japan (Funayama, 2011). Various total captures of ground beetles in our study can suggest that several species should be closely considered for their indicator value because of their abundance, such as *P. m. melas*; *C. coriaceus*; *M. minutulus*; *Harpalus (Harpalus) dimidiatus* (P. Rossi 1790) and *A. dorsalis*. According to Miñarro and Dapena (2003) the three most common beetles, *Pterostichus (Steropus) gallega* (Fairmaire 1859), *Pseudoophonus (Pseudoophonus) rufipes* (De Geer 1774) and *Poecilus (Poecilus) cupreus* (Linne 1758) represented more than 98% of the total captures. Those species were not present in our study, but on the other hand our three most abundant species represented more than 75% of the total captures. Similar results were found in a study from Miñarro et al. (2008) where 83.76% of all captures were for three of the most abundant species *P. rufipes* (55.84%), *P. cupreus* (17.62%) and *Carabus (Tachypus) cancellatus subsp. cancellatus* Illiger 1798 (10.30%), respectively. The most abundant species, which basically determined the composition of orchard carabid assemblages in study from Markó and Kádár (2005), were *Harpalus (Harpalus) froelichii* Sturm 181 *Harpalus (Acardystus) flavescens* (Piller & Mitterpacher 1783), *Calathus (Neocalathus) ambiguus subsp. ambiguus* (Paykull 1790), and *Harpalus (Harpalus) hirtipes* (Panzer 1796). Every agronomic

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practice may influence the environmental variables such as the substrate temperature or humidity, the availability of organic matter, food or shelter, and consequently carabid captures may fluctuate in accordance with their own needs (Miñarro et al., 2008). The exclusion nets also change microclimate conditions (e.g. temperature, relative humidity, light) (Brkljača et al., 2016; Fruk et al., 2016) beneath the net as well as on the soil surface beneath the trees. This practice could influence the richness and diversity of ground beetles. Applying exclusion nets for improving the utilization of solar radiation by fruit trees (Shahak et al., 2004) influences the total capture rate of some ground beetle species. The Agritenax yellow nets had the highest total ground beetle captures among all other nets and the control. Captures varied most likely due to solar radiation, as nets influenced the cover of ground plants or soil humidity beneath the trees, or due to an altered abundance of pests or other fauna. In the study of Miñarro and Dapena (2003) and Miñarro et al. (2008), it was found that ground beetles were captured in the greatest abundance in tilled and herbicide plots compared to mulched plots. In our study, the exclusion nets clearly attracted some ground beetles from the surrounding areas, though the whole orchard was mulched and no herbicides were applied. We observed water condensation on each type of net over the sampling period which implicates that water loss is lower than on the control plots, which is also corroborated by some authors (Wachsmann et al., 2014). Although soil moisture negatively affected carabid catches in cultivated fields (Cárcamo, 1995), in our study exclusion nets might have had the opposite impact. Our sampling period was summer and the beginning of autumn when water loss from the soil is the greatest and ground beetles might be seeking a humid microclimate. It is clear that exclusion nets change the microclimate, which can impact other organisms, such as weeds, which can improve the water balance beneath the apple trees. This could influence prey species richness for ground beetles. Exclusion nets influenced capture rates of three species (*A. dorsalis*, *M. minutullus* and *P. m. melas*). Species of *A. dorsalis* occur in open meadows and grasslands, usually on soil rich in gravel or clay, and often limestone soil. The species also inhabits arable land, particularly winter crops on heavy soil (Merivee et al., 2012). It is a spring breeder, meaning the species is the most numerous between May and June (Lindroth, 1986). The influence of exclusion nets could be explained mainly by their increase of the preferred (aphid) prey of this species, which is a known aphid predator of cereal crops (Thiele, 1977; Bilde and Toft, 1997). The second important species for this study was the eurytopic *M. minutulus.* It is predominately found at sites with pioneer vegetation or grasses (Brändle et al., 2000) as a typical predator, fully winged. The species is a spring breeder and in this study it was also influenced by exclusion nets. The presence of this species in this study was mostly under Agritenax yellow nets during September. The reason for this could be their preference for prey (aphids) and prolonged vegetation of weeds (grass) under this type of net. The capture of species *P. melas melas* as a predatory, autumn breeder species, that inhabits forests, forest edges, meadows and agricultural land, was also influenced by exclusion nets. Reasons for this, besides prey preference for aphids, might be humidity that attracted another prey, slugs, of the genus *Pterostichus* (Oberholzer et al., 2003). Slugs were observed in high abundance under exclusion nets. Funayama (2011) considered that only *Amara chalcites* Dejean 1828 adults can be used as bioindicators for pesticide use pressure in apple orchards in northern Japan, whereas the main diet of this species is probably C3 plants. The results of our study showed that, the selected omnivorous and predatory species of ground beetles are influenced by the exclusion nets, so that they have the potential as

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bioindicators for evaluating the use of netting for improving agricultural habitat. Furthermore, Miñarro et al. (2008) stated that in orchards, groundcover management affected the density and diversity of ground beetles, which is consistent with our study in which exclusion nets have influenced microclimate (humidity) and ground beetle prey attraction.

Conclusion

The main conclusions of the study are: a) one species of ground beetle could not be considered for bio-indicative value of cultivation impacts and b) most of the captures were for the several most abundant ground beetle species. Considering all of the above, the main hypothesis about the negative impact of the exclusion nets on ground beetles could be rejected as this study indicates a positive effect from exclusion nets on ground beetle abundance in an IPM apple orchard planted with different varieties of apples in north-west Croatia.

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USE OF THE EPIPHYTIC DIATOMS TO ESTIMATE THE ECOLOGICAL STATUS OF LAKE MOGAN

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Abstract. In this study, the seasonal succession of the diatoms on *Phragmites australis* community was investigated in Lake Mogan (Turkey), and the epiphytic diatom community was used to estimate the ecological condition of the lake. Five stations were chosen in the littoral region of the lake, and diatom samples were taken from the *P. australis* monthly between January and November 2013. Two stations were selected in the pelagial region and chlorophyll *a* concentrations were determined. Lake water temperature, pH, dissolved oxygen concentration, conductivity and Secchi depths were measured, and total nitrogen, total phosphorus and silicate were analyzed. A total of 61 epiphytic diatom species were identified in Lake Mogan. The dominant species were those with tolerance to α–β mesosaprobic conditions. According to the mean Secchi depth, chlorophyll and total phosphorus concentrations, the lake was eutrophic. The IDG, IPS, DESCY, WAT, IDP and TDI indices, calculated according to epiphytic diatoms, indicated an ecological quality between medium and poor (classes III-IV). Among them, IDG and IPS are recommended for monitoring of ecological status in the lake because these indices also integrated all diatom taxa within the samples. The occurrence of pollution-tolerant species and the fact that most indices showed an ecological quality of medium or poor indicated an increase of organic matter and eutrophication in Lake Mogan.
Keywords: *epiphyton, reed, shallow lake, water quality, chlorophyll*

Introduction

The most widespread algae, diatoms, can be found in all water resources, from small streams to sea, and from clean to polluted water. Diatoms include many different forms and can be found on all types of substrates; and they can easily be sampled in great numbers from small surfaces in aqueous environments. They can also be sampled from artificial surfaces, so that their rapid response to changes in water quality, together with a fast access to results by means of diatom indices, make them advantageous for use in monitoring water quality. Among their disadvantages are the need for taxonomic knowledge in identifying them, and the difficulty in separating dead and live cells. Diatoms have been used as an indicator of water quality since 1949, and for this reason many species have been classified according to their tolerance and sensitivity to pollution. The cells do not break up easily because they contain silica, and are indicators of long term as well as short term changes (Cox, 1991; Round, 1991; Smol and Stoermer, 2010). Diatoms are particularly used as indicators of pH, conductivity, salinity and trophic level (Cox, 1991).

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The Water Framework Directive, adopted by and implemented in the European Union in the year 2000, classifies water resources by their ecological qualities and aims to bring water to a good ecological quality. Phytobenthos, according to Water Framework Directive, is one of the indicators used to determine the ecological quality of standing and flowing waters (Anonymous, 2000). The use of epiphytic diatoms, which are a component of lake phytobenthos, is important to estimate the ecological quality of shallow lakes.

Because diatoms form a large proportion (90-95%) of benthic habitats of water ecosystems, they have become an important part of water quality monitoring studies (Ács et al., 2004). The occurrence of a particular diatom species in different bodies of water, from clean to polluted waters, enables the use of indices of diatom variety. Diatom indices are based on tolerance and/or sensivity of taxa (genus or species) and allow the assessment of water quality using the structure of diatom communities.

Although there have been many studies of the use of diatoms in determining water quality and/or ecological quality in flowing water (Atıcı, 1997; Eloranta and Soinien, 2002; Blanco et al., 2004; Johnson et al., 2006; Kalyoncu et al., 2009; Salomoni et al., 2011; Gün, 2011; Solak and Ács, 2011; Kıvrak et al., 2012; Tokatlı, 2012; Kalyoncu and Şerbetçi, 2013; Taş et al., 2015; Demir et al., 2017; Toudjani et al., 2017;), a limited number of such studies have been conducted on lakes (Poulíčková et al., 2004; Ács et al., 2005; Kovács et al., 2007; Lirika et al., 2013; Tokatlı, 2013; Kavya and Ulavi, 2014; Szabo et al., 2017). For this reason, studies of the use of diatoms to determine ecological quality in lakes are of great importance. Tokatlı (2013) reported that the TDI index had been calculated in Porsuk Reservoir using epipelic diatoms, and that according to this index it was mesotrophic.

Several studies have been conducted in limnology, water quality or phytoplankton in Lake Mogan (Akbulut and Akbulut, 2002; Fakıoğlu and Pulatsü, 2005; Atıcı et al., 2010; Yerli et al., 2012; Atıcı and Tokatlı, 2014; Şanal et al., 2015). Demir et al., (2014) researched the possibility of using Q index in assessing the ecological quality class of Lake Mogan. Estimates of the ecological quality made in connection with phytoplankton community structure in the lake were compared with the nutrient status. The results showed that Q index could be used in monitoring ecological quality in Lake Mogan. It is a shallow lake surrounded by a belt of reed *Phragmites australis* (Cavanilles) Trinius *et* Steudel on its shores. According to King et al., (2006), *P. australis* is recommended as an ideal plant for sampling epiphytic diatoms when determining ecological quality in lakes.

The aim of the present study was to determine the ecological quality of Lake Mogan, which is an important example of a shallow lake under anthropogenic pressure, using epiphytic diatoms found on *Phragmites australis*, and to determine the diatom composition in the lake.

Materials and Methods

Study Site

Lake Mogan is situated in Sakarya Basin, 20 km south of Ankara in Turkey, between 39˚44'45" and 39˚47'45" north, 32˚46'30" and 32˚49'30" east, at an altitude of 973 m above sea level. It has an area of 5.7 km², and an average depth of 2.4 m. The maximum depth is 4.25 m. The lake is 5.4 km long and approximately 1.7 km wide. Its shore length is approximately 16 km (Demir et al., 2014).

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Because they have ecological and recreational importance, the wetland-marsh areas around Lakes Mogan and Eymir have been designated as the Gölbaşı Special Environmental Protection Area (Anonymous, 2015). Lake has been heavily influenced by human actions ranging from agricultural and domestic to semi-industrial pollution sources. The lake under the pression of anthropogenic pollution due to the external phosphorus load resulting from detergents in waste water effluents and fertilizers in agricultural run-off from nearby areas (Fakıoğlu and Pulatsü, 2005; Topçu and Pulatsü, 2017).

Analysis

In this study, samples were taken once a month from Lake Mogan between January and November 2013. Samples of water and epiphytic diatoms were taken at five stations chosen in the littoral region of the lake (stations 1, 2, 3, 4 and 5), and other water samples were taken at two stations in the pelagial region (stations 7 selected at the deepest point of the lake and station 6 close to the entry) in order to determine the concentration of chlorophyll *a* (*Figure 1*). In December, samples could not be taken because the lake was frozen.

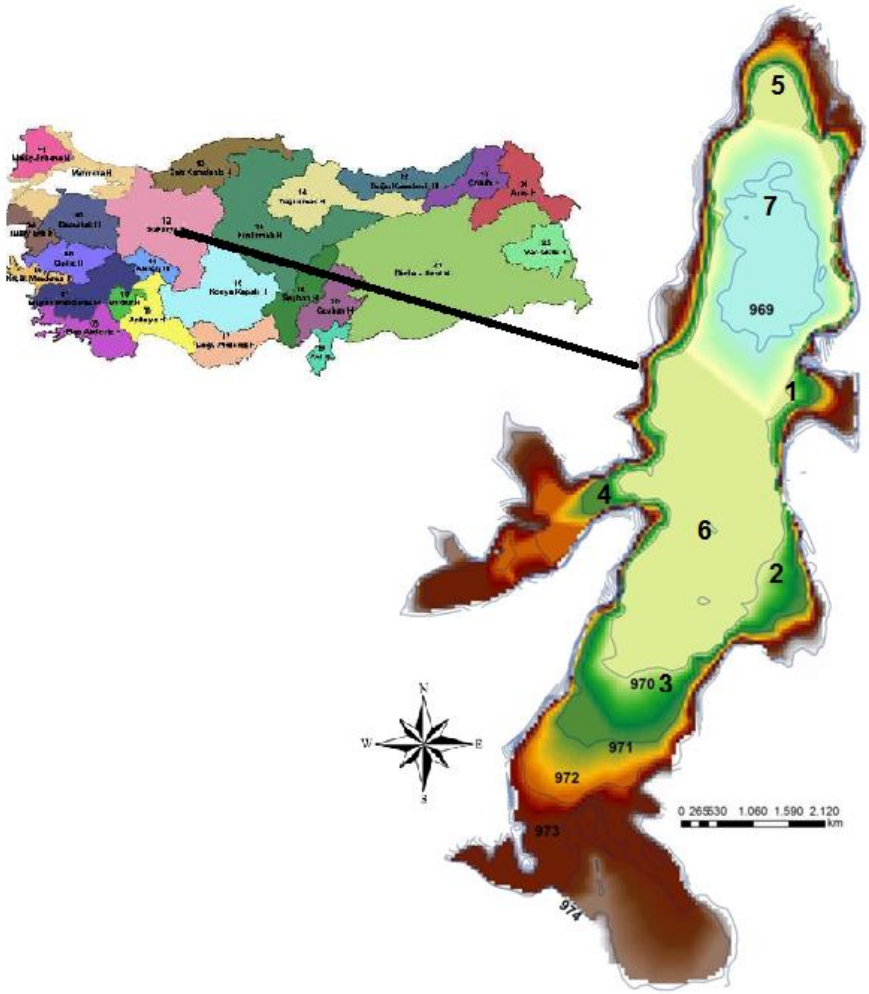


Figure 1. *Sakarya basin and sampling stations in Lake Mogan (1-5:littoral stations, 6- 7: pelagial stations)*

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Two independent parallel water samples were collected from below the lake surface with a 2 l Ruttner water sampler. At each station in the littoral region, five reeds (*Phragmites australis* (Cavanilles) Trinius *et* Steudel) were collected, and 20-cm sections were taken from below the water surface (King et al., 2006). The sections of reeds were placed in a container and agitated with 70% ethanol to separate the epiphytic algae from the plants and to bring them to a homogeneous state (TS EN 15708, 2010). The diatoms were treated with H_2O_2 to remove organic material, and triplicate stable preparations were made using Naphrax (CEN, 2004).

Identification of the diatoms was performed by measuring them with an imaging system using a Leica DFC295 camera and the LAS vs 4.8 program, and referring to the relevant taxonomic literature (Krammer and Lange-Bertalot, 1985, 1986, 1988, 1991a, 1991b; Cox, 1996; Lange-Bertalot, 2013). Also, all of the taxonomic data was updated in accordance with algae databases (Guiry and Guiry, 2018). Triplicate preparats were counted for each station. At least 400 cells were counted in each preparat, and SLA, DESCY, IDSE, SHE, TDI, CEE, IPS, IBD, IDAP, EPI-D, DI-CH, IDP, SID, TID, WAT, %PT, IDG, LOBO and TDIL diatom indices were calculated using the OMNIDIA vs 6.06 package (Lecointe et al., 1993). Most of the indices are modified from the weighted average equation of Zelinka and Marvan's index (Bellinger and Sigee, 2010). In addition, ecological characteristics were determined according to Denys (1991), Håkansson (1993), Hofmann (1994) and Van Dam et al., (1994). In the chlorophyll *a* analysis, spectrophotometric method was used. Dublicate water samples of 500 ml taken from below the surface at two stations were filtered with Whatman GF/C filter paper and extracted with acetone, after which the optical density of the extract was read on a spectrophotometer (Shimadzu UV 1240) at wavelengths of 630, 645 and 665 nm (Strickland and Parsons, 1972; APHA, 1998).

Measurements were made at the stations of Secchi depth using a 20 cm diameter Secchi disc, of dissolved oxygen, pH and electrical conductivity, using a YSI ProPlus Multiparameter. Total phosphorus, total nitrogen and silica were analyzed by standard methods (APHA, 1998).

Statistical analyses

The monthly and station-to-station variations in water temperature, Secchi depth, dissolved oxygen, pH, electrical conductivity, total phosphorus, total nitrogen and silicate values in Lake Mogan were analysed using a one-way variance analysis (ANOVA). In addition, the correlations between diatom indices and physico-chemical parameters were examined with Spearman correlation analysis using SPSS vs17 package. All statistical calculations and checks were conducted according to Kesici and Kocabaş (2007).

Results

The monthly variations in water temperature, Secchi depth, dissolved oxygen, pH, EC, total nitrogen and silicate in the lake were found to be significant ($p<0.05$), but the variations in these parameters between stations were found not to have statistical significance. An evaluation was made according to the average of the results of the monthly measurements in the lake and according to the Surface Water Quality Directive and trophic status criteria (Anonymous, 2016) (*Table 1*).

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Table 1. *Monthly values of physical and chemical water quality parameters in Lake Mogan between January and November 2013 (mean ± standard error)*[1]

Month	J n=14[2]	F n=14	M n=14	A n=14	M n=14	J n=14	J n=14	A n=14	S n=14	O n=14	N n=14	Mean± SE n=154	WQ I	WQ II	WQ III	WQ IV
Temp. °C	4.83 ±0,17	10.22 ±0.23	9.09 ±0.13	21.61 ±0.43	23.75 ±0.39	25.49 ±0.20	25.54 ±0.27	25.19 ±0.29	18.23 ±0.22	13.84 ±0.31	7.41 ±0.28	16.84 ±8.01	-	-	-	-
pH	8.20 ±0.02	8.01 ±0.05	8.44 ±0.05	8.47 ±0.02	9.62 ±0.01	9.49 ±0.03	9.85 ±0.02	9.78 ±0.04	10.96 ±0.22	10.87 ±0.03	10.94 ±0.14	9.51 ±1.11	6-9	6-9	6-9	6-9
EC µS/cm	1262.5 ±20.6	1265 ±10.2	1371.5 ±16.0	1300.1 ±29.9	1364.3 ±14.3	1284.1 ±42.5	1256.2 ±28.2	1280.2 ±23.3	1267.4 ±12.9	1291.0 ±13.1	1361.7 ±16.7	1300 ±45	< 400	1000	3000	> 3000
DO mgO_2/l	8.66 ±0.56	8.51 ±0.20	7.39 ±0.30	8.33 ±0.15	6.52 ±0.25	6.48 ±0.25	5.91 ±0.86	7.94 ±0.30	8.39 ±0.17	10.22 ±0.38	10.95 ±0.62	8.12 ±1.60	> 8	6	3	< 3
Silicate mg/l	7.60 ±2.07	4.21 ±1.22	3.14 ±0.98	5.00 ±0.57	5.36 ±1.02	8.71 ±1.52	6.29 ±1.18	7.93 ±0.67	8.43 ±1.13	8.43 ±1.20	8.43 ±2.22	6.68 ±1.91	**Oligo-trophic**	**Meso-trophic**	**Eu-trophic**	**Hyper-trophic**
TP µg/l	82.00 ±50.00	84.29 ±32.07	144.29 ±249.52	57.14 ±22.15	150.00 ±247.5	165.71 ±154.15	117.14 ±11.13	71.43 ±3.76	51.43 ±14.64	54.29 ±7.89	54.29 ±13.97	93.82 ±16.22	<10	10-30	31-100	>100
TN µg/l	620.00 ±170	542.86 ±83.81	507.14 ±156.60	542.86 ±127.24	450.00 ±100	707.14 ±216.85	557.14 ±142.68	492.86 ±151.19	435.71 ±69.01	378.57 ±75.59	350.00 ±0.00	507.7 ±105.4	<350	350-650	651-1500	>1500
Chl *a*[3] µg/l	15.33 ±1.59	24.22 ±7.20	26.29 ±5.06	23.50 ±1.48	27.99 ±0.86	28.26 ±0.50	32.07 ±1.18	35.62 ±3.59	19.11 ±0.38	7.63 ±2.24	4.28 ±0.68	22.05 ±10.12	<3.5	3.5-9	9.1-25.0	>25
SD M	1.07 ±0.27	0.89 ±0.14	0.91 ±0.16	2.36 ±0.62	1.78 ±0.63	2.21 ±0.32	1.49 ±0.5	0.96 ±0.23	1.08 ±0.14	1.24± 0.14	1.40 ±0.28	1.40 ±0.43	>4	4-2	1.9-1	<1

[1]Water Quality (WQ) and trophic status of the lake were evaluated according to Surface Water Quality Directive (Anonymous, 2016).
[2]The number of repetitions.
[3]Chlorophyll *a* was measured in two pelagial stations (n= 44).

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In the samples taken from Lake Mogan, 58 species from the class Bacillariophyceae were identified, two species from Coscinodiscophyceae, and one from Mediophyceae, totaling 61 species. The composition of the species of diatoms in the lake is given in *Table 2*.

It was found that *Achnanthidium minutissimum, Navicula cryptonella, Ulnaria acus, Encyonopsis microcephala, Rhopalodia gibba* and *Nitzschia dissipata* were dominant in the epiphytic diatom community of the lake. When percentage distribution of the epiphytic diatom species was considered, it was found that *A. minutissimum* was predominant with an average of 17%, followed by *N. cryptonella* with 8.5% and *U. acus* with 7.3% (*Table 2*).

Table 2. *Composition of epiphytic diatom species in the research period in Lake Mogan*

Species	**Composition %**
Achnanthidium minutissimum (Kützing) Czarnecki	17.00
Navicula cryptonella Lange-Bertalot	8.50
Ulnaria acus (Kützing) Aboal	7.31
Encyonopsis microcephala (Grunow) Krammer	5.14
Rhopalodia gibba (Ehrenberg) Otto Müller	4.15
Nitzschia dissipata (Kützing) Rabenhorst	3.56
Ulnaria ulna (Nitzsch) Compère	3.36
Diatoma moniliformis (Kützing) D.M.Williams	3.36
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	3.16
Gomphonema brebissonii Kützing	2.96
Cymbella excisa Kützing	2.77
Diatoma tenuis C. Agardh	2.77
Epithemia sorex Kützing	2.77
Navicula cryptocephala Kützing	2.37
Fragilaria capucina var. *vaucheriae* (Kützing) Lange-Bertalot	2.37
Nitzschia palea (Kützing) W.Smith	1.98
Amphora copulata (Kützing) Schoeman and R.E.M.Archibald	1.78
Mastogloia smithii Thwaites ex W. Smith	1.58
Fragilaria capucina Desmazières	1.58
Gomphonema olivaceum (Hornemann) Brébisson	1.58
Cymbella neocistula Krammer	1.58
Navicula tripunctata (O. F. Müller) Bory	1.38
Epithemia turgida (Ehrenberg) Kützing	1.19
Navicula radiosa Kützing	0.99
Encyonema auerswaldii Rabenhorst	0.99
Mastogloia elliptica (C. Agardh) Cleve	0.99
Gomphonema italicum Kützing	0.79
Nitzschia amphibia Grunow	0.79
Tryblionella hungarica Grunow Frenguelli	0.59
Gyrosigma acuminatum (Kützing) Rabenhorst	0.59

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Ulnaria capitata (Ehrenberg) Compère	0.59
Encyonema silesiacum (Bleisch) D.G. Mann	0.59
Fragilaria famelica (Kützing) Lange-Bertalot	0.59
Cymbella helvetica Kützing	0.40
Navicula cincta (Ehrenberg) Ralfs	0.40
Lindavia comta (Kützing) Nakov, Gullory, Julius, Theriot, Alverson	0.40
Amphora pediculus (Kützing) Grunow	0.40
Gomphonema intricatum var. *vibrio* (Ehrenberg) Cleve	0.40
Nitzschia sigmoidea (Nitzsch) W. Smith	0.40
Cymbella compacta Østrup	0.40
Gomphonema pseudoaugur Lange-Bertalot	0.40
Cyclotella meneghiniana Kützing	0.40
Anomoeoneis sphaerophora Pfitzer	0.40
Cocconeis placentula Ehrenberg	0.40
Melosira varians C. Agardh	0.40
Cocconeis pediculus Ehrenberg	0.40
Navicula gregaria Donkin	0.40
Craticula cuspidata (Kützing) D.G.Mann	0.20
Caloneis amphisbaena (Bory) Cleve	0.20
Eunotia bilunaris (Ehrenberg) Schaarschmidt	0.20
Campylodiscus bicostatus W.Smith in Roper	0.20
Cymbella lanceolata (C. Agardh) C. Agardh	0.20
Nitzschia tryblionella Hantzsch	0.20
Epithemia adnata (Kützing) Brébisson	0.20
Fallacia pygmaea (Kützing) Stickle and D.G.Mann	0.20
Pinnularia brebissonii (Kützing) Rabenhorst	0.20
Cymatopleura solea (Brébisson) W.Smith	0.20
Sellaphora pupula (Kützing) Mereschkovsky	0.20
Surirella brebissonii Krammer and Lange-Bertalot	0.20
Surirella ovalis Brébisson	0.20
Pinnularia viridiformis Krammer	0.20
Number of species	**61**

Table 3 shows the ecological evaluation of the diatom community in Lake Mogan. The diatom community in the lake was classified by its pH as alkaliphilic. Oxygen requirement was found to be high. There was a slight salinity, and in terms of nitrogen uptake, autotrophic nitrogen tolerance was found. The saprobity level was estimated as β-mesosaprobic and α-β mesosaprobic. The trophic condition was found to be eutrophic, as having numerous eutrophication-tolerant species (*Table 3*).

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Table 3. *Estimation of ecological parameters according to epiphytic diatom abundance in Lake Mogan*

Van Dam et al. (1994)	**1**	**2**	**3**	**4**	**5**	**6**	**7**				**DOMINANT**
pH	0	2	253	472	146	0					**4** alkaliphilous
Salinity	0	753	57	63							**2** fresh-brackish water
Nitrogen uptake	204	462	18	20							**2** N-autotrophic tolerant
Oxygen requirements	271	196	200	36	4						**1** high
Saprobity	67	575	126	40	20						**2** β-mesosaprobe
Trophic status	0	0	26	117	362	20	322				**5** eutrophentic
Moisture	109	261	443	6	0						**3** aquatic
Hofmann (1994)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	
Trophic state	172	0	8	0	103	292	405	0	20		**6** tolerant
Sabrobity	184	8	123	235	14	289	4	91	34	20	**5** alfa-beta mesosaprobe
Håkansson (1993)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**		
Classes pH	0	0	2	2	117	180	391	16	40		**7** alkaliphilic
Denys (1991)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		
Habitat	166	0	45	482	132	174	0	0	0		**3** tychoplanktonic, epontic origin
Current	184	0	0	32	773	12					**4** indifferent

Results were made of the variations by station of diatom indices in Lake Mogan (*Table 4*). In calculation of diatom indices, 100% of the species identified were used for IDG, 97-100% for IPS, 88-96% for EPI-D, 81-89% for IBD, 76-85% for TDI, 64-67 % for TDIL, 74-85% for TID, 70-83% for CEE, 63-73% for SID, 61-73% for IDSE, 60-73% for SLA, 58-70% for SHE, 53-67% for DI-CH, 44-57% for IDP, 38-50% for DESCY, 35-51% for LOBO, 27-43% for IDAP, and 20-33% for WAT.

Table 4. *The variation of diatom indices according to stations in Lake Mogan*

Station	**DESCY 1-5**	**WAT 0-100**	**TDI 0-100**	**IDG 1-5**	**IPS 1-5**	**IDP 0-4**	**IDP**
1	3.84	68.74	52.56	3.73	3.61	1.63	III
2	3.87	71.72	48.50	3.80	3.66	1.52	III
3	3.96	66.85	52.5	3.68	3.42	1.68	III
4	3.66	69.07	48.15	3.74	3.57	1.67	III
5	3.74	68.74	52.34	3.72	3.56	1.67	III

The correlation between diatom indices and the physical and chemical parameters measured in the lake was examined, and it was found that most of the diatom indices were correlated with the physical and chemical parameters (*Table 5*).

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Table 5. *Correlations between diatom indices and physical-chemical parameters in Lake Mogan (n=55)*

Indices	SLA	DESCY	IDSE	SHE	WAT	TDI	%PT	IDG	CEE	IPS	IBD	IDAP	EPI-D	DI-CH	IDP	LOBO	SID	TID
SD	0.006	-0.379**	0.005	0.277*	0.135	-0.073	-0.193	0.338*	0.168	0.125	0.096	0.073	-0.209	0.002	-0.084	0.234	-0.146	-0.051
Temp	-0.578**	-0.347**	0.521**	0.798**	0.466**	-0.298*	-0.487**	0.716**	0.523**	0.237	0.426**	0.387**	-0.691**	-0.468**	-0.444**	0.666**	-0.462**	-0.411**
pH	0.065	0.250	0.358**	0.378**	-0.388**	-0.673**	-0.626**	0.546**	0.417**	0.797**	0.504**	0.101	-0.011	-0.540**	-0.606**	-0.129	-0.645**	-0.422**
EC	0.257	-0.104	-0.347**	-0.195	-0.317*	0.121	0.311*	-0.151	-0.191	-0.074	-0.178	-0.182	0.122	0.224	0.262	-0.260	0.150	0.094
DO	0.550**	0.465**	-0.286*	-0.561**	-0.561**	0.061	0.132	-0.467**	-0.372**	0.149	-0.128	-0.379**	0.633**	0.163	0.090	-0.614**	0.191	0.205
TP	-0.170	-0.027	0.040	0.148	0.409**	0.112	-0.124	0.100	0.141	-0.051	0.068	0.068	-0.223	-0.120	0.021	0.208	0.043	0.072
TN	-0.180	-0.248	-0.059	0.091	0.414**	0.220	0.145	-0.068	-0.055	-0.235	-0.054	0.077	-0.194	0.128	0.143	0.248	0.097	0.124
SiO_2	0.182	0.201	0.139	0.160	-0.255	-0.380**	-0.568**	0.302*	0.164	0.593**	0.341*	-0.099	0.094	-0.363**	-0.362**	-0.195	-0.260	-0.195

[1]**SLA:** Sládečék Index, **EPI-D:** Eutrophication Pollution Index – Diatoms, **LOBO:** Lobo's Index, **TID:** Rott's Trophic Index**, SID:** Rott's Saprobic Index, **IDP:** Pampean Diatom Index, **DESCY:** Descy's Index, **IDSE:** Leclercq and Maquet's Index, **TDIL:** Trophic Diatom Index for Lakes, **SHE:** Steinberg and Schiefele's Index, **DI-CH:** Swiss Diatom Index, **IPS:** Specific Pollution Index, **IDG**: Generic Diatom Index, **IDAP:** Artois-Picardie Diatom Index, **IBD:** Biological Diatom Index, **CEE:** European Index, **WAT:** Diatom Assemblage Index for Organic Pollution, **TDI:** Trophic Diatom Index, **%PT:** % of Pollution Tolerant Taxa

*$p<0.05$ **$p<0.01$

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Discussion

In this study on Lake Mogan, the ecological quality of the lake was estimated in relation to epiphytic diatoms and various parameters of water quality. Considering the composition of species identified in the study, it was found that most were alkaliphilic species with high oxygen requirements according to Håkansson (1993) and Van Dam et al. (1994). This is supported by the pH and oxygen values of the lake. The saprobity level was estimated as β-mesosaprobic according to Van Dam et al. (1994), and α-β mesosaprobic according to Hofmann (1994). In addition, it was found to contain fresh and saltwater species which prefer water rich in electrolytes. Most species were found in β-mesosaprobic and α-mesosaprobic environments in terms of organic pollution, and in terms of trophic level, most species were eutrophic and organic pollution tolerant. The trophic condition was found to be eutrophic according to Van Dam et al. (1994), and according to Hofmann (1994) as having numerous eutrophication-tolerant species.

The mean IBD, EPI-D, DI-CH, SID, CEE, IDAP and IDSE indices calculated according to the epiphytic diatoms identified in the lake indicated that the water quality of the lake was in class II, and the mean TID index showed a water quality of class II-III. Lake Mogan showed class III (α-mesosaprobic) water quality according to WAT index. Values of 3 and 4 according to DESCY index and of 1.5 and 2.0 according to IDP index indicated medium nutrient-rich waters (Descy, 1979; Gómez and Licursi, 2001). Averages of 3.81 for DESCY index and 1.63 for IDP index were estimated. IDG, IPS, IDP, WAT, DESCY and TDI indices calculated according to epiphytic diatoms indicated class III water quality. According to these values, Lake Mogan is in class III of water quality (critic level pollution and eutrophication). TDIL, developed with the investigation of epiphytic diatoms on *P. australis* in shallow lakes in Hungary, was applied in Lake Mogan, and the proportion of the species used in the index was found to be 64-67%. The indices used in different countries have been developed for water sources in different typologies. It was determined that the greatest number of species was used by IDG. This is because in this index, taxonomy is at the genus level, and this greatly eases its application to many bodies of water. According to chlorophyll *a* and total phosphorus concentration, Lake Mogan is eutrophic. The occurrence of eutrophic and pollution-tolerant species and ecological quality varying from medium to poor on most indices indicated an increase in organic material and eutrophication in Lake Mogan.

According to SLA and SHE indices, Lake Mogan showed class I water quality, while according to the average LOBO index, the lake showed class V water quality. The results of SLA, SHE and LOBO index were not in accordance with water quality parameters and other diatom indices. Therefore, SLA, SHE and LOBO indices are not suitable for use in future water quality monitoring studies in Lake Mogan.

According to the results obtained in this study, it could be said that IDG, IPS, IDP, DESCY, WAT and TDI indices reflected water quality of lake. Various researchers have shown that diatom indices calculated with diatom compositions found in the littoral regions of lakes can be used to reflect their trophic conditions. Blanco et al. (2004) monitored the water quality of six lakes in Spain, and reported that there was a significant correlation between the total nitrogen concentration and IBD and IPS indices, and that this correlation was lower than with phosphorus concentration. Acs et al. (2005) calculated IBD, IPS and IDG indices in monitoring the water quality of Lake Velence in Hungary, and concluded that IBD index could be the most suitable. Lirika et al. (2013) used Trophic Diatom Index (TI_{DIA}) and Saprobic Index (SI) to determine the

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water quality of Lake Ohrid. Kavya and Ulavi (2014) used Louis Leclercq IDSE index in Lakes Karanji and Kukkarahalli in India. Blanco et al. (2004) found IPS suitable in six lakes in Spain, Acs et al. (2005) found IPS and IDG suitable in Lake Velence. In Turkey, Tokatlı (2013) used TDI index in calculating the water quality of Porsuk Reservoir, and reported that the water quality of the lake was mesotrophic.

A significant correlation was found in this study between total phosphorus and total nitrogen concentrations and WAT index. In studies conducted in Turkey on epilithic diatoms in running water, Solak et al. (2007) reported that in Muğla-Akçay (Great Meander river) SLA, DESCY, IDP**,** LMA, WAT, CEE and IDAP indices showed high correlation with chemical variables. Solak (2009), working on Felent river in Kütahya, and Akbulut et al., (2010) on Kızılırmak river, found that IDAP, WAT, CEE and IPS indices had the highest correlations with physical and chemical parameters. Kıvrak et al., (2012) calculated EPI-D, SID, TDI and IDP indices in Akarçay river in Afyonkarahisar, and reported that these four indices, used in Akarçay and sensitive to organic pollution, could be successfully used in evaluating water quality in running waters in Turkey. Gün (2011), used SLA, DESCY, IDSE, SHE, TDI, GENRE, IPS, IBD, EPI-D, DI-CH, IDP, SID and TID indices in the Değirmendere river. It was concluded that the indices which showed the most deviation from physical and chemical parameters were TDI and DESCY. Tokatlı (2012) reported the water quality of the Upper Basin of the Gürleyik river as meso-eutrophic according to TDI index, and as mesotrophic according to IBD index.

In Turkey, apart from a study by Tokatlı (2013) to determine the water quality of Porsuk Reservoir using Trophic Diatom Index (TDI), no study was found monitoring the water quality of a lake using diatom indices. As a result, IDG and IPS are the most suitable for use in future studies of Lake Mogan. IDG, IPS, IDP, DESCY, WAT and TDI indices reflected water quality of lake but IDG and IPS also integrated all diatom taxa within the samples.

Conclusion

In conclusion, epiphytic diatoms can be used to monitor ecological quality in shallow lakes such as Lake Mogan, which are rich in aquatic macrophytes and surrounded by reed belts. Lake Mogan is an important example of a shallow lake which is under threat from pollution even though it has a protected status. Biological monitoring studies must continue concerning the sustainable use of the lake, and steps must be taken to prevent its pollution. It is predicted that the research conducted will contribute to the pool of knowledge for the protection and restoration of the lake.

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IDENTIFICATION OF KEY-TRENDS AND EVALUATION OF CONTEMPORARY RESEARCH REGARDING URBAN ECOSYSTEM SERVICES: A PATH TOWARDS SOCIO-ECOLOGICAL SUSTAINABILITY OF URBAN AREAS

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Abstract. The challenges accompanying socio-ecological and demographic transformations in the urban areas necessitate for coordinated efforts to ensure urban ecological resilience. Trans-disciplinary analytical construct of urban ecosystem services (UES) empowers the policy makers and urban planners to synchronize the orientations of human impacts and resilience of ecological resources in urban areas. The current study provides a systematic overview about the research orientations, approaches and techniques used in the recent studies regarding UES. The study examined: what types of evaluation methods were adopted in the recent UES research? What is their spatial and temporal pattern? What types of UES were focused and environmental components relied upon for the assessment? To address these questions, 116 relevant publications were scrutinized by using a set of assessment criteria. The findings indicated a lesser focus in research towards UES in developing countries as compared to the volume and increasing share of their urban population. The study also establishes that an overwhelming proportion of the UES research was carried out in the industrialized countries of the northern hemisphere but rather skewed towards studying regulatory ecosystem services. The recommendations for improving the relevancy of contemporary research for stakeholders were made.
Keywords: *urban ecosystem services, ecosystem disservices, urbanization, socio-ecological systems, urban environment, resilience, urban ecology*

Introduction

The contributions of natural resources to social and economic systems are referred to as ecosystem services (ES) defined as the benefits humans draw from the functioning and processes of ecological systems (Costanza et al., 1997; Daily et al., 1997; De Groot et al., 2002). It is acknowledged that the life on the planet Earth is a product of and

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dependent upon the constant support and productivity of ecosystems and services arising thereby (De Groot et al., 2002). These benefits from the ecological resources are classified into four major groups or categories i.e. regulatory, cultural, provisioning and supporting services (De Groot et al., 2002; Millennium Ecosystem Assessment, 2005a).

The birth of new urban settlements and/or the increase in the size of existing urban centers at the cost of non-urban land usages pose(s) new social and environmental challenges (Haase et al., 2014b). The increase in the proportion of global urban population (Heilig, 2012; Haase et al., 2014b), transformations in the urban based economic activities and concomitant life style changes in the urban centers have their visible impacts on the natural environment. The conjectured estimations suggest that the process of urbanization will accelerate in the future (Cohen, 2004; United-Nations, 2014; Graça et al., 2017). The promising technological advancements are sometimes misconstrued as a replacement for the natural ecosystems of urban areas. These advancements can supplement the contributions of natural ecological systems in an urban area but are incapable of substituting their role (Honey-Rosés et al., 2014).

The term "ecosystem services", initially appeared in Ehrlich and Ehrlich's work in 1981 (Liu et al., 2010; Chaudhary et al., 2015) while highlighting the contribution of ecological systems for human life. Now the term ES is frequently used for assessing the material contribution of natural resources in human wellbeing. The concept provides a common vocabulary for evaluating tripartite linkages between ecological, social and economic systems to ensure their integrated management. The concept of ES has proven useful, firstly, to synthesize the efforts for linking human and ecological systems for coherence and sustainability (Costanza and Daly, 1987; Daily et al., 2009). Secondly, scientists and policy makers rely on the concept while evaluating economic and political tradeoffs between landscape development and conservation alternatives (De Groot et al., 2010; Mcshane et al., 2011; Bürgi et al., 2015).

The analytical construct of Urban Ecosystem Services (UES) is relied upon for the assessment, management and conservation of the urban ecological resources and their correlation with human life. The paradigm of UES seems to be a more pragmatic strategy to ensure that the impacts of urbanization, climate change and socio-ecological transformations on the urban environment are addressed.

The first studies on UES date back to the mid-1990s (Cairns Jr and Palmer, 1995) but the interest in this sphere had spread worldwide by the end of the last century (Bolund and Hunhammar, 1999). The researchers such as Alavipanah et al. (2017), supported the previous findings of (Gómez-Baggethun et al., 2010; Kronenberg and Hubacek, 2013; Haase et al., 2014b) that less than 10% of ES research in scientific publications was investigated in urban areas and also depicted a decline in the publication concerning UES after 2015.

It transpires from scholarly efforts that the demands for UES are mounting but paradoxically the efforts are less focused to address the challenges associated with the urban environment (Millennium Ecosystem Assessment, 2005b; Haase et al., 2014b). The situation demands a systematic review of UES studies based upon an innovative framework for postulating pragmatic measures to improve the orientations, trends and techniques in research regarding UES.

Keeping in view these expectations, the current study was designed to decipher various dimensions of UES research in recent years. In this connection, the present review was carried out on the basis of selected research publications to analyze: 1) The current spatial-temporal trends in UES research 2) To weigh research orientations,

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approaches, and techniques adopted in the recent studies 3) to evaluate the components of urban environment used for assessments in the reviewed publications. The outcomes of the current study will provide insights and innovative options for integrating efforts to ensure the sustainable provision of UES.

Material and method

The current review is a meta-analysis and is based upon the bibliographic information obtained from ISI web of Knowledge (www.webofknowledge.com). The study considered the articles published in English during the period (May 2007 - May 2017) against the search term "urban ecosystem services'. The search returned a total of 127 records. Out of these, only those records were considered for further scrutiny, where, the search term UES was included in either the Title, Abstract or Keywords of a publication. On these criteria, 116 records were identified for further processing and content analysis (*Appendix-1*).

The content analysis of selected papers, based upon a list of assessment criteria with the predetermined choices (*Table 1*), was carried out to determine the current orientations of or in the UES studies, techniques and measures (monetary/non-monetary) relied upon in the research for assessments and inferences. The information pertaining to environmental components used for evaluating UES was also extracted from the reviewed publications. A component was only included in this study, provided, it was used as a parameter for assessment to publish the article. The components with different appellations, used to assess analogous goals (such as Hedonic Pricing or Property Value; carbon absorption or storage etc.) amalgamated into a single category for brevity and analysis. In this way 10 classes were formulated to interpret trends to select component(s) for assessing UES. The quantitative findings of this analysis were cartographically displayed in *Figures 1-7* for estimations and inferences.

Table 1. *Selection and assessment criteria adopted for the review of selected research publicatons*

Criterion (question)	**Possible entries**
Which type(s) of ES were analysed?	i. Regulating Services ii. Provisioning Services iii. Cultural Services iv. Supporting Services v. Cumulative Assessment of ES vi. Ecosystem Disservices
From which country (city) empirical data/ contextual information were obtained? If required for assessments.	The name of country/city in which the site/situation of study is located.
What is the location of Principal/corresponding author of the study?	The location of author

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What kinds of evaluation methods/indicators were relied upon for assessments in these studies?	i. Monetary ii. Non-Monetary iii. Both (Monetary and Non-Monetary) iv. Not Applicable
What was the principal objective of the study?	The objectives of these publications were grouped into eight categories on the basis of homogeneity in focus: i. Planning and Management ii. Assessment study iii. Governance and Policy iv. Assessment of Method v. Climate Adaption vi. Landuse Planning vii. Urban Ecology viii. Environmental Justice
Which specific component(S) of urban environment was/ were assessed?	The name of component(s) assessed in a publication: i. Aesthetics ii. Biodiversity iii. Carbon Sequestration/Storage iv. Climate Regulation v. Energy vi. Food Fuel vii. Hedonic Pricing viii. Others ix. Socioecological x. Water Management
What type of enquiry technique was used in the study?	i. Assessment (technique)study ii. Experimental (technique)study iii. Exploratory (technique) method iv. Conceptual Framework
What was the principal consideration of the study?	The content analysis revealed that reviewed studies were inherently designed to address the impacts of the following challenge (s): i. urbanization, ii. climate change iii. Loss of urban biodiversity

Results

Spatio-temporal trends

The temporal analysis of these publications in *Figure 1* reflected an upward trend to use the term UES in title, abstract or keywords in the initial years of the selected timeframe. However, a decline in tendency to use the term UES in publications was noticed in the last two years of the selected period.

The *Figure 2* illustrates that a leading share of UES research was carried out in European (62.71%) and North American (23.73%) contextual surroundings. The contributions in UES research from other continents were found disproportionately less as compared to the proportion of people residing in the urban areas of these continents (*Figure 2*).

The spatial analysis of these selected publications revealed (*Figure 3*) that a predominant proportion of UES research (99%) was carried out in the contextual settings of the northern hemisphere while the share from the southern hemisphere was found to be the less than (1%).The significant intra-continental inequalities in the publications regarding UES were also observed (*Figure 3*). In this connection, the

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contributions from Germany (20) are significantly higher than those from other European countries; China (4) is at the forefront from Asia while the USA (24) is the leading country in North America. The city of Berlin (Germany) was most frequently assessed in (9) studies from different perspectives of UES and followed by New York (USA) in (7) and Stockholm (Sweden) in (6) studies (*Appendix-2*).

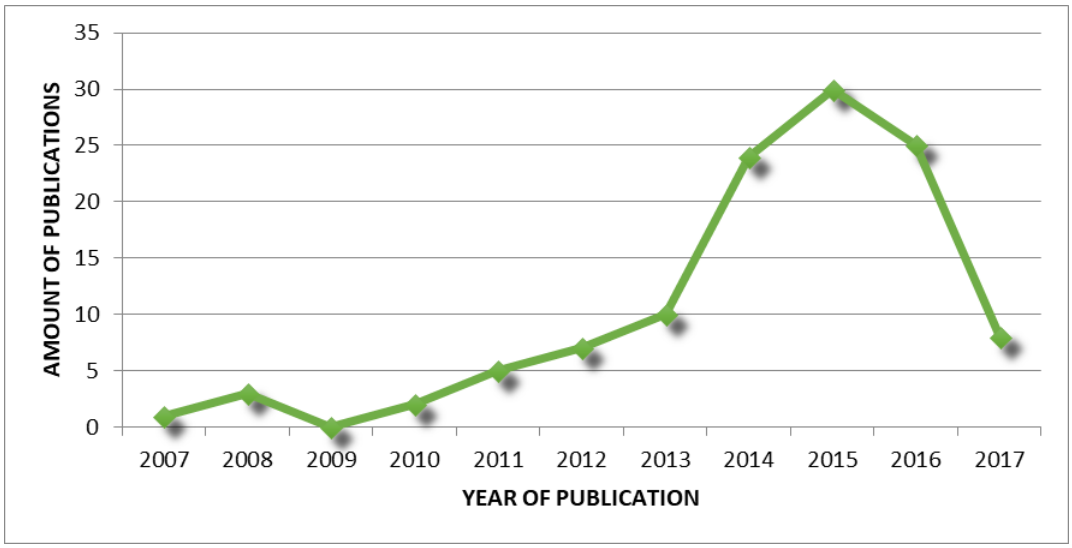


Figure 1. *The year wise distribution of publications which used the term UES during the period (2007-2017)*

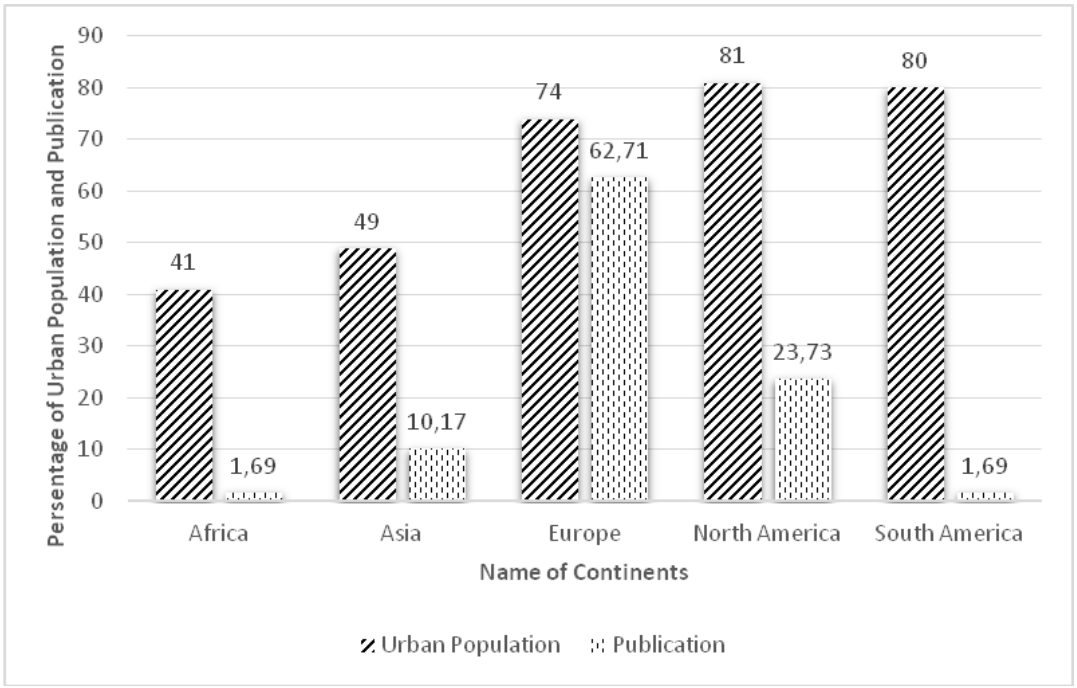


Figure 2. *Graph comparing the contribution in urban ecosystem research and the proportion of urban population of a continent (Urban population data Source: https://www.statista.com)*

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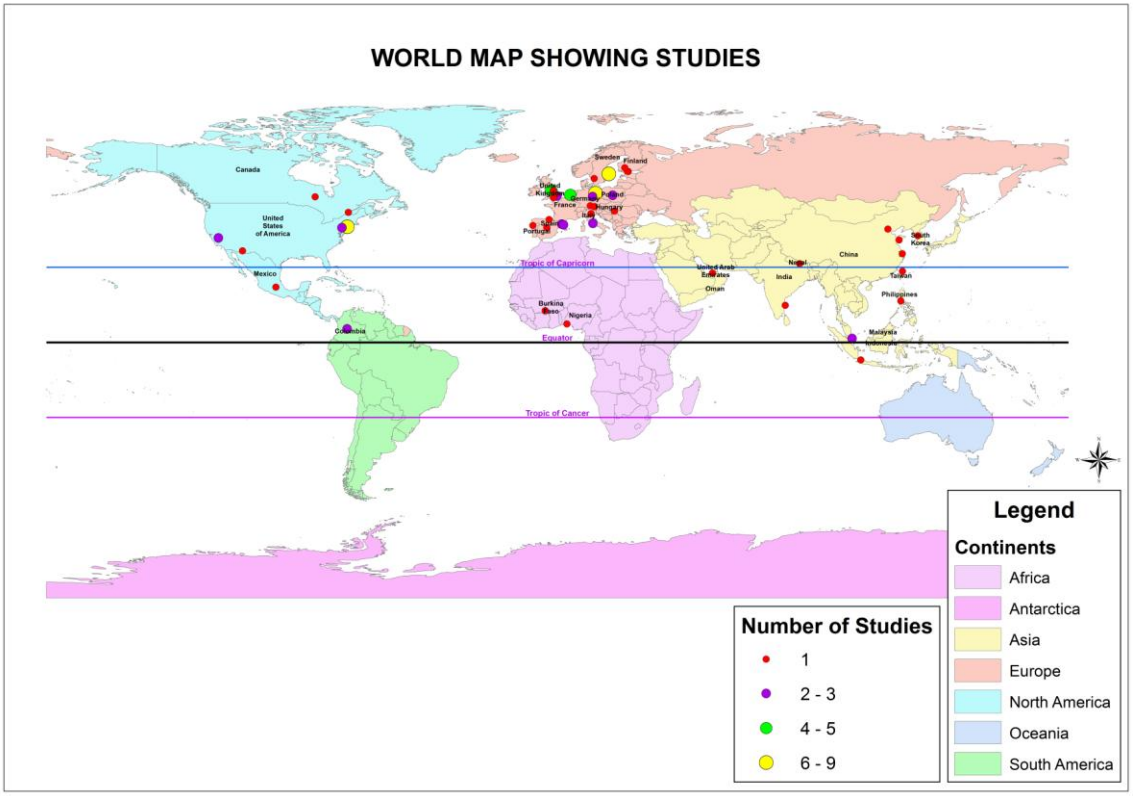


Figure 3. *World map showing locations and the numbers of UES studies*

The use of term UES in the title, abstract or keyword of a publication provides a measure to assess how much importance is given in research on highlighting the contributions of green infrastructure in urban social life. The *Figure 4* explicitly describes the frequency with which the term UES was used in the title, abstract and keywords of reviewed publications. The term urban ecosystem services was most frequently used in the abstracts (70), followed by keywords (56) and titles of (41) the reviewed publications.

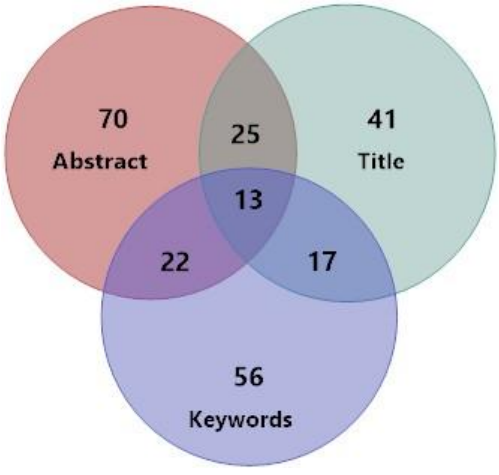


Figure 4. *Showing the use of the term UES in abstract, title and keywords*

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Types of UES and research trends

The intertwined and overlapping nature of contributions from UES makes it more intricate to catalogue a study into a specific category of services. As a pragmatic measure to overcome the problem, a specific study was simultaneously catalogued into different categories of ecosystem services provided these services were tested/evaluated in the publication. In the majority of publications, the focus of research was observed on the cumulative assessment of UES. It was followed by the deliberations on Regulatory services, cultural services and provisioning services. While the supporting services were assessed the least. However, urban ecosystem disservices (UESD) were also focused in (13) publications (*Figure 5*).

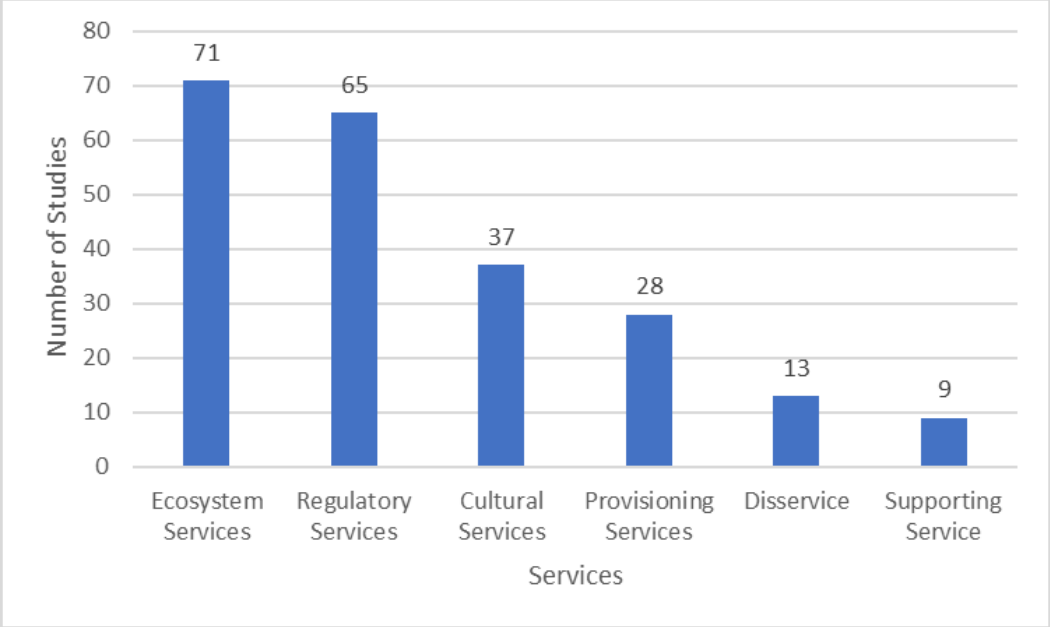


Figure 5. *Graph showing number of studies designed to focus the types of ecosystem services and disservices*

Study paradigms and UES

The impacts of urbanization, ecological degradations in urban areas and threats from global climatic changes are the potential stressors for UES. The content analysis of selected publications was carried out for assessing how much emphasis is being given to these stressors in UES studies. The findings in *Figure 6* revealed an intersecting nature of research inclinations. It also enumerates the number of studies designed to scrutinize the role of these stressors on the resilience of UES.

The trends in UES assessments

The findings of content analysis have been condensed in *Figure 7* to illustrate the numbers and proportion of studies focusing on a particular type of ES: nature of methods/indicators opted for evaluation, types of techniques relied upon for investigations, research or study objective(s) in contemporary research and selection of environmental component(S) for measurements (*Appendix-3*). These findings are indispensable for interpreting contemporary trends in UES studies.

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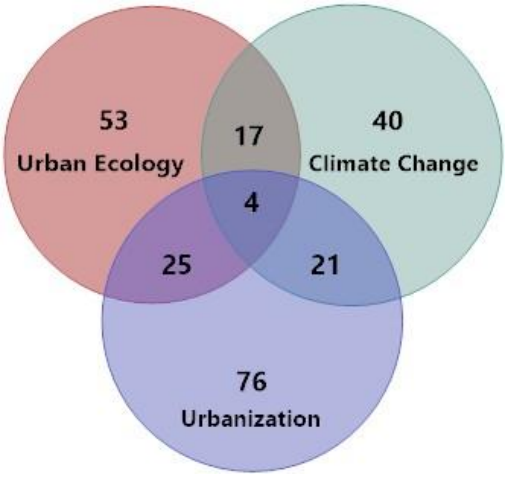


Figure 6. *Showing the focus of recent UES research*

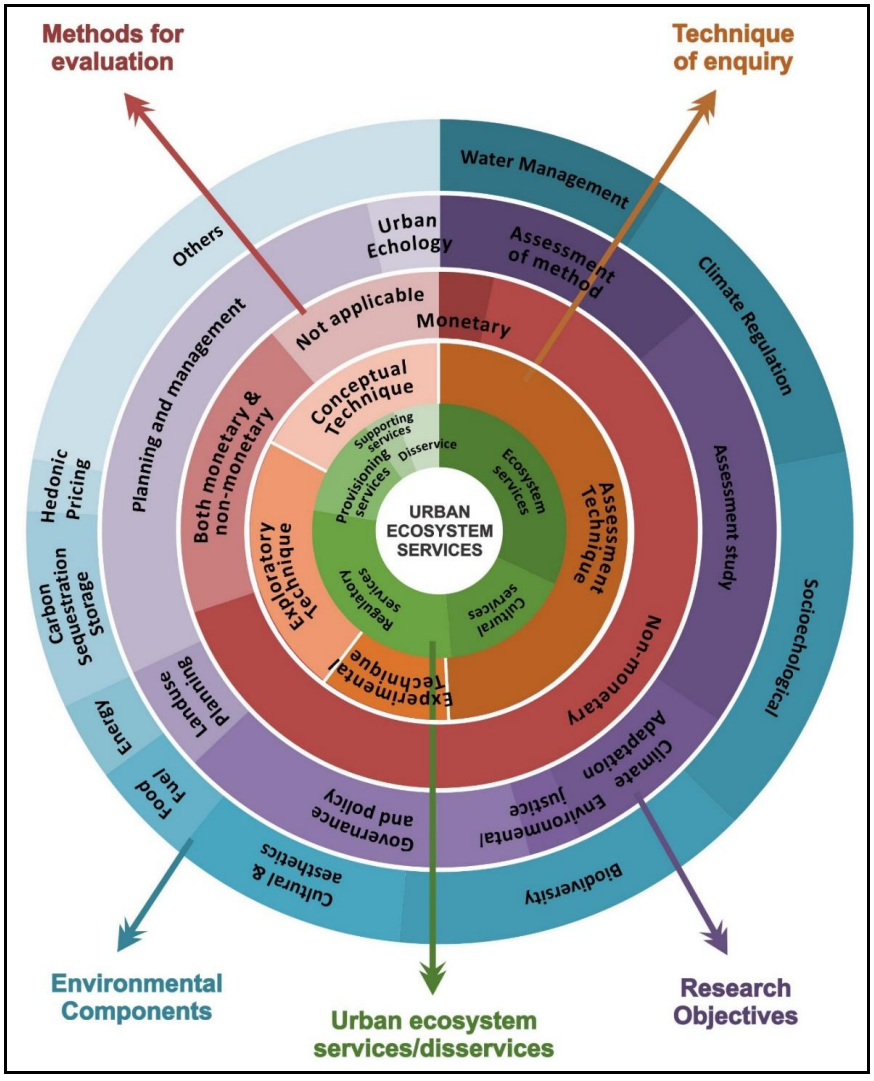


Figure 7. *The Multi-pie diagram showing types and proportion of environmental components, research objectives, evaluating methods, techniques for enquiry and types of UES relied upon in recent research*

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Discussion

Trends and techniques used in UES evaluation

The contingent valuation techniques proposed by Ciriacy-Wantrup in 1947, laid the foundations of Ecosystem Valuation (ESV) in modern times (Ciriacy-Wantrup, 1947; Spash, 2011; Mitchell and Carson, 2013) and a subsequent fervor for environmentalism in the 1960s, providing the much needed impetus to ESV (Liu et al., 2010; Salzman, 2011).

The findings in *Figure 5* indicated that Regulatory services (RS) are more in focus of the contemporary UES research compared to the other three types of ES or ESDS. It is followed by the focus of researchers on studying cultural services (CS) and Provisioning services (PS). In this connection, supporting services (SS) were observed as a lesser priority area of investigation. These dissimilarities in focus towards different types of UES are due to the nature of urban economic activities and socio-cultural life style of urban areas. Besides this, a recent surge in reported incidents of "urban heat island effect" and exacerbating climatic and environmental settings of urban areas are other plausible explanations for this skewedness in favor of RS and CS. Whereas, it is pertinent to mention that the societal acknowledgement of ecological resources meaningfully enhanced by the tangible contribution of PS. Besides this, the resilience of an ecological system is significantly determined by the performance of SS. Therefore more focus in UES research is required on assessing the contribution/role of SS and PS for ensuring sustainable provisions of UES in the face of mounting challenges to urban environment.

A marked emphasis in the majority of the reviewed publications (*Figure 6*) was observed on either 1) to measure the socio-ecological impacts of urbanization and concomitant behavioral changes on provision of UES for informed decision making 2) to assess the potentials of urban ecology for ensuring human wellbeing and urban environmental resilience or 3) to decipher the consequences of global warming and climate change on the supply of UES and socio-ecological sustainability of the urban areas. These propensities in research reflect the growing consciousness for ensuring urban ecological resilience in the face of imminent social, psychological, climatic and environmental vulnerabilities of urban areas.

The assessment or evaluations of an urban phenomenon, situation, policy or problem appeared a preferred technique of enquiry (49.14%) in the recent research (*Figure 7*). This analytical approach was adopted from different perspectives such as assessing the validity of a technological innovation/method (Lakes and Kim, 2012; La Rosa and Wiesmann, 2013; Schreyer and Lakes, 2016; Tigges et al., 2017), the effectiveness of a policy or planning instrument (Kaczorowska et al., 2016; Larondelle et al., 2016), or to estimate the impacts of socio-cultural factors on urban environment in studies (Kremer et al., 2013; Buchel and Frantzeskaki, 2015; Escobedo et al., 2015; Sutton and Anderson, 2016). As compared to this, the Experimental mode of enquiry was observed in a few studies (11.21%) for estimating the potentials of green infrastructure in the given urban context (Taylor et al., 2014; Cameron et al., 2015; Coma et al., 2017) and for assessing sthe role of building material (Capener and Sikander, 2015) in regulating the climate of urban areas. Experimentations with the help of Remote Sensing (RS) and Geographic Information System (GIS) were also made in studies (Tigges et al., 2013; Schreyer and Lakes, 2014) to evaluate their effectiveness for integrated planning of urban ecological resources. The experimentations with new options and opportunities

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are incumbent but resource dependent (financial and technological resources) however, the use of GIS and RS is gaining acceptance among the research communities (Kolanuvada et al., 2016) in the developing countries and in the less developed regions (Thapa, 2012) as well. These findings corroborate the notions that application and acknowledgement of RS and GIS as a research tool for integrated urban environmental managements will increase in the future.

The exploratory style of investigation was observed in (22.41%) studies. The method was specifically adopted in studies to understand patterns of urban land use changes (Haase et al., 2014a; Kain et al., 2016) or to explore nature based solution for the resilience of urban environment (Pataki et al., 2011; Kuittinen et al., 2016; Santiago Fink, 2016). This technique of investigation was also observed in the reviwed publications such as (Ernstson et al., 2008; Connolly et al., 2014; Kronenberg, 2015; Dennis and James, 2016b). These studies were designed to improve the environmental governance and management of urban areas.

The (17.24%) studies based their conclusions on conceptual discourses for postulating measures to ensure the resilience of urban ecosystems. This sort of orientation in UES research was specifically observed in the studies which were conceived to address the challenges of land use changes (Stott et al., 2015; Kain et al., 2016) and socio-cultural transformations in urban areas (Ernstson et al., 2010; Andersson et al., 2014). The researchers also contributed their scholarships through conceptual framework in the studies (Rozos et al., 2013; Wu, 2014; Mcphearson et al., 2015; Haas and Ban, 2017) to improve the precision and practicability of research regarding UES.

Monetary vs. non-monetary parameters for evaluating UES

The publications were assessed to study prevailing inclinations in UES research regarding the use of monetary and/or non-monetary parameters.

The monetary assessments of cost and benefits linked with UES are vital for informed decision making (Aevermann and Schmude, 2015). After the publication of The Economics of Ecosystems and Biodiversity (TEEB) in 2011 the use of monetary parameters in ES research are gaining more recognition. The use of monetary parameters for corroborating findings was found limited (3.45%) and restricted to the assessment mode of investigation. These findings are in line with the assertions of Sutton and Anderson (2016) that the monetary valuations of UES are more intricate and complex as compared to non-monetary valuations of ES. The overlapping nature of the benefits from UES: contestations over classification between benefits and/or services, controversies over methodology used for data acquisition and differences over spatial-temporal scale used for study are the conceivable explanations for less reliance on monetary parameters in the research concerning UES. However, the findings in monetary terms are readily and unambiguously understood in the present age of market economy and, thus, offer an effective technique for disseminating awareness about the contributions of ecological resources. Therefore, further investigations are needed to address the methodological/operational ambiguities responsible for discouraging the use of monetary parameters in UES research.

However, the majority of studies (66.38%), irrespective of their study design or orientation, preferred non-monetary parameters for evaluations of benefits arising from the ecological infrastructure of urban areas (Alam et al., 2016; Dennis and James, 2016b; Grafius et al., 2016; Kain et al., 2016; Kolanuvada et al., 2016; Kuittinen et al.,

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2016). As compared to this a substantial proportion of (18.97%) publications based their conclusions on the cumulative outcomes of monetary and non-monetary parameters (Elmqvist et al., 2015; Kiss et al., 2015; Langemeyer et al., 2015; Czembrowski et al., 2016). The added focus towards the cumulative assessments of UES is needed for holistic appraisal regarding the contributions of natural capital. The outcomes of such a research will ensure more support from social institutions and scientific communities for integrated ecological management of urban areas. Whereas, a number of publications (11.21%) extended their assertions through scholastic discourses or conceptual understandings rather than relying on either monetary or non-monetary measurements such as (Wu, 2014; Frantzeskaki and Kabisch, 2016; Corburn, 2017). These orientations, in the contemporary research, are indispensable for postulating out of the box strategies for uninterrupted supply of UES in the face of accelerating urbanization, ecological degradation and looming threats from climate change.

Study components and UES evaluation

The environmental component(s), relied upon for assessments in UES studies also reflect the inclinations of contemporary research. The findings in *Figure 7* indicate the proportion of studies which focused on a particular component of urban environment for assessment.

The anthropocentric orientations in UES research are stressed (Alberti et al., 2003; Graça et al., 2017) for integrated urban environmental management. In line with these demands a substantial number of studies (n=69) relied on the cumulative outcomes of socio-ecological parameters for drawing inferences and estimations regarding UES from diversified contextual settings such as (Ernstson et al., 2008; Buchel and Frantzeskaki, 2015; Escobedo et al., 2015; Vollmer et al., 2015; Graça et al., 2017).

It was followed by the selection of components related to urban biodiversity (n=64). The assessments about different forms of urban green infrastructures such as street trees (Kiss et al., 2015; Mcpherson et al., 2016), urban forests (Baró et al., 2014; Fusaro et al., 2015), parks (Sutton and Anderson, 2016) and impacts of urban vegetative diversity (Wang et al., 2015; Calderón-Contreras and Quiroz-Rosas, 2017) were made for different purposes ranging from the assessments of services and disservices of urban ecological infrastructure to urban planning.

A significant number of studies (n=57) in the 3rd category based their findings on atmospheric components of the urban environment. The measurements and quantifications of atmospheric temperature (Taylor et al., 2014; Di Leo et al., 2016) humidity (Capener and Sikander, 2015) and gaseous components (Manes et al., 2012) were made in these studies from various aspects while evaluating their impacts on the urban environment. The acquisition of accurate, cost effective and time efficient data related to atmospheric components at different spatial scale have become possible and easier due to advancements in the atmospheric and remote sciences (Larondelle and Lauf, 2016). In this connection a growing reliance on remotely sensed data for evaluating different components of urban climate was also observed.

The component of water was analyzed in the 4th category (n=42) from diversified perspectives for efficient use and management of urban blue infrastructure. The dominant orientations in the urban water studies were found towards accurate measurement of urban water resources (Larondelle and Lauf, 2016), to illustrate the importance of the rivers for urban residents (Vollmer et al., 2015; Weber and Ringold, 2015) and to postulate measures to ensure the efficient use of available water (Mccarthy

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et al., 2011). A growing interest to evaluate the impacts and complementing ability of technological innovations on urban water supply was also observed in studies (Rozos et al., 2013; Honey-Rosés et al., 2014). The components relied upon for assessment of cultural/aesthetic services such as the availability, role and importance of parks and urban green spaces were adjudged in the 5th category (n=42).

Urban climatic anomalies such as the urban heat island effect, smog, haze and resultant global warming are attributed to imbalance in the atmospheric carbon emanating from industrial, vehicular and domestic sources which are mostly located in the urban areas (Rosenfeld et al., 1998; Di Leo et al., 2016). These unwarranted climatic incidents induce researchers to strive for 'green oriented' solutions to control carbon emissions and concentration in the urban areas. In response to these challenges the focus of (n=35) studies in the 6th category was observed for finding plausible solutions to control and mitigate the adverse impacts of carbon concentrations in urban areas (Kuittinen et al., 2016; Tigges et al., 2017).

The assessments were made in (n=18) studies to evaluate the contribution of the urban ecological resources in providing food and fuel to urban residents and to weigh their contributions in managing the energy requirements of the urban areas in (n=14) studies. The market price of dwellings was also used as a proxy variable in (n=9) studies to assess the interrelationship between urban environments and worth of the property.

Besides these, a large number of other components (n=100) of urban environments from diversified settings were also used as parameters to evaluate the role and significance of UES for the urban areas.

Focus of research in UES

The studies in this review were designed to achieve multiple and diverse objectives, ranging from resolution of local environmental concerns to philosophical discourses for improved performance and resilience of urban ecological capital. The objectives found in the selected publications reflect the focus of these studies.The objectives set to achieve identical targets by using different linguistic expressions were condensed into eight groups for brevity and analysis (*Table 1*).The findings in *Figure 7* reflect the proportion of studies designed to achieve a specific target.

The sustainable planning and management of UES was observed as the most common objective of (28.45%) publications such as (Kronenberg, 2015; Stott et al., 2015; Vollmer et al., 2015; Alam et al., 2016; Dennis and James, 2016b). A closer scrutiny of the data indicates that such orientations in the research are closely associated with the industrialized nations. The appraisal of governance and policy associations with UES in (17.24%) studies appeared as the next important stream of investigation. The studies such as (Ernstson et al., 2010; Connolly et al., 2013; Connolly et al., 2014; Kronenberg, 2015; Frantzeskaki and Kabisch, 2016) were carried out to decipher the effects of environmental governance and policy framework on their contextual urban environment.

The assessments of context specific phenomena and practices are also stressed in the recent literature (Schreyer and Lakes, 2016) to get insights for optimal utilization and resilience of urban ecological resources. The focus of (20.69%) publications such as (Honey-Rosés et al., 2014; Youngsteadt et al., 2015; Dennis and James, 2016a; Kain et al., 2016) were found to estimate the impacts of contextual occurrences on the provisions of UES.

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To test the viability/application of new methods, techniques and approaches in the research are needed and validated in (13.79%) research initiatives regarding UES such as (Lundy and Wade, 2011; Lakes and Kim, 2012; Pincetl, 2012; Tigges et al., 2013). The use of GIS and RS for measuring, evaluating and planning of urban ecological resources is gaining acceptance. The researchers preferred to rely on the data retrieved through RS and GIS for detecting Urban Land use changes (Thapa, 2012; La Rosa and Wiesmann, 2013), estimating carbon storage/ sequestration (Baró et al., 2014; Tigges et al., 2017), assessing water availability (Yao et al., 2015) and for the measurements of urban vegetative cover (Banzhaf and Kollai, 2015; Schreyer and Lakes, 2015; Kolanuvada et al., 2016; Schreyer, 2016; Schreyer and Lakes, 2016). Besides this, a growing trend of computer-aided modelling for better utility and conservation of urban ecological resources was observed in this review (Grêt-Regamey et al., 2013). The scholastic efforts of (Huang et al., 2011; Inostroza, 2014; Martinico et al., 2014) for improvements in methodologies, techniques and approaches for better assessments of potentials and pressures on UES were also observed in this review.

The focus in studies were also observed on postulating measures to mitigate the consequences of creeping global warming for urban areas. The orientations of (8.62%) publications such as (Geneletti and Zardo, 2016) were found towards conjecturing on adaptive measures for the sustainability of urban environmental health, to address the challenges associated with global warming and consequential climate change. The researchers like (Mcwilliam et al., 2014; Estoque and Murayama, 2015; Grafius et al., 2016; Kaczorowska et al., 2016) tried to decipher the imprints of Urban Land use changes (5.17%) on sustainable provision of UES and highlighted the importance of land use planning for urban environmental resilience in their studies. The content analysis also indicated a growing emphasis in UES research on the accurate assessments of urban ecological resources in (3.45%) studies such as (Wu, 2014; Wang et al., 2015).

Besides, these dominant orientations in UES research a growing propensity in recent studies (2.59%) was also noticed in studies such as (Corburn, 2017) to ensure equitable distribution of UES among urban inhabitants under the influence of Environmental Justice debate.

Conclusions

This study is based on a systematic review protocol applied on a set of 116 scientific publications. It provides an overview of the evolving trends and gaps in UES research. The most obvious finding of this overview is that the concept of urban ecosystem services is gaining recognition as a component of informed decision making in urban planning and as a tool to monitor socio-ecological resilience of the urban areas. This review also establishes the fact that the research regarding UES is more focused in the technologically advanced and economically developed countries which have a really significant exposure to urban based industrial activities. The appearance of environmental externalities due to earlier industrialization, pressures from the society for healthy urban environment and availability of resources to fulfill these demands are the plausible explanations for more determined efforts to ensure resilience of UES in these countries. However, the insights of these studies are also immensely important for the regions where the research regarding UES is still in its embryonic stages.

The majority of publications based their appraisals on non-monetary parameters instead of monetary measurements. However, the evaluation of ecological contributions

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in monetary terms increases the acceptability of scientific findings and encourages policy makers/planners to adopt and utilize these findings.

A greater proportion of studies in this review either relied on assessment or an exploratory mode of investigations. These research techniques are frequently used in the social sciences and are comparatively easier, however an inherent element of subjectivity associated with them may compromise the objectivities in findings. As compared to it, a reliance on experimental mode of enquiry in UES will augment the credibility of findings. However, the conceptual style of investigation is imperative for postulating novice approaches for integrated management of UES in the face of emerging challenges for the urban areas.

The outcomes of this study stress on further investigations for devising standard protocols for the monetary measurements of UES. These initiatives should, hopefully, help to overcome the operational and methodological ambiguities for assessments of UES. The future research collaborations between and among nations based upon interdisciplinary research paradigm seems a viable option to achieve this objective. The trans-national research collaborations between the developed world and the less developed regions are also incumbent for postulating comprehensive strategies. These collaborations will also provide the opportunities to retrieve data from the contextual settings of these less investigated regions for conceptual discourses at the global level. Furthermore, these initiatives will directly and indirectly extend the much needed exposure and technical support to researchers investigating UES in these regions.

It is the considered opinion of the authors of this research contribution that the Sustained focus in research on UES is more needed in the less developed regions of the world. In these geographical regions the poor are the worst victims of urban ecological degradation. Thus, sustainable and equitable provisions of UES in these regions is a question of equity and justice debate and a real challenge for the researchers and urban planners to address.

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APPENDIX

Appendix 1. *116 selected and reviewed articles*

Author Name(s)	Title	Source Title	Year	Vol.	Issue	P. Start	P. End	Doi
Juntti Meri, Lundy Lian	A mixed methods approach to urban ecosystem services: Experienced environmental quality and its role in ecosystem assessment within an inner-city estate	Landscape and Urban Planning	2017	161		10	21	10.1016/j.landurbplan.2017.01.002
Dan Friess	Singapore as a long-term case study for tropical urban ecosystem services	Urban Ecosystems	2017	20		277	291	10.1007/s11252-016-0592-7
Chunglim Mak, Miklas Scholz, Philip James	Sustainable drainage system site assessment method using urban ecosystem services	Urban Ecosystems	2017	20		293	307	10.1007/s11252-016-0593-6
Jason Corburn	Urban Place and Health Equity: Critical Issues and Practices	International Journal of Environmental Research and Public Health	2017	14				10.3390/ijerph14020117
Jan Haas, Yifang Ban	Mapping and Monitoring Urban Ecosystem Services Using Multitemporal High-Resolution Satellite Data	IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing	2016	10		669	680	10.1109/JSTARS.2016.2586582
Jan Tigges, Galina Churkina, Tobia Lakes	Modeling above-ground carbon storage: a remote sensing approach to derive individual tree	Urban Ecosystems	2017	20		97	111	10.1007/s11252-016-0585-6

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	species information in urban settings							
Marisa S. Graca, Joao F. Goncalves, Paulo J.M. Alves, David J. Nowak, Robert Hoehn, Alexis Ellis, Paulo Farinha-Marques, Mario Cunha	Assessing mismatches in ecosystem services proficiency across the urban fabric of Porto (Portugal): The influence of structural and socioeconomic variables	Ecosystem Services	2017	23		82	93	10.1016/j.ecoser.2016.11.015
Rafael Calderon-Contrerasa, Laura Elisa Quiroz-Rosasb	Analysing scale, quality and diversity of green infrastructure and the provision of Urban Ecosystem Services: A case from Mexico City	Ecosystem Services	2017	23		127	137	10.1016/j.ecoser.2016.12.004
Sadroddin Alavipanaha, Dagmar Haasea, Tobia Lakes,Salman Qureshi	Integrating the third dimension into the concept of urban ecosystem services: A Review	Ecological Indicators	2017	72		374	398	10.1016/j.ecolind.2016.08.010
Julia Coma,Gabriel Perez,Alvaro de Gracia,Silvia Bures,Miguel Urrestarazu,Luisa F. Cabeza	Vertical greenery systems for energy savings in buildings: A comparative study between green walls and green facades	Building and Environment	2017	111		228	237	10.1016/j.buildenv.2016.11.014
Neele Larondelle, Steffen Lauf	Balancing demand and supply of multiple urban ecosystem services on different spatial scales	Ecosystem Services	2016	22		18	31	10.1016/j.ecoser.2016.09.008
Kaczorowska Annaa,Kain Jaan-Henrik,Kronenberg Jakub,Haase Dagmar	Ecosystem services in urban land use planning: Integration challenges in complex urban settings-Case of Stockholm	Ecosystem Services	2016	22		204	212	10.1016/j.ecoser.2015.04.006
Johannes Schreyer,Tobia Lakes	Deriving and Evaluating City-Wide Vegetation Heights from a TanDEM-X DEM	Remote Sensing	2016	11	8	777	780	10.3390/rs8110940

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Jaan-Henrik Kaina,Neele Larondelleb,Dagmar Haaseb, Anna Kaczorowskaa	Exploring local consequences of two land-use alternatives for the supply of urban ecosystem services in Stockholm year 2050	Ecological Indicators	2016	70		615	629	10.1016/j.ecolind.2016.02.062
Neele Larondelle,Niki Frantzeskaki,Dagmar Haasea	Mapping transition potential with stakeholder- and policy-driven scenarios in Rotterdam City	Ecological Indicators	2016	70		630	643	10.1016/j.ecolind.2016.02.028
Piotr Czembrowski,Jakub Kronenberg,Michał Czepkiewicz	Integrating non-monetary and monetary valuation methods - SoftGIS and hedonic pricing	Ecological Economics	2016	130		166	175	10.1016/j.ecolecon.2016.07.004
Theodore S. Eisenman	GREENING CITIES IN AN URBANIZING AGE The Human Health Bases in the Nineteenth and Early Twenty-first Centuries	Change Over Time	2016	6	1	216	246	10.1353/cot.2016.0014
Vaino Nurmi,Athanasios Votsis,Adriaan Perrels,Susanna Lehvävirta	Green Roof Cost-Benefit Analysis: Special Emphasis on Scenic Benefits	Journal of Benefit-Cost Analysis	2016	7	3	488	522	10.1017/bca.2016.18
Tom L. Green,Jakub Kronenberg,Erik Andersson,Thomas Elmqvist,Erik Go´mez-Baggethun	Insurance Value of Green Infrastructure in and Around Cities	Ecosystems	2016	19	6	1051	1063	10.1007/s10021-016-9986-x
Darren R. Grafius,Ron Corstanje,Philip H. Warren,Karl L. Evans,Steven Hancock,Jim A. Harris	The impact of land use/land cover scale on modelling urban ecosystem services	Landscape Ecology	2016	31	7	1509	1522	10.1007/s10980-015-0337-7
Matti Kuittinen,Caroline Moinel,Kristjana Adalgeirsdottir	Carbon sequestration through urban ecosystem services A case study from Finland	Science of the Total Environment	2016	563-564		623	632	10.1016/j.scitotenv.2016.03.168
Peleg Kremera,Zoé A. Hamsteadb,Timon McPhearsona	The value of urban ecosystem services in New York City: A	Environmental Science & Policy	2016	62		57	68	10.1016/j.envsci.2016.04.012

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	spatially explicit multicriteria analysis of landscape scale valuation scenarios							
Niki Frantzeskakia,Nadja Kabischb	Designing a knowledge co-production operating space for urban environmental governance-Lessons from Rotterdam, Netherlands and Berlin, Germany	Environmental Science & Policy	2016	62		90	98	10.1016/j.envsci.2016.01.010
Gregory McPhersona,Natalie van Doornb, John de Goede	Structure, function and value of street trees in California, USA	Urban Forestry & Urban Greening	2016	17		104	115	10.1016/j.ufug.2016.03.013
Paul C.Sutton,SharolynJ.Anderson	Holistic valuation of urban ecosystem services in New York City's Central Park	Ecosystem Services	2016	19		87	91	10.1016/j.ecoser.2016.04.003
Nestor Di Leo,Francisco J. Escobedo,Marielle Dubbeling	The role of urban green infrastructure in mitigating land surface temperature in Bobo-Dioulasso, Burkina Faso	Environment Development and Sustainability	2015	392	2	373	392	10.1007/s10668-015-9653-y
Helen Santiago Fink	Human-Nature for Climate Action: Nature-Based Solutions for Urban Sustainability	Sustainability	2016	8	3	254	264	10.3390/su8030254
Tommaso Sitzia,Thomas Campagnaro,Robert George Weir	Novel woodland patches in a small historical Mediterranean city: Padova, Northern Italy	Urban Ecosystems	2015	19	1	475	487	10.1007/s11252-015-0475-3
M. Dennis, P.James	Site-specific factors in the production of local urban ecosystem services: A case study of community-managed green space	Ecosystem Services	2016	17		208	216	10.1016/j.ecoser.2016.01.003
Sanna-Riikka Saarela, Janne Rinne	Knowledge brokering and boundary work for	Ecological Indicators	2016	61	1	49	62	10.1016/j.ecolind.2015.07.016

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	ecosystem service indicators. An urban case study in Finland							
Srinivasa Raju Kolanuvada,Muneeswaran Mariappan,Vani Krishnan	Demand-Based Urban Forest Planning using High Resolution Remote Sensing and AHP	Lidar Remote Sensing for Environmental Monitoring XV	2016	9879				10.1117/12.2223832
J.E. Taylor, R.W.F. Cameron, M.R. Emmett	The role of shrubs and climbers on improving thermal performance of brick walls during winter	International Conference on Landscape and Urban Horticulture and International Symposium on Sustainable Management in the Urban Forest	2016	1108		353	359	10.17660/ActaHortic.2016.1108.47
M. Dennis,P. James	User participation in urban green commons: Exploring the links between access, voluntarism, biodiversity and well being	Urban Forestry & Urban Greening	2016	15		22	31	10.1016/j.ufug.2015.11.009
Peleg Kremer,Zoé Hamstead,Dagmar Haase,Timon McPhearson,Niki Frantzeskaki,Erik Andersson,Nadja Kabisch,Neele Larondelle,Emily L. Rall,Annette Voigt ,Francesc Baró,Christine Bertram,Erik Gómez-Baggethun ,Rieke Hansen,Anna Kaczorowska,Jaan-Henrik Kain,Jakub Kronenberg,Johannes Langemeyer,Stephan Pauleit,Katrin Rehdanz,Maria Schewenius,Chantal van Ham,Daniel Wurster,Thomas Elmqvist	Key insights for the future of urban ecosystem services research	Ecology and Society	2016	21				10.5751/ES-08445-210229
Davide Geneletti,Linda Zardoa	Ecosystem-based adaptation in cities: An analysis of European urban climate adaptation plans	Land Use Policy	2016	50		38	47	10.1016/j.landusepol.2015.09.003
Mahbubul Alama,Jérôme Duprasb,Christian Messierba	A framework towards a composite indicator for	Ecological Indicators	2016	60		38	44	10.1016/j.ecolind.2015.05.035

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	urban ecosystem services							
Alison R.Holt,Meghann Mears,Lorraine Maltby,Philip Warren	Understanding spatial patterns in the production of multiple urban ecosystem services	Ecosystem Services	2015	16		33	46	10.1016/j.ecoser.2015.08.007
Ronald C. Estoque,Yuji Murayama	Intensity and spatial pattern of urban land changes in the megacities of Southeast Asia	Land Use Policy	2015	48		213	222	10.1016/j.landusepol.2015.05.017
Hua-Feng Wang,Salman Qureshi,Sonja Knapp,Cynthia Ross Friedman,Klaus Hubacek	A basic assessment of residential plant diversity and its ecosystem services and disservices in Beijing, China	Applied Geography	2015	64		121	131	10.1016/j.apgeog.2015.08.006
Ross W.F. Cameron ,Jane Taylor,Martin Emmett	A Hedera green facade - Energy performance and saving under different maritime-temperate, winter weather conditions	Building and Environment	2015	92		111	121	10.1016/j.buildenv.2015.04.011
Fangfang Yao,Chao Wang,Di Dong,Jiancheng Luo,Zhanfeng Shen,Kehan Yang	High-Resolution Mapping of Urban Surface Water Using ZY-3 Multi-Spectral Imagery	Remote Sensing	2015	7	9	12336	12355	10.3390/rs70912336
Iain Stott,Masashi Soga,Richard Inger,Kevin J Gaston1	Land sparing is crucial for urban ecosystem services	Frontiers in Ecology and the Environment	2015	13	7	387	393	10.1890/140286
Christopher Luederitz,Ebba Brink,Fabienne Gralla ,Verena Hermelingmeier,Moritz Meyer,Lisa Niven,LarsPanzer,Stefan Partelowb,Anna-LenaRau,Ryuei Sasaki,DavidJ.Abson,DanielJ.Lang,Christine Wamsler,Henrik von Wehrden	A review of urban ecosystem services: six key challenges for future research	Ecosystem Services	2015	14		98	112	10.1016/j.ecoser.2015.05.001
Marthe L. Derkzen,Astrid J. A. van Teeffelen,Peter H. Verburg	REVIEW Quantifying urban ecosystem	Applied Ecology	2015	52	4	1020	1032	10.1111/1365-2664.12469

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	services based on high-resolution data of urban green space: an assessment for Rotterdam, the Netherlands							
T Elmqvist,H Setala,SN Handel,S van der Ploeg,J Aronson,JN Blignaut,E Gomez-Baggethun,DJ Nowak,J Kronenberg,R de Groot	Benefits of restoring ecosystem services in urban areas	Environmental Sustainabilty	2015	14		101	108	10.1016/j.cosust.2015.05.001
D. Rodriguez-Rodriguez,J.H. Kain,D. Haase,F. Baro, A. Kaczorowska	Urban self-sufficiency through optimised ecosystem service demand. Autopian perspective from European cities	Futures	2015	70		13	23	10.1016/j.futures.2015.03.007
Derek Vollmera,Michaela F.Prescotta,Rita Padawangib, Christophe Girota,Adrienne Grêt-Regameyd	Understanding the value of urban riparian corridors: Considerations in planning for cultural services along an Indonesian river	Landscape and Urban Planning	2015	138		144	154	10.1016/j.landurbplan.2015.02.011
Kathleen L. Wolf, Alicia S.T. Robbins	Metro Nature, Environmental Health, and Economic Value	Environmental Health Perspect	2015	123	5	390	398	10.1289/ehp.1408216
Peleg Kremer, Erik Andersson, Timon McPhearson, Thomas Elmqvist	Advancing the frontier of urban ecosystem services research	Ecosystem Services	2015	12		149	151	10.1016/j.ecoser.2015.01.008
Timon McPhearson,Erik Andersson,Thomas Elmqvist,Niki Frantzeskaki	Resilience of and through urban ecosystem services	Ecosystem Services	2014	12		152	156	10.1016/j.ecoser.2014.07.012
Sophie Buchel,Niki Frantzeskaki	Citizens' voice: A case study about perceived ecosystem services by urban park users in Rotterdam, the Netherlands	Ecosystem Services	2014	12		169	177	10.1016/j.ecoser.2014.11.014
Johannes Langemeyer,Francesc Baró,Peter Roebeling,Erik Gómez-Baggethun	Contrasting values of cultural ecosystem	Ecosystem Services	2014	12		178	186	10.1016/j.ecoser.2014.11.016

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	services in urban areas: The case of park Montjuic in Barcelona							
Christine Bertram, Katrin Rehdanz	Preferences for cultural urban ecosystem services: Comparing attitudes, perception, and use	Ecosystem Services	2014	12		187	199	10.1016/j.ecoser.2014.12.011
Jakub Kronenberg	Why not to green a city? Institutional barriers to preserving urban ecosystem services	Ecosystem Services	2014	12		218	227	10.1016/j.ecoser.2014.07.002
Elsa Youngsteadt,Ryanna C. Henderson,Amy M. Savage,Andrew F. Ernst,Robertr.Dunn,Steven D. Frank	Habitat and species identity, not diversity, predict the extent of refuse consumption by urban arthropods	Global Change Biology	2015	21	3	1103	1115	10.1111/gcb.12791
Banzhaf E, Kollai, H.	MONITORING THE URBAN TREE COVER FOR URBAN ECOSYSTEM SERVICES - THE CASE OF LEIPZIG, GERMANY	The International Archives of Photogrammetry, Remote Sensing and Spatial Information Sciences	2015	XL.7	7	301	305	
Johannes Schreyer, Prof. Dr. Tobia Lakes	TanDEM-X & UAV data for modeling 3D vegetation information in urban areas REPEAT AT 84	Urban Remote Sensing Event	2015					10.1109/JURSE.2015.7120526
Jeannette Sieber, Manon Pons	Assessment of Urban Ecosystem Services using Ecosystem Services Reviews and GIS-based Tools	Procedia Engineering	2015	115		53	60	10.1016/j.proeng.2015.07.354
Carl-Magnus Capener,Eva Sikander	Green building envelopes - Moisture safety in ventilated light-weight building envelopes	Procedia Engineering	2015	78		3458	3464	10.1016/j.egypro.2015.11.179
Francisco J. Escobedo,Nicola	Socio-ecological	Urban Forestry & Urban	2015	14	4	1040	1053	10.1016/j.ufug.2015.09.011

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Clerici,Christina L. Staudhammer,Germán Tovar Corzo	dynamics and inequality in Bogota, Colombia's public urban forests and their ecosystem services	Greening						
Lina Fusaro,Elisabetta Salvatori,Simone Mereu,Federica Marando,Elisabetta Scassellati,Giovanna Abbate,Fausto Manes	Urban and peri-urban forests in the metropolitan area of Rome: Ecophysiological response of Quercus ilex L. in two green infrastructures in an ecosystem services perspective	Urban Forestry & Urban Greening	2015	14	4	1147	1156	10.1016/j.ufug.2015.10.013
Tim Aevermann,Jürgen Schmude	Quantification and monetary valuation of urban ecosystem services in Munich, Germany	The German Journal of Economic Geography	2015	59	3			10.1515/zfw-2015-0304
O. Adegun, S. Odunuga, O. S. Ajayi	Climate, runoff and landuse trends in the Owo River Catchment in Nigeria HYDROLOGIC NON-STATIONARITY AND EXTRAPOLATING MODELS TO PREDICT THE FUTURE	Proceedings of the International Association of Hydrological Sciences; Gottingen	2015	371		137	142	10.5194/piahs-371-137-2015
Kathleen L. Wolf,Marcus K. Measells,Stephen C. Grado,Alicia S.T. Robbins	Economic values of metro nature health benefits: A life course approach	Urban Forestry & Urban Greening	2015	14	3	694	701	10.1016/j.ufug.2015.06.009
Morton KISS ,Agnes TAKACS,Réka POGACSAS,Agnes GULYAS	The role of ecosystem services in climate and air quality in urban areas: Evaluating carbon sequestration and air pollution removal by street and park trees in Szeged (Hungary)	MORAVIAN GEOGRAPHICAL REPORTS	2015	23	3			10.1515/mgr-2015-0016

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Nadja Kabisch	Ecosystem service implementation and governance challenges in urban green space planning-The case of Berlin, Germany	Land Use Policy	2015	42		557	567	10.1016/j.landusepol.2014.09.005
Matthew A. Weber,Paul L. Ringold	Priority river metrics for residents of an urbanized arid watershed	Landscape and Urban Planning	2015	133		37	52	10.1016/j.landurbplan.2014.09.006
Wei Li,Jean-Daniel M. Saphores,Thomas W. Gillespie	A comparison of the economic benefits of urban green spaces estimated with NDVI and with high-resolution land cover data	Landscape and Urban Planning	2015	133		105	117	10.1016/j.landurbplan.2014.09.013
James J.T. Connolly,Erika S. Svendsen,Dana R. Fisher,Lindsay K. Campbell	Networked governance and the management of ecosystem services: The case of urban environmental stewardship in New York City	Ecosystem Services	2015	10		187	194	10.1016/j.ecoser.2014.08.005
Dagmar Haase,Annegret Haase,Dieter Rink	Conceptualizing the nexus between urban shrinkage and ecosystem services	Landscape and Urban Planning	2015	132		159	169	10.1016/j.landurbplan.2014.09.003
Yafei Wang,Frank Bakker,Rudolf de Groot,Heinrich Wörtche	Effect of ecosystem services provided by urban green infrastructure on indoor environment: A literature review	Building and Environment	2014	77		88	100	10.1016/j.buildenv.2014.03.021
Luis Inostroza	Measuring urban ecosystem functions through Technomass'-A novel indicator to assess urban metabolism	Ecological Indicators	2014	42		10	19	10.1016/j.ecolind.2014.02.035
Iris Lehmann,Juliane Mathey,Stefanie Rößler,Anne Bräuer,Valeri Goldberg	Urban vegetation structure types as a	Ecological Indicators	2014	42		58	72	10.1016/j.ecolind.2014.02.036

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	methodological approach for identifying ecosystem services - Application to the analysis of micro-climatic effects							
Jordi Honey-Rose,Nicholas Brozovic,Daniel W. Schneider	Changing Ecosystem Service Values Following Technological Change	Environmental Management	2014	53	6	1146	1157	10.1007/s00267-014-0270-6
Francesco Martinico, Daniele La Rosa, Riccardo Privitera	Green oriented urban development for urban ecosystem services provision in a medium sized city in southern Italy	Biogeosciences and Forestry	2014	7		385	395	10.3832/ifor1171-007
Neele Larondelle,Dagmar Haase,Nadja Kabisch	Mapping the diversity of regulating ecosystem services in European cities	Global Enviornmental Change	2014	26		119	129	10.1016/j.gloenvcha.2014.04.008
Jianguo Wu	Urban ecology and sustainability: The state-of-the-science and future directions	Landscape and Urban Planning	2014	125		209	221	10.1016/j.landurbplan.2014.01.018
Jack Ahern,Sarel Cilliers,Jari Niemelä	The concept of ecosystem services in adaptive urban planning and design: A framework for supporting innovation	Landscape and Urban Planning	2014	125		254	259	10.1016/j.landurbplan.2014.01.020
Dagmar Haase, Niki Frantzeskaki, Thomas Elmqvist	Ecosystem Services in Urban Landscapes: Practical Applications and Governance Implications	AMBIO	2014	43	4	407	412	10.1007/s13280-014-0503-1
Dagmar Haase,Neele Larondelle,Erik Andersson,Martina Artmann,Sara Borgstrom,Jurgen Breuste,Erik Gomez-Baggethun,Asa Gren, Zoe Hamstead,Rieke Hansen,Nadja Kabisch,Peleg	A Quantitative Review of Urban Ecosystem Service Assessments: Concepts, Models, and Implementation	AMBIO	2014	43	4	413	433	10.1007/s13280-014-0504-0

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Kremer,Johannes Langemeyer,Emily Lorance Rall,Timon McPhearson,Stephan Pauleit,Salman Qureshi,Nina Schwarz,Annette Voigt,Daniel Wurster,Thomas Elmqvist								
Erik Andersson,Stephan Barthel,Sara Borgstrom,Johan Colding,Thomas Elmqvist,Carl Folke,Asa Gren	Reconnecting Cities to the Biosphere: Stewardship of Green Infrastructure and Urban Ecosystem Services	AMBIO	2014	43	4	445	453	10.1007/s13280-014-0506-y
Daniel Wurster, Martina Artmann	Development of a Concept for Non-monetary Assessment of Urban Ecosystem Services at the Site Level	AMBIO	2014	43	4	454	465	10.1007/s13280-014-0502-2
Francesc Baro,Lydia Chaparro,Erik Gomez-Baggethun, Johannes Langemeyer,David J. Nowak,Jaume Terradas	Contribution of Ecosystem Services to Air Quality and Climate Change Mitigation Policies: The Case of Urban Forests in Barcelona, Spain	AMBIO	2014	43	4	466	479	10.1007/s13280-014-0507-x
Marek Giergiczny, Jakub Kronenberg	From Valuation to Governance: Using Choice Experiment to Value Street Trees	AMBIO	2014	43	4	492	501	10.1007/s13280-014-0516-9
Timon McPhearson, Zoe A. Hamstead, Peleg Kremer	Urban Ecosystem Services for Resilience Planning and Management in New York City	AMBIO	2014	43	4	502	515	10.1007/s13280-014-0509-8
Martina Artmann	Assessment of Soil Sealing Management Responses, Strategies, and Targets Toward Ecologically Sustainable Urban Land Use Management	AMBIO	2014	43	4	530	541	10.1007/s13280-014-0511-1
Anna Kaczorowska	Ecosystem Services and	ISOCARP International	2014			1080	1090	

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	Urban Resilience - Case of Stockholm	Planning Congress: URBAN TRANSFORMATIONS: CITIES AND WATER						
Johannes Schreyer, Tobia Lakes	Remote sensing-based approaches for modeling 3D vegetation information in urban areas	International Conference on Computational Science and Its Applications	2014	14				10.1109/ICCSA.2014.30
Wendy McWilliam,Robert Brown,Paul Eagles,Mark Seasons	Barriers to the effective planning and management of residential encroachment within urban forest edges: A Southern Ontario, Canada case study	Urban Forestry & Urban Greening	2014	13	1	48	62	10.1016/j.ufug.2013.08.002
Jürgen Breuste,Salman Qureshi,Junxiang Li	Applied urban ecology for sustainable urban environment	Urban Ecosystem	2013	16		675	680	10.1007/s11252-013-0337-9
Robert F. Young	Mainstreaming urban ecosystem services: A national survey of municipal foresters	Urban Ecosystem	2013	16	4	703	722	10.1007/s11252-013-0287-2
Peleg Kremer,Zoé A.Hamstead,Timon McPhearson	A social-ecological assessment of vacant lots in New York City	Landscape and Urban Planning	2013	120		218	233	10.1016/j.landurbplan.2013.05.003
Izaskun Casado-Arzuaga,Iosu Madariaga,Miren Onaindia	Perception, demand and user contribution to ecosystem services in the Bilbao Metropolitan Greenbelt	Journal of Environmental Management	2013	129		33	43	10.1016/j.jenvman.2013.05.059
Sarah Taylor Lovell,John R. Taylor	Supplying urban ecosystem services through multifunctional green infrastructure in the United States	Landscape Ecology	2013	28	8	1447	1463	10.1007/s10980-013-9912-y
Jan Tigges,Tobia Lakes,Patrick Hostert	Urban vegetation classification: Benefits of multitemporal Rapid	Remote Sensing of Environment	2013	136		66	75	10.1016/j.rse.2013.05.001

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	Eye satellite data							
Neele Larondelle,Dagmar Haase	Urban ecosystem services assessment along a rural-urban gradient: A cross-analysis of European cities	Ecological Indicators	2012	29		179	190	10.1016/j.ecolind.2012.12.022
Daniele La Rosa,Daniel Wiesmann	Land cover and impervious surface extraction using parametric and non-parametric algorithms from the open-source software R: an application to sustainable urban planning in Sicily	GIS Science and Remote Sensing	2013	50	2	231	250	10.1080/15481603.2013.795307
Erik Gómez-Baggethun,David N. Barton	Classifying and valuing ecosystem services for urban planning	Ecological Economics	2012	86		235	345	10.1016/j.ecolecon.2012.08.019
E. Rozos,C. Makropoulos, C Maksimovic	Rethinking urban areas: an example of an integrated blue-green	Water Science and Technology	2013	13	6	1534	1542	10.2166/ws.2013.140
Simon Farrugia,Malcolm D. Hudson,Lindsay McCulloch	An evaluation of flood control and urban cooling ecosystem services delivered by urban green infrastructure	International Journal of Biodiversity Science, Ecosystem Services & Management	2013	9		136	145	10.1080/21513732.2013.782342
Robert F. Young,E. Gregory McPherson	Governing metropolitan green infrastructure in the United States	Landscape and Urban Planning	2012	109	1	67	75	10.1016/j.landurbplan.2012.09.004
James J. Connolly,Erika S. Svendsen,Dana R. Fisher,Lindsay K. Campbell	Organizing urban ecosystem services through environmental stewardship governance in New York City	Landscape and Urban Planning	2012	109	1	76	84	10.1016/j.landurbplan.2012.07.001
Adrienne Grêt-Regamey,Enrico Celio,Thomas M. Klein,Ulrike Wissen Hayek	Understanding ecosystem services trade-offs with	Landscape and Urban Planning	2013	109	1	107	116	10.1016/j.landurbplan.2012.10.011

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	interactive procedural modeling for sustainable urban planning							
Kathleen Gail Radford,Philip James	Changes in the value of ecosystem services along a rural-urban gradient:A case study of Greater Manchester, UK	Landscape and Urban Planning	2012	109	1	117	127	10.1016/j.landurbplan.2012.10.007
Stephanie Pincetl	Nature, urban development and sustainability - What new elements are needed for a more comprehensive understanding?	Cities	2012	29	2	S32	S37	10.1016/j.cities.2012.06.009
Tobia Lakes,Hyun-Ok Kim	The urban environmental indicator "Biotope Area Ratio"-An enhanced approach to assess and manage the urban ecosystem services using high resolution remote-sensing	Ecological Indicators	2011	13	1	93	103	10.1016/j.ecolind.2011.05.016
Rajesh Bahadur Thapa	Monitoring landscape change in Kathmandu metropolitan region using multi-temporal satellite imagery	Earth Observing Missions and Sensors: Development, Implementation, and Characterization II	2012	8528				10.1117/12.978927
Fausto Manes,Guido Incerti,Elisabetta Salvatori,Marcello Vitale,Carlo Ricotta,Robert Costanza	Urban ecosystem services: tree diversity and stability of tropospheric ozone removal	Ecological Applications	2011	22	1	349	360	10.1890/11-0561.1
Heather R. Mccarthy,Diane E. Pataki,G. Darrel Jenerette	Plant water-use efficiency as a metric of urban ecosystem services	Ecological Applications	2011	21	8	3115	3127	10.1890/11-0048.1
L. Lundy, R. Wade	Integrating sciences to sustain urban ecosystem	Progress in Physical Geography	2011	35	5	653	669	10.1177/0309133311422464

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	services							
Shu-Li Huang ,Yu-Hwa Chen,Fei-Yu Kuo,Szu-Hua Wang	Emergy-based evaluation of pen-urban ecosystem services	Ecological Complexity	2010	8	1	38	50	10.1016/j.ecocom.2010.12.002
Diane E Pataki,Margaret M Carreiro,Jennifer Cherrier,Nancy E Grulke,Viniece Jennings,Stephanie Pincetl,Richard V Pouyat,Thomas H Whitlow,Wayne C Zipperer	Coupling biogeochemical cycles in urban environments: ecosystem services, green solutions, and misconceptions	Frontiers in Ecology and the Environment	2011	9	1	27	36	10.1890/090220
Henrik Ernstson,Stephan Barthel,Erik Andersson,Sara T. Borgström	Scale-Crossing Brokers and Network Governance of Urban Ecosystem Services: The Case of Stockholm	Ecology and Society	2010	15	4			
Klaus Lorenz,Rattan Lal	Biogeochemical C and N cycles in urban soils	Environment International	2008	35	1	1	8	10.1016/j.envint.2008.05.006
Henrik Ernstson,Sverker Sörlin,Thomas Elmqvist	Social Movements and Ecosystem Services-the Role of Social Network Structure in Protecting and Managing Urban Green Areas in Stockholm	Ecology and Society	2008	13	2			
Susannah E. Gill,John F. Handleya,A. Roland Ennosb, Stephan Pauleit,Nicolas Theuraya,Sarah J. Lindleya	Characterising the urban environment of UK cities and towns: A template for landscape planning	Landscape and Urban Planning	2008	87	3	210	222	10.1016/j.landurbplan.2008.06.008

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Appendix 2. *The table showing the name of urban centres and number of UES studies conducted*

City	Country	Number of Studies
Berlin	Germany	9
New York	USA	7
Stockholm	Sweden	6
Manchester	UK	5
Rotterdam	Netherland	4
Catalonia	Spain	3
Barcelona	Spain	3
Leipzig	Germany	3
London	UK	2
Singapore	Singapore	2
Lodz	Poland	2
Reading	UK	2
Bogota	Colombia	2
Rome	Italy	2
California	USA	2
Shanghai	China	1
Porto	Portugal	1
Mexico City	Mexico	1
Helsinki	Finland	1
Milton Keynes, Bedford, Luton	UK	1
Espoo	Finland	1
California	USA	1
Bobo-Dioulasso	Burkina Faso	1
Padova	Italy	1
Tampere	Finland	1
Chennai	India	1
Mascouche (MONTREAL)	Canada	1
Sheffield	UK	1
Manila, Bangkok	Philippines, Thailand	1
Beijing	China	1
Qingdao	China	1
Jakarta	Indonesia	1
Boras	Sweden	1
Munich	Germany	1
Lagos	Nigeria	1
Szeged	Hungary	1
Tucson (Arizona)	USA	1
Dresden	Germany	1
Salzburg	Austria	1
Ontario	Canada	1
Bilbao	Spain	1
Southampton	UK	1
Masdar City	Abu Dhabi	1
Seoul, Berlin	South Korea, Germany	1
Kathmandu	Nepal	1
Taipei	China	1

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Appendix 3. *The content analysis showing the number and proportion of reviewed publications focused a specific type of UES and also indicating the contemporary trends in research regarding techniques for enquiry, methods for evaluation, research/study objectives and environmental components relied upon for assessments*

The assessment criteria and research orientations of selected Publications	**No. of studies**	**(%)**
Type of Ecosystem services/ Disservices		
Ecosystem Services	71	31.84
Cultural Services	37	16.59
Regulatory Services	65	29.15
Provisioning Services	28	12.56
Supporting Service	9	4.04
Disservice	13	5.83
Technique of enquiry		
Assessment	57	49.14
Exploratory	26	22.41
Experimental	13	11.21
Conceptual framework	20	17.24
Method of Evaluation		
Monetary	4	3.45
Non-Monetary	77	66.38
Both (M - NM)	22	18.97
Not Applicable	13	11.21
Research/study Objective		
Landuse Planning	6	5.17
Planning and Management	33	28.45
Assessment of Method	16	13.79
Urban Ecology	4	3.45
Environmental Justice	3	2.59
Assessment study	24	20.69
Governance and Policy	20	17.24
Climate Adaption	10	8.62
Environmental Component(s) evaluated		
Water Management	42	9.33
Climate Regulation	57	12.67
Socioecological	69	15.33
Biodiversity	64	14.22
Aesthetics	42	9.33
Food Fuel	18	4.00
Energy	14	3.11
Human Health	24	5.33
Carbon Seqestration Storage	35	7.78
Hedonic Pricing	9	2.00
Others	76	16.89

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ESTIMATION OF MISSING STREAMFLOW DATA USING ANFIS MODELS AND DETERMINATION OF THE NUMBER OF DATASETS FOR ANFIS: THE CASE OF YEŞİLIRMAK RIVER

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Abstract. Good data analysis is required for the optimal design of water resources projects. However, data are not regularly collected due to material or technical reasons, which results in incomplete-data problems. Available data and data length are of great importance to solve those problems. Various studies have been conducted on missing data treatment. This study used data from the flow observation stations on Yeşilırmak River in Turkey. In the first part of the study, models were generated and compared in order to complete missing data using Artificial Neural Network Fuzzy Inference Systems (ANFIS), multiple regression and Normal Ratio Method. Thus, it is tried to define the usability besides the other model to complete the missing data. Likewise, in the study. It is aimed to define the minimum number of data necessary for the use age of ANFIS. For this purpose in the second part of the study, the minimum number of data required for ANFIS models was determined using the optimum ANFIS model. Of all methods compared in this study, ANFIS models yielded the most accurate results. A 10-year training set was also found to be sufficient as a data set.
Keywords: *Anfis, missing data, multiple regression, normal ratio method, Yeşilırmak*

Introduction

Both the growing population and the rapidly developing industrialization lead to an increased demand for water. The limited availability of resources results in a number of problems in meeting the demand. Exploitation of unused water resources or using existing water resources in an optimum way can be a solution to these problems. Optimal utilization of available water resources, in particular, requires a good analysis of data. Due to the small number of stations in project areas or insufficient data length, some studies have been undertaken to generate new data using existing measurement stations (Aissia et al., 2017). These hydrological studies have mainly focused on precipitation (Sun et al., 2017), evaporation (Güçlü et al., 2017) and river flows (Shiau and Hsu, 2016).

Studies on missing data treatment generally address data correlation (Bakis and Göncü, 2015; Britoa et al., 2017), back-propagation (BP) neural network using Artificial Intelligence (Gao and Wang, 2017), ANFIS models (Dastorani et al., 2010; Mpallas et al., 2010) and models using artificial neural networks (ANN) (Kim and Pachepsky, 2010; Kim et al., 2015). In addition, Fuzzy studies (Coulibaly and Evora, 2007), in which modeling is based on pure expert knowledge, are also important. Some studies on missing data treatment using ANFIS are the completion of missing flow data of the Middle Euphrates basin (Yilmaz and Muttil, 2014), completion of missing precipitation data in Serbia (Petkovic et al., 2016) and Malaysia (Nawaz et al., 2016),

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and completion of missing flow data and modelling of sediment transport of Terengganu River, Malaysia (Ismail and Zin, 2017) and Gediz River, Turkey (Ulke et al., 2009).

This study investigated the monthly data of the stations of Yeşilirmak River in the North of Turkey. In this study, Normal Ratio Method which is frequently used in literature and of which usability is proven and the Regression methods enable to reach the results by the relationship between the data are chosen to be able to compare the accuracy of affered ANFIS model. In the first part of the study, multiple regression tests based on interstation correlations were performed. In the second part of the study, an optimum data completion model was selected using ANFIS. In the last part of the study, the number of data required for a correct prediction was searched and the minimum number of data required for reliable estimates was discussed.

Material and Method

The first part of this section of the study will present information and statistics on Yeşilırmak River and its stations. The second part will provide information on the classical method, multiple regression method and ANFIS used in the study.

Yeşilırmak River and stations

The Yeşilırmak basin, one of the 25 basins in Turkey, is located between latitudes 39° 30' and 41° 21' and longitudes 34° 40' and 39° 48' (*Figure 1*). The basin is named after Yeşılırmak River. The main river channel of the basin is 519 km in length. The main tributaries of Yeşilırmak River are Kelkit, Çekerek, Çorum, Çat and Tersakan streams. Estimated to be about 3,8 million ha, Yeşilırmak basin is the third largest basin in Turkey (Kurunç et al., 2005).

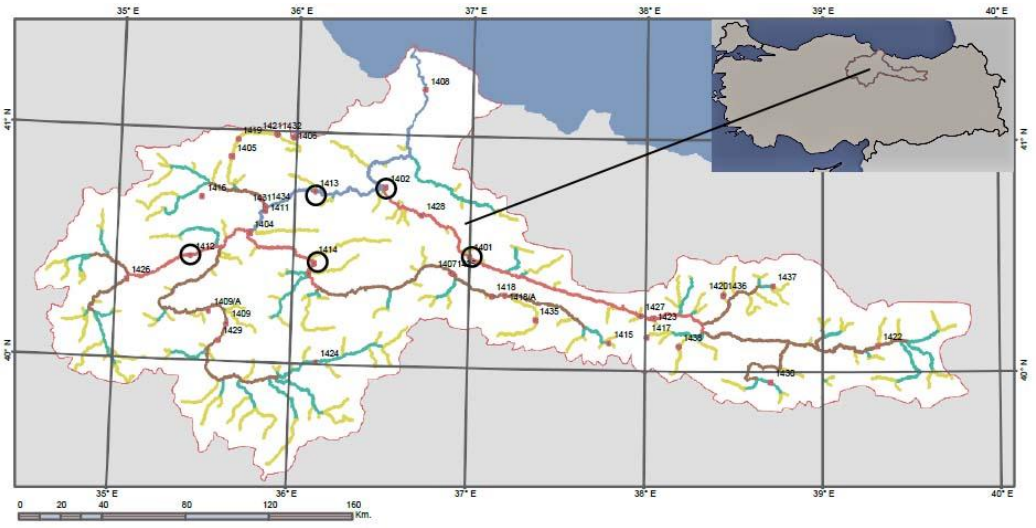


Figure 1. *Site location map of Yeşilırmak Basin*

Stations No 1401, 1402, 1412, 1413 and 1414 of Yeşilırmak River were used in the study. *Table 1* shows the statistics of the stations. *Table 2* summarizes the correlation between the stations.

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Table 1. *Statistical analysis of data from stations*

	1401	**1402**	**1412**	**1413**	**1414**
Y- coordinate	40°28'42''	40°46'18''	40°27'06''	40°44'40''	40°26'03''
X- coordinate	36°59'56''	36°30'45''	35°25'03''	36°06'43''	36°07'05''
Precipitation Area(km²)	10048.8	33904.0	3668.8	21667.2	5409.2
Altitude	375	190	530	301	510
Mean	71.96	150.01	7.35	63.88	25.68
Standard Error	3.07	5.17	0.37	2.34	0.84
Median	35.45	102.50	4.21	45.30	19.65
Standard Deviation	83.65	136.11	8.65	54.95	19.71
Kurtosis	4.27	2.81	6.10	3.41	4.79
Skewness	2.06	1.66	2.24	1.70	1.81
Max	5.23	13.50	0.02	2.47	2.46
Min	548.00	791.00	59.10	350.00	139.00
Number of Data	744	692	536	552	546
Confidence Interval (95,0%)	6.02	10.16	0.73	4.59	1.66

Table 2. *Correlation between data from stations*

Stations	**1401**	**1402**	**1412**	**1413**	**1414**
1401	1				
1402	0.909	1			
1412	0.516	0.784	1		
1413	0.702	0.926	0.885	1	
1414	0.539	0.565	0.432	0.518	1

Methods

Missing data treatment using Normal Ratio Method

In this method, each input data is divided by its annual average value, and these values are multiplied by the average of the station (average of data) whose missing data are to be completed. All input values obtained in the last stage of the calculation are summed, and divided by the number of inputs so that the missing data are completed (Ismail and Zin, 2017).

$$Q_e = (Q_1 * \frac{Q_{e(ort)}}{Q_{1(ort)}} + Q_2 * \frac{Q_{e(ort)}}{Q_{2(ort)}} + \cdots + Q_n * \frac{Q_{e(ort)}}{Q_{n(ort)}})/n \qquad \text{(Eq.1)}$$

where Q is the flow rate and n is the number of input stations.

Multiple regression analysis

Multiple regression analysis is a statistical method for determining the mathematical dimension of the relationship between variables affecting each other. The value to be estimated using the equation formulated based on multiple regression analysis is written in the form of a function of values affecting it (Sun and Trover, 2018).

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$$Y = \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_k X_k + U \quad \text{(Eq.2)}$$

where Y is the dependent (estimated) variable, X is the independent (explanatory) variable, β is the regression coefficient, k is the number of input parameters and U is the error term.

Multiple linear regression analysis can be used when data are normally distributed, the relationship between independent variables and dependent variable is linear, and error variance for each independent variable is constant (Hair et al., 2009).

ANFIS

Developed by Jang (1993), ANFIS is a modeling method that combines Fuzzy Logic and YSA models. Different from Fuzzy Logic, ANFIS is based on the use of data for the automatic acquisition of rules. ANFIS structure uses artificial neural networks' learning ability and fuzzy logic inference, and therefore, it is more successful than when artificial neural networks model or fuzzy logic is used alone. When input and output values are known, ANFIS determines all possible rules or allows them to be generated using input and output values (*Figure 2*). ANFIS structure consists of five layers: fuzzification layer, rule layer, normalization layer, defuzzification layer and summation layer (*Figure 3*). The first and fourth layers are adaptable (Nayak et al., 2004; Jang, 1993). ANFIS models can be especially used in the conditions if there are lots data and if they are organized.

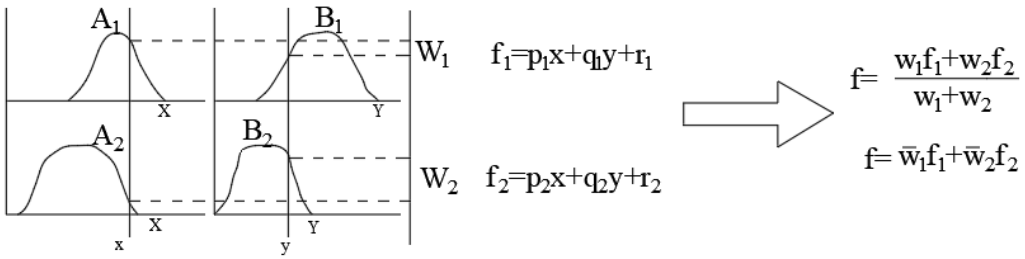


Figure 2. *Fuzzy Inference System*

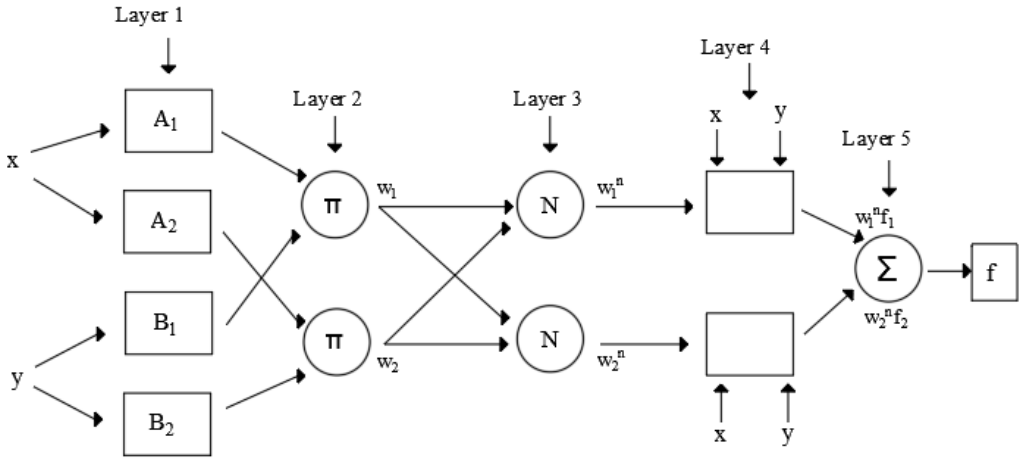


Figure 3. *ANFIS architecture*

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Results

Models were developed using Yeşilırmak River data for the estimation of missing data of stations 1402 and 1413. Two-input-one-output models and four-input-one-output models were developed to complete the missing data of station 1402. The two-input-one-output models were used as the output of station 1402. Stations 1413 and 1401 connected to station 1402 on the left- and right-hand sides, respectively, were used to estimate station 1402. In addition to these stations, stations 1412 and 1414 connected to station 1413 on the left-and right-hand sides, respectively, were used to estimate station 1413 in the four-input-one-output models. A two-input-one-output model was developed, and stations 1414 and 1412 were used for the estimation of missing data of station 1413. In the models developed for stations 1402 and 1413, classical and multiple regression models were constructed and compared as well as the ANFIS method. In the last part of the study, the minimum number of data required to reach the correct result using ANFIS models was obtained.

First data set models

The aim of these models was to complete the missing data of station 1402. For this, the data of stations 1413 and 1401 were used. Of 540 data, the first 400 were used for training and the remaining for testing. In addition to ANFIS models generated by changing the number of sets of input parameters, classical method and the multiple regression model were used to compare the results (*Table 3*).

The equation of the classical method is:

$$X_{1402} = (2{,}072 * X_{1401} + 2{,}215 * X_{1413})/2 \quad \text{(Eq.3)}$$

The equation of the multiple regression model is:

$$X_{1402} = 0{,}896 * X_{1401} + 1{,}178 * X_{1413} + 5{,}37 \quad \text{(Eq.4)}$$

Table 3. *Training and testing data results of models developed for the first data set*

Models		Training Data		Testing Data	
		R^2	Mean Squared Error %	R^2	Mean Squared Error %
ANFIS Models	3-3	0.976	11.73	0.977	17.36
	4-4	0.977	11.66	0.978	17.38
	5-5	**0.983**	**10.99**	**0.979**	17.55
	6-6	0.980	11.13	0.978	**16.60**
	7-7	0.979	11.12	0.961	17.00
	8-8	**0.983**	11.09	0.959	17.12
Normal Ratio Method		0,970	11.79	0.955	18.85
Multiple Regression		0,972	13.54	0.960	18.62

The results of this part of the study show that ANFIS models are not superior to the classical and multiple regression models but that all ANFIS models yield better results than the other two methods. The models in which each input has 5 subsets are the optimum models. The speed of the training phase of the model is also noteworthy. The

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comparison of the values obtained from the optimum ANFIS model with the observed values shows that the errors of both the minimum and maximum flow values are very few (*Figure 4*).

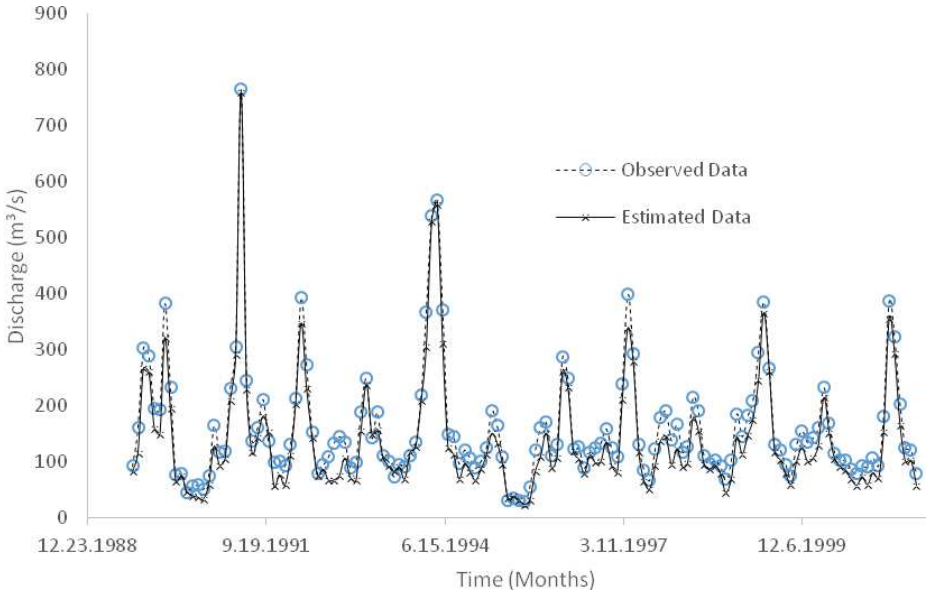


Figure 4. *Comparison of observed data and estimated data of Anfis (5-5) model test data for the first data set*

Second data set models

These models also aimed to complete the missing data of station 1402. To achieve this, the data of stations 1412 and 1414 as well as those of 1413 and 1401 were used. Of 504 data, the first 405 were used for training and the remaining for testing. In addition to ANFIS models generated by changing the number of sets of input parameters, classical method and multiple regression model were used to compare the results (*Table 4*).

The equation of the classical method is:

$$X_{1402} = (2{,}107 * X_{1401} + 19{,}53 * X_{1412} + 2{,}26 * X_{1413} + 5{,}44 * X_{1414})/4 \quad \text{(Eq.5)}$$

The equation of the multiple regression model is:

$$X_{1402} = 0{,}921 * X_{1401} + 1{,}036 * X_{1412} + 1{,}090 * X_{1413} - 0{,}251 * X_{1414} + 11{,}071 \quad \text{(Eq.6)}$$

The results show that ANFIS models provide more accurate results than the classical model and worse results than multiple regression models. The models with 4 inputs are quite slow, especially when the number of subsets of inputs is greater than 5. The models with an increasing number of inputs are much slower than multiple regression models. The comparison of the values of the optimum ANFIS model with the observed values shows that although the largest error is at the minimum flow values, this error is quite small at the maximum flow values (*Figure 5*).

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Table 4. *Training and testing data results of models developed for the second data set*

Models		Training Data		Testing Data	
		R^2	Mean Squared Error %	R^2	Mean Squared Error %
ANFIS Models	3-3-3-3	0.933	28.96	0.916	26.92
	4-4-4-4	0.986	11.27	0.919	16.94
	5-5-5-5	**0.991**	10.30	0.918	18.14
	6-6-6-6	**0.991**	**9.85**	0.919	19.09
Normal Ratio Method		0.873	33.79	0.863	28.61
Multiple Regression		0.976	18.26	**0.975**	**16.73**

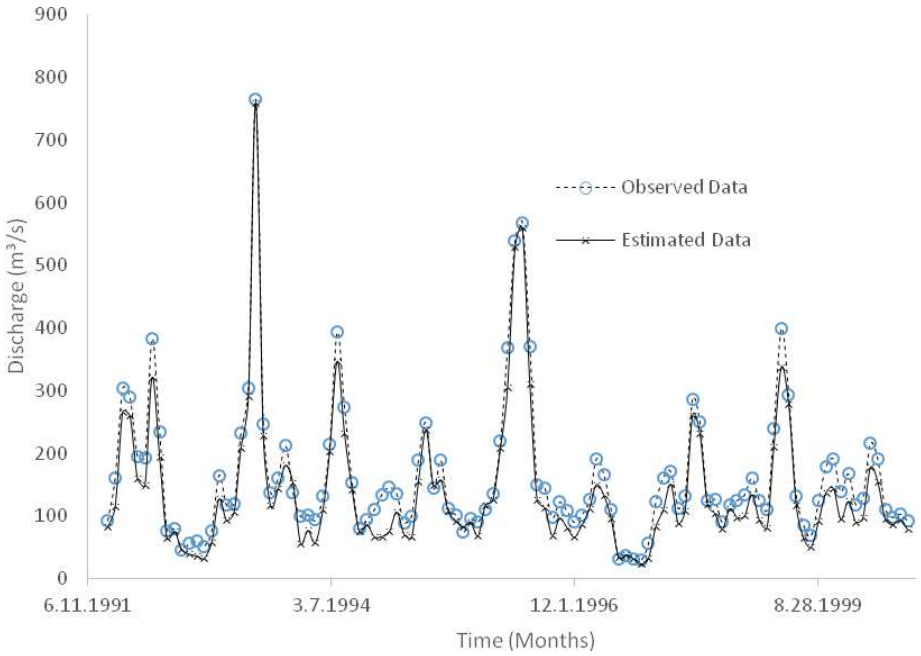


Figure 5. *Comparison of observed data and estimated data of Anfis (5-5) model test data for the second data set*

Third data set models

The aim of these models was to complete the missing data of station 1413. For this, the data of stations 1412 and 1414 were used. Of 504 data, the first 405 were used for training and the remaining for testing. The results were compared using the classical method and multiple regression model as well as ANFIS models generated by changing the number of sets of input parameters (*Table 5*).

The equation of the classical method is:

$$X_{1413} = (2{,}407 * X_{1414} + 8{,}641 * X_{1412})/2 \quad \text{(Eq.7)}$$

The equation of the multiple regression model is:

$$X_{1413} = 0{,}404 * X_{1414} + 4{,}904 * X_{1412} + 17{,}576 \quad \text{(Eq.8)}$$

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Table 5. *Training and testing data results of models developed for the third data set*

Models		Training Data		Testing Data	
		R^2	Mean Squared Error %	R^2	Mean Squared Error %
ANFIS Models	3-3	0.890	20.29	0.865	23.24
	4-4	0.892	19.35	0.872	22.93
	5-5	**0.881**	**18.64**	**0.879**	**22.56**
	6-6	0.865	19.88	0.854	23.07
Normal Ratio Method		0.764	27.41	0.694	36.60
Multiple Regression		0.905	34.26	0.757	46.74

The results show that ANFIS models provide more accurate results than the classical and multiple regression models. Although the results are not as good as those in the first data set, they remain within acceptable error limits. The models in which each input has 5 subsets are the optimum models. The comparison of the values of the optimum ANFIS model with the observed values shows that the errors of both the minimum and maximum flow values are very few (*Figure 6*).

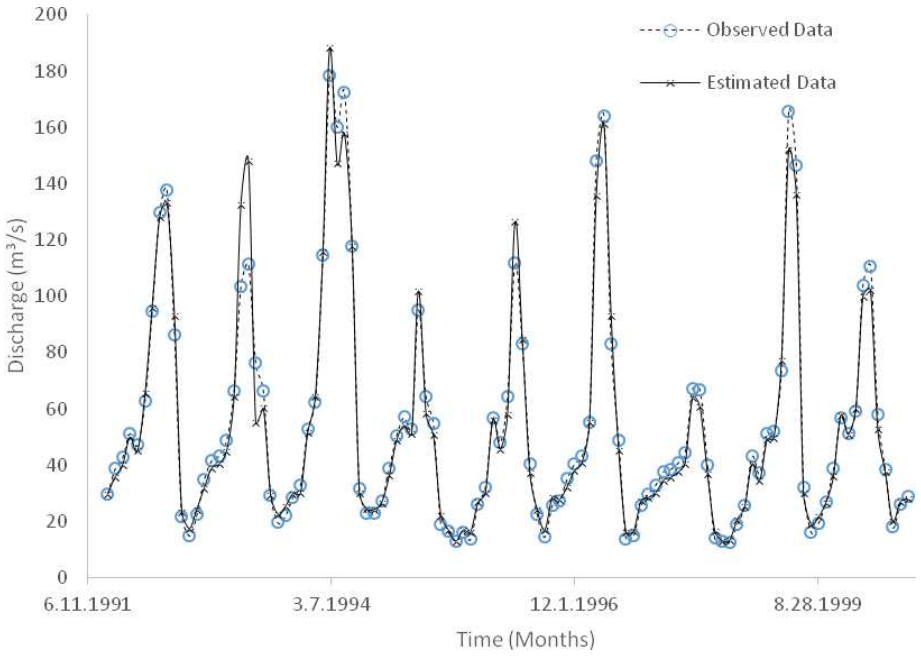


Figure 6. *Comparison of observed data and estimated data of Anfis (5-5) model test data for the third data set*

Determining the Minimum Number of Data for Anfis Model Training

This part of the study attempted to obtain the minimum number of data required for a reliable ANFIS model training. The model with a 5-5 set of the first data set (the optimum modelling) were the model of choice for this purpose. The models were trained using a 10-year data set and the procedure was repeated year by year. The evaluation of the results is summarized in *Table 6*.

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Table 6. *Regression and error values for the number of data used for ANFIS model training*

Number of Data	Training Data		Testing Data	
	R^2	Mean Squared Error %	R^2	Mean Squared Error %
1- year	0,999	0,74	0,036	169,27
2- year	0,993	13,42	0,071	118,02
3-year	0,992	16,79	0,139	111,06
4- year	0,985	21,38	0,198	36,91
5- year	0,980	22,39	0,628	30,44
6- year	0,981	21,23	0,738	21,92
7- year	0,982	19,77	0,803	20,12
8- year	0,983	18,76	0,844	17,77
9- year	0,984	16,88	0,898	17,74
10- year	0,985	12,27	0,967	17,65
33- year	0,983	10,99	0,979	17,55

The number of data used for ANFIS model training does not affect the regression coefficient of the training data very much (*Table 6*). However, the regression results obtained during the testing of the models show that the results of the 10-year data set and model are very similar to those of the 33-year data and training *(Figure 7*). The error values show that the 8-year data set is sufficient for training (*Figure 8*). In conclusion, a 10-year data set may be sufficient for Anfis model training.

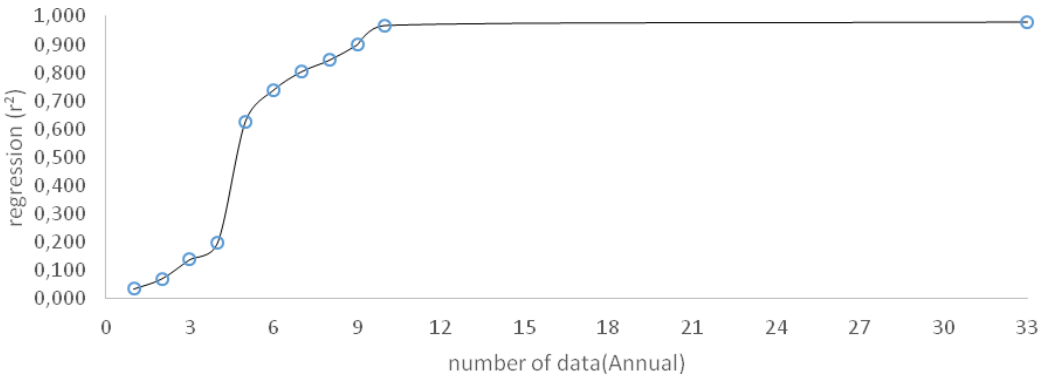


Figure 7. *Regression values for the number of data used for ANFIS model training*

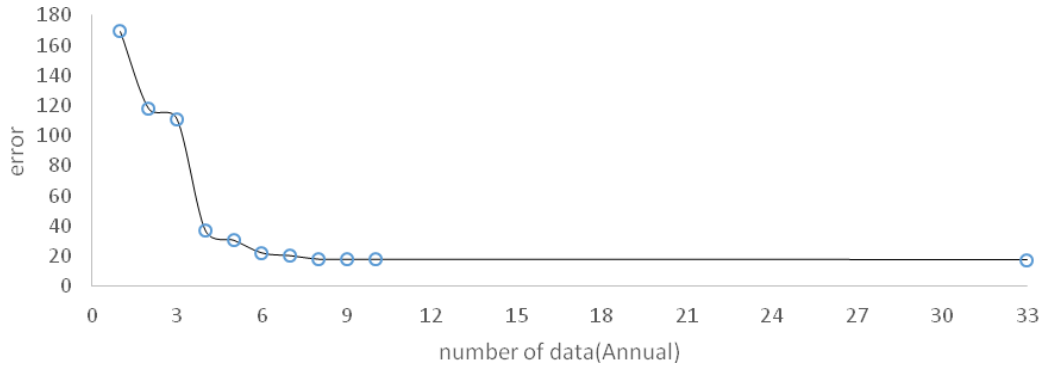


Figure 8. *Error values for the number of data used for ANFIS model training*

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Discussion

The study was repeated with 3 different data sets and ANFIS model behaviors is searched in different models. When the obtained results are analyzed it is seen that the increase in the number of the stations affects ANFIS modelsadversely; however, this effect isn't so big as Normal Ratio Method has. In contrast to this, it can be said that the increase in the number of the stations affect Multiple Regression Models in a positive way. While the proportion of error is more in the minimum discharge value, it is much smaller in the maximum discharge value. The changing of error in the maximum values isn't effected so much by the changings in the number of stations. One of the biggest lacknesses of ANFIS models is memorizing during the process of the training. To be able to overcome this lackness, some of the data should be allocated for testing. The accuracy rate which has %99 accuracy proportion during the process of training reaches to much lower degrees for testing date. This is a proof for the model that this model is composed via memorizing instead of learning and it can't be used. The closeness between the results obtained out of training and the test results shows that ANFIS is appropriate to complete missed data.

In the second part of the study, it is focused on the number of data which is necessary for being able to to use ANFIS models. Especially in the situation of having fewer data, the needed number of data is tried to determine to be able to get reliable results. In the study done for this, it is noticed that there is a difference less than % 1 between the models composed with a decade data and 8 composed with 33 years data. When error test data in inspected, it can be said that 8 years datacan be used safely. It is defined that the data is less and 3 years data can't be used. Annual data in the model is the biggest proof for showing tı trust the training results. Although the training results are extremely trustable, the test resultsare weak.

In the stuation of increasing the number of data, the results abtained via ANFIS move away the accuracy and so the time or elevation the results becomes longer. Additionally, every input parameter must be balanced in the number of membership functions. Number of membership functions is important for the accuracy of the model. Having many membership functions may affect the accuracy of the model in the negative way. The operation time of the models increases exponentially at the same time. For example while in a model having 2 inputs and having 5 sets of membership functions for each input, 25 rules composes; in amodel having 4 inputs and 10 sets of membership functions for each 10^4 rules are needed to be composed. However, this affects the model's operation performance adversely.

Conclusion

Sometimes it is not possible to collect long and coordinated data in order to optimally use water resources projects.The aim of this study was to develop ANFIS models for stations on Yeşilırmak River in order to solve this problem and improve the existing methods. Another aim of the study was to investigate how the number of input parameters and amount of data affect ANFIS models. For this purpose, 3 different data sets were analyzed.

Results show that besides classical and regression models, ANFIS models can be used to complete missing flow data. ANFIS models yield very accurate results especially when the number of input parameters is small. However, multiple regression models yield better results than ANFIS models when the number of input parameters is

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large. In addition, it takes ANFIS models longer to achieve results when the number of input parameters increases. Lastly, at least a 10-year data is required for a reliable ANFIS model training phase.

In conclusion, the ANFIS modelling yield accurate results and therefore can be used to complete missing data when the number of input parameters is small and data set is older than 10 years.

ANFIS models must be advocated by using the data of past and they can be used to complete the missing data which is going to happen in the future after proving their usage by looking at the test results. In addition, via this, newly opened stations past data can be supplied. In addition to completing the data of streamflow, it is thought that it can be used to complete the data having great importance in the projects of water sources. Such as rainfall, evaparation, water quality and sediment.

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PERSPECTIVE ON FOREST BIODIVERSITY INDICATORS FOR PROTECTED AREAS: A COMPARISON OF TURKISH AND SWEDISH FOREST EXPERT OPINIONS

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Abstract. This paper presents a comparative analysis of expert opinions on forest biodiversity indicators for protected areas, using a questionnaire given to forest experts in Turkey and Sweden. Experts were selected according to whether they had studied or worked in areas related to biodiversity, protected areas and sustainable forest management. A Mann-Whitney U test was used to determine the differences between the opinions of Swedish and Turkish experts regarding the indicators. The experts from both countries considered "endemic species" and "naturalness" as the most important indicators, while "overused species", "forest distribution and regeneration", "carrying capacity in terms of important species of area" and "the existence of different conservation status of protected areas" were considered equally as the least important indicators. The most important difference between the two groups was related to the indicators "dead wood" and "hollow trees", which Swedish experts found more important than their Turkish counterparts. Two other large differences were that the Swedish experts found "litter layer" much more important and Turkish experts instead found "plant species composition" much more important. The differences between the two groups reveal different perspectives regarding the planning and management of protected areas in each respective country.
Keywords: *sustainable forest management, dead wood, hollow trees, Turkey, Sweden*

Introduction

First introduced to the forestry literature in the 1990s, sustainable forest management criteria and indicators have been important tools for measuring the sustainability of forests (Mendoza and Prabhu, 2000; Brang et al., 2002). In 1992, the International Tropical Timber Association (ITTO) was the first to propose such criteria and indicators for tropical forests (ITTO, 1992, 2005). Subsequently, the annual Rio Summit highlights the importance of sustainable forest criteria and indicators, along with monitoring and reporting. (Castañeda et al., 2001; Rametsteiner, 2001; Mcdonald and Lane, 2004; Wolfslehner et al., 2005; Mrozek et al., 2006; Steenberg et al., 2013). Recently, the first step was taken towards the development of criteria and indicators for sustainable forest management for nine regional processes in the world. These processes include: the International Tropical Timber Organization (ITTO), Helsinki Process (MCPFE/Pan-European Process), Montreal Process, Tarapoto Proposal, Dry Zone Africa Process, African Timber Organization Process (ATO), Near East Process,

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Lepaterique Process of Central America and Dry Zone Asia Process (Akyol and Tolunay, 2014).

For these regional processes, seven key levels were identified as an overall framework for sustainable forest management. These levels include: the extent of forest resources, biodiversity, health and vitality of forest ecosystems, protective functions of forests, productive functions of forests, socio-economic functions, and legal, political and institutional framework (Castañeda, 2000; Siry et al., 2005; Purnomo et al., 2005; Grainger, 2012).

Among these levels, biodiversity criteria and indicators have been among the most debated since the Rio Summit. Until recently a great deal of effort has been undertaken to further develop the indicators for this criteria (Kotwal et al., 2008) because biodiversity refers to the dynamism, health, sustainability and energy of an ecosystem and its resilience against any kind of disturbance factor (Gülsoy and Özkan, 2008). In short, biodiversity is one of the fundamental bases for the continuity of life. Moreover, there are crucial challenges for ensuring the sustainability of biodiversity (Hagan and Whitman, 2006). These challenges arise because biodiversity is typically defined as the diversity of life measured at the levels of genes, species and ecosystems and the mutual interactions between these levels (Hagan and Whitman, 2006; Gaston, 1996; Claridge et al., 1997). Genetic structure is the primary component of vitality and is the basis of these levels.

The most important factor for the protection and sustainable use of biodiversity is the need to make a quantitative assessment of biodiversity (Duelli and Obrist, 2003). However, due to various challenges, the most practical approach for assessment is to use indicators related to biodiversity (Hagan and Whitman, 2006; Lindermayer et al., 2000). However, it is also difficult to identify and measure indicators for biodiversity (Heink and Kowarik, 2010) because the variables that can be practically surveyed and quantified are quite limited.

The main strategies developed to protect biodiversity and ensure its sustainability primarily include legal protection and the sustainable management of areas (Svancara et al., 2005). Protected areas that cover almost 12% of the Earth's surface are an important component of global protection strategies (Wells and McShane, 2004; Bajracharya et al., 2005). Although there are a broad spectra of indicators related to sustainable forest management, none of the abovementioned processes adequately describe protected areas (Blicharska et al., 2011; Tolunay and Akyol, 2015). This is because sustainable forest management processes utilize indicators that focus mainly on production forests across the world, which differ from protected areas.

Protected areas are of vital importance for biodiversity, and thus it is necessary to develop appropriate criteria and indicators. For example, one of the objectives of the "Integrated Approach to Management of Forests in Turkey, with Demonstration in High Conservation Value Forests in the Mediterranean Region" project implemented by the United Nations Development Programme is to develop biodiversity indicators in Turkey within the scope of sustainable forest management (UNDP, 2017).

The aim of this study was to evaluate biodiversity indicators that can be used for the sustainable management of protected areas, using opinions from experts. This data is then preliminarily presented for further discussion. From a global point of view, different or similar perspectives to various indicators are to be expected. Factors such as climate, geographical location, biodiversity, development level, and cultural features change the perceptions about and perspectives regarding protected areas in terms of

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nature conservation. To obtain some diverse perspectives, expert views were solicited about the protected areas in Turkey to represent Mediterranean forestry and from Sweden to represent Nordic forestry. We aimed to contribute to the protection and sustainable management of biodiversity in protected areas, which is of great importance at a global scale.

Materials and methods

The main materials of this study were comprised of indicators related to the protection, development and sustainability of biodiversity, which are among the criteria and indicators for sustainable forest management. The primary data was obtained from a survey conducted online in Turkey and Sweden. The secondary data was composed of information obtained from literature analyses and information, and documents and reports from various public entities and organizations (FAO, CIFOR, etc.).

The questionnaire was composed of multiple choice questions using a 4-point Likert scale (not important, less important, important and very important). The questionnaire contained a total of 27 questions divided into two parts. The first part asked about demographic features of the participating experts. The second part asked the experts to assess a set of biodiversity indicators developed from different sustainable forest management studies across the world (*Table 1*).

Table 1. *The set of indicators[1] prepared for the evaluation by the experts*

Indicators	Definition
Plant species composition	The number and composition of plant species per forest types and other wooded area types
Naturalness	The amount of forest types and other wooded area types divided in natural, semi natural or plantation
Forest distribution and regeneration	The rates of natural regeneration and survival of tree species
Sensitive and rare ecosystems	The amount and distribution of sensitive and rare ecosystems
Dead wood	The volume of standing and downed dead wood in different forest types and other wooded area types
Hollow trees	The number of hollow trees and the proportion of the total amount of trees or wood volume
Litter layer	The litter and decaying condition
Genetic resources	The condition of forest genetic resources in the areas managed for seed production, protected areas and other areas
Ecosystem structure	The spatial structure of forest covers in the ecosystem level
Endangered species	The number of endangered forest species according to Red List classification of International Union for Conservation of Nature (IUCN) in relation to total number of forest species. (Threatened, rare, vulnerable, endangered or extinct, mammals, birds other vertebrates or invertebrates)
Endemic species	The population condition of endemic forest species
Overused species	The population condition for overused forest species
Species whose lives depend on protected areas	The number of individuals and population trend for species dependent of protected forests

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Species whose distribution area is declining and habitat loss	The size of declining species distribution area and the existence and status of forest habitat losses within protected areas
Major distribution areas and population status of the species	The size of distribution area and population status for forest species
Carrying capacity in terms of important species of area	The carrying capacity for important species of protected areas
Water resources and wetland	The quality of water resources and wetlands in forests (Number and area)
Damaged areas	The size and proportion of forest areas damaged by various processes and factors (Pests, disease, fire and flood)
Human use of protected areas	The size and proportion of human uses of land within protected areas (Residential areas, agricultural areas, such as roads, etc.)
Effects of other sectors	The effect on forest from other sectors (Mining, agriculture, livestock, energy and infrastructure etc.)
Existence of contaminants around protected areas	The number, size and type of pollutants around protected forest areas
Differentiated conservation status within protected areas	The existence of areas with different conservation status within the protected forest area (Status, size, cause etc.)
Other issues important for sustainable management	-The availability and capacity of an institutional structure for the management and existence of functional legislation -The existence of economic policy framework and financial instruments -The educational and informational opportunities for implementation of the policy framework -the strengthening of knowledge about endangered species (inventory or research) -The management plans

[1]This set is based on the biodiversity indicators used in International Tropical Timber Organization (ITTO), Helsinki Process (MCPFE/Pan-European Process), Montreal Process, Tarapoto Proposal, Dry Zone Africa Process, African Timber Organization Process (ATO), Near East Process, Lepaterique Process of Central America, Dry Zone Asia Process, Center for International Forestry Research (CIFOR) processes at global level (FAO, 1996, 1997, 2003, 2004, 2010; CIFOR, 1999; ITTO, 2003, 2005; CCFM, 2004; MCPFE, 2006; MPCI, 2017)

In this study, the expert group consisted of specialists from universities and research institutions, experts at ministries of agriculture, forestry and environment related to protected areas, and NGO representatives in both Turkey and Sweden. Experts were selected according to whether they had studied or worked in areas related to biodiversity, protected areas and sustainable forest management. The interest areas and biographies of the experts were reviewed on their web sites.

Three-hundred participants who were assumed to be related to the subject were asked to participate in the study. The survey was submitted to Turkish and Swedish experts simultaneously between September and December 2016. The web link for the questionnaire was sent to Turkish and Swedish experts via email. During the survey, three reminders were given once a month. Ultimately, 122 experts from Turkey and 112 experts from Sweden participated in the survey. Therefore, the response rate was quite high (81.3% for Turkish, 74.6% for Swedish experts), and we assumed that this rate was rather satisfactory (Nulty, 2008). The profile characteristics of the experts participating in the study are demonstrated in *Table 2*. One third of the experts in the study were women and the proportion of women was higher in the Swedish group. The Swedish

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group had a slightly lower average age than the Turkish group. Nearly 70% of the experts belonged to universities and research institutes. There was a higher proportion of experts from universities and research institutes in the Turkish group. The Swedish group had a higher proportion of experts from governmental institutions, but a lower proportion from NGOs.

Statistical analyses were conducted using Statistical Package Program (SPSS 20.0) and the results were evaluated at a significance level of 5%. Cronbach's alpha coefficient was calculated as 0.856, and was greater than 0.8; thus, the scale had high statistical reliability. The data obtained from the questionnaire had a non-parametrical distribution (Kolmogorov-Smirnov test, $p < 0.05$); therefore, a Mann-Whitney U test (Nachar, 2008) was used to determine the differences between the opinions of Swedish and Turkish experts regarding the indicators. According to the average of the responses given by the experts, the indicators were ranked starting from indicators that were considered the most important

Table 2. *Profile characteristics of experts*

Characteristics	Turkey		Sweden		Total	
	f	%	*f*	%	*f*	%
Gender						
Female	34	27.9	46	41.1	80	34.2
Male	88	72.1	66	58.9	154	65.8
Age						
20-30	10	8.2	24	21.4	34	14.5
31-40	43	35.2	23	20.5	66	28.2
41-50	41	33.6	40	35.7	81	34.6
51-60	24	19.7	21	18.8	45	19.3
60<	4	3.3	4	3.6	8	3.4
Affiliation/Working Institution						
University	62	50.8	63	56.3	125	53.4
Research Institute	29	23.8	6	5.4	35	15.0
Agriculture, forest and environment ministries	16	13.1	25	22.3	41	17.5
Non-governmental organization (NGO)	9	7.4	3	2.7	12	5.1
Other	6	4.9	15	13.4	21	9.0

Results

Opinions of experts for biodiversity indicators related to forest species

The opinions about the importance of endangered and endemic species as indicators between the Turkish and Swedish experts did not significantly differ (*Table 3*). In other words, the experts in both groups found these indicators equally important (*Fig. 1*).

The evaluated level of importance for the other five species indicators (*Table 3*) was statistically different ($p < 0.05$) between the two groups. For all of these indicators, the level of importance given by Turkish experts was higher than that of Swedish experts (according to 4-point Likert scale) (*Fig. 1*). The least important species indicator in the evaluation was "overused species". It was also the indicator where the evaluation differed most between the groups. In total, 47% of Swedish experts found this indicator

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not and less important, while only 9% of the Turkish experts found this indicator less important.

Table 3. *Mann-Whitney U test results for the importance of forest species indicators*

Indicators	M-Whitney U	Wilcoxon W	Z	p
Endangered species	6325.000	12653.000	-1.798	0.072
Endemic species	6623.000	12951.000	-0.853	0.393
Overused species	4085.000	10413.000	-5.721	**0.000**
Species whose lives depend on protected areas	5889.500	12217.500	-2.494	**0.013**
Species whose distribution area is declining and habitat loss	5924.000	12252.000	-2.328	**0.020**
Major distribution areas and population status of the species	5985.000	12313.000	-2.059	**0.039**
Carrying capacity in terms of important species of area	5123.500	11451.500	-3.720	**0.000**

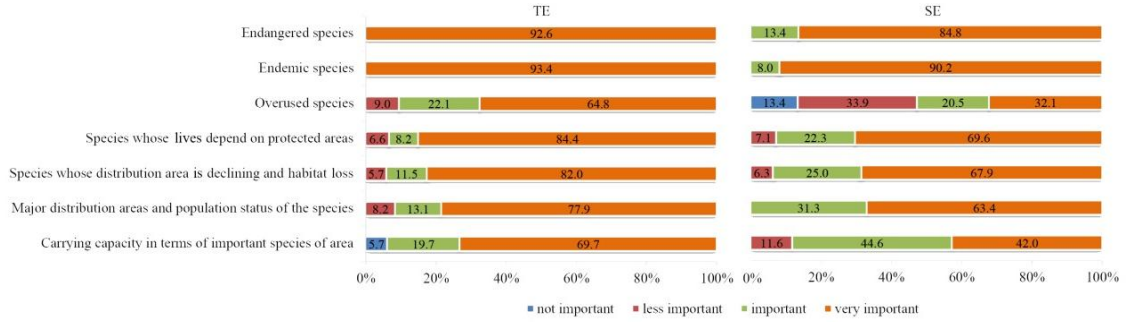


Figure 1. *Differences in given level of importance for the forest species indicators. (Turkish experts: TE, Swedish experts: SE, n_{TE} = 122, n_{SE} = 112, only values > 5%)*

Opinions of experts for indicators related to conservation of natural heritage, genetic resources and some forest structures

Genetic diversity refers to the diversity of genetic material and is assessed as the genetic difference in a specific species, population, variety, subspecies or race. The Turkish and Swedish experts found the indicators for genetic resources very important (*Fig. 2*), and there were no statistically significant differences found between the two groups (*Table 4*).

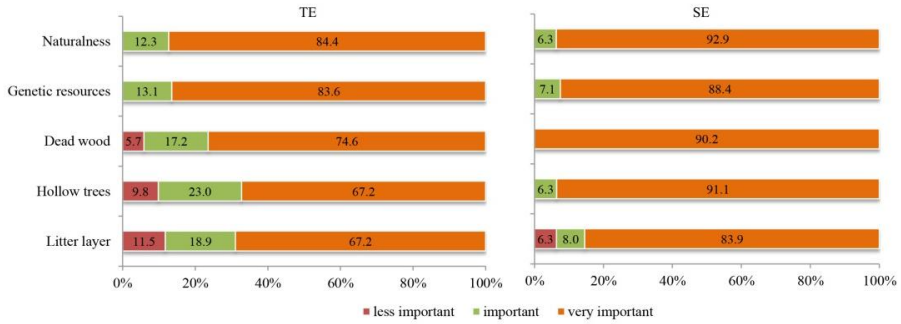


Figure 2. *Differences in given level of importance for the conservation of natural heritage, genetic resources and some forest structures indicators (n_{TE} = 122, n_{SE} = 112, only values > 5%)*

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Turkish experts found the indicators for dead wood and hollow trees less important than their Swedish counterparts (*Fig. 2*). The opinion about the importance of the dead wood and hollow trees indicator differed significantly between Turkish and Swedish experts (*Table 4*). Similarly, the "naturalness" indicator was found more important by the Swedish experts (*Fig. 2*). Another significant difference was that the Swedish experts found litter layer more important than did Turkish experts.

Table 4. *Mann-Whitney U test results for the importance of conservation of natural heritage, genetic resources and some important structures in forests indicators*

Indicators	M-Whitney U	Wilcoxon W	Z	p
Naturalness	6249.500	13752.500	-2.033	**0.042**
Genetic resources	6528.500	14031.500	-0.971	0.332
Dead wood	5801.000	13304.000	-2.984	**0.003**
Hollow trees	5202.000	12705.000	-4.411	**0.000**
Litter layer	5720.500	13223.500	-2.843	**0.004**

Opinions of experts for biodiversity indicators related to ecosystem

Regarding biodiversity indicators related to ecosystem, there were different opinions between the two expert groups for "plant species composition" and "forest distribution and regeneration" (*Fig. 3*). These indicators were found relatively more important by the Turkish experts and were statistically different (*Table 5*).

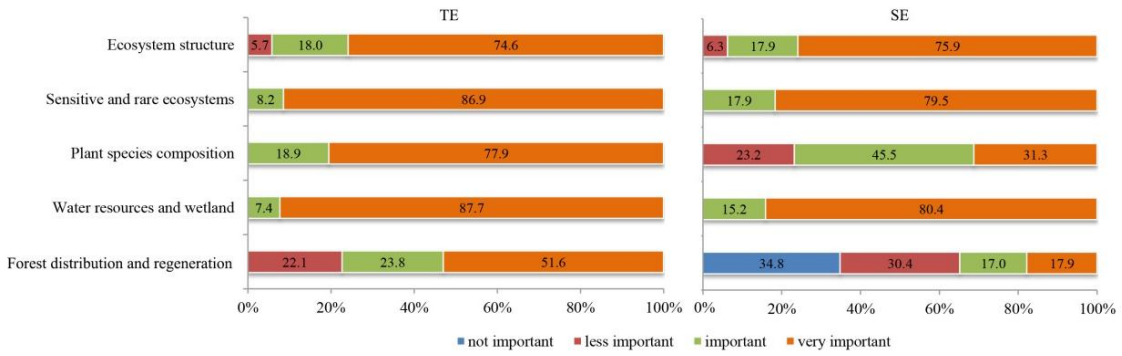


Figure 3. *Differences in given level of importance for ecosystem indicators (n_{TE} = 122, n_{SE} = 112, only values > 5%)*

Table 5. *Mann-Whitney U test results for the importance of ecosystems indicators*

Indicators	M-Whitney U	Wilcoxon W	Z	P
Ecosystem structure	6723.000	14226.000	-0.279	0.780
Sensitive and rare ecosystems	6374.500	12702.500	-1.366	0.172
Plant species composition	3450.000	9778.000	-7.333	**0.000**
Water resources and wetland	6358.500	12686.500	-1.444	0.149
Forest distribution and regeneration	3275.000	9603.000	-7.156	**0.000**

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Turkish and Swedish experts found "sensitive and rare ecosystems" and "ecosystem structure" indicators equally important. The "plant species composition" indicator was found less important by the Swedish experts (*Fig. 3*).

Opinions of experts for biodiversity indicators related to conservation status and external factors

There were no statistically significant differences between the opinions of the Turkish and Swedish experts regarding the importance of forest biodiversity indicators regarding conservation status and external factors (*Table 6*), and most of the experts deemed these indicators as "important" or "very important" (*Fig. 4*).

Table 6. *Mann-Whitney U test results for the importance of conservation status and external factors indicators*

Indicators	M-Whitney U	Wilcoxon W	Z	p
Existence of contaminants around protected areas	6467.500	12795.500	-1.057	0.290
Differentiated conservation status within protected areas	6226.500	12554.500	-1.341	0.180
Effects of other sectors	6788.500	14291.500	-0.110	0.913
Human use of protected areas	6392.500	12720.500	-1.009	0.313
Damaged areas	6650.000	14153.000	-0.450	0.653
Other issues important for sustainable management	6794.500	14297.500	-0.107	0.915

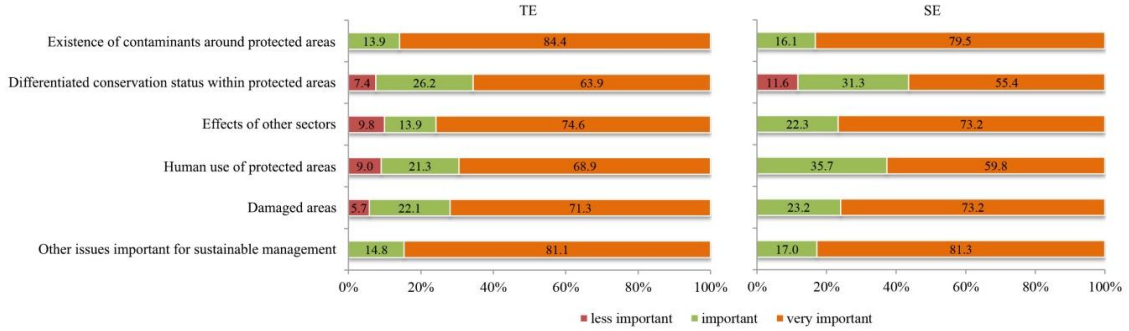


Figure 4. *Differences in given level of importance for forest biodiversity indicators related to conservation status and forest structure (n_{TE} = 122, n_{SE} = 112, only values > 5%)*

Ranking for forest biodiversity indicators

Table 7 presents the ranking of the scores and opinions of the experts for each indicator from the highest score to the lowest according to averages. The averages are calculated from the responses given on a 4-point Likert scale (not important = 1, less important = 2, important = 3 and very important = 4). Accordingly, the top five and last five indicators in the table are indicated with a grey colour. *Table 7* shows that only the indicator "forest distribution and regeneration" out of the 23 indicators had an average value of 2.74. The average value of the remaining 22 indicators was above 3. In general, this indicates that the experts found the indicators appropriate for the study.

The average values of the responses given by the Swedish and Turkish experts regarding the importance level of the indicators reveal that "endemic species" and

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"naturalness" were placed at the top amongst all indicators. This means that experts from both groups found endemic species and naturalness indicators equally important. The indicators for which there were the largest differences in opinion between the Swedish and Turkish experts included "dead wood" and "hollow trees". These indicators were ranked third and fourth, respectively, by Swedish experts, while Turkish experts ranked them fourteenth and eighteenth, respectively. The two indicators where both Turkish and Swedish experts gave the lowest rank and found equally less important were "overused species" and "forest distribution and regeneration". Additionally, "carrying capacity in terms of important species of area" and "the existence of different conservation status of protected areas" were found equally less important (*Table 7*).

Table 7. *Mean scores and rank for the evaluated forest biodiversity indicators*

Indicators	Turkish experts		Swedish experts		Total	
	Mean	Rank	Mean	Rank	Mean	Rank
Endemic species	3.90	1	3.88	2	3.89	1
Naturalness	3.81	5	3.92	1	3.86	2
Endangered species	3.89	2	3.83	6	3.86	3
Genetic resources	3.80	6	3.83	5	3.81	4
Water resources and wetland	3.83	3	3.76	9	3.79	5
Existence of contaminants around protected areas	3.83	4	3.74	10	3.79	6
Sensitive and rare ecosystems	3.80	7	3.77	8	3.78	7
Other issues important for the sustainable management	3.76	9	3.79	7	3.78	8
Dead wood	3.64	14	3.84	4	3.74	9
Hollow trees	3.57	18	3.88	3	3.72	10
Species whose lives depend on protected areas	3.76	8	3.61	15	3.69	11
Ecosystem structure	3.66	13	3.70	12	3.68	12
Species whose distribution area is declining and habitat loss	3.75	11	3.60	16	3.68	13
Damaged areas	3.64	15	3.70	13	3.67	14
Effects of other sectors	3.61	16	3.69	14	3.65	15
Major distribution areas and population status of the species	3.68	12	3.57	17	3.63	16
Litter layer	3.51	21	3.74	11	3.62	17
Human use of protected areas	3.58	17	3.55	18	3.57	18
Differentiated conservation status within protected areas	3.52	20	3.40	19	3.46	19
Plant species composition	3.75	10	3.08	21	3.43	20
Carrying capacity in terms of important species of area	3.53	19	3.27	20	3.41	21
Overused species	3.48	22	2.71	22	3.11	22
Forest distribution and regeneration	3.25	23	2.18	23	2.74	23

Other indicators with large differences were "plant species composition," as this was ranked tenth by Turkish experts and only twenty-first by Swedish experts. On the contrary, "litter layer" was ranked eleventh by Swedish experts and twenty-first by Turkish experts and was found less important than other indicators. The sometimes-diverging opinions between the groups are interesting, and can be helpful in defining the most important forest

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biodiversity indicators. The dynamic and varying structure of the indicators contribute positively to the identification of the best indicators for a given region.

Discussion

Dead wood and hollow trees represent structures of great importance for biodiversity and ecosystem health in forests. They provide important habitats for many species. Furthermore, they play a crucial role in increasing the resilience of forests and ensuring the continuity of ecological balance. Dead wood is a critical source for forest biodiversity and is commonly used as an indicator for sustainable forest management (Jonsson et al., 2016). Although the importance of dead wood for biodiversity is commonly known, directed strategies for protection of dead wood in forest ecosystems are implemented only in some of the regions across the world (Seibold et al., 2015). The most important difference among indicators is "dead wood" and "hollow trees" according to the experts in this study. Turkish experts found these indicators less important. One reason for this may be because of the common belief in Turkey that the existence of dead wood and hollow trees results in entomological pest species outbreaks (bark beetles, etc.) and may have implications for protected areas, for example by increasing the risk of forest fires. On the contrary, hollow trees have for a long time been known among Swedish foresters and biologists as very important for cavity nesting birds and many saproxylic invertebrates (Ehnström and Waldén, 1986; Nilsson and Baranowski, 1994; Ranius and Jansson, 2000). For Turkish conditions, similar results have been found in recent studies (Jansson and Coskun, 2008; Sama et al., 2011; Bergner et al., 2016), but this knowledge is still new and not commonly acknowledged in Turkish society.

Swedish experts found the "naturalness" indicator more important. This may be because of the opinion in Sweden that a desirable management regime in many protected forest areas is to make them look as "untouched" as possible (Steinwall, 2015). Turkish experts found the indicators for "plant species composition" and "forest distribution and regeneration" more important. One reason for this may be that the question of whether or not silvicultural treatments should be implemented in the protected areas of Turkey is still controversial. This debate exists because the affected areas are impacted to a certain extent by anthropogenic factors that are sometimes from entomological pest species outbreaks and an increased risk of forest fire (Colak, 2001; Alptekin et al., 2010). In Sweden there are lower risks for wild fires, and in some forest habitats wild fires are seen as positive and a part of the natural disturbance regime in the northern taiga forest ecosystem (Drobyshev et al., 2012).

The indicators for "sensitive and rare ecosystems" and "ecosystem structure" are used for monitoring, particularly in protected areas (Akyol and Tolunay, 2014). These ecosystems are jeopardized by the global climate change (Williams et al., 2015). Climate change may have multiple effects such as those related to the dynamics of species within ecosystems and abiotic factors that may have implications for ecosystems, such as forest fire dynamics (Costanza et al., 2016). Turkish and Swedish experts found these indicators equally important. It is also important to monitor "plant species composition" and put this in relation to these effects (Seidl et al., 2014; Pommerening et al., 2016), but in this study the Swedish experts found this less important than Turkish experts.

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Regarding biodiversity indicators related to conservation status and external factor, there were same opinions between the two expert groups and these indicators were found important or very important. Similar to most developing countries, a rigid and conservative approach is dominant in Turkey. This is the reason why local people are usually excluded from the planning and management of protected areas in Turkey. This approach is based on the assumption that a natural resource can be protected by prohibiting or restricting its exploitation as a natural resource by law (Rutagarama and Martin, 2006). This approach leads to a conflict between managers and users from the perspective of objectives set for the protected areas, since the aims of the resource managers are to minimize or prevent the utilization of these resources while users try to increase their utilization of the resources or at least maintain the same level of exploitation (Alkan, 2009; Alicia et al., 2018). Ultimately, rigid and passive protection approaches fail because there is dependence by local people on the natural resources in or around protected areas. This is especially the case in developing countries, where use could not be eliminated and alternative sources of income could not be provided to locals who are already in difficult socioeconomic situations. As such, protection often times exists only on paper (Akyol et al., 2017; Alkan et al., 2009; Alkan and Korkmaz, 2009; Thomas and Middleton, 2003; WWF, 2003; Arias et al., 2000).

Conclusions

It is important to monitor and assess biodiversity and sustainability in protected areas. Suitable indicators for protected areas have not been developed from the perspective of biodiversity and sustainable forest management. This study analysed a set of forest biodiversity indicators developed in international processes and solicited opinions from Turkish and Swedish forest experts to compare and analyse the indicators.

Although the overall ranking order differed, the first nine indicators were ranked the same by both expert groups and the top four indicators in total when adding the two groups together were among top six for both groups. These indicators were: “endemic species”, “naturalness” “endangered species” and “genetic resources”. The indicators with the lowest rankings in total and for both expert groups were “overused species” and “forest distribution and regeneration”.

Indicators with significant importance ranking differences were “dead wood” and “hollow trees”. This has important implications. Recent studies have shown very high biodiversity in old hollow oaks in Turkey, and thus these studies should be expanded to also cover other tree species and dead wood in general in Turkish forests. Naturally it takes time before results from scientific studies gains acceptance in society, and as such differences may be in part due to such a time lag in distributing knowledge.

The results also reveal different perceptions on the importance of such structures amongst people from different biogeographical regions, and hence environmental conditions may result in different perspectives on nature management. The differences should be considered an advantage in the process of developing improved biodiversity indicators as both groups could benefit from each other’s experiences in these matters.

There is a need for further studies to evaluate the measurability of these indicators and to identify indicators that are the most cost-effective and reliable for measurement and quantification. It is also preferable if these indicators can be monitored in a standardized manner to ensure non-biased long-term data regarding indicator dynamics

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and how they relate to external factors, such as climate change. The set of indicators assessed in this study are a first step in the process of developing appropriate indicators for biodiversity in protected areas. This research provides a solid base and a guideline for the next step in the process.

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EVALUATION OF AIR AND SOIL POLLUTION USING THE BIOMARKER *MORINGA OLEIFERA* IN JEDDAH CITY, SAUDI ARABIA

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Abstract. Physiological processes, photosynthesis, carbon and nitrogen metabolism, osmotic adjustment and secondary metabolites are known to be affected by air and soil pollutants. The present study was designed to evaluate the impact of air and soil pollution in Jeddah city, Saudi Arabia, on the physiological processes of *Moringa oleifera*. Four accessions differing in their pollution intensity were selected for this study (S1-S4). Elemental analysis revealed that Pd, Cu, Mn, Cr, Fe and Zn were the main soil pollutants in study accessions and trees growing in S2 have accumulated large quantities of these metals in their leaves. The physiological response to environmental pollution in *M. oleifera* tree was found to be dependent on pollution intensity. The heavily polluted accession showed a different response from the rest of accessions. As an adaptive strategy to environmental stress, this site has shown an increase in some metabolites such as lipids, flavonoids, free amino acids, alkaloids and saponins. However, some metabolites were decreased in response to pollution intensity such as soluble sugars and proteins, ascorbic acid, and terpenoids.
Keywords: *Moringa oleifera, heavy metals, phytotoxicity, physiological response, antioxidant capacity*

Introduction

Moringa (*Moringa oleifera* Lam.) is the most broadly cultivated species within Moringaceae family and is known as a rapidly-growing tree. It is commonly distributed in the sub-Himalayan parts of India, Pakistan, Bangladesh and Afghanistan. It has been introduced and become naturalized in many other parts of the world including Arabian peninsula. *Moringa oleifera* has many common names as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, and many other synonyms (Fahey, 2005). *Moringa oleifera* is widely cultivated for many purposes as efficient food stuff, biofuel production, and other implementations. Traditionally, it is used in therapeutic objectives around the world due to actual manifestations. These advantages have been related to metabolites like phenolics, vitamins and proteins (Health, 2005; Adedapo et al., 2009). *Moringa oleifera* offers a rich and unusual mixture of nutrients in its leaves, pods and seeds, amino acids that possess many therapeutic activities like antioxidant, anti-aging and anti-inflammatory. Moringa is sometimes called "Mother's Best Friend" and "Miracle Tree" as it has been advised as a complementary to treat malnutrition (Sreelatha and Padma, 2009).

In fact, moringa is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach (Rockwood et al., 2013). The leaves of *M. oleifera* has been established as a rich source of phenolics and glucosinolates (Amaglo et al., 2010), minerals like iron, calcium, phosphorus and copper, (Saini et al., 2014a), tocopherols (Saini et al., 2014b) and antioxidants (Dillard and German, 2000). It also contains high concentrations of

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ascorbic acid, estrogenic substances and beta-sitosterol, vitamins A, B and C, α-tocopherol, riboflavin, nicotinic acid, pyridoxine, β-carotene, protein, and essential amino acids such as methionine, cystine, tryptophan and lysine (Rajput et al., 2017). These components have pharmacological potentials like antimicrobial, anticancer, antihyperlipidemic, antidiabetic, antiulcer, analgesic, antifertility, anticonvulsant and hepatoprotective (Ganatra et al., 2012).

Global awareness of air pollution has been rising due to its massive danger to public health. World Health Organization (WHO) reported that annual concentration of ambient air pollution was increased worldwide by 8% during the recent five years (Nakao et al., 2017). In regard to plants, unrestrained use of fossil fuels in industries and transport sectors has led to the increase in concentrations of gaseous pollutants such as SO_2, NO_2, etc. (Rai et al., 2011). It is a known fact that 60 % of air pollution in city is caused by automobiles only. On sensitive species of both plants and animals, the effect of these pollutants is observed at acute level. Response of plants towards air is being assessed by the air pollution tolerance index (APTI). Some plant species and varieties are so sensitive that they can be easily used as biological indicators or monitors of specific pollutants. They can further assist the planner in managing the urban cities (Horaginamani and Ravichandran, 2010).

With the extension of the urban expansion of the city of Jeddah, Kingdom of Saudi Arabia (KSA), many human requirements led to the accumulation of various wastes containing hazardous and non-hazardous components. The lack of proper disposal of those wastes cause environmental disasters on all surroundings. Solid waste management studies have shown that the amount of solid household waste produced per individual in industrialized and developed countries ranges from 2 to 2.5 kg/ day. The World Health Organization classified the Arab Gulf countries among the rich countries characterized by the rise of solid waste for individuals, which led to the emergence of the problem of open and closed garbage dump sites. Due to the fact that plants play an effective role in purifying the environment of pollutants, *M. oleifera* was selected for this study. It was noted that its fruits and leaves are palatable by the expatriate workers, despite of it was planted in areas close to garbage dumps and in heavy traffic roads. This study provides an overview on the nutritional value, antioxidant capacity, secondary metabolites, as well as heavy metals exclusion capacity from air and soil by *M. oleifera* in four differentially polluted accessions in Jeddah city, KSA.

Materials and methods

Green leaves of *M. oleifera* tree were collected during the summer season of 2017 from four different accessions in Jeddah city, KSA. The first accession (S1) was a double public road passing by cars (21.33.32 N; 39.10.33 E), the second accession (S2) was a polluted area by flaming fires, factories wastes and random waste disposal (21.21.16 N; 39.13.09 E). The third accession (S3) was a narrow bystreet with rare traffic (21.33.29 N; 39.10.32 E) and the fourth accession (S4) was a secondary street with average traffic (21.33.49 N; 39.10.44 E). The map of different experimental sites is provided in *Photo 1*. The mature and fully expanded leaves were collected from the top branches of the trees in the study sites. A total of 250 leaves were collected from 5 trees at each experimental site. Leaf samples were washed with tap water several times and once with distillated water, placed in an air-forced oven at 60 °C for five days. Dried samples were grinded into fine powder by an electrical mixer, sieved through 0.2 mm

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sieve and stored in paper bags for analysis. Soil samples were collected at the time of leaves collections from the study sites. Soil samples were collected from the surface soil layers with a depth of about 0 to 20 cm at the tree rhizosphere. Soil samples were replicated three times per each experimental site. The collected soil samples air dried and sieved for physical and chemical characterization.



Photo 1. *A satellite image of the four experimental sites*

Mineral analysis of plant and soil samples

Plant and soil samples were digested with a mixture of 69% HNO_3 and 30% H_2O_2 (5:2 v/v). The mineral content concentrations in digested solutions were determined using inductively coupled plasma-optical emission spectroscopy (Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA).

Estimation of photosynthetic pigments

Total photosynthetic pigments; Chl a, Chl b and carotenoids, were extracted from moringa leaves by grinding in cold acetone (80%) using pre-chilled mortar and pestle. The extracted pigments were centrifugated at 3000 rpm for 15 min and quantified according to the method prescribed by Metzner et al. (1965). The quantified pigments were expressed as (µg/g dw).

Preparation of methanolic extract

The fine powdered plant tissues (5 g for each sample) were extracted with 50 ml of 95% methanol for 12 h at room temperature in an orbital shaker (Panasonic, MIR-S100, Japan). The extracts were filtered through Whatman No. 1 filter paper. The residues

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were extracted twice again as previously and extracts were combined. The combined extracts were concentrated under reduced pressure at 40 °C using rotary evaporator (Heidolph) and adjusted to 50 ml by methanol. Extracts were stored in glass vials at 4 °C until the time of analysis. All the spectrophotometric measurements were carried out using JENWAY 6315 UV/Visible Spectrophotometer (Japan).

Determination of total soluble carbohydrates

The total soluble carbohydrates content in the methanolic extract of plant powder was measured according to the phenol–sulfuric acid method using glucose as a standard sugar (Dubois et al., 1965) and expressed as mg/g dw.

Determination of total soluble proteins

Total soluble protein content of the extract was measured using bovine serum albumin (BSA) as a protein standard. The extract or the standard protein was mixed with Coomassie brilliant blue G250 reagent, the absorbance was measured at 595 nm and the results were expressed as mg/g dw (Bradford, 1976).

Lipid quantification

Total lipid content of leaf powders was extracted by the method reported by Bligh and Dyer (1959). The samples were stirred in a chloroform and methanol mixture (2:1 v/v) over 48 h, the powder was filtered off and washed with additional chloroform. This was repeated three times. The volatiles were removed under reduced pressure and the lipid content was expressed as mg/g dw.

Determination of flavonoids

Colorimetric aluminum chloride method was used for flavonoid determination (Chang et al., 2002). Leaf extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M K-acetate and 2 ml of distilled water. The absorbance of the reaction mixture was measured at 417 nm. Total flavonoids were calculated as quercetin from a calibration curve and expressed as mg/g dw.

Determination of ascorbic acid (AA)

Ascorbic acid was extracted by homogenization in 5% sulfosalicylic acid. The resultant homogenate was centrifugated at 5000 rpm for 15 min and 1 ml of the supernatant was incubated with 2 ml 2% Na-molybdate, 2 ml 0.15 N H_2SO4 and 1 ml 1.5 mM Na_2HPO_4 at 60 °C for 40 min. Absorbance was measured at 660 nm and ascorbic acid content was calculated as mg/g dw using a prepared calibration curve by ascorbic acid (Oser, 1979).

DPPH radical scavenging activity

The antioxidant capacity of the obtained methanolic extracts was measured by bleaching of the purple coloured solution of 1, 1- diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Sun et al. (1988). Methanolic extracts were added to 0.2 mM DPPH and incubated at room temperature for 30 min, then the absorbance was measured against a blank at 517 nm. DPPH scavenging activity was calculated from the formula: (%) = 100 [(A blank-A sample)/A blank].

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Free amino acids estimation

Amino acids content of the methanolic extracts was estimated by ninhydrin assay using glycine as a standard amino acid (Lee and Takahashi, 1966). Samples were mixed with ninhydrin-citrate buffer-glycerol mixture (0.5 ml 1% ninhydrin solution in 0.5 M citrate buffer pH 5.5, 1.2 ml of 55% glycerol and 0.2 ml of 0.5 M citrate buffer). The mixture was then shaken and boiled in a water bath for 12 min, cooled, shaken well, and the absorbance was measured at 570 nm and the result was calculated as mg/g dw.

Determination of phenolic compounds

The total phenolic content of methanolic extracts was determined using Folin-Ciocalteu's reagent (Jindali and Singh, 1975). Aliquot of extracts was mixed with 0.1 ml Folin-Ciocalteu's reagent and 1 ml 20% Na_2CO_3, then completed up to a known volume with dist. water. Thereafter, the absorbance was measured at 650 nm after 30 min and gallic acid was used as a standard phenol and phenol content was expressed as mg/g dw.

Determination of alkaloids

Alkaloids were determined using the method of Harbourne (1984). Alkaloids were extracted by a mixture of acetic acid and ethanol (1:9 v/v). The mixture filtered and the extract was concentrated on a water bath. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The precipitate was collected, washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed then expressed as mg/g dw.

Estimation of saponins

Saponin content was estimated quantitatively by the method described by Hiai et al. (1975) in the methanolic extract. Aliquot of 0.5 ml extract was mixed with 0.5 ml of 8% vanilline. The mixture was placed in an ice bath and mixed with 5 ml of 72% H_2SO_4, then heated in a water bath at 60 °C for 10 min followed by cooling in the ice-cold water bath. The content of saponins was calculated as mg/g dw using a standard curve by cholesterol.

Total tannins determination

The tannin contents of methanolic extracts were determined by method of Broadhurst and Jones (1978) using tannic as a standard. Aliquot of 400 µl extract is added to 3 ml of 4% vanillin in methanol and 1.5 ml of concentrated HCl. After 15 min of incubation the absorbance was read at 500 nm. Tannin content was calculated as mg/g dw.

Total terpenoids determination

Aliquot of 1.5 ml chloroform was added to 200 µl extract, the mixture was vortexed well, then 100 µl conc. H_2SO_4 were added and incubated at dark for 2 hs. The supernatant was decanted and 1.5 ml of 95% methanol was added, followed by vortexing until all the precipitate dissolved completely and the absorbance was measured at 538 nm. Total terpenoids were calculated using a standard curve prepared by linalool and expressed as mg/g dw (Ghorai et al., 2012).

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Statistical analysis

Results were statistically analyzed by one-way ANOVA using Costat under Windows software. Significant differences among accessions were calculated by Duncan's multiple range test at 0.05%. Results were represented as mean of three replications ± standard deviation (SD).

Results

Table 1 shows the physical properties of soil samples collected from *M. oleifera* various growth habitats (accessions). A highly significant change was observed in electrical conductivity (EC) as a measure of soluble salts in the soil and salinity level. The uppermost EC value was quantified for S4 and S1 soil samples (897 and 860 µS/cm, respectively). Meanwhile, the lowermost values were observed in soil samples of S3 and S2 (335.3 and 345.7 µS/cm, respectively). As for soil reaction, the results showed that S1 soil was slightly acidic (6.9), S2 was exactly neutral (7), however S3 and S4 soils were marginally alkaline (7.2).

Table 1. *Physical properties (EC and pH) of soil samples collected from growth accessions of M. oleifera trees in Jeddah city*

No	EC (µS/cm)	pH
S1	860.0±7.0c	6.9±0.2a
S2	345.7±0.6b	7.0±0.0ab
S3	335.3±0.6a	7.2±0.1c
S4	897.0±1.0d	7.2±0.1bc

The concentrations of various heavy metals (Cr, Pb, Cu, Fe, Mn and Zn), as well as some essential elements (P, Ca, Mg and K) in addition to Na in the soils and leaves of *M. oleifera* collected from four differentially polluted habitats, S1-S4, in Jeddah city, KSA significantly varied (*Table 2*).

Table 2. *Mineral analysis of soil samples and M. oleifera leaves (ppm) exposed to different pollution intensities in Jeddah city*

Metal concentration (ppm)	Soil samples				Leaf samples			
	S1	S2	S3	S4	S1	S2	S3	S4
Cr	1.15^{a}	0.98^{d}	1.06^{c}	1.12^{b}	0.02^{a}	0.10^{b}	0.02^{a}	0.01^{b}
Pb	0.50^{c}	3.63^{a}	1.13^{b}	0.21^{d}	0.25^{c}	1.95^{a}	0.41^{d}	0.01^{b}
Cu	0.74^{d}	1.97^{a}	1.22^{b}	1.08^{c}	0.06^{c}	0.29^{a}	0.06^{c}	0.11^{b}
Fe	865.0^{a}	818.0^{b}	766.0^{d}	785.0^{c}	6.27^{b}	18.00^{a}	0.19^{c}	6.71^{b}
Mn	15.4^{c}	26.4^{a}	15.90^{bc}	16.70^{b}	0.42^{c}	2.30^{a}	0.55^{b}	0.06^{d}
Zn	1.70^{d}	2.30^{b}	3.10^{a}	1.91^{c}	0.59^{b}	6.40^{a}	0.53^{b}	0.53^{b}
P	23.14^{a}	18.09^{c}	15.78^{d}	21.25^{b}	13.89^{c}	20.89^{a}	5.47^{d}	17.67^{b}
Ca	296.0^{a}	182.0^{b}	141.0^{c}	106.0^{d}	60.80^{b}	37.30^{c}	170.0^{a}	172^{a}
Mg	597.5^{b}	550.6^{c}	602.7^{b}	726.8^{a}	45.70^{c}	147.30^{a}	$64.6.0^{b}$	36.6^{d}
Na	22.23^{c}	17.30^{d}	25.47^{a}	24.05^{b}	13.92^{d}	83.73^{a}	17.61^{c}	24.65^{b}
K	84.80^{b}	73.10^{c}	73.30^{c}	116.0^{a}	53.90^{c}	70.10^{b}	90.20^{a}	54.1^{c}

Means within the same row followed by different letters are significantly different ($P < 0.05$)

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Difference in heavy metals transport from soils to leaves of *M. oleifera* was remarkable. The most pronounced concentration of metals in soil samples was recorded for Pb, Cu and Mn in S2 soil (3.63, 1.97 and 26.4 ppm, respectively), Cr and Fe in S1 soil (1.15 and 865.0 ppm respectively) and Zn in S3 soil (3.10 ppm). The results indicated that soil S4 is less contaminated with heavy metals than the rest of the soils under study. For essential elements, it has been proven that S1 soil was the richest with P and Ca (23.14 and 296.0 ppm, respectively) and S4 soil was the richest with Mg and K (726.8 and 116.0 ppm, respectively). However, the highest Na concentration was more remarkable in S3 soil (25.47 ppm).

Estimation of heavy metals and essential minerals in *M. oleifera* leaves distinguished trees grown in S2 accession (heavily polluted by flaming fires, factories waste and random waste disposal) as highly accumulative for these elements. The most prominent concentration in S2 leaves was observed for Pb, Cu, Fe, Mn, Zn, P, Mg and Na (1.95, 0.29, 18.0, 2.3, 6.4, 20.89, 147.30 and 83.73 ppm, respectively). However, the most prominent concentration of Ca and K was observed in S1 and S3, respectively (60.80 and 90.20 ppm). Therefore, *M. oleifera* could be used as a biomarker for air and soil pollution in heavily polluted areas like S2 one.

The ambient air and soil pollution profoundly affected photosynthetic pigments concentration in *M. oleifera* leaves. Chlorophyll a content significantly lowered in S2, S3 and S4 accessions (3.90, 3.31 and 3.65 µg/g dw, respectively) comparable to S1 (4.71 µg/g dw) (*Fig. 1*). The most pronounced reduction in Chl a was observed in leaves of S3. For Chl b, the same result was attained, as S1 was higher than other accessions (1.61 µg/g dw). The least Chl b content was observed in S2 and S4 (1.41 and 1.40 µg/g dw, respectively). In contrast to chlorophyll, carotenoids recorded the highest content in S2 leaves (1.46 µg/g dw).

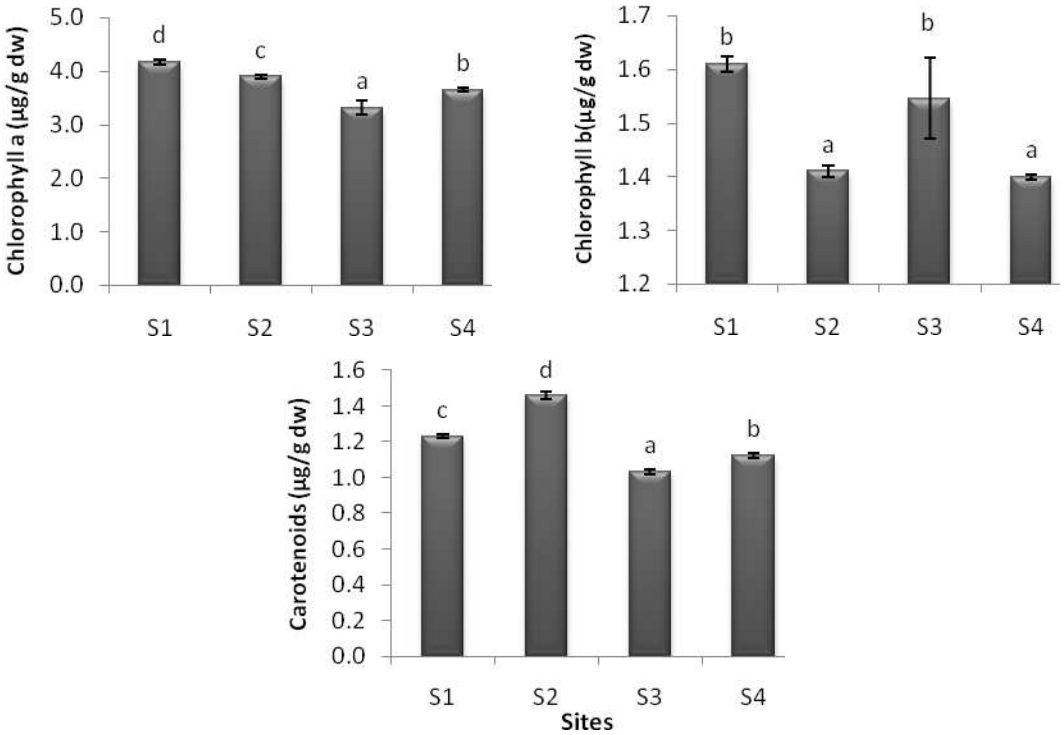


Figure 1. *Photosynthetic pigments of M. oleifera leaves (µg/g dw) exposed to different pollution intensities in Jeddah city*

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Total soluble sugars content in *M. oleifera* leaves was profoundly varied with the growth site (*Fig. 2*). The uppermost sugar content was detected in S4 leaves compared to other accessions. However, the least sugar concentration was in leaves of trees growing in S1 (77.6 mg/g dw). The same pattern of sugars concentration was perceived for total soluble proteins in the studied accessions. As for total lipids, the leaves of trees growing in S1 and S4 recorded the least content (58.0 and 61.5 mg/g dw, respectively), whereas those of S2 and S3 recorded higher levels (77.0 mg/g dw).

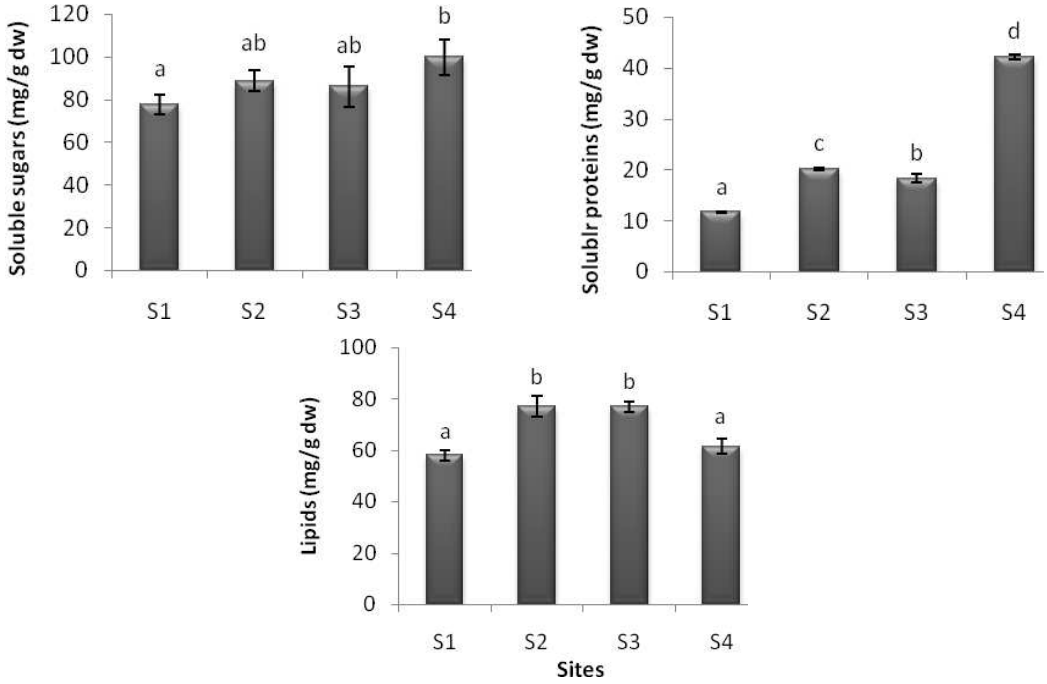


Figure 2. *Total soluble sugars, soluble proteins and total lipids of M. oleifera leaves (mg/g dw) exposed to different pollution intensities in Jeddah city*

Data in *Figure 3* illustrate the non-enzymatic antioxidant activities of *M. oleifera* leaves growing at different accessions in Jeddah city (KSA), represented in flavonoids, ascorbic acid and total free radical scavenging activity (DPPH). The highest flavonoids content (5.76 mg/g dw) was ascertained in S2 leaves, however the least content (2.57 mg/g dw) was in S1. Ascorbic acid level ranged from lower value (19.11 µmol/g dw) in S3 to higher value (23.68 µmol/g dw) in S1 leaves. Free radical scavenging (DPPH) activity differed in plant leaves with the difference in growth position. The most pronounced DPPH activity (35.7%) was detected in S4 leaves, meanwhile the least activity (24.7%) was recorded in S1 leaves.

Accumulation of free amino acids, phenolic compounds and alkaloids in *M. oleifera* leaves in response to pollution intensity in four geographical accessions in Jeddah city is summarized in *Figure 4*. Total content of free amino acids oscillated between 61.9 and 187.0 mg/g dw in the studied plant leaves. Accumulation of free amino acids was more pronounced in S2 (highest value), nevertheless the least content was detected in S1 leaves. Study results revealed a significant difference among study accessions in *M. oleifera* leaves phenolic content. The highest phenolic content was detected in the leaves of S4 and S2 (78.39 and 76.75 mg/g dw, respectively), however the lowermost

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content was in leaves of S1 (39.29 mg/g dw). For alkaloids content, the change with the growth accession was highly significant. Accession S2 recorded the maximum rate of alkaloids accumulation (51.0 mg/g dw) in *M. oleifera* leaves, while accession S3 showed the lowest alkaloids content (25.0 mg/g dw) in *M. oleifera* leaves.

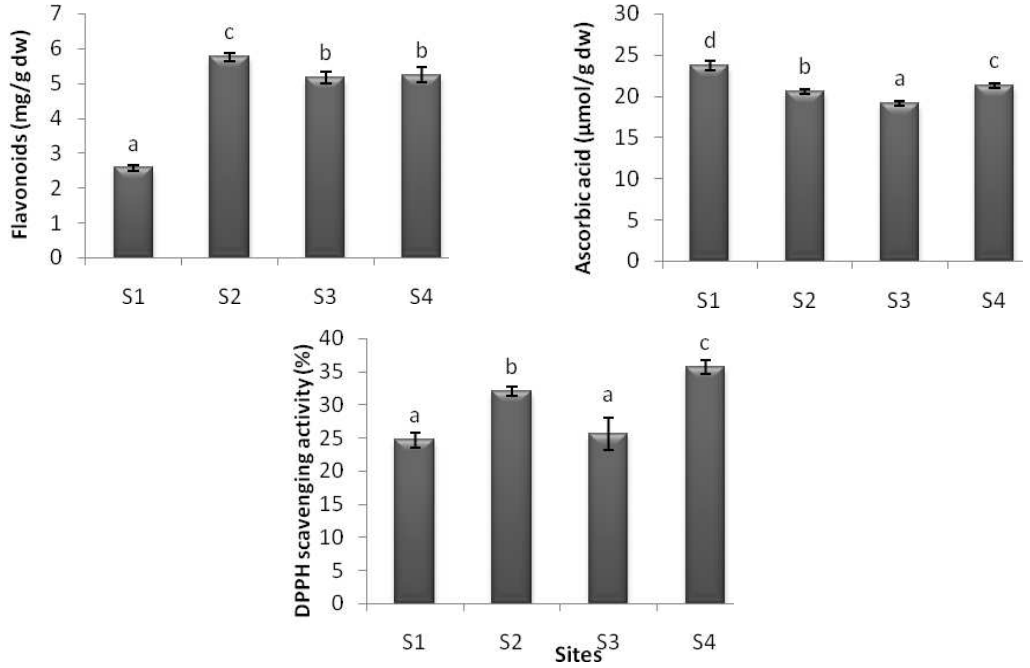


Figure 3. *Non-enzymatic antioxidant activities of M. oleifera leaves (flavonoids, ascorbic acid and DPPH activity) exposed to different pollution intensities in Jeddah city*

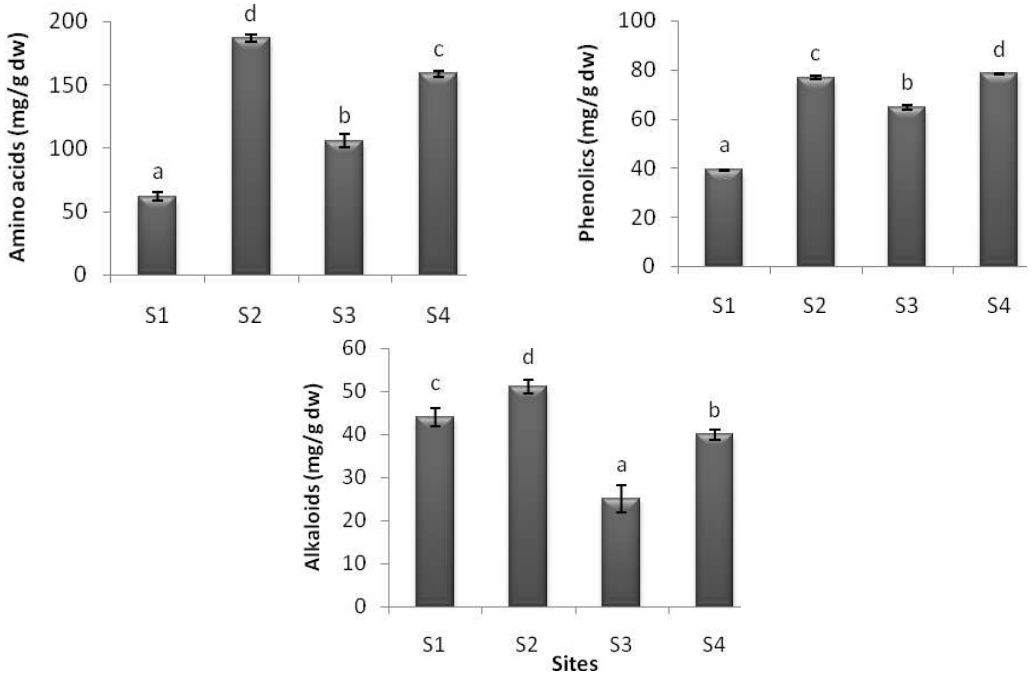


Figure 4. *Variations in amino acids, phenolic compounds and alkaloids contents (mg/g dw) of M. oleifera leaves exposed to different pollution intensities in Jeddah city*

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Variation in the content of some secondary metabolites (saponins, tannins and terpenoids) in *M. oleifera* leaves with the change in the intensity of air and soil pollution in Jeddah city, KSA, is represented in *Figure 5*. The heavy pollution in S2 by flaming fires, factories wastes and random waste disposal caused the accumulation of saponins in *M. oleifera* leaves with the uppermost value (21.3 mg/g dw) throughout this study. In contrast, the lowermost accumulation of saponins (11.6 mg/g dw) was documented in S2, which is exposed to air pollution by automobile exhaust. Tannins and terpenoids content was significantly varied with growth habitat from lowest value (4.35 and 1.59 mg/g dw, respectively) in S3, to highest one (6.06 and 5.90 mg/g dw, respectively) in S1.

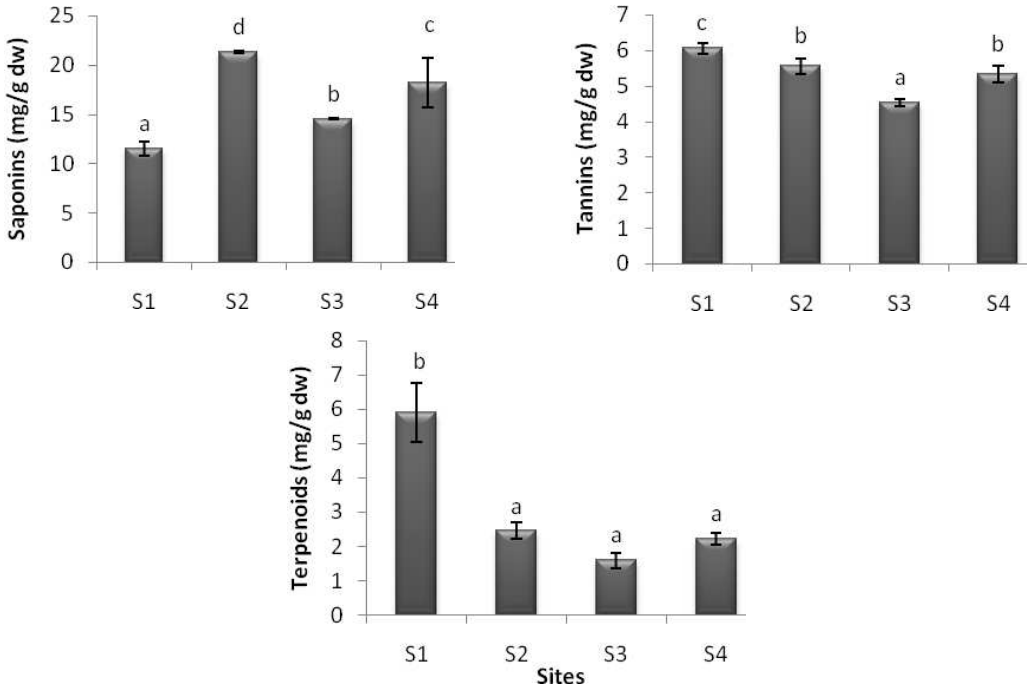


Figure 5. *Variations in secondary metabolites (saponins, tannins and terpenoids) content (mg/g dw) of M. oleifera leaves exposed to different pollution intensities in Jeddah city*

Discussion

The crisis of environmental pollution is one of the most important challenges facing man in our time. After the industrial revolution in the nineteenth century, the sources of water, air and soil pollution increased, which had a profound impact on the biosphere in which humans, animals and plants live. At the end, all sources of pollution affects human health negatively, either directly or indirectly. The plants are considered to be the most important indicators of evidence of pollution occurrence, and also contribute significantly to the removal of some environmental pollutants. In the present study, *Moringa oleifera* tree growing in differentially polluted habitats in Jeddah city, KSA was used as indicator on air and soil pollution. The variation in pollution intensity had significantly affected mineral content, photosynthetic pigments, primary and secondary metabolites and antioxidant status of *M. oleifera* leaves.

The present study showed that S2 soil (the most polluted site due to flaming fires and factories waste disposal) was more polluted with toxic metals as Pb, Cu and Mn. In the

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same time, trees growing in that area accumulated incredible quantities of heavy metals like Pb, Cu, Mn, Fe and Zn in their leaves. Anthropological activities release large amounts of pollutants due to industrial effluents leading to pollution of ground water through the infiltration by soil which is a major issue (Vasanthavigar et al., 2012). Presence of heavy metals in soil and water can cause bioaccumulation affecting the entire ecosystem and pose harmful health consequences to all life forms. The main effects of these metals are inhibition of seed germination, hindering growth and production, affecting the yield of crops and viability of soil microflora (Sethy and Ghosh, 2013).

Photosynthetic pigments of *M. oleifera* leaves were affected by the pollution intensity of the growth site. Chlorophyll a content significantly affected within study accessions, the highest Chl a content was recorded in S1 (double public road exposed to automobile pollutions). Such results are in agreement with those obtained by Giri et al. (2013), who reported that air pollution significantly lowered Chl a content in *Azadirachta indica*, *Mangifera indica*, *Nerium oleander* and *Dalbergia sissoo* leaves. On the other hand, S4 and S2 leaves showed the least Chl b content. This result suggested that Chl b was more sensitive to air pollution than Chl a in *M. oleifera* leaves. Accordingly, the reduction in Chl b content was compatible with the results of Giri et al. (2013) who concluded that polluted sites have less Chl b content than non-polluted ones. Such reduction in Chl b content in air-polluted sites could be attributed to the accumulation of some metals in leaf tissues that increase chlorophyll degrading enzymes activity, inhibit chlorophyll synthesizing activity, or replace Fe and Mn ions in chloroplast proteins reducing their activity. In addition, the non-neutralized heavy metal ions stimulate formation of an excess of free radicals and slow down the turnover of chloroplasts proteins, mostly D1 protein (Lin and Aarts, 2012).

Carotenoids accumulation in plant leaves is a stress marker in most plant species. Carotenoids recorded the highest content in S2 leaves of *M. oleifera*. As a normal consequence of air and soil pollution, generation of free radicals and active oxygen species is stimulated. To prevent the deleterious effects of these oxygen species, plants have developed antioxidative mechanisms to detoxify such molecules. Carotenoids accumulation in response to heavy metals was observed by (Pinto et al., 2011) in *Gracilaria tenuistipitata* macroalga as they play crucial role in membrane stability, specially thilakoid membranes, as well as being natural antioxidants.

Soluble sugars and proteins were significantly lowered in heavily polluted accessions, comparable to those less polluted. Such result is in agreement with those obtained by Tzvetkova and Kolarov (1996), who concluded that decrease in total sugar content of in polluted sites could be attributed to the photosynthetic inhibition or stimulation of respiration rate. Also, decrease of soluble sugars could be an indicator of starch hydrolysis disturbance or altered carbohydrate metabolism in metal and gaseous polluted sites. The reduced carbon and sugar metabolism due to heavy metals and air pollutants might result from their probable interaction with ribulose-bisphosphate carboxilase active centre (Stiborov'a et al., 1987). The reduced protein content in polluted area could be attributed to impairment of pollutants with nitrogen metabolism or disruption in protein synthesizing enzymes. Such findings are in agreement with those of Rai (2016), who attributed that the enhanced rate of protein denaturation and breakdown of existing protein to amino acids are the main causes of protein reduction in response to air pollution. In addition, prolonged exposure to environmental pollutants results in depletion of the antioxidant enzymes as a result of oxidative damage to

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biological molecules, such as protein and DNA (Bebianno et al., 2005). Furthermore, heavy metals disorder protein metabolism either by forming complex with functional side chains or by substituting essential metal ions in metalloproteins (Tamás et al., 2014).

High pollution level in S2 and S3 accession resulted in higher levels of total lipids accumulation in plant leaves comparable to other ones. The same result was obtained by Nouairi et al. (2006) in *Brassica juncea* plants exposed to cadmium stress. They attributed this to the increase in vacuolar area where toxic metals are frequently stored. Previous work of Djebali et al. (2005) revealed variable effects of heavy metals on different fatty acids, as they have negative impact on α-linolenic acid proportion, while other fatty acids were affected positively.

Our results revealed that the highest flavonoids content was found in the heavily polluted (S2) leaves. The results are in accordance with those of Robles et al. (2003) who reported that total flavonoids accumulation was indicator on ozone pollution in *Pinus halepensis* growing at five different polluted areas. Also, *Betula pubescens* leaves in strongly polluted areas significantly accumulated variability of some phenolics which might related to the effect of environmental contamination on shikimate and phenyl-propanoid pathways (Loponen et al., 2001). Such increase in flavonoids content could be associated with its defensive role under conditions of environmental stress.

Variations in ascorbic acid level with growth accession were not effective. Previous work of Zengin and Munzuroglu (2005) showed that metal-treated (lead, copper, cadmium and mercury) bean seedlings showed significant higher ascorbic acid content. Such increase in ascorbic acid in polluted areas could contribute in raising the antioxidant capacity of stressed plants. Heavily polluted areas (S2 and S3) showed moderate DPPH activity, however less polluted areas showed oscillation in DPPH activity. The variation in total antioxidant activity between growth accessions could be attributed to the variable response of plant leaves to pollution intensity, in addition to the selective impact of contaminant kind on the antioxidant molecules.

The highest leaf free amino acids content was accounted for *M. oleifera* growing in S2 accession (most polluted). The increased accumulation of amino acids under metal toxicity might be due to its role in detoxifying heavy metal through making complexes with it, consequently assisting in the protection of the leaf and photosynthetic machinery from heavy metal injury (Rascio and Navari-Izzo, 2011). A leading mechanism in metal detoxification is the chelation of metals by metabolites rich in -SH groups. Hence, the content of non-protein thiols, which include thiol-rich peptides, glutathione, or other -SH rich compounds. A recent work of Nadgórska–Socha et al. (2017) demonstrated that thiol-containing amino acids content in *Robinia pseudoacacia* and *Taraxacum officinale* leaves were higher in the high traffic and emission regions.

Phenolic content in the leaves of *M. oleifera* only slightly differed between the various contaminated study sites. The uppermost phenolic content was detected in the leaves of S4 and S2. Mir et al. (2009) reported that total flavonoids and phenolics in *Catharanthus roseus* and *Ocimum sanctum* may serve as biomarkers of urban auto pollution as they showed a positive relationship with the vehicular pollution load. Rai et al. (2007) have also reported significant increase in total phenol contents in wheat under air pollution stress. Increase in phenolics under environmental stresses was monitored in many plant species as they play a crucial role in defense mechanisms against ROS damage.

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With respect to alkaloids accumulation, accession S2 recorded the maximum rate in *M. oleifera* leaves. These results are consistent with those obtained by Singh et al. (2000), who reported that alkaloid content of *Datura innoxia* was considerably accumulated in various plant organs in response to coal-smoke pollution. They attributed the increase in alkaloid content to a chemical adaptation or a defensive/protective strategy under stress. Alkaloids accumulation under stress conditions was reported in various plant species as *Senecio longilobus* (Briske and Camp, 1982), *Lupinus angustifolius* (Jansen et al., 2009) and *Achnatherum inebrians* (Zhang et al., 2011).

On the same pattern, the heavy pollution in S2 caused the accumulation of saponins in *M. oleifera* leaves comparable to other study sites. Szakiel et al. (2011) reviewed that plants belonging to the same species in different geographical zones may differ significantly in their content of secondary metabolites. This phenomenon was observed in many saponin-containing plants, including *Panax ginseng*, *Panax quinquefolius* and *Panax notoginseng*. The accumulation of saponins and other secondary metabolites in response to environmental factors is a part of an adaptive strategy leading to enhanced tolerance (Ramakrishna and Ravishankar, 2011). Nevertheless, tannins and terpenoids along this study showed variable accumulation in response to pollution intensity.

Conclusion

Trees and plants in the surrounding environment are considered the lungs of our life, on which most other organisms rely to obtain oxygen and energy. Civilization and urban life have become a major burden on the environment as a result of pollution of water, air and soil. In this study, *Moringa oleifera* trees growing in differentially polluted accessions in Jeddah city, KSA, showed that the intense pollution has a deleterious impact on the metabolic machinery and the physiological processes of the trees. As a responsive mechanism in heavily polluted accession, *M. oleifera* showed a diminution in chlorophyll, soluble sugars, soluble proteins, ascorbic acid, and terpenoid contents. On the other hand, lipids, flavonoids, amino acids, alkaloids and saponins were accumulated in the tree leaves.

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SOCIOECONOMIC DETERMINANTS OF RURAL HOUSEHOLDS' FOOD CROP PRODUCTION IN OGUN STATE, NIGERIA

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Abstract. Agricultural production seems to hold the key to economic growth and development in the developing nations of the world. The study focused on the socioeconomic determinants of rural household's arable crop production in Ogun State, Nigeria. The specific objectives of this study were to estimate the gross margin of the farmers and determine the effect of their gender resource on food crop production in the study area. Multistage sampling technique was employed to select 210 respondents as population of the study. Budgetary analysis was used to determine their profit margin while Tobit regression analysis was modeled to determine the effect of household's gender resource(s) on food crop output. The result shows that 54.30% of the respondents have no formal education, 66.7% male and an average household size of 7 persons. Also, a mean age of 51 years and an average farm size of 9 hectares were derived in the study. Furthermore, the budgetary result revealed that food crop production is profitable with Gross margin of ₦205107.20/ha ($564.457/ha). Tobit regression result shows among others that gender of the household's head has a significant influence on output of the farm ($P < 0.05$). The study therefore recommended the provision of labour saving techniques to encourage female farmers in agricultural production in the study area.
Keywords: *gender resource, gross margin, socioeconomics, Tobit regression*

Introduction

The improvement in agricultural production is a result of more efficient use of the factors or production, viz physical, socio-economic, institutional and technological. The role of agriculture in economic development of most countries can hardly be overemphasized, the contribution of agricultural growth to overall poverty reduction has been documented (Sarris, 2005). A retrospective look into the Nigerian economy and its development reveals that agriculture was both the main stay of the Nigerian economy and the chief foreign exchange earner (Nwaiwu et al., 2017). In the 1960s, agriculture accounted for well over 80% of the export earnings and employment; about 65% of the GDP (gross domestic product) and about 50% of the government revenue (FRN, 2000). This contribution to the Nigerian economic growth has however declined over the years.

The contribution of agriculture to the GDP was about 50% in 1970 and 34% in 2003 (CBN, 2003). Although agriculture no longer serves as the leading contributor to

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Nigeria's gross national product and leading foreign exchange earner due to phenomenal growth in the petroleum sector of the economy, agriculture is still the dominant economic activity in terms of employment and linkages with the rest of the economy (Adebile and Amusan, 2011). The principal constraint to the growth of the agricultural sector is the fact that the structure and method of production have remained the same since independence more than four decades ago (Ukeje, 2005). The United Nations Food and Agriculture Organization rate the productivity of Nigeria's farmland as low to medium— but with medium to good productivity if properly managed (Parukayo and Umaru, 2012). To be effective, and attain higher level of productivity and growth in the agricultural sector there is a need to identify the major factors that determine its growth.

Agricultural production thus seems to hold the key to economic growth and development in developing countries. It is expected to stimulate increased crop production and growth into marketable surpluses, improve income earnings and employment opportunities, and enhance nutritional status, health care and household welfare in general. It is also expected to create changes in gender roles, imbalances within the household and a shift in the allocation of the resources available within the household. These changes will affect males and females differently in terms of costs and benefits generated and the efficient utilization of the household Resources. This necessitates the need for the incorporation of gender perspectives into development programmes. This is because gender related differences are expected to influence activity diversification and income generation in the household.

Problem statement

Farming activities in tropical Africa has always been dominated by rural women who grow a substantial amount of the staple foods, yet, they still have much less access to knowledge, technology, credit and land than their male counterpart (Adewumi and Omotesho, 2002). African women on small scale rain-fed farms produce up to 70-80% of the domestic food supply in most sub-Saharan African societies (WHO, 2003). On average, they also provide 46% of the agricultural labour. However, women's yields are too low by Green Revolution Standards (3 to 4 tonnes per hectare for food grains), and much lower than men's yields in societies where a comparison can be made (for example, where men grow the same crops on different fields or where yields of female headed households can be compared to those of male headed households).

Gender differences in productivity have been shown to be due to differences in the intensity of use of productive inputs (such as fertilizer, manure, land and labour, credit, extension training, and education) rather than in differences in the efficiency or management styles of men and women (Ganiyu and Omotayo, 2017). Because rural farmers faced problem of cash and/or credit to acquire modern yield-increasing inputs of production, they tend to produce less, and more of their crops are consumed within the family (Rahji, 2007). In Nigeria, the Structural Adjustment Programme (SAP) also tended to disproportionately burden women. The removal of subsidies, which have caused a rise in input prices, and liberalization measures, which have resulted in a flood of cheap imports, have further harmed local food producers, especially women farmers (Rahji, 2007).

Estimates by (Ukeje, 2005), show that if productive inputs like fertilizer, manure and labour could only be reallocated within the African household in some societies the results could mean an increase in the value of household output in the range of 10-20%.

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In response to the widespread gender disparity in terms of access to farm credit and technical logistics in rice production, the government of Nigeria has introduced pro-gender special crop interventions like block farms. In view of the above problems, this research will try to find answer to the following research questions such as how do we measure the gender impact of household resource allocation on agricultural production and what is the gross margin of food crop farmers in the study area?

Objective of the study

The general objective of the study is socioeconomic determinant of household food crop production in Ogun State, Nigeria. The specific objectives of the study are to:

1. Describe socio-economic characteristics of food crop farmers in Ogun State, Nigeria
2. Estimate profitability of food crop farmers in the study area
3. Determine the effect of household gender resource on food crop production

Hypothesis of the study

The hypothesis of the study stated in null form:

HO_1: There is no significant difference between female farming hour and male farming hour in agricultural production in Ogun state.

Materials and methods

Methodology

The study was carried out in Ogun State in South Western Nigeria because of her agricultural production pedigree. Ogun state is located in the south-west corner of Nigeria and was created in 1976 by the then Federal Military Government from the old western region (*Fig. 1*). It is neighboured by Oyo, Ondo, Lagos, Edo and Delta States. It is situated within the tropics and derives its name from the big "River Ogun". The state lies between 7°01' and 7°18' N and between 2°2'and 3°55' E. It has a tropical climate with rainforest vegetation on its southern part and a derived savannah on its northern end. It has an estimated land area of 16,409.26 km^2. The estimated human population is 3,751,140 (2006 population census) and it is characterized commercially by a dual economic focus, the bourgeoning industrial sector and a dominant agricultural sector. The study populations were mainly rural farming households who engaged in food crop production in the study area.

Sampling procedure and sample size

Sampling technique for this study was based on the four Agricultural zones by the Ogun state Agricultural Development Programme (OGADEP). These are Abeokuta, Ikenne, Ilaro and ijebu zones. Multistage random sampling technique was employed to select a total of 210 farming household in the study area. First stage involved the random selection of two zones which are Abeokuta and Ilaro. Second stage involved random selection of three (3) and two (2) blocks from each of the two ADP zones respectively. Stage three involved a random selection of four (4) cells from each of the blocks. While the last stage involved random selection of eleven (11) farming households from each of the cells. But a total of 210 sampled data were used for this

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study. Primary data were collected in 2014 from food crop farmers using structured questionnaire and interview schedule. Data were analysed by frequency count, percentage, mean, budgetary and Tobit regression analysis using Stata 11.

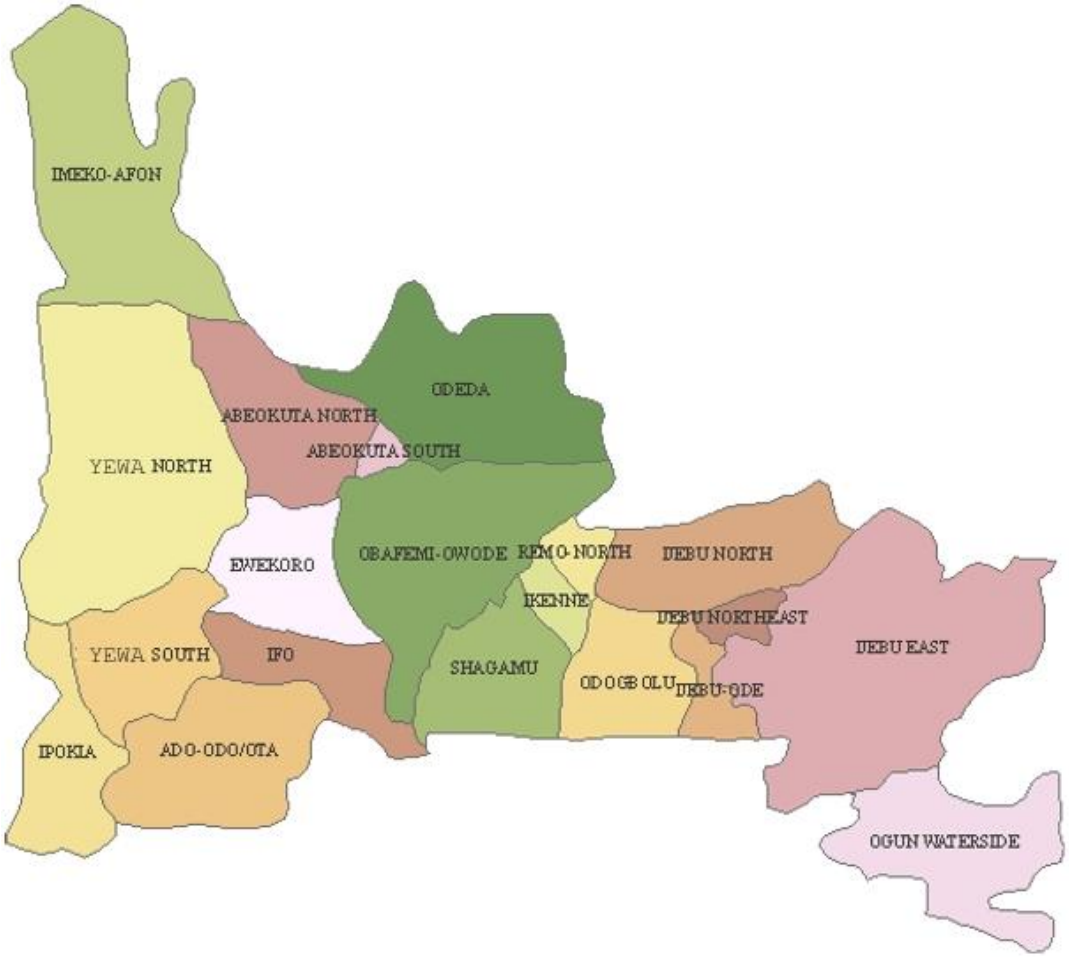


Figure 1. *Map of Ogun State showing local government areas*

Results and discussion

Socioeconomic characteristics of respondents

Socioeconomic factors of the respondents comprise of personal attributes of the respondents are presented in *Table 1*. Majorities (66.7%) of the respondents are male and 33.3% are female. In addition, majority (55.2%) of the respondents were married, this indicates that the respondents are adults and people who have mature orientation of life. Also, 4.8% of the respondents were single and about 30.0% of them had no formal education. This is quite a large proportion in this century as the implication of these results could be that higher number educational year is known to have a positive influence on the ability of the farmers (Omotayo, 2016). The household size as shown in *Table 1* revealed that 1.0% have household size that is less than 3, 46.6% of the respondents have household size that is less than 7, 49.5% of the rural farmers have household size that is greater than 6 and 2.9% of the rural farmers have household that is greater than 10. This is support of the findings of (Adelekan and Omotayo, 2017).

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Also, the duration of years spent on farming indicated that 22.9% have spent between 1-10 years in farming with mean farming experience of 23 years. Majority (54.3%) of the respondents rural reported that their spouse had no formal education, 31.4% had primary education and 11.4% had secondary education while 2.9% of them had tertiary education with mean farm size of 9 hectares.

Table 1. *Socioeconomic characteristics of rural food crop farmers. (Source: Field Survey, 2014)*

Characteristics	Frequency	Percentage	Mean
Age			
Less than 30	2	1	
30-40	42	20.2	
41-50	70	33.6	
51-60	56	26.8	51 years
60 above	40	19.4	
Religion			
Christianity	104	49.5	
Islam	106	50.5	
Marital status			
Single	10	4.8	
Married	116	55.2	
Divorced	20	9.5	
Widowed	30	14.3	
Separated	34	16.2	
Educational Level			
No formal education	62	29.5	
Primary education	106	50.5	
Secondary education	34	16.2	
Tertiary education	8	3.8	
Spouse education			
No formal education	114	54.3	
Primary education	66	31.4	
Secondary education	24	11.4	
Tertiary education	6	2.9	
Farming experience			
Less than 10	48	22.9	
Oct-15	44	21	
16-20	26	12.4	
21-25	10	4.9	223
25 above	82	39.3	
Farm size			
Less than 3 ha	40	19.1	
3-May	62	29.6	
6-Sep	32	15.3	9
Oct-13	24	11.5	
13 above	52	24.8	

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Gender			
Male	70	33.3	
Female	140	66.7	
Total house size			
Less than 3	2	1	
3-Jun	98	46.6	
7-Oct	104	49.5	7
10 above	6	2.9	
Total	**210**	**100**	

Budgetary analysis of farming households

The budgetary result for farming system of the farming household in the study area was presented in *Table 2*. The Total Variable Cost per hectare (TVC/ha) for food crop production such as maize, cassava, vegetable and pepper was estimated at ₦77131.80/ha ($212.268). Furthermore, Labour cost, Transportation cost, cost of planting materials, cost of herbicides, cost of fertilizer and cost of tractor service was accounted for 41.5%, 2.1%, 5.6%, 5.8%, 11% and 34% of the Total Variable Cost of production, respectively. This implies that labour constituted the larger proportion (42.0%) of Total Variable Cost of production of food crop farmers in the study area. Furthermore, Total Revenue (TR), Gross Margin (GM) for food crop production were estimated at ₦282239.00/ha ($ 789.114) and ₦205107.20/ha ($564.460) respectively in the study area. This implies that food crop farmers perform better in terms of margin between total revenue and total variable cost of food crop production.

Table 2. *Cost-return structure of food crop farmers in the study area. (Source: Field Survey, 2014)*

Variables	Value (N)	% of total variable cost
Revenue		
Value of maize	122255.00 ($336.447)	
Value of cassava	104654.00 ($288.009)	
Value of other	55330.00 ($154.697)	
Total revenue	**282239.00** ($789.114)	
Variable cost items		
Labour cost (N)	32037.00($89.5724)	41.535
Transportation cost	1639.80($4.51275)	2.126
Cost of planting materials	4290.00($11.8061)	5.562
Cost of herbicides	4454.00($12.4530)	5.775
Cost of fertilizer	8511.00($3,044,090)	11.034
Cost of tractor service	26200.00($9,370,840)	33.968
Total variable cost	**77131.80($215.653)**	
Gross margin	**205107.20 ($573.460)**	

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Parameter estimates of the Tobit regression

Table 3 below showed the result of Tobit regression to determine the effect of explanatory variable on the output of food crop farmers in Ibarapa Central Local Government Area of Oyo State. The Marginal effects of the independent Variables were estimated because they are very important for decision making. Among 14 variables considered in the model, Ten (10) were found to be statistically significant which includes gender, farm size, farm, fertilizer, planting materials and herbicide. All the coefficients have the expected sign. The parameters estimates of the Tobit regression of food crop farmers showed that farm size, fertilizer, hired labour, gender were positively significant at 1% respectively while male labour hour was positively significant at 5% confidence level. This implies that 1% increment in these variables will increase the farmers output by 15.7, 2.8, 0.01, 2.3 and 1.5 respectively. Also, herbicide, female off time and female labour hours were negatively significant to farmers output in the study area at 1%. The implication is that the herbicide was over utilized and female does not really contribute significantly to agricultural production in the study area.

Table 3. *Parameter estimates of the Tobit regression. (Source: Field Survey, 2014)*

Variables	Coefficient	Standard error	t-value
Constant	-66.97913	45.05613	-1.49
Gender (X_1)	15.69475	1.59054	10.08***
Marital status (X_2)	1.350062	6.245415	0.22
Educational level (X_3)	6.833226	9.187224	0.74
Male hour (X_4)	1.542199	0.7912725	1.95**
Spouse education (X_5)	-6.672392	8.030902	-0.83
Farm size (X_6)	2.837706	0.8978289	3.16***
Farm experience (X_7)	1.426395	1.393604	0.24
Fertilizer (X_8)	2.75065	1.073695	2.56***
Planting materials (X_9)	0.001723	0.000824	3.21***
Agrochemicals (X_{11})	-1.924005	0.583555	-4.14***
Male off time (X_{11})	-2.394674	1.930861	-1.24
Female off (X_{12})	-0.0002051	0.0000531	-3.39***
Female labour (X_{13})	-3.3124179	0.3488113	-9.90***
Hired labour (X_{14})	2.2542008	0.0186509	8.80***
Diagnostic statistics			
Pseudo R2	0.6294		
Log likelihood	-568.49311		
Chi square	34.66***		

***implies significant at 1%, **implies significant at 5%)

Measurement of mean difference between male labour hour and female labour hour in agricultural production

The measurement of mean differences between female labour hours and male labour hours was accomplished with the use of t-test of difference of mean and the result is presented in the *Table 4*. It was based on the hypothesis that there is no significant difference between male labour hours and female labour hours contributing to farming

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output in the study area. The results of the t-test showed that there is a significant difference between the male labour hour and female labour hours. This implies that male contribute more to farming output than their female counterpart in the study area. Therefore, the null hypothesis was rejected. This implies that there exist a variation in their respective contribution to farming output in the study area which was also buttressed by the result of Tobit regression.

Table 4. *Mean difference of between male and female farmer's labour hours. (Source: Field Survey, 2014)*

Category	Mean hour	Standard deviation	Number	t-value	Decision
Male labour hours	6.304541	0.9423509	105	7.6096	Reject H_O
Female labour hours	7.647619	1.990268	105		

Discussion

Various empirical works on household contribution on agriculture such as (Omotayo and Oladejo, 2016; Dipeolu et al., 2009; Ukeje, 2005): reported that male respondents are more involved in agricultural production than their female counterpart. This could be due to the nature of the job as well as the laborious nature of the rural agriculture in the study areas. Furthermore, labour constituted the larger proportion (42.0%) of Total Variable Cost of production of food crop farmers in the study with Total Revenue (TR), Gross Margin (GM) estimated at ₦282239.00/ha ($789.114) and ₦205107.20/ha ($573.460) respectively in the study area. Also, parameters estimates of the Tobit regression of food crop farmers showed that farm size, fertilizer, hired labour, gender were positively significant at 1% respectively while male labour hour was positively significant at 5% confidence level. Also, herbicide, female off time and female labour hours were negatively significant to farmers output in the study area at 1%. Ukeje (2005) was in the opinion that food crop output is proportional to gender of the household head, farm size, agrochemical used and labour cost.

Conclusion

The findings of this study revealed that majority of the farmers are still in their active age, married and are therefore expected to be productive for available resources. Also, food crop production is profitable in the study area. Furthermore, the Tobit regression shows that male contributes more to agricultural production than female in the study area because majority of the female in the study area venture most of their time on household core and agricultural marketing than food crop production. Based on the study findings and conclusions, the following recommendations are made:

1. The research on labour saving techniques for agriculture in the study area should be initiated to as to improve their production.
2. There should be incentive to encourage female farmers in agriculture.
3. Farm expansion policy that will make agricultural land available to households should be made and this will impact positively on food crop production in the study in Ogun state and Nigeria in whole.

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MORPHO-NUTRITIONAL RESPONSE OF LETTUCE (*LACTUCA SATIVA* L.) TO ORGANIC WASTE EXTRACTS GROWN UNDER HYDROPONIC CONDITION

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Abstract. Organic wastes are utilized as source of nutrients to grow vegetables and reduce environmental degradation. This research aimed to study influence of different concentrations of organic waste extracts (Goat Manure Extract, GME; Banana Peel Extract, BPE) as T_o: 0% GME and BPE (control: irrigation water), T_1:1% GME, T_2: 2% GME, T_3: 3% GME, T_4: 3% GME + 1% BPE, T_5: 3% GME + 2% BPE on morphological and nutritional traits of loose-leaf Lettuce through hydroponic experiment at Gilgit city in Pakistan. The number of leaves, fresh weight, dry weight, carbohydrates, fat, fiber, calcium, phosphorus, sodium, potassium, chromium, thiamine, niacin, and energy were higher by 74, 7, 72, 13, 157, 100, 6, 15, 9, 4, 33, 150, 68, 53 and 52%, respectively, under T_5, whereas protein, ash, iron and riboflavin were greater by 86, 200, 105 and 292%, respectively, under T_4 compared to T_o treatment except moisture contents. Number of leaves, leaf fresh weight, leaf dry weight, and leaf moisture are strongly related to and are good predictors (R^2 = 0.67-0.97) of energy production from Lettuce. Integrated utilization of organic waste extracts (T_4-T_5) increased Lettuce leaf yield components and its nutritional value for human consumption.
Keywords: *banana peel, goat manure, minerals, nutrients, organic extracts, vitamins*

Introduction

Organic hydroponic production of leafy vegetables under controlled environment is one of the most appropriate technologies to accomplish human food requirements in soilless mountainous and urban areas. This production technology is economically viable to curb food insecurity in urban and rural hardship locations (Croft et al., 2017). Organic productions are preferred owing to inherent health benefits and lack of side effects for human body. Agricultural wastes (farmyard and fruits) are abundantly produced worldwide. Improper management and utilization of these wastes result in environmental degradation and the spreading of diseases (Manahan, 2005). These organic wastes especially manures and fruit peels (banana) serve as potential sources of macro- and micronutrients for crop production (Golabi et al., 2004; Kowalska et al., 2017). Under conventional soil based methods of vegetable production, success of accepted organic

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fertilizer component is dependent on soil decomposer which convert these wastes into useable form of nutrients which are absorbed by plants (Clark et al., 1998). Hydroponic production technology provides an opportunity to grow diverse type of leafy and fruity vegetables using organic sources of plant nutrients (El-Kazzaz and El-Kazzaz, 2017).

Leafy vegetable like Lettuce (*Lactuca sativa* L.) is an annual crop having fibrous root system that survives under diverse temperature situations (Manolopoulou and Varzakas, 2016) and respond to nitrogen levels positively (Gonzalez et al., 2016). It is a rich source of nutrients, minerals and vitamins for human body (Roy and Chakrabarti, 2003; Orech et al., 2007). The vegetable is being grown hydroponically in greenhouses using both salt based fertilizers and organic extracts (Rosik-Dulewska and Grabda, 2002). Though, greenhouse vegetable production is an intensive system mainly used in commercial farming (Kittas et al., 2013). This cultivation is achieved through modifying nutrient medium to optimum levels through testing and comparing different manure extracts. In hydroponics, nutrients which are required for plant growth are provided directly while eliminating needs for soil and soil organisms completely. Direct nutrition results have been noted in the form of higher growth rates and yields compared to soil cultivation (Raviv and Lieth, 2008). But consumers want to buy produce which is not tainted with hazardous chemicals or poisons as result of inorganic methods of nutrition.

Merits and demerits are associated with both organic and salt-based fertilizers. In salt-based fertilizers, nutrient concentration per unit weight is higher, availability is uniform and immediate, and precision of nutrient analysis is rapid compared to organic fertilizer. Mineral nitrogen is productive three-fold more than an organic nitrogen source (Masclaux-Daubresse et al., 2010). Yields were not different for a range of vegetables produced with organic N fertilizers compared to inorganic N fertilizers (Fabio et al., 2007). Gradual diminishing resources of world shows concerns about disadvantages of salt-based fertilizers. This includes excessive energy needs for formation, pollution due to mining and processing, and potential dwindling of finite resources of phosphorous and potassium (Fischer, 1986). The main disadvantage to salt-based fertilizers is that these are synthetically prepared which means the composition of material is chemically or genetically changed by combining, extracting or refining through human activity. Comparatively, organic fertilizers derived from animal or plant wastes are utilizable and recyclable (Elamin and Elagib, 2001). Moreover, organic produce has greater sale price compared to conventional produce (Sgroi et al., 2015). The nutritional value of organic foods compared to traditional ones is twofold the elemental composition on fresh weight basis (Matt et al., 2011; Orman and Kaplan, 2017). Hence the objective of this research was to explore and evaluate prospect of utilization of organic wastes of banana peel and goat manure as source of nutrients for hydroponic production of loose leaf Lettuce vegetable in food insecure soilless mountainous and urban areas.

Material and methods

Experimental site and layout

Research was conducted from March 25 to June 10, 2016 inside a greenhouse (GH) of 16 ft width (bottom), 8 ft height and 40 ft length located at Karakoram International University, Gilgit, Pakistan, having coordinates of 35.91 Latitude and 74.31 Longitude (*Fig. 1*). Temperature remained inside GH was between 20 and 30 °C and was greater by a few degrees compared to outside. Average relative humanity was remained around 65%. Inside GH structure, eighteen sets each made of PVC pipe (3 ft) fitted on an iron

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stand and were arranged to accommodate six treatments with three replications. Within each set, pipe was connected with water inflow pipe and a drainage pipe using connection pipes. Each pipe is closed on both ends with cups and had inlet on one end and an outlet on the other end. Each treatment set was connected with main pipes for supply of water at top and with a drainage pipe at bottom. Main water supply pipe was connected to water storage tank where water supply was controlled with a valve, while drainage pipe served as an outlet from the GH. Each small piece of pipe within a treatment set had five holes to accommodate a gutter containing growth medium (perlite) to anchor a Lettuce plant. Water flow from the tank was maintained to each set (treatment) at the rate of 0.5 L per minute (*Fig. 2*).

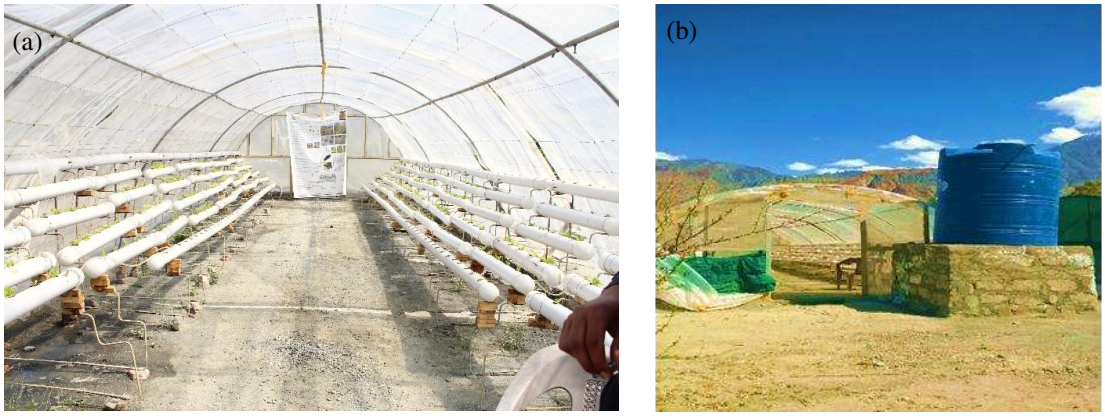


Figure 1. *Experimental site (a: inside; b: outside) for hydroponic production of loose leaf lettuce under greenhouse condition*

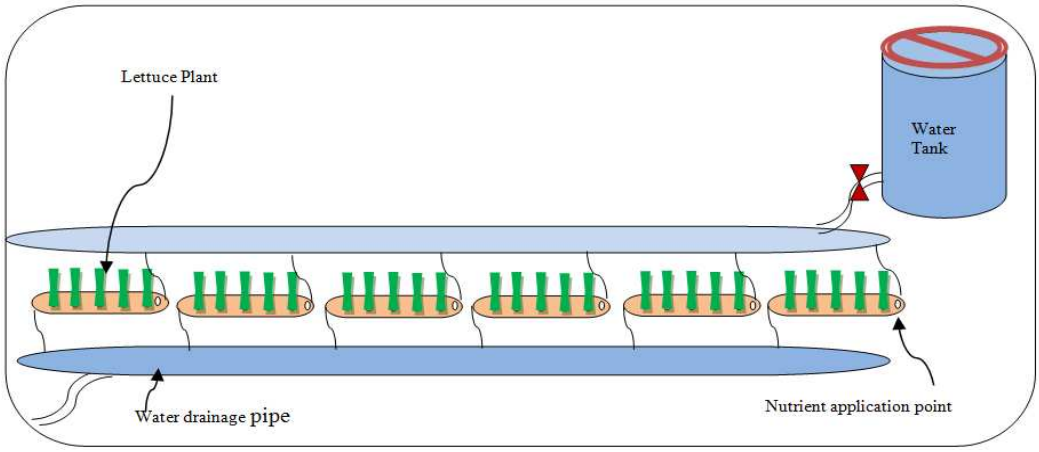


Figure 2. *Schematic diagram of hydroponic system for lettuce production*

Pre-experiment nutrient solution preparation

Two types of extracts were prepared one from goat manure (GM) and the second from Banana peel (BP). Banana peel of fully matured fruit (yellow colored with black spots) was procured from market and goat manure was obtained from a goat farmer in composted condition. Five kilograms of each organic material was boiled into 20 L of

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water in a container over a gas stove till a concentrated solution of 5 L after filtration was obtained.

Experimental organization and management

Experimental plant loose-leaf Lettuce (*Lactuca sativa* var. crispa) of family Asteraceae was seeded in five pots placed in five holes of a pipe of 3 ft long. Six concentrations prepared from concentrated extracts of GM and BP (T_o: 0% GME/BPE, T_1: 1% GME, T_2: 2% GME, T_3: 3% GME, T_4: 3% GME + 1% BPE, T_5: 3% GME + 2% BPE) in total volume of 0.5 L. These treatments/ concentrations were assigned to eighteen sets randomly on the basis of CRD statistical design. The nutrient formulations (diluted) were applied to assigned treatment sets on alternate days. Treatment concentrations were analyzed for major and some minor plant nutrients i.e., Nitrogen, Phosphorus, Potassium, Calcium, Sodium, Chromium, Iron, pH, and EC using standard methods (*Table 1*).

Table 1. *Nutrient analysis of treatment solutions prepared from organic extracts*

Nutrient	**Plant nutrition treatment**					
	T_o: Irrigation water (0% GME/BPE)*	**T_1: 1% GME**	**T_2: 2% GME**	**T_3: 3% GME**	**T_4: 3% GME + 1% BPE**	**T_5: 3% GME + 2% BPE**
Nitrates (N), mgL^{-1}	1.5	1.6	1.7	2.0	2.1	2.2
Phosphorus (P), mgL^{-1}	0.001	0.003	0.005	0.006	0.008	0.010
Potassium (K), mgL^{-1}	2.0	2.5	2.8	2.9	3.1	3.2
Calcium (Ca), mgL^{-1}	58	62	66	72	76	80
Sodium (Na), mgL^{-1}	26	55	69	80	98	115
Chromium (Cr), mgL^{-1}	0.01	0.02	0.02	0.03	0.04	0.05
Iron (Fe), mgL^{-1}	0.1	0.5	0.6	0.8	2.0	2.6
pH	5.3	5.6	5.8	6.2	6.4	6.5
EC, dSm^{-1}	0.6	1.5	2.6	3.4	4.2	5.2

*GME_goat manure extract; BPE_ banana peel extract

Morpho-nutritional measurement and analysis

After sixty-five days of seeding, leaves of three plants of Lettuce were harvested from each replicate leaving behind the root portion. Number of leaves was counted and fresh weight of leaves was taken. Thereafter, harvested leaves were kept in an oven to dry at 105 °C till constant weight. Dry weight of the leaves was measured using an electronic balance and moisture contents were computed. The dried samples from each replicate were ground and sieved. Powdered triplicate samples were analyzed for proximate and mineral composition for nutritional evaluation of food material in accordance with AOAC (2000). Further, the samples were examined for vitamins, i.e., Thiamin, Riboflavin, Niacin, and Ascorbic acid. Thiamine and riboflavin were found by quantitative analysis as described by Hucker et al. (2011), Niacin was investigated by colorimetric method (Kawila, 2011) and Ascorbic acid (Vitamin C) was found through dichlorophenol Indophenol dye reduction method (Smirnoff, 2000). Kjeldhal method was adopted to find crude protein through determining nitrogen contents of food material. Sohxtech was used to determine crude fat through ether extraction process

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(Thiex et al., 2003). Crude fiber contents were found by acid digestion, alkali digestion and fibertech (Busuttil-Griffin et al., 2015). Ashing of samples was performed in a muffle furnace at 550 °C for 6 hours. For all these determinations powdered and oven dried samples were used in triplicate. Food energy of protein and carbohydrates was derived by multiplying with 4.1 and energy from fat was computed by multiplying with 9.3 (Khalil and Saleemullah, 2004; Arshad and Ranamukhaarachchi, 2012). Analysis of macro-minerals i.e., calcium, phosphorus, potassium, and sodium was carried by digestion of samples with $HClO_4$ and HNO_3 as proposed by Khattak and Rahman (2016) whereas for micro-minerals i.e., chromium and iron, dry ash method was adopted for digestion as described by Abbruzzini et al. (2014). Following the digestion, samples were analyzed for selected minerals using U.V. Spectrophotometer (ORION), Atomic Absorption Spectrometer (VARIAN) and Flame Photometer (SHER WOOD).

Statistical analysis

Data pertaining to number of leaves, leaf fresh weight, leaf dry weight, nutritional composition, mineral contents and vitamins were analyzed using statistical program SAS version 9.0 for one-way Analysis of Variance (ANOVA) and Fisher protected Least Significant Difference (LSD) test was performed to compare treatment means (Gelman, 2005). Pearson Correlation and Regression analysis were performed to know relationship among yield parameters and prediction of energy production.

Results and discussion

Growth and yield performance

Lettuce plant produced number of leaves in the range of 10-18 leaves per plant, leaves fresh weight (LFW) as 239-256 g and leaves dry weight (LDW) as 15-25 g. There was a significant influence of different concentrations (T_o: 0% GME and BPE (control), T_1: 1% GME, T_2: 2% GME, T_3: 3% GME, T_4: 3% GME + 1% BPE, T_5: 3% GME + 2% BPE) of organic extract on number of leaves produced, LFW and LDW. The highest number of leaves per plant (18) was produced under T_5 and reduced ($p = 0.001$) in T_4 and rest of the treatments including control (*Fig. 3A*). The LFW (256 g) and LDW (25 g) of Lettuce were higher under T_4 and T_5 and reduced ($p = 0.001$) in T_3, T_2, T_1, and T_o (*Fig. 3B, C*). Compared to control, yield components of Lettuce were increased with increasing quantity of GME and BPE. Increased supply of extracts carried greater quantity of plant nutrients which have enhanced the yield components of Lettuce. The elements required in trace amounts are easily carried in organic extracts. The results are in agreement with Haggag et al. (2014, 2015) and where a manure compost tea was prepared and utilized for olive seedlings growth. Mowa et al. (2017) also noted increased growth and yield performance of tomato grown on goat manure.

Moisture contents

Leaf moisture of Lettuce was in the range of 90-94% and was influenced significantly due to concentration of organic extracts. It was reduced ($p = 0.001$) in T_1, T_2, T_3, T_4, and T_5 compared to T_o (*Fig. 3D*). Under organic nutrition, moisture in Lettuce leaves was reduced and leaves became more hardy and crispy. Higher moisture in Lettuce leaves causes yellowing and reduces shelf life and market quality.

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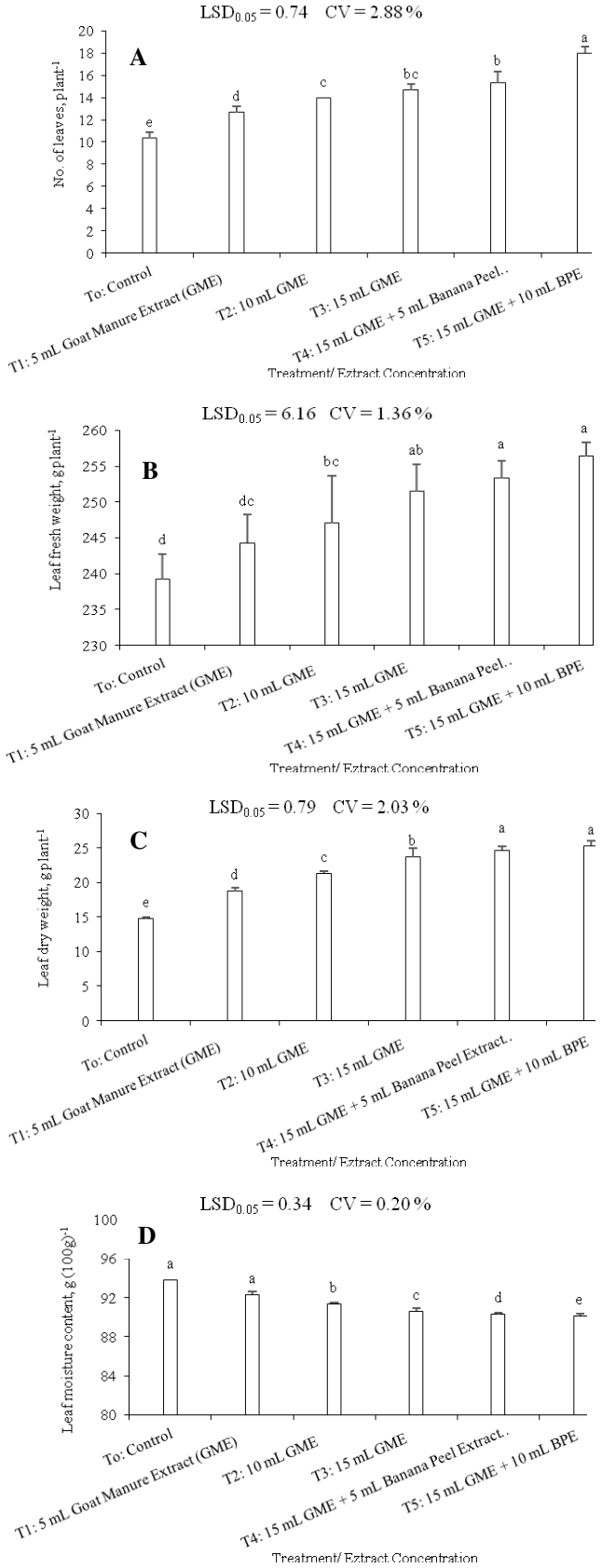


Figure 3. *Effect of nutrient extracts of goat manure and banana peel extracts on number of leaves (A), leaf fresh weight (B), leaf dry weight (C) and leaf moisture content (D) of lettuce*

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Nutritional evaluation

Protein, fat, carbohydrates, fibers, and ash contents in Lettuce leaves were in the range of 1.5-2.7, 0.3-0.7, 2.9-3.3, 0.7-1.4, and 0.5-1.5 g, respectively, and were varied significantly under different plant nutrition treatments (*Table 2*). The fat contents were increased ($p = 0.001$) in T_3, T_4 and T_5 (0.8 g) compared to that of T_o, T_1 and T_2 (0.3-0.6 g). Leaf carbohydrates increased ($p = 0.001$) under T_5 compared to rest of the treatments including control. Leaf protein, fibers and ash contents were increased ($p = 0.001$) under T_4 and T_5 conditions compared to rest of the treatments.

Food nutrition of Lettuce leaves was enhanced under higher concentrations of GME and BPE owing to greater efficiency of organic culture carrying high nutritional value. Organic extracts have enhanced bioaccumulation of nutrients. This is in congruence with the findings of Bourn and Prescott (2002), Custic et al. (2003) and Uusiku et al. (2010) where greater nutritional value of organic foods was highlighted compared to conventional inorganic production.

Table 2. *Effect of nutrient extracts of goat manure and banana peel on protein, fat, carbohydrates, fiber and ash contents of lettuce leaves*

Extract concentration	Protein, g	Fat, g	Carbohydrate, g	Fiber, g	Ash, g
T_o: Irrigation water (0% GME/BPE) control	$1.5\pm0.2^{e*}$	0.3 ± 0.0^{c}	2.9 ± 0.1^{c}	0.7 ± 0.1^{d}	0.5 ± 0.1^{e}
T_1: 1% GME	1.8 ± 0.1^{d}	0.4 ± 0.1^{bc}	3.1 ± 0.1^{ab}	1.0 ± 0.2^{c}	1.0 ± 0.1^{d}
T_2: 2% GME	2.0 ± 0.1^{c}	0.6 ± 0.2^{b}	3.2 ± 0.1^{ab}	$1.2\pm.01^{b}$	1.3 ± 0.1^{c}
T_3: 3% GME	2.5 ± 0.1^{b}	0.8 ± 0.1^{a}	3.2 ± 0.1^{ab}	1.3 ± 0.1^{ab}	1.4 ± 0.1^{bc}
T_4: 3% GME + 1% BPE	2.7 ± 0.1^{a}	0.8 ± 0.1^{a}	3.1 ± 0.1^{bc}	1.4 ± 0.1^{ab}	1.5 ± 0.0^{a}
T_5: 3% GME + 2% BPE	2.7 ± 0.1^{a}	0.8 ± 0.1^{a}	3.3 ± 0.1^{a}	1.4 ± 0.1^{a}	1.5 ± 0.1^{ab}
LSD ($p \leq 0.05$)	0.16	0.14	0.17	0.17	0.11

*Different lower-case superscript letters with the column indicate significant difference between the mean values at $p \leq 0.05$

Mineral composition

Calcium, phosphorus, sodium, potassium, chromium, and iron in leaves of Lettuce were in the range of 42-45, 32-37, 4.3-4.7, 58.3-60.6, 0.003-0.004, and 0.83-1.7 mg $(100\ g)^{-1}$ on dry weight basis, respectively, and were significantly influenced under varied plant nutrient management conditions. Contents of all these minerals were higher ($p = 0.001$) under T_3, T_4 and T_5 conditions compared to T_o, T_1 and T_2 treatments (*Table 3*). Compared to control, treatments of organic extracts increased the mineral contents in Lettuce leaves. This shows potential of Lettuce for the take up of minerals supplied by the organic extracts. This is in agreement with the findings of Olle and Williams (2012) where mineral contents were increased compared to soil or inorganic nutrient based cultures.

Vitamins production

Thiamin, riboflavin, niacin and ascorbic acid analyzed in Lettuce leaves were noted in the range of 0.04-0.1, 0.12-0.50, 0.4-0.7, and 7.0-10.7 mg $(100\ g)^{-1}$ on dry weight basis, respectively (*Table 4*). Contents of all vitamins in leaves responded differently to treatment concentrations tested. Contents of thiamin and ascorbic acid were greater ($p = 0.001$) under T_4

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and T_5 compared to rest of treatment conditions. Riboflavin and niacin were higher ($p = 0.001$) under T_3 and T_4 treatments compared to rest treatment environments. Increase of vitamins in organically produced vegetables has also been reported by Matt et al. (2011).

Table 3. *Effect of nutrient extracts of goat manure and banana peel extracts on calcium, phosphorus, sodium, potassium, chromium, and iron contents of Lettuce leaves on dry weight basis*

Extract concentration	Calcium mg $(100g)^{-1}$	Phosphorus mg $(100g)^{-1}$	Sodium mg $(100g)^{-1}$	Potassium mg $(100g)^{-1}$	Chromium mg $(100g)^{-1}$	Iron mg $(100g)^{-1}$
T_o: Irrigation water (0% GME/BPE) control	$2.7\pm1.5^{b*}$	32.0 ± 1.0^{d}	4.3 ± 0.1^{c}	58.3 ± 0.1^{d}	0.003 ± 0.001^{bc}	0.8 ± 0.2^{d}
T_1: 1% GME	42.0 ± 1.0^{b}	34.3 ± 0.6^{a}	4.5 ± 0.1^{ac}	60.0 ± 0.2^{c}	0.003 ± 0.001^{bc}	1.3 ± 0.1^{c}
T_2: 2% GME	43.0 ± 1.0^{b}	35.3 ± 0.6^{bc}	4.6 ± 0.1^{ab}	60.3 ± 0.1^{b}	0.003 ± 0.001^{ab}	1.4 ± 0.1^{bc}
T_3: 3% GME	45.0 ± 1.0^{a}	36.0 ± 1.0^{ab}	4.6 ± 0.1^{a}	60.5 ± 0.1^{ab}	0.004 ± 0.001^{ab}	1.5 ± 0.1^{ab}
T_4: 3% GME + 1% BPE	45.3 ± 0.6^{a}	35.0 ± 1.0^{bc}	4.7 ± 0.1^{a}	60.6 ± 0.1^{a}	0.004 ± 0.001^{a}	1.7 ± 0.1^{a}
T_5: 3% GME + 2% BPE	45.3 ± 0.6^{a}	36.7 ± 0.6^{a}	4.7 ± 0.1^{a}	60.5 ± 0.2^{a}	0.004 ± 0.001^{bc}	1.7 ± 0.1^{a}
LSD ($p \leq 0.05$)	1.98	1.47	0.16	0.20	0.001	0.18

*Different lower-case superscript letters indicate significant difference between the mean values at $p \leq 0.05$

Table 4. *Effect of nutrient extracts of goat manure and banana peel on thiamine, riboflavin, niacin, ascorbic acid and energy contents of lettuce leaves on dry weight basis*

Extract concentration	Thiamine mg $(100g)^{-1}$	Riboflavin mg $(100g)^{-1}$	Niacin mg $(100g)^{-1}$	Ascorbic acid mg $(100g)^{-1}$	Energy calories
T_o: Irrigation water (0% GME/BPE) control	$0.04\pm0.01^{e*}$	0.12 ± 0.07^{c}	0.40 ± 0.10^{b}	7.00 ± 1.00^{c}	20.67 ± 0.23^{d}
T_1: 1% GME	0.07 ± 0.01^{d}	0.27 ± 0.06^{b}	0.50 ± 0.10^{b}	7.33 ± 0.57^{c}	24.27 ± 0.90^{c}
T_2: 2% GME	0.07 ± 0.01^{dc}	0.33 ± 0.06^{b}	0.47 ± 0.06^{b}	7.33 ± 0.55^{c}	26.73 ± 1.16^{c}
T_3: 3% GME	0.08 ± 0.00^{bc}	0.37 ± 0.06^{ab}	0.53 ± 0.06^{ab}	8.65 ± 0.56^{b}	30.50 ± 1.28^{a}
T_4: 3% GME + 1% BPE	0.09 ± 0.01^{ab}	0.47 ± 0.06^{a}	0.67 ± 0.06^{a}	9.66 ± 0.55^{ab}	30.90 ± 0.89^{a}
T_5: 3% GME + 2% BPE	0.10 ± 0.01^{a}	0.33 ± 0.06^{b}	0.67 ± 0.06^{a}	10.67 ± 0.54^{a}	31.43 ± 0.67^{a}
LSD ($p \leq 0.05$)	0.01	0.11	0.14	1.22	1.59

*Different lower-case superscript letters indicate significant difference between the mean values at $p \leq 0.05$

Food energy production

Sum of energy supplied by food items is the ultimate goal that keep human body healthy and active for day to day work. It is a good parameter for comparing and evaluating different food products as beneficial for human health. Total energy production of Lettuce leaves computed from its carbohydrates, protein and fats contents was in the range of 20.7-31.4 calories. Energy value was influenced due to different waste extract concentrations (*Table 4*) and was improved ($p = 0.001$) under T_4 and T_5 treatments compared to T_o, T_1, T_2 and T_3 treatments. Food energy was strongly related with number of Lettuce plant leaves ($r^2 = 0.89$), LFW ($r^2 = 0.82$), LDW ($r^2 = 0.98$) and leaf moisture ($r^2 = -0.98$) (*Fig. 4*). Regression analysis ($r^2 = 0.98$) showed that food energy in Lettuce leaves is predictable using number of leaves, LFW, LDW, and leaf moisture content (*Eq. 1*). The higher nutritional quality of Lettuce leaves makes it better source of food energy as also observed

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by Iyaka et al. (2014). Crude carbohydrates, protein and fat contents were quite high in the vegetable samples, thus serve as good source of digestible energy. Furthermore, high potassium, sodium and calcium contents make it good food especially for hypertensive patient as potassium decreases high blood pressure. The proximate and mineral analysis indicates that consumption of Lettuce along with cereals in sufficient quantity could contribute immensely towards attainment of human nutritional needs for normal body growth, development and sufficient protection against diseases caused by malnutrition.

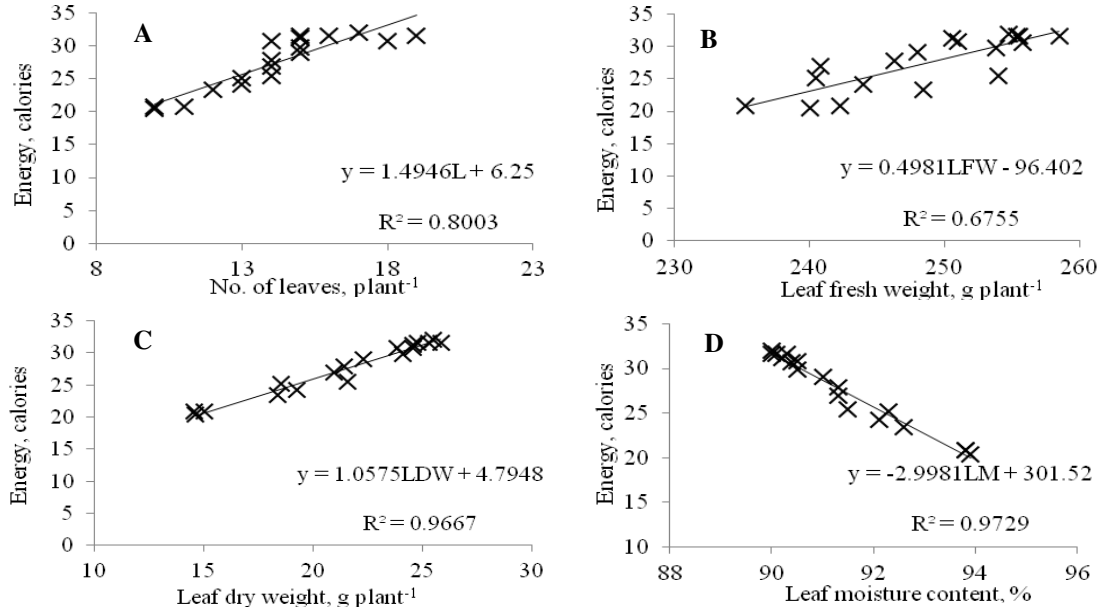


Figure 4. *Relationship of energy contents with number of leaves (A), leaf fresh weight (B), leaf dry weight (C) and leaf moisture content (D) in lettuce*

$$Engergy\ (Calories) = -563.5 - 0.132LFW + 4.016LDW + 6.55LM \quad \text{(Eq.1)}$$

Conclusions

The research results indicated the usefulness of organic extracts of goat manure and banana peel for hydroponic production of loose-leaf Lettuce in soilless mountainous and urban conditions. Lettuce grown under organic extracts had value added characteristics that can be marketed at higher prices. Bioaccumulation of food nutrients, minerals and vitamins in Lettuce leaves has been observed that encompass special significance for human nutrition and health benefits. Furthermore, this research suggests utilization of organic wastes especially goat manure and banana peel for the development sophisticated nutrient solutions for onward application in hydroponic systems. Soilless culture coupled with integrated use of evaluated organic waste extracts appears to be quite successful for gardeners and small farmers for intensive production of leafy vegetables or other high value crops for household use and income generation. It is strongly recommended for further research on use of goat manure and banana peel extracts for other leafy vegetables i.e., spinach, and also research studies on preparation and utilization of other animal and fruit wastes for the production of different leafy vegetables under hydroponic conditions.

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APPENDIX

Supplementary Table 1. *Simple statistical data*

Variable	No. of observations	Mean	Standard deviation	Minimum	Maximum
No. of leaves, plant^{-1}	18	14.17	2.48	10.00	19.00
Leaf fresh weight, g plant^{-1}	18	248.61	6.83	235.30	258.50
Leaf dry weight, g plant^{-1}	18	21.40	3.86	14.60	25.90
Leaf moisture, %	18	91.42	1.36	90.00	93.90
Protein, g	18	2.20	0.49	1.30	2.80
Fat, g	18	0.60	0.20	0.30	0.80
Carbohydrates, g	18	3.13	0.15	2.80	3.30
Fiber, g	18	1.15	0.26	0.60	1.50
Ash, g	18	1.20	0.36	0.40	1.50
Calcium, mg (100)$^{-1}$	18	43.89	1.64	41.00	46.00
Phosphorus, mg (100)$^{-1}$	18	34.89	1.68	31.00	37.00
Sodium, mg (100)$^{-1}$	18	4.56	0.16	4.20	4.80
Potassium, mg (100)$^{-1}$	18	60.07	0.85	58.20	60.80
Chromium, mg (100)$^{-1}$	18	0.00	0.00	0.00	0.01
Iron, mg (100)$^{-1}$	18	1.41	0.31	0.70	1.80
Thiamine, mg (100)$^{-1}$	18	0.07	0.02	0.03	0.10
Riboflavin, mg (100)$^{-1}$	18	0.31	0.12	0.07	0.50
Niacin, mg (100)$^{-1}$	18	0.54	0.12	0.30	0.70
Ascorbic acid, mg (100)$^{-1}$	18	8.44	1.50	6.00	11.00
Energy, calories	18	27.42	4.14	20.40	32.00

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SPATIAL VARIABILITY OF STREAMBED VERTICAL HYDRAULIC CONDUCTIVITY AND ITS RELATION WITH STREAM MORPHOLOGIES, CASE STUDY: ZIYARAT RIVER, GOLESTAN PROVINCE, IRAN

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Abstract. Streambed vertical hydraulic conductivity (K_v) is a key parameter in the analysis of interactions between streams and aquifers, and that of stream ecosystems. However, the relationship between streambed hydraulic conductivity and stream morphologies has remained untouched. An in-situ standpipe permeameter test method was used to determine the spatial variability in streambed K_v measured along 36 km of stream reach in Ziyarat River, Golestan Province, Iran. To triangulate the data and obtain more reliable results, both rising and falling head methods were used. There was great spatial variability in K_v among the six test reaches and three K_v distribution patterns have been accomplished. Test locations in meandering reaches showed a more heterogeneous distribution of vertical hydraulic conductivity than what was observed in straight reaches. The difference in the K_v of data obtained from meandering reaches was greater than that of the straight reaches which is an indication of a change in the morphology of the river in these reaches due to the erosion and deposition. In other words, morphologic changes of the river in meandering reaches caused evident changes in the streambed which, eventually, caused the erosion and deposition of sediments.
Keywords: *erosion and deposition, groundwater/surface-water relations, geomorphology, hypoheic zone, Iran, geomorphology*

Introduction

Precise measurement of streambed hydraulic conductivity (K_v) is crucial in analyzing the magnitude and extent of water exchange and contaminant transfer between surface water and groundwater. It is even important when coping with a number of geotechnical problems involving the management of groundwater and surface water (Cardenas et al., 2004; Chen et al., 2008; Song et al., 2009; Sebok et al., 2014).

In-stream, the slug test, and the seepage meter coupled with hydraulic gradient measurement, have been widely used. The slug test quantifies only the horizontal hydraulic conductivity. However, it could be employed to specify the permeability of the deeper sediment layer (Chen, 2004; Cheng et al., 2011). The streambed vertical hydraulic conductivity (K_v) is known to constantly vary spatially. It has been found by some studies that the K_v varies greatly along a river transect (Chen, 2005; Min et al., 2013).

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In the analysis of the hydraulic relationship between streams and aquifers, the channel morphologic features are key considered as important (Sebok et al., 2014). Variable pressure heads control Hyporheic interaction in the streambed sediments as well as its environmental conditions (Koch et al., 2011).

The interaction of streams and aquifers can be improved by means of growth of meander length and can be decreased by a reduction in river sinuosity (Boano et al., 2006). Because of the transport of fine materials, the hydraulic conductivity of shallow alluvial aquifers under condition of sinuosity-driven hyporheic flow is not fixed (Nowinski et al., 2011). A meandering stream is one of the key sedimentological forms that links the regional aquifers to the streams (Dong et al., 2012). Sebok et al. (2014) showed that there are more variable streambed characteristics in the meandering than in the straight section. The existence of several orders of magnitude in the range of streambed anisotropy values has been seen close to stream banks (Sebok et al., 2013). The water exchange in the main channel can move into slower pools by anabranch diversions, and enhances the exchange process into the subsurface (Koch et al., 2011). A study which combined the K_v and erosion and deposition processes of streambed sediments has been conducted by Genereux et al. (2008) and Levy et al. (2011).

(Jiang et al., 2015) A few studies were conducted on the Biloo River in Shanghai, China in four sites and three different morphologies of hydraulic conductivity (K_v). The data were limited to a small area of the river – around 40 to 50 m of the river which were either straight or meandering. In this study, larger reaches were selected to gain more reliable results. For instance, several bends existed in a meandering reach. To better understand the morphologic effects of the river on the hydraulic conductivity (K_v) of the streambed, the reaches were selected from three parts of the river: upstream, midstream, and downstream. This was done in order to investigate reaches that represent all parts of the river.

In another research conducted by Lopez et al. (2015), the scholars have gathered over 50 samples from Wadi Khulays dune field, located in western Saudi Arabia, while they also measured the parameters, including grain size distribution, porosity and hydraulic conductivity. They attempted to modify the Beyer equation by adding a parameter linked to the mud concentration and for quantifying the fitting parameter, an inverse optimization process was applied. The obtained equation was promising in predicting the saturated hydraulic conductivity, as its estimations present the lowest error of any empirical equation.

Previous studies recommended that there are many effects on hydraulic conductivity such as stream morphology and geomorphology attributes, river- bed forms, erosion and deposition process, and the riverbed sediments. The knowledge of the streambed hydraulic conductivity for different stream mmrophologies relatively is, however, scarce. The present study investigates the spatial variability in streambed K_v spanning 36 km of stream reach in the Ziyarat River, Golestan Province, Iran. An in-situ field standpipe permeameter was utilized to collect 189 total measurements using 6 test sites in 2 stream morphologies: one in the straight stream channel and one in meander reach. All sediment samples were collected after the K_v tests and they were used for grain-size analysis.

This study had three objectives: (1) investigating the hydraulic conductivity (K_v) of the streambed from upstream to downstream; (2) finding the morphologic effects of the river, including the meandering and straight forms, on the hydraulic conductivity (K_v) of the river from upstream to downstream; and (3) analyzing the sediment grain size in

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straight and meandering reaches and examining the relationship between sediment grain size and hydraulic conductivity (K_v). (4) Finding the best empirical methods used to estimate hydraulic conductivity based on the grain-size distribution.

Materials and methods

Study area

Ziyarat River, originating from Alborz mountain range, is located in Golestan Province, which is a northeastern area in Iran. It flows to the north and after 36 kms, merges into Gharesoo River. The drainage basin in upstream is about 100 km^2 with dimensions of 19.64 km in length and 5.11 km in width. The mean of water abstraction rate at stream gauge is about 10.5 m^2. The selected six reaches were all covered by these three categories (*Fig. 1*). The study area is located in a temperate climate. The mean of annual precipitation is equal to 601 mm, and mean temperature is 13.4 °C. The variation in seasonal precipitation is distinctive and not uniform. The temperature of stream water ranges from 19.04 to 29.2 °C. Although, the weather is rainier in winter, the majority of floods occur from August to November. The streambed sediments were alluvial coarse, sand-gravel, and clay and silt in downstream. The highest height of Ziyarat stream gauge is 3068 m and the beginning of main river is 2620 m. The length of the river to Naharkhoran is 21 km with the slop average rate of 10.1% and the remainder of river to Ghorbanabad is 15 km with the slop average rate of 4.6%.

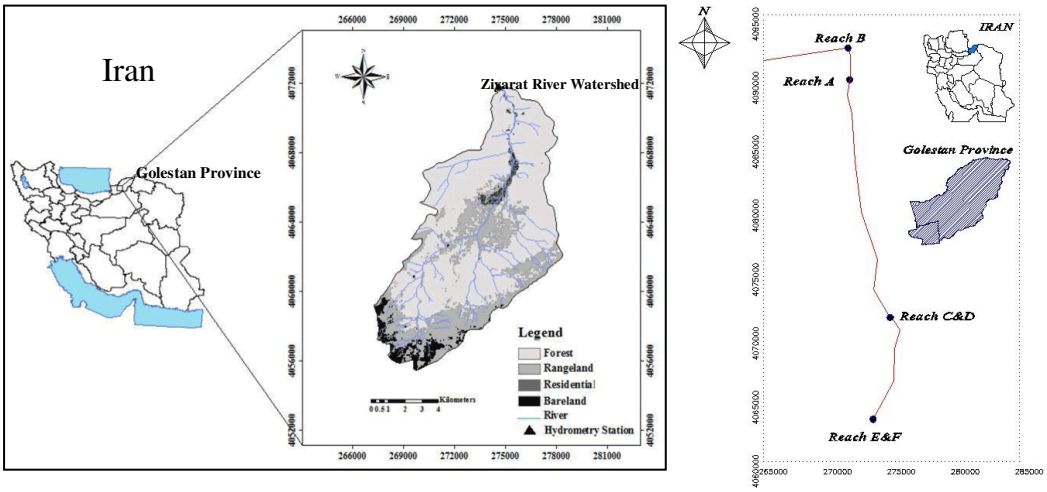


Figure 1. *The location of the study area along the Ziyarat River*

Reach selection

The basis of the triple division follows Leopold and Wolman (1957) which, arbitrarily, identified the rivers with a bend coefficient (sinuosity index) larger than 1.5 as meandering and the ones with a bend coefficient lower than 1.5 as straight rivers. We first explain how reaches were determined in this study and then discuss the way we divided each reach into straight and meandering forms.

For each reach, the following field protocol was used to estimate reach hydraulic conductivity using point measurements (Datry et al., 2014). The length of the reach was around 200 to 220 m and, then, each reach was divided into 10 equal parts using the 11

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transverse sections which had a distance of twice the size of the wetted width from each other. The hydraulic conductivity is obtained from three parts of these 11 sections: the left, center and left sides.

As mentioned above, the straight reach was determined by the sinuosity index which is varied between 1 and 1.5. In addition, the meandering reach was identified by using a sinuosity index of 3 to 4.5 (Gordon et al., 2004). Six reaches were selected in this study: two from the upstream, two from midstream, and two from downstream (*Table 1*). To better investigate the relationship between the hydraulic conductivity and morphology of the river, the selected reaches were straight and meandering. Therefore, in the upstream, one reach is straight and one is meandering. The same can be applied to the reaches in the midstream and downstream.

Table 1. *Hydrological condition and geomorphologies of test reaches*

	Reach A	**Reach B**	**Reach C**	**Reach D**	**Reach E**	**Reach F**
Reach name	Ghorbanabad straight	Ghorbanabad Meander	Neharkhoran Straight	Neharkhoran Meander	Ziyarat straight	Ziyarat Meander
Test date	May 27, 2018	May 28, 2018	May 29, 2018	May 30, 2018	May 31, 2018	June 1, 2018
End point UTM (X,Y)	(271764, 4090027)	(272887, 4092667)	(274756, 4071500)	(274925, 4071368)	(273662, 4063515)	(273556, 4063317)
Reach length (m)	200	220	200	200	200	200
Number of measurement	33	33	33	33	33	33
Number of sediments sampling	9	9	9	9	9	9
Average water depth (cm)	46.8	28.3	14.4	13.9	13.5	13.6
Stream gradient (m/m)	0.9%	0.49%	4.25%	4.18%	5.74%	6.9%
Average thickness of measured sediment (cm)	44	43	39.5	39	38	38.5
Reach description	Streambed sediment contains medium sand, silt and clay particles	Streambed sediment contains silt and clay articles	Streambed sediment contains large part of sand, coarse silt and a small part of gravel	Streambed sediment contains large part of sand and a small part of gravel	Streambed sediment contains large part of sand and a small part of gravel	Streambed sediment contains large part of sand and a small part of gravel

Vertical hydraulic conductivity

A falling-head test is conducted by rapidly raising the water level in the control well and subsequently measuring the falling water level. Slug-in test is another term for falling-head test. A field permeameter method has been adopted for measuring vertical hydraulic conductivity in previous studies (see e.g. Genereux et al., 2008; Song et al.,

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2010; Dong et al., 2012). The purpose of this method is to determine the streambed hydraulic conductivity (K_v), and as it is illustrated in *Figure 2,* it operates by vertically inserting a pipe into the streambed, filling the pipe with water, and calculating the amount of decline of the water level inside the pipe. After repeating this process for a few times, the K_v can be measured using this rate. In the present study, steel standpipe with an inner diameter of 4.8 cm and length of 120 cm was used. The pipe was injected into the sediments of the streambed and ensured that the length of the sediment column was nearly equal to 40 cm. A sensor (Onset HOBO Water Level-U20L-Series) with an accuracy of 1 mm was also employed to measure the water levels. Water was added during the test at the top of the pipe to create a hydraulic head. Then, the head was passed to fall in the pipe. In the current research, a water-level declining interval of 1 cm was used to record the hydraulic head and time. The hydraulic head estimations were obtained more than 3 times.

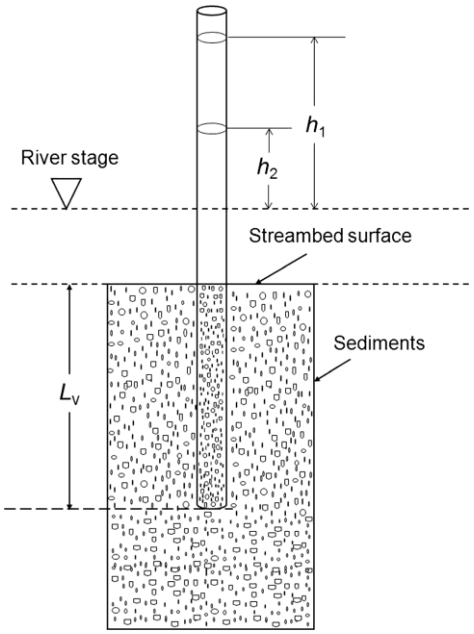


Figure 2. *Schematic diagram showing an in situ permeameter test to determine streambed*

To measure the K_v value using the equation proposed by Hvorslev (1951), all pairs of measured data of hydraulic head and time were used based on *Equation 1.*

$$K_v = \frac{\frac{\pi D}{11m} + L_v}{t_2 - t_1} ln(h_1/h_2) \quad \text{(Eq.1)}$$

where D denotes the inner diameter of the pipe, m denotes the square root of the ratio of the horizontal conductivity K_h represents the vertical conductivity K_v (i.e., $m=\sqrt{\frac{Kh}{Kv}}$), L_v refers to the length of the sediment column, h_1 and h_2 are hydraulic head inside the pipe measured at times t_1 and t_2, respectively. Overall, K_h is greater than K_v. An adapted Hvorslev solution has been proposed by Chen (2004) and Song et al. (2009) in order to specify the K_v when L_v is greater than D:

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$$K_v = \frac{L_v}{t_2 - t_1} ln(h_1/h_2) \quad \text{(Eq.2)}$$

However, the error in K_v from the formula provided by the adapted Hvorslev equation (*Eq. 2*) is connected to the ratio (L_v/ D) of the estimated sediment length (L_v) to the inner diameter (D) of the steel pipe. When $1 < m < 5$, if the ratio (L_v/D) is greater than 5, the error of the adapted estimation will be less than 5% (Chen, 2004).

Moreover, to triangulate the data, the rising-head method was used. A rising-head test is initiated by rapidly lowering the water level in the control standpipe and then taking measurements of the rising water level in the standpipe. The Pneumatic Hi-K Slug provides high-quality data for accurate slug testing of hydraulic conductivity (K_v) (*Fig. 3*).

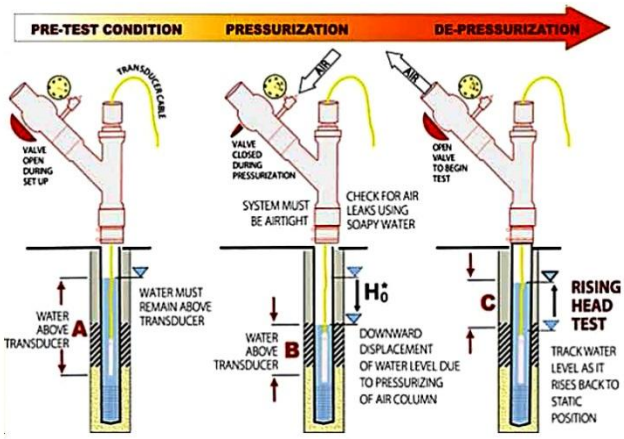


Figure 3. *Anatomy of the pneumatic slug test*

The following instruments were used: Pneumatic 'HI-K' SLUG from Midwest GEOSCIENCES group, Onset HOBO Water Level-U20L-Series, and Universal Optic-USB Base Station.

The experiment was once accomplished using the falling-head method and once using the rising head. Finally, the values of hydraulic conductivity obtained from these two methods were compared and in the cases where there were significant differences, the K_v obtained from the rising-head method was retained. The method of KGS and BOWER was employed particularly for the up and midstream where the K_v was high.

Sediments sampling

Due to limitations in analyzing grain size of sediments for all the 189 data, we decided to delimit the sampling from the streambed for each reach to 9 samples using systematic sampling. In other words, 54 samples from six reached were chosen. Indeed, the nine samples opted (*Table 1*) for sediment sampling were a portion of the 33 points which were measured (*Fig. 4*)

Three points which were selected for each reach were obtained from the middle, right, and left sides (transverse). These three points were located at the beginning, in the middle and at the end of each reach.

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Hand Operated Auger instrument was used to sample and transfer undisturbed sediments. Due to sediments resistance and coarseness, common instruments could not properly enter streambed sediments and, particularly, seal. This justifies our employed instrument in this study that was used in hyporheic zone (40-45 cm bellow the river surface). As a result, this led the whole auger to move into streambed sediments. Eventually, the samples were sent to Golestan Province soil mechanic laboratory. Moreover, for measuring hydraulic conductivity based on particle-size distribution (PSD), the 54 sets of soil samples were collected. To estimate particle size distribution (grain size curve), AASHTO-T27 method was applied. The finest sieve size was 0.075 mm and the largest one was 9.5 mm. Particle size < 0.075 mm was grouped into silt and clay, particle size between 0.075–2.0 mm into sand, and particle size > 2.0 mm into gravel (Song et al., 2010).



Figure 4. *Principle of the sediment sampling method*

Normality test

A Kolmogorov-Smirnov test was employed to check whether the distribution of the data was normal or not. This test was conducted using SPSS version 22.0. If $p < .05$, it rejects the null hypothesis that the K_v value does not belong to the normal or lognormal distribution. However, it accepts that the K_v values belong to the normal distribution if $p > .05$.

The results, as displayed in *Table 2*, showed that the values for reach B did not have a normal distribution while the reaches of A, D, and F had a normal distribution. In addition, the reaches of C and E were not normally distributed although their values were close to the significance level for normality. All the 189 data were not normally distributed as indicated by the Kolmogorov-Smirnov test. However, when they were classified into reaches, the results indicated that some of them were normal.

As the extracted data for K_v were in the quantitative form (Ratio Data) and their distribution was approximately normal, while the number of these samples was greater than any other group (33 samples from each group) and only a quantitative variable of six groups was compared with each other, hence we used One-Way ANOVA to

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compare the mean of the data at different reaches. First, a comparison was made between the groups in term of variance, so called Test of homogeneity of variance and it was observed that the associated variances were not identical ($P < 0.05$). Therefore, unequal variances tests were used for making a comparison between the mean of six groups; for this purpose, Dunnett tests were utilized. As a result, the obtained significance level was equal to $P < 0.05$, hence the H_0 hypothesis was rejected, while H_1 hypothesis was confirmed with 95% confidence, implying that there is a significant difference between the K_v mean for six reaches level.

Table 2. *Results of the Kolmogorov-Smirnov test for the six reaches*

Test site	Kolmogorov-Smirnov Test Sig.	Number of data
Reach A	0.2	33
Reach B	0.000	33
Reach C	0.047	33
Reach D	0.2	33
Reach E	0.048	33
Reach F	0.102	33

Therefore, the six reaches were analyzed through one-way ANOVA. *Table 3* shows that there was not any significant difference between the reaches of C and D. Similarly, no significant difference was observed between the reaches of E and F. The correlations between the other reaches were, however, significant.

Table 3. *Result of one-way ANOVA for comparing six reaches*

	Reach A	Reach B	Reach C	Reach D	Reach E	Reach F
Reach A	-------	*	*	*	*	*
Reach B		-------	*	*	*	*
Reach C			-------	√	*	*
Reach D				-------	*	*
Reach E					-------	√
Reach F						-------

Results

The values of K_v in the 189 measured data for six reaches of the streambed were between 0.109(m/d) and 152.37(m/d). According to *Table 4*, overall, the highest value of K_v, as expected, was found in the upstream within reach F, which was a meandering type of reach. The least amount of hydraulic conductivity was obtained in downstream in reach B, which is also a meandering type. To better analyze the hydraulic conductivity in these six reaches, K_v was obtained for 11 intervals which had a distance of 20 m. In fact, in each reach, the K_v was measured once in the middle of the streambed, once in the right side, and once in the left side of the streambed. Therefore, from each reach of the streambed in the total 11 intervals, 33 data of hydraulic conductivity were obtained.

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Table 4. *Statistics for K_v values from permeameter tests*

	Range (m/d)	Mean (m/d)	Median (m/d)	Std. error of mean	Std deviation
Reach A	0.245-4.860	1.798	1.632	0.235	1.351
Reach B	0.109-5.041	0.687	0.233	0.204	1.172
Reach C	71.48-142.2	104.55	105.68	2.57	14.76
Reach D	63.56-125.56	102.46	103.99	2.604	14.6
Reach E	73.76-146.67	118.56	122.47	3.304	18.98
Reach F	63.01-152.37	118.96	122.25	3.66	21.05

Reach A

Geographical position of reach A is presented in *Table 1* which illustrates that this reach is located in the downstream. This reach has a straight form and since the grains of the sediments in the streambed are tiny, the hydraulic conductivity has been low, within the range of 0.245(m/d) and 4.86(m/d), as shown in *Table 4*. The distribution of the data was normal, and the results also showed that the mean of K_v in the middle of the streambed is more than the right or left sides of it as shown in *Table 5*. In addition, the mean of the data in the middle of the canal is closer to the first quarter.

Table 5. *Mean of K_v values for three sides test reaches*

	K_v (left)	K_v (center)	K_v (right)
Reach A	1.700	2.68	1.01
Reach B	1.098	0.549	0.414
Reach C	97.79	113.75	102.11
Reach D	110.56	103.064	93.76
Reach E	114.89	127.36	113.43
Reach F	128.68	119.40	108.81

Reach B

Reach B which is a meandering type of reach, is located in downstream, as mentioned in *Table 1*. It was previously highlighted that the K_v obtained from this reach did not have a normal distribution. Since this reach was in the lower side of the streambed, its range of K_v was remarkably such low that it was between 0. 109 (m/d) and 5.041 (m/d), and its mean was 0.687 (m/d), as shown in *Table 4*. As it was illustrated in *Figure 5b,* the rate of changes to the hydraulic conductivity in the left side of the streambed was more than the middle and right side of it. The grains of the sediments were mostly made of silt and clay which, in some parts, acted like an impermeable layer.

Reach C

This reach was located in the middle part of the streambed and had a straight form. Its geographical position is described in *Table 1* According to *Table 4,* the range of changes of K_v is between 71.48 (m/d) and 142.2 (m/d) which shows a permeable layer. Moreover, the median and mean of K_v data in the middle of the streambed are more

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than the left and right sides of it (see *Figure 5c*). The mean of K_v, as shown in *Table 4*, is 104.55. Although the data were not normal, its distribution was close to normal (see *Table 2*).

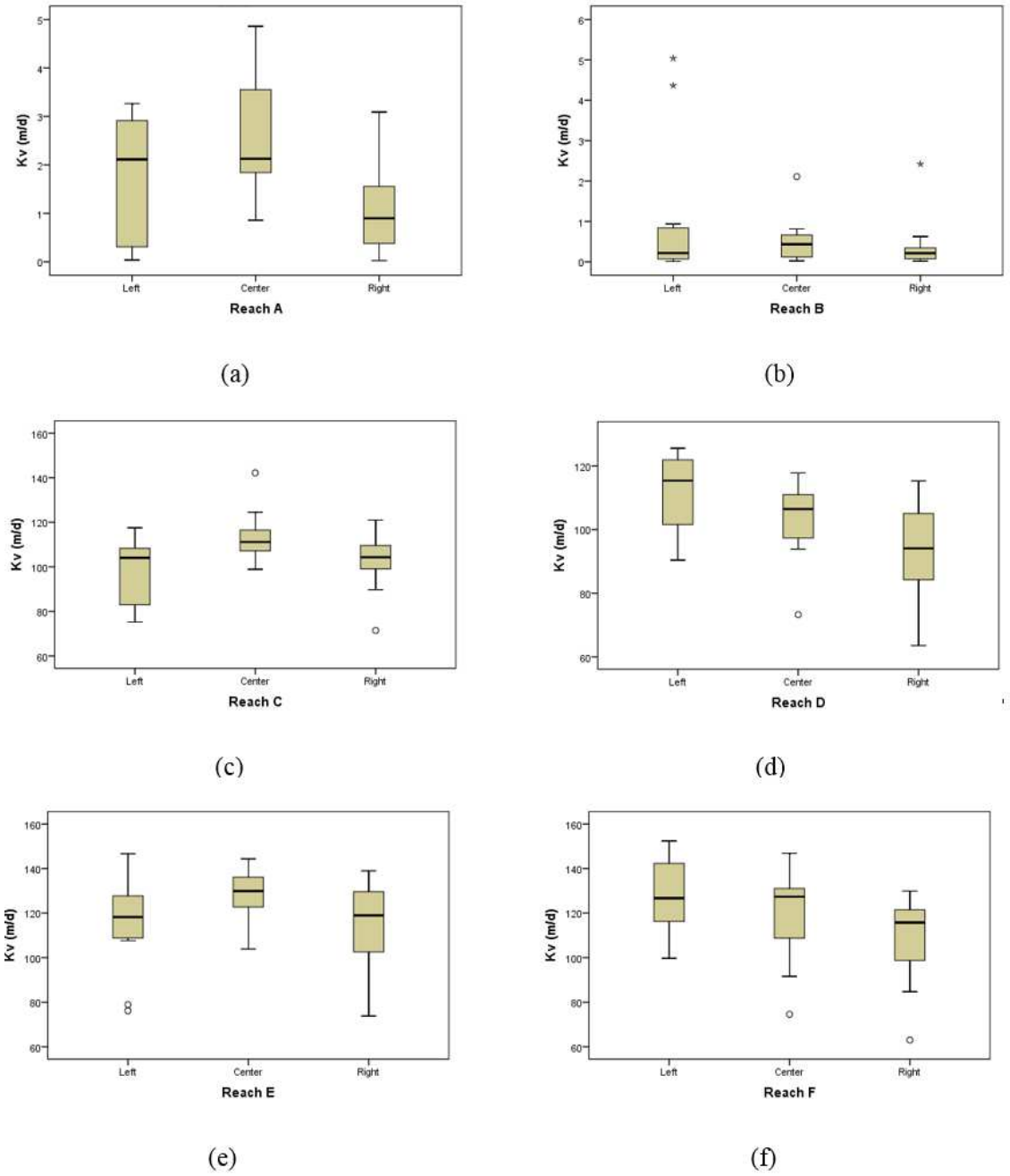


Figure 5. *Box plots of streambed K_v for each test reach*

Reach D

This reach, which has a meandering shape, is located at the geographical position of *Table 1*. The range of K_v, as shown in *Table 4,* is between 63.56 (m/d) and 125.56 (m/d) and the mean of the K_v of the streambed is 102.46 (m/d). In addition, the mean of the K_v in the left side of the streambed is more than the right side. According to what is shown in

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the box plot, the range of the changes of the K_v in the right side of the streambed is more than the other two parts. The data were normally distributed, as indicated in *Table 2*.

Reach E

This reach of the river, which has a straight shape, is located in the upper part of the river. The range of the changes of K_v (*Table 4*) is between 73.76 (m/d) and 146.67 (m/d). Moreover, the K_v for the riverbed is equal to 118.56 (m/d). The results indicated that the K_v for the streambed in the middle part of the river is more than the other two parts. The range of the changes in the middle part, however, was less than the other two sides. This range, as displayed in *Fig. 5e*, has decreased the normality of the data, though they were so close to normality. The plot of frequency (*Fig. 6e*) shows the normality of the data.

Reach F

This reach of the river, which has a meandering shape, is located in the upper side of the river and based on *Table 1*, the range of its changes is between 63.01 (m/d) and 152.37 (m/d) (*Table 4*). Also, (*Table 5*)shows that the hydraulic conductivity (K_v) in the left side of the river is more than the other two sides. Similarly, the range of hydraulic conductivity in the left side is higher than the middle and right sides of the river (see *Fig. 5f*). The distribution of the data was also normal, as demonstrated in the frequency plot (see *Fig. 6f*).

Grain size analysis

Grain size is the main controlling factor for streambed hydraulic conductivity (Song et al., 2010). After investigating the six reaches, it became evident that the physical features in the downstream and, in particular, the meandering reach (Reach B), were different from the other reaches, as shown in *Table 6*. The sediments in the bed of these two reaches (A and B) were completely small and consisted of silt and clay (*Table 6*).

The average distributions of grain size in sediments are significantly different at each position of the channel at all the tested reaches (*Fig. 7*).

It becomes evident after comparing the data and distributions of K_v values (*Table 4*) collected from In situ permeameter tests at each position (L/C/R) at all test reaches that a higher average value of cumulative percentage in weight of silt and clay (< 0.075 mm) is correlated with higher hydraulic conductivity.

According to *Table 5*, the process of erosion in the left side of the channel is stronger than in the right side. Erosional areas mainly occur in the left towards the central channel which has caused the mean K_v in the left side of the reach to be more than the right side. Therefore, the mean of the grain size in the left side of the reach is expected to be more than the right side (*Fig. 7b*). In fact, in Reach A, the central area has more erosion than the left or right sides, and it is shown that the erosional process increases from the center bank to the right and left banks. The hydraulic conductivity in the middle part of reach A is more than the other parts and this is directly related to its larger grain size. For better analysis of the 54 sediment samples, obtained from six selected reaches of the entire riverbed, we were obliged to determine the percent of sediments particles size in accordance with *Table 6*. *Table 6* is useful for gaining an insight into the impact of erosion and sedimentation on hydraulic conductivity within

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six reaches, resulted from difference between the morphological properties of the reaches, from upstream till downstream.

Table 6. *Sediment grain size distributions for the 54 samples collected*

Particle size	Test reaches					
	Reach A	**Reach B**	**Reach C**	**Reach D**	**Reach E**	**Reach F**
% < 2 mm	58	100	40.8	47	40.8	42.1
Average value of < 0.075 mm cumulative percentage in weight	2.7	18	1.11	2	1.12	1.4
Average media d_{50} (mm)	1.2	0.17	3.1	2.1	3.1	2.9

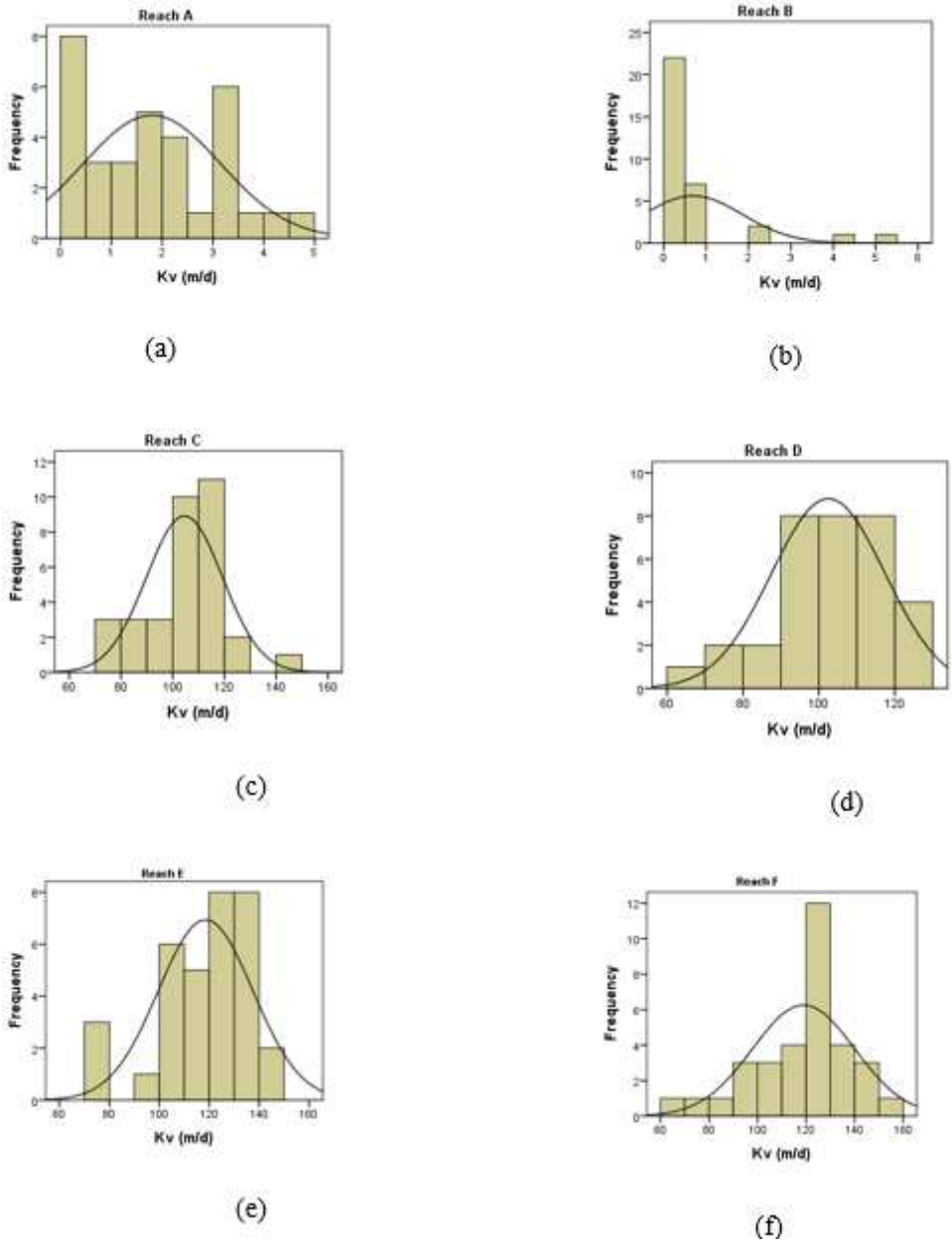


Figure 6. *Histogram of K_v values pooled together from each test reach*

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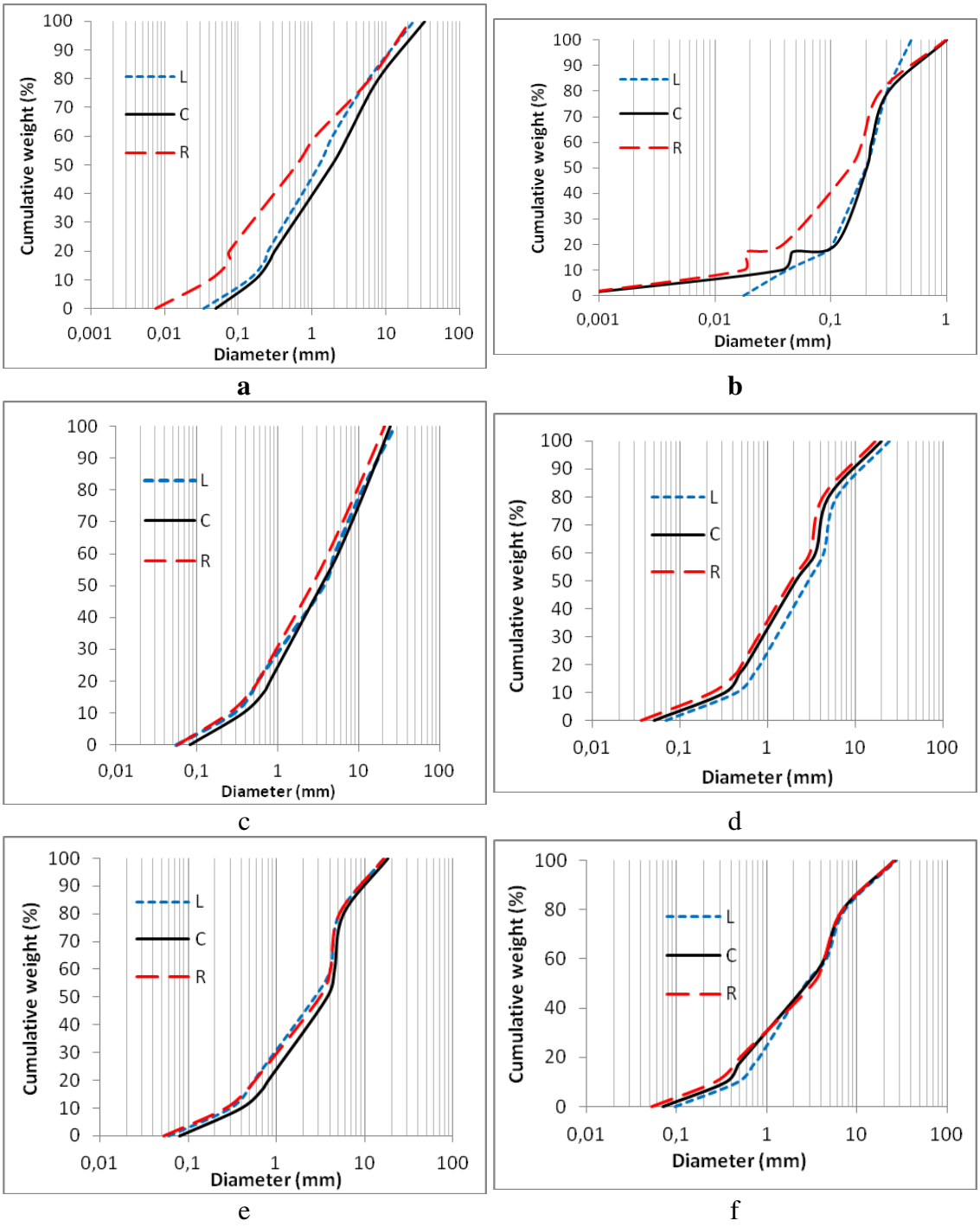


Figure 7. *Average grain size distributions of streambed from each test reach along the Ziyarat River.* ***a.*** *Straight reach A,* ***b.*** *Meandering reach B,* ***c.*** *Straight reach C,* ***d.*** *Meandering reach D,* ***e.*** *Straight reach C,* ***f.*** *Meandering reach F*

This difference can be attributed to the difference between meandering reaches and straight reaches, such that with more erosion in the left parts of the reach due to the curvature of the streambed. This difference affects the grain size of the sediments and, eventually K_v.

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When the particles in the upstream are compared with the ones in the downstream, it becomes evident that the larger grains exist in the upstream and the smaller ones exist in the downstream and even the silt and clay particles (<0.075 mm) cover the whole river in this part. This could be a reason for the high hydraulic conductivity (K_v) in the upstream and a low hydraulic conductivity (K_v) in the downstream as well as the impermeability of the streambed in the downstream. To better understand this issue, the correlation between K_v and d_{10} was investigated. d_{10} is the soil particle diameter (mm) such that 10% of all soil particles are finer (smaller) by weight.

Correlation between K_v and grain size

The statistical analysis of the values of K_v and the grain size of the sediments in the streambed showed that the K_v had the highest correlation with d_{10} and d_{60}.

Where d_{10} and d_{60}, are the soil particle diameter (mm) that 10% and 60% of all soil particles are finer (smaller) by weight.

Pearson correlation analysis was used to verify whether the two normally distributed variables were significantly correlated. However, when all the data from correlation coefficients between grain size and the vertical hydraulic conductivity are taken into account for each test reach. The results showed that the correlation between K_v and d_{10} was higher (r = .906) than the one between K_v and d_{60} (r = .0.748). The results for all the test reaches showed that the correlations were significant at 95% confidence level. The analysis indicated that the significance level was less than 0.05 which is an indication that the two variables were statistically correlated and had a linear correlation.

Empirical methods used to estimate hydraulic conductivity from grain-size distribution

The available soil properties such as particle-size distribution revealed many different relationships. As shown in *Tables 7* and *5* empirical methods; USBR, Shepherd, Hazen, Beyer, Slichter and Kozeny, were selected from Vukovic and Soro (1992) to measure Ks, hydraulic conductivity. The applied parameters are as follow: g denotes the acceleration of gravity; ν denotes the kinematic viscosity for a given temperature; in this study, at 20 °C, $\nu = 1.004 \times 10^{-6}$ m^2/s (Kasenow, 2002).

β represents a dimensionless coefficient, All equations have the porosity (n) function, and d_w refers to the effective grain diameter (mm) at w cumulative weight percentage of smaller grain size. Porosity can be estimated by (Vukovic and Soro, 1992; Kasenow, 2002) $n = 0.255(1 + 0.83^c)$, where c is the coefficient of grain uniformity and is represented by $c = d_{60}/d_{10}$. Here, d_{60} and d_{10} are the particle diameters derived from cumulative distribution curves and represent size fractions at which they are equal to 60% and 10%, respectively, of the sample by weight composed of grains of a smaller size (Ayers et al., 1998).

Results are clearly shown in *Figure 8,* illustrates the mean score between the obtained Hydraulic conductivity data from empirical formulas and permeameter tests in six reaches from upstream to downstream. It is evident that the mean of these five formulas in first and second reaches from downstream are completely near. Therefore, it indicates that empirical formulas are good predictors for estimating the hydraulic conductivity, especially in fine sediments.

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Table 7. *Methods used to estimate hydraulic conductivity grain size data (m/d)*

Formula	β	Equation	$\bar{x}_a$	$\bar{x}_g$	$\tilde{x}$
USBR	4.8×10^{-4}	$\beta \frac{g}{v} d_{20}^{2.3}$	127.67	31.31	83.37
Shepherd	142	$d_{50}^{1.65}$	141.91	45.04	174.62
Hazen	6×10^{-4}	$\beta \frac{g}{v}[1 + 10\,(n - 0.26)]d_{10}^2$	42.26	11.39	35.46
Beyer	6×10^{-4}	$\beta \frac{g}{v} \log(\frac{500}{c})\, d_{10}^2$	56.54	0.00	45.49
Slichter	0.01	$\beta \frac{g}{v} n^{3.287} \times d_{10}^2$	8.53	2.35	6.99
Kozeny	8.3×10^{-4}	$\beta \frac{g}{v} \frac{n^3}{(1-n)^2} \times d_{10}^2$	19.21	5.36	15.62
K_v			65.47	14.41	78.85

Also shown are the arithmetic mean $\bar{x}_a$, the geometric mean $\bar{x}_g$ and median hydraulic conductivity $(\tilde{x})$ calculated by the methods K values are given in md^{-1}

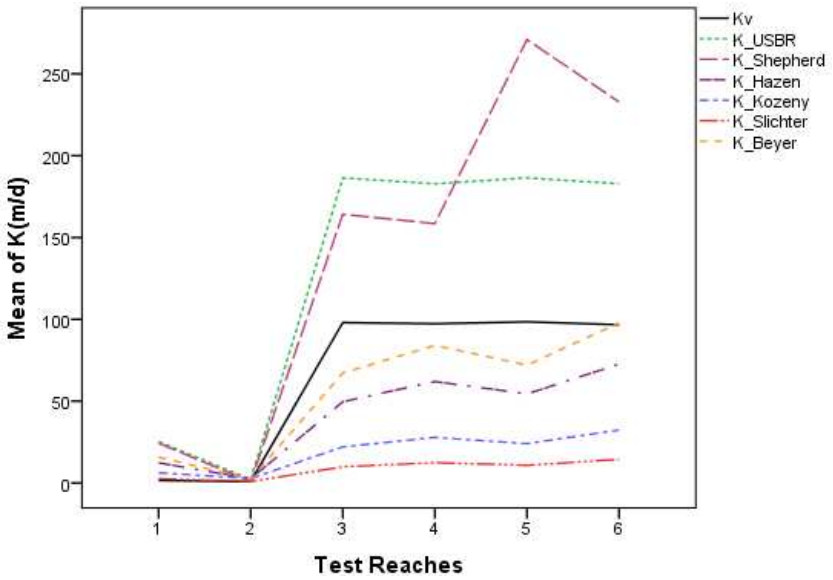


Figure 8. *Mean values of K from each of the five empirical grain size methods for these six reaches*

We should bear in mind that *Table 7* shows the the arithmetic mean $\bar{x}_a$, the geometric mean $\bar{x}_g$ and median hydraulic conductivity $(\tilde{x})$. The obtained medians from USBR formula and permeameter test were closer. Box plot Odong (2007) proved that Kozeny formula is the best estimator. Comparing different formulas, Song et al. (2009) stated that the obtained data from USBR were closer to hydraulic conductivity measured from Falling-Head standpipe method.

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Discussion

The amount of K_v in the total 189 samples analyzed in the six reaches was between 0.109 and 152.37. According to *Table 4*, overall, the highest measured K_v in the upstream was obtained for reach F and the lowest range of hydraulic conductivity was measured in downstream in the meandering reach B. In addition, this study shows that in straight reaches, whether in the upstream, midstream, or upstream, the highest K_v was found in the central part of the river.

In the straight-shaped reaches (see *Fig. 7a, c, e*), the curve intersection of the right, middle and left sediments with X axis was close to each other, while in the meandering reaches such difference was evident due to the high potential of the flow in sedimentation, particularly within the left section of the river channel (see *Table 5*) and this fact leads to a relative increase in the hydraulic conductivity of the mentioned section. That is, the impact of meandering reach properties within B, D and F reaches on the sediment particles transfer led to the lack of a uniform distribution for the particles percentage. The interesting aspect of the difference reaches to its peak in the downstream of the river (see *Fig. 7b*), where the relative increase in the sedimentation within the right section of the reach and existence of erosion within the left section not only lead to formation of variable percent of silt and clay fine particles, but also these factors lead to formation of an impermeable layer and mitigation in the hydraulic conductivity, as well as they lead to a high mean difference of K_v between the left and right sections of the river.

The findings of streambed K_v distribution indicated a large spatial variability for each reach test and grain size in streambed sediments (Song et al., 2010). Sebok et al. (2014) found that the streambed attributes in meandering reaches were more different than in straight ones. The variability of K_v values was related to the erosion and deposition of streambed sediments, as found in some other studies (e.g. Genereux et al., 2008; Levy et al., 2011).

The results obtained in this research are in line with the afore-mentioned results, but with the innovation in choosing the reaches throughout the river, the effect of meandering and straight reaches on the hydraulic conductivity has been clarified, so that the morphological variation of the river are more evident in the downstream of the river, so that the variation ranges for K_v are closer at the upstream, even in the meandering reaches, but this case is contrariwise in the downstream section, and changes in the K_v are obvious.

Regional variations in streambed characteristics, including sedimentary texture and sedimentary structure, have been among other attributes (Min et al., 2013). As a result, the grain size of streambed sediment, which can be related to various types of morphologies, bed forms, and erosional and depositional regimes, is correlated with K_v values.

With regard to the investigation applied in the present study, it was observed that each test reach has different stream morphology. Reach B, which is a meandering reach, is composed of silt and clay particles which has caused the hydraulic conductivity to be low and, in other words, it causes an impermeable layer to exist in the bed of the river. Another point is related to the speed of the stream. Since, this reach has the lowest speed of stream compared to other reaches; its steep is close to zero.

Previous studies which have been accomplished by Jiang et al. (2015) and Sebok et al. (2014) have somewhat clarified the role of river morphology in the hydraulic conductivity, but in the present research it was attempted present novelty in the methods

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section, especially a Rising Head test method was added with the advanced instrument, and most importantly, the morphological properties of the river and their impact on the K_v were studied. Moreover, the role of straight and meandering reaches in the entire river along the whole path from upstream and downstream was considered

This study indicates that the special stream morphologies have caused bed forms that relate to erosion and deposition of channel at each test reach. Those different erosion and deposition patterns resulted in different sediment distributions and spatial variability for K_v values at each test reach (*Fig. 7*), while the erosional side can probably have greater grain size contrast due to depositional conditions. These are the reasons for hydraulic conductivity variability and the spatial distribution patterns. In fact, the steep of the streambed can be considered as one of the factors leading to the erosional changes in the sediments of the river.

The consistent distribution of the composing materials of the river in the upstream and due to the fact that they approach to each other in the midstream, but in the downstream, especially in the meandering reach of Ghorbanabad (Reach B), this distribution is not consistent and these changes on the shape of the river have led to irregular changes in K_v. In addition, it has caused the distribution of particles to be mixed with sand, as illustrated in the image. In other words, due to the existence of silt and clay, the streambed has changed into an impermeable bed with a very low K_v. Erosion and deposition in of sediments in the meandering reaches D and F have led to a higher difference in their hydraulic conductivity.

It should be also noted that, this study attempted to cover the whole streambed so upstream, middle stream and downstream samples, which were selected. The result showed Beyer as the best predictor, however, other formulas might have been the best if all streams had not been selected. On other words, if only downstream sampling with its fine sediments (silt and clay) had been selected, slitcher formula might have been the best. Since, this formula is the strongest in fine sediments. Overall, to have better generalization to all such alluvial rivers (starting from mountain then releasing to plain and/or sea), six reaches were selected.

Due to the selected river morphology, being mostly coarse sediments, Beyer and USBR were more successful in predicting Ks. That is, rivers' fine sediments in downstream were fewer than coarse ones in the whole streambed.

The meticulous pints which is obvious in the present research is related to the fact that hydraulic conductivity difference relative to the estimation of experimental formals is located at downstream and upstream of the rover. So, this fact is highly essential due to the special morphology of the riverbed within this range, in which the bed sediments consisted of coarse grains. In the Future studies, the selected ranges considering whether the region is located in downstream or upstream should be scrutinized independently due to the critical role of d_{10} factor for estimating the hydraulic conductivity of the riverbed.

Conclusion

In this study, an in-situ permeameter test method was conducted using both falling and rising-head methods to determine the spatial variability in streambed. The K_v in a 36-km stream reach in Ziyarat River, located in Golestan Province, Iran, was studied. Collectively, the data from 189 measurements at 6 test reaches were analyzed. There were two different stream morphologies: straight reach and meandering reach. The

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results showed that the difference in the K_v of meandering reaches was more than the straight reaches, and this difference shows a change in the morphology of the river in these reaches as a result of erosion and deposition. To put it simply, the morphologic changes in the meandering reaches have caused evident changes in the streambed which have, eventually, led to the erosion and deposition of sediments and fluctuations in the values of K_v.

The K_v distributions in the straight reach show that the K_v values are greater in the center of the river. The analysis showed that the significance level was less than 0.05, which is an indication that the two variables are statistically related and have a linear correlation. The distributions of morphologies in streambed from each test reach along the Ziyarat River indicated that the stream morphology is a key variable which affects erosional and depositional conditions and bed forms. The erosional and depositional stream hydrologic processes winnowed the sediments and mobilized the sand and other particles contained in the streambed to form deposits of different grain size.

Streambed hydraulic conductivity may be related to the grain size. The results of grain size analysis showed that the average grain size distribution of streambed sediments in each right, central, and left side of the section in the downstream (Ghorbanabad Reach) have significant differences, especially in the meandering reach of the downstream.

Due to the dynamic sedimentation and scouring processes in the streambed, K_v showed higher temporal variability – 0.5 m below the streambed surface occurred in a relatively stable environment. The significant difference between the K_v of streambed in the upstream and downstream stems from the form of the sediments composing the streambed such that with an increase in the silt and clay in the downstream, a layer with low impermeability is formed. In addition, the K_v obtained from the two straight and meandering reaches in downstream had a significant difference whereas the same corresponding data were different from each other in the upstream and midstream. Therefore, the effect of the river morphology on K_v is evident. This indicates that in a meandering stream, more measurements are necessary for the thorough characterization of hydraulic parameters than in a straight stream.

Indeed, the importance of d_{10} factor relation with hydraulic conductivity cannot be ignored due to the key role of extremely fine particles in the riverbed, including slit and clay. Especially, the afore-mentioned relation is evident within the meandering reaches throughout the river and downstream regions. Moreover, the prediction of hydraulic conductivity using the experimental formulas for data validation was highly affected when the capability of the each experimental formula within upstream to downstream was tested in terms of direct and meandering reaches. The findings obtained for the present article will be useful for other rivers incorporating the similar morphological properties whose bank originates from mountain of steep slope and finally it reaches to see after passing through a plain.

Declarations. There is not any conflict of interest.

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LONG-TERM EFFECT OF ORGANIC AND CONVENTIONAL FARMING PRACTICES ON MICROBIAL BIOMASS CARBON, ENZYME ACTIVITIES AND MICROBIAL POPULATIONS IN DIFFERENT TEXTURED SOILS OF HARYANA STATE (INDIA)

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Abstract. The aim of the present study was to evaluate the impact of management practices on soil biological properties of texturally different soils of Haryana. Surface soil samples were collected from 25 organic farms and their adjoining conventional farms at 11 districts of Haryana and assayed for microbial biomass carbon (MBC), dehydrogenase and phosphatase activities along with total bacterial, actinomycetes and fungi populations in soil. The overall dehydrogenase and phosphatase activities at different locations were found to increase from 35.3 to 56.1 µg TPF g^{-1} soil 24 hr^{-1} and from 275.8 to 364.1 µg PNP g^{-1} soil, respectively under organic farming i.e. an increase of 57.5 and 32.01%. Overall a significantly higher MBC (69.1 %) was observed under organically managed soils (274 mg kg^{-1}) compared to conventional (162 mg kg^{-1}). The total bacterial, fungal and actinomycetes counts increased by 56.9, 55.2 and 49.5%, respectively, in comparison to those in the conventionally managed soils. The study concluded that soils under organic farming were found to be superior in terms of biological properties than conventionally managed soils which are essential for enhancing soil production and other functions in the given ecosystem.
Keywords: *management practices, microbial growth, organic farming, soil quality*

Introduction

Land management practices in present day agriculture are getting more and more important where food grain production and fertilizer use run parallel to meet the food requirements of over increasing population of India and leading to over exploitation of our natural resources like soil and water and therefore, soil is degrading day by day with respect to its fertility and productivity. This may be attributed to the fact that removal of nutrient resources from the soil strata is higher than their replenishment, so soil is becoming deficient in major available macro and micronutrients, which consequently leads to the decline of organic matter in soil and the overuse of chemical fertilizers and pesticides are adversely affecting the ecosystem (Yang et al., 2000). Thus, most soils are far from being ideal in fertility and should therefore be improved only by adopting appropriate management practices which includes amendments, like organic manures for enhancing the activity of "soil life". Enzymes and microorganisms like bacteria, actinomycetes and fungi present in soil are sensitive to changing soil environment and hence are considered to be one of the best soil health indicators. These microbes found to have direct role in sustaining soil fertility and its production by playing a crucial role in transformation of various nutrients and thereby improving the soil functioning in a

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given ecosystem. Thus, the study of the microbes and responses of enzymatic activities to disturbing agricultural activities is vital to understand how management practices contribute to sustaining soil fertility and productivity to improve soil (Wardle et al., 1999). Microbial populations and their activity are of immense importance in maintaining or sustaining soil health. Providing habitat and transformation of nutrients are complex processes brought about by succession of different micro-organisms in the soil which affect the soil fertility and over use of pesticides in conventional farming may inhibit these vital processes (Baum et al. 2003; Jolankai et al., 2006). Moreover, when soil applied pesticides fail to reach the target, they affect adjacent ecosystems through leaching or aerial drift affecting the diversity and abundance of non-targeted microorganisms causing negative effects on ecosystem processes and trophic interactions (Pimentel and Edwards, 1982). Different land management practices and continuous cultivation without any addition to soils are imposing variations in organic matter levels of soils and subsequent loss of soil organic carbon from the fields under intensive agricultural practices and leads to the alteration of microbial biomass (Srivastava and Singh, 1989). Therefore, there is a growing intention among farmers and researchers to adopt alternative farming practices that enhance or improve the biological properties of soils and thereby sustain soil health and productivity. Among different farming systems, organic farming is popular and its adoption level is increasing day by day to achieve the target of sustainability. In India, it has been estimated that cultivated area under certified organic farming has grown almost 17 fold in the last decade (0.042 mha in 2003-04 to 1.49 mha in 2016-17). The State of Haryana, one of the major agriculture based state of the country situated in the NCR region. It has about 4000 ha of land under organic farming (Anonymous, 2016). The key characteristics of organic farming include protecting the long-term fertility of soils by maintaining organic matter, fostering soil biological activity, careful mechanical intervention, providing nitrogen self-sufficiency through the use of legumes and biological nitrogen fixation, effective recycling of organic materials including crop residues, livestock wastes and weed, and diseases and pest control relying primarily on crop rotations, natural predators, diversity and resistant varieties. At present, the most optimistic estimates show that about 25-30 percent of nutrient needs can be met by various organic sources. Many researchers reported that in organically managed fields activity of earth worms is higher than in inorganic field (Edwards and Lofty, 1974; Kotcon, 2011; Rai et al., 2014; Velmourougane, 2016). In the biodegradation process, earthworms and microbes work together and produce vermicompost, which provides macro (N, P, K, Ca, and Mg) and micro nutrients (Fe, Mn, Zn, and Cu) (Amir and Fouzia, 2011). In organic farming, use of manures and crop residues instead of a synthetic fertilizer is reported to reduce groundwater contamination, improve microbial activity, recycle dairy/poultry wastes, and improve soil properties (Poudel et al., 2002). However, the information regarding the long-term effect of organic farming practices on soil biological properties and its feasibility are very scanty. Keeping in view the above concerns, the present study was planned to evaluate the long-term impact of management practices on soil biological properties like microbial biomass carbon (MBC), dehydrogenase and phosphatase activities along with bacterial, actinomycetes and fungi populations in fields particularly of vegetables and horticultural crops with texturally different soils in Haryana.

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Materials and Methods

Study area

Organic farms under different cropping systems, vegetables and horticultural crops and their adjoining conventional farms were identified at eleven districts of Haryana, namely, Sirsa, Fatehabad, Hisar, Jind, Kaithal, Karnal, Kurukshetra, Ambala, Panchkula, Yamunanagar and Panipat and their soil properties were studied. Locations of different sampling sites are presented in *Figure 1*. A total of 150 soil samples (three replications) of 0-15 cm depth were collected from the two types of farming systems, which were pooled to make 50 composite samples and analysed for microbial biomass, dehydrogenase and alkaline phosphatase activity, total bacterial, fungal and actinomycetes counts. The texture of soils varied between sand to clay loam, representing almost all the soil types of the State and is presented in *Table 1*.

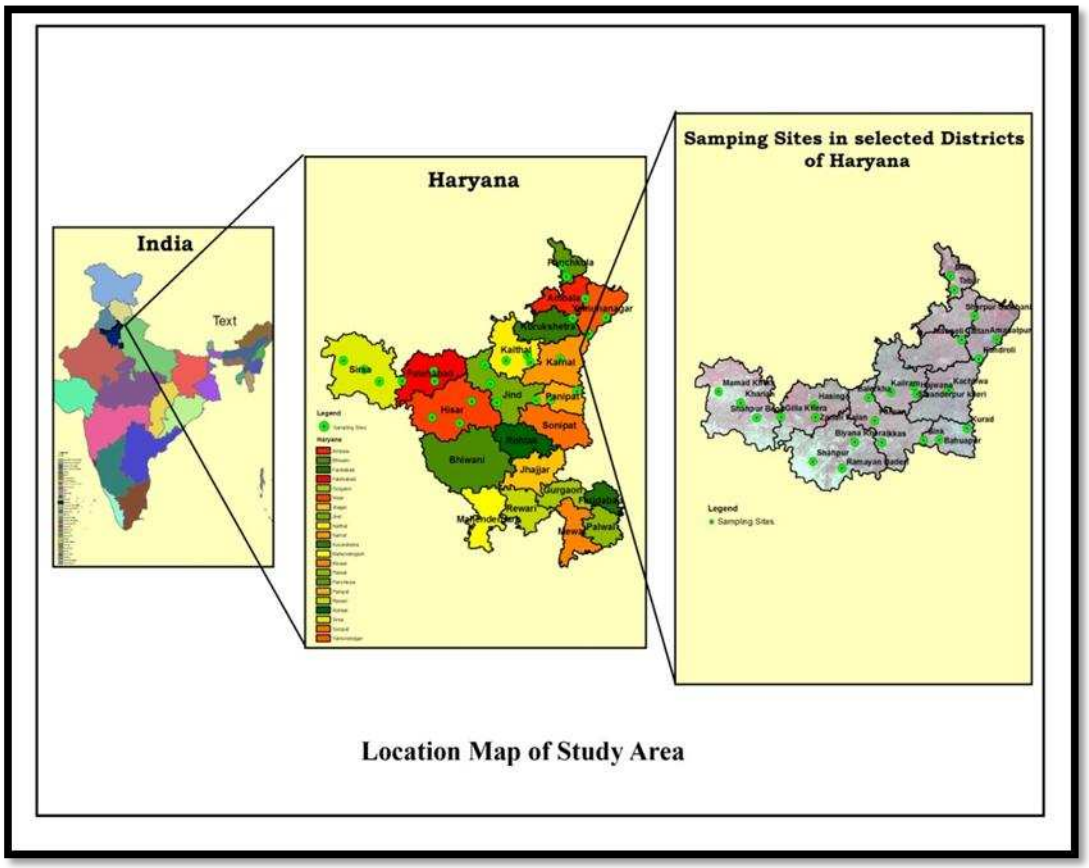


Figure 1. *Sampling sites in selected districts of Haryana*

Collection of soil samples

The soil samples collected were representative of the area sampled. A field was treated as a single sampling and recently fertilized fields, bunds, channels, areas near trees, farm ways, buildings, wells, compost piles or other non-representative locations were avoided during sampling. Three soil samples were taken randomly from field and finally one composite sample was prepared.

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Table 1. *Soil texture at different locations under conventional and organic farming systems*

Location	District	Village	Texture
L_1	Panchkula	Billa	Sand
L_2	Sirsa	Mamad Khera	Sand
L_3	Panipat	Kurad	Loamy sand
L_4	Panchkula	Tabar	Loamy sand
L_5	Sirsa	Kharian	Loamy sand
L_6	Fatehabad	Zandli Kalan	Loamy sand
L_7	Fatehabad	Hasinga	Loamy sand
L_8	Hisar	Shahpur	Loamy sand
L_9	Hisar	Biyana Khera	Sandy loam
L_{10}	Panipat	Bahuapur	Sandy loam
L_{11}	Jind	Ikkas	Sandy loam
L_{12}	Yamunanagar	Amadalpur	Sandy loam
L_{13}	Jind	Balerkha	Loam
L_{14}	Kaithal	Kailram	Loam
L_{15}	Kurukshetra	Mangoli Jattan	Loam
L_{16}	Kaithal	Hajwana	Loam
L_{17}	Hisar	Ramayan Daderi	Loam
L_{18}	Panipat	Sink	Silt loam
L_{19}	Yamunanagar	Kandroli	Silt loam
L_{20}	Fatehabad	Gilla Khera	Silt loam
L_{21}	Sirsa	Shahpur Begu	Sandy clay loam
L_{22}	Kaithal	Sikanderpur kheri	Sandy clay loam
L_{23}	Jind	Palwan	Clay loam
L_{24}	Karnal	Kachhwa	Clay loam
L_{25}	Ambala	Sherpur Sulkhani	Clay loam

Processing of soil samples

After collection soil samples were kept refrigerated at 0°C for analysis of the microbiological properties. Growth media used for the total bacterial, fungal and actinomycetes counts are given in *Table 2*.

Table 2. *Growth media used for microbial analysis*

Sr. No.	Microbe	Medium
1.	Bacteria	Nutrient Agar
2.	Fungi	Potato Dextrose Agar
3.	Actinomycetes	Ken-Knight‘s Medium

Soil enzymatic activities

Dehydrogenase activity

Dehydrogenase activity was determined by using the method of Casida et al. (1964). Five gram soil was taken and mixed with 3% solution of 2,3,5-triphenyl tetrazolium chloride and 2.5 ml of distilled water, and incubated for 24 h at 37°C temperature in an incubator. After incubation, 10 ml methanol was added and shaken for 1 minute and filtered through Whatman No. 1 filter paper. The absorbance was measured at 485 nm by using a spectrophotometer.

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Alkaline phosphatase activity

Alkaline phosphatase activity was determined by using the method of Tabatabai and Bremner (1969). Enzyme activity was measured by taking 1 g of soil in a flask and adding 0.2 ml of toluene, 4 ml of modified universal buffer (MUB), 1 ml of p-nitrophenyl phosphate solution to it. After incubating for 1 hour, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added. After stirring for a few seconds, suspension was filtered through Whatman No. 1 filter paper and absorbance of the filtrate was measured at 420 nm using a spectrophotometer.

Microbial biomass carbon (soil fumigation-extraction method)

Microbial biomass carbon (MBC) was determined by soil fumigation-extraction method (Vance et al., 1987). 10 g of soil was fumigated with 20 ml of $CHCL_3$ in vacuum desiccators for 24 hours in dark and another 10 g of the same soil sample was refrigerated, then both samples (fumigated and non-fumigated) were extracted with 0.5 M K_2SO_4 for half an hour and then the extract was treated with H_2SO_4 and orthophosphoric acid, and heated on hot plate at 120 ^oC for 30 minutes. After diluting to 250 ml with distilled water, 2-3 drops of ferroin indicator was added and titrated against 0.05 N FAS (Ferrous Ammonium Sulphate) and MBC was calculated:

$$MBC(\mu g/g\ soil) = \frac{OC_F - OC_{UF}}{K} \quad \text{(Eq.1)}$$

where,

- OC_F = Total amount of EC (Extractable C) in fumigated soil.
- OC_{UF} = Total amount of EC (Extractable C) in non-fumigated soil.
- K = The proportion of microbial C evolved as CO_2= 0.45 for 10 days of incubation at 25°C (Jenkinson and Ladd, 1981).

Microbial counts

Total bacterial, fungal and actinomycetes counts were estimated using serial dilution plate technique. One gram of soil was transferred into 10 ml of sterilized water in 250 ml flask. Sample was mixed by vigorous shaking and now the sample was diluted ten-fold (10^{-1} dilution). From this, 1 ml of soil suspension was transferred to another 9 ml water blank with the help of sterile pipette and hence sample was diluted to 10^{-2}. Similarly, dilutions were made up to 10^{-7}. Different dilutions were made to estimate the different microbial parameters (*Table 3*).

Table 3. *Dilution used for different microbes in the appropriate nutrient media*

Sr. No.	Microbe	Dilution factor
1.	Bacteria	10^{-6} -10^{-7}
2.	Fungi	10^{-3} -10^{-4}
3.	Actinomycetes	10^{-4} -10^{-5}

Now, 1 ml of different diluted samples i.e.10^{-6}-10^{-7} were transferred in appropriate pre-sterilized labeled Petri-plates in front of laminar flow. Then 25-30 ml of appropriate media was poured into each dilution plate and shaken to ensure uniform dispersal of the

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cells/suspension in the media. These plates were allowed to solidify on working table of the laminar. Further, the plates are incubated in an inverted position (upside down) for 2-5 days at 28 ± 2°C temperature in an incubator and number of colonies on different dilution media plates were recorded and populations per gram soil were enumerated.

Statistical analysis

The significance of treatment effects was analyzed using two factorial RBD analysis with OP Stat, CCS HAU Hisar software (Sheoran et al., 1998). Spatial variability graphs were prepared by using ArcGIS software.

Results and Discussion

Dehydrogenase and phosphatase activity

Data on dehydrogenase activity in soils under the two farming systems given in *Table 4* revealed that organic farming led to significant improvement in dehydrogenase activity over conventional practice. The dehydrogenase activity under organic farming was observed significantly higher than in conventional practice at all the locations with different soil textures. The soil dehydrogenase activity in different locations varied between 18.5 to 46.0 µg TPF g^{-1} soil 24 hr^{-1} in conventional farming while it ranged from 23.8 to 78.6 µg TPF g^{-1} soil 24 hr^{-1} under organic farming. The extent of increase in dehydrogenase activity under organic farming was, however, found to increase with fineness of the texture at different locations. For example, the increase in dehydrogenase activities were 28.6 % in sand (L_1), 55.1 % in loamy sand (L_8), 68.1% in sandy loam (L_{12}), and 76.6% in clay loam soil at location L_{24}. The overall dehydrogenase activity which was 35.3µg TPF g^{-1} soil 24 hr^{-1} under conventional farming at different locations was found to increase to 56.1 µg TPF g^{-1} soil 24 hr^{-1} under organic farming i.e. an increase of 57.5%. The interactive effect of location and farming system on dehydrogenase activity was also found significant. The significant increase in the dehydrogenase activity in texturally different soils of different locations under conventional and organic farming is presented in *Figure 2*.

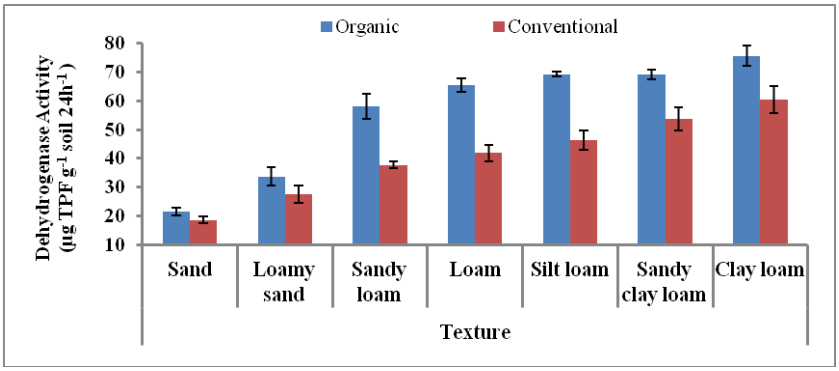


Figure 2. *Dehydrogenase activity in different textured soils under conventional and organic farming*

Moreover, adoption of organic farming showed a significant increase of alkaline phosphatase in different textured soils over the conventional farming. In general, both

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the farming systems were found to enhance the phosphatase activity with increase in clay content of soil but the extent of increase of alkaline phosphatase was greater under organic farming system than in conventional practice .The values of phosphatase activity varies from 132.6 to 453.1 µg PNP g^{-1} soil hr^{-1}among different locations under conventional system of farming while it ranged from 151.8 to 642.8 µg PNP g^{-1} soil hr^{-1} under organic system of farming. The conversion of conventional system of farming to organic farming resulted in a significant increase in phosphatase activity from 275.8 to 364.1 µg PNP g^{-1} soil hr^{-1}. The per cent increase in the phosphatase activity under organic farming over conventional in different textured soils showed an increase of 22.8 % in sand which further increased to 36.8% in clay loam soil. The phosphatase activity in different textured soils of different locations under conventional and organic farming is represented in *Figure 3*.

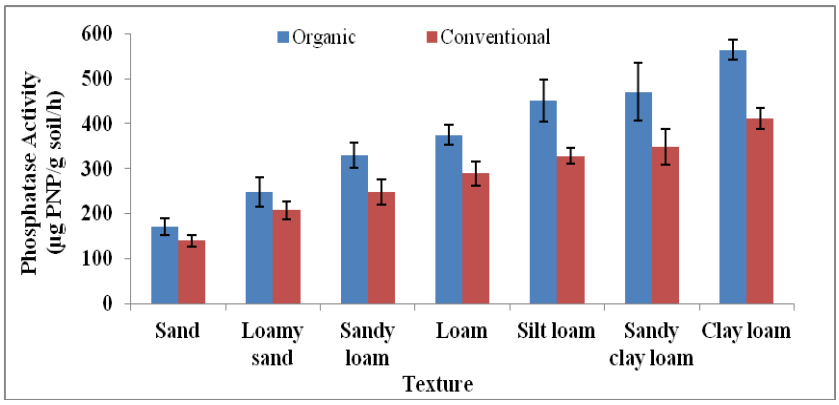


Figure 3. *Phosphatase activity in different textured soils under conventional and organic farming*

The study confirmed the positive influence and higher microbial activity indices of organic farming (higher enzymatic activity, higher soil microbial biomass carbon content) compared to conventional farming system and the higher enzyme activities under organic practices might be attributed to enhanced nutrient availability from added organics, increase in release of root exudates due to improved crop growth and favorable environment for microbial growth (Burns et al., 2013; Tamilselvi et al., 2015).

Farmyard manure and crop residues provide a source of organic matter, and when returned to soil they increase organic C in soil (Banger et al., 2010) and further, the prevalence of organic substrates stimulated soil microorganisms to produce enzymes responsible for the conversion of unavailable nutrients to plant available forms. Most importantly, a closer look at the relationship between physicochemical and biological indicators of soil health evidenced the significance of organic matter to enzyme activities suggesting enhanced nutrient cycling in systems receiving organic amendments (Sihi et al., 2017). Moreover, phosphatase activity in the soils occurs either in response to plant roots exudates or from microbes such as bacteria or fungi (Dinkelaker and Marschner, 1992) and changes in phosphatase activity can indicate changes in the soil phosphoryl substrates (Rao and Tarafdar, 1992).

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Table 4. *Dehydrogenase, phosphatase activities and soil microbial biomass carbon under conventional and organic farming at different locations*

Location (L)	Dehydrogenase (µg TPF g^{-1} soil $24hr^{-1}$)			Alkaline phosphatase (µg PNP g^{-1} soil hr^{-1})			Microbial biomass carbon (mg kg^{-1})		
	Conventional	**Organic**	**Mean**	**Conventional**	**Organic**	**Mean**	**Conventional**	**Organic**	**Mean**
L_1	18.5	23.8	**21.1**	146.4	191.5	**168.9**	132	152	**142**
L_2	28.7	24.2	**26.5**	132.6	151.8	**142.2**	87	105	**96**
L_3	29.6	36.7	**33.1**	196.2	211.7	**204.0**	99	166	**132**
L_4	22.6	37.9	**30.2**	195.2	286.4	**240.8**	137	173	**155**
L_5	27.4	43.6	**35.5**	236.7	242.6	**239.6**	122	199	**160**
L_6	25.4	38.0	**31.7**	226.7	230.8	**228.7**	114	173	**143**
L_7	28.7	41.5	**35.1**	203.3	244.2	**223.7**	101	198	**149**
L_8	23.3	36.1	**29.7**	165.6	219.7	**192.6**	129	167	**148**
L_9	36.1	52.8	**44.4**	249.1	321.3	**285.2**	111	211	**161**
L_{10}	38.1	56.7	**47.4**	274.8	334.6	**304.7**	109	209	**159**
L_{11}	28.4	59.4	**43.9**	209.4	292.8	**251.1**	146	258	**202**
L_{12}	37.6	63.2	**50.4**	237.6	365.4	**301.5**	137	328	**232**
L_{13}	42.3	62.7	**52.5**	209.2	349.2	**279.2**	132	278	**205**
L_{14}	41.8	67.8	**54.8**	335.1	407.4	**371.2**	176	293	**234**
L_{15}	40.3	63.9	**52.1**	278.6	366.8	**322.7**	268	402	**335**
L_{16}	46.3	67.9	**57.1**	267.3	378.2	**322.5**	212	391	**301**
L_{17}	38.8	60.3	**49.5**	289.4	382.7	**336.0**	183	394	**288**
L_{18}	42.4	68.7	**55.9**	367.2	497.6	**432.4**	145	343	**244**
L_{19}	40.5	70.3	**55.4**	321.1	452.8	**386.9**	168	355	**261**
L_{20}	39.5	68.7	**54.1**	314.1	402.7	**358.4**	176	300	**238**
L_{21}	41.7	64.3	**53.0**	402.2	425.2	**413.7**	204	344	**274**
L_{22}	43.2	69.2	**56.2**	453.1	516.4	**484.6**	176	305	**240**
L_{23}	46.0	76.4	**61.2**	368.2	627.4	**497.3**	269	404	**336**
L_{24}	44.5	78.6	**61.5**	412.3	642.8	**527.5**	256	310	**283**
L_{25}	39.8	71.9	**55.8**	403.7	561.8	**482.5**	271	411	**341**
Mean	**35.3**	**56.1**		**275.8**	**364.1**		**162**	**274**	
CD at 5%	Location (L) = 1.6, Farming system (F) = 4.7, L x F = 7.5			Location (L) = 9.9, Farming system (F) = 2.8, L x F = 14.1			Location (L) = 7.9, Farming system (F) = 2.2, L x F = 11.1		

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These findings agree with several research studies (Mader et al., 2002; Dinesh et al., 2010; Lazcano et al., 2013; Sunita, 2016) where higher microbial biomass and enzyme activities were reported even in a single growing season in response to short-term incorporation of organic manures in organics compared to conventional systems.

This indicates that instant increase in soil enzymatic activities which are associated with a rapid increase in availability of organic substrates required for microbial growth.The dehydrogenase (*Figure 4a*) and phosphatase (*Figure 4b*) activities in soils plotted for organic farming against adjoining soils under conventional farming (intercept set at zero) also showed clearly that organic farming is very effective in enhancing dehydrogenase (y = 0.663x) and phosphatase (y = 0.738x) over conventional system in Haryana (*Figure 4*). Moreover, the spatial variability maps for different location are also presented in *Figure 5* and *Figure 6*.

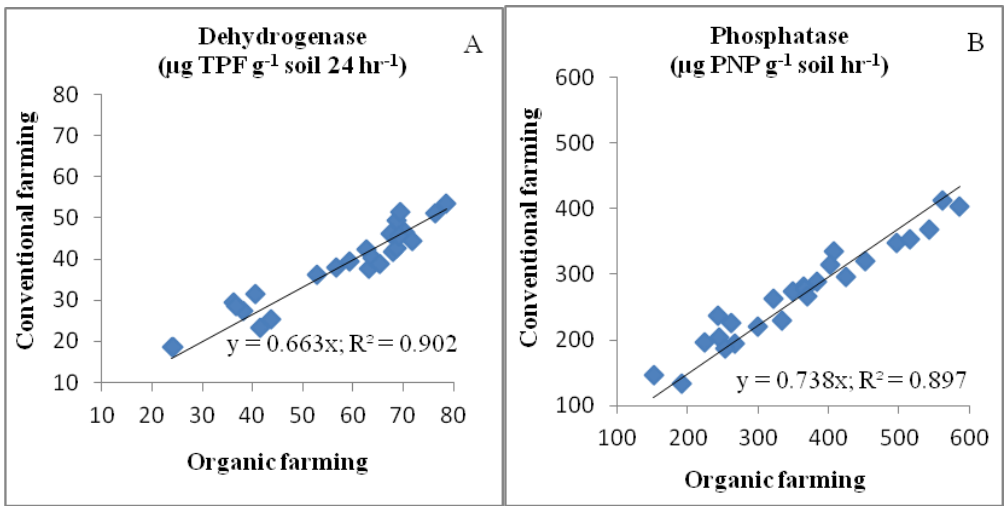


Figure 4. *The relationship of dehydrogenase (A) and phosphatase (B) activities in soils under organic and conventional farming systems*

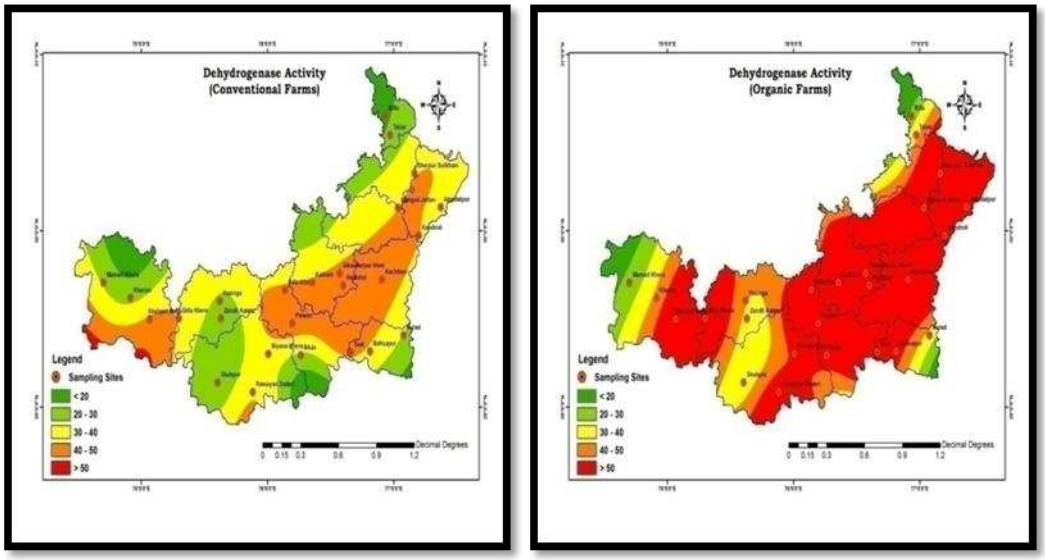


Figure 5. *Spatial variability map of dehydrogenase activity in soils under conventional and organic farming systems at different locations of Haryana*

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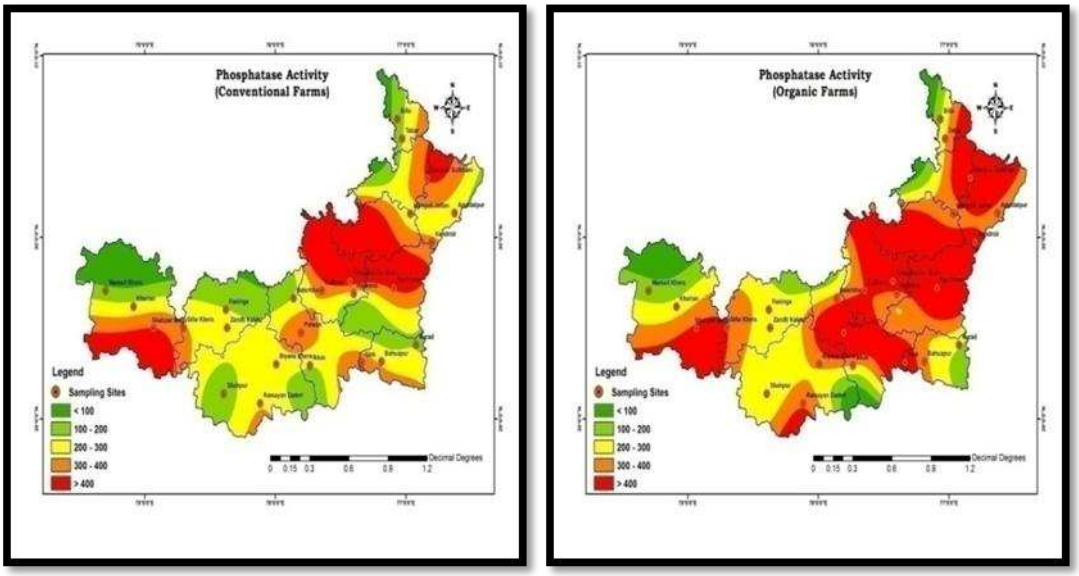


Figure 6. *Spatial variability map of phosphatase activity in soils under conventional and organic farming systems at different locations of Haryana*

Microbial biomass carbon (MBC)

The MBC is one of the fractions of SOC which constitutes a very small portion but considered as a good indicator of soil health. Organic farming had significant effect on soil microbial biomass carbon in texturally different soils at different locations (*Table 4*). Locations with fine textured soils have significantly higher amount of MBC as compared to locations with light textured soils i.e. L_1 to L_{25}. Under conventional farming, the MBC was lowest (87 mg kg^{-1}) in sand at location L_2, while it was highest in clay loam soil at location L_{25} (271 mg kg^{-1}). Similarly, the values of lowest and highest MBC were found to be 105 and 411 mg kg^{-1} in sand (L_2) and clay loam soil (L_{25}) under organic farming. Overall a significantly higher MBC (69.1 %) was observed under organically managed (274 mg kg^{-1}) as compared to conventional (162 mg kg^{-1}). The interactive effect of location and farming system was also found significant and the highest MBC content (411mg kg^{-1}) was observed at location L_{25} under organic farming. Variations in MBC in different textured soils under conventional and organic farming have also been presented in *Figure 7*. MBC serves as an important reservoir of plant nutrients (Marumoto et al., 1982; Okur et al., 2009) and soil microbial C decrease with decrease in fineness of soil texture due to declining C input but observed to be higher in organically managed soils than in conventionally managed due to the permanent input of organic residues with high C/N ratio (Schjonning et al., 2002; Crecchioa et al., 2004; Melero et al., 2006; Araujo et al., 2009; Amaral et al., 2011). Furthermore, higher soil microbial biomass carbon in organic farming is mainly due to the regular application of farmyard manure and large carbon inputs in the form of organic amendment. Additionally, farmyard manure supplies readily available N, resulting higher plant biomass. Consequently, more crop residues are incorporated in soil and thereby higher organic matter levels are maintained (Roldan et al., 2005). This also provides a favorable environment for microorganisms, contributing to a highly diverse and stable microbial community structure in organic farming systems (Wada and Toyota, 2007).

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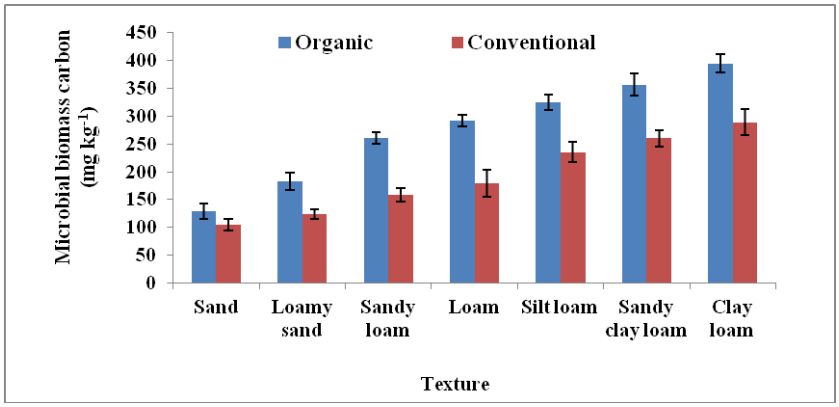


Figure 7. *Microbial biomass carbon in different textured soils under conventional and organic farming*

The plot of the microbial biomass carbon of soils under organic farming against the microbial biomass carbon of the adjoining soils under conventional farming (intercept set at zero) showed that organic farming in general increases microbial biomass carbon (y = 0.638x) of soils (*Figure 8*). The spatial variability maps of MBC for different location are also presented in *Figure 9*.

Total bacterial, fungal and actinomycetes counts

Wide variation of soil microbial populations has been recorded at different locations under organic and conventional farming systems as well (*Table 5*). The consistent increase was noticed in the population of bacteria, fungi and actinomycetes at all the locations under organic farming but the extent of increase was more pronounced in heavy textured soils subjected to organic manures such as FYM, neem cake or vermicompost.

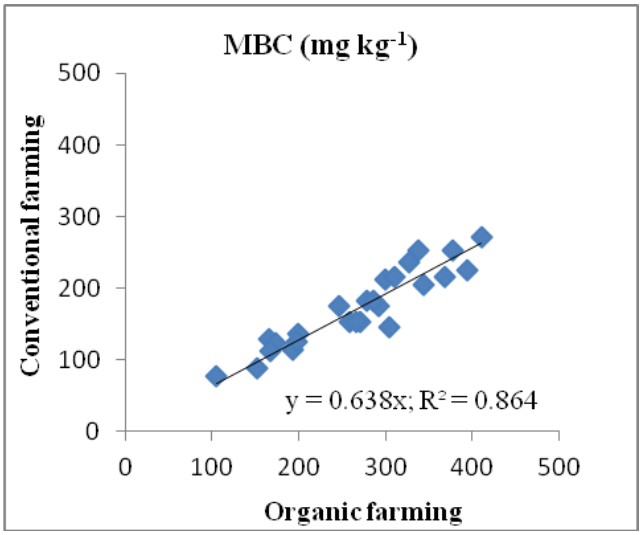


Figure 8. *The relationship of microbial biomass carbon activities in soils under organic and conventional farming systems*

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Table 5. *Effect of organic and conventional farming practices on microbial population in soil at different locations*

Location (L)	Bacteria (CFU g^{-1} soil × 10^6)			Fungi (CFU g^{-1} soil × 10^3)			Actinomycetes (CFU g^{-1} soil × 10^4)		
	Conventional	**Organic**	**Mean**	**Conventional**	**Organic**	**Mean**	**Conventional**	**Organic**	**Mean**
L_1	6.00	8.75	**7.38**	5.81	6.23	**6.02**	7.89	11.55	**9.72**
L_2	5.75	6.75	**6.25**	4.54	5.69	**5.12**	5.84	9.62	**7.73**
L_3	9.89	13.11	**11.50**	8.01	10.62	**9.32**	8.11	12.59	**10.35**
L_4	11.61	17.84	**14.73**	9.40	14.45	**11.93**	12.08	17.94	**15.01**
L_5	10.96	16.77	**13.87**	8.88	13.58	**11.23**	10.18	14.23	**12.20**
L_6	12.42	20.16	**16.29**	10.06	16.33	**13.19**	10.35	13.28	**11.82**
L_7	14.19	21.92	**18.06**	11.50	17.75	**14.63**	13.63	21.56	**17.59**
L_8	8.81	15.94	**12.38**	7.14	12.91	**10.03**	9.31	15.61	**12.46**
L_9	13.33	17.84	**15.59**	10.80	14.45	**12.63**	14.32	19.84	**17.08**
L_{10}	16.72	24.37	**20.54**	13.54	19.74	**16.64**	17.02	23.12	**20.07**
L_{11}	12.26	19.99	**16.13**	9.93	16.20	**13.06**	14.16	20.87	**17.52**
L_{12}	14.53	21.10	**17.82**	11.76	17.10	**14.43**	12.85	20.32	**16.59**
L_{13}	14.86	22.04	**18.45**	12.04	17.85	**14.94**	10.35	21.17	**15.76**
L_{14}	16.12	25.70	**20.91**	13.06	20.81	**16.94**	16.44	26.57	**21.50**
L_{15}	18.49	29.03	**23.76**	14.98	23.51	**19.24**	15.35	23.29	**19.32**
L_{16}	13.61	22.58	**18.10**	11.03	18.28	**14.66**	14.66	24.32	**19.49**
L_{17}	14.90	21.07	**17.98**	12.07	17.07	**14.57**	16.96	22.62	**19.79**
L_{18}	14.26	19.35	**16.80**	11.55	15.68	**13.61**	19.49	27.43	**23.46**
L_{19}	16.81	24.51	**20.66**	13.62	19.85	**16.74**	14.83	25.53	**20.18**
L_{20}	18.49	30.74	**24.62**	14.98	24.90	**19.94**	18.78	27.77	**23.28**
L_{21}	15.48	31.39	**23.44**	12.54	25.42	**18.98**	20.46	29.64	**25.05**
L_{22}	18.49	33.81	**26.15**	14.98	27.39	**21.19**	20.87	31.39	**26.13**
L_{23}	20.37	35.47	**27.92**	16.50	28.74	**22.62**	26.15	43.99	**35.07**
L_{24}	28.16	40.42	**34.29**	22.81	32.74	**27.78**	24.53	35.66	**30.09**
L_{25}	25.16	39.13	**32.15**	20.38	31.69	**26.04**	22.60	41.06	**31.83**
Mean	**14.87**	**23.19**		**12.08**	**18.76**		**15.09**	**23.24**	
CD at 5%	Location (L) = 1.57, Farming system (F) = 0.44, L x F = 2.22			Location (L)= 1.34, Farming system (F) = 0.38, L x F = 1.90			Location (L)= 1.91, Farming system (F) = 0.54, L x F = 2.7		

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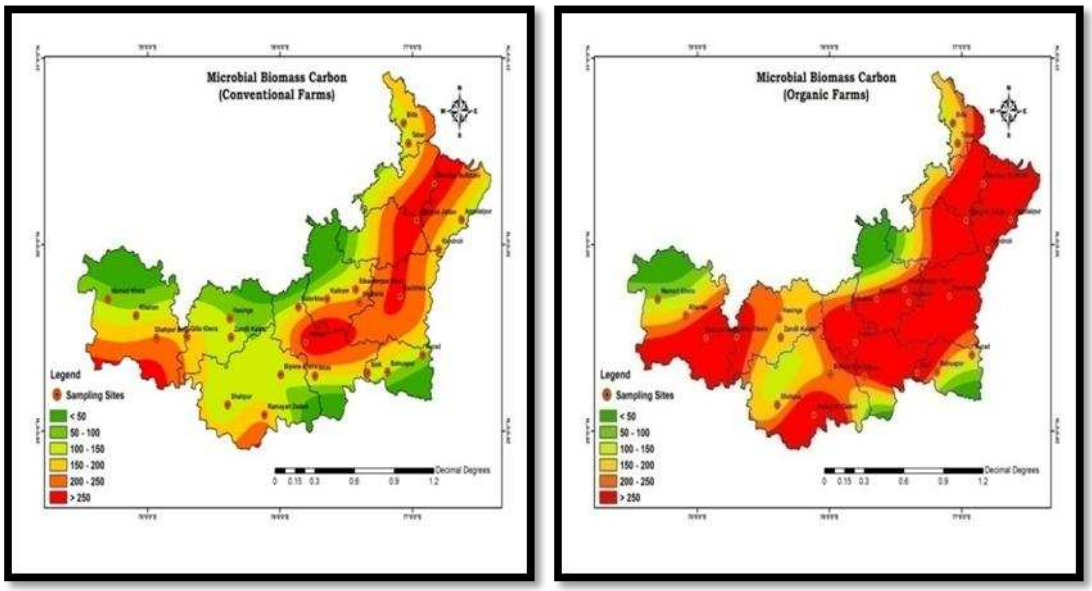


Figure 9. *Spatial variability map of MBC in soils under conventional and organic farming systems at different locations of Haryana*

The mean value of different locations pertaining to population of bacteria was observed to be 14.87×10^6 CFU g^{-1} soil under conventionally managed soils which was significantly increased to 23.34×10^6 CFU g^{-1} soil upon conversion to organic farming. Similarly, fungi population was significantly increased from 12.08×10^3 CFU g^{-1} soil to 18.76×10^3 CFU g^{-1} soil and actinomycetes population from 15.09×10^4 CFU g^{-1} soil to 22.57×10^4 CFU g^{-1} soil under organic farming as compared to conventional farming practices. The average populations of bacteria, fungi and actinomycetes in the soils at different locations under organic farming were 56.9%, 55.2% and 49.5%, respectively, higher than those in the conventionally managed soils. In different textured soils, the bacteria showed variations from 6.25×10^6 in sand to 24.56×10^6 CFU g^{-1} soil in clay loam soil whereas fungal and actinomycetes population varied from 5.17×10^3 in sand to 23.58×10^3 in clay loam soil and 6.86×10^4 in sand to 28.89×10^4 CFU g^{-1} soil in clay loam, respectively, under conventional farming (*Figures 10 to 12*).

Addition of various nutrient inputs through various sources such as FYM, neem cake and vermicompost under organic farming practices exhibited huge variation and resulted in rapid stimulation of microbial growth and subsequently, the increase in bacteria, fungi and actinomycetes population was observed to be 7.75×10^6, 5.96×10^3 and 8.28×10^4 CFU g^{-1} soil in sand to 38.34×10^6, 31.06×10^3 and 35.89×10^4 CFU g^{-1} soil, respectively, in clay loam soil. The significant increase in population of these microorganisms under organic farming, however, observed to be significant only in loamy soils as compared to conventional farming system.

Growth of bacteria, fungi and actinomycetes on different nutrient media has also been shown in *Plates 1 to 3*. The average populations of bacteria, fungi and actinomycetes in the soils at different locations under organic farming were 56.9%, 55.2% and 49.5%, respectively, higher than those in the conventionally managed soils.

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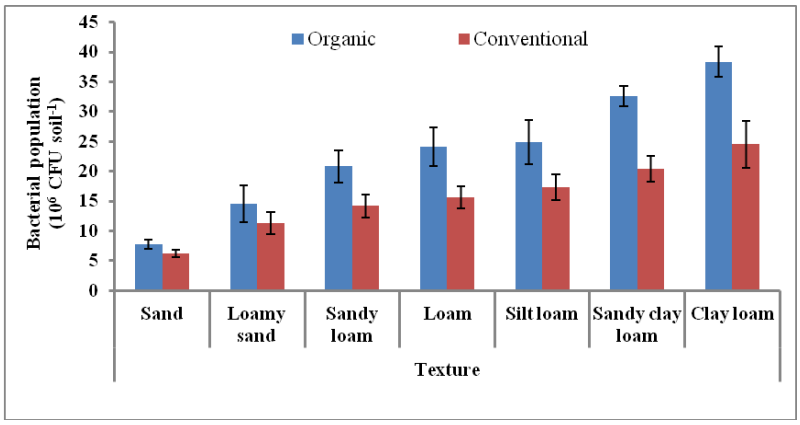


Figure 10. *Bacterial populations in different textured soils under conventional and organic farming systems*

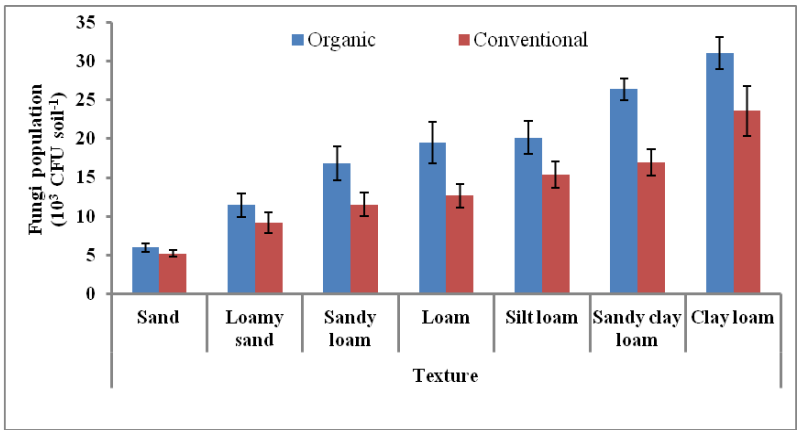


Figure 11. *Fungal populations in different textured soils under conventional and organic farming systems*

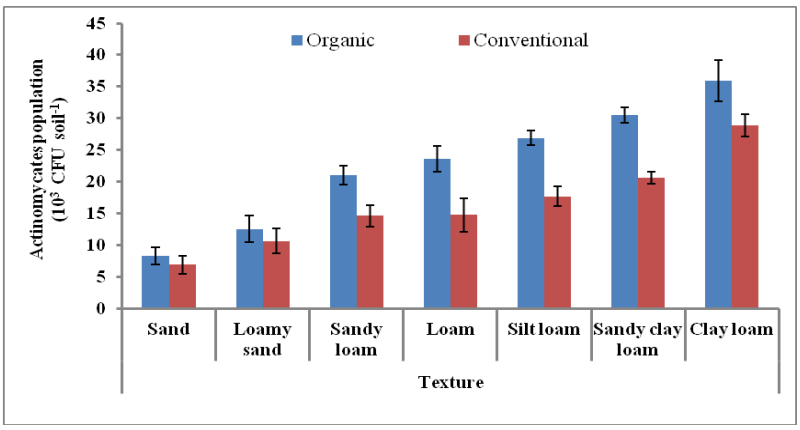
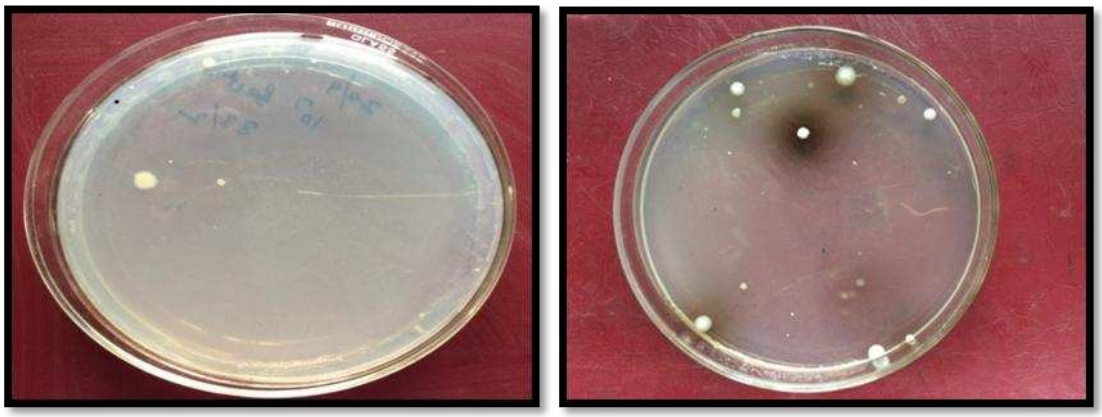


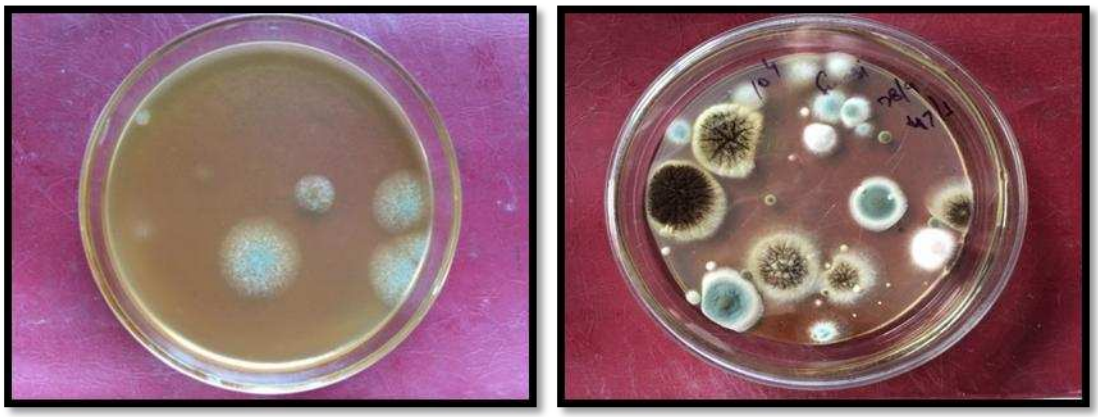
Figure 12. *Actinomycetes populations in different textured soils under conventional and organic farming systems*

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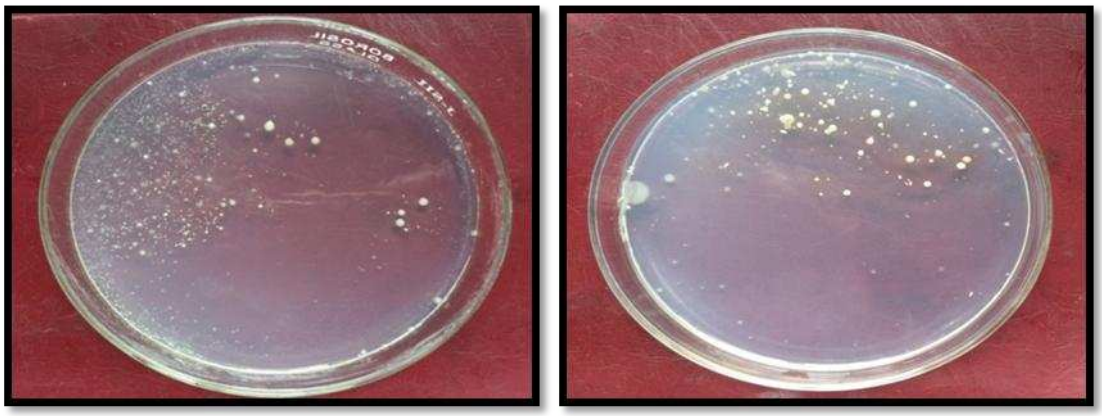
Conventional *Organic*

Plate 1. *Growth of different bacterial species on nutrient agar media*



Conventional *Organic*

Plate 2. *Growth of different fungal species on potato dextrose media*



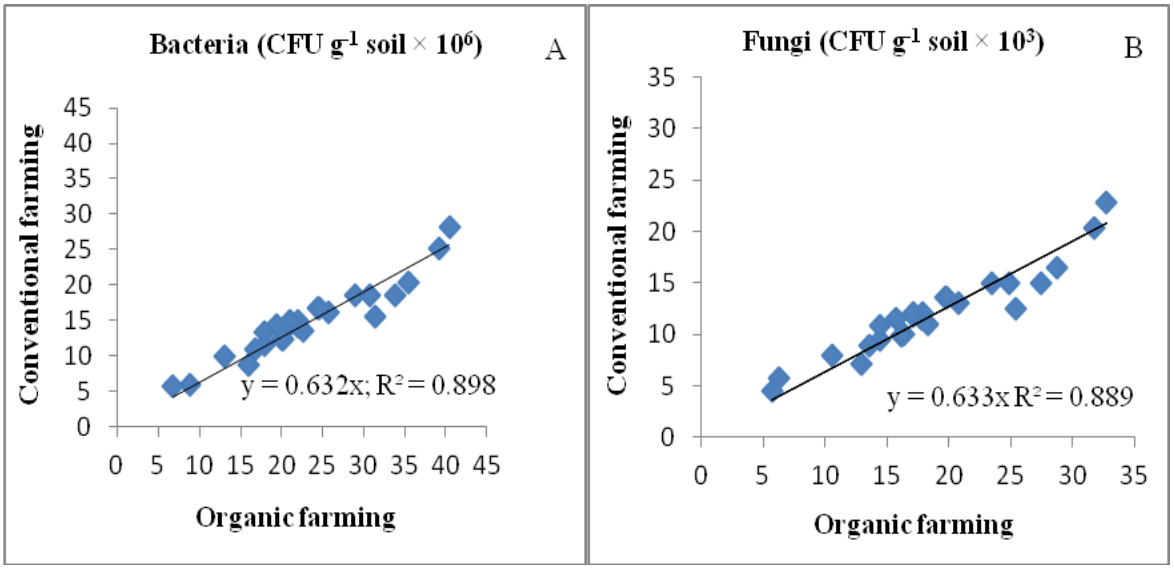
Conventional *Organic*

Plate 3. *Growth of different actinomycetes species on kenknight media*

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The addition of FYM, neem cake, vermicompost and other organic sources of nutrients enhanced growth of microbial populations which may be attributed to the increment in organic matter in soil that serves as a food and energy source for microbes. These results are supported by the findings of Bobulska et al. (2015). In recent years, multiple studies comparing conventional and organic agriculture have reported differences in soil properties, increased microbial activity and diversity in organically managed soils, or distinct microbial profiles between the two systems (Wu et al., 2008; Moeskops et al., 2010; Bobulska et al., 2015). Results from our study clearly indicated that soil texture significantly affected bacteria, fungi and actinomycetes populations, and higher growth of microbial populations was observed in heavy textured soils (clay loam) than light textured soils (sand). Among six textural classes, the highest growth of microbial populations was observed in clay loam and the lowest in sand and there was positive a correlation between soil organic carbon and growth of microbial populations. Several researches documented that fine soils were found to be more suitable for bacteria, fungi and actinomycetes survival because smaller size particles provide a protective habitat for microorganisms through pore size exclusion of predators (Sessitsch et al., 2001 and Zhang et al., 2007). Alternatively, higher microbial population in clay loam soils may be due to a higher organic matter content, water retention characteristics and nutrient availability (Grayston et al., 2004). Microbial activity in soil is strongly influenced by the clays and humates which bind organic chemicals, inorganic ions, and water films to their surfaces (Van et al., 1996).

The bacterial (*Figure 13a*), fungal (*Figure 13b*) and actinomycetes (*Figure 13c*) populations of soils plotted for organic farming against adjoining soils under conventional farming (intercept set at zero) also showed clearly that organic farming had a promising effect in increasing bacterial (y = 0.632x), fungal (y = 0.633x) and actinomycetes populations (y = 0.642x) over conventional system in Haryana (*Figure 13*). The spatial variability maps of bacteria, fungi and actinomycetes at different locations are also presented in *Figures 14 to 16*.



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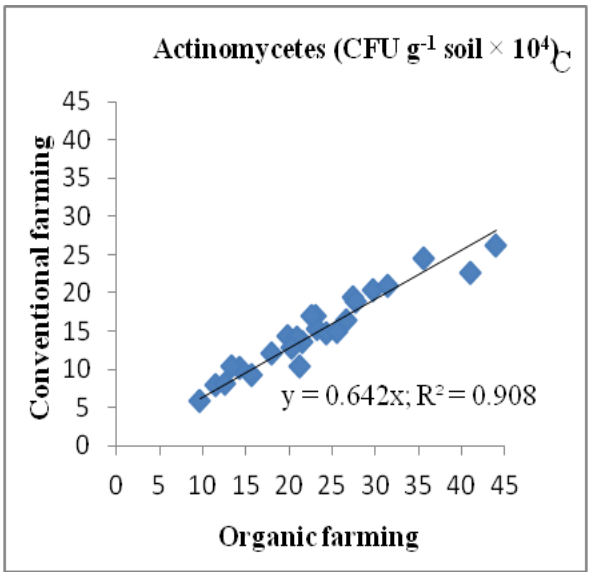


Figure 13. *The relationship of bacteria, fungi and actinomycetes populations in soils under organic and conventional farming system*

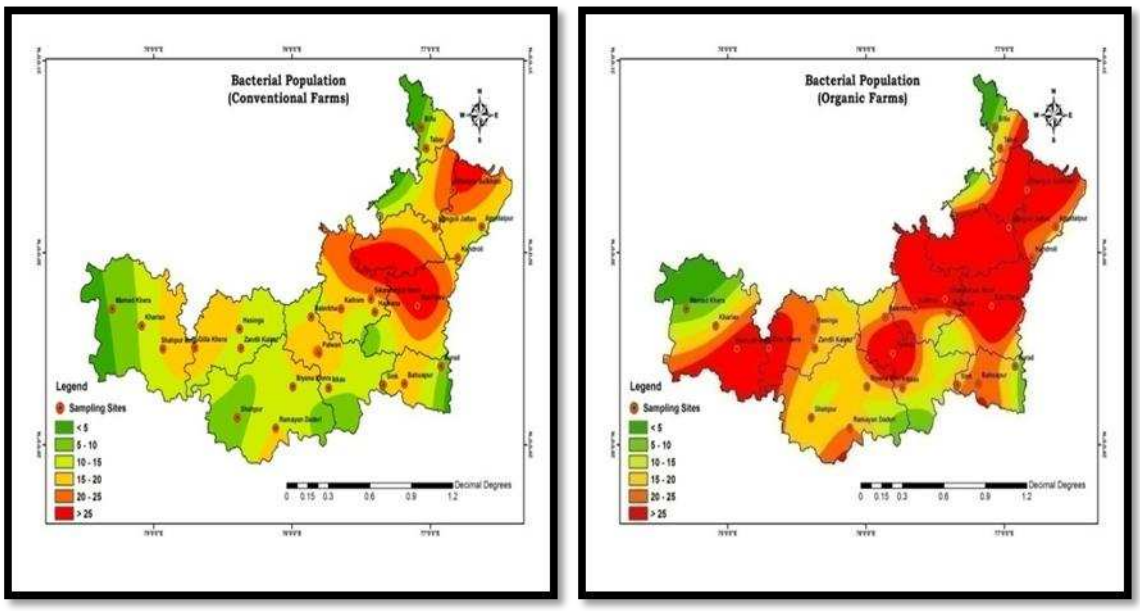


Figure 14. *Spatial variability map of bacterial populations in soils under conventional and organic farming systems at different locations of Haryana*

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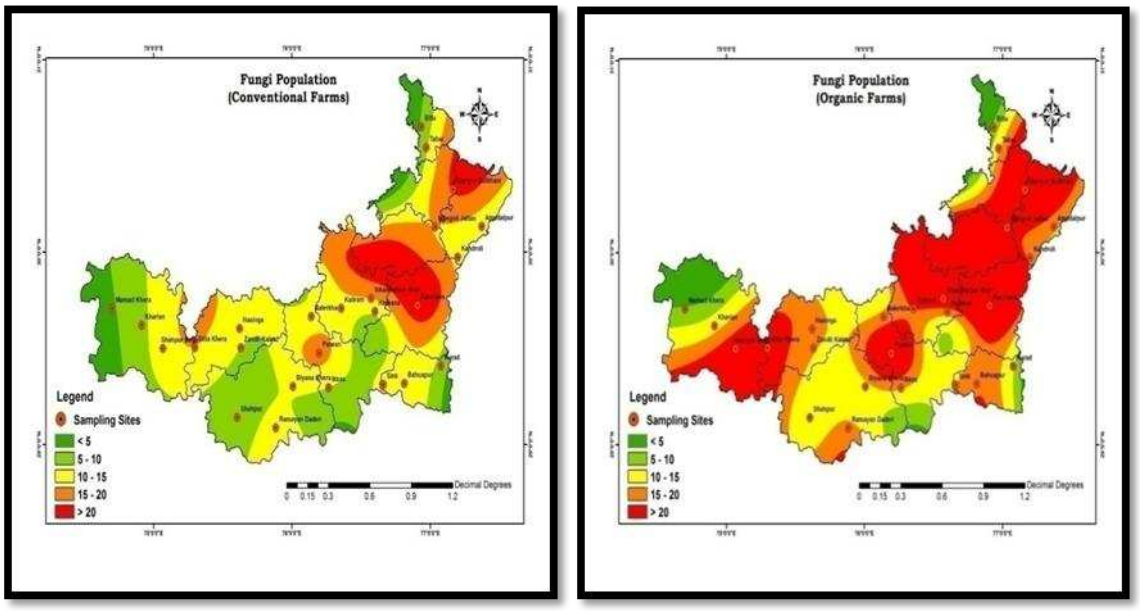


Figure 15. *Spatial variability map of fungal populations in soils under conventional and organic farming systems at different locations of Haryana*

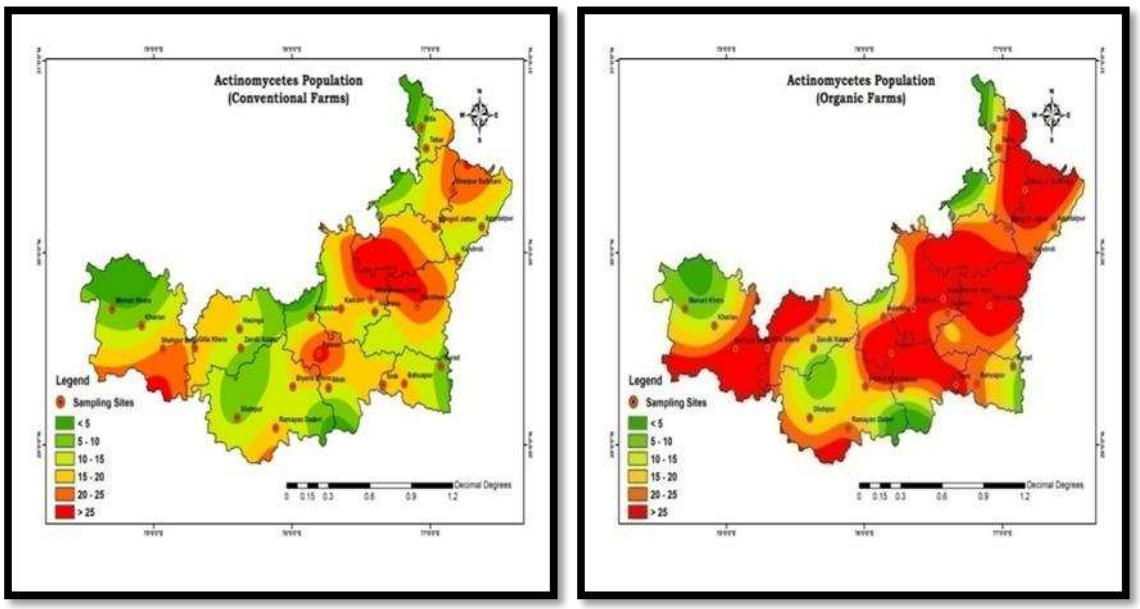


Figure 16. *Spatial variability map of actinomycetes populations in soils under conventional and organic farming systems at different locations of Haryana*

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Conclusion

The biological properties of soils in terms of enzymatic activities, microbial biomass carbon and total CFU of bacterial, fungal and actinomycetes populations were studied for the organic and conventional farming practices and they were found to be superior in the case of organic farming practices as compared to their counterparts. Higher organic matter input through application of various organic manures under organic farming practices is the key factor in enhancing the enzymatic activities and soil microbial biomass carbon along with higher growth of microbial populations in soil. The results from the study indicate that the management practices have clear positive and significant effects on various biological properties of soils. Enzymatic and microbial responses to chemical fertilizers and pesticides under conventional farming indicated the resource limitation and substrate availability to these biological communities in soil. Thus, microbiological properties of soil can serve as potential heath indicator of soils as being affected by different management practices.

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QUANTITATIVE ECOLOGICAL RISK ASSESSMENT OF SHORTFIN MAKO SHARK (*ISURUS OXYRINCHUS*): PROPOSED MODEL AND APPLICATION EXAMPLE

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Abstract. Most of the world's catches of sharks are incidentally taken by tuna fishing gear, constituting bycatches that increase the extinction risk of several species of shark. This not only alters ecosystem functions by removing top predators, but may also hinder the industry production itself due to cutoff measures set by authorities. This paper focuses on the female population abundance of a very important bycatch species, the shortfin mako shark (*Isurus oxyrinchus*), and proposes a stochastic model for quantitative risk assessment under varying harvest regimes and control measures. The proposed model can be applied to any shortfin mako shark population by changing initial conditions and harvest parameters. The flexibility of the model makes it practicable to simulate hundreds of scenarios, analyse and compare the most relevant results such as the risk of extinction caused by a given harvest regime, median time to extinction, expected minimum biomass, risk of low harvest, and risk reduction caused by control measure; these outcomes are useful for rational decisions under uncertainty. We present an application example as a means to demonstrate how the proposed model can be used to drive management decisions for sustainable tuna harvest.
Keywords: *stochastic model, probabilistic systems, sensitivity analysis, monte carlo simulation, ecological modeling*

Introduction

Fishes represent the only major food source still harvested from wild populations (Ryman et al., 1995). In 2014, fish harvested from aquaculture amounted an estimated first-sale value of U$160.2 billion (FAO, 2016). Tunas and tuna-like species are an important food source, used mostly for canning and sashimi, and, because of their high economic value and extensive international trade, are an important global commodity. The total catch of tuna and tuna-like species was about 7.4 million tons, representing 9% of the global catch in 2013. Tuna fishing is undertaken by thousands of vessels in

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the Pacific, Indian and Atlantic oceans, using a range of gear types (e.g., longline) (Clarke, 2008; FAO, 2016; Gilman and Lundin, 2008; Hamilton et al., 2011).

Most of the world's catches of sharks are incidentally taken by various types of tuna fishing gear, constituting bycatch that is either discarded at sea (without fins or dead) or landed for sale (Carvalho et al., 2011). Bycatches increase the risk of extinction of several species of shark and alters ecosystem functions by removing top predators (Myers and Baum, 2007). Moreover, bycatches cause economical risk to the industry because of conservation limits set by the International Commission for the Conservation of Atlantic Tunas (ICCAT), i.e., a cutoff threshold at which fishing should stop, often set at 20% of the unfished equilibrium abundance of relevant species such as the mako shark (Smith et al., 1993). In other words, if mako shark population declines more than 80% of its unfished equilibrium abundance, tunas' harvest must be forbidden. Thus, we focus here on a shark species mainly because there has been increasing concern about the status of some shark stocks and the population-level effects caused by their exploitation (Anon, 2009, 2013; Carvalho et al., 2011; FAO, 1999; Myers and Baum, 2007).

The adult shortfin mako shark (SMA) (*Isurus oxyrinchus*) is an apex predator at the top of the marine environment food web and it is not a prey to any marine animal. These sharks are long lived, slow growing, reach sexual maturity late in their life history, and produce few offspring (Levesque, 2007). They have a common total length of 270 cm, maximum total length of 445 cm (Weigmann, 2016), maximum published weight of 50.,8 kgs and maximum reported age of 32 years (Natanson et al., 2006).

This work focuses on the SMA for several reasons (Anon, 2009, 2013; Cosandey-Godin and Morgan, 2011; Levesque, 2007): (i) its relatively high abundance and presence in multiple and widespread fisheries; (ii) it is captured in great numbers in all oceans and ranks as one of the most dominant species caught in pelagic longlines and gillnets; (iii) it is considered an apex predator at the top of the marine environment food web; (iv) female SMAs are being caught below the size of maturity; (v) unlike most shark species, the SMA is economically important due to both its quality of meat and fins; (vi) it is a valuable product of the pelagic longline swordfish and tuna fisheries; (vii) failures in conservation management of SMA can be significant and costly.

Therefore, we propose a stochastic population model for Quantitative Ecological Risk Assessment (QERA) of SMA bycatch that results from the industrial tuna fishing. Our model is a useful tool for both conservation of SMA and the sustainable management of tuna industry. Moreover, it is capable of describing the dynamics of SMA population under varying conditions (harvest regime and control measures), for evaluating the role of such conditions, and for producing meaningful conclusions that can be used to drive conservation and management decisions. The model is thought to be generic so that it can be applied to any SMA population (e.g., South Atlantic, North Atlantic, North Pacific) by making minor changes in parameters and initial conditions.

We exemplify the application of the model for the SMA South Atlantic population and describe means of evaluating risk of extinction and yield associated with alternative decisions about harvest rates and cutoff threshold. The methodology, used for QERA in both the model building and application example, is similar to the approach used in (Duarte et al., 2013, 2014; Duarte and Droguett, 2015), but here it is specifically tailored for solving the SMA case.

The remainder of the paper is organized as follows. First, we provide a brief literature review. Second, we propose a model structure to describe the dynamics of the

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system in any ocean. Next, by means of an application example, we demonstrate how to conduct a QERA of SMA bycatch implied by tuna fishing based on the proposed model. Finally, we conclude remarks by presenting the model benefits and shortcomings.

Materials and Methods

Literature Review

Even though the SMA is physiologically unique, economically important, and exploited in relatively large numbers (Levesque, 2007), there are few population models for this species (Anon, 2009, 2013). These approaches aimed at diagnosing the population status over the last 30 years by quantifying measures such as past and current biomass, and abundance. Conversely, we aim at describing the population dynamics under varying scenarios over time so that the proposed model can be a useful tool for rational decisions under uncertainty.

There are some applications of stochastic models for risk assessment of marine fish (Duarte et al., 2013; Fu et al., 2004; Hart and Cadrin, 2004; Smedbol and Stephenson, 2004) that have the goal of describing the population dynamics over time and under predefined conditions by using past data for estimating the model parameters and setting the initial conditions. They include uncertainty in parameters, and thus results are given in terms of probability. However, at the best of authors' knowledge, there is no such approach tailored for mako shark populations.

Yet, our modelling approach is similar to the two models of herring (Fu et al., 2004; Smedbol and Stephenson, 2004) in the following aspects: (i) it evaluates the risk of extinction caused by harvest; (ii) includes uncertainty in parameters; (iii) has the flexibility to include a cutoff measure; and (iv) includes low-frequency events that may cause recruitment failure, which occurs when individuals at age 0 (pups) have poor survival due to hypothetical events such as pollution, construction or any human impact in breeding areas on the coast; or environmentally induced recruitment failure such as hurricanes, earthquakes, tsunamis. The specific cause of recruitment failure remains a mystery and there is no research about its effects in SMA, but we assume recruitment failure may also occur with SMA as happens with other marine fishes (Payne et al., 2009; Smedbol and Stephenson, 2004).

In spite of the similarities, our approach departs from the abovementioned works in the following aspects: (i) the proposed model describes a population of a shark species, which is the utmost difference because sharks and herrings have remarkably different life histories; (ii) it models females only; (iii) it measures uncertainty about the future harvest regime through a plausible range of scenarios; and (iv) it evaluates the risk caused by conservative scenarios of harvest regime (Levesque, 2007). Then, in the next section, we present the characteristics of the proposed model.

The Proposed Model

The proposed model only considers females because they are a key indicator to control. In fact, females produce new individuals and keep the life cycle on. Thus, keeping track of females is more effective to evaluate extinction risk. Furthermore, most available data are given only for females, probably because of the same aforementioned reason. Thus, hereafter, "population", if not specified, refers to "female population".

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Then, the purpose of the model is to describe the SMA female abundance over 100 years under varying conditions. Indeed, we consider: (i) a benchmark scenario (Scn-0) that simulates the natural population dynamics under no harvest; (ii) varying harvest scenarios with different harvest parameters and (iii) harvest scenarios with and without a cutoff threshold for which no harvest occurs if abundance falls below it.

Now, let $N_i(t)$ be the abundance of females in stage *i*; N the total abundance of female population; s_i the survival rate (per year) of females in stage *i*; p_i the permanence rate (per year) of females in stage *i*; *f* the fecundity rate (pups per year); a_{ij} the transition rate (per year) from stage *j* to stage *i*, where *i* is the line and *j* the column in the matrix; h_i is the harvest rate for stage *i*; N^{cutoff} is the abundance threshold for cutoff; and F^{rf} is the frequency per year that recruitment failure occurs. Note that $a_{10} = s_0$; $p_1 = s_1 - a_{21}$; $p_2 = s_2 - a_{32}$; and $p_3 = s_3$. Then, the algorithm described below constitutes our proposed generic model for SMA female abundance that can be parameterized to describe the dynamics of the system in any ocean. It represents one replication for simulating the population model and involves the following steps:

i)If $N < K$, then make

$$\begin{bmatrix} N_0(t+1) \\ N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} = \begin{bmatrix} 0 & 0 & f & 0 \\ a_{10} & p_1 & 0 & 0 \\ 0 & a_{21} & p_2 & 0 \\ 0 & 0 & a_{32} & p_3 \end{bmatrix} \begin{bmatrix} N_0(t) \\ N_1(t) \\ N_2(t) \\ N_3(t) \end{bmatrix} \quad \text{(Eq.1)}$$

If $N > N^{cutoff}$, then make

$$\begin{bmatrix} N_0(t+1) \\ N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} = \begin{bmatrix} N_0(t+1) \\ N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} * \begin{bmatrix} 1-h_0 \\ 1-h_1 \\ 1-h_2 \\ 1-h_3 \end{bmatrix} \quad \text{(Eq.2)}$$

ii) Otherwise:

$$\begin{bmatrix} N_0(t+1) \\ N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} = [K] \begin{bmatrix} 0.169 \\ 0.678 \\ 0.086 \\ 0.068 \end{bmatrix} \quad \text{(Eq.3)}$$

iii)Generate random number *U* from a uniform distribution.

If U < F^{rf}, then

$$\begin{bmatrix} N_0(t+1) \\ N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} = \begin{bmatrix} N_0(t+1) \\ N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{bmatrix} * \begin{bmatrix} M^{rf} \\ 1 \\ 1 \\ 1 \end{bmatrix} \quad \text{(Eq.4)}$$

Table 1 defines the model variables, parameters and initial conditions. The estimation of parameters and the setting of initial conditions will be explained in next

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sections. *Eq. 1* is a Lefkovitch matrix (Lefkovitch, 1965), which describes the natural dynamics of the population. We consider a four-stage structure for SMA population: pups, juveniles, mature adults, and post-reproductive adults. *Eq. 2* models the effect of harvest, which was considered in the simulation to reflect stage selectivity by the fishery. Note that harvest strategies do not affect survival rates. Instead, for each scenario, harvest was included by removing a certain proportion of the stage-specific population every year, and a cutoff threshold was included to simulate a control measure in which no harvest occurs if the abundance declines belowthat limit. *Eq. 3* describes the Density Dependence (DD) effect, which represents a change in the influence of any factor that affects population growth as the population density changes (Forbes et al., 2010; Pauwels, 2002). The constants in *Eq. 3* refer to the steady-state population distribution, i.e., the proportion of individuals in each stage (16.9% age 0, 67.8% juveniles, 8.6% mature adults and 6.8% post-reproductive adults) when the population dynamics reach a stationary state, which is based only on the stage matrix for Scn-0 and is a result of eigenvalue analysis. For more details, see references (Akçakaya et al., 1997; Akçakaya and Root, 2013; Burgman et al., 1993). Finally, *Eq. 4* allows to include known and/or suspected variations in vital rates (survival and fecundity) caused by low-frequency, high-consequence events such as recruitment failure. This is modelled via Bernoulli trials (Burgman et al., 2012).

Note that, although the proposed model structure is thought to be generic, it can be tailored to incorporate many realistic and case-specific features such as: (i) a stage-structured SMA population (pups, juveniles, mature adults, and post-reproductive adults); (ii) stage-specific initial abundance; (iii) uncertainty in parameters such as survival and fecundity; (iv) Density Denpendency; (v) low-frequency events that may cause recruitment failure; (vi) stage-specific annual harvest as a proportion of the stage-specific abundance; and (vii) cutoff threshold for which no harvest occurs if abundance is below it.

The four stages (pups, juveniles, mature adults, and post-reproductive adults) considered for female individuals in the population are structured based on characteristics such as weight, survival, fecundity and harvest rate. A summary of the female SMA life history in our model is as follows (*Fig. 1*) (Levesque, 2007). Pups (stage 0) are born at age 0. Those who survive until age 1 become juveniles (stage 1). The juveniles, who survive until the age of 19-21, reach maturity and become mature adults (stage 2). Mature adults who survive produce new pups until the age of 25-27 years, when they reach sexual mortality and become post-reproductive adults (stage 3). Post-reproductive adults naturally die at an average age of 32 years. By doing so, we can project the stage-specific population abundance using a Lefkovitch matrix (also known as Stage matrix) (Lefkovitch, 1965).

The model also considers DD. Authors of fish case studies (Fu et al., 2004; Smedbol and Stephenson, 2004) had reported difficulty for estimating the DD parameters. Most assumed Ceiling-type DD (Akçakaya et al., 1999), according to which, on one hand, vital rates are not affected until the population reaches the carrying capacity K that is the level of abundance above which population tends to decline. On the other hand, other types of DD increase vital rates at low density. Thus, Ceiling-type DD is more conservative to assess extinction because production of new individuals is likely to be underestimated at low density and, hence, the risk of extinction will be overestimated (Burgman et al., 1993).

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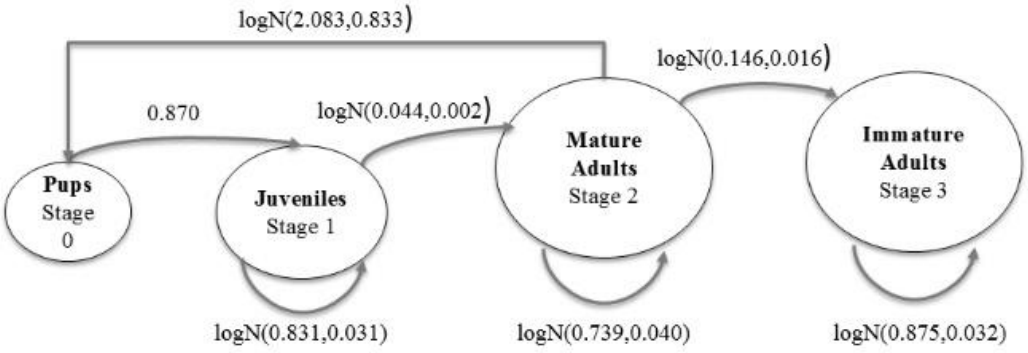


Figure 1. *Female SMA life cycle. The values above the arrows are estimates for transition and permanence rates*

Then, our model also assumes Ceiling-type DD. If the population significantly grows so that it reaches *K*, then it remains at that level until the population decreases below this level. In this case, *K* acts as a population ceiling (Akçakaya et al., 1999). These conditions are modeled in the first and second steps of the algorithm.

The proposed model also includes uncertainty. The effect of parameter uncertainty on outcomes can be bounded by fitting a Probability Distribution Function (PDF) (e.g., Normal or Lognormal) to each parameter chosen from their average, lower and upper values. For a given scenario, one should define initial conditions and fit a PDF to each parameter using data, literature information, and/or expert opinion. After that, a chosen number of replications of the model (say 10,000) can be simulated via Monte Carlo approach (Kalos and Whitlock, 2008). For a given scenario, the result is a dataset of abundances, $N_i(t)$, for each stage *i* and for each time-step *t*. From such dataset, the following summarized outputs can be provided:

- PDFs for the proportion of abundance decline within 100 years.
- Cumulative Distribution Functions (CDFs) for the time to quasi-extinction (i.e., 80% population decline) within 100 years.
- point estimates originated from either the PDFs or CDFs, i.e.:
    - year-specific risk of quasi-extinction (i.e., probability of 80% abundance decline).
    - median time to quasi-extinction.
    - expected minimum abundance (i.e., an estimate of the smallest population size that is expected to occur within 100 years).
    - expected total harvest weight at the end of simulation.
    - year-specific risk of low harvest (i.e., the probability that the annual harvest will be at or below a threshold measure).

For the same population, one can conduct a sensitivity analysis of harvest scenarios and control measures by varying parameters such as h_i and N^{cutoff}, while keeping all other parameters the same. For example, by keeping all other parameters the same as in Scn-0 and varying parameters related to harvest, we aim at evaluating the added/reduced risk caused by each harvest scenario. Likewise, by keeping all other parameters the same and varying the cutoff threshold, we aim to assess the reduced risk caused by this limit that is, in fact, a control measure to satisfy conservation objectives.

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Thus, the proposed model provides the following outputs: risk categories and ranking of scenarios for better risk communication to stakeholders; the quantified reduced risk caused by varying control measures; suggest harvest regime that not only reduces conservation concerns, but also achieves maximal benefits by increasing yield over the long term at acceptable risk of low harvest.

Application Example

We conduct an application example and parameterize the proposed model to the South Atlantic Ocean. The next subsections are useful as a guide on how to apply the model and interpret results. The outcomes are useful to validate the model, yet they should not be used to drive real political decisions with regard to conservation of SMA since data are still uncertain. However, in the future, with more accurate SMA catch data available, a new round of scenarios can be simulated and the outcomes may be used for this purpose.

Materials and Data Sources

Data used here are gathered from several public sources. Estimates of parameters related to the harvest distribution of stages and sex were obtained from onboard observer's data provided under request by the Brazilian Ministry of Fisheries and Aquaculture. An onboard observer after each set filled out the logbooks. Data included individual records of 33 vessels in the South Atlantic that registered 241,776 SMAs catches between December/2004 and February/2009. Useful information to this work also considered: the vessel identification, onboard observer identification, date, location of fishing ground (latitude and longitude), effort (number of hooks), fork length (cm) and sex (male, female or not available).

To transform data given in terms of fork length into weight or age and the inverse, we use the length-weight correlation (Kohler and Casey, 1995) (*Eq. 5*) and the 3-parameter Gompertz growth function for SMA, (Natanson, 2002) (*Eq. 6*), which produced the most biologically reasonable estimates for females.

$$WT = 5.2432 \times 10^{-6} \times (L)^{3.1407} \quad \text{(Eq.5)}$$

where: WT = Total weight; L = Fork length (i.e., the length of a fish measured from the tip of the snout to the end of the middle caudal fin rays and is used in fishes in which it is difficult to inform where the vertebral column ends (Froese and Pauly, 2011)).

$$L(t) = L_0 e^{G(1-e^{(-kt)})} \text{ and } G = \ln {}^{L_\infty}/_{L_0} \quad \text{(Eq.6)}$$

where: t is the age (years); L_0 = 88 cm; $L_\infty = 366$ cm; and k = 0.087 $year^{-1}$.

From the female total catches, only 7,359 had their fork length measured so that we transform fork length into age, then into stage, and calculate (respectively for age 0, juvenile, mature adult and post-reproductive adult) the annual catch percentage for each stage i, AC_i, as 5.95%, 92.21%, 0.38% and 1.46% and the average weight in kg of each stage as 6.7, 113.9, 329.5, 417.2. Thus, stage-specific harvest rates are estimated for a specific scenario as follows:

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$$h_i = \frac{(population\ harvest\ rate\ per\ year\ for\ the\ specific\ scenario) \times \sum_{\forall i} N_i(0) \times AC_i}{N_i(0)} \quad \text{(Eq.7)}$$

Bycatch of SMAs results in substantial number of SMAs being discarded dead. Quantifying total harvest from bycatch is challenging because comprehensive data on these discards are unavailable (Cosandey-Godin and Morgan, 2011). Then, we assume that mortality of SMAs is greater than catch estimates (Anon, 2013) for two reasons. Indeed, estimates may not include uncertainty about (i) post-release mortality (Cosandey-Godin and Morgan, 2011; Gilman et al., 2008), and (ii) the catches of sharks that were illegally discarded at sea to make space for tunas in the freezers.

Estimates of initial abundance and carrying capacity were obtained from a stock assessment conducted by ICCAT (Anon, 2013) and estimates of parameters related to the ecology and population dynamics (i.e., natural mortality, age and growth, reproduction, stage-specific life span, age at first maturity, age-weight) were gathered from literature. *Table 1* describes the model parameters and their source of information. For the cases, where there is more than one source for the same parameter, we assume the one with widest confidence interval in order to be more conservative. Note that for the Carrying Capacity parameter, to extrapolate data that do not differentiate sex (Anon, 2013) into females only data, we consider the common male:female ratio in the population that is equal to 1 (Garcia-Cortes and Mejuto, 2002).

We adopted software RAMAS Metapop v.6.0 (Akçakaya and Root, 2013). This software is a computational tool for population model construction and probabilistic simulation via Monte Carlo methods (Kalos and Whitlock, 2008).

Parameters, Uncertainty and Error

Table 1 presents the parameters ω governing the dynamics of the system. They were given either as a mean value $\bar{\omega}$ or as a triplet $(\omega_L, \bar{\omega}, \omega_U)$, which represents lower, mean and upper values. For the latter case, we consider the parameters have a truncated normal distribution and that the error $(\max\{\bar{\omega} - \omega_L, \omega_U - \bar{\omega}, 0.5(\omega_U - \omega_L)\})$ corresponds to a 3σ interval; thus, such parameters will be randomly selected to fall between their limits in 99.865% of replications.

It is believed that one can make good use of a Gaussian approach in vital rates because there is a reasonable reason for random values not to be too far away from the average, i.e., there are biological limitations preventing very large deviations and natural forces of equilibrium bringing vital rates back to their average values (Taleb, 2007). For probabilistic simulation, the RAMAS software converts the normal distribution of vital rates into a corresponding lognormal distribution. This conversion avoids bias resulting from truncation because $\omega \geq 0$ (Burgman et al., 1993; Ferson and Akçakaya, 1990).

We assume that the population is exposed each year to a 10% probability of recruitment failure (i.e., $F^{rf} = 0.1/year$ - *Eq. 4*) that decreases stage 0 abundance to 5% of the value expected if such event had not occurred. Carrying capacity (K) was estimated (*Table 1*) based on the population biomass provided by the stock assessment as 956,777 mt (from Scenario-6 in reference (Anon, 2013)) under virgin conditions, i.e., the current population abundance if there was no fishing. Most simulation scenarios included annual harvest that prevented abundance from reaching the ceiling defined by K.

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Table 1. Definition of variables and parameters. The discrete time unit is 1 year

Variable, Parameter or Initial condition	Symbol	Description and assumptions	Mean	Max and/or Min	SD
Number of female SMA at time *t*	$N(t)$	Assessment endpoint. Population abundance at time *t*. The sum of the number of females in all stages.			
Number of female SMA in stage *i* at time *t*	$N_i(t)$	Abundance of female SMA at stage *i*.			
Transition rate from stage 0 to stage 1 (per year)	a_{10}	Survival of age 0 class (0.87) (Anon, 2013).	0.87		
Permanence rate in stage 1 (per year)	p_1	Annual survival rate for age 1+ (i.e., 1 year or more) is in the range of 0.78-0.97 (Anon, 2013), thus we assume mean = 0.875. Median (50%) age at maturity for females is in the range of 19-21 years (Bishop et al., 2006), thus we assume mean = 20 years.	= 0.875*(20-1)/20 = 0.831	Max = 0.97*(21 - 1)/21 = 0.924; Min = 0.78*(19-1)/19 = 0.739	= (0.924 – 0.739)/6 = 0.031
Transition rate from stage 1 to stage 2 (per year)	a_{21}	Annual survival rate for age 1+ is in the range of 0.78-0.97 (Anon, 2013), thus we assume mean = 0.785. Median (50%) age at maturity for females is in the range of 19-21 years (Bishop et al., 2006), thus we assume mean = 20.	= 0.875/20 = 0.044	Max = 0.97/19 = 0.051	= (0.051 – 0.044)/3 = 0.002
Permanence rate in stage 2 (per year)	p_2	Annual survival rate for age 1+ is in the range of 0.78-0.97 (Anon, 2013), thus we assume mean = 0.875. Age that female SMA reach sexual mortality is within the range of 25-27 years (Stevens, 1983), thus we assume mean = 26. Average duration in stage 2 = 26 – 20 = 6 years. Max duration in stage 2 = 27 – 19 = 8 years.	= 0.875*(6 – 1)/6 = 0.729	Max = 0.97*(8 – 1)/8 = 0.849;	= (0.849 – 0.729)/3 = 0.04

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Variable, Parameter or Initial condition	Symbol	Description and assumptions	Mean	Max and/or Min	SD
Fecundity rate from stage 2 to 0 (per year)	f	Litter size ranges from 4 to 27.5 and mean litter size is 12.5 (Mollet et al., 2000). Reproductive periodicity (3 years) (Anon, 2013). Probability of producing a female pup = 0.5.	= (12.5/3)*0.5 = 2.083	Max = (27.5/3)*0.5 = 4.583	= (4.583 – 2.083)/3 = 0.833
Transition rate from stage 2 to stage 3 (per year)	a_{32}	Annual survival rate for age 1+ is in the range of 0.78-0.97 (Anon, 2013). Age that female SMA reaches sexual mortality is within the range of 25-27 years (Stevens, 1983). Average duration in stage 2 = 26 – 20 = 6 years. Max duration in stage 2 = 27 – 19.	= 0.875/6 = 0.146	Min = 0.78/8 = 0.098	= (0.146 - 0.098)/3 = 0.016
Permanence rate in stage 3 (per year)	p_3	Annual survival rate for age 1+ is in the range of 0.78-0.97, mean = 0.875 (Anon, 2013).	0.875	Max = 0.97	= (0.970 - 0.875)/3 = 0.032
Carrying Capacity	K	The SMA biomass under virgin conditions (956,777,000 kg) (from S6, Table 19, Anon (2013)) Sex ratio in the population (1 female: 1 male) (Garcia-Cortes and Mejuto, 2002) Female SMA mean weight (205.031 kg) (from length-weight correlation and growth function in Materials and Data Sources section).	= (956,777,000/205.031)*0.5 = 2,333,248		
Population cutoff/quasi-extinction threshold	N^{cutoff}	Unfished equilibrium abundance (2,333,248) (Anon, 2013). Cutoff threshold is often set at 20% of unfished equilibrium abundance (Smith et al., 1993).	= 2,333,248*0.2 = 466,650		
Frequency (per year) of recruitment failure	F^{rf}	Based on population models of other marine fishes (Payne et al., 2009; Smedbol and Stephenson, 2004).	0.1		

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Variable, Parameter or Initial condition	Symbol	Description and assumptions	Mean	Max and/or Min	SD
Stage 0 abundance multiplier if recruitment failure occurs	M^{rf}	Based on population models of other marine fishes (Payne et al., 2009; Smedbol and Stephenson, 2004).	0.05		
Initial abundance of female pups	$N_0(0)$	Refers to Initial Conditions section	381,279		
Initial abundance of female juveniles	$N_1(0)$	Refers to Initial Conditions section	1,431,337		
Initial abundance of female mature adults	$N_2(0)$	Refers to Initial Conditions section	194,529		
Initial abundance of female post-reproductive adults	$N_3(0)$	Refers to Initial Conditions section	151,271		
A harvest weight threshold for which harvest is considered low if below this threshold	H_{low}	Estimated catches of SMAs in 2010 (2,496 mt) (Anon, 2013). Sex selectivity by the fishery (1 female: 1 male) (onboard observers data). Proportion of minimum harvest weight in 2010 to consider that harvest is low (20%) (from expert opinion).	250 mt		

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Initial Conditions

First, we estimate the initial population abundance from the stock assessment by ICCAT (Anon, 2013), which simulated 13 scenarios with varying parameters and provided the SMA biomass in 2010 as result of each simulation. We consider the effect of uncertainty in initial biomass by using the result of the scenario with the lowest value, i.e., 885,085 mt from Scenario-6, Table 19 (Anon, 2013). Next, we assume the initial stage distribution is equal to the steady stage distribution in *Eq. 3*; this results in the initial conditions in *Table 1*.

Harvest Scenarios

The real number of SMA catches is uncertain. The landing data only show known catches, but the unknown catches (e.g., illegal discards at sea) may be even greater than the known ones. As suggested by (Taylor et al., 2002), the effect of parameter uncertainty on outcomes can be considered by consolidating scenarios with parameters chosen from the range of plausibility. We interviewed an onboard observer and data monitor that worked in a tuna longline vessel that had capacity to stock 200 tons of fish meat. From that discussion, we deliberated a range of playsibility for real catches, i.e., we assumed real catches are not smaller than Catch Estimates (CE) in 2010 and not greater than thirty-two times CE. We simulated hundreds of harvest scenarios by varying catch paremeters whitin this range of plausibility. For the sake of illustration, *Table 2* only presents the parameters of six scenarios that we thought are the most relevant to present in this work. We selected these scenarios for presentations purpose for the following reasons: Scn-0 is a base scenario that reflects natural population growth without any bycatch; Scn-CE is one extreme in the range of plausability which reflects catch estimates without unknown bycatches; Scn-4 is another extreme that reflects total catches (known and unknown) being thirty-two times greater than CE; and Scn-1. Scn-2, Scn-3 are well spaced intermediate scenarios withint the range of plausibility in which total catches are, respectively, four, seven and twenty-one times greater than CE.

Table 2. *Harvest parameters*

Harvest scenario	**Scn-0**	**Scn-CE**	**Scn-1**	**Scn-2**	**Scn-3**	**Scn-4**
Total harvest per year	0	0.29%	1%	2%	6%	9%
h_0	0	0.10%	0.34%	0.67%	2.02%	3.03%
h_1	0	0.40%	1.39%	2.87%	8.34%	12.51%
h_2	0	0.01%	0.04%	0.08%	0.25%	0.38%
h_3	0	0.06%	0.21%	0.41%	1.24%	1.87%
Number of female catches at first year	0	6,259	21,584	43,168	129,505	194,257
Number of times that simulated catches are greater than catch estimates (Anon, 2013)	0	1	4	7	21	32

By simulating Scn-0, we aim at evaluating the natural population dynamics, quantify its background risk and the added risk caused by all relevant scenarios when compared to it. By simulating Scn-CE, Scn-1, Scn-2, Scn-3 and Scn-4 we aim at propagating the

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effect of uncertainty about real catches. We also simulate all harvest scenarios with and without cutoff in order to evaluate the maximum reduced risk caused by this management threshold that is, in fact, a control measure to comply conservation objectives.

Risk Categories

By categorizing risks, results can be better interpreted by risk managers, society, and other interested parties. We use the risk categories proposed by (Duarte et al., 2013, 2014; Duarte and Droguett, 2015). It is important to note that the following categories are more conservative than the International Union for Conservation of Nature (IUCN) categories (IUCN, 2001). The latter are used to classify species affected by a whole range of environmental changes and human disturbance at regional (IUCN, 2003) or global-levels, whereas the former is proposed to classify risks caused by a single stressor (harvest) to a single population, which is our case.

- CRITICALLY ENDANGERED (CR): more than 50% additional probability of quasi-extinction within 3 generations (i.e., median time to quasi-extinction is shorter than 3 generations).
- ENDANGERED (EN): more than 20% additional probability of quasi-extinction within 5 generations.
- VULNERABLE (VU): more than 10% additional probability of quasi-extinction within 5 generations.
- NEGLIGIBLE (NE): less than 10% additional probability of quasi-extinction within 5 generations.

We assume that female SMAs have a generation time of 20 years (Froese and Pauly, 2011) and harvest scenarios that cause NE risks are considered sustainable.

Results and Discussion

Fig. 2 illustrates how female SMA average abundance in the South Atlantic varies over time (100 years) for several harvest scenarios (Scn-0, Scn-CE, Scn-1, Scn-2, Scn-3 and Scn-4, respectively, 0%, 0.29%, 1%, 2%, 6% and 9% of the population removed every year). The Scn-0 curve represents the population dynamics if no fishing occurs. Scn-0 curve is superimposed on Scn-CE, which shows the abundance trajectory if real catches were as described by landing data. The abundance reaches a dynamic equilibrium at the carrying capacity, which is around 2,33E+6.

Note that population growth rate when unfished (Scn-0) is almost nil and the same happens to Scn-CE. Obviously, the population abundance for Scn-CE is overestimated because landing data do not consider unkown bycatches discarded at sea. Catch estimates from landing data is so optimistic that, for practical purposes, the population dynamics under these catch estimates (Scn-CE) is the same as under no fishing (Scn-0). Nonetheless, Scn-CE is a positive scenario that is presented to highlight model accuracy.

Because of the great uncertainty about real catches, we also consider Scn-3 or Scn-4 (6% or 9% annual harvest, respectively), which show overfishing situations in which the population rapidly declines to, respectively, 1,260,764 (258,496 mt) and 519,322 (106,477 mt) females. Scn-3 causes an additional 10.6% risk of quasi-extinction within 5 generations; then, it is classified as VU, whereas Scn-4 causes 56.2% probability of

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quasi-extinction within 3 generations; thus, it is categorized as CR. These two scenarios simulate more conservative situations, but, given the great importance of SMA to the ecosystem, it is worth dealing with uncertainty in this way. In the case of more accurate SMA catch data being available in the future, interpretation of results may change.

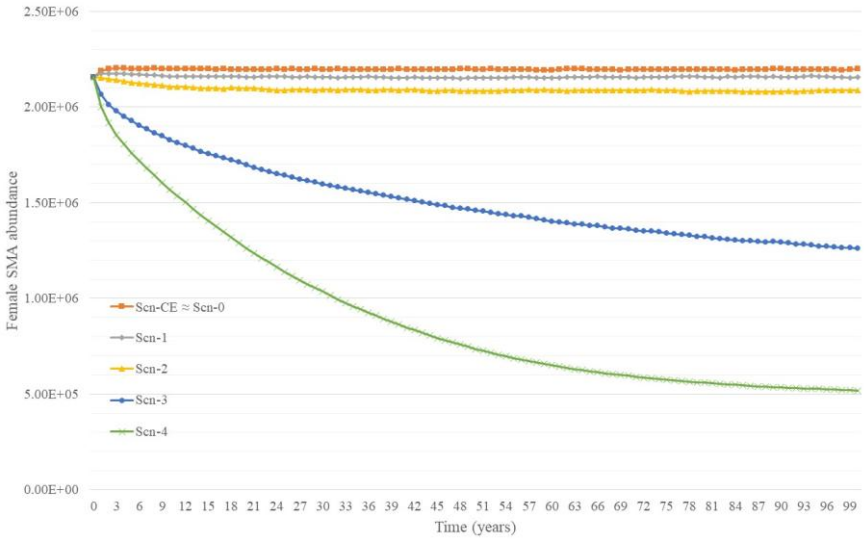


Figure 2. *Female SMA average abundance for relevant scenarios.*

Table 3 summarizes the main results for each harvest scenario. Values with a + or – symbol mean that they are being compared to the benchmark Scn-0, except for the maximum effect of cutoff measure, which is compared against the same scenario without cutoff. The risk of harvest scenarios is indicated both as additional risk of quasi-extinction compared to Scn-0 and as reduced expected minimum abundance also related to Scn-0. The median time to quasi-extinction is calculated from the CDF for the time that the population size will fall below the quasi-extinction threshold. Only Scn-4 had the median of its distribution shorter than 100 years, i.e., 50% probability of quasi-extinction within 56 years. All other scenarios had less than 50% probability of quasi-extinction within 100 years.

Table 2. *Risk results*

Harvest scenario	Scn-0	Scn-CE	Scn-1	Scn-2	Scn-3	Scn-4
Risk of quasi-extinction	0	0	0	0	+0.106	+0.878
Risk category	NE	NE	NE	NE	VU	CR
Median time (years) to quasi-extinction	>100	>100	>100	>100	>100	56
Expected minimum biomass (mt)	332,785	327,778	316,081	297,773	170,477	80,731
Maximum effect of cutoff measure	Not applicable	Not significant	Not significant	Not significant	-0.057 risk of 83% population decline	-0.721 risk of 87% population decline
Expected yield (total harvest weight (mt))	Not applicable	73,660	241,554	469,821	1,000,000	886,045
Risk of low harvest (≤ 250 mt)	Not applicable	0	0	0	0.132	0.887

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The Effect of the Cutoff Threshold Measure

We quantify the maximum effect caused by the cutoff management threshold. The maximum effect of cutoff is measured as the maximum reduced risk for a scenario with and without cutoff. The reported number is the Kolmogorov-Smirnov test statistic D, which is the maximum vertical difference between risk curves, based on a two sample Kolmogorov-Smirnov test (Akçakaya and Root, 2013). The approximate location (point X at which maximum reduced risk occurs, i.e., percentage of decline) is also given.

Thus, we simulate all harvest scenarios with and without cutoff, compare their risk of population decline and measure the maximum reduced risk and the point at which it occurs (*Table 3*). For Scn-4, the cutoff measure reduces 72% the risk of 87% population decline, whereas for Scn-3, it reduces 5.7% the risk of 83% population decline. For all other scenarios, risk reduction is insignificant.

Harvest Results

The yield of SMA is not only important as a secondary product, but also as an indicator for yield of tuna, since SMA are bycatched by various types of tuna and tuna-like fishing gear. Thus, changes in SMA harvest means proportional changes in tuna harvest. The number of SMA catches as a proportion of tuna catches depends on several specific factors such as the vessel fishing gear and fishing grounds. Thus, the vessel interested in how the presented SMA harvest results impact their yield of tuna should make their own estimates.

Fig. 3 shows, for each harvest scenario, the expected total weight of harvest as a function of time. The harvest is calculated based on the average weight of each stage (see Materials and Data Sources section). Scn-0 is not applicable here since it is not a yielding scenario. Total harvest for 100 years of simulation and risk of low harvest are presented in *Table 3*.

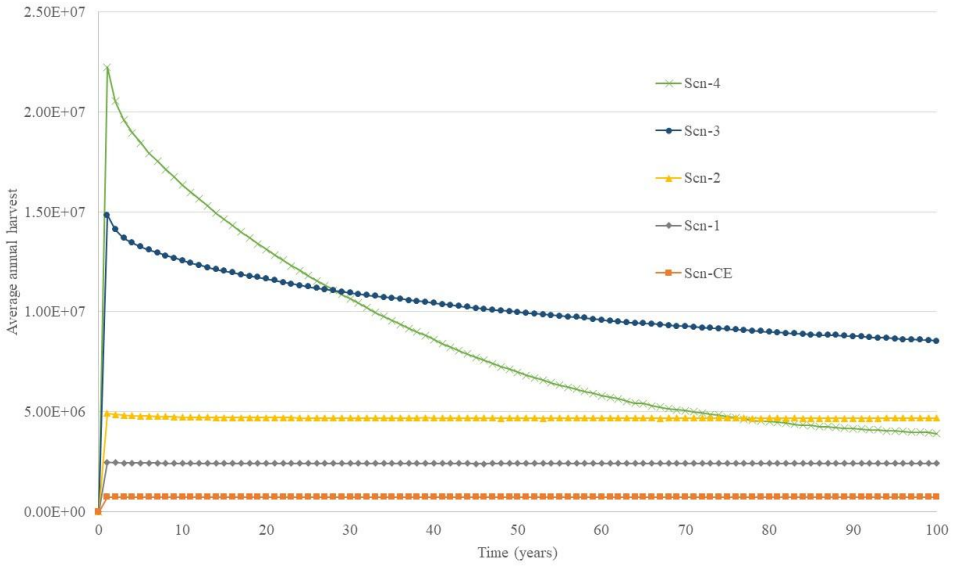


Figure 3. *Average weight of female SMA harvest as a function of time for each harvest scenario (Scn-CE, Scn-1, Scn-2, Scn-3 and Scn-4)*

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It is worth focusing on Scn-3 or Scn-4 to deal with uncertainty because of inaccurate SMA catch data. These scenarios are not good for both conservation and production because: (i) they are not sustainable, i.e., Scn-3 is categorized as VE and Scn-4 as CR; (ii) although Scn-3 maximizes yield, it causes a considerable risk of low harvest (13%); and (iii) Scn-4 does not provide maximum yield and risk of low harvest is significantly high (89%).

Conclusions

This paper dealt with a very important bycatch species of worldwide tuna fisheries. Its capture is of major conservation concern, thus this subject is of great interest and relevance for pelagic fisheries and their management. Yet, the methodology to approach the problem is innovative because it is capable of: (i) quantifying the added risk of extinction caused by harvest scenarios and the reduced risk caused by cutoff measures; (ii) dealing with uncertainty; (iii) including low frequency events; and (iv) quantifying risk of low harvest.

The proposed model can be applied to any SMA population by changing initial conditions and harvest parameters. We conducted QERA for the SMA South Atlantic population in order to exemplify the application of the model and consistent results were obtained.

The model is most useful for risk managers as an aid for rational decisions about harvest regimes under uncertainty. The flexibility of the model made it practicable to simulate and evaluate hundreds of scenarios and analyze their results. The risk results of relevant scenarios were transformed into risk categories for easier communication to stakeholders. The model also allowed for update of parameters as new data become available. Companies interested in sustainable production can thus register data of SMA bycatch and use them as an input for the model.

For the application example, great uncertainty on input data also caused great uncertainty in results. Without accurate SMA catch data, which is caused for example by unknown illegal discards, uncertainty about the real scenario is great. Consequently, risk results for SMA quasi-extinction varies from categories NE to CR.In this sense, we recommend future studies that can reduce data uncertanty, e.g.: improving the current methods for data monitoring onboard; new data gathering methods other than the traditional onboard observer (e.g., fin trade data); and new statistical approaches for catch estimates (e.g., bayesian statistics). In this way, our proposed model can be used to simulate less uncertain scenarios in order to aid decision makers. For now, the application example described in this article only works for the sake of illustration.

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