IMPACTS OF CLIMATE CHANGE ON NET PRIMARY PRODUCTION: A MODELLING STUDY AT PAN-EUROPEAN SCALE

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Abstract. Identification of the net primary production capacity of European vegetated areas has been becoming the meaning since last decades. Responses of carbon uptake by autotrophs and storage in terrestrial ecosystems under environmental changes is quite important to understand and predict the biogeochemical cycles, and thus the interactions between atmosphere and terrestrial biosphere in the future. Remote sensing of the Earth systems has been having very important roles for calibration of the modelling results during last 20 years. In this paper, we simulate the impacts of the climate change and elevated CO_2 in the atmosphere on net primary production by autotrophs by using Community Land Model vers. 4.5 (CLM4.5) with remarkable high grid resolution (i.e. 25x25 km) at pan-European scale. We especially focused on the time period in the future when the global warming reaches the 2ºC (i.e. 2034-2063) in Europe. The CLM4.5 model performs quite good in Western and Southern Europe. Although the model predicts the NPP ca. 2 times higher than the remote sensed NPP by MODIS, the analysis between in-situ data and CLM4.5 shows better correlation than between in-situ data and remote sensed NPP in 19 study areas. Despite of the higher correlation of the model with in-situ data, it is still needed long-term observation studies needed from different biome types and plant functional types.
Keywords: *biogeochemical cycle, COP21, CLM4.5, MODIS, remote sensing*

Introduction

Net Primary Production (NPP) is one of the most important keywords for investigation of the climate change effect on carbon uptake and storage by photosynthetic organisms. Numerous studies focused on determining the climate change impacts on NPP in the terrestrial ecosystems (Melillo et al., 1993; Cao and Woodward, 1998; Bonan, 2008; Ummenhofer et al., 2015). Plant productivity is a very important aspect in the global biogeochemical cycles especially in global carbon cycle due to the absorption of a part of anthropogenic emitted CO_2 from the atmosphere (Esser et al., 2012). NPP is also a quite essential parameter for all ecosystems, since it can illustrate the quality and quantity of absorbing the incoming solar energy, and also indicates the fundamental energy source for all heterotroph organisms in the ecosystems. The potential change in the primary production in the terrestrial and marine ecosystems under global 2 ºC average temperature increase has been discussing since the last decades (Chust et al., 2014; Guanter et al., 2014; Danelichen et al., 2015). Among others, Kirschbaum (2000) studied the responses of vegetation growth, photosynthesis, and respiration to the change climate from pre-industrial time (i.e. 1900) up to 2100. In his study, it has been mentioned that a 2 ºC increase in global average temperature affects the physiological and biological processes of various plant species that especially distribute in a narrow temperature niches. On the other hand, a 20% of yield increase in crop plants was reported under global average temperature increase up to 2 ºC within the fourth assessment report of the Intergovernmental Panel on Climate

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Change (IPCC) (Easterling et al., 2007). In the assessment report, it was also pointed to a decline in crop yield with increasing temperature after 2 ºC increasing period. Wan et al. (2005) quantified a similar impact of temperature increase in semi-natural vegetation (i.e. grassland). In their field study, they monitored about 19% increase in above ground net primary production under 2 ºC temperature warming. Certainly, not only the temperature but also precipitation, solar energy, humidity and wind speed have significant impacts on the NPP (Wan et al., 2005). In a Chihuahuan desert grassland, Thomey et al. (2011) studied the effect of precipitation on net primary production. They quantified a substantive increase in NPP due to an increase in precipitation in the study areas. Approximately 1.3% of incoming solar energy is absorbed by plants during the growing season. A substantial accumulation of net biomass takes in some vegetated regions from decades to centuries, which indicates that such vegetated regions actually points to net sink of carbon (Dixon et al., 1994). For instance, temperate and boreal forests are the main green areas for sink of carbon in pan-European region. Kauppi et al. (1992) referenced to a ~0.12 Pg C estimated annual carbon fluxes in that forests.

Estimation and measurement of NPP are carried out by various methods in the terrestrial ecosystem (Lieth, 1975; Esser, 1998; Zhao et al., 2005). For such aims, enhanced remote sensing of NPP has been widely using to study, quantify, and understand the carbon uptake and storage capacity of the terrestrial ecosystems since last decades (Liu et al., 1997; Turner et al., 2004; Maselli et al., 2013; Pachavo and Murwira, 2014; Wang, 2016). Although remote sensing performs good results for NPP at global or regional scale, the satellite or radars have some technical difficulties in estimating of NPP under cloudy or snowy days. That leads often to under- or overestimation of NPP in the terrestrial biosphere (Zhao et al., 2005, 2006, 2010; Pan et al., 2006; Turner et al., 2006). Compared to the remote sensing methods, earth system models can deliver often quite good results for NPP in terrestrial biosphere (Prieto-Blanco et al., 2009; Donmez et al., 2011).

In this paper, we aimed to define the carbon storage capacity in high grid resolution at pan-European scale, and investigate the behaviour of carbon sink areas under climate change. Those produce new aspects to estimate of net carbon storage in European terrestrial biosphere. This is an important issue to find out how will the carbon storage capacity of terrestrial ecosystem be affected by changing the combination of the relevant climate parameter in the 2 ºC global warming period in the future. It also gives data about the carbon storage capacity of European vegetation in the future when the global average temperature increases up to 2 ºC.

Also the main objectives of the study were: (*i*) to describe the spatio-temporal heterogeneity in NPP by using remote sensed and modelled data, (*ii*) to quantify the difference between the remote sensed and modelled NPP, (*iii*) to identify the relationship between the climate conditions and modelled NPP, (*iv*) to indicate the change in NPP during the 2 ºC global average temperature increase period.

Material and Methods

Model Initialization

For estimating of net primary production, the Community Land Model version 4.5 (CLM4.5) was established on 25x25 km grid resolution at pan-European scale. The model was run with bias corrected climate data for 800 years in ad-hoc (accelerated method) mode to get the main carbon pools of the terrestrial biosphere (e.g. soil

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carbon, vegetation carbon, total ecosystem carbon etc.) in steady state. The accelerated method of the model, which based on the acceleration of decomposition rates for a spin-up phase in CLM4.5, describes the steady state process of the model. By this method, the main aim is an approximation of steady state conditions for the CLM4.5 model by using specific characteristics of the model dynamics for producing individual time processes via the model condition space, and methods of multivariate minimization that repeatedly investigate multiple time processes by condition space in searching of reasonable equilibrium solutions. The detailed description of the algorithms for the ad-hoc method and steady state runs (spin-up) was published by Thornton and Rosenbloom (2005).

We first run the model with the ad-hoc method for 800 years to get the carbon pools in the ecosystems in equilibrium. Within this run, we used stable climate conditions that were taken from the ensemble average of 30-year historical run of the used regional climate model in monthly resolution. After reaching the carbon pools of the ecosystems (i.e. carbon pools in soil and vegetation) the steady state we switched of the ad-hoc method and run the model with the normal decomposition rates but the same climate data up to 1950. Thereafter, we forced the model with bias corrected monthly climate data from 1950 to 2100. For the future period (i.e. from 2004 to 2100), the bias corrected climate data were simulated by used regional climate model under consideration the Representative Concentration Pathway 4.5 (RCP4.5) (see Sec. 2.2).

Atmospheric Forcing Data

In this study, we used six climate parameters (see *Tab. 1*) from outputs of the Rossby Centre Regional Atmospheric Model (SMHI-RCA4), which was driven by EC-EARTH General Circulation Model (GCM), to force the CLM4.5 for the study periods. The RCM model was used to downscale transient global climate projections, i.e. EC-EARTH's outputs as boundary conditions, over Europe at a 25 km spatial resolution (Jacob et al., 2014; Strandberg et al., 2014). For this aim, the RCM considered the Representative Concentration Pathway 4.5 (RCP4.5) for prediction the climate parameter from 2004 to 2100.

Table 1. *The used climate variable for atmospheric forcing of CLM4.5 model*

Code	**Variable name as daily mean value (unit)**
tas	Surface temperature at 2 m (*°C*)
pr	Sum of precipitation (*mm*)
rlds	Surface downwelling longwave radiation ($\frac{W}{m^2}$)
rsds	Surface downwelling shortwave radiation ($\frac{W}{m2}$)
huss	Near surface specific humidity ($\frac{kg}{kg}$)
sfcWind	Near surface wind speed ($\frac{m}{sn}$)

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Model Description

After spinning up of the model, we run it with required climate data from 1970 to 2100. According to the simulations which show the start of 2ºC global average temperature increase in ca. 2030 and the end in ca. 2060, we take two 30 year periods (1970-2000 and 2030-2060) for investigation the change of the NPP in past observed period (1970-2000) and future projected period (2030-2060). The version of the model uses the 17 plant functional types (PFTs) from the study of Lawrence & Chase (2007).

The NPP is formulated in the CLM4.5 as

$$NPP = GPP - (M_R + G_R) \qquad \text{(Eq.1)}$$

where M_R is for maintenance and G_R is for growth respiration. Maintenance growth respiration is mainly calculated by sum of carbon fluxes in leaf, fine root, live steam and live root (see Eq. 1).

$$M_R = CF_{leaf} + CF_{froot} + CF_{livestem} + CF_{livecroot} \qquad \text{(Eq.2)}$$

where CF_{leaf}, CF_{froot}, $CF_{livestem}$, and $CF_{livecroot}$ is maintenance respiration costs for leaf, fine root, live stem, and live coarse root, respectively.

Growth respiration is also calculated as 30% of the total carbon in new growth (Larcher, 1995 "Physiological Plant Ecology").

$$G_R = 0.3\ x\ GPP \qquad \text{(Eq.3)}$$

In its simplest form, GPP is modelled in CLM4.5 by considering the carboxylation as:

$$GPP = \min\left(A_c, A_j, A_p\right) \qquad \text{(Eq.4)}$$

The RuBP carboxylase (Rubisco) limited rate of carboxylation Ac (μmol CO2·m^{-2}·s^{-1}) is

$$A_c = \begin{Bmatrix} \frac{V_{cmax}(c_i - \Gamma_*)}{c_i + K_c\left(1 + \frac{o_i}{K_o}\right)} & for\ C_3\ plants \\ V_{cmax} & for\ C_4\ plants \end{Bmatrix} \quad c_i - \Gamma_* \geq 0. \qquad \text{(Eq.5)}$$

The light limited maximum rate of carboxylation that allows to regenerate RuBP Aj (μmol CO2·m^{-2}·s^{-1}) is

$$A_j = \begin{Bmatrix} \frac{V_{cmax}(c_i - \Gamma_*)}{4c_i + 8\Gamma_*} & for\ C_3\ plants \\ \alpha(4.6\phi) & for\ C_4\ plants \end{Bmatrix} \quad c_i - \Gamma_* \geq 0. \qquad \text{(Eq.6)}$$

The product-limited and PEP carboxylase-limited rate of carboxylation for C3 and C4 plants Ap (μmol CO2·m^{-2}·s^{-1}) is

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$$A_p = \begin{Bmatrix} 3T_p & for\ C_3\ plants \\ k_p \frac{c_i}{P_{atm}} & for\ C_4\ plants \end{Bmatrix} \quad \text{(Eq.7)}$$

In the equations of the carboxylation, c_i is the partial pressure of CO_2 in internal leaf (Pa), O_i is the partial of O_2 (Pa), K_c and K_o are the Michaelis-Menten constants (Pa) for CO_2 and O_2. Γ_* is the CO_2 compensation point. V_{cmax} is the maximum rate of C assimilation ($\mu mol \cdot m^{-2} \cdot s^{-1}$). J stands for electron transport rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$), T_p for triose phosphate utilization rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$). ϕ is the absorbed photosynthetically active radiation ($W \cdot m^{-2}$) and k_p is the initial slope of C_4 CO_2 response curve. The detailed description of the parameters can be found in the study by Oleson et al. (2013).

Model Simulations Design

The simulation of the NPP was continuously done by the model from 1950 to 2100. We analyzed two 30-year time periods, i.e. 1971-2000 and 2031-2060 as historical and future period, respectively. The selected 30-year time periods were chosen according to the IPCC AR5 report (Kirtman et al., 2013), i.e. 1971-2000 as a base line for depicting near term climate change affects, and the period in that the global average temperature reached the 2 ºC in the RCP4.5 scenario, i.e. 2031-2060 as a base line for depicting future climate change impacts on the NPP at pan-European scale.

Observation Data

The comparison and correlation analysis of the model results were done by using in-situ measurement data from six different data sources. We selected the locations with the NPP data from the data sources by considering the same biome and plant functional types (PFT) similar as in grid cells of the CLM4.5 model. We also considered the time periods of the in-situ measurements for comparison of the simulated and measured NPP in a grid cell.

Intercomparison of the Model

For intercomparison of the modelled NPP, we used the available monthly NPP data from MODIS (Moderate Resolution Imaging Spectro-radiometer) satellite for the time range between 2000 and 2014. The data were released within MOD17 project and provided continuous estimates of NPP in ca. 4 km resolution across the Earth's vegetated land surface (Zhao and Running, 2010). We used bilinear interpolation method of Climate Data Operators (CDO) to upscale MODIS results from 4x4 km to model output grid resolution (i.e. 25x25 km) for this study (CDO, 2015). The detailed description of the bilinear interpolation method and module is published by Schulzweida (2015). The correlation analysis between the in-situ data, and predicted and remote sensed data were carried out with IBM SPSS statistic software version 23.

Result and Discussion

Net primary production (NPP) was obtained from the MODIS sensor at 4 km spatial resolution for the pan-European terrestrial surface. In *Fig. 1-left*, the original 4x4 km resolution NPP and *Fig. 1-right* the from 4x4 km to 25x25 km up-scaled NPP are shown. It is to see that the upscale method does not change the quality and distribution

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of the NPP data in domain of the study. As it is expected, the highest 15-year average NPP value (~2200 $\frac{gC}{m^2\,yr}$ from 2000 to 2014) was taken by MODIS in temperate broadleafed and mixed forests in pan-European region (see *Fig. 1*). In temperate biomes, the dominant plant species are generally *Fagus spp.*, *Quercus spp.*, *Betula spp.* and *Carpinus spp.* (Schmithüsen, 1976; Olson et al., 2001). NPP was quantified between 300 and 600 $\frac{gC}{m^2\,yr}$ by the satellite in boreal forests of northern and eastern Europe (see *Fig. 1*). A long the Mediterranean cost, where vegetated areas are dominated by sclerophyllous shrub formation and evergreen seasonal dry forests with *Q. ilex* ranges the NPP between 200 and 1050 $\frac{gC}{m^2\,yr}$ (see *Fig. 1*).

In the *Table 2*, we summarize NPP values from seven different published sources from different times for three main biome types of pan-European vegetated regions. In that biome regions, the NPP values between 153 and 550 $\frac{gC}{m^2\,yr}$. Lieth (1975) was published quite similar NPP value for regions with temperate broadleafed and mixed, and boreal forests (see *Tab. 2 col. 1*). The value of NPP ranged from 600 to 2500 $\frac{gC}{m^2\,yr}$ for temperate and 200 to 1500 $\frac{gC}{m^2\,yr}$ in boreal biomes. Esser (2008) and Chapin et al. (2011) published similar NPP data to the estimation of NPP by MODIS in temperate and boreal regions (see *Table 2 col. 2 and 5*). Compared to considered data in the table, Del Grosso et al. (2008) and Huston and Wolverton (2009) addressed the minimum range for NPP in all three biome types (see *Table 2 col. 3 and 4*).

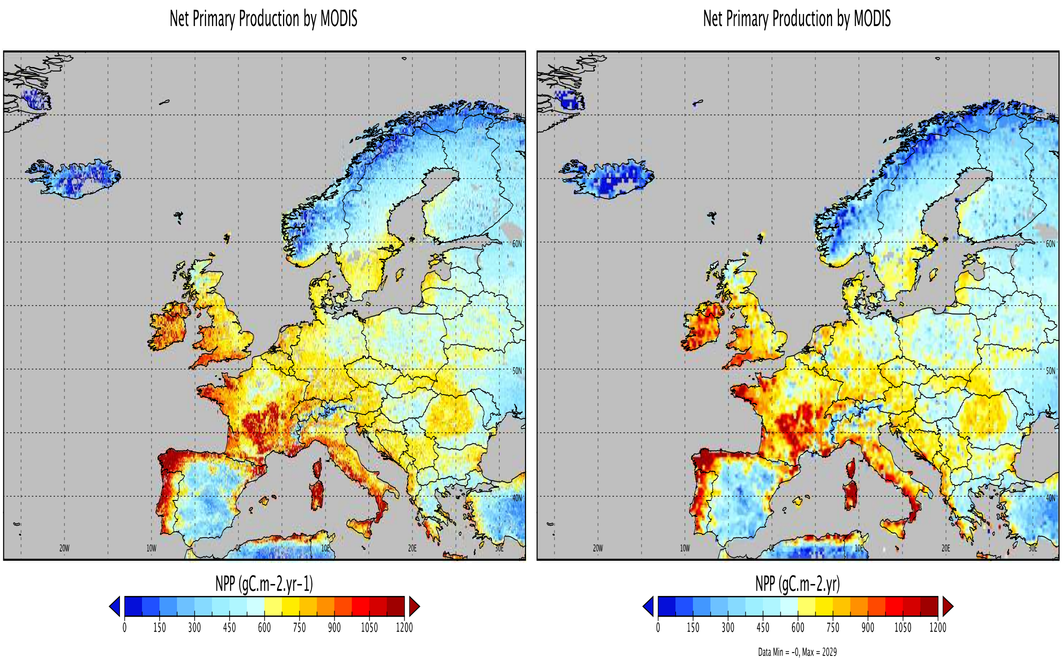

Figure 1. *The spatial distribution of NPP averaged over the period of 2000-2014. The left sub-figure represents NPP in 4x4 km resolution and the right shows the up-scaled NPP to 25x25 km spatial resolution*

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Table 2. *Net Primary Production (in $gC{\cdot}m^{-2}{\cdot}yr^{-1}$) from different sources for the three main climate zones in pan-European region. The climate zones include broadleafed and needleleaf with deciduous and evergreen plant functional types. The sources were chronological order.*

	1	2	3	4	5	6	7
Temperate	600-2500	960-1280	400-800	625-779	1550	615-2200	550-1890
Boreal	200-1500	161-348	100-600	190-234	375	155-550	320-630
Mediterrenean	200-1000	-----	50-600	------	1000	310-1030	355-980
Grassland	100-1500	58-1623	50-800	298-641	750-1080	150-1050	260-940

1. *Lieth, H., 1975*
2. *Esser, G., 1998*
3. *Del Grosso et al., 2008*
4. *Huston and Wolverton, 2009*
5. *Chapin et al., 2011*
6. *MODIS, 2000-2014 this study*
7. *CLM4.5, 2000-2014 this study*

In general, CLM4.5 model predicts the NPP quite similar to the MODIS estimations in all 3 biome types in pan-European domain (see *Table 2 col. 6*). We compare the distribution of 15 years' average NPP in pan-European domain. In *Figs. 2 and 3*, we illustrate the distribution of NPP by MODIS and CLM4.5 in pan-European domain.

Although the NPP by CLM4.5 and MODIS have similar pattern, there is particularly some differences in temperate broadleafed and mixed forests. In Eastern Europe the CLM4.5 predicts the NPP in most of the regions over 1000 $\frac{gC}{m^2\,yr}$, however the MODIS supplies the NPP between 300 and 700 $\frac{gC}{m^2\,yr}$. We plotted spatial distribution of 15 years (2000-2014) average of all six climate variables in the *Fig. 4*.

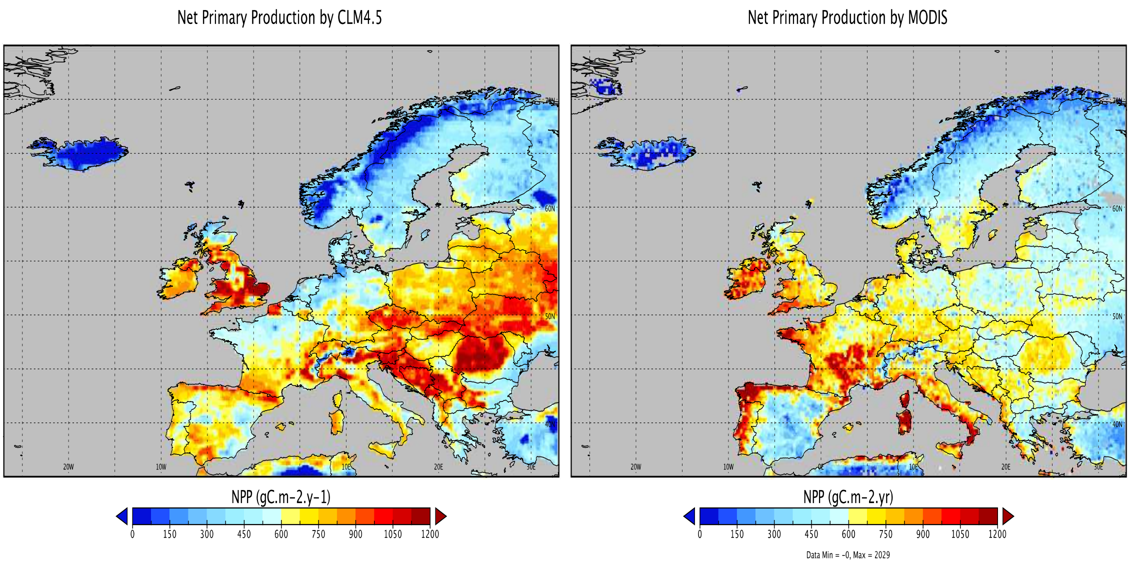

Figure 2. *Spatial distribution of 15 years (2000-2014) average predicted by CLM4.5 and observed by MODIS at pan-European level*

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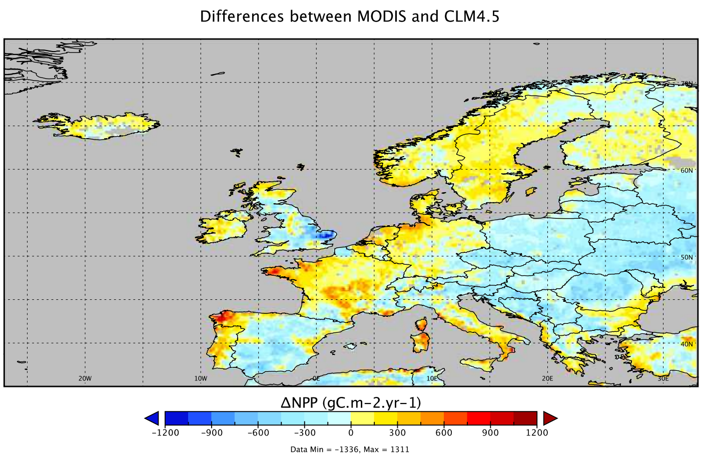

Figure 3. *Spatial distribution of the difference between 15 years average of by MODIS observed and by CLM4.5 predicted NPP at pan-European scale*

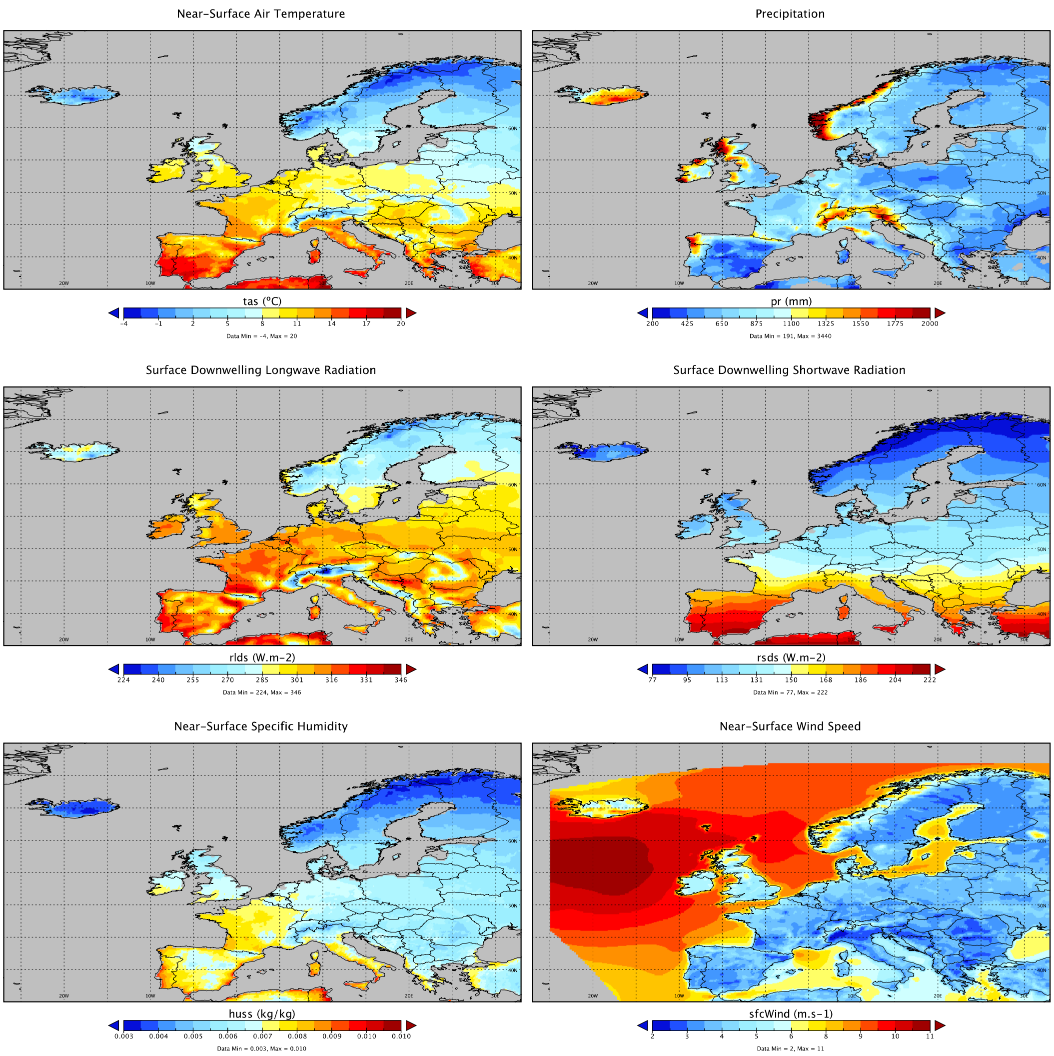

Figure 4. *Distribution of 15 years (2000-2014) average of six climate variables at pan-European scale*

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The multi regression analysis shows no correlation between the NPP and used six climate parameter at the time range 2000-2014 (see *Tab. 3*). But, by visual comparison the climate plots in the regions with high differences in NPP between the model and obtained satellite data, it’s to see that the differences are mainly due to combination effect of temperature and precipitation in eastern Europe (see *Fig. 2*).

Table 3. *Multi regression correlation between predicted NPP and six climate variable at the time range 2000-2014*

	NPP	huss	pr	rlds	rsds	sfcWind
huss	.270					
pr	-.044	-.125				
rlds	.374	.870**	-.360			
rsds	.204	.653**	-.562*	.863**		
sfcWind	-.105	-.643**	.421	-.740**	-.907**	
tas	.329	.806**	-.539*	.904**	.904**	-.733**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
N:15

That confirms that the NPP distinctions between the predicted and observed in the 4 regions are mainly depending on climate parameter and not on the parametrization of NPP for PFTs in the model. It is quite important to mention that the differences between the modelled and observed NPP does generally not mean that the quality of NPP modelling has substandard quality. Zhao et al. (2006) published data about uncertainties in quantifying the GPP (Gross Primary Production) and NPP by MODIS. They highlighted that the quantifying of NPP includes more uncertainties then GPP by MODIS. To clarify the origin of differences between CLM4.5 and MODIS, we validated the NPP products (CLM4.5 and MODIS) with in-situ data of 19 study areas from Oleksyn et al. (2000). They measured the NPP from *Pinus sylvestris* in 19 study areas. We selected the predicted and from satellite obtained NPP values for each study site. Since a grid cell of CLM4.5 can have several PFTs and *P. silvestris* is a needleleaf tree and distributes in temperate and boreal zones, we selected the percentage of two PFTs (i.e. Needleleaf Evergreen Temperate and Boreal Forests) for each grid cell, where the in-situ studies were done, and multiplied with the total NPP value of each 25x25 km grid cell. In the *Table 4*, the study sites, the NPP values from the sources of Oleksyn et al. (2000), CLM4.5 and MODIS 4x4 km resolution and the total percentage of the two PFTs are presented. Comparing of the NPP values of in-situ studies with the results of CLM4.5 and MODIS shows that the CLM4.5 has a better correlation with the NPP from Oleksyn et al. (2000) than MODIS (see *Table 4 col. 2, 5 and 6*). The scatter plot in *Fig. 5* presents the correlation between in-situ and CLM4.5 and MODIS with correlation coefficient R^2. The correlation of NPP between CLM4.5 and in-situ studies equates to 63% and between MODIS and in-situ studies ca. 1%, respectively (see *Fig. 5*). Certainly, it has to be mentioned that the correlation analysis includes in-situ data from one plant species in different study areas and climate zones.

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Table 4. *In-situ NPP data from 19 study sites that collected from Oleksyn et al. (2000), predicted NPP for 25x25 km grid resolution and PFT's percentage of each grid according to the PFT in the study sites, and the observed NPP by MODIS.*

	NPP ($gC·m^{-2}·yr^{-1}$)				
Lat, Lon	Oleksyn et al. (2000)	CLM4.5 25x25 km	PFT (%)	CLM4.5x PFT	MODIS 4x4 km
53.60, 20.00	820	1222	0.65	794.3	642
53.20, 23.37	740	1172	0.91	1066.52	608
53.00, 13.90	1070	1062	0.43	456.66	711
52.50, 10.50	690	945	0.56	529.2	633
51.60, 20.20	870	1519	0.46	698.74	507
51.10, 17.92	980	1288	0.83	1069.04	530
50.80, 4.43	900	1302	0.78	1015.56	611
49.5,8.5	930	727	0.92	668.84	344
48.8,7.78	580	877	0.69	605.13	804
48.77,17.05	470	1333	0.34	453.22	306
47.30,16.47	940	1207	0.88	1062.16	701
44.1,17.35	220	1300	0.19	247	746
43.2,19.5	100	1456	0.16	232.96	672
40.0,31.17	80	854	0.08	68.32	439
60.25,29.9	260	766	0.51	390.66	413
60.18,15.87	180	626	0.29	181.54	572
59.97,33.50	350	928	0.61	566.08	490
58.83,29.12	410	942	0.56	527.52	477
55.75,26.67	700	1165	0.67	780.55	427

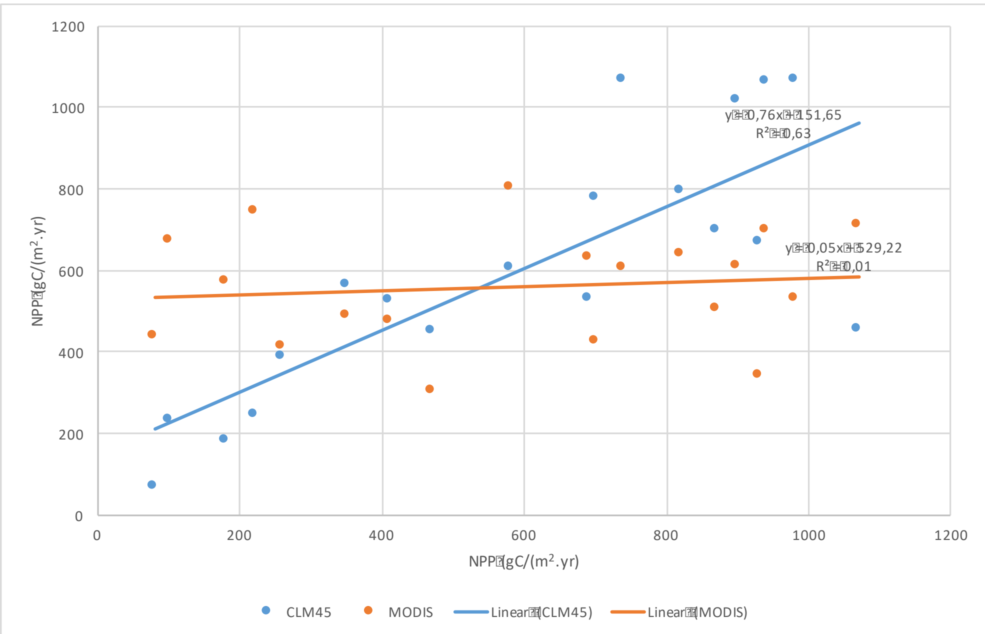

Figure 5. *Scatter plot for the correlation analysis between in-situ collected NPP data from Oleksyn et al. (2000) and by CLM4.5 predicted, and by MODIS observed data. The lines show the linear correlarion.*

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This study shows that CLM4.5 model predicts NPP fairly good for 25x25 km spatial resolution in pan-European scale. In 30 Nov. 2015, United Nations came together in Paris (France) (*United Nations Framework Convention on Climate Change, 21st Conference of the Parties* (*COP 21*)) to discuss the effect of 2 ºC average global temperature increase on ecosystems, economies, human health and adjust preferences for a road map for reducing anthropogenic factors, which caused the temperature increase globally. In last decade, the meaning of 2 ºC global average temperature has been increased and its effects on terrestrial biosphere, especially on carbon assimilation processes, has become increasingly important for all nations. The future climate predictions show that the 2 ºC average temperature increase in pan-European scale under consideration the Representative Concentration Pathways 4.5 (RCP4.5) emission scenario earliest in 2033 and latest 2065 (Kirtman et al., 2013). The RCP4.5 is consistent with a possible change in future anthropogenic greenhouse gases emissions (GHG). The RCP4.5 is named after the potential change of radiative forcing value +4.5 $\frac{W}{m^2}$ in 2100 relative to 1860 (pre-industrial) value (Meinshausen et al., 2011). The GHG emissions within the RCP4.5 scenario peaks close to 2040, and then stats to decline up to 2100. We decided to predict the NPP for 30-year period between 2034 and 2063 to identify the climate change effect on net primary production of the vegetation in pan-European scale. In the *Fig. 6*, we show the distribution of 30 years (2034-2063) average of NPP, the change between 2000-2014 and 2034-2063 and also the trend of NPP change up to 2100 in pan-European scale.

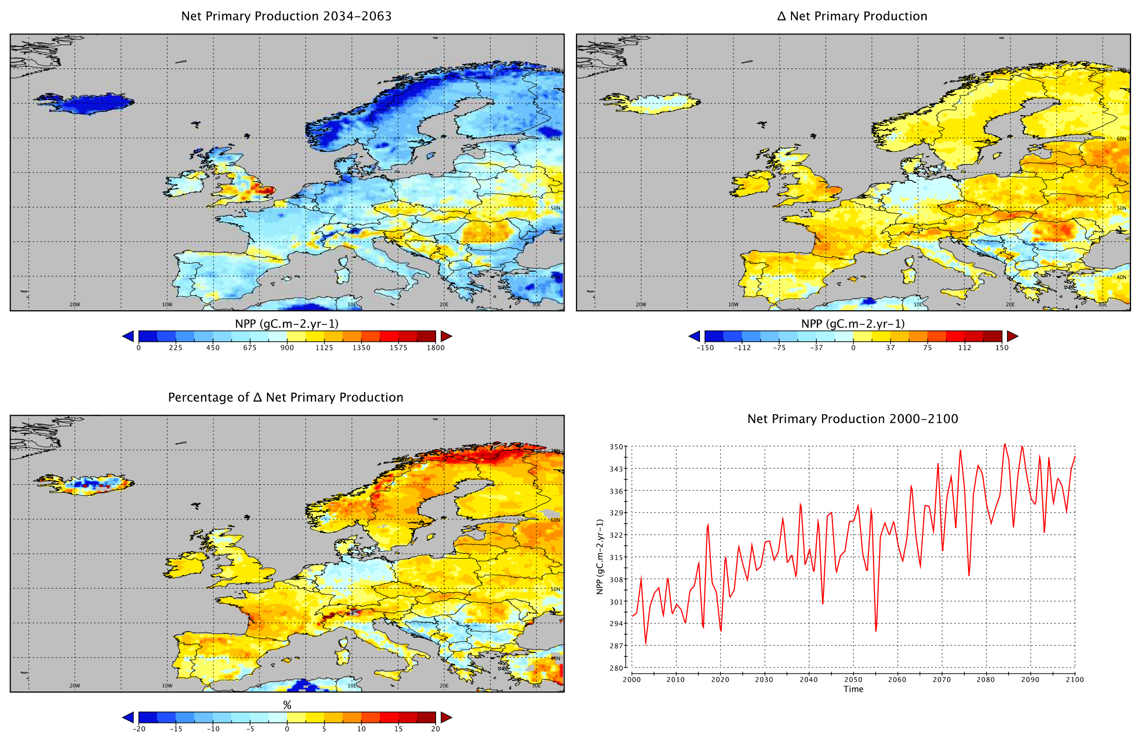

Figure 6. *By CLM4.5 predicted NPP for the future period (2034-2063) (upper left), the difference between 30 years (1971-2000) average of past period and future period (2034-2063) (upper right), the percentage of the difference between two periods (bottom left), and the NPP of the run of the model from 1971 to 2100 (bottom right).*

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Generally, NPP shows an increasing up to 30% in pan-European scale during the peak period of GHG emissions (2034-2063) (see *Figs. 6 upper right and bottom left*). However, the future prediction of NPP shows a decreasing in most of the regions in Germany, Italy and south-east Europe. The model illustrates up to 18% of reduction by NPP in that regions (see *Fig. 6 bottom left*). It is quite important to investigate not only spatial distribution of NPP but also inter-annual variability of NPP. The sub *Fig. 6 (bottom right)* shows the trend of average NPP variability from 1971 to 2100. NPP has a distinct increasing trend between 1971-2100. From 1971 to 2100 there is almost 15% an increase in NPP (see *Fig. 6 bottom right*).

Conclusion

This study compares predicted NPP by using CLM4.5 model with remotely sensed NPP in pan-European scale with in-situ measured data. The comparison was done for 25x25 km high resolution gridded data sets. It reveals that there are differences between modelled and observed NPP in eastern and middle Europe. NPP is either mis-quantified by MODIS or mis-predicted by CLM4.5. Since we do not have field studies in the regions, we could not detect the site of the failure in this study. But the high correlation between by CLM4.5 predicted and observed data in 19 study locations indicates that the model is most probably able to predict NPP in pan-European scale. According to the results in past observed period, we assume that the prediction of NPP quite acceptable for the future period. It shows that NPP will increase ca. 15% in average at pan-European level. Furthermore, northern and high altitude regions show most response to climate change with highest increase of NPP in the future period. Although NPP shows an increasing trend in most of the vegetated areas at pan-European level, NPP will minimally change in few regions e.g. in Germany, Italy and most of the Balkans.

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PREDICTING THE SUITABLE HABITAT OF THE INVASIVE *XANTHIUM STRUMARIUM* L. IN SOUTHEASTERN ZIMBABWE

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Abstract. Biological invasions have negatively affected virtually all ecosystems on earth. Thus, reducing the spread of such invasive species is important. A few broad strategies are available on how to reduce the spread of invasive species. The aim of this study is to predict the suitable habitat of the invasive species *Xanthium strumarium* and to identify the factors associated with its spread in order to improve efforts against invasive species. Presence (53 plots) and absence (52 plots) data for building the prediction were collected from Gonarezhou National Park and the adjacent Malipati communal land, Zimbabwe in April 2013. Ten modelling techniques were employed namely; Generalized linear model, General additive model, Classification tree analysis, Surface range envelope, Multivariate adaptive regression splines, Random Forest, Gradient boosting machines, Maximum entropy, Artificial neural networks and an ensemble model. Environmental factors related to the establishment success of *Xanthium strumarium* were also identified. Results of this study indicate that machine learning based techniques performed better at predicting the occurrence of *Xanthium strumarium*, although the ensemble prediction outperformed all the individual models. *Xanthium strumarium* was predicted to occur in areas that receive direct radiation from the sun, thus, aspect was identified as a critical factor in habitat selection for *Xanthium strumarium.*
Keywords: *area, climate change, plant population, bioclimatology, prediction, habitat, invasive*

Introduction

Rough cocklebur (*Xanthium strumarium* L.), is a broadleaved, tap rooted, forb whose stems are erect, ridged, rough and hairy (Hare, 1980). The species belongs to the Asteraceae family and it reproduces annually (Venodha, 2016). Rough cocklebur often appears in thick mono-specific stands usually in low-lying riparian areas and in agricultural fields (Marwat et al., 2010). The native habitat of *Xanthium strumarium* is North America and Argentina (Nel et al., 2004; Oksanen et al., 2016). Several studies have identified *Xanthium strumarium* to be invasive in southern African savannas, (Ekeleme et al., 2000; Holmes et al., 2008; Sithole et al., 2012). The success of *Xanthium strumarium* as an invasive species has been attributed to competitive genetic makeup (Gray et al., 1986) among other adaptive capabilities that the species possesses. However, the extent of *Xanthium strumarium* invasion in southeastern Zimbabwe is yet to be established.

Studies on species invasion have highlighted that the invasion process is reliant upon both the invasive capabilities of the species and the invasibility of the ecosystem. Hence it is important to consider the factors which are associated with habitat invasibility when predicting the occurrence of an invasive species. Given the fact that introduced species are more successful in disturbed ecosystems where competition has either been reduced or totally eliminated (Dukes, 2001; Eppstein and Molofsky, 2007), it is vital to consider

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the natural and human factors that are associated with the occurrence of *Xanthium strumarium* .

Information on the potential distribution of invasive species is essential as it may improve the quality of control efforts against invasion. Single model species distribution models are often affected by bias (Araújo and New, 2007). Thus more recently, researchers have ventured into multi-model approaches and ensemble forecasting (Ochoa-Ochoa et al., 2016). The use of several models could remove model-based bias and improve the chances of model suitability. Although multi algorithm species distribution modelling has gained popularity among spatial geographers, more work still needs to be done in southern African savannas, especially for invasive species.

A limited number of studies exist that attempt to identify the potential distribution of invasive species, *Xanthium strumarium* included. The predictions are especialy important given that savannas play a vital role in human welfare and economic development. Half of the biome's human population is directly dependant on it for their sustenance (Sankraan et al., 2005). Also, in the southeastern low-veld of Zimbabwe cattle farming is the main economic activity supporting livelihoods (Zengeya et al., 2011; Chigwenhese et al., 2016), and the area hosts a considerable number of wild mammals in the Gonarezhou national park, information on the suitable habitat of the invasive species is critical. *Xanthium strumarium* is unpalatable to both livestock and wildlife, thus, if left unmanaged the invasive species could cause major economic and biological losses. In that regard, this study aims to predict the occurrence of invasive species *Xanthium strumarium* in southeastern Zimbabwe. This prediction will be made by using an ensemble model which will incorporate the best performing algorithm among: Gradient boosting machines (GBM), Artificial neural networks (ANN), Maximum entropy (MaxEnt), Random forests (RF), General linear model (GLM), General additive machines (GAM), Multivariate adaptive regression splines (MARS) Surface range envelope (SRE) and Classification tree analysis (CTA). In this study, the ensemble model was also used to identify the environmental factors that are associated with the distribution of *Xanthium strumarium.*

Literature review

Invasive species are species that have an ability to spread on their own, causing harm to other species and their environment when introduced to new environments (Rejmánek et al., 2013; Richardson and Pyšek, 2006). These species are in most cases 'alien' in the sense that they are not native in the regions that they become invasive. Mostly they would have been transported by humans(Vitousek et al., 1997). The transportation of organisms by humans through global trade and globalization which has now increased has led to the breaking of natural biogeographic barriers (Clout and Williams, 2009). Organisms have found a way through humans to migrate from geographic areas of superior competition to areas where they can easily dominate and cause enormous damage. It is important however, to note that not all introduced species have the potential to become invasive. In the case that they become invasive, they often spread irreversibly, and damages increase over time (Keller et al., 2009) non-indigenous species (e.g., plants or animals) that adversely affect the habitats they invade economically, environmentally or ecologically (Dassonville et al., 2008). In recent years, economists are also making efforts to understand how invasive species affect

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economic systems, and in how invaders can be controlled to increase societal welfare(Keller et al., 2009). Such is the extent of the harm caused by invasive species.

Ever since 1958, scholars have been active in the field of invasion science (Rejmanek et al., 2005) and management has been one of the popular topics. Managing biological invasions involves identifying passageways of transport or the methods of transport and the attributes of the species that guarantees success when faced with barriers. It also involves using appropriate strategies to prevent, eradicate, or control the species of concern (Clout and Williams, 2009). It is important to obtain accurate assessments of location and abundance of invasive species so that managers can set these priorities and have the information to quickly and effectively combat the invaders, (Clout and Williams, 2009). It is also important to identify barriers to invasion and habitats where an invasive species cannot persist or cause much harm, (Clout and Williams, 2009). Given that invasive alien species represent a global threat, it is necessary to make the best use of all information available(Capdevila-Argüelles and Zilleti, 2005) to manage their spread.

More recently, research has ventured into modeling the potential habitats of invasive species using species distribution models. A species distribution model (SDM) is a model that relates species distribution data (occurrence or abundance at known locations) with information on the environmental and/or spatial characteristics of those locations (Elith and Leathwick, 2009). SDMs provides insight not only into the mechanisms but also potential hotspots for invasion. They explore the species-environment relationship further and thus provide useful information for protecting environments against invasive species. However, if species distribution models are to be used for early detection and management of invasive species in conservation practice, their accuracy and correct interpretation is crucial to minimize the ecological impact and economic cost of biological invasions.(Václavík and Meentemeyer, 2009). Thus, it becomes crucial to assess several species distribution modeling algorithms in order to determine the most accurate.

Although studies date back to the 1950's, most studies, have however been geographically biased (Pysek et al., 2008) towards regions in Britain, the Americas and closer to home South Africa. This bias has been a setback for the development of globally representative data sets for the management of invasive species. Hence the need to address the problem of invasive species for local ecosystems, which is the aim of this study.

Methods

Study site

This study was conducted in the Gonarezhou National park (GNP) and the adjacent Malipati communal land (MCL). The study area is located in the southeastern low veld region of Zimbabwe between latitudes 21° 00′ and 22° 15′ south and longitudes 32° 30′ and 33° 15′ east (*Fig. 1*). The southeastern low veld is semi-arid, with average monthly maximum temperatures of 25.9 C in July and 36 C in January and average monthly minimum temperatures range between 9 C in June and 24 C in January (Mott et al., 1984). Characteristic vegetation in the area is savanna woodland dominated by *Colophospermum mopane*, interspersed with *Androstachys johnsonii* and *Combretum apiculatum* on ridges and in riverine areas.

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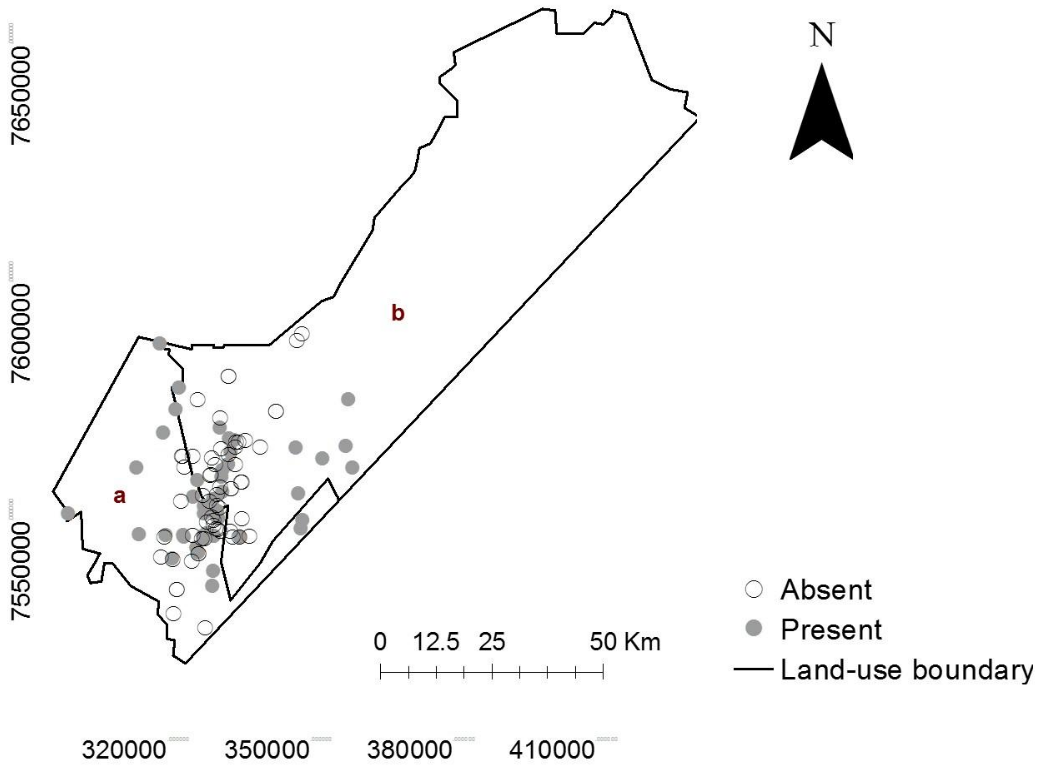

Figure 1. *Distribution of presence and absence data points in (a) Malipati communla lands and (b) Gonarezhou National Park.*

The study was conducted in these separate but adjacent sites so as to account for the effect of land use on the distribution of *Xanthium strumarium*. The sites, GNP and MCL have similar biophysical characteristics (Mombeshora and Bel, 2009) albeit they differ in their main land uses and, therefore, disturbance regimes. GNP is a protected area where wildlife conservation is the main land use activity while MCL is a communal land where cropping and livestock grazing are the main economic activities.

Presence and absence data

Species presence and absence data was collected in April 2013. The study area was stratified into two strata namely a protected landscape (GNP) and communal landscape (MCL). Thereafter, sampling units were randomised in the sites within a GIS environment. Vegetation surveys were conducted in 105 randomised plots. In each plot, all present rooted species were recorded. Most species were identified with expert knowledge in the field with the help of the staff from the Zimbabwe Parks and Wildlife Management authority. The remainder of the species were identified using the help of the National Botanical gardens database and experts. 53 locations were identified as present locations for *Xanthium strumarium,* and 52 were absent locations *(Fig 1).*

Environmental variables

Figure 2 presents maps of four environmental variables namely distance from homesteads, distance from roads, normalised difference vegetation index (NDVI), and

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aspect selected to predict the distribution of *Xanthium strumarium.* The selection of these variables was based on the ecological niche theory and the invasive species' dependency on disturbance regimes.

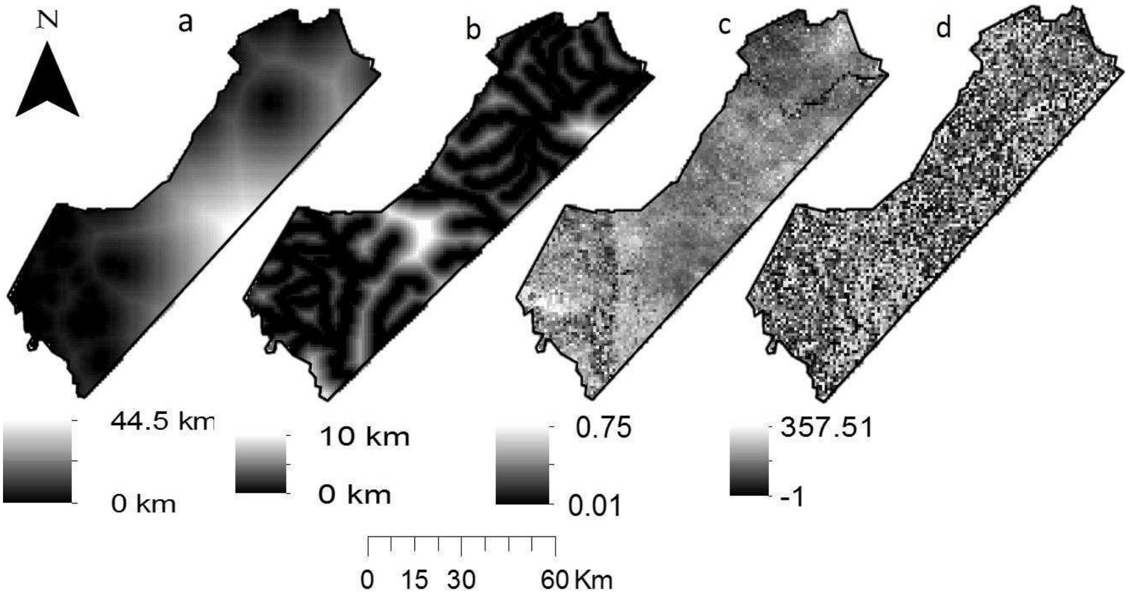

Figure 2. *Environmental variables used to build a predicted location of Xanthium strumarium. (a) Distance from homesteads, (b) Distance from rivers, (c) NDVI and (d) Aspect.*

To capture the potential role of human and livestock disturbance in the distribution of *Xanthium strumarium*, we calculated inverse the distance from settlements. Many studies identify anthropogenic disturbance as one of the major drivers of invasive species establishments in most ecosystems (Barrat-Segretain, 2001; Rejmanek and Sandlund, 1999; Foxcroft et al., 2011; Mortensen et al., 1993; Kumschick et al., 2012). The use of distance from settlements as a proxy of anthropogenic disturbance was justified on the basis that human-mediated disturbance activities such as crop production and livestock production occur in close proximity to where people live and keep their livestock. A point map of all settlements (that is dwelling units) in the study area was created by digitizing these as point features using a Landsat 8 image made available via Google Earth (www.googleearth.com) as the base layer. Next, the Euclidean distance from each dwelling unit was calculated using the Spatial Analyst tool in ArcGIS 9.2 (Wang et al., 2001). Then, an inverse distance map was derived from the Euclidean distance map using the in-built inverse distance Algorithm in Spatial Analyst tool. The weighted map of distance from homesteads was used as a proxy for human and livestock disturbance.

Distance from rivers was used as a proxy for moisture availability, which is crucial to the establishment of *Xanthium strumarium* given that the study site is a semi-arid savanna hence moisture availability is a limiting factor. Distance from rivers also served as a surrogate for propagule dispersal since it is known that the burs that carry the seeds of *Xanthium strumarium* are buoyant and can therefore be transported in flowing water,(Davies and Johnson, 2012). Also considering the fact that the burs of *Xanthium strumarium* can be dispersed by animals via attachment it makes ecological sense to

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assume that the propagules of *Xanthium strumarium* are more likely to be dispersed more efficiently along riparian areas as animals both domestic and wildlife come to drink water. The inverse distance from rivers was calculated from rasterized polylines of the river network using the standard Euclidean distance tool in ESRI ArcGIS 9.2 (Wang et al., 2001).

The third environmental predictor used for modelling the distribution of *Xanthium strumarium* is NDVI, which is a widely used proxy for primary production, which in turn represents the competitive ability of species already established in the study site (Hobbs and Huenneke, 1992). Previous studies have highlighted a negative relationship between competition and invasion (Kauffman et al., 1983; Mortensen et al., 1993). After the image was proccesses for atmospheric corrections and calibrated NDVI was calculated from a Landsat TM image acquired in April 2013. Formally, NDVI was calculated as:

$$NDVI = \frac{(NIR - R)}{(NIR + R)} \quad \text{(Eq.1)}$$

Where NIR is the Near Infrared band and R is the red band of the Landsat TM image.

Aspect was included because previous studies have highlighted that *Xanthium strumarium* establishes more successfully in areas where there is light, (especially the red band of light), as opposed to shaded areas (Sharkey and Raschke, 1981). Thus aspect was included as a proxy for light availability. Aspect was derived from a 30 m shuttle radar topography mission (SRTM) digital elevation model with Spatial Analyst-surface tool in ArcGIS 9.2 (Wang et al., 2001). The DEM was downloaded from (www. usgs.gov).

Prediction of suitable habitat

The study used a range of machine learning based, classification based, regression based and surface range envelope techniques to predict the distribution of *Xanthium strumarium* based on the presence/absence data and four environmental factors described earlier. Three regression-based modelling techniques were used in this study, namely, the General linear model (GLM), Generalized additive model (GAM) and the Multivariate additive regression splines (MARS). GLM predicts from the linear relationship of a given variable to a set of explanatory variables. GAM is almost similar to GLM except it adopts a smoothing effect which improves the output. MARS derives its predictions from recursive partitioning to derive a good set of basic functions. Thus each of these techniques were included for their individual competences.

Also, four machine learning techniques were used in this study. These were: Artificial neural networks (ANN) which were developed from the mathematical models of the human nervous system. Prediction is accomplished by adjusting to cause the overall network to output appropriate results (Abraham, 2005). The Random forests (RF) which operate with a set of trees called 'forests'. To make a classification each tree gives a classification and the 'forest' votes on the classification. Random forests chooses the classification which received the most votes (Cutler et al., 2007). Maximum entropy (MaxEnt) which uses the maximum entropy theory which estimates the maximised probability distribution given a set of constrains (Phillips et al., 2006).

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The above mentioned regression-based and machine-learning techniques were complemented by a classification based technique, Classification tree analysis (CTA). Classification methods consist of recursive partitions of dimensional space defined by predictions into groups that are as homogeneous as possible, as such classification have their strengths.The ninth modelling technique used was the surface range envelope modelling technique (SRE). It involved the use of interpolation of various climatic parameters to produce a model of the best fit of those parameters (Beaumont et al., 2005).

All of the nine modeling algorithms were implemented with the default settings of the BIOMOD2 package (Thuiller et al., 2014) in the R computing environment (Regnier and Harrison, 1993). BIOMOD2 was also set to split 80% of the data to be used for model calibration and 20% for model validation. Presence and absence data points were set such that they contributed equally towards the prediction. BIOMOD2 was set to run 3 permutations in estimating variable importance.

Ensemble modelling

A consensus or ensemble model (EM) which incorporated results from the best performing models was then executed, the results from which were regarded the final prediction. This was done within the Ensemble Modeling environment in the R statistical computing software. The total consensus model (Thuiller et al., 2014) uses the datasets used in building individual models, as well as the individual models. A TSS threshold of 0.6 was used to determine models that were included in building the ensemble model. Thus, models that had TSS of <0.6 were not included in the EM, thus ensuring that the consensus was robust. The weighted mean of probabilities algorithm which gives more weight to individual models with better TSS scores was adopted as the final prediction of the location of *Xanthium strumarium* in this study.

Model evaluation

Ten model evaluation metrics namely the Receiver operating curve (ROC), Cohen's Kappa (KAPPA), True kill statistic (TSS), False alarm ratio, (FAR) Success ratio (SR), Accuracy (fraction correct), Bias score (frequency bias), Probability of detection (POD), Critical success index (CSI), and Equitable threat score (ETS) are available in BIOMOD2 and can be used to determine the models that provided accurate predictions, given that a species distribution model is only useful if it is robust (Václavík and Meentemeyer, 2009). However, to strike a balance between the need for multiple test statistics which improves the robustness of assessments (Elith and Graham, 2009) and to avoid redundancy by using all of them, two of the commonly used model evaluation metrics namely ROC and TSS were used. ROC measures the ability of a model to discriminate between presence and absent points. Values of the ROC statistic range from 0 to 1, where < 0.5 represent predictions which are not better than random. The TSS statistic ranges from random (−1) to perfect agreement (+1) (Guisan and Thuiller, 2005). The advantage of using TSS is that it has all the good properties of KAPPA yet it is not sensitive to prevalence (Allouche et al., 2006).

Variable importance

The relative importance of each predictor variable used in building the ensemble model was also calculated using the BIOMOD2 package (Thuiller et al., 2014). In the evaluation procedure, BIOMOD first created a standard prediction by using all the four

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environmental variables. Thereafter, it generated other predictions, with one independent variable randomised. Thus, each variable was evaluated using the correlation cores of the standard prediction and each of the random predictions (Capinha and Anastácio, 2011). If the standard and random predictions are highly correlated, then the variable's relative importance is low (Capinha and Anastácio, 2011). The relative importance values ranged from 0 (lowest importance) to 1 (highest importance).

Curves representing the predictive ability of each environmental variable were also used to assess the response of the model to the variable. The BIOMOD2 response.plot2 function (Thuiller et al., 2014), was used to plot the response curves. To build the response curves, all other variables except the target variable were set constant to a fixed value (the mean) while the target variable for which the curve was being plotted was the only one that was allowed to vary. Thus, the variations and the curve attained showed how the model was sensitive to that specific predictor variable.

Results

Predicted occurrence of Xanthium strumarium

Model performance varied among the nine individual modelling techniques used, (*Fig 3*). The random forest (RF) and GBM, performed well in predicting the occurrence of *Xanthium strumarium* in Gonarezhou National Park. These two modelling techniques had high TSS and ROC values exceeding 0.6. Consequently, these algorithms were selected for building the ensemble projection, which is shown in *Figure 3* performed best with a TSS score of 0.87. The predictions made by MARS, MaxEnt, GLM, and SRE, were not better than outcomes of random predictions, given their TSS scores were less than 0.5.

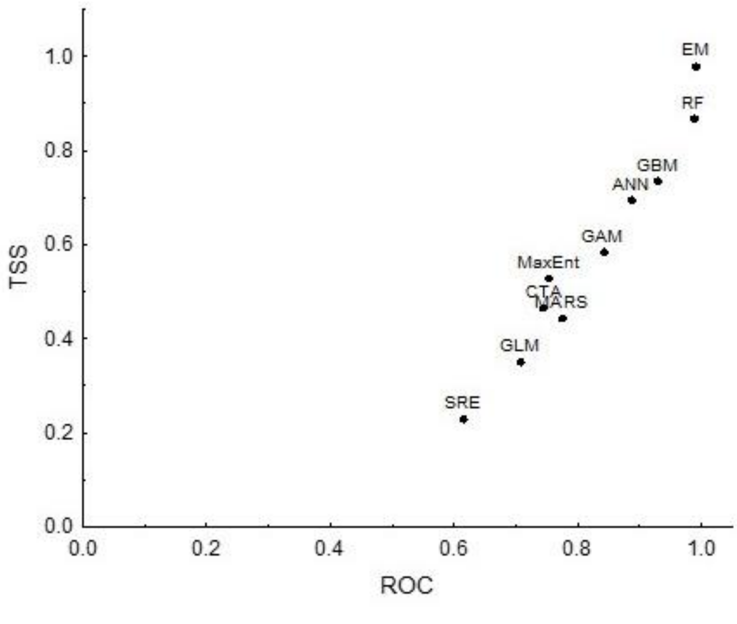

Figure 3. *Scatterplot showing the performance of ten modelling techniques used to predict the occurrence of the invasive species Xanthium strumarium in Gonarezhou National Park of Zimbabwe. The x-axis represents True skills statistic (TSS) and the y-axis shows Receiver operating curve (ROC).Distance from homesteads, (b) Distance from rivers, (c) NDVI and (d) Aspect.*

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The ensemble projection illustrates that probability of occurrence of *Xanthium strumarium* was overall higher in the protected national park compared to the adjacent communal farmland (*Fig.4).*

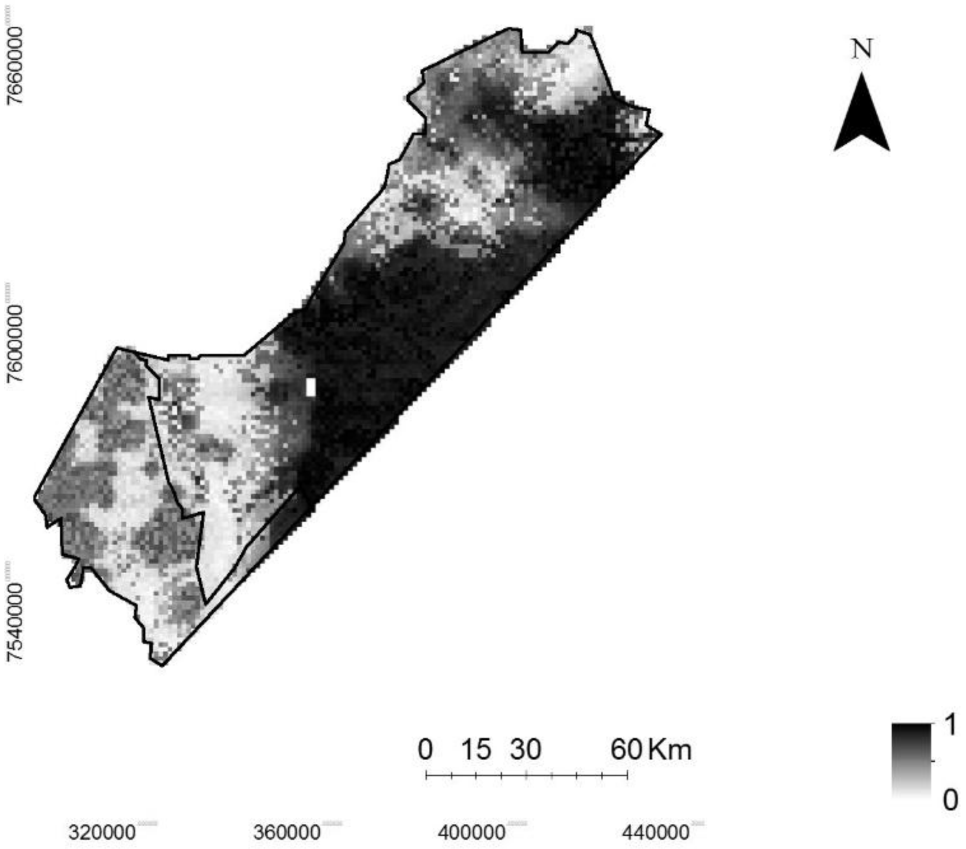

Figure 4. *The predicted Xanthium strumarium suitable habitat in Malipati Communal lands and Gonarezhou national park derived from the Ensemble*

Predicted occurrence of Xanthium strumarium

Results show that aspect, with a relative importance of 0.486 was the most important variable influencing the results of the ensemble prediction, followed NDVI and distance from settlements with relative importance values of 0.303 and 0.277, respectively. The predictor variable with the lowest relative importance of 0.021 was distance from rivers.

Results (*Fig. 5*) illustrate the response curves of *Xanthium strumarium* to the four factors considered important in predicting the species' probability of occurrence in Gonarezhou National Park. It can be observed that in general, probability of occurrence of *Xanthium strumarium* increases with aspect and there is a local maximum centered around 100o. The response curves for distance from settlements indicates that the highest peak in the probability of *Xanthium strumarium* occurred within a distance of 2-km from settlements. After this distance, the probability of *Xanthium strumarium* fluctuates but an increasing trend is discernible up to a distance of 15 km from settlements.

With regard to NDVI, results in *Figure 5*, illustrate that the probability of occurrence of *Xanthium strumarium* decreases with increasing NDVI.

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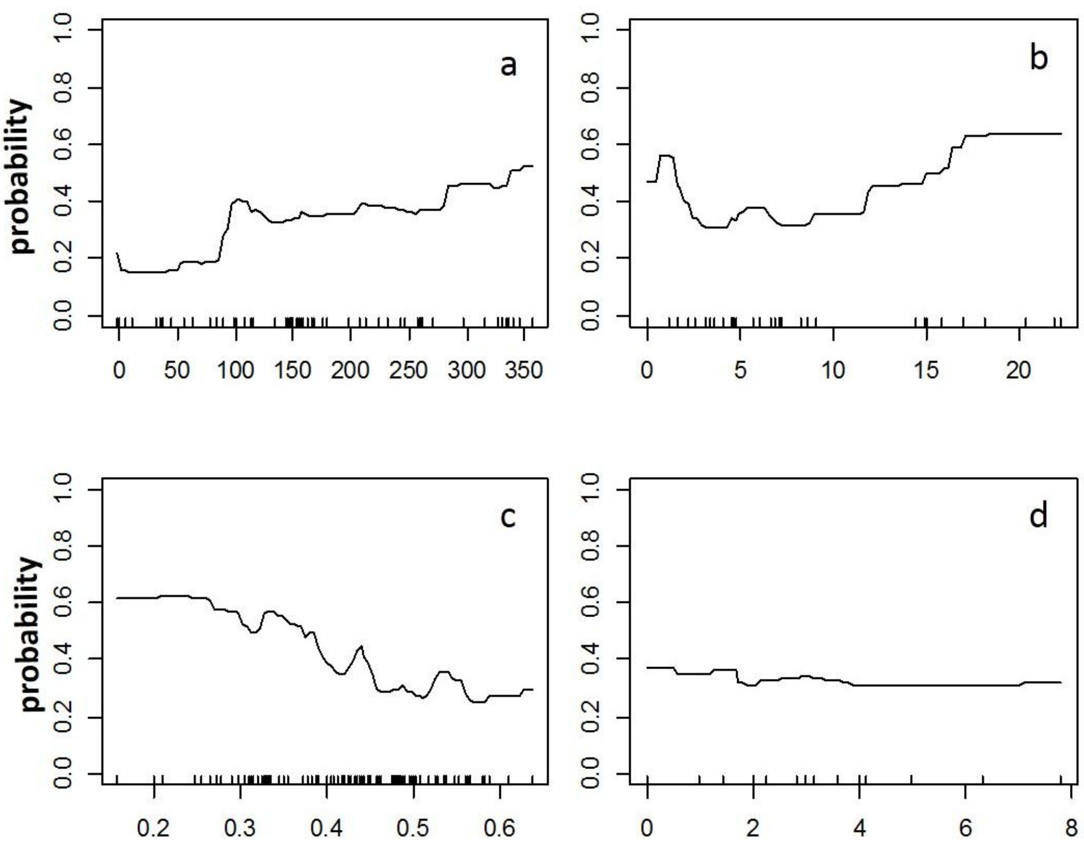

Figure 5. *Response curves of Xanthium strumarium (a) aspect (b) distance from settlements (c) NDVI (d) Distance from rivers*

Discussion

The aim of this study was to make a prediction of the locations where an invasive species *Xanthium strumarium* may occur within and around a protected area in southeastern Zimbabwe. Our hypothesis stated that since human disturbance is known to promote invasion (Chown et al., 2005; Addo-Fordjour et al., 2009; Holland et al., 2011), factors associated with human disturbance in conjunction with biotic factors improve the performance of models in predicting the occurrence of exotic species. The prediction produced by the ensemble model was fairly accurate with both the TSS and ROC more than 0.8 (*Fig. 2*). The prediction suggested that areas within the protected area have a higher probability of *Xanthium strumarium* occurrence than those in the communal area (*Fig. 4*). This result implies the need for improved efforts in managing the spread of *Xanthium strumarium* inside the protected area since conditions inside the national park is conducive to the growth of the species.

The study also intended to make a comparison of the performance of modeling techniques when predicting the location of *Xanthium strumarium* in and around a protected area in southeastern Zimbabwe. As expected, the ensemble model provided a superior prediction to all the other algorithms in this study. The superior performance of the consensus model could be attributed to the fact that it combines the outcomes of several good predictions to come up with one that has a better accuracy (Mestre et al., 2015; Ochoa-Ochoa et al., 2016). According to the accuracy assessment parameters employed in this study, RF performed better than the other algorithms. In fact, the top

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two algorithms RF and GBM are machine learning techniques. The success of machine learning algorithms could be attributed to the fact that learning based models learn from the existing data thus, they do not require an a priori model (Kasabov, 1996). Hence their predictions are unbiased and mostly turn out to represent nature more accurately.

The success of machine learning techniques in this study is not unique. Other studies, (Guisan et al., 2006; Austin, 2002; Austin et al., 2006) which also made comparisons of species distribution modelling techniques have also cited the strength of machine-based algorithms. Some of the algorithms, however, produced poor predictions regardless of the fact that similar variables were used for all the models. The differences in the predictions of the ensemble model and SRE model illustrates the importance of accurate species distribution modelling of invasive species. Accurate predictions of invasive species locations allow for targeted application of limited resources; especially in African savannas were resources are constrained. Therefore, from the results, it is deductible that the use of appropriate algorithms may improve efficiency in invasive species management. The poor performance of these algorithms in this study regardless of previous success in other studies proves that no single model works best for all species, in all areas, at all spatial scales, and over time (Jarnevich et al., 2015). Thus, from these results, we deduce that a multi-model approach is important for eliminating problems associated with model bias in species distribution modelling.

Another objective of this study was to investigate the environmental variables that were most influential in predicting the occurrence of *Xanthium strumarium*. The results suggest that aspect was the most important factor in building the predicted location of *Xanthium strumarium*. This finding implies that among the factors that were considered, the direction in which the slope is facing is most critical for the success of *Xanthium strumarium*. Several studies have established that quality of light reaching a *Xanthium strumarium* plant is important for its growth (Tucker and Mansfield, 1971; Wang et al., 2001; Mott et al., 1984). Therefore, given its preference for direct sunlight, management strategies could prioritize surfaces that have direct access to sunlight to reduce the survival rate of new introductions.

It is not surprising that the results suggest distance from homesteads to be the second most important variable in building the model for predicting the occurrence of *Xanthium strumarium*. In fact, the response curve for the distance from homesteads shows a rise of probability within 1-2 km of homesteads. Other studies on invasive species have identified humans as a source of propagules (Mack and Lonsdale, 2001; Brown and Peet, 2003; Farji-Brener and Ghermandi, 2008; Thêbaud and Simberloff, 2001; Wichmann et al., 2009) thus, the human environment acts as the epicentre for the spread of invasive species. Again, *Xanthium strumarium* is an agricultural weed, land tillage activities that occur in the communal area adjacent to Gonarezhou systematically creates niches for the establishment of *Xanthium strumarium*.

Also, given that the SELV of Zimbabwe is dry savanna, and animal husbandry is the main economic activity, the livestock numbers in the area may promote the establishment of *Xanthium strumarium* in two ways. The first way is through overgrazing (Roath and Krueger, 1982) which may disturb the natural vegetation and create niches for potential establishment of *Xanthium strumarium*. Secondly, is through propagule dispersal. *Xanthium strumarium* seeds are dispersed by water and by attaching to animal fur. Thus large amounts of livestock improve the transport of *Xanthium strumarium* hence it spreads over large areas quickly. It is thus critical to

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consider the critical role that humans are playing in facilitating the establishment of invasive *Xanthium strumarium*.

Xanthium strumarium responded negatively to competition just as previous studies (Stachowicz et al., 1999; Currie and Paquin, 1987; Foxcroft et al., 2011) have established the competition avoidance nature of most invasive species. Studies on ecosystem invasibility have established that invasive species generally avoid competitive patches as a competition avoiding strategy (Stachowicz et al., 1999; Bartomeus et al., 2012). As such, the response of *Xanthium strumarium* to NDVI may imply that *Xanthium strumarium*'s strength is its capability to utilise niched left over by disturbance events. This is evident in the fact that the species follows an annual lifecycle (Venodha, 2016) and also one plant can produce up to several hundreds of propagule. Thus, it can regenerate quickly and invade communities in very short amounts of time. To that end, it is important to acknowledge the role that disturbance plays in the establishment of invasive *Xanthium strumarium* in order to improve efforts of managing the spread of this invasive species.

Although rivers had the least influence in building the ensemble model, the response curve shows that highest probability of occurrence is observed within 2 km of rivers. This area is commonly known as the riparian zone. Riparian zones are plant communities on or near the banks of river channels. A variety of natural disturbances usually creates a unique mosaic of vegetation in riparian zones, which may be parallel to vegetation in the larger landscape (Didham et al., 2007). Adding to that, rivers play a second significant role of propagule transportation to terrestrial plants. The riparian zone mostly consists of palatable forbs (Roath and Krueger, 1982; Kauffman et al., 1983), thus there is the effective transport of *Xanthium strumarium* to and from riparian zones when they come to for food and water. Therefore, it is important to target these riparian areas when implementing management strategies of *Xanthium strumarium*, given the role riparian areas play in the species' establishment.

This study differs from other spatial modelling studies in the southern African savanna (Colgan et al., 2012; Baldeck et al., 2014; Mashintonio et al., 2014) in that it adopted a consensus approach to species modelling targeting an invasive species. In fact, previous studies in the biome have only been limited to single models (Stevens et al., 2014). Using the consensus modelling framework, robust predictions were made to aid pinpointing of potential hotspots *Xanthium strumarium* invasion in Gonarezhou national park and the adjacent communal landscapes.

Caution should be practiced when interpreting the results of this study since the prediction is only based on one site. More study sites could be necessary to further test their validity. Also, this study focused on only one invasive species which may have unique characteristics. Focus on more than one species could lead to more generalised conclusions. In this regard, it is recommended, that future studies focus on more than one study species, as well as on multiple study sites while using multiple distribution models.

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BIOREMEDIATION OF WASTEWATER FOR REUTILIZATION IN AGRICULTURAL SYSTEMS: A REVIEW

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Abstract. The bioremediation is a technique used in descontamination water, soil, o sludge, which consist of the use of living microorganisms and plant for degradation of pollutants by metabolic processes. Wastewater is often used in agriculture due to the limited availability of water resource, but also because wastewater provides nutrients for crops, and reduces cost production for some agricultural regions. However, its reuse as a source of irrigation in agriculture must first involve the use of bioremediation techniques. Until 2012, in Mexico, treated municipal wastewater accounted for about 50% of all wastewater generated. This suggests that greater efforts are needed to generate efficient alternatives to increase the volume of treated wastewater, and thus meet the demand for this resource in the agricultural sector. This review discusses in general the use and reuse of treated wastewater through bioremediation techniques. The objetive of this research is to review the state of the art bioremediation and wastewater reuse for agriculture in Mexico.

Keywords: *pollution, irrigation, phytoremediation, agroenvironment, agroecology*

Introduction

A major problem at the global and national levels is related to the demand and quality of irrigation water (Sierra, 2011; Rulkens, 2008). This creates conflicts because, on one hand, there is little availability of drinking water and, on the other, mismanagement of wastewater leads to economic, social and environmental problems (Monroy, 2013; Peña, 2013). In Mexico, between 1998 and 2007, the generation of wastewater from urban centers (municipal) increased from 239 to 243 $m^3 s^{-1}$, while industrial (non-municipal) discharges increased from 170 to 188.7 $m^3 s^{-1}$ in the same period. Of the total volume of wastewater generated in those years, only 25.3% was treated (INEGI, 2012). Seventy-seven percent of all water is used in agriculture, but 15% of all aquifers are already overexploited; thus, there is the need for substituting first use water with treated wastewater in this sector (Mejía et al., 2013).

Crop irrigation with wastewater can bring economic benefits by taking advantage of their contents of N and P, providing a supply of nutrients for plants and minimize the use of fertilizers, and supporting local food production (Plevich et al., 2012). In Mexico, in the Valle del Mezquital (Hidalgo), the volume of wastewater used for irrigation annually is between 10000 and 20000 $m^3 ha^{-1}$, with concentrations of 20 to 40 mg N L^{-1}; thus, irrigation with this water is equivalent to apply doses of 200-800 kg N ha^{-1} $year^{-1}$. In many cases, these amounts can cover the needs for N of some crops (Ramos, 1998).

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Two decades ago, the same dose of N represented an investment of between \$335.00 to \$1340.00 kg N ha^{-1} in the Valle del Mezquital (Hernández et al., 1993).

However, it is important to be cautious because wastewater not only contains nutrients, but also biological and chemical contaminants, such as heavy metals (Siebe, 1994; Méndez-García, 2000; Olmos and Alarcón-Herrera, 2014), which can damage human health (Rivera-Vázquez et al., 2007; Hernández-Acosta et al., 2014). According to the official standards established by SEMARNAT, the use of wastewater is defined in accordance to the sector or infrastructure that benefits from it; for example, urban use (irrigation of green areas, golf courses). However, the use of wastewater in agriculture depends on the type of treatment to which wastewater is subjected for use in irrigation of certain crops (*Table 1*). Westcot and Ayers (1984) indicate some fundamental criteria that urban wastewater must meet to be used in irrigation of agricultural and green areas (*Table 2*).

Table 1. *Criteria for the use of wastewater in Mexico (Pescod, 1992).*

Treatment	Vegetation type
Storage and retention for a few months	Not suitable for irrigation of crops that are eaten raw
Secondary	Suitable for irrigation of public parks Suitable for use in green areas along roads

Like other countries, Mexico has implemented programs for wastewater treatment since 2008, such as PROTAR (wastewater treatment program) and others, which seek to increase the volume of treated water and to improve the processes used for wastewater treatment. Under these projects, 2287 treatment plants were built throughout the country until December 2013 (CONAGUA, 2014); they operate according to the regulations of the General Law of National Waters (Atlas Digital del Agua, 2012). The quality of treated water must meet a series of physical, chemical and biological requirements. To this purpose, wastewater treatment involves a series of steps for recovering contaminated water its reuse. The disposal such treated water has effects on soil and on water bodies because wastewater treatment removes some contaminants, but not all, and many of them are toxic, such as heavy metals, which can be incorporated into the food web, causing direct or indirect damages to human health (Cañizares-Villanueva, 2000; Corinne et al., 2006; Rooney et al., 2006). For this reason, new alternatives for wastewater treatment have been developed, seeking to increase its reuse potential through different remediation techniques (CONAGUA, 2011, 2013a; Ferrera-Cerrato et al., 2006; Van Hamme et al., 2003).

Bioremediation refers to the optimization of naturally occurring remediation processes carried out by living organisms that degrade, alter or remove toxic organic compounds (Van Hamme et al., 2003). This biological strategy depends on the catabolic activities of organisms and on their ability to contribute to the degradation of contaminants of organic origin when using them as a source of food and energy (Pilon-Smits., 2005).The application of bioremediation techniques is an alternative for the disposal of non-treated wastewater during crop irrigation; the efficiency of bioremediation techniques depends on a number of factors that should be considered when choosing a treatment that provides the water quality that meets the needs of crops.

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First, one should consider the properties of the contaminants, since these define its potential for biodegradation, as well as the side effects that may affect the places from which contaminants have to be removed (Vassilev et al., 2004).

Table 2. *Requirements that treated urban wastewater must meet for use in irrigation of agricultural and green areas.*

Water quality	Type of crop or area to be irrigated	Irrigation method that can be used	Other conditions that must be met
Number of intestinal nematodes (*): <1 L^{-1} Number of fecal coliforms: <200/100 mL^{-1}	Irrigation of sports fields and green areas open to the public	Any	Irrigation should not be performed during public attendance hours
Number of intestinal nematodes (*): <1 L^{-1} Number of fecal coliforms: <1000/100 mL^{-1}	Irrigation of crops that are eaten raw	Any	
Number of intestinal nematodes (*): <1 L^{-1}	Irrigation of industrial, timber, fodder, cereal and oilseed crops, plant nurseries, crops for canning, plant products that are consumed cooked, and fruit trees	Any except: Spraying and flooding to irrigate vegetables Spraying to irrigate fruit trees	The irrigation of fruit trees with this type water should be suspended at least two weeks before harvest, and the fruit should not be collected from the ground The irrigation of grass for green fodder must be suspended at least two weeks before the cattle is allowed to graze
No limits are set but a treatment of at least primary sedimentation is required	Irrigation of industrial, timber, fodder, cereal and oilseed crops, as well as green areas not accessible to the public	Localized	

(*) *Ascaris, Trichuris* and *Ancylostoma* (Westcot and Ayers, 1984).

General considerations about wastewater

Wastewater discharge has increased in the last decades as a result of the of urban population. Water has become a central element of current environmental and economic policies, as well as a key development factor; all this has given urgency to the need to clean polluted water bodies in order to meet the needs of the population (Peña, 2013). The treatment of wastewater started during the industrial revolution; since then, it has been considered an environmental and social problem. In the nineteenth and twentieth centuries, many studies have tried to understand the composition of wastewater with the aim of devising better ways to remove and dispose wastewater contaminants (Tang, 2004; Parreiras, 2005).

Wastewaters are defined by the NOM-001 (1996) as waters of varied composition from industrial and municipal discharges that can have agricultural and domestic use. In

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addition, these waters contain inorganic elements that may have very different composition, from nutrients such as nitrogen and phosphorus, to toxic and hazardous substances (Domínguez-Mariana, 2004; Sierra, 2011). SEMARNAT (2002) defined wastewaters as waters from municipal, industrial, commercial, service, agricultural, livestock and domestic discharges, or from any other use, including a mixture of them. The classification of wastewaters is based on their origin; they are generally divided into urban and industrial. Urban wastewaters include sewage and domestic washing water, which contain detergents are always somewhat homogeneous in composition.

Industrial wastewaters come from any production, processing or handling process in which water is used; their composition may vary. These waters are more polluted than urban wastewaters, and, therefore, their contaminants are more difficult to remove (Félez-Santafe, 2009).

Purification of municipal wastewater

The treatment of urban and industrial wastewater is the most effective solution for the purification of effluents. However, it is very important to understand the toxic action of certain substances that can slow the purification process and even nullify the action of the microorganisms involved in the purification of water (Osorio et al., 2010). The treatments aimed at wastewater purification involve a number of physical, chemical and biological steps that seek to recover polluted water and, in many cases, return it to clean water (De la Peña et al., 2013). However, returning treated water to these channels is not recommended because these purification processes do not completely eliminate compounds that may cause damage to ecosystems, such as heavy metals.

The purification process is divided according to the biodegradability of wastewater components (Osorio et al., 2010): 1) the preliminary treatment removes large solid residues such as cans, rags, bottles, etc., as well as fatty contaminants (EPA, 2004); 2) the primary treatment removes suspended solids and organic matter by physical separation using methods such as gravity or chemical sedimentation, or filtration (CRA, 2000); 3) the secondary treatment removes 90% of the organic matter, turning it into a sedimentable biological floc (an agglomeration of organic matter, bacteria and minerals). This process is carried out using techniques such as activated sludge, aerated lagoons, trickling filters, biodiscs, and stabilization ponds (*Figures 1 and 2*) (Rulkens, 2008; Muñoz, 2011). Post-treatments, or tertiary treatments, consist in several anaerobic (for the degradation of organic matter); aerobic or facultative treatments used as a final complement for the removal of organic material and suspended solids. In addition, aerobic processes help remove heavy metals, nutrients and pathogens (CEPIS, 2002; Nelson et al., 2004).

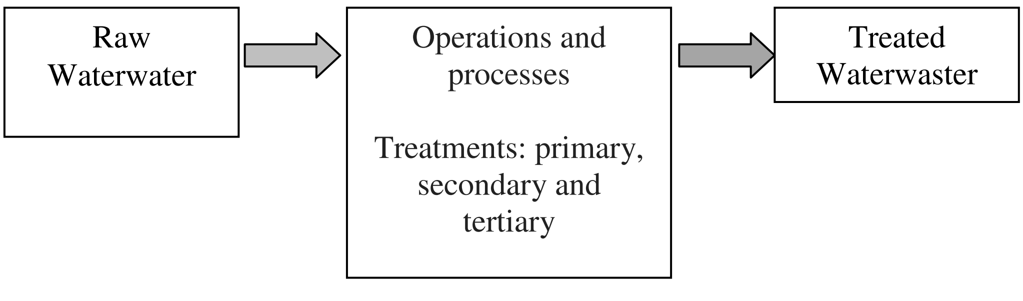

Figure 1. *General scheme of wastewater treatment (Crittenden et al., 2005).*

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<table>
<tr><td rowspan="6">Natural Processes</td><td rowspan="2">Unit Operations (Physical processes) Primary Treatment</td><td>Gravity precipitation</td><td>Silting</td></tr>
<tr><td>Impermeability of bodies</td><td>Filtration grids</td></tr>
<tr><td rowspan="4">Unit Processes (Chemical Processes and biological) Secondary Treatment</td><td rowspan="2">Separation layers polar and non polar</td><td>Defatting</td></tr>
<tr><td rowspan="2">Flocculation and coagulation</td></tr>
<tr><td>Abiotic agents</td></tr>
<tr><td>Biotics agents Tertiary Treatment</td><td>Degradation and metabolic absorption</td></tr>
</table>

Figure 2. *Operations and unit processes in the treatment of municipal wastewater (Tchobanoglous and Burton, 2005).*

In the coagulation process, wastewater contains suspended matter in the form of gels; and by adding metal salts, such substances tend to become unstable and begin a process of aggregation of dissolved organic matter. This promotes the aggregation of organic matter or bacteria, making it easier to remove them from wastewater (Ramírez, 2004).

The tertiary treatment may be aerobic or anaerobic, involving bacteria and protozoa that can be found suspended in the water or, on the contrary, fixed to a surface. In the aerobic processes, microorganisms degrade organic compounds such as sugars, fatty acids and proteins (Tchobanoglous and Burton, 2005). Under constant oxygenation, bacterial communities and suspended solids flocculate first and then precipitate as sludge (activated sludge and lagoons); once they are fixed to a surface, they can be removed by trickling filters and biofilters. In the anaerobic process, organisms act in two stages, a facultative stage and a strict one. In the first stage, known as acidogenic, the organic matter (OM) is broken down to its simplest organic acids; in the second stage, or acetogenic, fatty acids are broken down to its simplest parts and reduced to methane (CH_4) (Noyola, 1999). However, as in the aerobic process, reactors and anaerobic lagoons can be used in this process, while anaerobic filters can be used in fixed surfaces (Muñoz, 2011).

Wastewater treatment in Mexico

According to the National Water Commission (CONAGUA, 2007), the wastewater treatment regardless of the treatment of industrial discharges, is an indicator that refers to the percentage of wastewater collected from municipal sewer systems that is treated with activated sludge, and this percentage was 47.5% for 2012. However, CONAGUA (2011, 2013a) has recorded that stabilization ponds are the most used treatment for purifying municipal wastewaters in 732 treatment plants in Mexico, followed by activated sludge in 698 plants, while anaerobic upflow reactors are used in 188 plants, and the primary process is only utilized in 21 treatment plants (*Figure 3*).

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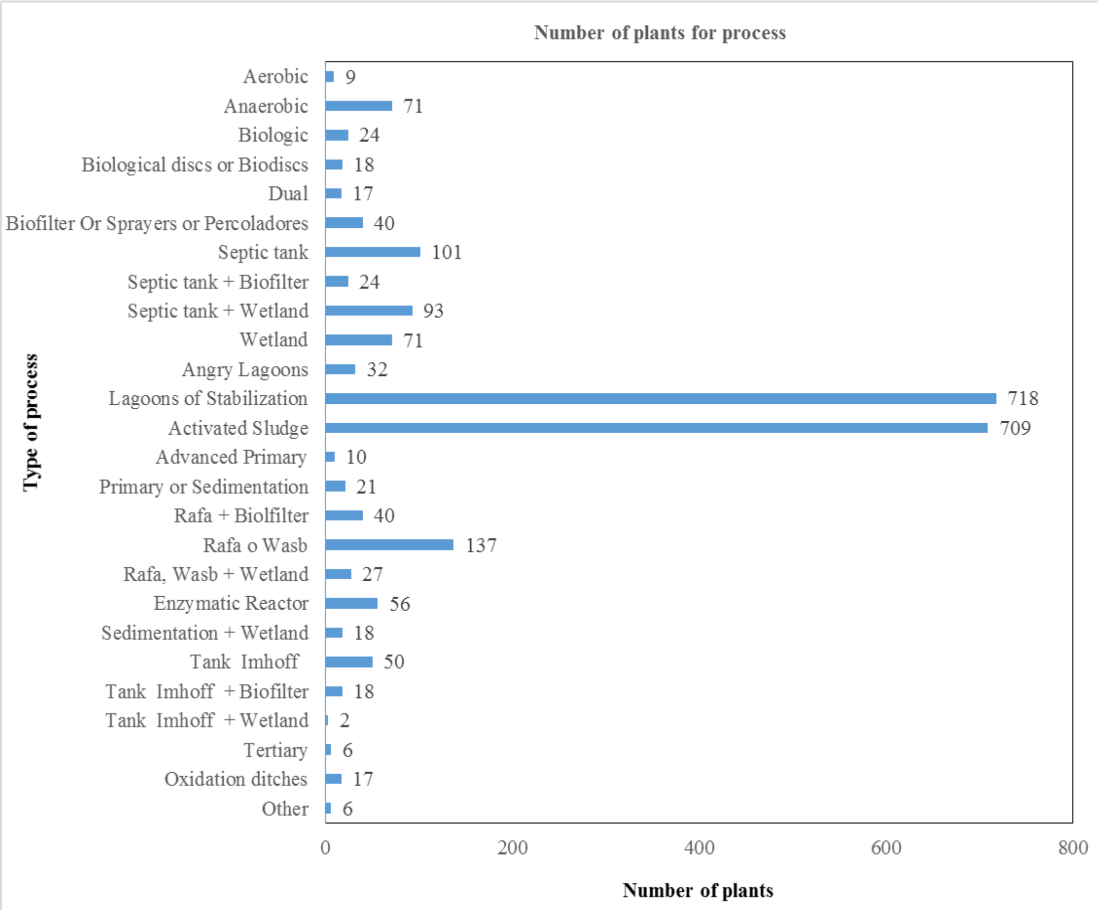

Figure 3. *Taken de:Inventario Native of Municipal Plants of Potabilización and of Treatment of Waste water in Operation (CONAGUA, 2014).*

Overview of wastewater reuse

Reusing wastewater is intended to take advantage of this water resource in other activities than those from which it was originated, but by previously subjecting it to a process of purification (Gutiérrez, 2003; Osorio et al., 2010).

In Latin America, about 400 $m^3 s^{-1}$ of wastewater are discharged into surface water sources; for example, in Colombia, 1,230,193 ha are irrigated with wastewater, of which 27% is treated wastewater, and 73% untreated but usually diluted with surface water (Silva et al., 2008). However, there is no complete and reliable information about wastewater reuse in Latin America (Cepis, 2003), and only 8% of the total wastewater that is generated daily, is treated (WSP, 2007). Thus, irrigation of agricultural areas is done mostly with untreated wastewater; Mexico generates more than half of the wastewater of all Latin America (Post, 2006).

The irrigation infrastructure in Mexico is one of the largest in the world, covering 6.46 million ha, but water availability is increasingly under pressure due to population growth. The distribution of water is based on the demand from the industrial, agricultural and municipal sectors, since there is the need to produce food and provide basic services to increasing human population (Elizondo, 2004). CONAGUA (2009) reported that, in 2007, Mexico generated 243 $m^3 s^{-1}$ of urban wastewater, of which 32.6% were treated; however, only 0.53 km^3 were treated properly, and at least 6.8 km^3

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of untreated wastewater were discharged into the environment. In 2009, the 2029 water treatment plants operating in the country treated 42%, of watewater, which is equivalent to 88.1 m^3 s^{-1}, of the total of 209.1 m^3 s^{-1} of the wastewater collected in sewer systems (Mejía et al., 2012). In 2011, 36% of all municipal wastewater was treated, removing 1.34 million tons of MO, which suggests that the generation of polluting matter will always be above the capacity to remove it; even if there was a notable increase in the number of the water treatment plants, which could reach 2709 by the year 2030, but the volume of wastewater treated would always be well below the total volume generated (SINA, 2012).

The agricultural sector requires the largest volume of water, ranging from 76.7% to 80% of the total available water; however, it only uses 46%, meaning that slightly less than half of the water is used for crop production. In contrast, the allocation of public water distribution service is about 14% (Mejia et al., 2012). Sainz-Santamaria and Becerra (2007) note that not all wastewater is discarded, since the agricultural sector reuses 2.43 km^3 each year. However, removing diluted contaminants from untreated and unused wastewater is extremely important. CONAGUA, 2014 brings the conditions that use in the agriculture residual water once (*Table 3*). In 2006, the World Health Organization issued new guidelines regarding the use of wastewater, excreta and greywater; these guidelines represent a tool for the preventive management of wastewater in agriculture and, thereby, for maximizing public health safety (WHO, 2006). The use of wastewater by farmers reveals an environmental tension between soil pollution and the emission of gases to the atmosphere, as well as jeopardizing human health, due to the toxic substances and pathogens that could enter the food chain. The contribution of N and P contained in wastewater consists on reducing the cost of fertilization and increasing the crop production (Monroy, 2013).

Table 3. *Plants of treatment of residual water for agricultural reuse for State, in Mexico.*

State	Number of plants of treatment	Total of flow treated in the plants (L s^{-1})
Aguascalientes	14	424.66
Baja California Sur	4	101
Coahuila	9	2,369
Chihuahua	2	8
Distrito Federal	5	2,319
Durango	16	2,258.2
Jalisco	1	3
Michoacán	2	105
Morelos	1	100
Nayarit	2	19
Nuevo León	9	265.8
Oaxaca	1	1
Puebla	23	697.4
San Luis Potosí	4	1,295.2
Sinaloa	8	278.8
Sonora	7	459.9
Tabasco	1	10
Zacatecas	7	130.5

Table realized from the National Inventory of Municipal Plants of Potabilización and of Treatment of Waste water in Operation (CONAGUA, 2014).

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In Mexico, urban wastewater has been used in agriculture for more than 100 years in the Valle del Mezquital, Hidalgo, the largest (100,000 ha) and oldest agricultural region irrigated with wastewater in the world (DFID, 1998). The wastewater generated by Mexico City and surrounding areas is the main source for the agricultural development of the Mezquital valley, in where there is paradoxically low availability of drinking water (Flores et al., 1997). The organic matter contained in the wastewater improves soil conditions and plant productivity, but also contributes to soil pollution, and endangers plants, environment, and human health (León-Suematsu, 1995).

Furthermore, the use of wastewater should be restricted due to its high content of salts, heavy metals, bacteria and viruses (*Table 4*) (Zekri and Koo, 1994), by which several developed and developing countries have regulated its utilization. From the environmental point of view, the reuse of wastewater in desert areas, in where water resources are scarce, also offers several advantages (Crook, 1984). This raises the need to develop appropriate management and monitoring techniques for allowing better control of this resource (Bouwer, 1992).

Table 4. *General composition of wastewater (DSEUA, 2000).*

Parameter	Units	Symbol
Chemical demand of oxygen	mg L^{-1}	DQO
Biochemical demand of oxygen	mg L^{-1}	DBO
Total suspended solids	mg L^{-1}	SST
Suspended solids	mg L^{-1}	SSV
Ammonia nitrogen	mg L^{-1}	$N\text{-}NH_3$
Organic phosphorus	mg L^{-1}	PO_4^{-2}
Total coliforms	NMP /10 mL	
Fecal coliforms	NMP /100 mL	
Helminth eggs	HH L^{-1}	

MPN=Most Probable Number.

Some case studies carried out in Mexico on the use of wastewater in the agriculture

Wastewater used for irrigation includes all possible degrees of epuration, from untreated water to those from tertiary treatment. In Mexico, some studies have been conducted to determine the quality of wastewater used for irrigation and the effect that this irrigation produces on the environment. Among them are the works performed at the Mezquital Valley by Siebe and Cifuentes (1993) who evaluated the epidemiological impact of wastewater used in agriculture at irrigation districts 03 and 100. The aim was to compare rain fed agricultural areas, at the State of Hidalgo. It was found prevalence of *Ascaris lumbricoides*, *Giardia lambia* and *Entamoeba histolytica* among the population. They also reported that the wastewater contains high concentrations of indicator organisms: 108 fecal coliforms/100 mL and 70 eggs of L-^{1}. It was concluded that fecal coliform concentration varies over time. Chilton et al. (1996) reported that the infiltration of wastewater has changed the hydro geochemical along the Valle del Mezquital. Castañón et al. (1995) reported that in the Valle de León, which is irrigated with wastewater with high chromium contents, it generates negative environmental impacts by contamination of groundwater, soil and the increase of infectious diseases among the population because the wastewater is used without any purification treatment. On the other hand, the same area has been irrigated with wastewater for over

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30 years on crop rotation of sorghum, wheat and alfalfa (Esteller, 2002). On the positive side it should be noted the increase of agricultural production in these areas and the improving of soil characteristics.

Sarabia-Meléndez et al. (2011) found that due to the limited availability of groundwater for irrigation use, some farmers in the municipalities of San Luis Potosi and Soledad de Graciano Sánchez, SLP, Mexico have long been used for irrigation of peri-urban agricultural areas, wells located in deep and shallow aquifers in the valley, and wastewater from domestic and industrial discharges. This causes a complex picture of crop production and public health risk by the presence of fecal coliforms.

Lucho-Constantine et al. (2005a) assessed the accumulation and distribution of the major elements in agricultural soils from 03 (DR03) District in the state of Hidalgo, Mexico, irrigated with untreated wastewater for an average of 20 years. They concluded that there is a significant correlation between the run time, the contents of Pb, Cd, Cr, B, M. O., organic C and easily exchangeable fraction. Meanwhile, Flores-Magdaleno et al. (2011) also conducted a study in Hidalgo on the effect of heavy metals in wastewater in soils of the municipality of Mixquihula. They found that when considering the limits established in Spain, concentration of As, Ni and Cd exceeds permissible level in 20, 60 and 60% respectively of the analyzed samples of wastewater and concentration of extractable metals in agricultural soils is present as follows: Pb> Ni> Cd> Como> Cr> Hg. The irrigation water presents no problems for use depending on the concentration of Hg, Cr and Zn. However, Pb concentration exceeds the maximum allowable.

Use of bioremediation techniques for wastewater treatment

The term "treatment technology" refers to any unit operation or series of unit operations that alter the composition of a hazardous or pollutant substance through chemical, physical or biological actions, so as to reduce the toxicity, mobility or volume of the contaminated material (EPA, 2004).

Bioremediation is used for the restoration of ecological damage through the optimization of certain living organisms (plants, fungi and bacteria , for instance) which are able to remove, degrade or transform toxic organic compounds into harmless or less toxic metabolic products (Van Hamme et al., 2003; Garbisu et al., 2002). The most common bioremediation techniques are phytoremediation, electrobiorremediation, leaching, chelation, methylation and precipitation (Martínez-Prado et al., 2011).

Phytoremediation consists on the use of plants and associated rhizosphere microorganisms that may favor the recovering contaminated water, soil and sludge. This biotechnology can be used *in situ* by which has no expensive costs nor secondary consequences, and consequently is an environmentally and ecologically friendly technology (Barceló and Poschenrieder, 2003; Nuñez et al., 2004; 2006; Delgadillo-López et al., 2011). Phytoremediation comprises mechanisms that reduce, *in situ* or *ex situ*, the concentration of several toxic compounds through physiological and biochemical processes performed by plants and microorganisms that remove (phytoextraction, phytodegradation or phytovolatilization), or immobilize (phytostabilization and rhizofiltration) contaminants from soil or water (Bernal et al., 2007). It should be noted that not all of these mechanisms are used in phytoremediation of wastewater (*Table 5*) (Delgadillo-Lopez et al., 2011).

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Table 5. *Bioremediation techniques commonly used for wastewater.*

Process	Mechanism	Contaminants
Phytoextraction	Hyperaccumulation	Inorganic
Phytodegradation	Using plants and associated microorganisms to degrade contaminants	Organic
Rhizofiltration	Using roots to absorb and adsorb contaminants from water	Organic and inorganic

Taken from Ghosh and Singh (2005).

The plants recommended for phytoremediation should be fast-growing, have high biomass production and tolerance, and accumulate metals; and they should preferably be local species, representative of the ecosystem and, finally, easily harvestable (Nuñez et al., 2009; Delgadillo-Lopez et al., 2011). Phytoremediation of heavy metals has been successful due to the ability of plants to absorb and accumulate these inorganic contaminants (Adriano, 2004; Lopez-Delgadillo et al., 2011), but plants differ on their capability for absorbing and accumulate toxic metals from water, soil and sludge (Delgadillo-López et al., 2011). Thus, plants have specific strategies against the presence of metals in their environment; some base their resistance on possessing efficient mechanisms for metal exclusion that restrict their transport to aerial parts; but others accumulate metals in the aerial parts as nontoxic chemical species (Llugany et al., 2007). The most common plants used for decontaminating heavy metals are *Impatiens balsamina* (L.), *Calendula officinalis* (L.), *Alcea rosea* L., *Spirodela polyrrhiza* (L.) Schleid., *Brassica juncea* (L.) Czern., *Phragmites australis* (Cav.) Trin. ex Steud., *Zea mays* (L.)., *Medicago sativa* (L.)., and *Lupinus luteus* (L.).

Some of these species are characterized as hyperaccumulator plants due to their capability to accumulate high amount of heavy metals in their tissues (Arenas et al., 2011).

Rhizofiltration is a technique similar to phytoextraction that can be used to decontaminate wastewater by filtering it through a mass of roots of hydroponically grown plants, so that the dissolved contaminants are adsorb or absorbed and accumulated (Nuñez et al., 2009; Cherian and Oliveira, 2005). Rhizofiltration processes have been directed to the wastewater treatment in Mexico, and some studies have used hydroponic *Helianthus annuus* (L.). and *Mentha deriva* (L.)., but the process has not been 100% effective, as these plants achieved only a partial removal of nitrates (60%) and phosphates, 40% (Torres-Calderón, 2009). Other studies have shown that, under hydroponic conditions, *Vetiveria zizanioides* (L.) (Seguier.), reduced the total nitrogen levels from 100 mg L^{-1} to 6 mg L^{-1} (94% efficiency), while the total phosphorus level was diminished from 10 mg L^{-1} to 1 mg L^{-1} (90% efficiency) (Truong et al., 2000).

The use of macrophytes to remove heavy metals from wastewater has produced good results. Nuñez et al. (2004) indicate that *Salvinia minima* (Seguier.), a tropical water fern, is very efficient for removing Pb and Cd. Furthermore, Alvarez et al. (2004) indicate that the water lily, despite being a problematic weed, has the ability to absorb heavy metals through its root system; and its efficiency of removing Cr from tanneries

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is about 75%, exceeding the provisions of Norm 002 for the content of chromium in wastewater. Bustamante and González (2013) report that *Myriophyllum aquaticum* (L.) can remove 53. 5% of Cr (VI) and 30% of Cu on average. Shahabaldin et al. (2015) described several sucessful studies of phytoremediation using *Eichhornia crassipes* (Mart) (water hyacinth) for removing heavy metals.

Among the studies conducted with *E. crassipes* (Mart) in wetlands, Lissy et al. (2011) reported up to 65% removal of Cr and Cu, while there are reports that the removal of Zn, Cu, Cd and Cr decreases in industrial wastewater (Yapoga et al., 2013).

The roots of *E. crassipes* (Mart) have the ability to quickly adsorb metals from an artificial wastewater system with different concentrations of $NiCl_2$ (1, 2, 3 and 4 mg L^{-1}), indicating that Ni was adsorbed through the roots in greater proportion rather than allocated in the aerial part (Gonzalez et al., 2015). The accumulation of metals by *E. crassipes* (Mart) is affected due to the age of the plant, temperature, pH, light intensity, the concentration of toxic metals, and competition with other ions and, furthermore, the absorption of Cr (IV) is favored by the presence of phenol, which is used as carbon and energy source (Gupta and Balomajumder (2015). Ajayi and Ogunbayo (2012) described that *E. crassipes* (Mart) is more efficient for removing Cd (94% removal) than Cu and Fe from wastewater from textile, metallurgical or pharmaceutical industries.

The use of aquatic plants to remove radioactive metals such as uranium has shown good results. *Callitriche stagnalis* (Scop).,*Potamogeton natans* (L.)., and *Potamogeton pectinatus* (L.)., native plants from central Portugal with high biomass accumulation are efficient species for being utilized for phytofiltration of uranium from water contaminated (500 g U L^{-1}), obtaining significant decreases in the content of U, and good accumulation of in plant tissues (Pratas et al., 2014). Ferrera-Cerrato et al. (2006) indicate that plants used in phytoremediation are characterized for their tolerance to extreme concentrations of organic or inorganic compounds, and for their physiological mechanisms involved in such resistance, tolerance, and survivability in contaminated water.

Some tree species can also be used in phytoremediation of wastewater. Guidi et al. (2014) demonstrated the high potential of willow plants (*Salix alba L.*) for phytoremediation of large volumes of wastewater in short rotations. For instance, willows plants were used to treat 5200 m^3 ha^{-1} of water with a high content of $N\text{-}NH_4^+$, achieving high decontamination efficiency mainly due to the high evapotranspiration rate and nutrient retention.

Microalgae is another biological alternative to remove N and P from wastewater (Unnithan, 2014). *Chlorella* and *Scedesmus* are common microalgae used for removing heavy metals (Garza et al., 2010; Canizares et al., 2013). Travieso et al. (2004) reported that the use of *Chlorella vulgaris* for tertiary treatment of swine wastewater in which the content of N and P was reduced, while *Scenedesmus dimorphus* was able to remove NH_4^+ and P in secondary treatments under bioreactor processes (Gonzalez et al., 1997). Other experiments under laboratory conditions reported that the removal of Cd was higher with *C. vulgaris* when compared with *Scedemus acutus*, but both species achieved a removal efficiency of Cr (VI) up to 80% (Canizares et al., 2013). The use of *Chlorella* sp. for bioremediation of aquaculture wastewater has also shown good results. Modh et al. (2015) reported a correlation between the growth kinetics of *Chlorella* sp. and the nutrients $N\text{-}NH_4^+$ and $P\text{-}PO_4$ contained in aquaculture wastewater; indicating that the optimal inoculation of *Chlorella* was 30% (v/v), by which the removal of $N\text{-}NH_4^+$ and $P\text{-}PO_4$ was 98.5 and 92.2 4%, respectively. In addition, the *Azolla-Anabaena*

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symbiosis produced some benefits in the bioremediation of wastewater. Some studies reported that this symbiosystem accumulate 13.3 g kg^{-1} of Cd, 2.27 g kg^{-1} of Se, and 3.36 g kg^{-1} of Cu (Sánchez-Viveros et al., 2013). The *Table 6* lists several of bioremediation studies conducted with wastewater in Mexico, and it is important to note that, until 2014, the research on bioremediation in Mexico mainly focused in soils contaminated with hydrocarbons or heavy metals.

Table 6. *Research lines carried out in various institutions and phytoremediation projects for wastewater in Mexico.*

Research line and projects being developed	Contaminant	Institution	State	Year
Phytoremediation of wastewater from pig farms and coffee processing plants.	Organic matter and nutrients	INECOL	Veracruz	1994
Biosorption of Pb Cd, cadmium and As using two tropical floating aquatic plants (Spirodela polyrrhiza and *Salvinia minima*) in plug flow lagoons	Heavy metals	INECOL	Veracruz	2001
Development of a bioadsorbent from the biomass of *Spirulina sp.*	Heavy metals	INECOL	Veracruz	2001
Removal of Ar from mining effluents and groundwater with native accumulating plants through the implementation, at pilot-scale, of constructed wetlands	Heavy metals	CIMAV	Chihuahua	2001
Electrochemical removal of metals from plant biomass or biosorbents.	Heavy metals	CIDETEQ	Querétaro	2001
Use of water hyacinth (*Eichhornia crassipes*) for the removal of heavy metals from contaminated water.	Heavy metals	CIDETEQ	Querétaro	2001
Production of phytochelatins by *Salvinia minima* exposed to Pb and As.	Heavy metals	CICY	Yucatán	2001
Enhancement of the removal capacity of Pb and As by aquatic plants	Heavy metals	CICY	Yucatán	2001
Removal of contaminants and pathogens from wastewater by the vertical root method, using plants from the region	Organic matter and pathogens	UADY	Yucatán	98-02
Phytoremediation of heavy metals in the Tenorio tank and its environmental impact	Heavy metals	UASLP	San Luis Potosi	2002
Phytoremediation and bioadsorption for the sustainable use of water	Heavy metals	INECOL	Veracruz	2004
Phycoremediation (using algae) and phytoremediation (using plants) for removing contaminants from wastewater		INECOL	Veracruz	2006

Modified from Nuñez et al., 2004.

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Perspectives and conclusions

In general, the Mexican agricultural sector is the main consumer of water resources, whose availability is scarce in some areas, making it necessary to direct bioremediation techniques to reuse wastewater from municipal sources. However, it is advisable to treat wastewater before using it, but in practice, it is used without prior treatment. Thus, bioremediation should be considered as an efficient technology for cleaning and reusing wastewater.

Bioremediation is a low-cost technology that does not require complex infrastructure, and can be used to treat large volumes of wastewater. In developed countries, bioremediation is highly efficient for removing, transforming or degrading different types of contaminants found in wastewater, particularly using the mechanisms of phytoextraction and rhizofiltration.

Wastewater is mostly used to irrigate horticultural crops for direct consumption, due to its contribution on plant nutrition, which reduces crop costs due to limited fertilizer application. However, this practice has potential risks to public health, and thus, it should be reconsidered, and the prior treatment of wastewater must be encouraged.

Plants are able to adsorb, absorb, metabolize, accumulate, stabilize, or even volatilize organic or inorganic contaminants. As a biotechnology, phytoremediation offers advantages over conventional methods of wastewater remediation. However, further research is needed to reassess and validate the benefits and the effectiveness of bioremediation/phytoremediation systems for cleaning and detoxifying wastewaters, particularly in countries like Mexico.

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GEOLOGIC STRUCTURAL CONTROLS ON COALBED METHANE CONTENT OF THE NO. 8 COAL SEAM, GUJIAO AREA, SHANXI, CHINA

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Abstract. Gas contents are highly heterogeneous in coalbed methane (CBM) reservoirs of the Gujiao area, Shanxi province, China. Diverse geologic data and stimulate methods were used to provide insight into the origin of this variability and the consequences of gas content in the No. 8 coal seam. The results show that the No. 8 coal seam experienced two gas-generating periods and one fluctuant period, and has stepped into migration period since the Late Cretaceous. The distribution of gas content of the No. 8 coal seam in the Gujiao area is mainly dominated by structural form, water level and burial depth, all of which are structural related parameters. The gas content in the subarea A is much lower than that in the subarea B because the subarea A is deformed severely and the subarea B is a gentle monocline. The gas content first increases and then decreases with increasing burial depth, and the turning burial depth is about 600.0 m. Strong runoff area is harmful for preserving gas due to the washing effect in it. The analyzing results show that: gentle and simple monocline blocks with burial depth ranges from 500.0 m to 800.0 m, water level lower than 900.0 m is the most important “sweet spots” for CBM development in the Gujiao area.

Keywords: *coalbed methane; gas content; structural controls; Gujiao area; Xishan coal field*

Introduction

With the decline in conventional natural gas reserves and increased demand and price of natural gas, industry has shown great interest in coalbed methane (CBM) resources in recent decades, which requires accurate estimation of CBM potential and recoverable reserves to assist in its development (Karacan, 2009; Zhang et al., 2010). Basin-scale investigation of the CBM resource have been performed in the Piceance and Matanuska basins in the United states (Johnson and Flores, 1998; Payne and Ortoleva, 2001), the Bowen and Sydney basins in Australia (Boreham et al., 1998; Faiz et al., 2007), and the Qinshui and Ordos basins in China (Wang et al., 2013; Cai et al., 2014). Previous reports and papers show that geologic structure is the most important control factor of reservoirs of CBM. The generation, migration and preservation of CBM during geologic history are dominated by tectonic evolution movements (Scott et al., 1994). Plutonic and thermal metamorphisms determine the gas generation (Liu et al., 2005), and the generated gas

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content continuously increases with increasing $R_{o, max}$ (the maximum vitrinite reflectance) (Qin et al., 1997). Tectonic subsidence is helpful for preserving gas, but uplifting movement will cause the migration of gas (Song et al., 2012). Structural deformation, burial depth and hydrodynamic condition are key parameters controlling the gas distribution (Meng et al., 2014a). Despite the great number of papers and reports, important questions about controls of geologic structure on gas content remain unanswered, and what have mainly mentioned by former researchers are the distribution and evolution of gas content and permeability, which are the most important factors affecting gas production. However, previous resource evaluations haven't taken the geologic structure into consideration. Moreover, the researches of resource evaluation and geologic structure in China mainly have concentrated in the famous southern Qinshui basin (SQB) and eastern Ordos basin (EOB) (Lv et al., 2012; Meng et al., 2014b; Zhao et al., 2015). Related research is necessary but absent in many CBM-producing basins in China, especially in the Xishan coal field (XCF).

Compared with the SQB and EOB, the XCF has very short CBM exploration and development history (the CBM development was started in 2011) and much more complicate structural conditions, in which Gujiao is the only one area has been put into CBM development (Wang et al., 2015a). However, the CBM development in the Gujiao area is unsuccessful (the average gas production is about 277 m^3/d per well) due to its' complicate geologic structure and the absent of resource evaluation (Xia et al., 2016).

Herein, the effects of geologic structure on the distribution of the gas content in the No. 8 coal seam were analyzed based on the coal core testing data, logging interpretation results, coalmine geology data, field geology data and water chemical measurements. Then the favorable geologic structural conditions for preserving gas were recognized. This study will be helpful for the CBM development, and will give instructions for adjustment of CBM development plan in the Gujiao area.

Geologic background

The XCF (*Figure 1*), located in the center of Shanxi province, China, extends eastward into the Lvliang uplift, westward into the Taiyuan fault depression, northward into the Wutai mountain and southward into the Jinzhong fault depression. The XCF subjected to four orogenies; the Indosinian, Early Yanshanian, Late Yanshanian and Himalayanian orogenies (Wang et al., 2007). The Pennsylvania Taiyuan and Permian Shanxi Formations are the main coal-bearing strata (*Figure 2*) in the XCF. The Gujiao area, distributed in the northwest of the XCF, is the only area put into CBM development. The No. 8 coal seam in the Taiyuan formation is the primary target zone for CBM development with a thickness range of 0.56 m - 4.95 m (the average is 3.15 m), burial depth is 180.6 m - 905.8 m (the average is 611.1 m), maximum vitrinite reflectance ($R_{o, max}$) is 1.23 % - 2.22 %. The gas content of the No. 8 coal seam ranges from 4.0 m^3/t to 16.5 m^3/t, and the CH_4 concentration changes between 79.34 % and 99.34 % (Pang et al., 2015).

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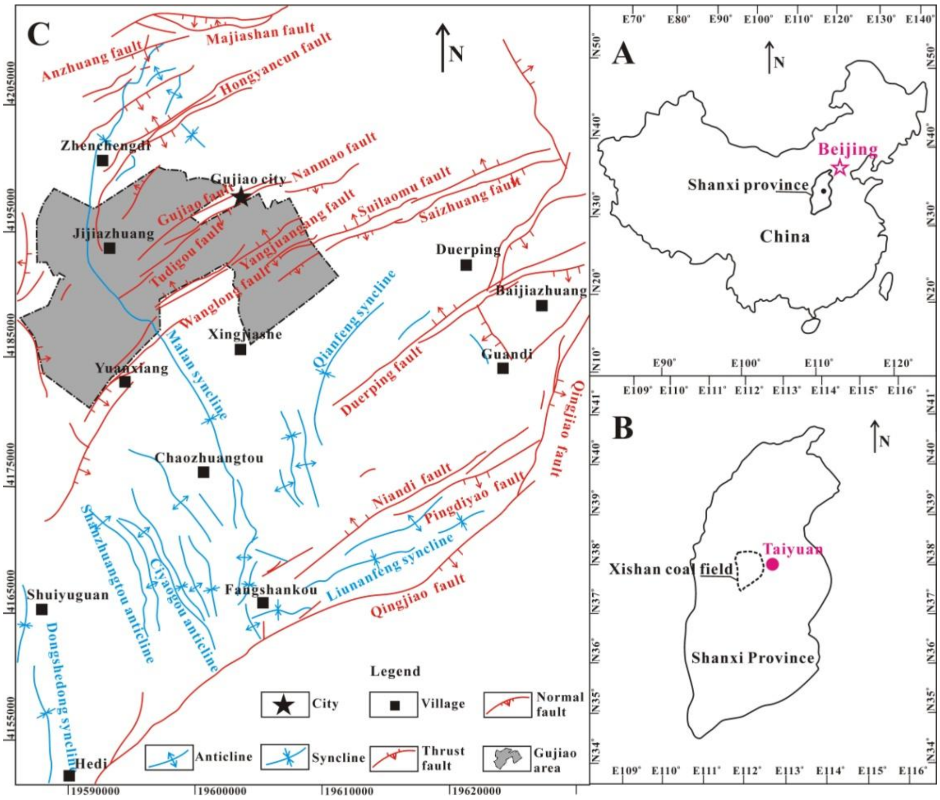

Figure 1. *Study area. (A) Location of Shanxi province in China. (B) Location of the XCF in Shanxi province. (C) Location of the Gujiao area in the XCF.*

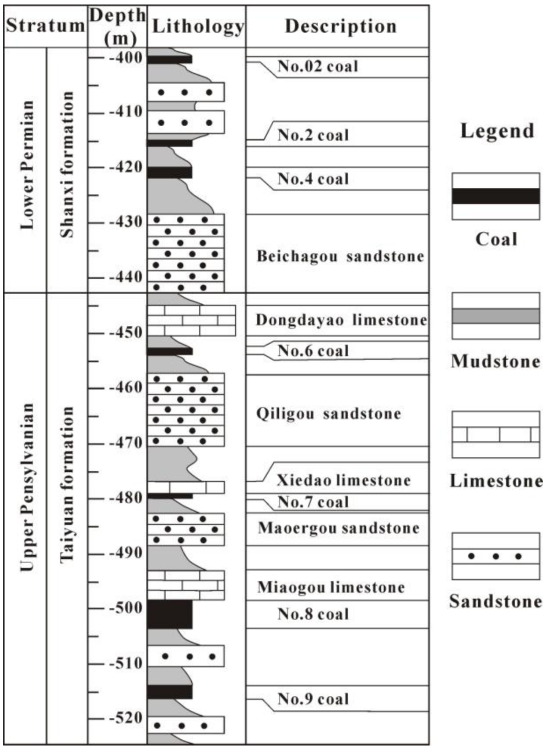

Figure 2. *Lithology column of the coal-bearing formations in the Gujiao area, XCF.*

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Samples and experiments

A total of 45 core coal samples were collected from the No. 8 coal seam from 45 CBM wells in the Gujiao area following the Chinese National Standard GB/T 19222-2003 (*Figure 3*). The samples were immediately sealed up in desorption canisters, in which the coalbed gas naturally desorbed from the coal matrix surface. The volume and composition of the desorbed gas were measured following the Chinese National Standards GB/T 19559-2008 and GB/T 13610-2003, respectively. The methane isothermal adsorption experiment and the maximum vitrinite reflectance ($R_{o,\ max}$) test of 3 coal core samples (E006, C046 and W195) were conducted using isothermal adsorption and desorption instruments IS-300 following the Chinese National Standard GB/T 19560-2004, and using polarizing microscope Leica DM4500P following the Chinese National Standard GB/T 6948-1998, respectively. Moreover, 30 produced water samples were collected from 30 CBM wells in the Gujiao area. Considering the comparability between gas distribution and water composition, many water sampling points were coincident with the coal core sampling points. The water sample containers were carefully washed more than 3 times before collecting water. The water samples were immediately sent to laboratory to measure their chemical composition. Finally, the body structure of the No. 8 coal seam were observed and described in active underground coalmines in the Gujiao area (*Figure 4*). All testing results are documented in *Table 1 to Table 3*.

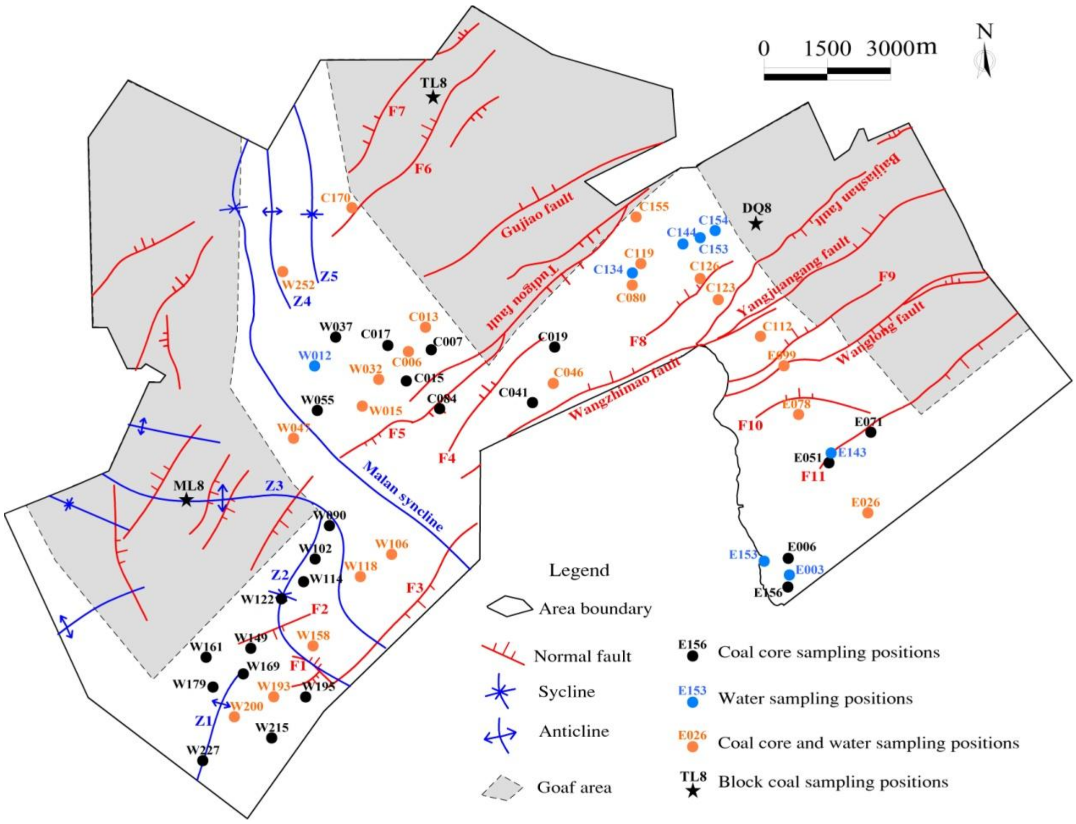

Figure 3. *Structural sketch of the Gujiao area showing sampling points.*

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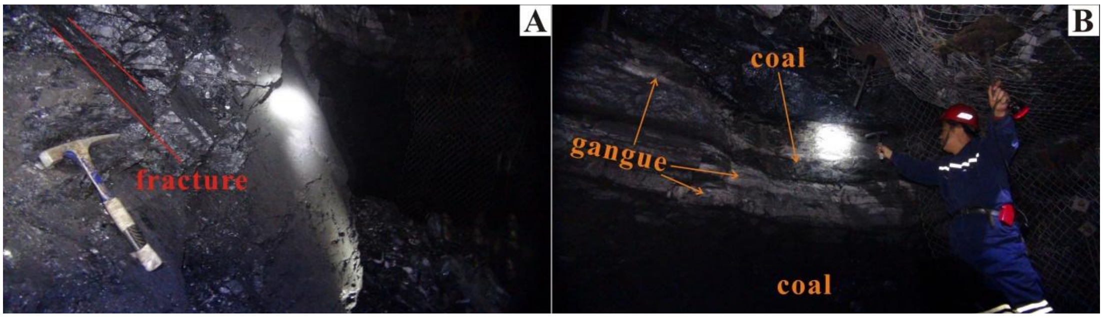

Figure 4. *The photos of coal seams took in underground coalmines by anti-explosion camera (the person in B is Prof. Xiaoxia Song, who is a co-author of this work).*

Table 1. *Burial depth, gas content and gas composition of the No. 8 coal seam in the Gujiao area*

Sample ID.	Burial depth (m)	Gas content (m^3/t)	Proximate analysis (%)			Gas composition (%)		
			M_{ad}	A_{ad}	V_{ad}	N_2	CO_2	CH_4
E006	483.8	7.9	1.02	36.13	20.14	2.01	0.28	97.71
E026	564.1	10.2	0.66	23.47	18.39	5.26	2.26	91.81
E051	502.5	12.4	0.49	11.53	14.23	5.69	1.11	93.2
E071	452.1	9.8	0.51	22.21	17.59	0.99	0.53	97.91
E078	444.4	7.6	0.72	30.17	17.87	17.31	0.53	82.15
E099	554.5	12.2	0.58	19.94	16.01	6	1.42	92.58
E156	473.9	9.2	0.64	19.49	17.95	2.6	0.28	97.12
W015	785.5	12.2	0.83	10.36	19.25	0.84	1.61	97.55
W032	853.7	12.2	0.6	14.39	18.58	3.09	1.8	95.11
W037	859.6	4.8	0.58	19.61	23.11	9.1	1.42	88.32
W047	773.7	5.4	0.7	35.26	25.15	12.62	1.46	85.39
W055	830.9	5.5	0.58	13.25	23.42	9.93	2.02	85.55
W090	491.7	9.6	0.56	15.19	22.87	3.98	0.54	95.48
W102	556.6	5.1	0.92	38.12	26.16	9.73	1.33	86.03
W106	709.7	4.1	0.53	47.03	27.48	23.43	1.63	74.94
W114	565.3	5.5	0.54	14.84	22.23	2.83	1.05	96.12
W118	615.4	8.1	0.9	26.28	22.46	0.98	0.44	98.42
W122	651.3	7.4	0.84	20.85	24.53	19.11	1.5	79.34
W149	620.6	6.4	0.75	6.43	18.93	11.05	0.74	87.95
W158	681.1	6.2	0.76	26.69	22.69	15.46	2.03	81.31
W161	546.2	5.0	0.54	36.64	25.34	10.44	0.85	88.55
W169	673.2	6.6	0.82	24.02	19.94	5.42	0.67	93.91
W179	576.3	4.9	0.87	24.85	17.36	9.89	0.74	87.65
W193	644.5	7.9	0.74	21.27	19.09	4.85	1.72	93.3
W195	699.2	10.3	0.82	10.26	18.63	1.86	0.52	93.34
W200	653.7	4.3	0.75	31.84	18.39	x	x	x

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W215	598.8	6.5	0.86	20.85	15.98	20.89	1.22	77.79
W227	428.8	5.4	1.25	25.64	11.93	2.21	0.33	97.32
W252	460.5	8.1	0.68	8.39	23.72	6.84	0.82	92.34
C006	783.0	9.9	0.4	11.22	21.14	5.59	1.51	92.9
C007	760.4	12.5	0.48	15.61	19.6	0.4	1.1	98.5
C013	716.2	13.1	0.41	14.82	18.48	0.94	1.04	98.02
C015	751.5	10.2	0.76	31.09	22.01	14.02	1.73	84.26
C017	802.5	12.2	0.72	12.24	18.74	1.46	1.42	96.98
C019	610.0	16.1	0.66	25.97	18.63	4.79	1.13	93.93
C041	680.0	15.4	0.59	12.26	17.23	11.13	1.07	87.79
C046	593.1	13.9	0.74	14.66	16.86	1.22	2.13	96.66
C080	508.5	9.7	0.49	20.75	17.89	0.34	0.32	99.34
C084	853.6	12.7	0.62	11.38	17.63	0.36	1.47	98.17
C112	486.0	10.1	0.61	8.69	13.92	6.24	0.86	92.9
C119	461.6	10.0	0.8	14.56	16.75	0.98	0.29	98.73
C123	446.2	12.1	0.6	13.96	15.41	1.06	0.41	98.53
C126	379.9	8.2	0.4	10.19	14.71	3.26	0.3	96.44
C155	471.9	7.5	0.65	30.58	21.59	1.8	0.23	97.98
C170	441.6	6.2	0.48	24.89	23.14	13.73	0.77	83.4

Note: M_{ad}, Moisture content (as air dry basis); A_{ad}, Ash content (as air dry basis); V_{ad}, Volatile matter content, (as air dry basis); x, not analyzed.

Table 2. *$R_{o, max}$, Langmuir parameters and coal structure of the No. 8 coal seam in the Gujiao area*

Sample ID.	Coal lithotype	Coal structure	$R_{o, max}$ (%)	Coal macerals (%)			P_L (MPa)	V_L (m^3/t)
				V	I	M		
E006	semi-bright	un-deformed	1.83	x	x	x	1.69	23.33
C046	semi-bright	un-deformed	1.89	x	x	x	1.63	27.91
W195	semi-bright	un-deformed	1.91	x	x	x	1.55	22.34
DQ8	semi-bright	un-deformed	1.85	88.6	10.4	1.0	x	x
TL8	semi-bright	un-deformed	1.76	78.9	18.4	2.7	x	x
ML8	semi-bright	slight-deformed	1.96	85.6	12.9	1.5	x	x

Note: V, vitrinite; I, inertinite; M, minerals; P_L, Langmuir pressure; V_L, Langmuir volume; x, not analyzed.

Table 3. *Statistical data of water type in the Gujiao area*

Sample ID	Cation (mg/L)			Union (mg/L)			M (mg/L)	PH	Water type
	K^+	Na^+	Ca^{2+} $+Mg^{2+}$	Cl^-	$SO4^{2-}$	HCO_3^- $+CO_3^{2-}$			
C006	5.82	1054.64	12.09	335.68	99.20	1892.53	2512.0	8.38	$NaHCO_3$
C013	3.65	914.70	8.08	136.16	52.89	1771.01	1955.0	8.48	$NaHCO_3$

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C046	2.17	999.18	9.19	325.78	103.52	1774.41	2405.0	8.69	$NaHCO_3$
C080	1.85	483.80	10.16	121.14	33.14	1022.81	1224.0	8.31	$NaHCO_3$
C112	0.94	207.60	6.83	92.58	45.48	931.50	1162.0	8.81	$NaHCO_3$
C119	1.31	471.87	7.44	140.12	54.54	803.59	1065.0	8.48	$NaHCO_3$
C123	0.94	402.12	7.45	155.46	49.60	640.02	923.0	8.36	$NaHCO_3$
C126	1.45	526.04	8.69	114.86	49.18	1041.68	1230.0	8.49	$NaHCO_3$
C134	1.35	359.20	6.63	136.89	19.97	718.74	906.0	7.82	$NaHCO_3$
C144	0.89	248.00	7.05	123.12	30.26	410.22	634.0	7.73	$NaHCO_3$
C153	1.14	272.20	16.07	116.22	32.31	556.00	736.0	7.97	$NaHCO_3$
C154	0.94	231.40	9.22	111.30	35.61	450.91	632.0	7.82	$NaHCO_3$
C155	1.23	393.34	6.92	141.60	46.30	635.91	904.0	8.30	$NaHCO_3$
C170	4.71	743.14	6.73	64.85	37.25	1556.36	1570.0	8.65	$NaHCO_3$
E003	1.70	251.20	8.81	99.48	37.25	488.20	664.0	8.19	$NaHCO_3$
E026	3.95	817.13	20.32	242.60	54.12	1654.24	2027.0	8.35	$NaHCO_3$
E078	3.18	820.61	6.40	106.44	80.46	1564.04	1814.0	8.56	$NaHCO_3$
E099	2.05	273.63	11.76	101.99	47.54	1322.86	1512.0	8.32	$NaHCO_3$
E143	5.02	898.17	8.26	137.64	61.94	1731.38	1966.0	8.50	$NaHCO_3$
E153	3.30	327.60	7.15	124.10	40.14	681.44	862.0	8.13	$NaHCO_3$
W012	2.97	458.00	12.04	63.03	31.48	1138.41	1192.0	8.36	$NaHCO_3$
W015	3.88	777.94	15.92	82.68	51.66	1560.58	1690.0	8.47	$NaHCO_3$
W032	0.57	156.78	35.52	52.97	53.72	413.96	510.0	7.37	$NaHCO_3$
W047	6.03	926.56	12.86	56.94	64.41	1931.06	2032.0	8.49	$NaHCO_3$
W106	4.38	872.19	4.77	56.44	63.18	1735.74	1904.0	8.50	$NaHCO_3$
W118	3.57	866.85	10.64	60.90	79.64	1747.83	1930.0	8.39	$NaHCO_3$
W158	2.46	859.24	8.39	60.90	120.80	1724.53	1931.0	8.44	$NaHCO_3$
W193	4.13	743.55	13.11	49.02	61.12	1673.69	1670.0	8.37	$NaHCO_3$
W200	3.09	383.03	5.68	19.30	74.30	784.00	886.0	8.43	$NaHCO_3$
W252	3.89	710.80	9.73	72.78	64.00	1419.85	1596.0	8.57	$NaHCO_3$

Note: M, mineralization.

Results and discussions

Geologic evolution

Geologic evolution history

After the Middle and Late Ordovician, the North China platform was overall uplifted due to the Caledonian crustal movements, which resulted in unconformity in the sedimentary record from the Late Ordovician to the Early Carboniferous (Kusky et al., 2007). Subsidence began again in the Middle Carboniferous due to the Hercynian crustal movements and the Permo-Carboniferous coal-bearing strata were deposited during this stage (Yang et al., 2005).

Based on the results in previous research in the XCF (Su et al., 2005; Wang et al., 2007; Sun et al., 2014; Liu et al., 2014), and logging interpretation data, the burial history

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of the Taiyuan formation at CBM wells E006, C046 and W195 were built in the 1D package of Petromod software by back-stripping method in this study. The Taiyuan formation has experienced seven tectonic evolution stages. As can be seen in *Figure 5*, stage I and stage IV were two slow subsidence stages from the Late Carboniferious to the Late Permian and from the Middle to the Late Jurassic, respectively. Stage II was a rapid subsidence stage from the Late Permian to the Triassic, during which the Taiyuan formation was rapidly buried with the maximum burial depth is about 4000.0 m. This formation was uplifted transitorily and slowly during stage III. Stage V and stage VI were two rapidly uplifting stages, during these two stages, the Taiyuan formation was uplifted and of which the Upper formation was eroded heavily. Stage VII is a stage where uplifting and subsidence have coexisted since the Paleogene. As a result of the evolution of burial history, the present burial depth of the No. 8 coal seam in the Gujiao area ranges from 180.6 m to 905.8 m.

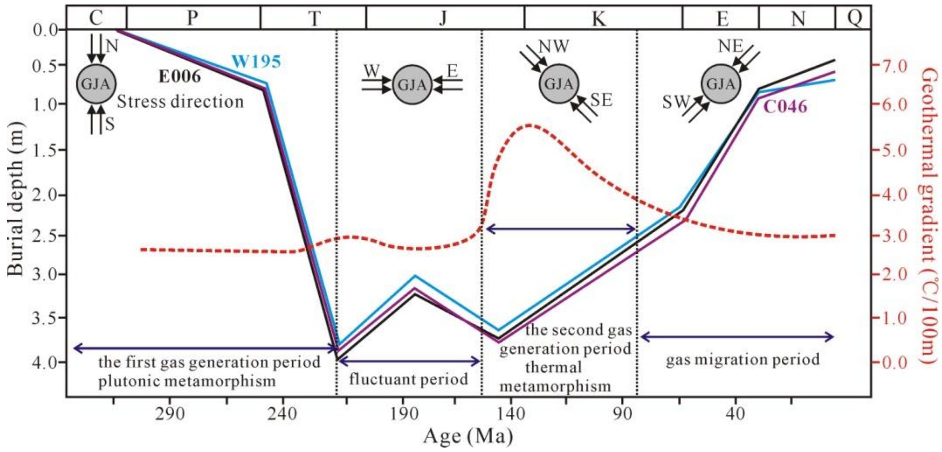

Figure 5. *Burial and geothermal gradient curve of the No. 8 coal seam in the Gujiao area (data of red line from Liu et al., 2005).*

Before the Yanshanian orogeny movement, the XCF was a craton basin with a slightly lower geothermal gradient of 2.6 ^{o}C/100m - 2.9 ^{o}C/100m (Sun et al., 2014). During the Yanshanian orogeny, which occurred in the early stage V, the igneous intrusions in the south part of the XCF caused abnormal high geothermal gradient (5.0 ^{o}C/100m – 6.0 ^{o}C/100m), and the geothermal gradient has decreased to a normal value after the Yanshanian orogeny movement (Wang et al., 2007).

Evolutionary process of CBM generation and accumulation

Based on the geologic evolution history of the Taiyuan formation, four periods of gas generation and accumulation can be divided considering the metamorphic types of coal and the preservation condition of CBM. The coal thickness, roof thickness, $R_{o,\ max}$, gas content and proximate components of the No. 8 coal seam in well E006, C046 and W195 were used (*Table 1 and 2*) to simulate the evolution history of gas content by referenced

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the experiment results in Qin et al., (1997), and the simulation method in Wei et al. (2007 and 2010). The results are shown in *Figure 6*.

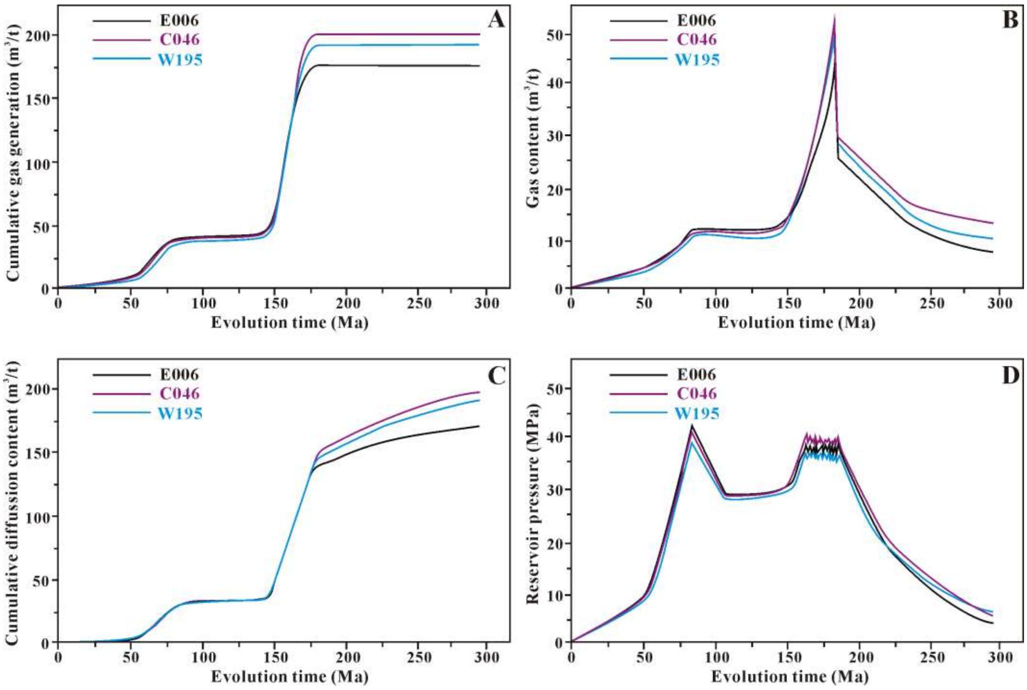

Figure 6. *Coalbed methane evolution history curves of the No. 8 coal seam in the Gujiao area.*

The first period ranged from the Late Carboniferous to the Triassic (*Figure 5*), during which the XCF was compressed by the NS trending stress due to the collision between the North China plate and the South China plate (Lin, 1991), and the plutonic metamorphism improved the maturity of coal and generated a certain amount of gas (the first gas generation period) (Liu et al., 2005). The $R_{o,\ max}$, cumulative gas generation and gas content of the No. 8 coal seam could as higher as 0.70 %, 50.0 m^3/t and 15.0 m^3/t, respectively, at the end of this period. The second one was a fluctuant period for gas accumulation, in which the coal rank and cumulative gas generation of the No. 8 coal seam were slightly increased, and the gas content didn't change notably. Owing to the Lvliang uplifting movement under the WE trending stress during this period, the rudiment of the XCF was formed (Guan and Li, 2001). The third period was the second gas generation period from the Late Jurassic to the Early Cretaceous, during which the plutonic metamorphism ended because of the uplifting movements (Wang et al., 2007). However, the thermal metamorphism caused by the igneous intrusion improved greatly the coal rank of the No. 8 coal seam, and a great amount of gas was generated. The $R_{o,\ max}$, cumulative gas generation and gas content of the No. 8 coal seam could reach 2.00 %, 200.0 m^3/t and 30.0 m^3/t, respectively. The last period is the gas migration period since the Late Cretaceous, during which, continuously uplifting movements destroyed the gas equilibrium again and again owing to the release of overburden stress (Su et al., 2005). As

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a result, the coalification and gas generation are ended, and the previous gas is desorbed and migrated. The residual gas content in the No. 8 coal seam ranges from 4.0 m^3/t to 16.5 m^3/t. The Jinzhong and Taiyuan grabens developed during this period because the effects of the Himalaya orogeny, and these two grabens separated the XCF from the Qinshui basin completely (Fu, 2008).

The geologic evolution history has determined the coal rank, gas generation and migration, but could not individually explain the present distribution of gas content, because which is affected by various parameters, especially by structural form, burial depth and hydrodynamic condition (Bustin and Clarkson, 1998; Jiang et al., 2010; Wang et al., 2013), and hydrodynamic condition and burial depth are controlled by structural form (Cai et al., 2014; Meng et al., 2014c).

Structural geometry

Structural geometry characteristics

The structural map of the No. 8 coal seam in the Gujiao area was drew (*Figure 7*) based on the data of coalmining geology and CBM well-logging interpretation, and previous research (Guan and Li, 2001; Fu, 2008; Wang et al., 2015a), which reflects that the Gujiao area is a NS axial striking syncline (Malan syncline). The structural geometry of the east wing of the Malan syncline is far different with the west one. The east wing is a gentle monocline including many large normal faults with average dip angle is 4.6^o. However, the west wing is a steep monocline containing many secondary folds with average dip angle is 24.5^o. According to the completely different structural characteristics between the two wings of the Malan syncline, the Gujiao area has been subdivided into two subareas (subarea A distributes in the west wing and B in the east) by the axial of the Malan syncline. Burial depth and water level are two structure-dominated parameters, and usually higher elevation corresponds to shallower burial depth and higher water level, and vice versa (Perrier and Quiblier, 1974).

Structural form and gas content

The CBM is mainly adsorbed in the coal matrix, only a little CBM (less than 10 %) are free or dissolved gases (Bustin and Clarkson, 1998). The structural deformations of coal seams during geologic evolution have released the tectonic stress and then the adsorbed gas has been desorbed and migrated (Meng et al., 2014a). As a result, the gas content in the coal seams decreases continuously. The No. 15 coal seam in the SQB has nearly same rank, composition and burial depth with the No. 8 coal seam in the XCF, however, the gas content of the No. 15 coal seam is much higher than the No.8 (Lv et al., 2012; Wang et al., 2015a) duo to that the SQB is a relatively complete and uniform tectonic block, and the XCF is much complicate and multiform. The tectonic stress from the east to the west on the Gujiao area during the Early Yanshanian orogeny creates the Malan syncline, and because the obstruction of the Lvliang mountain, the subarea A was the passive region and deformed heavily. However, the subarea B was the driving region and in which the

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plastic deformations were few but brittle fractures were developed (Gui, 1986). As a result, the subarea B is a relatively uniform block and the subarea A is a multiform block in the Gujiao area.

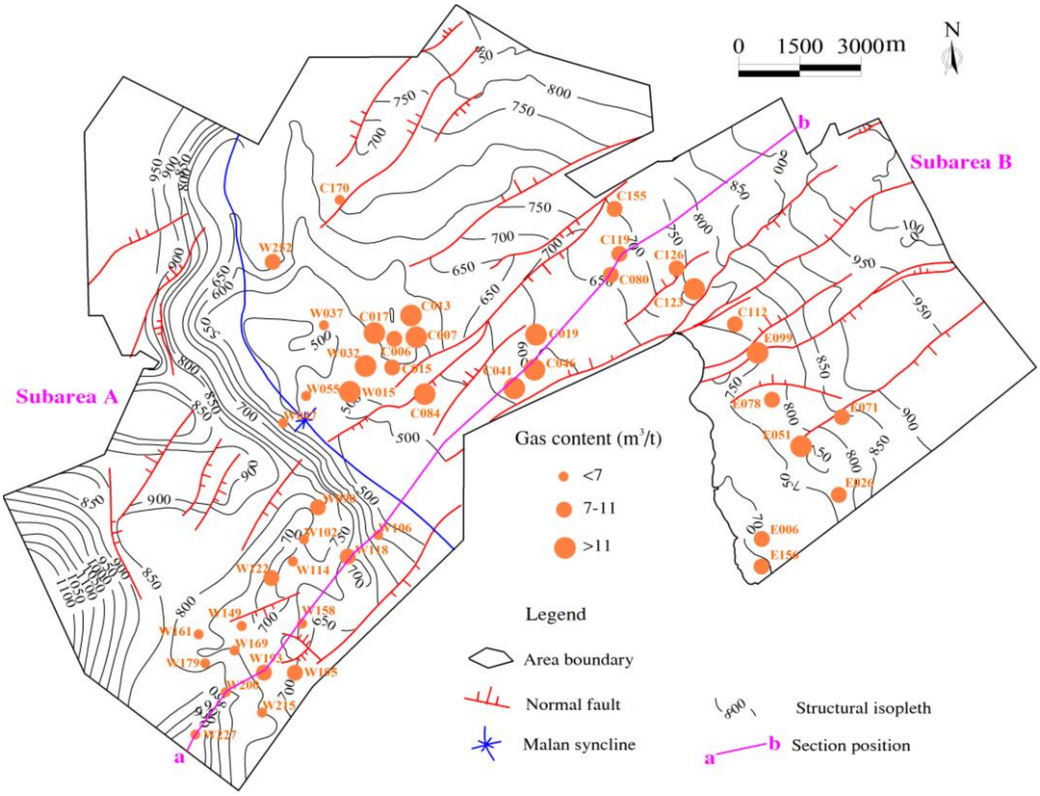

Figure 7. *Structural top of the No. 8 coal seam in the Gujiao area (structural map was drew based on the coalmining geology data in the goaf area, CBM-well logging interpretation data in CBM-producing area).*

Gas content of coal is dominated by many parameters, among which coal composition has been considered to be the most important one. Coal with higher fixed carbon content has stronger adsorption capacity and better gas generation potential (Walker et al., 2001). In the Gujiao area, the relation between composition and gas content of the No. 8 coal seam is weak due to the effect of structural form. As documented in *Table 4*, the No. 8 coal seam in the subareas A and B have similar average composition and burial depth, however, the average gas content in the subarea B (10.7 m^3/t) is much higher than that in the subarea A (6.4 m^3/t). The severe structural deformation in the subarea A caused that a large amount of gas was desorbed and migrated. Sedimentary parameters (including coal composition and thickness) mainly determine the gas-generating potential (Yao et al., 2014), and the present actual gas content mainly depends on the tectonic evolution history and structural form. Moreover, the adsorbability of main gases is in the order of CO_2 > CH_4 > N_2, and the stress release caused by structural deformation will increase the content of gas which has weak adsorbability.

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Table 4. *The average composition, burial depth and gas content of the subarea A and B in the Gujiao area*

Subarea	Burial depth (m)	Gas content (m^3/t)	Proximate analysis (%)			Gas composition (%)		
			M_{ad}	A_{ad}	FC_{ad}	N_2	CO_2	CH_4
A	619.9	6.4	0.76	23.56	54.30	9.53	1.09	88.60
B	604.6	10.7	0.62	18.47	62.57	4.62	1.04	94.15

Note: M_{ad}, Moisture content (as air dry basis); A_{ad}, Ash content (as air dry basis); V_{ad}, Volatile matter content, (as air dry basis).

Faults and gas content

As with structural deformation, fault is the result of stress release, which will induce the gas desorption and migration (Pashin and Groshong Jr, 1998). The effect of structural deformation on gas content is broad since the deformation is a large-area action, however, the effect of fault on gas content is local, which only distribute near fault. Which have induced that the subarea B still has much higher gas content than the subarea A even though many large normal faults developed in the subarea B. The faults in the Gujiao area were mainly induced by NE directional tensile stress (Guan and Li, 2001), however, these tensile faults were subsequently closed under NE directional compressive stress, and these activities were clearly recorded by the cleavages (*Figure 9*). Closed faults have prevented the gas migration and are helpful for preserving gas. As a result, the gas content near the faults in the Gujiao area hasn't decreased notably (*Figure 7*).

The effects of burial depth on gas content

Great number of papers and reports in various CBM-producing basins around the world show that the gas content increases with the increasing burial depth in the shallow buried area, but decreases with the burial depth increasing in the deep buried area (Bustin and Clarkson, 1998; Faiz et al., 2007; Wang et al., 2013; Cai et al., 2014). The coal reservoir pressure usually increases with the increasing burial depth. On the one hand the increasing pressure will improve the adsorption capacity of the coal reservoir, but on the other which will change the matrix and pore structure of it when the pressure is high enough and then decrease its' adsorption capacity (Xiang et al., 2011; Li et al., 2015). The distribution of the burial depth of the No. 8 coal seam in the Gujiao area can be seen in *Figure 10A*, which reflects that the burial depth inclines to increase from the wings to core of the Malan syncline. In the Gujiao area, as can be seen in *Figure 11A*, the relation between burial depth and gas content of the No. 8 coal seam is much complicate and irregular compared with that in the SQB (Lv et al., 2012) and EOB (Cai et al., 2014). However, when considering the subarea B individually, the relation between burial depth and gas content still notable and the critical burial depth is about 600.0 m. It is odd, and hasn't been mentioned and explained by previous studies.

Structural form has superposed effect on gas content. Theoretically, the gas content in syncline region is higher than that in anticline region because syncline has relative deeper

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burial depth and higher reservoir pressure than anticline (Faiz et al., 2007). However, the core regions of syncline and anticline have been deformed severely, as a result, the flank regions of syncline and anticline tend to have higher gas content. The relation between gas content and burial depth has been weakened by structural form in the subarea A. The subarea B is a gentle monocline, in which the structural form is uniform, so the effect of burial depth on gas content can be reflected.

The effects of hydrodynamic condition on gas content

Hydrodynamic condition is one of the most important parameters dominating the distribution of gas content (Meng et al., 2014c). In strong runoff area, flowing underground water will take the dissolved gas and free gas away. To maintain the dynamic balance among adsorbed gas, dissolved gas and free gas, adsorbed gas will partly desorbed and become dissolved gas and free gas (Wang et al., 2015b). As a result, the gas content will decrease gradually in strong runoff area. The stronger the hydrodynamic force is, the lower the gas content has been retained. In stagnant area, the hydrostatic column pressure improved the coal reservoir pressure and adsorption capacity, which is helpful to preserve gas.

Runoff area and stagnant area are relative concepts. To reflect the hydrodynamic characteristics in the Gujiao area, equivalent water level was calculated by referencing reduced water level formula in Wang et al. (2015b), based on the data of the No. 8 coal reservoir pressure measured in production practice. The result shows that the water level ranges from 648.0 m to 1187.0 m and the main hydrodynamic flowing direction is parallel to the dip direction of the coal-bearing formation (*Figure 8*). The core of the Malan syncline is the main catchment area, and in the wings the water level decreases with increasing elevation of the No. 8 coal seam (*Figure 10B*). Chemical composition analyses of water samples reflect that they are $NaHCO_3$ type, and the mineralization ranges from 510 mg/L to 2512 mg/L (*Table 3*). Water mineralization has close relation with water level. As can be seen in *Figure 8*, high mineralization distributes in the low water level region and vice versa.

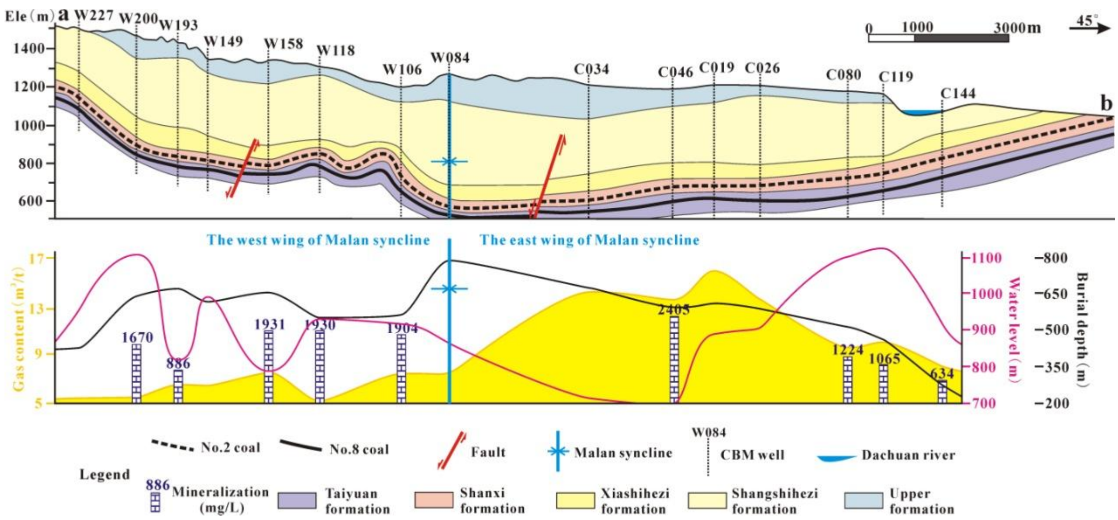

Figure 8. *Well section in Gujiao area showing burial depth, gas content of the No. 8 coal seam*

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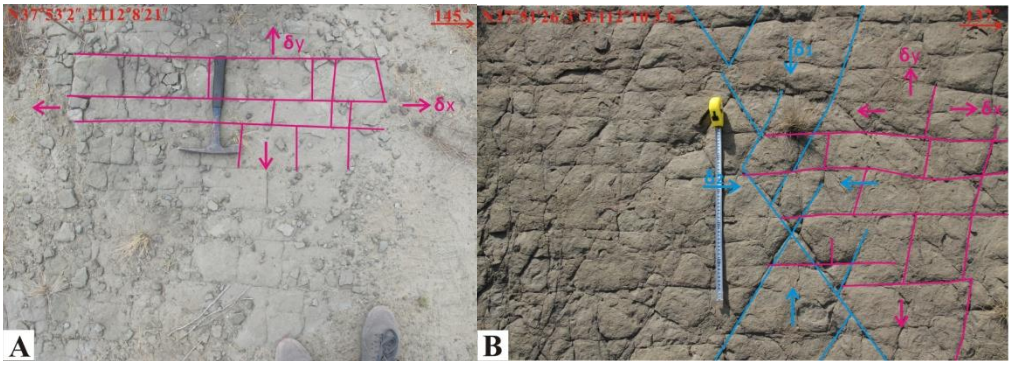

Figure 9. *Field pictures of cleavages in the XCF. (A) Outside the Gujiao area. (B) In the Gujiao area.*

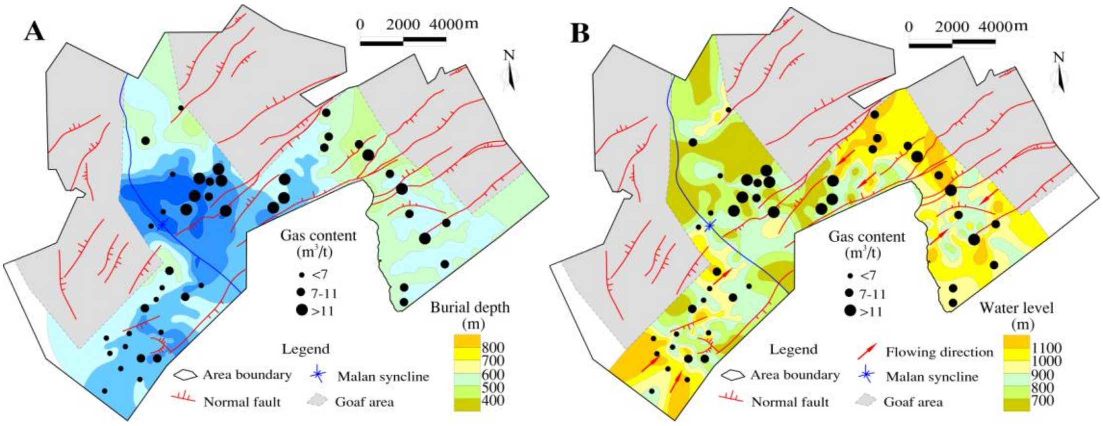

Figure 10. *(A) The burial depth isopleths map of the No.8 coal seam. (B) The water level isopleths map*

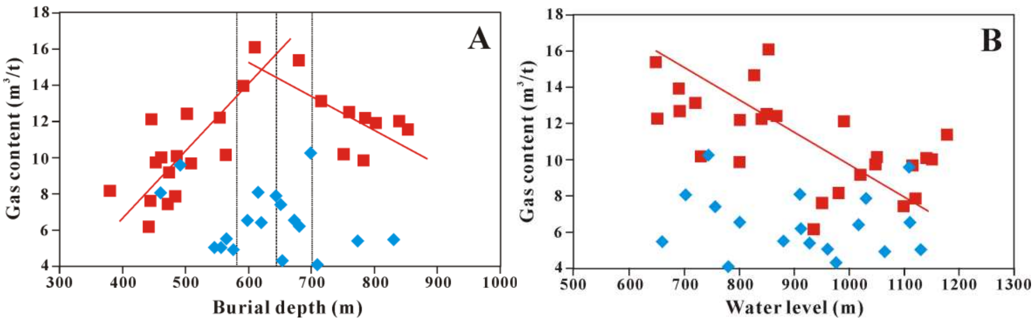

Figure 11. *(A) Relationship between burial depth and gas content. (B) Relationship between water level and gas content.*

The relation between water level and gas content is shown in *Figure 11B*. In the subarea B, the gas content decreases with the increasing water level. However, their relation is weak in the subarea A because the superposed effect from structural form. In the Gujiao area, the gas content is less than 10.0 m^3/t when the water level is higher than

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1000.0 m, and more than 12.0 m^3/t when it is lower than 900.0 m, and which reflect that weak hydrodynamic force is more favorable for gas preservation.

"Sweet spots" for CBM development

In the Gujiao area, based on the analyses in section 4.2, it can be concluded that gentle monocline is much helpful for preserving gas compared with deformed subarea and the effect of faults on gas content is weak because these faults are closed. Regions with burial depth from 500.0 m to 800.0 m and water level lower than 900.0 m are favorable regions for producing CBM. According to the effects of structural form, burial depth and hydrodynamic condition on the gas content of the No. 8 coal seam, the "sweet spots" of CBM development in the Gujiao area were recognized and showed in *Figure 12*, which will be instructive for CBM development and instructing CBM development plan in the Gujiao area.

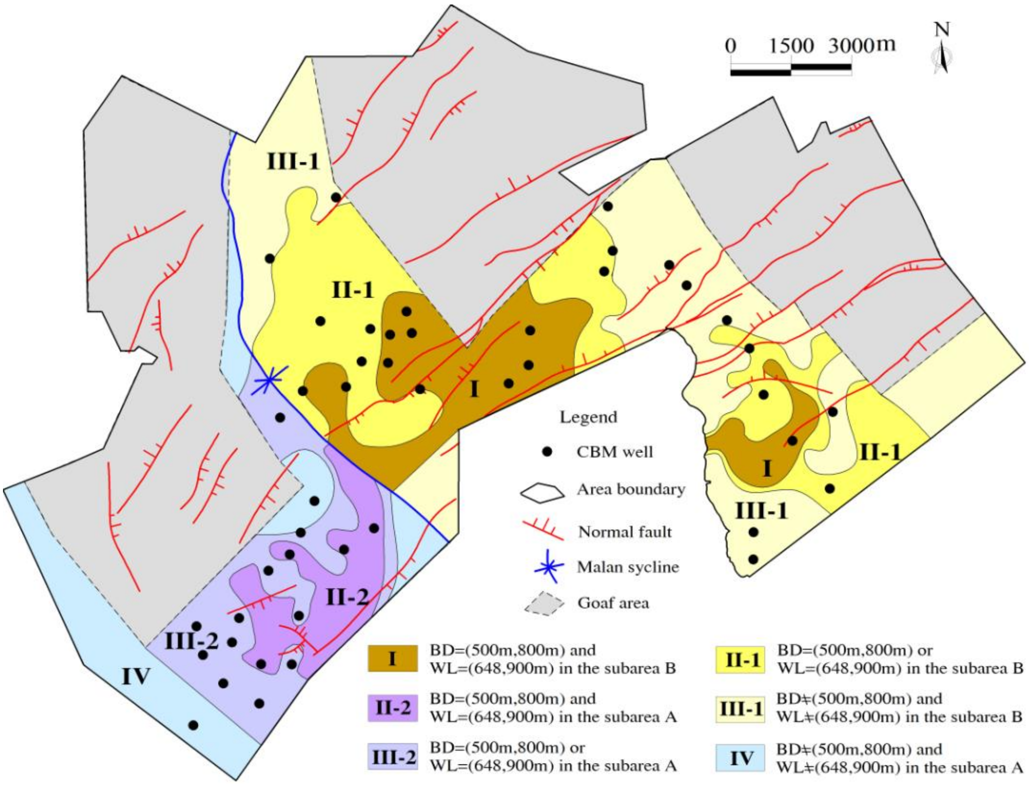

Figure 12. *Evaluation regions for CBM concentration potential and trapping classification of the No. 8 coal seam in the Gujiao area*

A total of 6 types of regions were subdivided (*Figure 12*). The type I blocks are the most prospective blocks, and the type II-1 and II-2 blocks take second place. The type III-1, III-2 and IV blocks are deemed to be unfit for CBM development, and the type I, II-1 and II-2 blocks are "sweet spots" in the Gujiao area.

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Conclusions

In the Gujiao area, the No. 8 coal seam experienced two gas-generating periods and one fluctuant period, and has entered migration period since the Late Cretaceous. The distribution of gas content of the No. 8 coal seam in the Gujiao area is mainly dominated by structural form, water level and burial depth, all which are structural related parameters. The gas content in the subarea A is much lower than that in the subarea B because the subarea A is deformed severely and the subarea B is a gentle monocline. The gas content first increases and then decreases with increasing burial depth, and the turning burial depth is about 600 m. Strong runoff area is harmful for preserving gas due to the washing effect in it. The analyzing results show that: gentle and simple monocline blocks with burial depth ranges from 500.0 m to 800.0 m, water level lower than 900.0 m is the most important “sweet spots” for CBM development.

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PROCESS OF WETLAND LOSS IN THE LOWER NAKDONG RIVER, SOUTH KOREA

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Abstract. The historical distribution of wetlands, the process of wetland loss, and the factors responsible for it in South Korea during the past century were investigated to identify trends in wetland loss. Numerous wetlands were lost because of human activities associated with economic development, such as agricultural development, industrialization, and urbanization. The process of wetland loss and alternation were categorized into five types. (1) Wetlands were directly lost by reclamation and development of agricultural land. (2) Levees were constructed on wetlands to prevent flood damage to agricultural land. (3) Houses for farmers were built on the wetlands after reclamation. (4) Roads constructed as infrastructure for farms fragmented the wetlands into small parts. (5) Factories were built on wetlands and paddy fields.
Keywords: *conservation; historical change; restoration; wetland loss*

Introduction

Wetlands are a complex habitat with both aquatic and terrestrial characteristics. Many species are well adapted to live in these habitats (Keddy, 2010). Humans have also relied upon wetlands as a long-term source of natural resources. Wetland components such as water, land, and wildlife have made huge contributions to human livelihoods (Assessment, 2005). Various wetland components should coordinate harmoniously with each other to retain the services of the wetland ecosystem. However, an attitude prevails wherein only a one-sided functioning is expected of a wetland, based on the habitat condition—either aquatic or terrestrial. In a wetland that has served only as an aquatic habitat, filtration, and water resource could be degraded due to aquaculture, drainage, and run-off from fertilized crops and pesticides (Brinson and Malvárez, 2002). In contrast, a wetland that has only served as a terrestrial area that restricts human activities owing to its wet soil and water could be directly transformed via reclamation for use as agricultural land and as a residential area (Dahl, 2000; Hefner and Brown, 1984). Consequently, numerous wetlands are degraded and are being converted for human overuse and misuse more rapidly than any other ecosystem. Additionally, wetlands are the most threatened habitats among the various ecosystems (Dudgeon et al., 2006).

A common understanding of the causes of wetland loss and alteration is dominated by simplifications that, in turn, are the basis for many environment-development policies (Finlayson and Eliot, 2001). Institutional factors mediate responses of people to

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economic opportunities and it further affects condition of wetlands (Gardner, 2011; Lambin et al., 2001). Industrial, residential, and areas for maintenance of the society have substituted agricultural land and wetlands (Giblett, 1996). Urbanization, as a major cause of wetland loss, is also included in this process. Wetland loss and its causes have reflected the phases of time (Finlayson, 2012; Salafsky et al., 2002).

In the present study, we examined the historical distribution of wetlands, the process, and causes of wetland loss in South Korea during the past century. South Korea started developing rapidly since the 1960s (Levy and Kuo, 1991). The rapid industrialization over the past 40 years also resulted in a steep increase in its demand for natural resources (Lim and Tang, 2002). As a result, South Korea today has one of the largest relative ecological deficits of any country. In particular, many natural habitats and their biodiversity, including wetlands in South Korea, have been lost and degraded as a result of human activities even before realizing their ecological and economic functions. We focused on a regional scale where floodplain wetlands are widely developed along the river channel. This approach provides more detail on the wetland and on the process and causes of wetland loss at different times.

Our study had three main objectives: (1) to identify the historical distribution of wetlands, (2) to track the wetland area and its conversion to alternative land-use types, and (3) to examine the driving factors (causes) behind wetland loss. We attempted to summarize the trends and processes of wetland loss using historical maps and aerial photography.

Material and methods

Study area

We traced the distribution of wetlands from 1918 to 2011 in the lower part of the Nakdong River in South Korea (*Figure 1*). Historically, numerous wetlands have been distributed in this area (Do et al., 2012a) because there are large rivers (Nakdong River) and their tributaries (Nam River) flowing through a flat valley with fluvial soil (Son and Jeon, 2003). Approximately 20% of all the wetlands in South Korea are concentrated within this area (Do et al., 2012b). Although the area is dominated by forest (52.18%), agricultural and urbanized areas account for 31.97% and 5.07%, respectively.

Identifying wetland distribution

Changes in the historical distribution of wetlands over the past 100 years were determined using 1:25,000 topographic maps. The oldest topographical map drawn to scale for this area was published in 1918 by the General of the Chosun Dynasty belonging to the Japanese Government. However, at that time the map had been produced only at a 1:50,000 scale. Seven topographical maps since 1963 (1963, 1978, 1986, 1995, 1998, 2004, and 2009) and an aerial map (2011) published by the National Geographic Information Institute of Korea were used to identify wetland distribution. All topographical maps were digitized after scanning at 600 dpi. Digitized maps were geometrically corrected based on a digital 1:25,000 topographic map produced in 2011. Geometric corrections were made possible based on Ground Control Point (GCP), which are fixed points and artificial landmarks that undergo little change over time (e.g., roads and bridges). The maps were corrected using the polynomial method in ERDAS IMAGINE 9.1 (Intergraph Corporation, USA). A minimum of 12 GCPs were selected for the transformation. Image transformation minimized the errors in root mean square

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values to less than 2.1 m. Wetlands were identified using the legend (wetland, surface of water, rivers are not included) of the map, and each wetland was delineated using the vector editor tool in ArcMAP 9.3 (ESRI, USA).

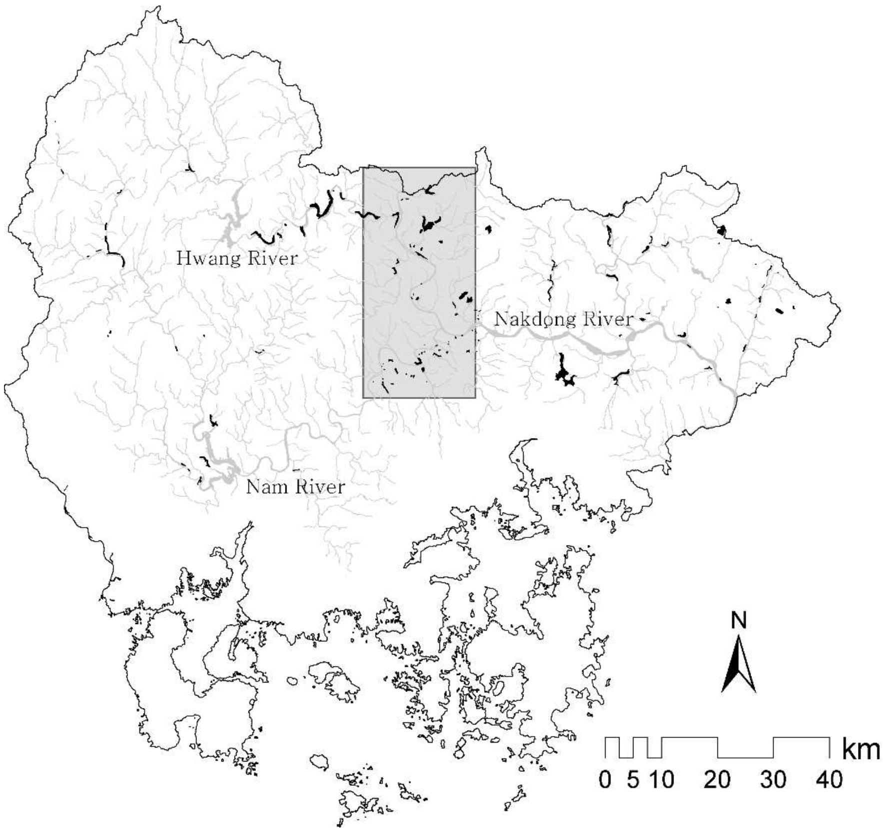

Figure 1. *Wetland distribution in the study area (study area = grey color square with black line, black color = wetland, grey color = river)*

Change of wetland distribution and loss of wetland

The causes of wetland loss or conversion were classified into six categories (agriculture land, levee, road, bare land, industrial area, and residential area). The areas of wetland loss or conversion were identified by comparing with the land condition of the following year. In addition, we confirmed the changes by visiting all wetlands in the study area from May 2011 to July 2012. Wetland boundaries obtained from the map were corrected with data collected during this field survey. The number of wetlands, the area that they cover, and the mean nearest-neighbor distance (MNN) between wetlands were calculated each year for identifying the process of wetland loss and changes in the distribution pattern of wetlands at a large scale. MNN is the minimum distance between wetlands, based on the shortest distance between their edges. Additionally, the landscape diversity indices, including Shannon's diversity index (SDI) and Shannon's evenness index (SEI), were calculated based on the number and area of wetlands each year. SDI is a measure of relative patch diversity and is equal to zero when there is only one patch in the landscape, and it increases as the number of patch types or the proportional distribution of patch types increases. SEI is a measure of patch distribution and abundance. It is equal to zero when the observed patch distribution is low, and it

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approaches one when the distribution of patch types becomes more even. The mean perimeter-area ratio (MPAR) and the mean patch fractal dimension (MPFD) for each year were chosen to assess the alternation of wetland shape at the habitat level. MPAR is a simple ratio of patch perimeter to area, in which patch shape is confounded with patch size; holding the shape constant, an increase in patch size will cause a decrease in the perimeter-area ratio. MPFD is a measure of the ratio of perimeter per unit area, and it increases as the wetlands become more irregular. All spatial and temporal analyses and data processing were performed using FRAGSTATS Version 3.3 for GIS analysis (McGarigal et al., 2002).

Linear regression analysis was used to examine the relationships between the number of wetlands, area, mean distances between wetlands, wetland shape across the years using PASW Statistics 18. The r^2 and standardized beta (B) of linear regression were used to identify the positive and negative relationship between variables and years.

Result

Wetland loss

In the past century, the number of wetlands and the total wetland area within the study area declined by approximately 65% and 70%, respectively. However, the reduction ratio of the number of wetlands in comparison with the previous year, declined steadily with each year. In particular, the number of wetlands and wetland areas adjacent to contributories (Nam River) decreased more when compared with wetlands adjacent to a main river (Nakdong River, *Figure 2*). The reduction of wetland areas ($B = -0.99$, $r^2 = 0.98$, $p < 0.001$) was steeper than the reduction in the number of wetlands ($B = -0.924$, $r^2 = 0.85$, $p < 0.001$).

At the landscape level, MNN among wetlands was significantly more each year, indicating the increased isolation of the wetlands (*Table 1*). The Shannon diversity index (SDI) and the Shannon evenness index (SEI) decreased significantly, as the years passed. In particular, the reduction ratio of SDI was higher than that of SEI. This also means that the reduction in the number of wetlands was more effected by a decrease of SDI. At the habitat level, MPAR and MPFD increased significantly indicating that the area of each wetland decreased continuously, and the physical dimensions of each wetland became more complex.

Table 1. *Change of landscape indices and their trends in the study area*

Year	SDI	SEI	MNN	MPAR	MPFD
1918	3.83	0.78	685.97	244.62	1.30
1963	3.40	0.70	652.23	436.83	1.33
1978	3.27	0.75	873.12	328.44	1.32
1986	3.26	0.74	785.81	351.29	1.32
1998	2.43	0.60	1,081.31	382.80	1.33
2004	2.76	0.68	965.01	364.16	1.32
2009	2.65	0.63	913.34	534.81	1.35
2011	2.51	0.65	1,050.28	568.05	1.35
B	-0.913	-0.775	0.792	0.716	0.828
r^2	0.834	0.601	0.627	0.512	0.685
p	0.002	0.024	0.019	0.046	0.011

* SDI = Shannon diversity index, SEI = Shannon evenness index, MNN = mean nearest-neighbor distance, MPAR = mean perimeter-area ratio, MPFD = mean patch fractal dimension

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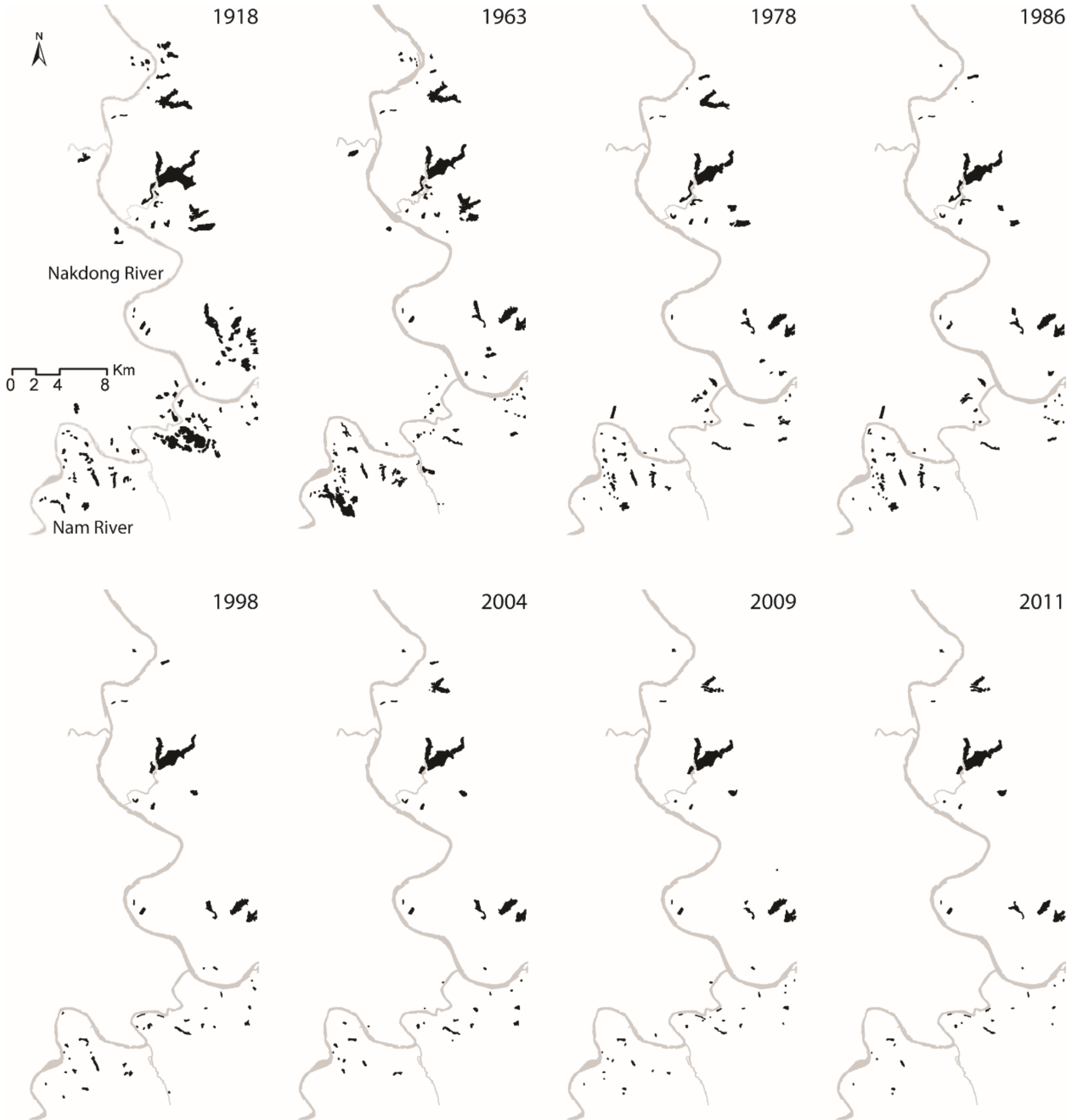

Figure 2. *Change of wetland distribution in the study site (wetlands = black, river channel = grey)*

Causes of wetland loss

The major cause of wetland loss was associated with agricultural development in the region. Approximately 42% of lost wetlands were diminished due to conversion from wetlands to agricultural lands. On the floodplain, which is a major type of wetland in this area, levees were constructed for flood control. Approximately 39% of the wetland area was lost or altered by the construction of levees. Development of roads, factories, and residential areas associated with industrialization and urbanization accounted for approximately 12% of the total loss of wetland area. The bare land that was converted from wetlands accounted for a further 7% of the total area of wetland that was lost. These areas were converted to specific land-use types, especially levees and agricultural lands. In the early period (1960–70), agricultural development was the dominant cause of wetland loss. In contrast, loss caused by industrialization and urbanization has been

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recorded since the 1990s, although agricultural lands and levees have always been major causative factors (*Figure 3*).

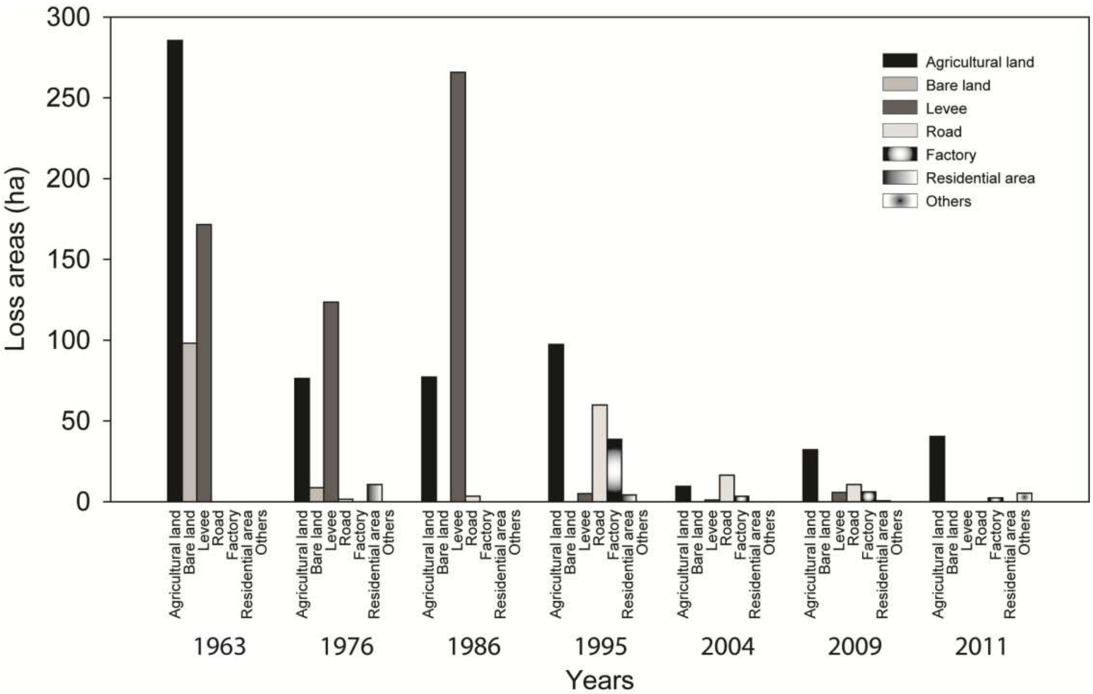

Figure 3. *Changes in the contribution of various factors to wetland loss over the years*

Discussion

Process of wetland loss

Numerous wetlands have been lost as a result of human activities associated with economic development, such as agricultural development, industrialization, and urbanization. The process of wetland loss and alternation appeared to be divided into five stages (*Figure 4*). (1) Wetlands were directly lost by reclamation for development of agricultural land. (2) Levees were constructed on wetlands for prevention of flood damage to agricultural land. (3) Houses for farmers were built on the wetlands after reclamation. (4) Roads constructed as infrastructure for the farms fragmented the wetlands into small parts. (5) Factories were built on reclaimed paddy fields and wetlands.

The wetlands were a major focus for agricultural development (Liu et al., 2004). In Asian countries where rice is cultivated along with aquaculture, the wet soil, alluvial soil, and flat land of wetlands are attractive to farmers (Zhang et al., 2010). The construction of levees will have a serious impact on wetland distribution. Many wetlands were directly destroyed owing to the construction of levees, and these levees may further isolate the remaining wetlands by reducing natural flooding, which is a critical variable to maintain the wetland aquatic condition and biota (Casanova and Brock, 2000; Middleton, 1999). Recently, local governments have been facilitating reclamation and relocation of industrial complexes to agricultural areas (including rice fields) to increase industrial development in the rural areas.

Although partial wetlands remain after land reclamation, they undergo further physical modification. The size becomes smaller, the shape more complex, and the

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wetlands become isolated. Most of the remaining wetlands were largely used for irrigation. Do et al. (Do et al., 2012b) argued that only the wetlands that had specific usages remained.

During field survey, we also confirmed that most remaining wetlands were used for irrigation, and they were designated as a reservoir by the land use policy of the local government. They were also designated as a protected area by acts associated with wetlands and wildlife. However, the size and shape of these wetlands can be modified by any degree of change in usage. The construction of roads was a major cause of wetland fragmentation. Although the impact of fragmentation may be less severe than wetland loss, fragmentation is also critical to migrating species and regional biodiversity (Rosenzweig, 1995). In comparison with small habitats, large habitats are more likely to intercept potential colonists, and to have lower extinction rates owing to their larger population size (McArthur and Wilson, 1967; O'Connell et al., 2013). A more modified habitat contains more periphery-adjacent area. Species that depend on resources found in the interior habitat may avoid the habitat at the edges because of its less favorable quality, predation risk, and microhabitat conditions (Fletcher et al., 2007; Sebastián-González and Green, 2014).

The loss by conversion from wetland to agricultural land, especially paddy fields, might have different ecological pathways from those for agricultural land with dry farming practices (i.e., corn field, wheat field, etc.). The paddy field is a temporary wetland that harbors many of the species that breed in natural temporary ponds (Lawler, 2001). Additionally, the paddy field has the potential to help sustain the regional diversity of many invertebrates and vertebrates that depend on aquatic/semi-aquatic habitat (Bambaradeniya and Amarasinghe, 2004). However, to maintain the ecological function as a wetland, low impact farming practices are required. The intensive farming practices with insecticide and fertilization have a critical effect on the biodiversity in paddy fields (Hegde et al., 2014; Simpson et al., 1994). Further, intensive farming requires more agricultural infrastructures (e.g., levee and pipe channel) that leads to degradation of hydrological conditions of wetlands. Recently, winter flooding has been suggested as an important management practice to improve biodiversity in the paddy field. Winter flooding maintains the wet condition similar to a permanent wetland and supports aquatic biota (Fujioka et al., 2010; Kim et al., 2011).

On the other hand, areas of abandoned paddy fields have increased with the changes in the structure of rural economy and a decrease in the rural population (Taylor and Martin, 2001). The abandoned paddy fields are good potential areas for wetland restoration. In particular, paddy fields converted from wetlands are one of the best sites for restoring historical wetlands (Nakamura et al., 2006). Such abandoned paddy fields generally maintain a soil seed bank of wetland species (Middleton, 1999; Middleton, 2003). Further, such fields require fewer modifications as they maintain similar hydrologic conditions with the floodplains in the region. More retrospective research on land use change in the floodplain area will provide essential understanding for implementing an effective wetland restoration and management plan.

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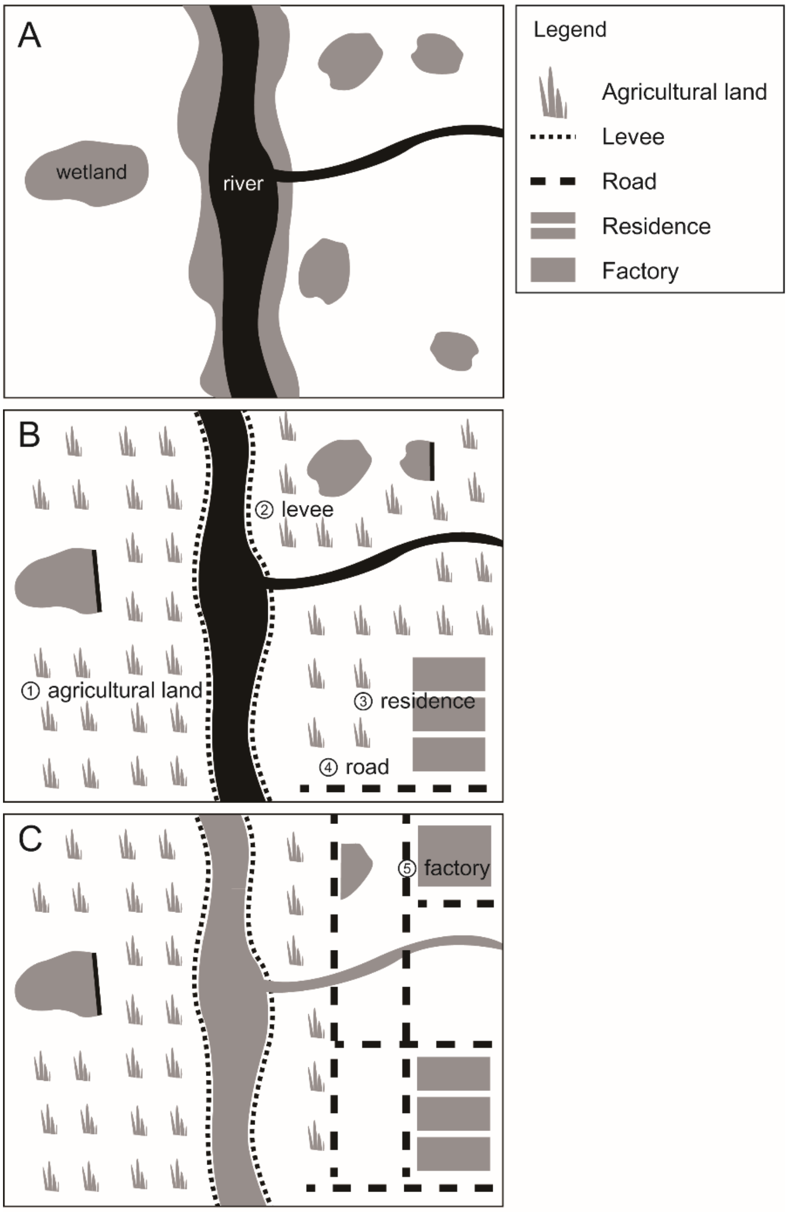

Figure 4. *Schematic representation of the process of wetland loss; the numbers in circles represent the process stages; A: natural floodplain, B: initial reclamation for agricultural field (early-middle stage), C: construction of urban infrastructure (late stage)*

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DIFFERENCES IN THE MICROBIAL POPULATION ASSOCIATED WITH THREE WETLAND TYPES IN THE SANJIANG PLAIN, NORTHEAST CHINA

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Abstract. The Sanjiang Plain is the largest freshwater wetland area in Northeast China and plays an important role in regulating climate stability for this region. However, agricultural activity has decreased wetland coverage with about 84 % since the1950s.This has resulted in lowered water tables and degraded wetlands, with dryer marsh meadows and dry meadow coverage replacing wet marshlands. Here, we report investigations towards the soil microbial community composition and diversity in the different degeneration wetlands types. Bacterial and fungal communities in the soil types were compared using high resolution bar-coded pyrosequencing technology. The results revealed that the bacterial and fungal diversity was lower in wet marshland than in drier marsh meadow and dry meadows.The distribution of sequence reads into different bacterial and fungal phyla further differed between the soil samples. The wet marsh soil displayed a higher abundance of Proteobacteria but lower abundance of Acidobacteria, while the higher abundance of unclassified fungi but smaller fractions of Ascomycota and Basidiomycota than the other soil types.The results reported here demonstrate that soil bacterial and fungal communities change as a result of differences in the soil environment in the Sanjiang Plain.
Keywords: *community structure, microorganism; Miseq; wetland degradation; bacterial diversity; fungal diversity*

Introduction

Wetlands are the most important terrestrial ecosystems in the world, having crucial environmental functions such as regulation of the carbon cycle (Keller, 2011), maintaining fresh water capacities (McJannet et al., 2012) and protecting biodiversity (Burton and Uzarski, 2009). Wetlands are areas with land and shallow water bodies, where the water tables permanently or periodically higher than surface level and with specific wetland ecological communities (Mausbach and Parker, 2001; Mitsch and Gosselink, 2007). Although natural wetlands occupy only 5–8 % of Earth's land surfaces (Mitsch and Gosselink, 2007), they are regarded as the "kidney" of the Earth, playing several key roles in biogeochemical processes such as pollutant degradation,

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nitrification, denitrification, methanogenesis, methanotrophy, and iron and sulfate reduction (Davidsson et al., 1997; Gutknecht et al., 2006).

The Sanjiang Plain, covering an area of 10.89 million hectare, contains the largest freshwater wetland in Northeast China. It is also named *Deyeuxia angustifolia* (Kom.) wetland because this plant is the dominant species in the area.The local wetland environment is inevitable for climate stability, biodiversity protection, and greenhouse gas emission reduction in Northeast Asia. However, growing human populations have resulted in a decline of the wetlands. Whereas half of the Sanjiang Plain was covered by freshwater in 1950 (Zhao et al., 1999; Liu and Ma, 2000), approximately 84% of the wetlands have since been converted to farmland, especially to paddy fields (Liu and Ma, 2000). As a consequence of agricultural water use and decreased precipitation, the amount of water and water-covered surface area has decreased steadily, resulting in degraded wetlands. The original wet marshlands have changed to drier marsh meadows (recognized as a transitional state in wetland degeneration) and eventually into dry meadows, a fully degenerative state of wetland. Ecosystems have changed as well, with a decreased vegetation diversity, a fall in methane emission and a rise incarbon dioxide and nitrous oxide emission being prominent.

Several studies have evaluated the effects of wetland degeneration in the Sanjiang Plain on greenhouse gas emission (Song et al., 2013), nitrogen cycling between the atmosphere, vegetation and the soil (Sun, 2007), or soil microbial biomass and soil respiration (Huang et al., 2012), but little attention has been paid to the composition and diversity of soil microbial communities and how the microbial composition responses to wetland degeneration.With the complete array available from pristine wetlands to severely degenerative states, including three well-recognized wetland types along a water decline gradient within a small area, the site of the Sanjiang Wetland Experimental Station provided ideal site to study the effects on soil microbial ecosystems.

We hypothesized that changes in the soil microbial communities would be visible between wet marshlands, drier marsh meadows and dry meadows in Sanjiang Plain, and further that the variation in composition of soil fungi might exceed that of bacteria. To test these hypotheses, we collected soil samples from the three wetland types and estimated the composition, diversity and phylogeny of soil bacterial and fungal communities in these samples using high-throughput sequence analysis.

Material and methods

Site description and soil sampling

The study was conducted at the Sanjiang Wetland Experimental Station (47˚35’N, 133˚31’E), property of the Institute of Nature and Ecology of Heilongjiang Academy of Sciences, China (*Figure 1*).The local average monthly temperature ranges from −21.6 °C in January to 21.5 °C in July, with an annual average of 1.9 °C. The average annual precipitation is about 560 mm, with approximately 80% occurring between May and October. Three sites inside the station were selected for this study. Wet marshland was designated K0, a drier marsh meadow as K1 and dry meadow was called K2. The K0 site was approximately 20 m away from K2, and these were approximately 1 km away from the K1 site. All three sites are characterized by Quaternary sediments, and their soils were classified as Albic Boric Luvisols with a silty clay texture (Xi, 1993).

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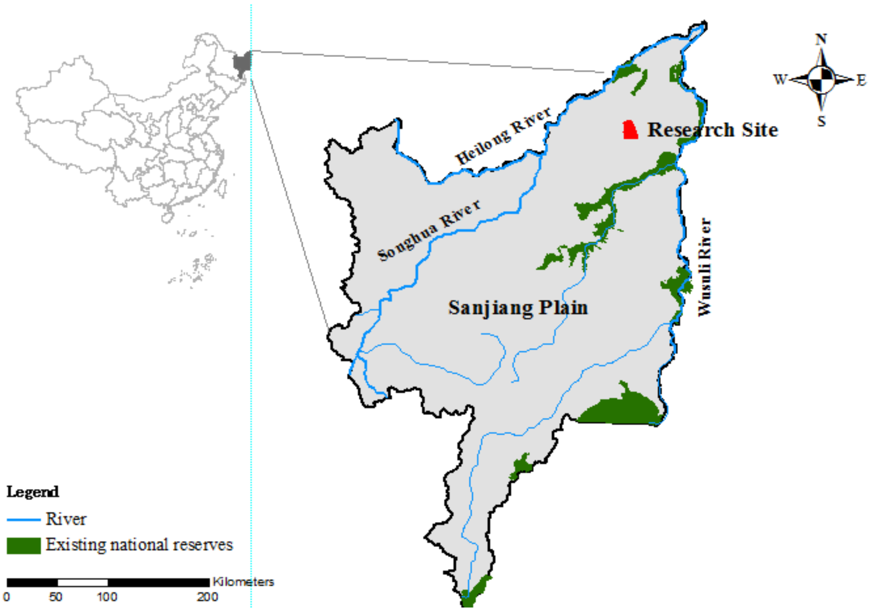

Figure 1. *Map of the Research Site in the Sanjiang Plain, China*

Soil samples were taken at a depth of 0–20 cm on 15 June 2014. Approximately 1kg soil was collected from five locations within each site and stored in polyethylene bags, placed in a container with ice and immediately transported to the laboratory. Upon arrival, approximately 2g of each soil sample was placed in a sterile micro centrifuge tube (2 mL) and stored at −80 °C for DNA extraction. The remaining of the samples was air-dried with the weight difference used as a measure of soil water content. Dried soil was used for the determination of other physiochemical properties: Soil pH was measured using a pH meter after mixing the soil with water (1:5 w/v) for 30 min. The soil total carbon (TC) and total nitrogen (TN) composition was determined using an Elemental analyzer (Vario EL III, Elementar Analyses system, Hanau, Germany). Nitrogen fractions NH_4^+-N and NO_3^--N were measured by use of FLAstsr 5000 analyzer (Foss Tecator AB Sweden Supply Company, Hoganas, Sweden).

Soil DNA extraction and high-throughput sequencing

DNA was extracted from 0.5g of each frozen soil sample with a MOBIO PowerSoil DNA Isolation Kit (USA) according to the manufacturer's instructions. The extracted DNA was diluted in 100 μL TE (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and stored at −20 °C until used.

For PCR amplification and pyrosequencing we selected the V1-V3 region of bacterial 16S rRNA and the ITS1 region of fungal rDNA (Chakravorty et al., 2007; Bellemain et al., 2010). High-throughput sequencing was performed by the Shanghai Majorbio Biotechnology Company, Shanghai, China.

Data analysis

Obtained high-throughput sequences were analysed by using Mothur Software. The UniFrac statistical analysis tool was used to compare bacterial and fungal community compositions (Lozupone and Knight, 2005).

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Statistical analysis of effective and optimized gene sequences

Using the simultaneous sequencing method for multi-samples, a barcode labeled gene sequences and forward primer sequences were introduced in the sequences of all samples. Sequences containing both barcode and forward primer sequences were then selected as effective sequences, after which the sequencing connectors and barcode sequences were removed. Subsequent data analyses were performed using the treated, effective sequences.

To obtain high-quality, accurate results from bio-informatic analyses, sequences were optimized by discarding sequences with lengths less than 150bp, those containing imprecise base calling, or those for which primer bases contained more than two mismatching sequences. The optimized sequences were used for subsequent statistical analyses.

OTU-based analysis

All sequences were identified to operational taxonomic units (OTU) for bio-informatic statistical analysis. Optimized gene sequences with gene lengths greater than 350bp were selected, compared with the SILVA database and then clustered. Clustering analysis was performed using the software packages mothur and chopseq (http://www.mothur.org/wiki/Main_Page).

Bacterial community diversity and rarefaction curve

Species richness and diversity of the bacterial community were characterized by Chao1 and the Shannon index, and the sequencing depth index was expressed as Coverage. Alpha-diversity of the bacterial community was measured at significance levels of 97% (0.03).The estimates were calculated by employing the tools Aligner, Complete Linkage Clustering, and Rarefaction of the RDP pyrosequencing pipeline**.**

The optimized gene sequences were randomly sampled. The sub-sampled sequences and the number of OTUs present in each were used to calculate a rarefaction curve. If the rarefaction curve tends to be flat then the sampling process is considered to be rational and further sampling is likely to produce few new OTUs, otherwise increased sampling will produce more new OTUs.

Analysis on whole-sample similarity

The Jest algorithm was used to compare the differences in OTUs from the three soil samples and to calculate the number of sequences from each OTU, thus obtaining a similarity relation between the samples. The selected OTUs had a similarity level of 0.03. Canonical Correlation Analysis for bacteria was conducted by R software.

Results

Soil Physicochemical Properties

Soil characteristics such as pH, organic carbon content,total nitrogen,ammonium nitrogen, nitrate nitrogen, and soil water content of the three wetland types K0 (wet marshland), K1 (drier marsh meadow) and K2 (dry meadow) are listed in *Table 1*. All but one determined variables followed an increasing trend, from K0 to K2, while, as expected, soil water contentwas lower in K2 than in K0.

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Table 1. *chemical properties of soil samples*

Sites	pH	Organic C(g.kg-1)	Total N (g.kg-1)	Ammonium nitrogen(mg.kg-1)	Nitrate nitrogen (mg.kg-1)	Soil water content (%)
K0	5.56±0.01^{A}	42.32±0.12^{A}	2.27±0.01^{A}	17.47±0.56^{A}	4.25±0.07^{A}	185±0.11^{C}
K1	5.66±0.02^{B}	44.23±0.19^{B}	2.70±0.02^{B}	18.51±0.56^{B}	4.41±0.08^{B}	86±0.08^{A}
K2	5.82±0.01^{C}	47.91±0.16^{C}	2.88±0.02^{C}	20.17±0.56^{C}	5.15±0.05^{C}	75±0.10^{A}

Different capital letters in the same column identify significant differences at 0.05 level among parameters.K0, K1, K2 represent wet marshland, drier marsh meadow and dry meadow, respectively.

Diversity of bacterial and fungal communities

The diversity of bacterial and fungal communities was calculated from 16S rRNA sequences and ITS rDNA sequencesat the 3 %level, respectively, among the three samples. In total 660, 683,and 636 OTUs for bacteria and 199, 291, and 260 OTUs for fungi were identified in samples K0, K1 and K2, respectively (*Table 2*). The diversity indices showed that the drier marsh meadow K1 had the highest Shannon's diversity index and the lowest Simpson's index compared with the other samples. The S_{chao} estimator of the three samples was in the order K1>K2>K0. Thus, all diversity indices showed that the bacterial and fungal community compositions varied between the three types in that they were most diverse in drier marsh meadow soil (*Table 2*).

Rarefaction curves of bacterial and fungal sequences

Rarefaction curves were calculated by plotting the number of OTUs at the 3% level (*Figure 2*). At that level, the curves were increased at the rate of OTUs detection, indicating that the reads analysis evaluated almost the full extent of taxonomic diversity at the species level, and the coverage of the reads of bacterial and fungal sequences was estimated as above 98% (*Table 2*).

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Table 2. *Diversity indices for obtained bacterial and fungal sequences from three wetland soils in Sanjiang plain, NE China.*

	Bacteria						Fungi					
	Reads	**OTUs**	**Coverage (%)**	***H'***	***D***	S_{chao}	**Reads**	**OTUs**	**Coverage (%)**	***H'***	***D***	S_{chao}
Wet marsh wetland(K0)	17417	660	99%	5.19	0.0156	709	18375	199	99.8%	2.33	0.0920	206
Drier marsh meadow wetland (K1)	10816	683	98%	5.26	0.0129	750	18299	291	99. 8%	3.25	0.0782	301
Dry meadow wetland (K2)	8955	636	98%	5.22	0.0131	745	22953	260	99.8%	3.49	0.0762	268

OTUs:Operational taxonomic units, *H'*: Shannon's diversity index, D:Simpson'sindex

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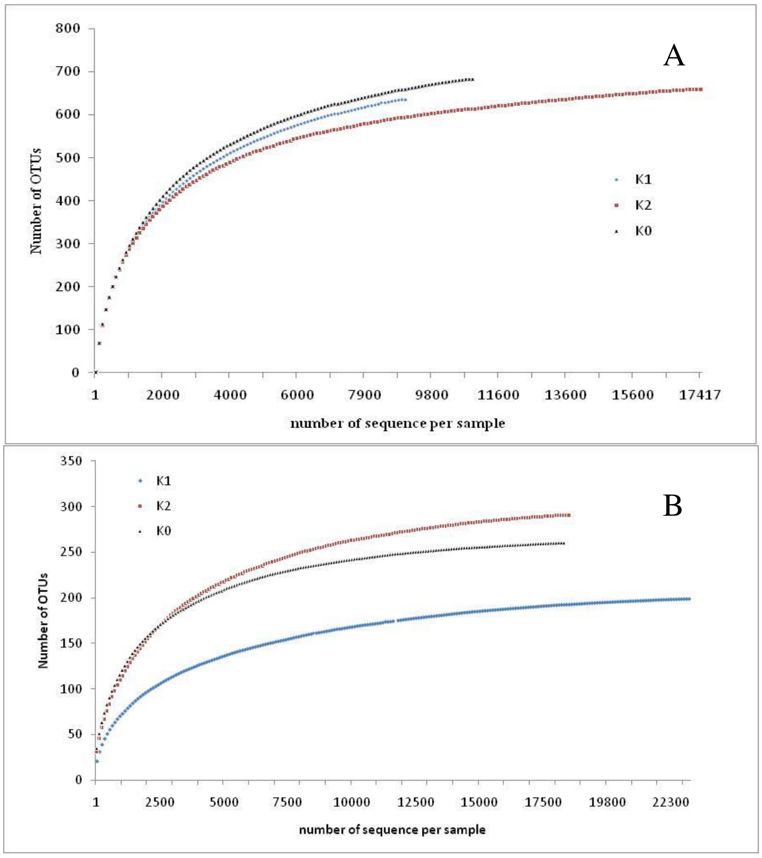

Figure 2. *Rarefaction curves for the bacterial 16S (panel A)and fungal ITS (panel B) rRNA sequences obtained. Operational taxonomic units(OTUs) were calculated based on the 3% level.*

Compositions of bacterial and fungal communities

Bacterial phyla were determined using the classifier tool at the RDP website. The obtained sequences were classified into (in decreasing order):15432 Proteobacteria, 12118 Acidobacteria, 1984 Bacteroidetes, 1533 Chloroflexi, 1530 Planctomycetes, 924 Firmicutes, 899 Nitrospirae, 471 Chlorobi, 332 Gemmatimonadetes and 150 Elusimicrobia; 933 clones remained unclassified. About 90% of all 37, 188 bacterial clones belonged to six taxonomic phyla: Proteobacteria (42%), Acidobacteria (33%), Bacteroidetes (5%), Chloroflexi (4%), Planctomycetes(4%), Firmicutes(3%) and Nitrospirae(2%).

The distribution of clones into the different bacterial divisions among the three high through put clone libraries was uneven, as shown in *Figure 3A*. Notably, Proteobacteria were highly over represented in wet marshland, while the abundance of Acidobacteria, Chloroflexi and Chlorobiwas higher in the drier marsh meadow soil (K1) than in the other two types. In contrast, Planctomycetes, Nitrospirae and Gemmatimonadetes were more abundant in dry meadow soil K2 in comparison to the other two types. Highest proportions of Bacteroidetes, Firmicutes and unidentified bacteria were observed in wet marshland (K0).

Fungal sequences were divided into 19470 Ascomycota, 23384 Basidiomycota, and these were again unevenly distributed among the three sample types (*Figure 3B*). The abundance of Ascomycota(56.56%) and Zygomycota(72.65%) was higher in the drier

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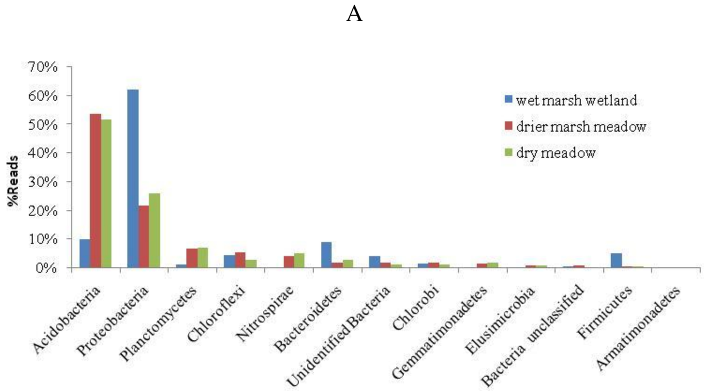
marsh meadow soils(K1) than in the other two types, while Basidiomycota were remarkably abundant in dry meadow samples(K2), and the highest proportion of unidentified fungal sequences(75.12%) was obtained from wetmarshland (K0). Thus, the dominant fungal phylum was different in each type of wetland.



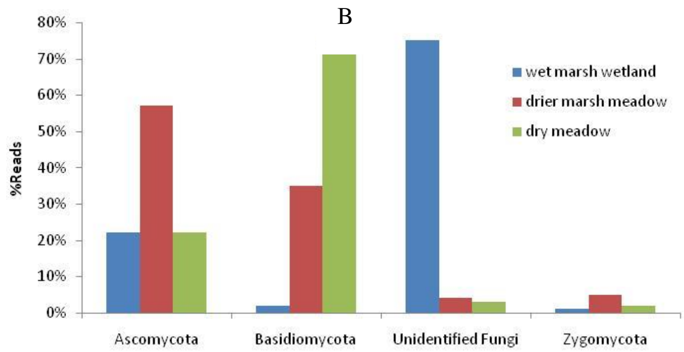


Figure 3. *Distribution of thebacterial (panel A) and fungal(panel B) rRNA genesequences in phyla obtained fromwetmarsh wetland (K0), drier marsh meadow wetland (K1) and dry meadow wetland (K2)*

Comparison of bacterial and fungal community composition betweenthe three wetlands

Shared OTUs of bacteria and fungi among the three wetlands are shown in a Venn diagram in *Figure 4*. The analysis identified that 52 fungal and 373 bacterial OTUs were shared among all three wetland samples, while 41 OTUs of fungi and 178 OTUs of bacteria were shared between drier marsh meadow and dry meadow samples, 34 fungal and 82 bacterial OTUs were found in both wet marshland and drier marsh meadows, and 25 fungal and 48 bacterial OTUs were shared between wet marshland and dry meadow soils. Numbers of unique OTUs were highest in wet marshland K0, both for bacteria (180) and fungi (157), and lowest in dry meadow K2 (81 and 37, respectively).

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Bacteria Fungi

Figure 4. *Venn diagram showing shared and unique OTUs identified for bacteria (left) and fungi (right) obtained from wet marsh wetland(K0),drier marsh meadow (K1) and dry meadow soils (K2).*

Long-term wetland degeneration alters soil microbial community composition

The statistical significance of differences in bacterial and fungal community compositions for all sequences was analysed by UniFrac (*Table 3*).The difference between total clones obtained from K0 and K1 was highly significant for bacteria. Highly significant differences at the phyla level are indicated in bold in the table. Only one difference was highly significant for Fungi (Agaricomycetes between K0 and K2).

Finally, a Canonical Correlation Analysis was performed, the results of which are presented in *Figure 5*. A negative correlation was observed between abundance of Acidobacteria and pH and soil organic carbon content. Thus, soil pH and carbon content are the major factors governing the abundance of Acidobacteria in the wetlands of Sanjiang Plain.

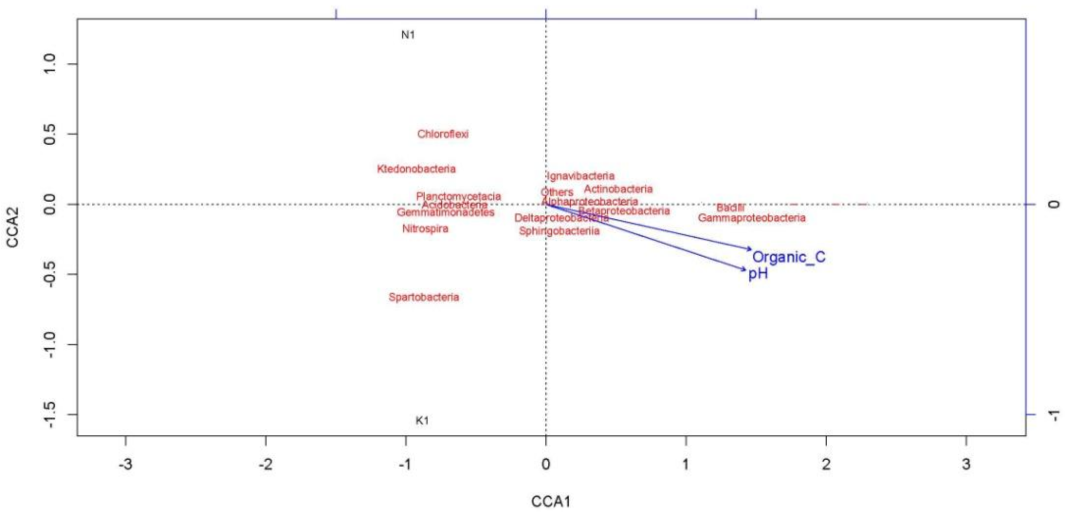

Figure 5. *Canonical Correlation Analysis for bacteria in Sanjiang Plain*

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Table 3. *UniFrac P-test values for bacterial and fungal sequences at total clones and individual phylum level for the three wetland soils in the Sanjiang Plain, NE China*

Bacteria												
Sample	**Total clones**		**Acidobacteria**		**Alpha-proteobacteria**		**Planctomycetacia**		**Delta-proteobacteria**		**Beta-proteobacteria**	
	K1	K0	K1	K0	K1	K0	K1	K0	K1	K0	K1	K0
K1	-	**0.001**	-	0.007	-	**0.001**	-	0.010	-	**0.003**	-	0.022
K2	0.313	0.012	0.461	**0.002**	0.532	**0.002**	0.767	0.031	0.695	**0.004**	0.632	0.017
Fungi												
Sample	**Total clones**		**Dothideomycetes**		**Sordariomycetes**		**Agaricomycetes**		**Leotiomycetes**		**Zygomycota**	
	K1	K0	K1	K0	K1	K0	K1	K0	K1	K0	K1	K0
K1	-	0.823	-	0.415	-	0.151	-	0.069	-	0.812	-	0.620
K2	0.779	0.996	0.047	0.095	0.322	0.537	0.162	**0.005**	0.274	0.237	0.447	0.339

Statistically significant findings at or below the 0.005 level are given in bold.

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Discussion

The diversity of bacterial and fungal communities as determined in this study was lower in samples from wet marshland than in the degraded wetland types, as estimated by the number of OTUs,and as indicted by the Shannon's diversity, Simpson's and S_{chao} indices at phylum level (*Table 2*).This suggests that during the process of marsh degenerating into dry meadow the diversity of soil bacteria and fungi increases. Marsh wetland is characterized by perennial water levels that are characteristically low in oxygen. This restricts the growth of soil microbes and limits microbial diversity. Drier marsh meadow and dry meadows, however, provide better conditions for a diversity of microbes because their soils have lower water content and much more oxygen, which promoted soil microbial diversity. This is consistent with the results of Li (2011), who found that increased water content in wetlands decreased the oxygen amount and restrained the growth of fungi. Xu (2004) also found that a high soil water content restrains fungal diversity of valleys wetlands in Changbai Mountains, China (Xu et al., 2004). The results presented here also suggest that soil microbial diversity increases as a result of degeneration of wetlands in the Sanjiang Plain.

The difference in bacterial and fungal community compositions between the three wetland types was shown in *Fig. 1*, and their statistical significance presented in *Table 3*, suggest that the degeneration from marsh wetlands todrier environments significantly alters the soil bacterial and fungal community compositions. Liu (2014) also observed changes in the bacterial composition between such environments. Several studies have been conducted on soil microbial composition of various kinds of wetlands around the world (Dedysh, 2011).The majority of available research demonstrated that the proportion of Acidobacteria and Proteobacteria was higher than any other bacterial phyla in wetlands (Hartman et al., 2008; Ausec et al., 2009; Pankratov et al., 2011). For example, Ausec et al. (2009) observed Acidobacteria as the dominant phylum present in bog soils (41.6%) and in fen soils (23.7%) sampled in Slovenia. Hartmanet al.(2008) determined 38.1% Acidobacteria, 17.4% Alpha proteobacteria and 9.7% Actinobacteria as the major bacterial phyla in wetland soils of a North Carolina coastal plain, but they did not detect any Chloroflexi (Hartmanet al., 2008). Kanokratana et al. (2011) found that Acidobacteria (35.0%) and Proteobacteria (37.9%) dominated in soil from a tropical peat swamp forest in Thailand, by analyzing 280 clones of full length 16s rRNA sequences.The result of our research is consistent with those studies, as we found about 90% of all bacterial clones belonged to six taxonomic phyla, of which Proteobacteria dominated (*Figure 3*). The proportion of Proteobacteria was higher in the wet marshland than in the degraded types, but the fraction of Acidobacteria was higher in the two dryer types. This indicates that the composition of bacteria has changed during wetland degeneration. Li (2015) pointed out that Proteobacteria was the dominant phyla in the wetland of the Wuliangsuhai eutrophic lake, with an even higher fraction of Proteobacteria detected than in our findings (Li et al., 2015). Proteobacteria seem to dominatein water-covered surfaces but their numbers decrease while Acidobacteria increase during transition to drier environments. The results presented here strongly suggest that the composition of bacterial communities in the three wetland types of the Sanjiang Plain have under gone changes due to a fall of the water table.

The composition of the fungal community seems to be less complex than that of bacteria. The proportions of the three fungal phyla Ascomycota, Basidiomycota, and

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Zygomycota as well as unclassified fungi all varied in the three analysed wetland types. The dominant fungi in the types differed, from unclassified fungi in the wet marshland to Ascomycota in the drier marsh meadow and Basidiomycota in the dry meadow samples.

We thus infer that: 1) the soil nutrients in the three wetland types are different. For example, the wet marshland soil had the highest water content, which combined with low oxygen levels does not support fungal growth very well. This would explain the low fungal diversity and relatively large fraction of unclassified fungi observed. In contrast, a lower soil water content and higher oxygen levels in the drier marsh meadow and dry meadow sites better supported fungal growth and stimulated fungal diversity. 2) The composition of dominant vegetation correlated with the soil fungal composition. Wang (2016, unpublished) observed that with the decrease of the wetland area of the Sanjiang Plain the composition of plants significantly changed due to agricultural development and other human activities. Although *Deyeuxia angustifolia* was the dominant species in all sampled environments, its proportion was different for each type. As a result, the vegetative was the composition and decomposition ratio differed as well. For example, the litter composition was relatively simple and decomposed rather slowly in the marsh wetland. This correlated with a simple fungal composition and low diversity. The drier marsh meadow and dry meadow lands had a better soil environment to promote higher plant diversity, with a more complex litter composition that decomposed faster, correlating with a fungal composition dominated by Ascomycota and Basidiomycota. Tang (2012) found that soil microbial community compositions change according to water logging time, plant diversity and altitude. Zhao (2011) inferred that soil microbial community compositions were different in different plant diversity wetlands. Nevertheless, other studies reported that plant communities, waste composition and soil nutrients did not affect the compositionof soil microbial communities (Tscherko, 2005; Andersen, 2010). Hence, the underlying causes for variation of soil microbial community composition can becomplex and would require more research to be explained.

As reported by others, the abundance of Acidobacteria correlated negatively with soil organic carbon levels (Smit et al., 2001; Fiereret al., 2007) and soil pH (Jones et al., 2009; Rousk et al., 2010); the Canonical Correlation Analysis presented here also suggest this negative correlation. Thus soil pH and soil carbon are major factors governing the abundance of Acidobacteria in the wetlands of the Sanjiang Plain.

In conclusion, the conversion from wet marshlands to dry meadow increased the diversity of bacterial and fungal community and altered the community composition. The permanent conversion of submerged marsh wetland into dry meadow increased the abundance of Acidobacteria, Planctomycetes, Ascomycota, and Basidiomycota but decreased the abundance of Proteobacteria and unclassified fungi. These changes correlated with soil pH and organic carbon content, which were considered main impact factor on soil microbial composition.

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DETERMINATION OF THE BEST GEOSTATISTICAL METHOD FOR CLIMATIC ZONING IN IRAN

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Abstract. Zoning using climatic indices is of significant value in climate studies and climate- related planning. The aim of this study is to assess De Martonne aridity index and to select the best model to draw Iran‘s complete map based on 150 station‘s temperature and precipitation data over a 25-year period (1986-2010). Kolmogorov - Smirnov test (K–S test) was used to check the normality of the data. In order to assess De Martonne aridity index, annual temperature and precipitation data were collected from properly-distributed stations in the study period. Using De Martonne aridity index formula, this index was calculated for all the stations. In the next step, using different geostatistical methods, De Martonne aridity index map was drawn. Semivariogram was used to show the spatial correlation between aridity index data in which linear semivariograms of 0.84 was the best interpolation model to show the correlation. To estimate De Martonne aridity index, inverse distance weighting (IDW), global polynomial interpolation (GPI), radial basis function (RBF), local polynomial interpolation (LPI), as well as Kiriging methods were used. Root mean square error (RMSE), and mean absolute error (MAE) were used to select the best interpolation method. Our results showed that simple kriging method shows the highest correlation with the observed data (R2=0.77). Moreover, it is shown that Iran's central regions due to locating in low lands and being far from the northern and western mountain ranges (Alborz and Zagros) has the lowest De Martonne aridity index (< 5, 5 – 10) and is classified as arid and semi-arid areas while Iran's northern regions has the highest De Martonne aridity index (> 55) is classified as very humid area showing a wide climate range of arid to very humid in Iran.
Keywords: *GIS, rainfall, interpolation, temperature, semivariogram*

Introduction

Aridity which is defined as the lack of moisture is a climatic phenomenon that is based on the average climatic conditions of a consistent region (Tabari et al., 2014). Increased aridity and subsequent desertification, is one of the environmental problems that affect people's living conditions in the world‘s arid areas. Paying attention to different climatic regions and its understanding is quite essential. Understanding climatic condition is the most essential step in studying different human activities such as agriculture, environment, urban planning, transportation, tourism, etc., (Adnan and Haider, 2012). Climatic parameters are useful tools to characterize the status of the climatic system and to perceive its different involving mechanisms (Deniz et al., 2011).

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Aridity index is a climatic index that can be used to monitor and predict aridity (Nastos et al, 2013). So far, several climatic indices have been developed and used utilized in order to study the climatic condition of various regions among which Emberger, Torrent White (Alizadeh et al., 2001), De Martonne., etc. can be mentioned (Tabari et al., 2014). Determination of the most suitable interpolation method in an area and explanation of its spatial distribution is necessary to estimate the spatial distribution of the climatic parameters. There are various methods to assess and estimate such parameters. All these methods are computationally fast and easy. For example, the classical methods, such as Thiessen and the arithmetic mean can be mentioned. There are several methods to estimate the spatial data among which the most common ones include arithmetic mean, gradient, and Thiessen (Corwin et al., 1992; Hosseini et al., 1993). But for some reasons such as not considering the spatial data, and correlation between observations, such methods are not sufficiently accurate. Of course, there are other methods that due to consideration of the spatial correlation of data are highly significant. Deficiencies of the mentioned methods specify the necessity of using geostatistical methods. Geostatistical methods due to considering the spatial correlation of data are of particular importance in assessment of the spatial distribution of the geological data, and gives a better estimate of the underlying parameter in the areas that are not sampled yet (Karimi Nazar et al., 2009). One of the currently used alternative methods to the classical statistical methods (such as regression, weighted inverse square distance, etc.) is the geostatistical methods. Nowadays, these methods are used for interpolation of rainfall stations and other spatial variables (Shabani et al., 2011). Many researchers have been involved in comparison and assessment of various interpolation methods which represents the importance of this issue to reduce the errors of method selection. To interpolate the annual rainfall and temperature in an area of five thousand square kilometers in Portugal, Goovaerts (2000) considered simple kriging method more appropriate as compared to inverse-square method, linear regression with height, Thiessen and kriging methods. Tabari et al. (2014) used data from 40 stations in a time period of 1965 to 2005 to assess De Martonne aridity index. Using the ordinary kriging climate zoning method, these researchers concluded that 88% of Iran involves arid and hyper-arid areas. Furthermore, De Martonne aridity index was decreased by 18% to 54 % in the western and north-western areas. The aim of this study was climate zoning and assessment of De Martonne aridity index using a variety of geostatistical methods and data from 150 rainfall stations in Iran. The aridity index De Martonne modeling was carried out using Arc GIS 9.3 and GS^+ softwares.

Materials and methods

Study area

Iran, with an area of 1648195 square kilometers has been located between latitude of 25° to 40° N and longitude of 44° to 63° E (*Fig.1*). So in terms of latitude southern parts are located in tropical areas, and northern parts are located in subtropical regions. Iran has different climatic conditions due to its geographical position which means fifteen degrees' latitude dispute between the most southern and the most northern point, as well as folds and ups and downs that can be observed on its surface. Apart from these two factors, the combination of the air masses that originate from different regions and collide on Iranian plateau, is one of the most important factors to determine Iran‘s climatic conditions. Proximity to the Persian Gulf and Oman Sea on one side and the

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impact of the Mediterranean Sea on the other side, along with the presence of dry deserts of Arabia and Africa and northeastern great Siberian Plain is effective on type of the air masses that reach Iran.

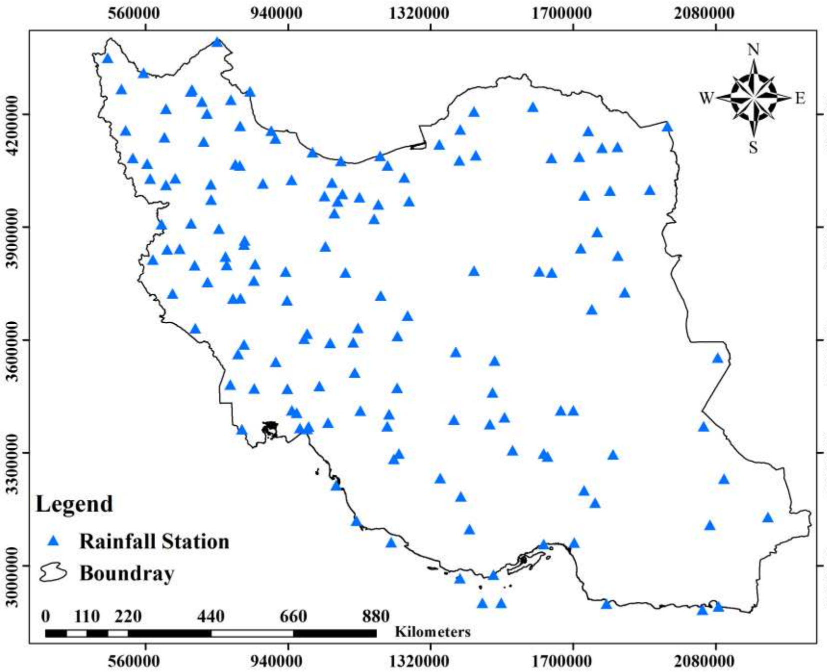

***Figure 1.** The positions of the rainfall stations*

Statistical analysis

Kolomograve - Smirnov test was used to evaluate the normality of the data in the present study. Rainfall data were used following normalization. The histogram (*Figure 2*) and the statistical analysis of De Martonne aridity index from 150 stations in the study area over a period of 25 years (1985-2009) was analyzed in SPSS V.21 software. The results are presented in *Table 1*. *Figure 1* shows the positions of the rainfall stations.

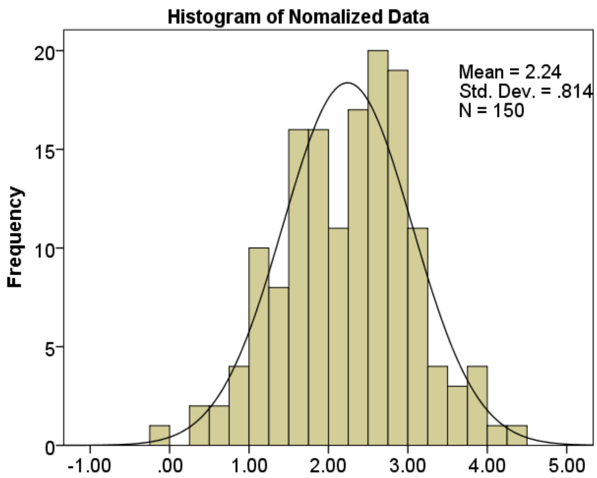

***Figure 2.** Histogram of the normalized De Martonne aridity index*

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Table 1. *Statistical analysis of the data from the study area*

maximum	minimum	skewness	variance	mode	standard deviation	the mean standard error	mean	parameter
71.26	0.82	2.42	134.56	3.34	11.60	0.94	12.93	value

De Martonne aridity index (I)

De Martonne climate classification is one of the conventional methods in climate classification which is used in most climatology projects, particularly in dam construction, agriculture, etc. The basis of this method is presented in Equation (1) and *Table 2* (Tabari et al., 2014).

$$I = \frac{P}{T+10} \qquad \text{(Eq.1)}$$

Where P is the average annual rainfall in mm, T is the average annual temperature in Celsius, and I is De Martonne aridity index.

Table 2. *De Martonne aridity index climate classification (Tabari et al., 2014)*

I_{DM} value	Climatic condition
$I_{DM} < 5$	Hyper- arid
$5 < I_{DM} < 10$	arid
$10 < I_{DM} < 20$	Semi- arid
$20 < I_{DM} < 24$	Mediterranean
$24 < I_{DM} < 28$	Sub-humid
$28 < I_{DM} < 35$	humid
$35 < I_{DM} < 55$	Very humid
$55 < I_{DM}$	Extremely humid

Therefore, annual rain falls and temperature data from 150 rain stations in a time period of 1985 to 2009 were extracted. Statistical analysis was carried out in SPSS V.21. De Martonne aridity index for each station was calculated according to equation I in Excel 2013. The best geostatistical method for climate zoning was selected using Arc GIS V.9.3 software.

Geostatistical analysis

Geostatistics according to the simplest definition is an interpolation method in which the interpolation index or estimation is the minimization of estimation variance (Hohn, 1998). Interpolation is actually the estimation of continuous unknown variable based on a known sample in the region (Lu and Wong, 2008). Geostatistical estimation is one of the most accurate estimation method, since it examines several factors including the distance between points, anisotropy, and spatial variability. But this method has a high volume of calculations that increases the calculation time in large operations (Hirsche et al., 1998). Geostatistics is the study of phenomena which vary in space and time. It

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deals with analysis of samplings with different positions in order to make a continuous level (Johnston et al., 2001). Geostatistical analysis is looking for a way to characterize the spatial continuity and to collect statistical tools and to model such changes. The basic assumption of this spatial- statistical analysis is that nearby observations as compared to distant observations show more statistical correlation. It should be mentioned that the possibility of achieving accurate and efficient results through this type of analysis, is achieved when data are normally distributed and possibly fixed and their mean and variance do not vary spatially (Bohling, 2005).

Variogram

Geostatistics is used to determine the spatial structure of the variables by the average of probabilistic models. This spatial structure is characterized by variogram (Zamani-Ahmad Mahmoodi et al., 2014). Actually variogram is the first step to model spatial structure for Kiriging method. The main aim of semivariogram establishment is to be able to characterize the structure of variable based on the spatial distance. Variogram is calculated using the following equation (Webster and Oliver, 2000: 56):

$$\gamma(h) = \frac{1}{2n(h)} \sum_{i=1}^{n(h)} [z(x_i) - z(x_{i+h})]^2 \qquad \text{(Eq.2)}$$

Where, γ (h) is semivariogram for a pair of points that are located with h distance from each other, n is the number of pair of points that are located with distance h from each other, z (xi) is the observed value for the variable in point X, and z (xi+h) is Observed value for the variable with distance h from *x* (Webster and Oliver, 2000: 56). In variogram curve with increasing distance (h), the amount of γ (h) increases, and this situation will continue until a certain distance from which the value remains constant. In the present study, De Martonne aridity index was estimated using different interpolation methods including inverse distance weighting (IDW), global polynomial interpolation (GPI), radial basis function (RBF), local polynomial interpolation (LPI), as well as Kiriging methods.

Results

Variogram was used to show the spatial correlation between De Martonne aridity index data. The results are presented in *Fig.3*. Then variogram Y (h) was used to fit the data as it shows the spatial correlation between observed De Martonne aridity index data better than other variograms. For this matter the ratio between the nugget effect and sill was used (Co+C) (Li et al., 2015). If the value is <0.25 it indicates high spatial correlation, if the value is in the range of 0.25 – 0.75 it indicates medium spatial correlation, and if the value is > 0.75 it shows either low spatial correlation or no spatial correlation between data (Khodakarami et al., 2011).

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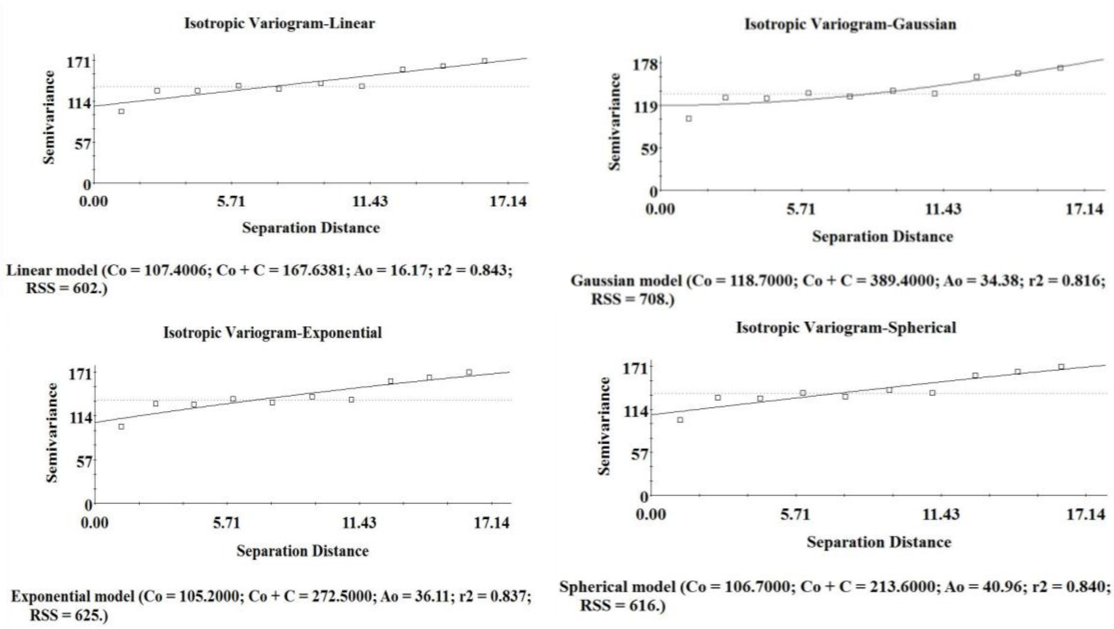

Figure 3. *Variogram to fit De Martonne aridity index data*

Therefore, this ratio was used to select the best variogram. Based on the obtained parameters for the variogram Y (h), linear variogram with the ratio of the 0.83 modeled the best correlation between data and was used as the best interpolation method. Following linear variogram, Gaussian, spherical, and exponential variograms are placed with the amount of 0.82, 0.84, and 0.83, respectively. Different types of the applied variograms to fit data are presented in Table 3.

Table 3. *The nugget effect and sill obtained for the fitted variograms*

Model type	Nugget effect (mm^2) Co	Sill (mm^2) Co+C	C /Co+C ratio	Distance (km) Ao	R^2
Gaussian	118	389	0.69	34	0.81
Linear	107	167	0.35	16	0.84
spherical	106	213	0.50	40	0.84
exponential	105	272	0.61	36	0.83

The results on different models evaluation is presented in *Table 4*. Our results showed the highest correlation between simple- kriging method and the observed data (R2=0.77).

Table 4. *De Martonne aridity index data correlation based on different statistical methods*

Method	Linear	Power	Exponential
Emprical	0.54	0.70	0.46
LPI_1	0.53	0.69	0.45
LPI_2	0.54	−	−
LPI_3	0.54	−	−

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GPI_1	0.23	–	–
GPI_2	0.22	–	–
GPI_3	0.25	–	–
IDW_1	0.43	0.63	0.37
IDW_2	0.42	0.66	0.42
IDW_3	0.41	0.66	0.43
RBF	0.50	0.70	0.45
K_O_Ga	0.55	0.73	0.48
K_O_St	0.55	0.73	0.48
K_Si_Ga	0.57	0.69	0.45
K_Si_St	0.57	0.69	0.45
K_Si_Ex	0.72	0.77	0.56
K_Si_Sp	0.65	0.73	0.50
K_Un_Ga	0.55	0.72	0.47
K_ Un _St	0.55	0.72	0.47

Q-Q Plot of the original and normalized data in SPSS V.21 is presented in *Fig.4*. The plot representing the observed and predicted De Martonne aridity index data using simple – kriging method is presented in *Fig.5*.

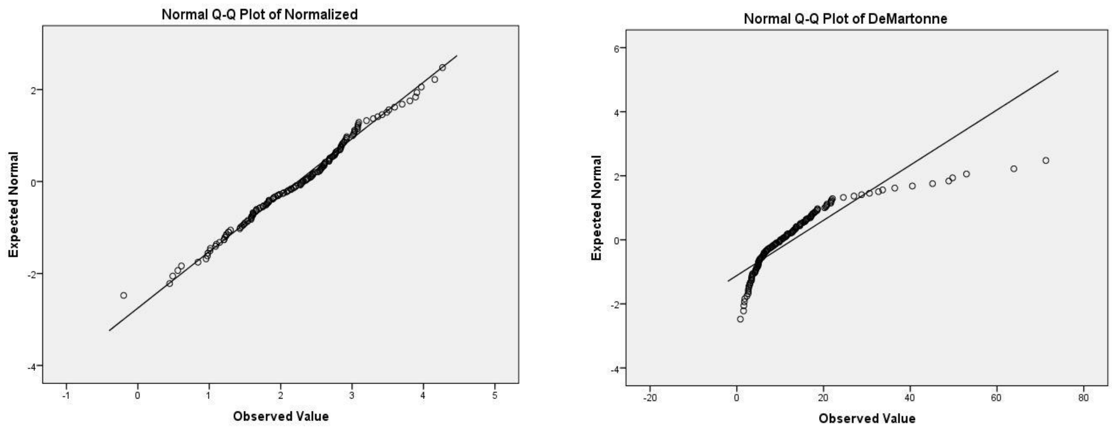

Figure 4. *Q-Q Plot of the original (right side), and normalized (left side) De Martonne aridity index data.*

Fig.6. represents the zoning map of De Martonne aridity index using the simple-kriging method which shows the highest statistical accuracy than other methods. Because of the high number of stations in the area and their appropriate distribution, a complete zoning map of the area can be prepared. Also *Figure 6* represents the map of the difference between the average maximum and minimum temperature ($\Delta\theta$), average maximum and average minimum temperature. It was statistically predicted that a high correlation may be observed between $\Delta\theta$ and De Martonne aridity index, however, this correlation was very low at the level of approximately 10%. Comparison of $\Delta\theta$ and De Martonne aridity index maps showed a high correlation between these parameters on their maps, so that in the central and eastern parts of the country with the lowest De Martonne aridity index, ($\Delta\theta$) shows the highest value. Furthermore, as we getting

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closer to the northern strip of the country, De Martonne aridity index is increased, while ($\Delta\theta$) is decreased. Unlike the central and northern strip of the country, a good spatial correlation is not observed between these two indices in the west and south of the country. Because of the low rainfall in the southern strip, De Martonne aridity index is also low, but because of the proximity to the sea as a source of moderating temperatures and also proximity to the equator, the difference between the minimum and maximum average temperature is not too much. Also, due to the high rainfall in the west of the country, De Martonne aridity index is high, and the temperature difference is too much.

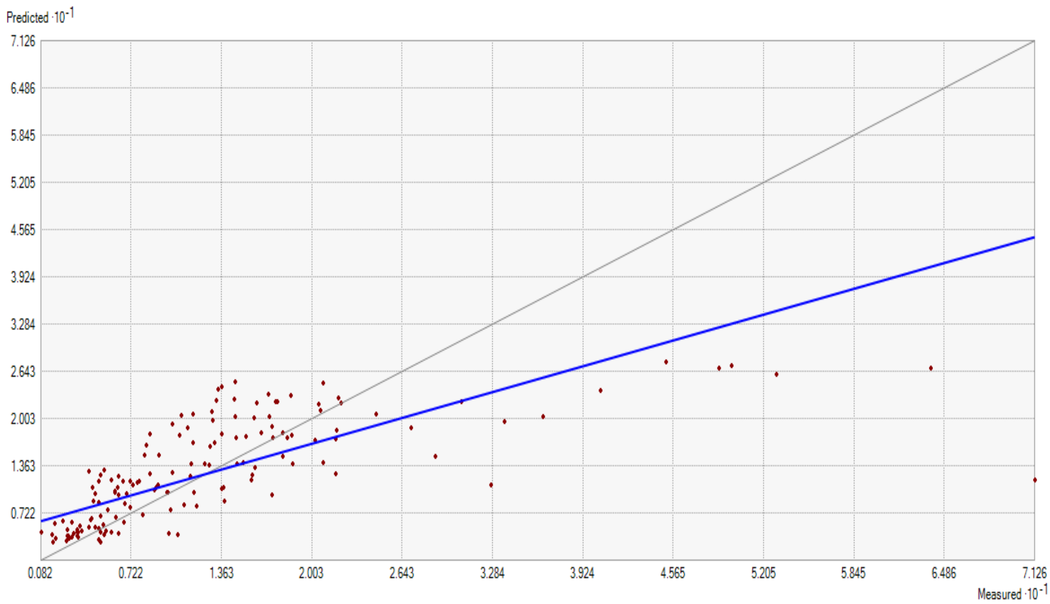
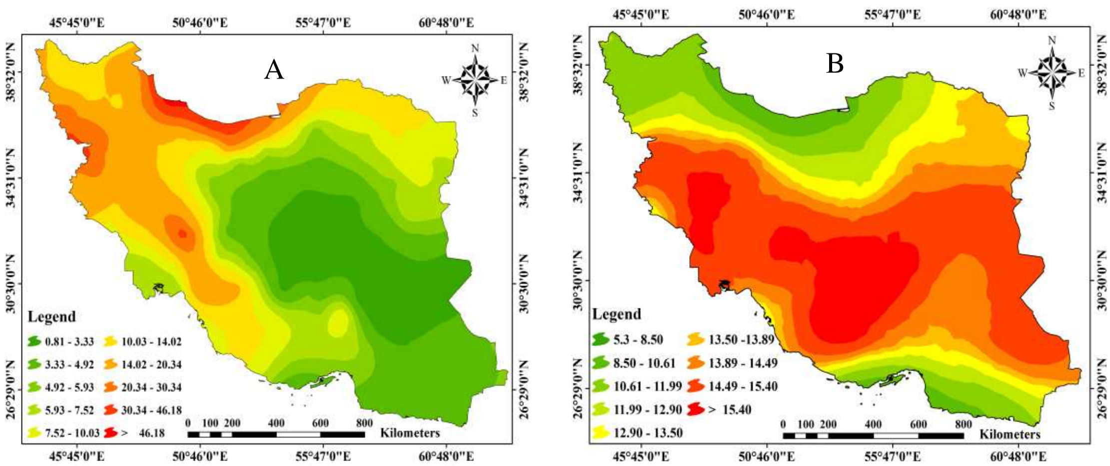


Figure 5. *Assessment of the relation between the observed and predicted De Martonne aridity index data using simple – kriging method.*



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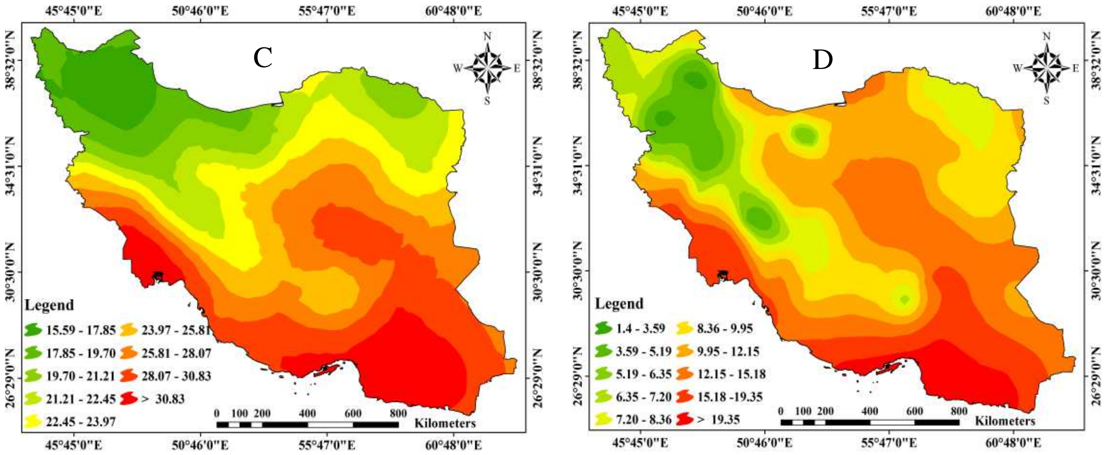

Figure 6. *Map of De Martonne Aridity index (A), difference between the average maximum and minimum temperature ($\Delta\theta$) (B), average maximum temperature (C) and average minimum temperature (D)*

In the present study, 17 interpolation methods including IDW, GPI, RBF, LPI, as well as Kiriging was used to assess De Martonne aridity index in Iran. Variogram used in this study clearly showed that semivariograms besides demonstration of the spatial correlation between De Martonne aridity index data are able to model the changes in the spatial correlation in various aspects. Our results showed the highest correlation between Simple-Kriging method and the observed De Martonne aridity index data. Furthermore, it was shown that linear variogram with the nugget effect /sill ratio of the 0.83 modeled the best correlation between observed De Martonne aridity index data and was used as the best interpolation method. Following linear variogram, Gaussian, spherical, and exponential variograms are placed with the amount of 0.82, 0.84, and 0.83, respectively. Moreover, the relation between the observed and predicted De Martonne aridity index data (R2=0.77) was assessed to validate data (*Fig.5*). The strong correlation between the observed and expected data shows high performance of Simple-Kriging method as the interpolation method in the present study. Musburger et al. (2012) used the correlation between the observed and predicted data (R2 = 0.69) to validate the data series.

Conclusion

Interpolation of data along with GIS is of particular importance in spatial analysis, since many of the maps used in GIS operations are provided through interpolation. In fact, providing continuous models from spatial and temporal distribution of data is possible through interpolation. De Martonne aridity index is a linear relationship, and as it has a direct relation with rainfall value, the classification of this index is highly correlated with rainfall value (R2=0.96). The lowest De Martonne aridity index belongs to central, eastern and south-eastern areas which are actually Kavir plain (central Iran) and Lut plain (Southeast). Getting away from these areas in all geographical directions, the aridity is decreased, and as a result De Martonne aridity index is increased. The highest De Martonne aridity index is observed in northern areas, next to the Caspian Sea. Astara with the mean annual rainfall of 2000 mm is located in this area (40 times

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more than the mean annual rainfall of the central areas). Iran's climate is highly affected by the presence of the Alborz and Zagros Mountains. Alborz Mountains, particularly its northern slopes devote a high portion of the northern rainfall to itself and prevents the passage of the northern stream to the central areas. Zagros Mountain which is located in north-west /south-east direction devotes the Mediterranean rainfall to it and prevents the rainfall to be passed to the central parts. As a result, a vast part of central areas in Iran has an arid and semi-arid climate.

Other factors which contribute to the excessive dryness of these areas include being away from the moisture sources and being placed in high pressure mid-latitudes. The major factors that can affect climate classification within a country are topography and mountain as other factors such as being away from the moisture sources and being placed in high pressure mid-latitudes are fixed for a country.

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NOT ALL OF THE RARE OPERATIONAL TAXONOMIC UNITS (OTUs) PLAY THE SAME ROLE IN MAINTAINING COMMUNITY STABILITY

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Abstract. The role of rare biosphere in maintaining the community diversity and metabolic activity has recently been highlighted. However, it is still unclear whether the rare species play the same role in maintaining the community diversity. Considering different responses of microbial species on environment changes, we speculate rare species played different roles in maintaining the α-diversity. To verify the speculation, we analyzed the prokaryotic species in three eutrophic rivers and one eutrophic lake via Miseq sequencing of 16S rDNA amplicons. Although more than 50 phyla were identified from 20 samples, only seven were dominant. The dominant species were strictly restricted. The rare operational taxonomic units (OTUs) belonging to the dominant phyla played a crucial role in maintaining the community diversity. These results intensified our knowledge on the role of rare species in maintaining the diversity of microbial community.
Keywords: *Chaohu Lake*; *community diversity*; *microbial ecology*; *rare species*; *stochastic*

Introduction

Enumerating microbial community diversity (MCD) and analyzing the mechanisms that maintain the diversity are two basic ecological and environmental issues. The application of DNA-based molecular tools, especially high-throughput sequencing technologies, greatly enhances our understanding of the MCD as these techniques circumvent the problems in classical culture-based techniques (Caporaso et al., 2011; Rees et al., 2004; Sergeant et al., 2012). Lots of microbial communities that exist in various habitats, such as soil (Sul et al., 2011; Vasileiadis et al., 2012), ocean (Gilbert et al., 2009), human and animal guts (David et al., 2014; Lee et al., 2011; Ni et al., 2014) and freshwater (Kara et al., 2013; Widder et al., 2014), have been investigated by these

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techniques. Based on the investigations, a lot of ecological principles that maintain the MCD are described, such as the taxa-area relationship (Horner-Devine et al., 2004), the cosmopolitan distribution of microbial subgroups (Chaffron et al., 2010) and the distance-decay relationship (Bell, 2010). These ecological principles extend our knowledge about the mechanisms that maintain the MCD (Ni et al., 2016). However, although we know microbial communities are extremely diverse in natural environments, and they are typically composed of a few dominant species followed by a large number of rare species (Zhou et al., 2015), the roles of the rare species on maintaining the community α-diversity are still largely unclear.

Recently, the role of rare species on maintaining the MCD and the metabolic activity has been highlighted (Campbell et al., 2011; Coveley et al., 2015). Coveley et al. (2015) point out a fraction of the rare species acts as a backup system for maintaining ecosystem resilience in face of perturbation. When environment is disturbed, some rare species rise to dominant species to maintain the diversity and metabolic activity of microbial community. However, whether every rare species in a specific microbial community plays the same role on maintaining the MCD is still unclear.

Shannon's index and Simpson index are the generally accepted indexes to indicate the community α-diversity. Since both Shannon's index and Simpson index consider the abundance of each species, the influences of species with different relative abundances are distinct. Therefore, when the dominant species is absent causing by environmental disturbance, the ability becoming to dominant species was a rational agent to character the role of rare species on maintaining the community α-diversity. According to this inference, the rare species that can become to dominant species under environmental disturbance play more important roles than those cannot become to dominant species. Therefore, through analyzing the fluctuation of species in different habitats, we can infer the roles of rare species on maintaining the α-diversity.

To address the question that whether every rare species in a specific microbial community plays the same role on maintaining the α-diversity, we analyzed the fluctuation of prokaryotic species in three eutrophic rivers and one eutrophic lake via Miseq sequencing of prokaryotic 16S rRNA gene amplicons.

Materials and Methods

Sampling sites and sample collection

Samples were collected in four seasons: September 2013 (autumn), December 2013 (winter), March 2014 (spring), and June 2014 (summer). In each season, five sites were set at Nanfei River (NF, N31°51′00.11″, E117°18′34.27″), Zhigao River (ZG, N31°46'32.77", E117°44'55.04"), Hangbu River (HB, N31°25′19.08″, E116°56′48.32″), East Chaohu Lake (EC, E117°35′52.2", N31°36′21.0") and West Chaohu Lake (WC, E117°17′44.7", N31°40′26.5") (*Fig. 1*). All sites were sampled within one day. Water of 500 ml and water of 1L was collected from each sampling site for chemical characterization and for microbial DNA extraction, respectively. DNA extraction was carried out within 24 h after sampling collection.

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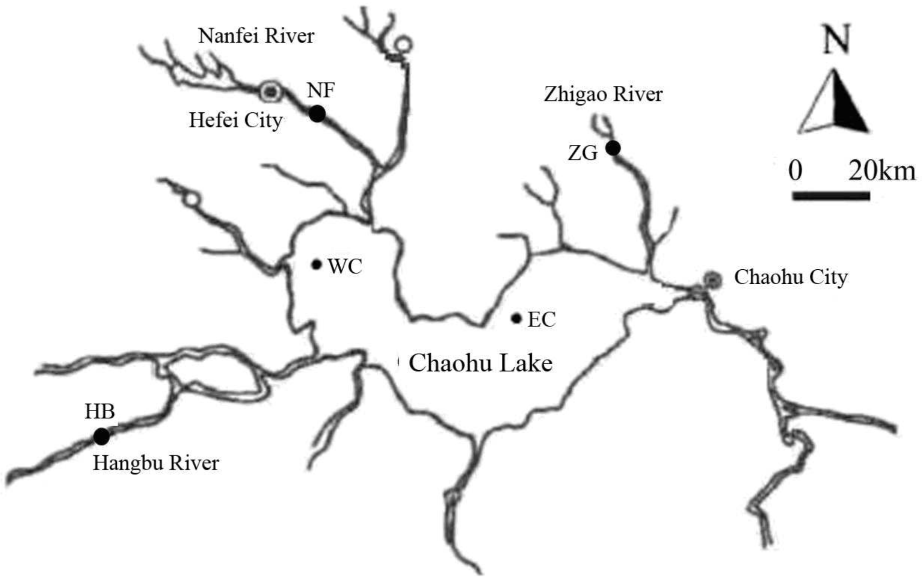

Figure 1. *Sampling sites of Chaohu Lake and three rivers. ZG, Zhigao River; HB, Hangbu River; NF, Nanfei River; WC, West Chaohu Lake and EC, East Chaohu Lake.*

Measurement of physical and chemical indexes

Water temperature (WT) was measured in at field simultaneously with sample collection. Total phosphorus (TP), pH, total nitrogen (TN), nitrate (NO_3-N) and ammonia nitrogen (NH_4-N) were measured according to standard methods (Huang, 2000). The trophic level index (TLI) was calculated based upon the concentration of TP according to the standard method (OECD, 1982).

DNA extraction and MiSeq sequencing of 16S rRNA gene amplicons

The microbial DNA was extracted using the standard phenol-chloroform method with some modifications (Ni et al., 2010). The water samples for microbial DNA extraction were filtered by polycarbonate membranes with 0.22 μm pore size. The filter membranes were cut into pieces and transferred into 2.0 ml sterile centrifuge tubes. Then the tubes were added 600 μL of extraction buffer (100 mM Tris-HCl (pH 8.0), 100 mM phosphate (pH 8.0), 100 mM EDTA (pH 8.0), 150 mM NaCl, 1% (wt/vol) CTAB and 2 mg Lysozyme) and were incubated at 37°C for 90 min. After incubation, cells were disrupted by three cycles of freezing (-80°C for 30 min) and thawing (65°C for 30 min). Cells were then incubated at 65°C for 30 min with 100 μL 20% SDS and 10 mg proteinase K. The supernatant was treated with 0.2 volumes 8 M potassium acetate to remove polysaccharides followed by treatment with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) to remove protein and cell debris. Residual phenol was removed by the addition of an equal volume of chloroform-isoamyl alcohol. Nucleic acids were precipitated from the supernatant by adding approximately an equal volume of isopropanol and maintaining the samples at 4°C for at least 2 h. The solution was centrifuged at 14,000 ×g for 20 min. Residual of isopropanol were completely removed by adding 70% (v/v) alcohol and centrifugation at 14,000 ×g for 20 min. Extracted genomic DNA samples were dissolved by 50 μL of Tris-EDTA (TE) buffer. DNA concentration and quality were checked using a NanoDrop Spectrophotometer.

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The extracted DNA was diluted to 10 ng/μL and stored at -40°C for downstream use.

V4-V5 hypervariable region of prokaryotic 16S rDNA was amplified using universal primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT -3') with 12 nt unique barcode and sequenced as a previous report (Li et al., 2016). The sequencing was performed on an Illumina Miseq system using paired-end technology provided by Dongguan Meikang BioScience Inc., China.

Data analysis

The raw reads were spliced using FLASH 1.2.8. The merged sequences were processed using QIIME Pipeline (Caporaso et al., 2010). All sequence reads were trimmed and assigned to each sample based on their barcodes. The sequences with high quality (length > 300 bp, without ambiguous base 'N', and average base quality score > 30) were used for downstream analysis. Chimera check was conducted using the Uchime algorithm (Edgar et al., 2011). Non-chimera sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold. Taxonomy was assigned using the Ribosomal Database Project classifier (Wang et al., 2007). Canonical correspondence analysis (CCA) with Monte Carlo permutation test was carried out by vegan package in R platform (Dixon, 2003).

The merged sequences were deposited into the NCBI short-reads archive database (Accession Number: SRR2079561-SRR2079595 and SRR2079602).

Results and Discussion

A total of 320554 high-quality sequences from 20 samples were obtained in this study. At the phylum level, except 0.7-3.8% reads were unclassified into archaeal and bacterial phyla, 2 distinct archaeal phyla and 49 distinct bacterial phyla were identified. However, only seven bacterial phyla, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Planctomycetes, Proteobacteria and Verrucomicrobia were dominant phyla (their relative abundance is more than 10%), and their reads occupied 88.7-95.7% of total reads. Four of these seven phyla, i.e. Actinobacteria (13 sampling sites), Bacteroidetes (14 sampling sites), Cyanobacteria (14 sampling sites) and Proteobacteria (20 sampling sites) were the dominant phyla in more than ten sampling sites. These results implied that although there were high biodiversity, the dominant phyla in eutrophic rivers and lake were strictly restricted. In addition, there was no consistent seasonal changing pattern among different sampling sites. For instance, in the sampling site ZG, the relative abundance of Verrucomicrobia in winter was obviously higher than other seasons, while those in autumn were obviously higher than others in the sampling site HB (*Fig. 2*).

To analyze the randomness and succession of microorganisms in species (OTU) level, we compared the trends of the dominant OTUs (their relative abundance is more than 1%) among different seasons. A total of 14,746 OTUs were detected from the 20 sampling sites. However, only 83 OTUs were dominant. All of the dominant OTUs were classified into Bacteria. Except one dominant OTU was classified into phylum Chlorobi and one dominant OTU was not classified into any phylum, others were classified into the seven dominant phyla, i.e. Actinobacteria (7 OTUs), Bacteroidetes (18 OTUs), Cyanobacteria (15 OTUs), Firmicutes (2 OTUs), Planctomycetes (4 OTUs),

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Proteobacteria (29 OTUs) and Verrucomicrobia (6 OTUs) (*Fig. 3*). It was consistent with the dominant phyla. The result showed that even though there were more than 14,700 OTUs detected in the present study, the dominant OTUs were strictly restricted into the seven dominant phyla. This result implied that although the role of rare species in maintaining the community diversity has been highlighted in a highly diverse ecosystem (Coveley et al., 2015), it did not mean that all of the rare OTUs played the same role in maintaining the community α-diversity. The rare species belong to the seven dominant phyla were more important in maintaining the community α-diversity. The role of those rare OTUs that were not classified into the seven dominant phyla still needs to illustrate.

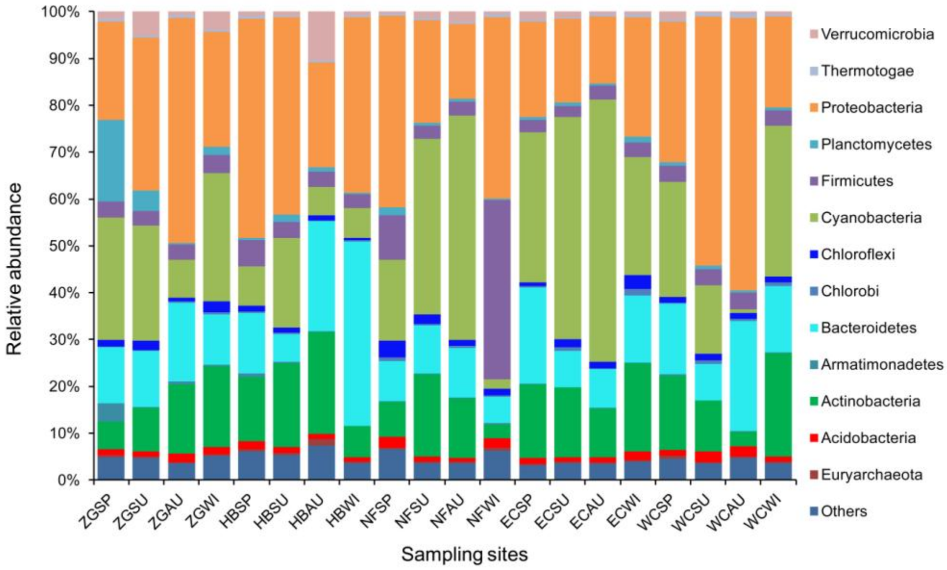

Figure 2. *Relative abundances of prokaryotic phyla harboring in eutrophic rivers and Lake Chaohu. ZG, Zhigao River; HB, Hangbu River; NF, Nanfei River; WC, West Chaohu Lake and EC, East Chaohu Lake.*

To elucidate the reasons causing the turnover of dominant OTUs, we analyzed the relevance between the compositions of the dominant OTUs and the environmental physicochemical factors containing WT, pH, TN, NH4, NO3, TP and TLI. According to the TLI, ZGWI, HBWI, WCWI, WCSP were eutrophic, the other sites were hypereutrophic. CCA with Monte Carlo permutation test showed neither total of environmental factors (Permutation test with 999 permutations, Pseudo-F = 1.058, p = 0.404) nor each environmental factor significantly impacted on the dominant OTU turnover.

To analyze fluctuating details of the dominant OTUs, we artificially differentiated the dominant OTUs between two distinct factions according to their occurrence frequency as dominant OTU in the samples: high-frequency dominant OTUs that appear more than or equal half number of samples ($\geq$10) with the relative abundance more than 1% and low-frequency dominant OTUs that appear less than half number of samples (< 10) with the relative abundance more than 1%. A total of 5 OTUs were detected as high-frequency dominant OTUs (*Fig. 4*) and they belonged to different phyla, i.e. each two OTUs belonged to Proteobacteria and Actinobacteria, and one OTU belonged to

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Cyanobacteria. Seventy-eight OTUs were detected as low-frequency dominant OTUs, in which 43 dominant OTUs were only detected from one samples (*Fig. 4*). This result implied that stochastic process was a non-ignorable factor that influences the microbial community structure, just as other evidences provided by previous reports (Chase, Myers, 2011; Zhou et al., 2014).

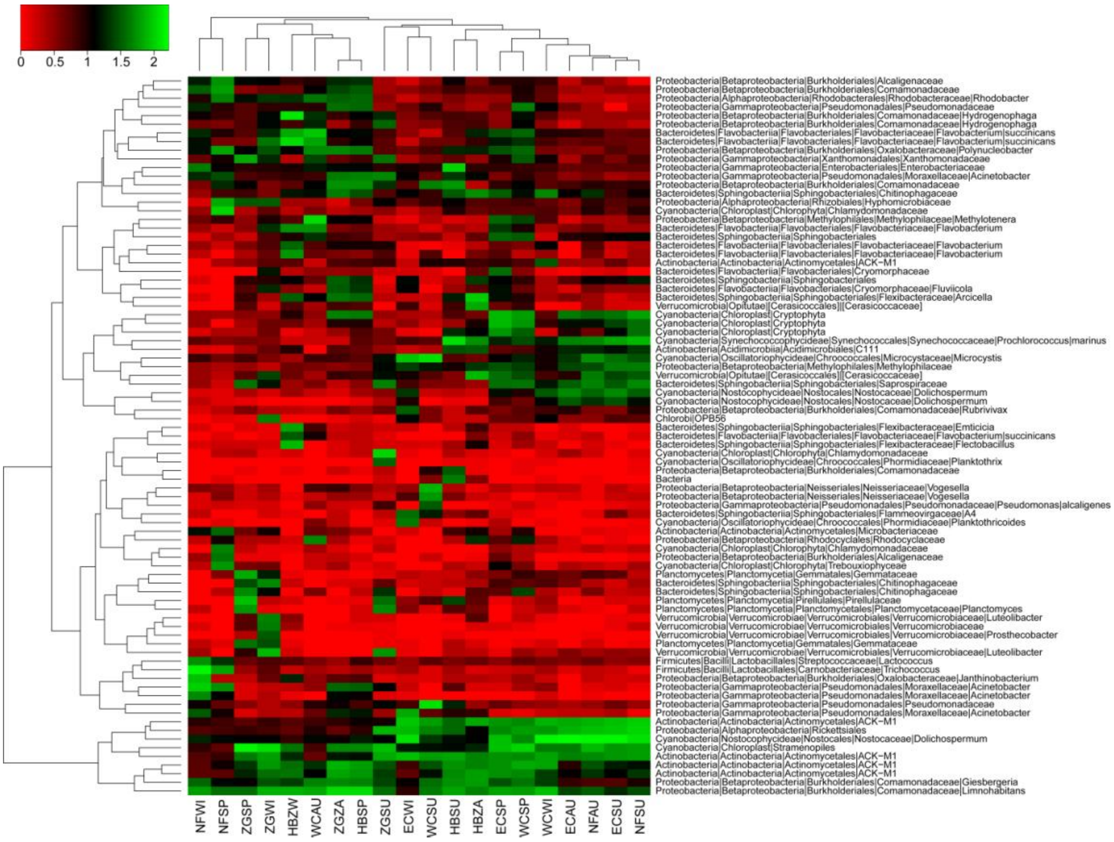

Figure 3. *Heatmap profile of dominant OTUs. The data were transformed according to the formula as follows, log_{10}(relative abundance of each otu * 100 + 1). ZG, Zhigao River; HB, Hangbu River; NF, Nanfei River; WC, West Chaohu Lake and EC, East Chaohu Lake.*

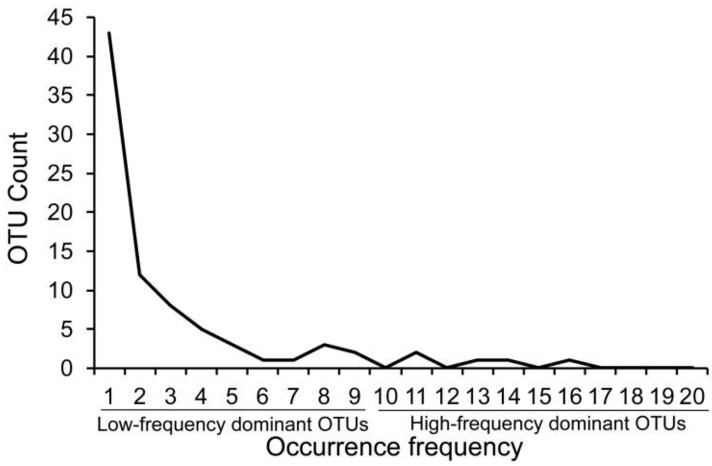

Figure 4. *Occurrence frequency of the dominant OTUs in the samples*

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Conclusions

It was concluded that not all of the rare OTUs played the same role in maintaining the community diversity. The rare OTUs belonging to seven dominant phyla played a crucial role in maintaining the microbial community diversity and which OTUs in the seven dominant phyla could become the dominant OTUs were conditionally stochastic.

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LOCAL CLIMATE AFFECTS GROWTH AND GRAIN PRODUCTIVITY OF PRECISION HILL-DIRECT-SEEDED RICE IN SOUTH CHINA

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Abstract. Temperature fluctuations at critical growth stages of rice may cause crop yield loss, shifts in crop growth periods as well as in sowing and harvesting times. Direct seeded rice has been proved as a the best alternative to transplanted rice and has been adopted successfully in many regions over the globe. This study was carried out to evaluate the productivity of rice sown under different environments with precision hill-direct-seeding method. Four rice varieties viz. Peizataifeng, Yuxiangyouzhan, Huahang 31 and Huayou 213 were sown in 6 different environments (E1-E6) by following split-plot design with four replications. Results showed that environments (E), varieties (V) and their interactions (E × V) significantly ($p \leq 0.01$) affected seedling survival rate, grain yield and yield related traits like panicles m^{-2}, spikelets $panicle^{-1}$ ($p \leq 0.05$), fully filled grain % , 1000-grain weight, harvest index as well as daily dry matter and grain productivity. Differential varietal response under different environments depicted that all environments remained productive regarding rice growth and yield except environment 3 (E3) that caused grain yield reduction up to 45.86%. The overall performance and yield response of rice varieties under different environments was recorded as: *Huahang 31 > Yuxiangyouzhan > Peizataifeng > Huayou 213.* Further, correlation analysis showed significant and positive relationships of rice grain yield with yield contributing factors ($r > 0.47$) except panicles m^{-2} and grains $panicle^{-1}$. Hence, fluctuations in regional/local climatic conditions may cause shifts in sowing/planting times, poor stand establishment and seedlings survival rates that may lead to yield penalty in rice.
Keywords: *environment, growth, rice cultivars, temperature fluctuations, yield*

Introduction

Rice is one of the world's most important cereal and a major food for more than half of the global population (Farooq et al., 2011; Krishnan et al., 2011; Ashraf et al., 2014).

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In China, more than 65% population solely relies on rice that serves as staple food (Zhang et al., 2005; Mo et al., 2016a). So for maintaining food security in China and to save the world population from hunger, it is necessary to increase the rice productivity by adopting better management strategies (Fang and Cheng, 2009; Ashraf et al., 2015). In general, about 0.6 to 0.9% increase in rice production per annum is required to meet the consumption rate of increasing population worldwide (Carriger and Vallee, 2007). On the other hand an increase of crop productivity per unit of land area is needed due to the effects of urbanization and industrialization on agricultural lands (Takai et al., 2006).

Climate change is one of the external factors that affect crop productivity worldwide, and may have significant impacts on growth and yield formation of rice. Predictions about climate change through general circulation models warrant an increase in global temperature of 4 ºC by the end of 21st century (IPCC, 2007) which confirms that future rice production will be in warmer climate.

Thus, adjustment of crop planting dates is an important strategy to avoid the effects of environmental severities in order to maintain crop growth and productivity (Sacks et al., 2010; Yao et al., 2011). Sowing dates affect rice growth duration, thus affecting rice response to light, temperature and other environmental factors (Wang et al., 2001a; Wang et al., 2001b; Yao et al., 2012; Ehsanullah et al., 2014). Low temperature in spring is the main factor that affects sowing date of direct seeding rice, for example, sowing too early may result in low temperature stress, whilst late sowing may face difficulty in the coordination of temperature and light growth period and ultimately the productivity of rice (Yi et al., 2010). The suitable temperature indices to get higher paddy yields during early-season rice in Guangdong province (China) are as follows: whole growth period is 23-24 °C, whereas from germination $\rightarrow$ tillering $\rightarrow$ booting $\rightarrow$ heading $\rightarrow$ physiological maturity are 18-21 °C, 21-25 °C, 24-28 °C and 27-30 °C, respectively (Wang et al., 2012). It has also been reported that rice should be sown earlier in Guangdong early-season rice cropping systems than their normal sowing (most probably in early March) in the fields due to the variable rainfall pattern and temperature fluctuations caused by a special climatic character of this region called "*Dragon Boat Water*" (Chen et al., 2006; Chen et al., 2010; Huang et al., 2011; Wang, Chen and Huang, 2011; Li et al., 2016).

Precision hill-direct-seeding of rice is a new planting technique developed by Luo et al. (2007) which has been used in China and some other countries in Asia (Kargbo et al., 2016). It showed advantages when compared to manual transplanting of rice nursery in the field and/or manual direct seeding (Luo et al., 2008; Tang et al., 2009). Moreover, precision hill-direct-seeding of rice requires optimal climatic conditions in the field, especially suitable temperature. In the best of our knowledge, to date, no experimental proof has yet been reported on the effects of different temperature regimes on seedling survival rate, growth and yield of precision hill-direct-seeded rice in the agro-climatic conditions of South China. Therefore, we have conducted a field experiment with four popularized rice cultivars sown under six different environments to study their effects on rice seedling survival rate, yield and yield components of precision hill-direct-seeded rice under local climatic conditions of the Guangdong province of South China where

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temperature fluctuations (low at early seedling establishment phase and high at flowering and grain filling stage) limits rice growth and productivity. The main objective of this study was to ascertain the impacts of changes in the local climatic conditions on the performance and yield formation of rice under field conditions.

Materials and methods

The field experiment was conducted at Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou (23°09′N, 113°22′E and 11 m above the sea level), Guangdong Province, P. R. China, during 2011. The experimental soil was sandy loam soil with following properties: soil pH = 5.44; organic matter = 19.65g kg^{-1}, total nitrogen = 0.97g kg^{-1}, available phosphorus = 31.74 mg kg^{-1}, available potassium = 189.48 mg kg^{-1}. This region has a humid subtropical monsoonal climate with warm winters and hot summers with an annual average temperature range lies between 21 to 29 °C (Mo et al., 2016b). Four popularized rice cultivars of this region i.e., *Peizataifeng*, *Yuxiangyouzhan*, *Huahang 31* and *Huayou 213* with 125-130 days of growth were obtained from College of Agriculture, South China Agricultural University Guagzhou, China. Among these cultivars, *Peizataifeng* and *Huayou213* were hybrid rice, whereas *Yuxiangyouzhan* and *Huahang 31* were inbred rice.

Field preparation and crop management practices were followed according to the standard practices for this region (Tang et al., 2008). Before sowing, field was fully irrigated and then cultivated with tractor-mounted cultivator thrice, and two days later raked with laser land leveling machine. Seeds of all four rice cultivars were sown on 6 different dates (*Table 1*) using a precision hill-direct-seeding machine (2BD-10) at spacing of (35 cm + 15 cm) × 14 cm with a seed rate of 15 kg ha^{-1} in the well prepared land by following split-plot design with four replications. The meteorological data regarding temperature range and relative humidity during the whole rice growing season has been shown in *Figure 1*. ‘Super rice special fertilizer’ 1200kg ha^{-1} (N 12.5%, P_2O_5 6.0%, K_2O 10.0%, organic matter 15.0%) was applied 25 days after sowing. Mature crop was harvested on 10th and 15th July to record paddy yield and harvest index. Standard agronomic practices were followed with respect to irrigation, pest management, and weed control as recommended by the Guangdong Province, South China.

Table 1. *Development of four rice cultivars as affected by a wide range of environmental conditions*

Environments	Sowing dates	Dates of 50% flowering	Days to harvest	Mean max./min./ Avg. temperature (°C) ^{a}	Mean max./min./ Avg. temperature (°C) ^{b}
E1	24-Feb	2-Jun	137	25.7/15.3/18.9	33.2/23.7/27.9
E2	1-Mar	6-Jun	132	22.0/13.4/16.9	33.5/25.6/28.9
E3	5-Mar	9-Jun	132	17.7/12.9/14.8	32.8/25.8/28.7
E4	10-Mar	11-Jun	127	22.5/13.4/17.0	32.6/25.5/28.5
E5	15-Mar	12-Jun	122	17.4/11.9/14.2	32.3/25.5/28.3
E6	20-Mar	12-Jun	117	20.3/13.9/16.3	32.3/25.5/28.3

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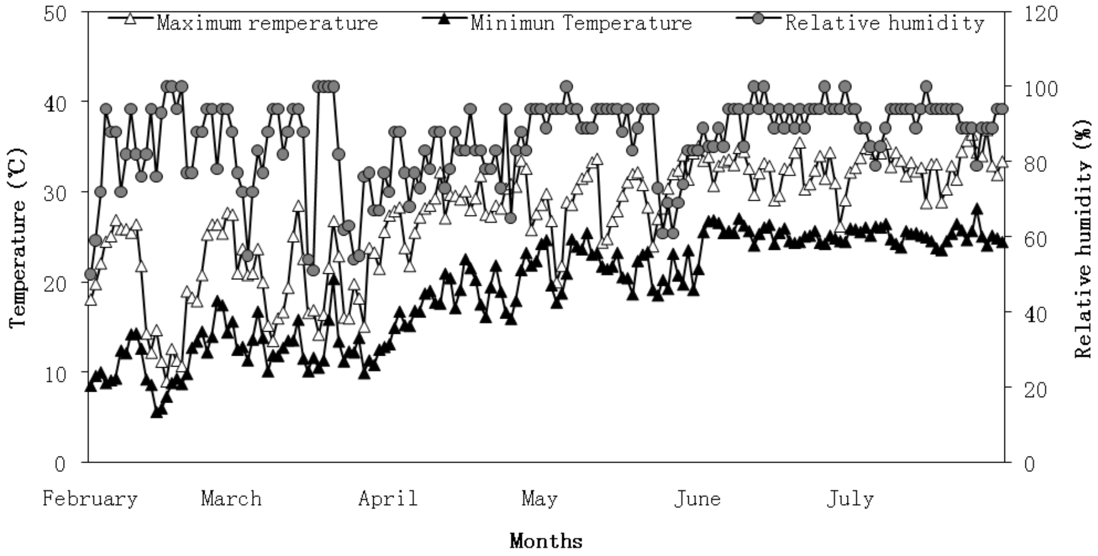

Figure 1. *Daily maximum and minimum temperature (°C) and relative humidity (%) during the course of study*

Seed number were counted immediately from a randomly selected unit area (1 m^2) at four different locations in each plot after sowing and recorded by marking each location; the survival seedling number was counted at each marked location at the four leaf stage to calculate the seedling survival rate by following formula: (survived seedlings / total rice seedlings) × 100.

At maturity, four hills were uprooted randomly, oven-dried at 70 °C till constant weight to determine of rice dry biomass. Panicle numbers were counted from a randomly selected unit area (1 m^2) at four different locations in each plot then averaged. Five hills were randomly selected from each plot to calculate total spikelets per panicle, grains per panicle and fully filled grain percentage. Five samples of thousand grains were taken randomly from each seed lot, weighed and averaged to record 1000-grain weight. One unit area (1 m^2) of plants was harvested, threshed manually, sun dried, weighed and then adjusted to 14% moisture content to recorded grain yield.

Harvest index (HI) was calculated as: (grain yield/biological yield) × 100. Daily grain and dry matter productivity was calculated as the ratio between grain yield and dry matter accumulation at maturity to whole crop growth period and expressed in kg grain per hectare per day and kg dry weight per hectare per day (kg ha^{-1} d^{-1}), respectively.

A split plot design with a plot size of 30 m^2 was followed in which sowing dates and cultivars were randomized in main and subplots, respectively with four replications. Statistical analyses were performed using analysis of variance (ANOVA) by using Statistix 8 (Analytical software, Tallahassee, FL, USA). The differences amongst treatments were separated using least significant difference at the 5% probability level. Figures were generated by using Microsoft Excel 2007.

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Results

After seed sowing, the highest values for mean maximum temperature were recorded in E1 while lowest values for mean maximum, minimum and average temperatures were recorded in E5 environment (*Table 1*). Days to 2-leaves stage in rice seedlings for E2 was exposed to temperatures less than 12 °C (around March 15) (*Fig. 1; Table 1*). Days to 50% flowering were shorter with late sowing (E5 and E6) than with early sowing (*Table 1*). Besides, days to harvest were shorter with late sowing than with early sowing, excluding a similar growth period in E2 and E3. The environment from E2 to E3, i.e., from March 2 to March 5 sowing seems to be the environment period that separated the six environments into the two ranges of environments. The mean maximum, minimum and average temperature of 50% flowering were in the range of 32.3-33.5 °C, 23.7-25.8 °C and 27.9-28.9 °C, respectively. However, high temperatures (~35 °C) were observed in E3 (June 9) environment (*Fig.1*). Grain yield was significantly different for environments, variety their interaction (E × V) (*Table 2*). Among six different environments, E6 on average produced the highest grain yield, with E1-E5 affected by cooler temperature during seedling stage, and/or higher temperature during flowering and/or grain filling stage, resulting in significantly lower grain yields. For rice varieties, maximum and minimum grain yield was obtained for *Huahang 31* (6.75 t ha^{-1}) and *Huayou 213* (4.75 t ha^{-1}), respectively (*Fig. 3a*).

Table 2. *Effect of growing environment on grain yield, yield related traits, seedling survival rate and productivity of rice among varieties in terms of F-value*

Traits measured	**Environment（E）**	**Variety （V）**	**E × V**
Grain yield	2457.78**	138.86**	16.13**
Panicle number per m^2	6.15**	9.45**	3.11**
Number of spikelets per panicle	3.22*	14.27**	2.85**
Fully filled grain percentage	26.36**	15.28**	2.82**
1000-grain weight	437.26**	242.52**	92.19**
Seedling survival rate	82.51**	8.99**	12.72**
Daily dry matter productivity	48.89**	6.02**	5.28**
Daily grain productivity	3673.68**	137.33**	16.56**
Harvest index	4.67**	25.50**	8.75**

Date with no asterisk were non-significant, * and ** mean significance at the 0.05 and 0.01 probability level, respectively

Yield related traits, seedling survival rate and productivity of rice varied significantly among varieties, environment, and E × V interactions (*Table 2*). Overall, maximum temperature had significant and positive effects on all yield related traits, seedling survival rate and productivity of rice except for number of grains per panicle and harvest index. Across environmental analysis revealed that low seedling survival rate in E2 and E3 (due to low temperature at this stage, especially at 2 leaf stage) than other environments led to reduced panicle number per m^2 (*Fig. 1, 2c and 3a*). Moreover, the

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maximum (76.13%) and the minimum (31.33%) seedling survival rate were recorded at E5 and E1, respectively (*Fig. 3a*). Grain filling percentage was affected by high temperature during E2-E4, which resulted in significant reduction in grain filling % than other environments (*Table 1, Fig. 2b*). On average, E6 exhibited the highest daily grain and dry matter productivity (62.80 and 151.05 kg ha^{-1} d^{-1}, respectively) than other environments, while E3 was observed to decline in grain and dry matter productivity by 30.14 and 86.68 kg ha^{-1} d^{-1}, respectively. Furthermore, values for daily grain (37.47 to 53.02 kg ha^{-1} d^{-1}) and dry matter (109.23 to 119.86 kg ha^{-1} d^{-1}) were recorded in both inbred and hybrid rice cultivars (*Fig. 3b and d*). Further, *Huahang 31* and *Yuxiangyouzha* produced higher grain yield while substantial decrease in grain filling percentage was observed in *Peizataifeng* and *Huayou 213* and reduced grain number per panicle in E3. Additionally, both E1 and E6 had 42.77% and 41.93% higher harvest index, respectively as compared to other environments. Among rice varieties, the highest harvest index was recorded in the following order: *Huahang 31* (53.02%) > *Yuxiangyouzhan* (40.08%) > *Peizataifeng* (36.28%) > *Huayou 213* (34.93%) (*Fig. 3c*).

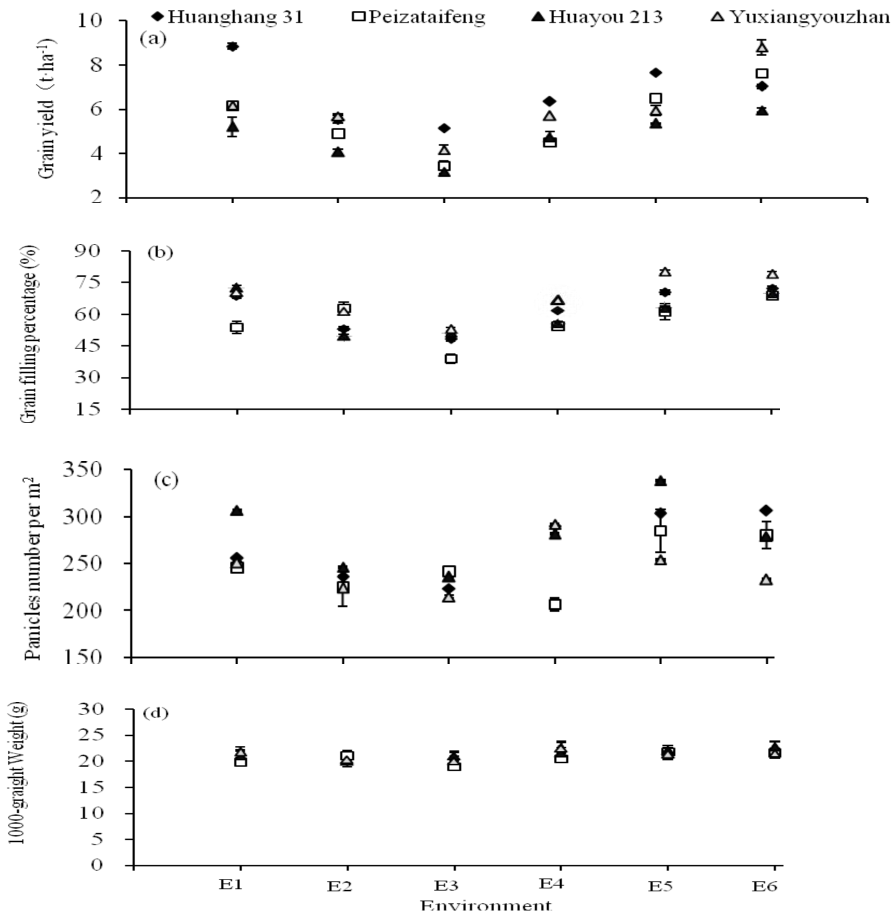

Figure 2. *Effect of series of six different environments on (a) grain yield (t ha^{-1}), (b) grain filling %, (c) panicles number per m^2, (d) 1000-grain weight (g) of four different rice cultivars. Capped bars indicate means of three replicates ± SE.*

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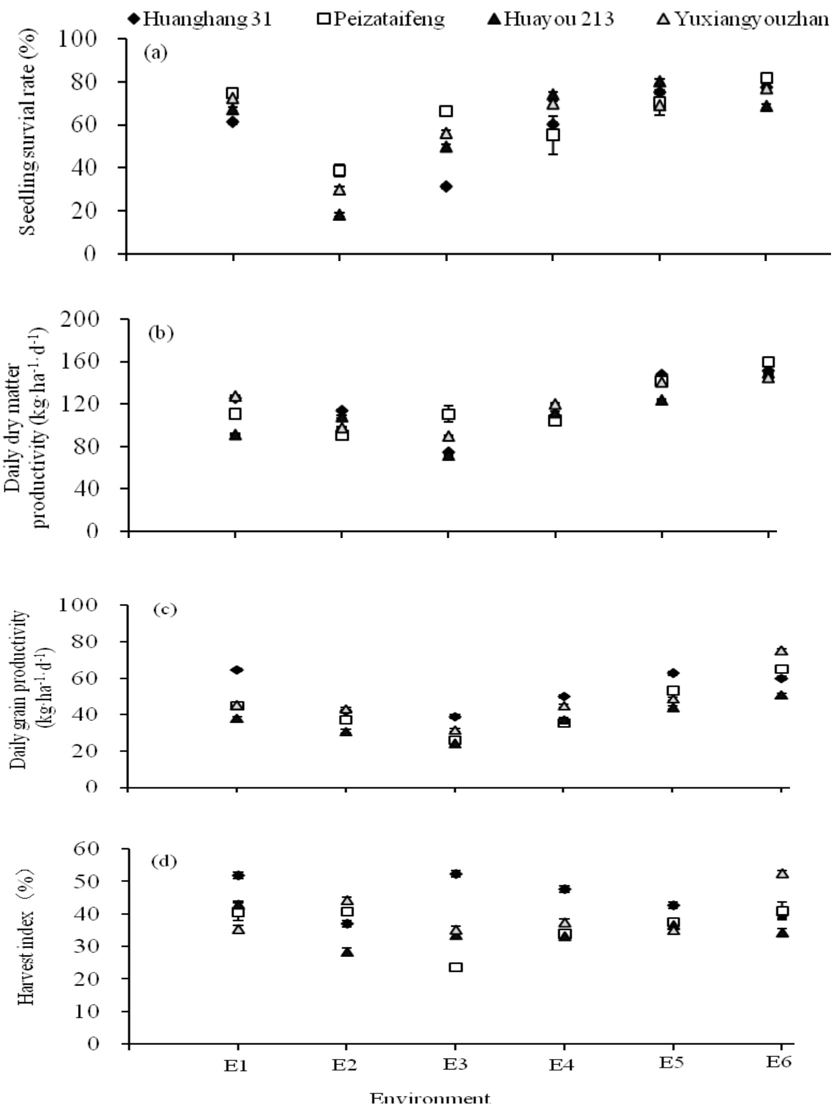

Figure 3. *Effect of series of 6 different environments on (a) seedling survival rate (%), (b) daily dry matter productivity (kg ha^{-1} d^{-1}), (c) harvest index (%), (d) daily grain productivity (kg ha^{-1} d^{-1}) of four different rice cultivars. Capped bars indicate means of three replicates ± SE.*

Correlation analysis depicted that grain yield significantly and positively correlated with filled grain % (r = 0.722), 1000-grain weight (r = 0.478), daily grain productivity (r = 0.978), dry matter accumulation (r = 0.718), harvest index (r = 0. 692) and daily dry matter productivity (r = 0.703), however, panicles m^{-2} and grains panicle^{-1} were remained non-significant regarding grain yield (r = 0.303 and r = 0.115), respectively. Furthermore, panicles m^{-2} was negatively correlated with grain panicle^{-1} while grains panicle^{-1} also showed a negative correlation with filled grain %, 1000-grain weight, dry matter accumulation and daily dry matter productivity (*Table 3*).

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Table 3. *Correlation analyses for seedling survival rate, grain yield, yield related traits and productivity of rice among varieties and treatments*

Traits	Grain yield	Panicles number per m^2	Grains per panicle	Filled grain percentage	1000-grain weight	Daily grain productivity	Dry matter accumulation	Harvest index	Daily dry matter productivity
Grain yield	1								
Panicles number per m^2	0.303	1							
Number of grains per panicle	0.115	0.561**	1						
Filled grain percentage	0.722**	0.425*	–0.128	1					
1000-grain weight	0.478*	0.649**	–0.365	0.641**	1				
Daily grain productivity	0.978**	0.351	0.069	0.746**	0.542**	1			
Dry matter accumulation	0.718**	0.450*	–0.013	0.604**	0.424*	0.753**	1		
Harvest index	0.692**	0.019	0.191	0.431*	0.285	0.627**	0.009	1	
Daily dry matter productivity	0.703**	0.485*	–0.064	0.633**	0.510*	0.784**	0.969**	0.023	1

*Significant at p≤ 0.05; **Significant at p≤ 0.01; values without asterisks are non-significant at both p≤ 0.01; p≤ 0.05

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Discussion

Enhancing grain yield has long been a main purpose of rice researchers to meet the needs of increasing population; however climate change threatens to decrease the crop productivity. In the process of rice yield formation, rice growth period is confronted with different light and temperature conditions. Differences in sowing dates might result in changed growth periods thereby affecting plant biomass accumulation through altering the length of photosynthetic time (Yao et al., 2012). Thus, sowing dates affect grain yield by influencing the whole rice growing process. Sowing or planting dates for crops like rice, wheat, maize etc. were studied worldwide in coping with global climate change or for gaining high yield and quality (Sacks et al., 2010; Yao et al., 2011; Hussain et al., 2012). Sowing dates significantly affected the seedling survival rate, grain yield, yield related traits and rice productivity of plants growing in different environments (*Table 2*). Similarly, Sun et al. (2012) reported a significant effect of sowing dates on yield and some yield related traits (spikelets and seed setting rate) under low-light stress at heading stage. E1 and E6 supposed to increase yield due to increase in panicle number per m^2, fully filled grain percentage and 1000-grain weight, yet different rice varieties performed differently in this connection *(Fig. 2)*.

Grain yield productivity on each sowing date (E1 to E6) was 48.14 kg ha^{-1} d^{-1}, 38.21 kg ha^{-1} d^{-1}, 30.14 kg ha^{-1} d^{-1}, 41.86 kg ha^{-1} d^{-1}, 52.16 kg ha^{-1} d^{-1} and 62.80 kg ha^{-1} d^{-1}, respectively (*Fig. 3d*). Thus, an increase in rice yield by more than 30.14 kg ha^{-1} d^{-1} for each day may be achieved, if rice sowing was earlier before and after E3. Rice sown on both E2 and E3 had a lower seedling survival rate due to extensive low temperature which caused a gradual reduction in panicle number per m^2 (*Fig. 2c*). Higher grain filling percentage and 1000-grain weight was observed on rice sown earlier before E3 and might be due to prolonged rice photosynthesis time in early sowing and warmer climate in late sowing enjoyed by crop resulting in higher daily dry matter productivity. Under delayed sowing, rice spikelet number per panicle and fully filled grain percentage decreased, and no difference in panicle and grain weight was noticed (Yao et al., 2010, 2011; Huo et al., 2012; Abid et al., 2015). Shorter rice photosynthesis time and higher temperature at panicle initiation stage might be the possible reasons for the fewer grain number per panicle, observed in late sowing environment (E4-E6). These findings corroborate with Chen et al. (2003) who argued that in single-season rice, delayed sowing reduced the grain number per panicle, but increased seed setting rate and 1000-grain weight, improved late leaf area index, increased photosynthetic products and improved rice quality. In Philippines, Peng et al. (2004) observed 10% reduction in paddy yield for each unit rise in minimum temperature limits during one-season of rice cultivation whereas rice yield variability and poor seed quality due to extreme temperatures at reproductive stage was also reported by Martínezeixarch and Eills (2015).

All rice cultivars were remained significantly different for seedling survival rate, grain yield, and yield related traits and rice productivity (*Table 2*). Variation in grain

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yield and other parameters for different rice genotypes might be due to their genetic diversity and morphological characters (Yang et al., 2007; Shahidullah et al., 2009; Ashfaq et al., 2012). Higher grain yield recorded in rice variety *Huahang 31* (6.75 t ha^{-1}) might be attributed to higher yield related traits (panicle number per m^2 and 1000-grain weight) *(Fig. 2)*, daily dry matter productivity and harvest index (*Fig. 3b and c*). *Yuxiangyouzhan* had a higher grain number per panicle, grain filling percentage and daily dry matter productivity, but reduced panicles per m^2, 1000-grain weight and harvest index led to lower grain yield than *Huanghang 31*. The lowest grain yield was noted in *Huayou 213* due to the reduction in yield related traits (except panicles per m^2), daily dry matter productivity and harvested index (*Fig. 2 and 3*).

E × V interaction had significant effect on rice yield, yield related traits and other parameters (*Table 1*). *Huahang 31* and *Yuxiangyouzhan* (sown on 24th February and 20th March, respectively) provided higher grain yield that might be due to higher dry matter productivity and harvest index in both these cultivars (*Fig. 2 and 3*). The substantial decrease in grain filling percentage observed in *Peizataifeng* and *Huayou 213* in E3 (sown on March 5) and along with reduced grain number per panicle observed in *Huayou 213* in E3 (sown on March 5) resulted in minimum grain yield in both these cultivars. Genetic variations among rice varieties might also be responsible for differential response regarding grain yield, dry matter accumulation and harvest index under the same or different environmental conditions (Yao et al., 2010). For instance, delayed sowing resulted in yield decline due to reduced spikelet number per panicle and seed setting rate, but degree of yield reduction was different among different rice varieties (Nagarajan et al., 2010). Similarly, differential yield response and environmental sensitivity of different rice cultivars under different environments was also reported by Wang et al. (2001). We further found that different rice varieties produced different yields for E × V interaction which indicates a strong relationship among local climatic conditions including temperature fluctuations, photosynthetic and respiratory activities of plants and yield formation and other morpho-physiological and biochemical mechanisms that affect growth and productivity of rice.

Conclusion

Conclusively, among all environments, only E3 caused yield reduction by 30.14 kg ha^{-1} d^{-1}. Compared to other environments, the grain yield declined by 14.45- 45.86% in E3, however, different rice varieties performed differently in this regard. Hence, the entire environment was considered favorable for rice production in Guangdong province (China) except for E3.

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INVASION OF ABANDONED AGRICULTURAL FIELDS BY *ACACIA MEARNSII* AFFECT SOIL PROPERTIES IN EASTERN CAPE, SOUTH AFRICA

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Abstract. Abandoned agricultural fields are susceptible to alien plant invasion because they experienced intensive anthropogenic activities. However, there is limited studies on soil physico-chemical changes in alien plant invaded abandoned agricultural fields in comparison to uninvaded abandoned agricultural fields and natural sites. This comparative study explores soil physico-chemical changes in ten-year-old abandoned agricultural fields invaded by *Acacia mearnsii* in comparison to ten-year-old uninvaded abandoned agricultural fields and natural sites in Eastern Cape, South Africa. Soil physico-chemical properties were measured on topsoil collected from invaded and uninvaded abandoned agricultural fields as well as natural sites over three months. Results on soil nutrient changes between invaded and uninvaded abandoned agricultural fields compared to natural sites were varied, with significantly higher total C and cations of Na and Mg reported in *A. mearnsii* invaded abandoned agricultural fields, whereas soil pH and P were significantly higher in uninvaded abandoned agricultural fields. Soils underneath invaded abandoned agricultural fields were compact, had high litter mass, reduced infiltration and were repellent compared to soil in uninvaded abandoned agricultural fields and natural sites. It appears that the invasion of *A. mearnsii* in abandoned agricultural fields has triggered changes in soil physico-chemical properties.
Keywords: *invasive alien plant; nutrient cycling; litter; soil water repellency; land use legacy*

Introduction

Areas of abandoned agricultural fields are increasing globally (Chambers et al., 2014), due to geo-bio-physical, socio-economic and management factors (Rey Benayas et al., 2007). Agricultural fields are abandoned when they cease to generate an income or when opportunities for resource adjustments are exhausted (MacDonald et al., 2000). Abandoned agricultural fields characterize a change in land use and cover, which can affect ecosystem structure and function (Jiao et al., 2013). Lack of ecosystem recovery to the original condition following abandonment can result in loss of biodiversity, decreased structural heterogeneity, loss of soils and cultural values (Chambers et al., 2014). Hobbs and Cramer (2007) reported that abandoned agricultural fields can remain in a degraded state for decades leading to invasion by alien invasive plants. Factors that promote the invasion of abandoned agricultural fields by alien plants include elevated soil nutrients that favour productivity of alien plants, better competitive ability of alien invasive plants, increased seed productivity and dispersal of alien invasive plants and poor seed bank of native species (Hobbs and Cramer, 2007). Once alien invasive plants have invaded abandoned agricultural fields, they have been shown to modify biotic and abiotic soil properties (Hobbs and Cramer, 2007). The presence of alien species in abandoned agricultural fields has been shown to affect soil nutrients, nutrient cycles and soil microbial communities (Milton, 2004; Ruwanza et al., 2012).

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Acacia mearnsii (Black wattle) is one of the most invasive alien plants in South Africa (Dye and Jarmain, 2004). The plant is native to Australia, but has invaded most of the high rainfall regions of South Africa (Dye and Jarmain, 2004). The plant has been reported to cause biodiversity loss, reduced water supply, increased soil erosion and increased fire intensity (Le Maitre et al., 2011). Its success as an invasive alien plant is attributed to its ability to produce huge amounts of seed, persistent seed banks, capacity to fix nitrogen and ability to out-compete natives for resources like water and soil nutrients (Le Maitre et al., 2011). Several management interventions to control *A. mearnsii* in South Africa are underway, including measures to prevent introductions, mechanical and biological control (Le Maitre et al., 2011). However, these interventions have yielded mixed results when it comes to control and restoration efficacy (Esler et al., 2008). Therefore, improved and clearer understanding of *A. mearnsii* invasions, especially plant-soil interactions in abandoned agricultural fields which are susceptible to invasion, will help implementation of an effective *A. mearnsii* management strategy.

Most studies on the effects of alien invasive plants on soil properties in South Africa have concentrated on comparisons between invaded, natural and cleared sites (Witkowski, 1991; Yelenik et al., 2004; Le Maitre et al., 2011), but not on abandoned agricultural fields. Also, most of the above mentioned studies concentrate on changes in soil chemical properties, leaving soil physical properties less studied. The aim of this study was to assess if soils underneath ten-year-old abandoned agricultural fields invaded with *A. mearnsii* have different soil properties compared to soils in adjacent ten-year-old uninvaded abandoned agricultural fields and natural sites in the Eastern Cape province of South Africa. The main research question was whether *A. mearnsii* invasion of abandoned agricultural fields changes soil properties, namely soil physico-chemical properties, penetration resistance, infiltration, hydraulic conductivity, soil repellency and litter mass.

Methods and materials

Study area

The study was conducted at Glen View farm (33°26'32.57"S; 26°19'14.12"E) which is located approximately 40 kms outside Grahamstown along the N2 highway in the Eastern Cape Province of South Africa (*Fig. 1*). The farm currently produce livestock mostly cattle, but abandoned agricultural fields were used for chicory cultivation. Vegetation is classified as Bhisho Thornveld which is characterised by small trees and a short-to-medium, dense sour-grass (Mucina and Rutherford, 2006). Soils are generally loamy and sandy and are of the Adelaide subgroup (Mucina and Rutherford, 2006). Although rainfall in this area falls throughout the year, most of it falls in summer between October and April. Mean annual rainfall approximate 750 mm, whilst mean annual temperature approximate 16°C (Mucina and Rutherford, 2006).

Site selection

Soils were collected within the farm at three different invasion treatments, namely *A. mearnsii* invaded abandoned agricultural fields, uninvaded abandoned agricultural fields and natural sites. The above mentioned three invasion treatments were approximately one km apart. Agricultural activities were abandoned ten years ago in both the *A.*

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mearnsii invaded and the uninvaded abandoned agricultural fields. At each of the above mentioned invasion conditions, three sites were selected for soil sampling. Sites within each invasion treatment were approximately 200 m apart to provide a measure of independence (Galatowitsch and Richardson, 2005). All *A. mearnsii* invaded abandoned agricultural fields were dominated by heavy invasions, with canopy cover greater than 70 % and had no underneath vegetation. The uninvaded abandoned agricultural fields were dominated by grasses and herbs. The natural sites were dominated by native trees and shrubs.

At each site a 50 m transect (from west to east) with the first transect point being underneath the vegetation canopy (in *A. mearnsii* invaded and natural sites), was established comprising five soil collecting points spaced 10 m apart. At each point, two soil cores (30 cm apart) were collected monthly in August, September and October of 2015. This resulted in 90 soil cores being collected per month, thus 10 soil cores from 3 invasion treatments and 3 site replicates per treatment. Half of the soil cores collected per sampling point were used for gravimetric soil moisture measurement and the other half for soil repellency measurements. During the month of August, an additional 45 soil cores (one additional soil core per collection point) were collected for soil chemical analysis which was conducted once due to lack of finances. Soils were collected using a soil auger at a depth of 10 cm after the removal of the overlaying debris. Collection of soils at the above mentioned depth was based on results by Jovanovic et al. (2009) who reported that soil nutrients were represented at a depth of 0 – 5 cm underneath *Acacia* sites. After soil collection, soil nutrient analyses, pH, soil resistivity, gravimetric soil moisture and soil repellency were assessed under laboratory condition at Rhodes University in Grahamstown. Soil penetration resistance, infiltration and litter mass measurements were conducted at each of the soil collecting point for the three above mentioned months.

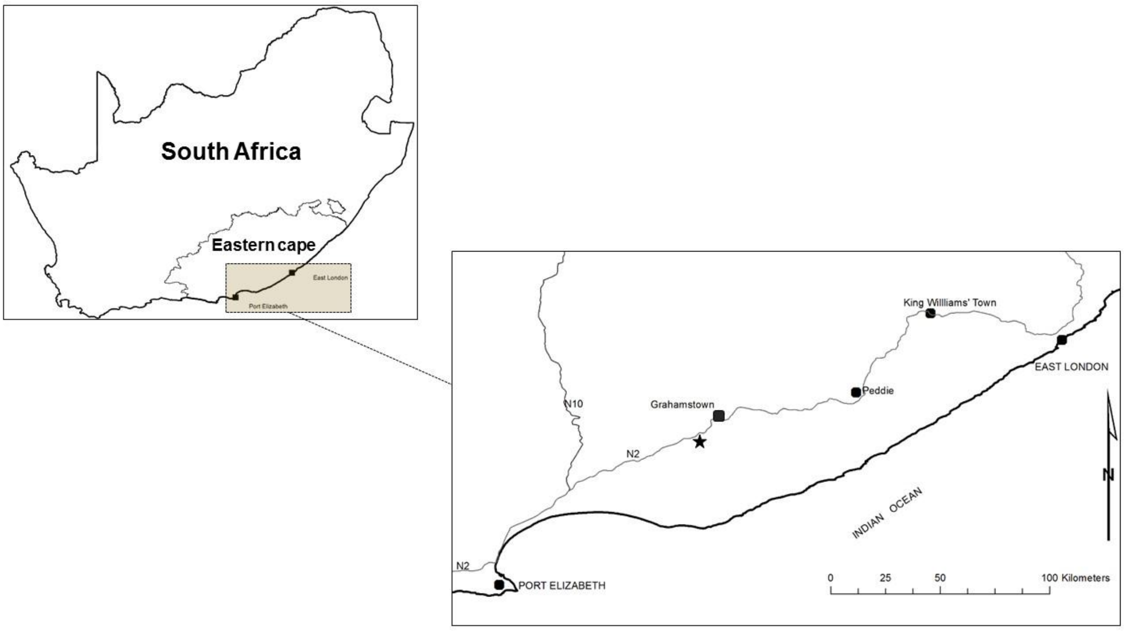

Figure 1. *Location of the study area (in asterisk) approximately 40 kms south west of Grahamstown along the N2 highway in the Eastern Cape province of South Africa.*

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Soil chemical properties measurements

Soil P was determined using the Bray-II extraction method (Bray and Krutz, 1945), whilst total carbon was determined using the Walkley-Black method (Chan et al., 2001). Total nitrogen was determined by complete combustion using a Eurovector Euro EA Elemental Analyser, whilst nitrate and ammonium were determined using an auto analyser. All 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, USA).

Soil physical properties measurements

Soil moisture was measured in terms of gravimetric soil moisture expressed as a percentage. Collected soil cores were first sieved to remove stones and plant materials. They were then weighed wet and dried in a drying oven at 105^{o}C for 72 hours, then re-weighed to obtain the water content (Black, 1965). Soil pH was measured in 1:5 soil-KCl extract (Rhoades, 1982), whilst soil resistivity was measured using a resistivity meter. Soil penetration resistance which measures soil compaction was measured using a mini penetrometer (SOILTEST, Inc.). Litter mass was measured at all soil collection points using a 100 cm^2 metal template placed at the litter collection area. The leaf litter within the template was collected down to the humus soil layer. All collected litter samples were first sieved to remove soils, then oven dried at 70^{o}C for 72 hours to reduce variability that result from wet litter. The dry litter was then weighed to determine the mass per 100 cm^2.

Infiltration rate was measured on all soil collection points using a Mini Disk Infiltrometer (MDI, Decagon Devices, Pullman, WA). The infiltrometer measures the volume of water (mL) that passes from the Infiltrometer into the soils. Infiltration rate was measured from the drop of water in the lower chamber at 30 second intervals for five minutes. Using the method of Zhang (1997), infiltration rate was determined from the measured cumulative infiltration rates over time. Hydraulic conductivity was measured from the infiltration measurements using the van Genuchten-Zhang method (Zhang, 1997).

Soil repellency was measured using the Water Droplet Penetration Time (WDPT) method, which measures how long repellency persists on soils (Bisdom et al., 1993; Doerr et al., 2000). Soils collected from each point were first sieved using a 2 mm sieve then air-dried over seven days. After drying, 20 grams of sample soils were placed into petri dished and were levelled. The WDPT test was conducted by placing five drops of distilled water on the soil surface and record the time taken for each water droplet to penetrate the soil. The water drops were applied using a hypodermic syringe. The average penetration time of the five drops was taken as representative of the WDPT for each sample. The WDPT categories were (i) wettable when the water drop infiltrated within 5 s, (ii) slightly repellent when the water drop infiltrated between 5 and 60 s, (iii) strongly repellent when the water drop infiltrated between 60 and 600 s, (iv) severely repellent when the water drop infiltrated between 600 and 3600 s and (v) extremely repellent when the water drop infiltrated above 3600 s (Bisdom et al., 1993).

Statistical analysis

Data were analyzed using STATISTICA version 12 (Statsoft Inc, 2012). Proof of normality and proof of homogeneity of variances were tested using Kolmogorov–

Smirnov tests and Levene's test respectively and data were normally distributed. Soil chemical properties, pH and resistivity comparisons between *A. mearnsii* invaded abandoned agricultural fields, uninvaded abandoned agricultural fields and natural sites were analyzed using one-way ANOVA since data was collected only once in August. Gravimetric soil moisture content, soil penetration resistance, litter mass and soil hydraulic conductivity, which were all collected monthly on the same transect were analyzed using repeated measures ANOVA. Soil repellency categories were analyzed using the Chi-squared test. Where ANOVAs were significant, the Tukey's HSD unequal *n* test was used to determine differences between invasion treatments at $P < 0.05$.

Results

Soil chemical properties

Significant ($p < 0.01$) differences between the three different invasion treatments were found in soil P, total C and exchangeable cations of Na and Mg (*Table 1*). Of the above mentioned significant differences only soil P was significantly higher in uninvaded abandoned agricultural fields compared to *A. mearnsii* invaded abandoned agricultural fields and natural sites (*Table 1*). Site comparisons of the observed significant differences in soil P showed that the differences were more visible in all three sites. Total C and exchangeable cations of Na and Mg were significantly higher in *A. mearnsii* invaded abandoned agricultural fields than in natural sites and uninvaded abandoned agricultural fields (*Table 1*). Significant differences in total C, Na and Mg were visible in sites two and three than in site one. Soil total N, NO_3-N, NH_4-N and the exchangeable cations of K and Ca showed no significant ($p > 0.05$) differences between the three different invasion treatments (*Table 1*).

Soil physical properties

Soil moisture, pH and resistivity

Gravimetric soil moisture content was significantly ($p < 0.001$) higher in uninvaded abandoned agricultural fields compared to *A. mearnsii* invaded abandoned agricultural fields and natural sites (*Table 2*). Similarly, monthly comparisons on gravimetric soil moisture content as well as interactions between invasion and months showed significant ($p < 0.01$) differences (*Table 2*). The months of August and September had high (mean: 11.87% and 11.81% respectively) gravimetric soil moisture content in uninvaded abandoned agricultural fields compared to the month of October (mean: 9.47%). Contrary, the months of September and October (mean: 4.71% and 4.20% respectively) had high gravimetric soil moisture content in *A. mearnsii* invaded abandoned agricultural fields compared to the month of August (mean: 2.45%). Similarly, the months of September and October (mean: 3.73% and 3.87% respectively) had high gravimetric soil moisture content compared in natural sites compared to the month of August (mean: 1.88%). Site comparisons for gravimetric soil moisture content showed significant ($p < 0.001$) differences in all three sites for all the three months.

Soil pH was significantly ($p < 0.001$) higher in uninvaded abandoned agricultural fields compared to *A. mearnsii* invaded abandoned agricultural fields and natural sites (*Table 1*). Site comparisons for soil pH showed that significant differences were more

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visible in all the three sites. Soil resistivity, showed no significant ($p > 0.05$) differences between the three different invasion treatments (*Table 1*).

Table 1. *Soil physico-chemical properties of soil samples taken from invaded abandoned agricultural fields, uninvaded abandoned agricultural fields and natural sites. Data are means ± SE and one-way ANOVA results are shown (*p < 0.05, **p < 0.01, ***p < 0.001). NS = not significant at p > 0.05. Values within columns with different letter superscripts are significantly different.*

Soil Properties	Invaded abandoned agricultural fields	Uninvaded abandoned agricultural fields	Natural sites	One-way ANOVA
pH	4.05 ± 0.06^{c}	5.29 ± 0.08^{a}	4.64 ± 0.017^{b}	28.18***
Soil Resistivity (Ohm)	4136.73 ± 942.20^{a}	5025.33 ± 159.03^{a}	5001.40 ± 840.04^{a}	0.48ns
Total nutrient concentrations (mg/kg)				
P Bray II	7.73 ± 1.98^{c}	26.87 ± 1.90^{a}	18.13 ± 3.00^{b}	16.63***
Total C	10226.67 ± 1270.86^{a}	4813.33 ± 479.07^{b}	7080.00 ± 961.11^{ab}	8.01**
Total N	262.00 ± 53.16^{a}	311.33 ± 58.34^{a}	295.33 ± 62.52^{a}	0.19ns
NO_3-N	1.09 ± 0.19^{a}	2.05 ± 0.59^{a}	1.29 ± 0.17^{a}	1.87ns
NH_4-N	8.98 ± 1.24^{a}	8.35 ± 0.56^{a}	8.81 ± 1.11^{a}	0.10ns
Exchangeable cations (mg/kg)				
K	52.00 ± 6.18^{a}	39.00 ± 1.40^{a}	49.01 ± 5.05^{a}	2.17ns
Na	29.90 ± 3.52^{a}	12.88 ± 0.38^{b}	21.93 ± 3.46^{ab}	8.87***
Ca	156.27 ± 17.74^{a}	214.00 ± 15.27^{a}	179.60 ± 16.42^{a}	3.09ns
Mg	55.92 ± 5.62^{a}	38.00 ± 1.03^{b}	45.12 ± 3.28^{ab}	5.63**

Soil penetration and litter mass

Soil penetration resistance levels were significantly ($p < 0.001$) higher in *A. mearnsii* invaded abandoned agricultural fields compared to uninvaded abandoned agricultural fields and natural sites (*Table 2*). Site comparisons for soil penetration resistance levels showed significant ($p < 0.001$) differences in all three sites for all the three months. However, monthly comparisons and interactions between months and invasion on soil penetration resistance levels showed no significant ($p > 0.05$) differences (*Table 2*).

Litter mass was significantly ($p < 0.001$) higher in *A. mearnsii* invaded abandoned agricultural fields compared to uninvaded abandoned agricultural fields and natural sites (*Table 2*). Site comparisons for litter mass showed significant ($p < 0.001$) differences in all three sites for all the three months. Monthly comparisons on litter mass showed no significant ($p > 0.05$) differences between the three different invasion treatments, whereas, interactions between invasion and months showed significant ($p < 0.01$) differences (*Table 2*).

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Table 2. *Comparison of gravimetric soil moisture content, soil penetration resistance levels and litter mass between soils underneath invaded abandoned agricultural fields, uninvaded abandoned agricultural fields and natural site. Data are means ± se and repeated ANOVA results are shown (*p < 0.05, **p < 0.01, ***p < 0.001). NS = not significant at p > 0.05. Values within columns with different letter superscripts are significantly different.*

	August 2015			**September 2015**			**October 2015**			**Repeated ANOVA (F-values)**		
	Invaded abandoned agricultural fields	Uninvaded abandoned agricultural fields	Natural sites	Invaded abandoned agricultural fields	Uninvaded abandoned agricultural fields	Natural sites	Invaded abandoned agricultural fields	Uninvaded abandoned agricultural fields	Natural sites	Invasion	Months	Invasion x months
Gravimetric soil moisture content (%)	2.45 ± 0.38^b	11.87 ± 0.40^a	1.89 ± 0.34^b	4.71 ± 0.62^b	11.81 ± 0.61^a	3.73 ± 0.38^b	4.20 ± 0.37^b	9.47 ± 0.37^a	3.87 ± 0.37^b	303.11***	7.45**	8.10***
Soil penetration resistance levels	2.33 ± 0.11^a	0.11 ± 0.02^c	0.85 ± 0.12^b	2.40 ± 0.13^a	0.14 ± 0.01^c	0.90 ± 0.13^b	2.42 ± 0.09^a	0.12 ± 0.01^c	1.01 ± 0.13^b	403.51***	0.60ns	0.16ns
Litter mass ($g/100m^2$)	132.85 ± 14.64^a	27.49 ± 2.46^b	42.69 ± 4.44^b	154.30 ± 12.42^a	20.03 ± 1.36^c	63.01 ± 6.16^b	121.77 ± 4.49^a	26.98 ± 1.58^c	67.31 ± 4.98^b	183.92***	1.87ns	3.39*

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Soil infiltration and conductivity

Infiltration rates were significantly ($p < 0.001$) higher in uninvaded abandoned agricultural fields compared to *A. mearnsii* invaded abandoned agricultural fields and natural sites for all the three months. Average infiltration rate after five minutes was 15.47 cm in August, 14.67 cm in September and 12.67 cm in October in uninvaded abandoned agricultural fields compared to 4.47 cm in August, 3.47 cm in September and 1.60 cm in October in *A. mearnsii* invaded abandoned agricultural fields agricultural fields and 5.60 cm in August, 5.73 cm in September and 4.00 cm in October in natural sites (*Fig. 2A, B, and C*). Site comparisons for infiltration rates showed significant ($p < 0.001$) differences in all sites for all the three months.

Soil hydraulic conductivity was significantly ($p < 0.001$) higher in uninvaded abandoned agricultural fields compared to *A. mearnsii* invaded abandoned agricultural fields and natural sites (*Fig. 2D*). However, monthly comparisons for soil hydraulic conductivity as well as interactions between months and invasion showed no significant ($p > 0.05$) differences (*Fig. 2D*).

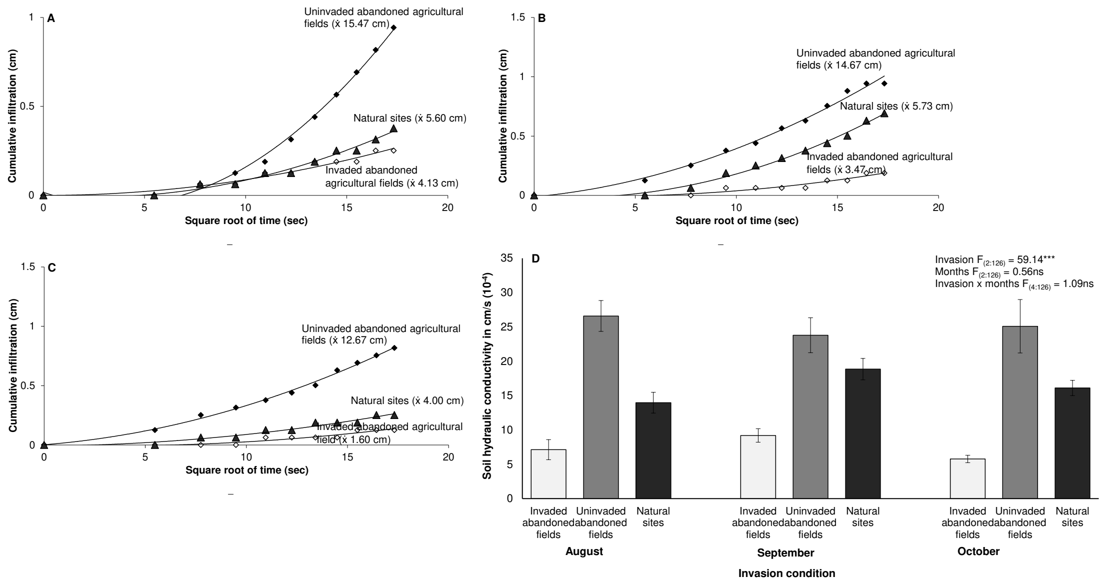

Figure 2. *Comparison of cumulative infiltration in the months (A) August, (B) September, (C) October and (D) soil hydraulic conductivity for all three months between soils underneath invaded abandoned agricultural fields, uninvaded abandoned agricultural fields and natural sites. Points and bars are means and repeated ANOVA results are shown (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). NS = not significant at $p > 0.05$.*

Soil repellency

All soils collected from uninvaded abandoned agricultural fields were wettable for all the three months (*Fig. 3*). Soils from *A. mearnsii* invaded abandoned agricultural fields were slightly, strongly or severely repellent for all the three months (*Fig. 3*). Five (33%) of the 15 soil samples collected from *A. mearnsii* invaded abandoned agricultural fields in August and October as well as six (40%) of the soil samples collected from the same above mentioned fields in September were slightly repellent (*Fig. 3*). Seven (47%) of the soils collected from *A. mearnsii* invaded abandoned agricultural fields in September

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and October as well as eight (53%) of the soils collected from the same above mentioned fields in August were strongly repellent (*Fig. 3*). Some 20% in October and 13% in August and September of the soils collected from *A. mearnsii* invaded abandoned agricultural fields were severely repellent (*Fig. 3*). Ten (67%) of the soils collected in natural sites in August and October as well as seven (47%) of the soils collected from the same above mentioned sites in September were wettable. The remaining five (33%) soil samples collected in natural sites in August and October as well as eight (55%) of the soils collected from the same above mentioned sites in September were slightly repellent (*Fig. 3*). Chi-squared test showed significant ($p < 0.001$) differences in WDPT categories between the three different invasion treatments for all the three months (*Fig. 3*).

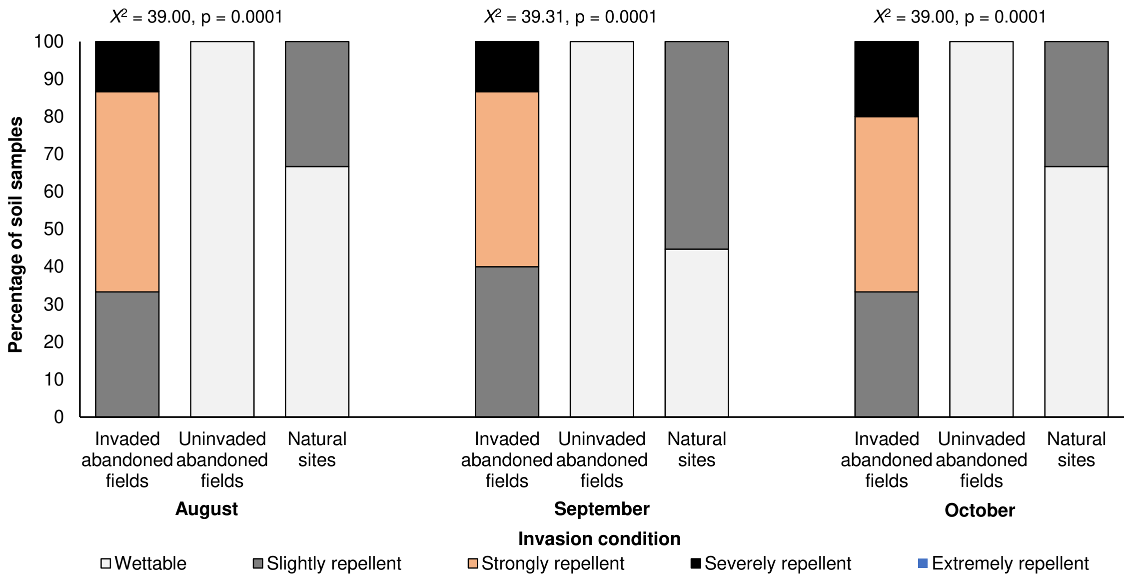

Figure 3. *Distribution of soil repellency classes using the Water Droplet Penetration Time (WDPT) method in soil samples taken from underneath invaded abandoned agricultural fields, uninvaded abandoned agricultural fields and natural sites.*

Discussion

Results on soil chemical properties were varied, with some nutrients e.g. total C being higher in *A. mearnsii* invaded abandoned agricultural fields, whilst soil P was higher in uninvaded abandoned agricultural fields. The study expected soil chemical properties in uninvaded abandoned agricultural fields to be high due to past fertilization (Jiao et al., 2013); however, this was only true for soil P. The fact that measured soil chemical properties in natural sites and uninvaded abandoned agricultural fields were lower than in *A. mearnsii* invaded abandoned agricultural fields points to the presence of *A. mearnsii* as the possible cause of the changes in soil chemical properties.

Indeed, several studies have reported that invasion by alien *Acacias* cause changes in soil nutrients (Yelenik et al., 2004; Marchante et al., 2008). Musil (1993) and Yelenik et al. (2004) reported high soil nutrients e.g. soil N, Ca, Mg, K, Mn and B concentrations in *A. saligna* invaded sites compared to natural fynbos sites. Marchante et al. (2008) showed that *A. longifolia* invaded sites have high soil C and N pools than uninvaded sites in Portuguese coastal dunes. Contrary, Rodríguez-Echeverría et al. (2013) reported

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converse effects of *Acacia* species, showing higher organic matter, soil K and N in soils collected underneath native *Pinus pinaster* species than in soil collected from *A. dealbata* invaded sites. The above mentioned examples seem to show mixed results regards the effect of *Acacia* invasions on soil nutrients.

Several factors can explain the observed changes in soil nutrients underneath *A. mearnsii* invaded abandoned agricultural fields e.g. litter accumulation and litter decomposition. The accumulation of litter underneath *Acacia* trees has been shown to be one of the drivers of soil nutrients increase, especially N and C (Yelenik et al., 2004). In this study litter mass were three times higher in *A. mearnsii* invaded abandoned agricultural fields compared to uninvaded abandoned agricultural fields and natural sites. *Acacia* litter has been shown to have high N concentrations which in turn results in increased soil N, NO_3-N and NH_4-N (Yelenik et al., 2004). However, in this study the reported high litter content in invaded abandoned agricultural fields did not result in high soil N, NO_3-N and NH_4-N. This could be a result of slow litter decomposition rates as suggested by Witkowski (1991) who reported low litter decomposition rates in *A. saligna* sites compared to *Leucospermum parile* sites, which is a fynbos indigenous shrub. The suspicion of slow litter decomposition at *A. mearnsii* invaded abandoned agricultural fields is supported by the fact that soils underneath *A. mearnsii* were compact and with little moisture, this known to affect bacterial and fungal activities (Pupin et al., 2009).

A. mearnsii invasion of abandoned agricultural fields seem to have triggered several changes in the physical nature of the soils compared to uninvaded abandoned agricultural fields and natural sites. The above mentioned changes in the physical nature of the soils were probably related to litter and the organic matter content in the soils which affects soil development, aggregation, macro-pores, bacterial and fungal activities (Jeddi et al., 2009). Although the litter of *A. mearnsii* has been reported to have less sources of hydrophobic substances (Scott, 2000), it still can contain molds, fungi and other agents of decomposition that cause the litter layer to be hydrophobic. Ens et al. (2010) reported that *A. longifolia* leaves possess hydrophobic compounds which causes soil hydrophobicity, which in turn triggers soil compaction, reduced water infiltration and hydraulic conductivity. Also, plant canopy has been reported to influence the intensity and duration of both light and water received by the soils (Kahi et al., 2009), which in turn affect soil moisture and soil temperature. Soils in uninvaded abandoned agricultural fields were not compact and had high infiltration rates. This observation can be explained by previous tilling. Indeed, previous soil tilling has been reported to reduce soil compaction (Hamza and Anderson, 2005) and improve infiltration (Olson et al., 2013).

Soils underneath *A. mearnsii* invaded abandoned agricultural fields were more repellent compared to soils in uninvaded abandoned agricultural fields and natural sites. This could be a result of the reported changes in soil physical properties as well as litter availability. Neinhuis and Barthlott (1997) reported that Mimosaceae species e.g. *A. dealbata* and *A. glaucoptera* have water repellent leaves. These leaves contain hydrophobic organic substances that when in the soils can cause water repellency (Neinhuis and Barthlott, 1997). These hydrophobic organic substances have been reported to cover soil particles and reduce the attraction between soil and water therefore causing repellency (Doerr et al., 2000) which also led to decrease infiltration which was observed in this study. Scott (2000) reported that soils underneath both *Eucalyptus* and *Acacia* plantations were repellent compared to soils underneath

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indigenous forest of South Africa and attributed it to canopy cover and plant litter. Soils in uninvaded abandoned agricultural fields were wettable this likely because of tilling that was done in the past. Blanco-Canqui (2011) reported that no-tillage triggers soil repellency, whereas, Eynard et al. (2004) found no significant differences in soil repellency between no-tillage and tillage.

The study concludes that the invasion of abandoned agricultural fields by *A. mearnsii* ten years ago has triggered changes in both soil physical properties and some chemical nutrients (total C). The presence of these invasive trees and their ability to produce huge amounts of litter which is suspected to contain hydrophobic substances seem to be the main driver of the reported changes in soil properties.

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EFFICIENCY OF PHYTOREMEDIATION ON THE SEDIMENTS CO-CONTAMINATED BY HEAVY METALS AND ORGANIC COMPOUNDS AND THE ROLE OF MICROBES IN THE REMEDIATION SYSTEM

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Abstract. This paper presents the findings of a one-year research project, in which participants explored the function of microbe remediation efficiency and plant growth. Corn and rye grass were planted separately and in mixed quantities to remediate sediments co-contaminated by heavy metals and typical polycyclic aromatic hydrocarbons (PAHs). The study revealed that both the number and the activity of microbes were affected during the sediment remediation process. It also showed that the overall trend at first increased and then decreased, with the microbial numbers reaching their highest levels during the second week of soil cultivation. In the heavy-metal, organic-compound, co-contaminated matrix, the researchers found that mono-cropping the corn removed more lead, with the removal rate reaching a high of 12.96%. They found further that in the mixed-planting pattern, the removal rate of zinc reached a maximum level of 31.4%. The results of the study showed conclusive evidence that organic matters play an important role in the plant-microbe remediation of contaminated sediments.
Keywords: *combined contamination, PAHs, corn, rye grass, microorganism*

Introduction

There has been great progress in environmental research in recent years and, in the course of these studies, much attention has been drawn to the damaging effects of pollutants on vegetation (Hou et al., 2014). Researchers have found that great quantities of contaminated sediment exist in organic compounds (Charrasse et al., 2014). The water quality in most cities has deteriorated, and the rivers into which much of the wastewater is deposited has become malodorous, This is due to the high level of alluvial-contaminated sediment which remains in the water long after the pollutants have been introduced (Wang et al., 2015). Too, the re-release of pollutants from the contaminated sediment has become a secondary source of pollution in the affected rivers (Kadhum et al., 2015; Varjo et al., 2003; Prajith et al., 2015). It is imperative that these issues be addressed, governed, and corrected with dispatch.

The very nature of heavy metals is that they remain stable (Tian et al., 2015). Once they have been deposited into the soil, they cannot be decomposed by microorganisms. Heavy metals not only pollute groundwater (Zhao et al., 2006), but they also inhibit plant

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growth and development. Besides this, they promote premature crop aging, resulting in poor agricultural product quality and smaller crop yields (Wang et al., 2013). Moreover, heavy-metal pollutants pass through the food chain and, ultimately, put the health of consumers at risk. Finally, polycyclic-aromatic-hydrocarbon (PAHs) pollutants wreak havoc on both the biosphere and the environment.

Given these well-documented facts, it comes as no surprise that studies on organic pollution removal have become a "hot topic" in research circles. Integrative sediments are not only quite contaminated, they also have a high-quantity (Xin et al., 2014), high-moisture content, and they contain objectionable constituents like heavy metals, pesticides and organic pollutants. In tandem, these elements are inflicting great damage upon the environment and will continue to do so if they are not properly dealt with (Bastami et al., 2015).

The status of soil health determines its ability to be productive. Researchers have found that phytoremediation is a cost-effective, environmentally-friendly approach to remediating soil that has been defiled by a mixture of organic and metal contaminants (Chirakkara et al., 2015; Lombi et al., 2001). Phytoremediation uses plants to degrade, immobilize, and extract contaminants from the soil and from water sources (Sharma et al., 2004; Ghosh et al, 2005). The capability of these plants to uptake contaminants while continuing to survive in the tainted soil, and the bioavailability of the contaminants in the soil are the limiting factors that influence phytoremediation efficiency (Kaewtubtim, 2016). Heavy metals tend to decrease in the presence of organic contaminants and can thus become a limiting factor in the phytoremediation of mixed- contaminated soil.

In past studies, researchers have tested the combination of phytoremediation with micro-organisms in mixed-contaminated soil (Dhal et al., 2013). Vouillamoz and Milke, for example, have shown convincing evidence that rye grass is effective in removing mixed contaminants from soil (Vouillamoz and Mike, 2001). The most common contaminants found in mixed-contaminated soil (Cheng et al., 2008), such as co-contaminated river sediment are pyrene, phenanthrene, lead and zinc (Wang et al., 2014., Akcil et al., 2015; Nguyen et al., 2015). To date, studies on enhanced phytoremediation have not yet addressed the rhizosphere effect on mixed contaminants (Priha et al., 1999). The existing techniques – phytoremediation and microbial remediation (Wang et al., 2008) have been developed through studies using soil that has been contaminated by either metals or organic contaminants; but no such research has been done using co-contaminated soil. The co-contaminated soil is closer to the actual condition. While some studies have explored the key role of microbial remediation in phytoremediation, they have not addressed the relationship between removal rates and microbial remediation.

This study aimed to investigate the relationship between phytoremediation efficiency and the rhizosphere effect. The researchers who took part tested the phyto-microbial remediation effects on combinational contaminated sediments by using corn and rye grass in potted plant experiments. Tests were conducted to measure both the plants' removal rates and their growth rates and to compare their biomass in soil which had been contaminated by a mixture of organic compounds (pyrene and phenanthrene) and heavy metals (lead and zinc).

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Experiment

Experimental materials and equipment

The primordial matrix was prepared by mixing dry river sediment screened with a 2 mm griddle and soil taken from a Beijing suburb at a ratio of 2:1. The primordial matrix was regarded as the control group (CK). The testing matrices that were added contained different proportions of lead, zinc, phenanthrene and pyrene. These were used as the contaminated-sediment samples (A and B) of different pollution levels, and the phenanthrene and pyrene were used to represent the exogenous organic pollutants (PAHs). The moisture content and nutrition level of the samples were adjusted in the laboratory. All of these procedures were followed by a natural decaying process which lasted 10 days, and the physical and chemical properties of the experimental soil was tested in the laboratory (*Table 1*).

Table 1. *The basic characteristics of the testing matrix and sample*

	pH	Lead (mg/kg)	Zinc (mg/kg)	Total organic matter (mg/kg)	EP (mg/kg)	EN (mg/kg)	Moisture content (%)
CK	6.70	33.96	103.38	745.04	1.23	104.95	21.89
A	6.68	260.33	301.29	770.65	1.24	132.93	11.76
B	6.54	570.02	605.02	895.43	1.12	118.92	9.95

EP: effective phosphorus; EN: effective nitrogen

The researchers chose corn and rye grass as the repairing plants (The Institute of Xinnong Agricultural Technology of Beijing) in the experiment to study the effects of phyto-microbial remediation and different planting patterns on sediments subjected to heavy-metal and organic-compound pollution. The experiments were divided into three categories: Corn Only, Rye Grass Only, and Mixed Planting.

The experimental equipment included; ICP-MS（Inductively Coupled Plasma Mass Spectrometry） (ELAN DRC-e, Perkin Elmer Limited Company); A Soxhlet extractor (SXT-02, Shanghai Hongji Instrument Limited Company); and an analytical balance (OHAUS- AR2140, Ohaus Limited Company).

Analysis methods

Heavy metal determination

0.04 g of dry soil was screened through a 200-mesh sieve and boiled in a high-pressure digestion tank, and then 2 mL HNO_3 and 200 μL H_2O_2 were added to the mix. A violent reaction followed which generated a large amount of yellow smog. The sample was then sonicated for 30 minutes and placed on a hot plate (130°C) until it was almost dry. Next, HNO_3 and HF were put into the tank at a ratio of 1:1 and covered with the lid.

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Finally, the tank was placed into an air oven overnight at a temperature of 170°C. When the digestion was finished, the digestion liquid was diluted to 80 g and reserved for the ICP-MS assay later.

Total organic compound determination

The soil sample was passed through a 2 mm sieve. About 10 g of the soil sample was mixed with a small amount of anhydrous magnesium sulfate and ground together thoroughly with a mortar and pestle. The sample was then wrapped in a paper filter and placed into an extractor into which about 80 mL of chloroform was injected. Adjusted the position to make the condenser pipe, extractor, and extraction bottle keep good contact to each other. Next, the extractor was switched on, and the temperature was set at 75°C for 12 hours. A weight measurement was then taken to calculate the total organic content of the soil sample.

Biomass determination

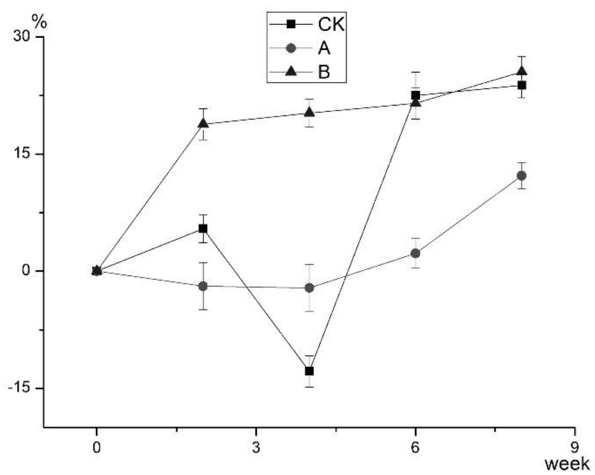
The determination of the EN and EP of soil sample was developed based on the alkali treatment, and the biomass of soil sample was detected using the most probable number (MPN) (Yu et al., 1990; Jia et al., 2004). A beef-extract-peptone medium (10g/L beef extract, 3g/L peptone) was used to quantify the total microbial density, MPN tubes were cultured for 72 h at 37°C.

Results and discussion

The removal effects under the mono-cropping planting pattern

The remediation effects of corn

The removal efficiencies and degradation rates of lead, zinc, phenanthrene and pyrene were investigated at different pollution levels (*Fig. 1*).



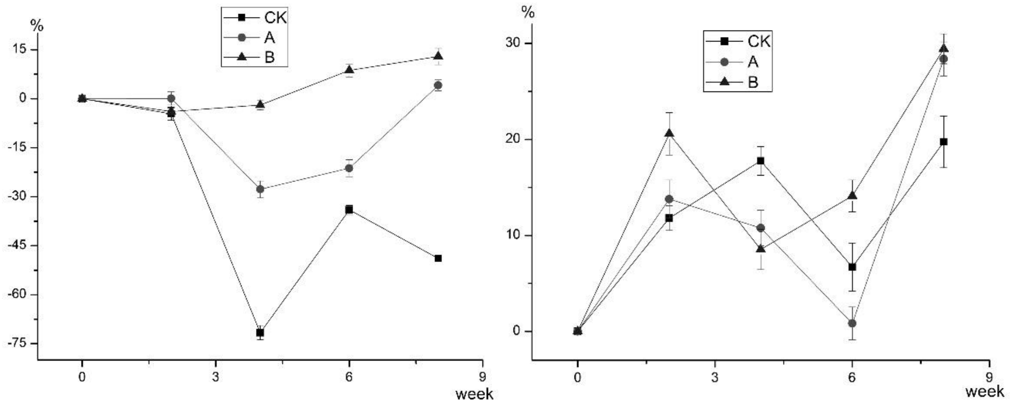


Figure 1. *The removal effects of corn on the pollutants. (a) The removal effects on the organic compounds in the sediment; (b) The removal effects on lead in the sediment; (c) The removal effects on zinc in the sediment.*

The effects of mono-cropping corn on the combined pollutants of different concentrations are shown in *Figure 1*. The removal rates of lead in the sediments containing different pollutant concentrations (A and B) were, respectively, 4.08% and 12.96%. The removal rates of zinc in the sediments were 28.4% and 29.5%, respectively. Test results showed that the removal of zinc was more effective than that of lead. During the 8th week, the removal rates of organic pollutants were, respectively, 12.24% and 25.53% at different concentration levels of pollutants (A and B). The researchers found that with the increase of the pollution level, the removal of organic matters was enhanced. They concluded that this may have been due to the increase of the common stresses of heavy metals and organic matters which are known to promote the absorbance of rhizosphere sediment and the accumulation of organic contaminants by the roots of corn (Ding et al., 2011). The also decided that these organic pollutants could serve as the nutrients which are important for the growth of plants and which the plants may oxidize into CO_2 and H_2O. The mycorrhizal fungi and algae in the rhizosphere of corn may not only decompose the organic matter directly, but may also promote the removal of organic pollutants by stimulating the secretion of root exudates of the plants (Zhao et al., 2005).

The remediation effects of rye grass

The rye grass was grown separately to test its remediation effects. The removal rates and the analytical results are presented in *Figure 2*.

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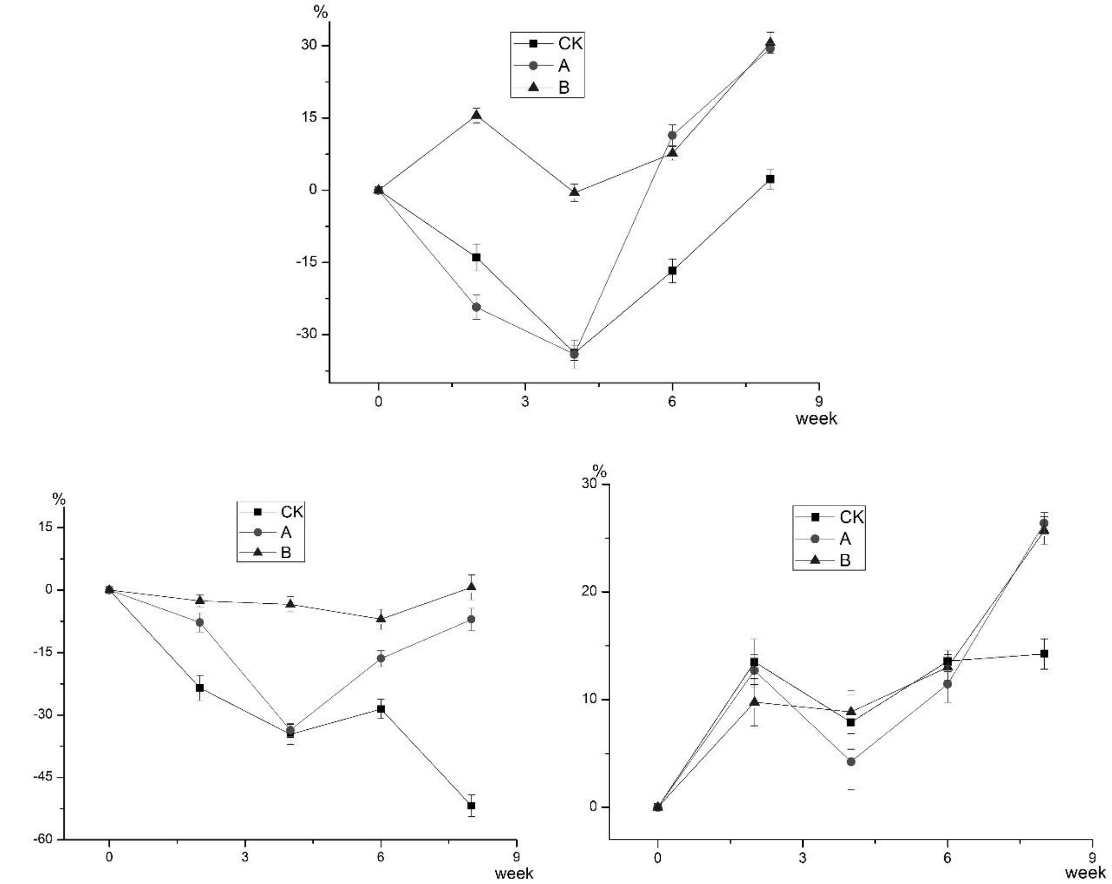

Figure 2. *The removal effects of combined pollutants on the treatment of rye grass. (a) The removal effects on the organic matters in the sediment; (b) The removal effects on lead in the sediment; (c) The removal effects on zinc in the sediment.*

The experiments conducted on rye grass revealed that the removal rates of lead in the sediment with different levels of pollution (A and B) were -7.07% and 0.68%, respectively, while those of zinc were 26.40% and 25.69%, respectively. Once again, it was found that the removal of zinc was more effective than that of lead. The removal rates of organic matters in the sediments with different concentrations of contaminants reached 29.43% and 30.62%, respectively. The removal rates of organic pollution from rye grass showed a better efficiency than corn, which was consistent with the research on corn. In addition, the removal rate of zinc was superior to that of lead in the given concentrations. This may have been due to the fact that zinc is an essential element in stimulating plant growth, while lead contains elements of biological toxicity.

In comparison to the tests conducted on corn, rye grass showed lower zinc-removal rates at both concentrations: A. 26.40% *vs.* 28.4% and B. 25.69% *vs.* 29.5%. This suggests that the removal efficiency of corn is slightly higher than that of rye grass and may have something to do with the biomass. The mono-cropped rye grass produced a smaller biomass than the corn as was indicated in the biomass tests results.

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The removal efficiency of organic contaminants by rye grass was superior to that of corn. Perhaps this was because perennial rye grass has a higher tolerance of organic matters and to the pH range of the sediment than corn.

The removal of combined pollutants by a mixed-planting pattern

The corn and rye grass were planted in a mixed pattern to test the remediation effects on the sediments containing combined pollutants. The removal rates of heavy metal on mixed plants are shown in *Figure 3*. At concentrations A and B, the removal rates of zinc were 21.2% and 31.4%, respectively, while those of lead were, respectively, 2.6% and 4.5% by the 8th week. These figures fell mid-way between the rates of the two single planting patterns.

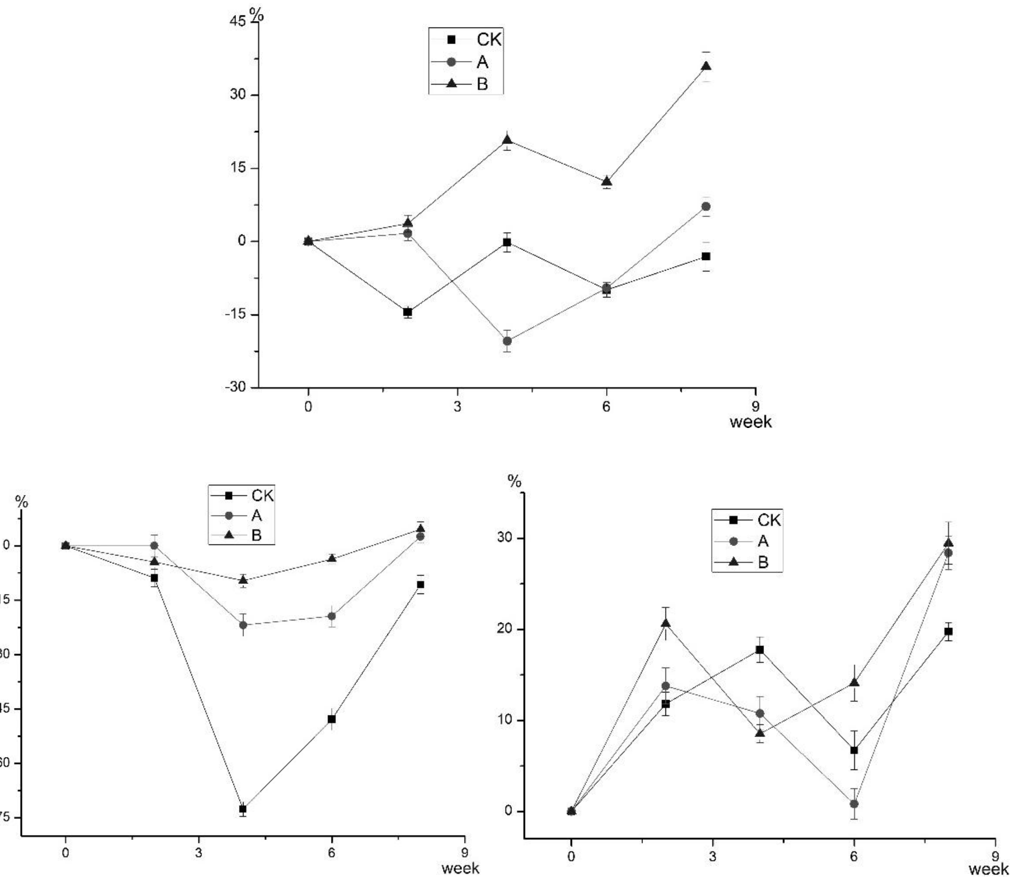

Figure 3. *The removal of combined pollutants under mixed plants. (a) The removal effect of organic matters in the sediment; (b) The removal effect of lead in the sediment; (c) The removal effect of zinc in the sediment*

At concentrations A and B, the removal rates of organic pollutants under mixed plants were, respectively, 7.15% and 35.86% by the 8th week. The removal rate of the organic pollutant at concentration A was much lower than that at concentration B. This suggests

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that when the level of the organic pollutant concentration was relatively high the mixed planting pattern was the better choice.

The study showed that phytoremediation resulted from the biological changes that prompted the rhizosphere soil enzyme activity as well as an increase of the microbial quantity. The study showed further that the removal of organic matters by plants is largely due to the plant-rhizosphere effect. On the one hand, the rhizosphere effect increases the removal rate of organic matters by bacteria. On the other hand, the plants secrete matrix substrates for microbial co-metabolism, and they stimulate the microbial mineralization of organic pollutants.

The Effects of the Biomass on Remediation and Plant Growth

The biomass was the more important factor in the decomposition of pollutants, and the biomass of bacteria in the contaminated soil was various at different concentration of pollutants, and the planting pattern had great effect on the biomass.

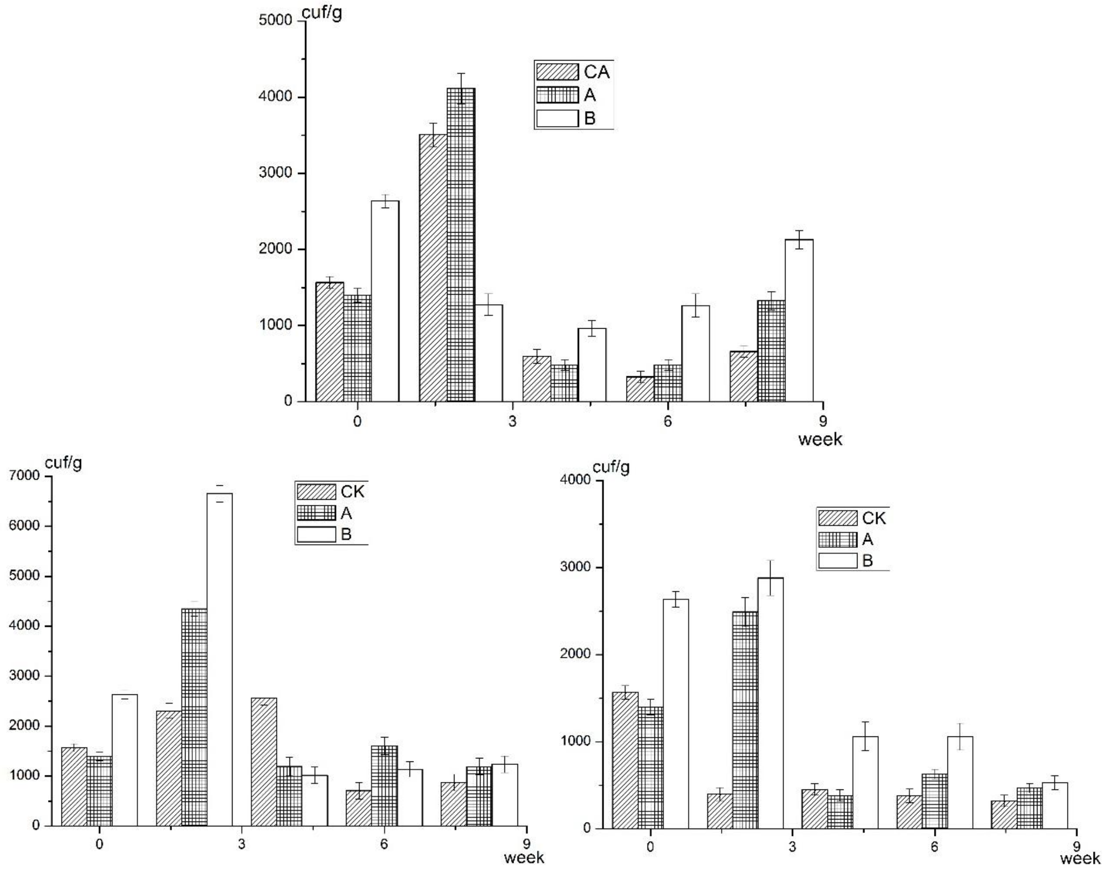

Figure 4. *The effects of biomass under different planting patterns. (a)The effect of biomass under mixed planting; (b) The effect of biomass in corn system; (c) The effect of biomass in rye grass system.*

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Figure 4 shows that the microbial quantity remained low in the early stages of the test after the pollutants were added to the matrix. After two weeks, the bacteria in the sediment increased dramatically. This may be due to pollution stress, which rapidly generates tolerant bacteria and would be consistent with the removal rates of pollutants In regard to the effects of heavy metals, it could stimulate the microbial proliferation at low concentrations. The bacteria in the sediments reached a maximum high during the 2nd week of the study but fell to a dramatic low during the 4th week and maintained this level until the 8th week. This may have been due to the adaptation of the selection, and the fact that only those tolerant bacteria survived. The heavy metals in the sediments stimulated the multiplication of microbes in the early stages of the experiment, but the microbial biomass in the sediments eventually dropped, because the heavy metals' toxic effects proved heartier than their promotion effects. Thus, the heavy metal' toxic effects negatively affect the sediment microbial flora thereby reducing the sediment microbial biomass.

The organic matters in the sediments also affected the biomass, because the organic matters could be used as carbon source for bacteria. Comparing with CK, addition of exogenous organic matters made the biomass increase at concentration A and B. The might be that the organic matters could serve as the carbon source, which promotes the microbial growth and reproduction.

Conclusions

In conclusion, the project participants found that both the biomass and bioactivity were affected by the addition of pollutants, as the biomass at first increased and then decreased in the presence of the exogenous pollutants. These results may have been due to the rhizosphere effect and the stimulation of exogenous pollution. They also found that as the duration of the pollution exposure time increased, a small number of tolerant microorganisms survived and accumulated in quantity. Over time, the variation of microorganisms affected the remediation efficiency.

In the co-contaminated soil, different plant species showed that they had different removal capabilities of lead. In the single-planting patterns, the removal efficiency of corn was better than that of rye grass. The removal rate of zinc by corn was slightly higher than that of rye grass. In addition, the removal rate of heavy metals at concentration B was better than that at concentration A. Within limits, a high concentration of pollutant resulted in positive removal efficiency. The mixed-planting patterns showed a wide range of applications in complex heavy-metal-polluted soil.

The removal effects of combined pollutants is associated with the biomass of lead-zinc-phenanthrene-pyrene combined pollution. They also found that the biomass of tolerant bacteria is critical for the remediation of combined pollution and that organic pollutants could be a crucial carbon source to promote the growth and reproduction of microorganisms.

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APPENDIX

Appendix 1. *The photographs of planted rye grass*

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INFLUENCES OF CHLORIDES ON VFA DISTILLATION DETERMINATION IN ANAEROBIC REACTION

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Abstract. In an experiment, an IC reactor was used to treat straw-washing wastewater from a paper mill; there appeared an acidification trend in this reactor due to accumulation of volatile fatty acid (short for VFA). In this paper, the influences of chlorides on VFA determination were investigated by detecting VFA in actual wastewater samples and imitated samples. The results showed that when wastewater samples were fed with sodium chloride standard solution, the determined VFA value increased by 12.9～38.4 mg/L relative to the samples not fed, and the addition of silver sulfate could shield the interference of chlorides on VFA detection. When standard samples containing different amounts of acetic acid but fed with the same amount of sodium chloride standard solution were used, chlorides showed an influence on VFA determination, and the increment of VFA value was between 35.1～40.3 mg/L, which did not linearly correlate with the acetic acid concentration. When standard samples containing the same amount of acetic acid (500 or 700 mg/L) but fed with different amounts of sodium chloride were used, the VFA value increased by 13.8～39.5 mg/L or 10.2～35.8 mg/L, which linearly correlated with the amount of additional sodium chloride.

Keywords: *anaerobic decomposition, chloride ions, IC reactor, wastewater treatment, distillation titration*

Introduction

High concentration organic wastewaters, such as pulping and paper mill wastewater (Liu, 2012), soybean protein wastewater (Zeng et al., 2011) and starch desizing (Pei et al., 2015) wastewater, are generally treated with anaerobic biochemistry reactors such as UASB, IC, HABR (Pei et al., 2015; Yu, 2015; Wang et al., 2015). In anaerobic reaction, organic matter is usually broken into low-molecular organic matter (Michael et al., 2015) or volatile fatty acids (short for VFA). VFA is a semi-finished matter in anaerobic decomposition, from which the methanogen can produce methane. It is noticed that the methane produced by methanogen only accounts for a small fraction of methane produced by H_2 and CO_2. However, the generation of H_2 and CO_2 also occurs during the process where macromolecular organic compounds convert into VFA. Therefore, VFA is commonly used as a monitoring index of anaerobic reactor (Boonsawang et al., 2015; Kardos et al., 2011). When VFA accumulates in anaerobic reactors, methanogenic bacteria tend to be in an inactive situation, and then it is said that the reactors are undergoing acidification (Yu et al., 2011). Considering an IC

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reactor always requires a high organic loading rate (short for OLR) and a high level of methanation, it is necessary to control the VFA concentration more strictly (Zhao and Mu, 2014; Zeng et al., 2011).

Detection methods for VFA content mainly include colorimetric method, chromatography, titration method and distillation. Colorimetric method is suitable to detect single organic acid that is not mixed with organic acids, owing to its great interference and complex pretreatment (Jiang, 2010). Chromatography is difficult for use in a factory, because of its large investment cost, complex operating steps, difficult temperature control and high demand for technical staffs (Wang et al., 2008). Titration method for VFA, based on equilibrium theory of buffer solution, requires special test target and needs investigation for applying (Wang et al., 2008; Lutzhoft et al., 2014). By contrast, distillation or distillation titration method does not need complex delicate devices, and can be operated simply and reliably (Jiang, 2010), so that it has been generally used in running monitor of anaerobic treatment project.

Anaerobic treatment (Liu, 2012) in this paper was carried out by using the pilot scale apparatus and laboratory in a pulping and papermaking factory. An IC anaerobic reactor with a height of 5.5 m and an effective volume of 6 m^3 was used to treat the straw-washing wastewater, and the influent flow in the experiment apparatus was 450～550 L/h. Effluent VFA samples from this reactor were analyzed three times each day. When the running volume loading rate was 1.95～4.69 kg COD/(m^3·d), the effluent VFA concentrations in the reactor were 192.0～533.0 mg/L; when the volume loading rate was 3.34～6.57 kg COD /(m^3·d), the VFA concentrations were 724.0~1211.0 mg/L. Obviously, the VFA concentrations were so high that a trend of acidification appeared in the reactor.

When treating straw-washing wastewater by an IC reactor, the main reasons why VFA values diverged and remained at a high level can be attributed to wastewater quality, running temperature, volume loading rate and pH. However, it is not clear whether some other factors have influences on the VFA determination process. Liquid chlorides are commonly used to blend pulp in pulping and paper mills, and they can get into the paper mill wastewater through water supply, drainage and wastewater treatment system. The concentration of chlorides in this kind of wastewater is 3000 mg/L approximately. In the distillation of water samples containing VFA, chloride ions and hydrogen ions can combine in the water form of hydrogen chloride under the acidification condition by adding phosphoric acid. With acidity and heating, hydrogen chloride is volatilized to gaseous state (Fritz and Fuget, 1956; Liu et al., 2012; Jiang, 2011), and then condenses and dissolves in the water in the condenser pipe, thus flowing into the receiving flask. Then, may these chlorides affect VFA detection? To the best of our knowledge, there is no relevant research paper on this topic up to now.

Experimental methods and materials

Wastewater samples were taken from the effluent of the anaerobic reactor in a factory (Liu, 2012). VFA standard solution and NaCl standard solution were prepared in laboratory as follows.

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VFA standard solution

Pipet 100 mL 99.5% acetic acid of analytical reagents (AR) into a volumetric flask of 1 L and bring to the rated volume by distilled water, thus obtaining 10% acetic acid solution. Suck 50 mL such acetic acid solution into a volumetric flask of 500 mL and bring to the rated volume by distilled water, thus obtaining 1% acetic acid solution. The density of acetic acid is 1.0492 kg/L, and then 1% acetic acid solution contains acetic acid of 1.044 g/ L, or 1.000 g/ 0.9579 L.

NaCl standard solution (Chinese state administration of environmental protection, 2002)

Put sodium chloride of guaranteed reagents (GR) into a crucible and heat it for 40~50 min at 600°C. After cooling down, weigh 8.2400 g this sodium chloride and dissolve it in a proper amount of distilled water. Then, put the solution into a volumetric flask of 1 L and bring to the rated volume by distilled water. Pipet 100 mL new solution from this flask into a volumetric flask of 500 mL and bring to the rated volume by distilled water. Now, the standard solution contains sodium chloride of 1.00 g/L.

Experimental method

Different amounts of sodium chloride standard solution were fed into the real or imitated samples respectively, and the VFA values of the samples fed with chlorides or not were determined respectively. Thus, by comparing the VFA data, it was possible to analyze whether chlorides fed in the water samples had influences on VFA determination. In the contrast experiment, wastewater or water samples were also prepared, but without addition of sodium chloride standard solution.

Distillation and titration were carried out simultaneously on the parallel samples, and the determination data of VFA content was the available value (or the average value).

VFA determination method

There is no national standard method for VFA determination in water samples (Lutzhoft et al., 2014), and it usually refers to the distillation method in books (He, 1998). Main steps of the determination are as follows:

1) Put 100 mL water sample in which VFA concentration is less than 30 mmol into a distillation flask, and add several drops of phenolphthalein.

2) Put 10% NaOH into the distillation flask to make the sample liquor alkaline, and the addition of NaOH should be slightly in excess.

3) Distill the liquid volumes in the flask to 50~60 mL and then stop distillation.

4) Dilute the remaining fluid to original volume, and add 10 mL 8.5% phosphoric acid. Then, place the receiving flask with 10 mL distilled water under the condenser pipe of the distillation device, and ensure the tube of condenser pipe to be immersed in the liquid level of the receiving flask. Continue to distill until the remaining liquid is 15~20 mL. After the distillation flask cools down, add 50 mL distilled water in the distillation flask, and starts to distill again until the remaining liquid is 10~20 mL.

5) Add 10 drops of phenolphthalein into the received liquor, and then titrate the liquor with NaOH standard solution. At endpoint, the ideal indicator color is a barely detectable shade of pale pink.

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Materials and apparatus

Analytical balance and glass devices used in VFA detection were delicate, and chemicals were analytical reagents (AR) or guaranteed reagents (GR) (Liu, 2012). The environment of laboratory was very exquisite. *Table 1* shows the related apparatus, devices and chemicals.

Table 1. *Apparatus, devices and chemicals*

Name of apparatus, devices or chemicals	Grade	Name of suppliers
Analytical balance	FA2004N	Shanghai JinHai instrument Co., Ltd
PH meter	PHB-4	Shanghai Precision & Scientific instrument Co., Ltd
Constant temperature drying ovens	202	Beijing ZhongXing WeiYe instrument Co., Ltd
Acetic acid	AR	Beijing ShiJi TuoXin fine chemical Co., Ltd
Sodium chloride	GR	Luoyang HaoHua chemical reagent Co., Ltd
Sodium hydroxide	AR	Beijing ShiJi TuoXin fine chemical Co., Ltd
Phosphoric acid	AR	Beijing ShiJi TuoXin fine chemical Co., Ltd
Silver sulfate	AR	Luoyang HaoHua chemical reagent Co., Ltd

Wastewater samples fed chlorides and detect VFA

Effluent samples of the anaerobic reactor, in which VFA values were 160.0～293.5 mg/L, were supplemented with sodium chloride standard solution of 200.0～800.0 mg/L. The VFA values of the samples with or without chlorides were determined, and the differences between the values were calculated. The results are illustrated in *Fig. 1*, where C-CL represents the additional amount of sodium chloride standard solution, C-VFA represents the VFA concentration of effluent samples without adding sodium chloride, and I-VFA represents the increment of VFA determination values due to adding sodium chloride. Obviously, I-VFA = VFA values of effluent samples with adding sodium chloride - VFA values of these samples without adding sodium chloride. These notations are used in the whole paper, and the units are mg/L for all.

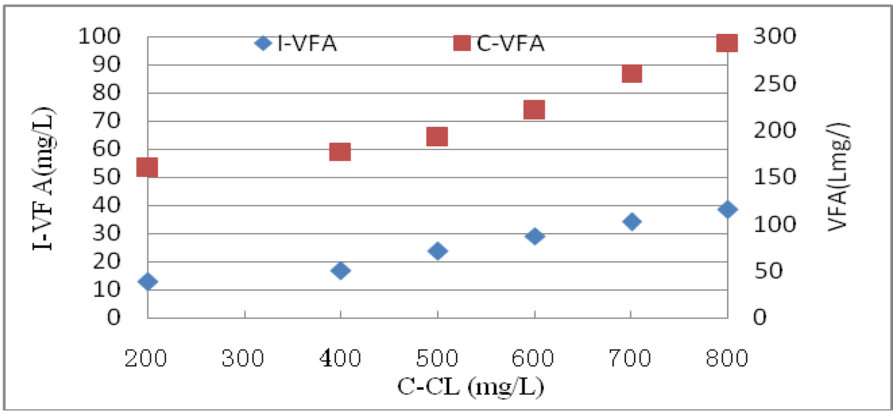

Figure 1. *VFA increment for the wastewater samples with different amounts of chlorides*

As can be seen from *Fig. 1*, when sodium chloride standard solution of 200.0, 400.0, 500.0, 600.0, 700.0 and 800.0 mg/L is respectively added into the effluent samples, the

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determined VFA values of the samples increase continuously, with an increment of 12.9～38.4 mg/L. It is believed that when there are chloride ions in wastewater, the influences of chlorides in water quality test can be shielded by adding silver sulfate or mercuric sulfate (Liu and Wu, 2011). Here, three samples were prepared to investigate the shielding effect: Sample A was confected with acetic acid of 1 g/L; Sample B was confected by feeding 3000 mg/L sodium chloride standard solution into Sample A; Sample C was confected by feeding 0.5 g/100 mL silver sulfate (Dong and Jin, 2014) into Sample B. The VFA of the three samples were determined, and the results are provided in *Table 2* (units are mg/L).

Table 2. *VFA determination values of samples added with silver sulfate*

	Sample A	Sample B	Sample C	B - A	B - C
Test 1 (mg/L)	986.0	1016.2	997.9	30.2	18.3
Test 2 (mg/L)	995.8	1031.0	1010.0	35.2	21.0

As shown in *Table 2*, when Sample A is added with sodium chloride, the determined VFA values increase by 30.2 and 35.2 mg/L in Tests 1 and 2 respectively; when Sample B is added with silver sulfate, the determined VFA values decrease by 18.3 and 21.0 mg/L in Tests 1 and 2 respectively. Hence, for the detection of VFA, silver sulfate can be added to shield partially the influences of chlorides when there are chlorides in samples. Despite of this, it can however be seen from *Fig. 1* and *Table 2* that chlorides in samples always have influences on the determined VFA values.

In the detection of VFA, further research is needed to determine whether the method used in the determination of COD, which is based on shielding the influence of chlorides by adding silver sulfate, can be adopted (Liu and Wu, 2011). On this basis, the additional amount of silver sulfate can be estimated for optimizing the shielding reaction as well as the reducing effect.

Distillation and detection of VFA values in samples with different acidity degrees

Before the second distillation in VFA detection, water samples must be acidified by adding phosphoric acid. In order to find out the influences of chlorides with different acidity degrees on VFA determination, a group of contrasting tests were performed. Three imitated samples, in which the concentration of acetic acid was 100 mg/L, were fed with different amounts of sodium chloride and phosphoric acid with a concentration of 8.5%. For each chloride concentration, two adding dosages of phosphoric acid were used: 10 mL phosphoric acid (Dosage 1) and 20 mL phosphoric acid (Dosage 2). *Table 3* shows the contrasting results, of which the units are mg/L.

Table 3. *Increment of VFA values for differment amounts of phosphoric acid*

Chloride concentration	Dosage 1	Dosage 2
500	21.4	22.7
1000	25.7	27.5
2000	35.4	36.6

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As can be seen, with increasing chloride concentration from 500 to 2000 mg/L, the detected VFA value presents an increasing trend. However, the distinction of VFA increment is slight when increasing the additional amount of phosphoric acid. It seems that phosphoric acid addition has no significant effect on VFA determination. In these experiments, however, it should be noticed that the additional amount of phosphoric acid is small, so that it cannot cause large variation of acidity to the distillate solution. Hence, further experiments are necessary to examine the influences of chlorides on VFA determination at different acidity degrees. However, theorists consider that the volatilization of hydrogen chloride from hydrochloric acid depends on the character, concentration and vapor pressure of hydrochloric acid, instead of the acidity degrees of hydrochloric acid.

VFA detection of confected standard water samples

VFA detection of the samples containing different amounts of acetic acid

A series of confected standard water samples fed with sodium chloride standard solution of 2000 mg/L but containing different amounts of acetic acid were tested for VFA determination, and their VFA values were compared with those samples containing different amounts of acetic acid but without sodium chloride. The differences in VFA values difference of these two series of samples, I-VFA, were deduced, and the relationship between the VFA increment and the acetic acid concentration is illustrated by *Fig. 2*, in which C-VFA represents the acetic acid concentration of the standard water samples, with units of mg/L; I-VFA/C-CL represents the ratio of the VFA increment and the amount of additional sodium chloride.

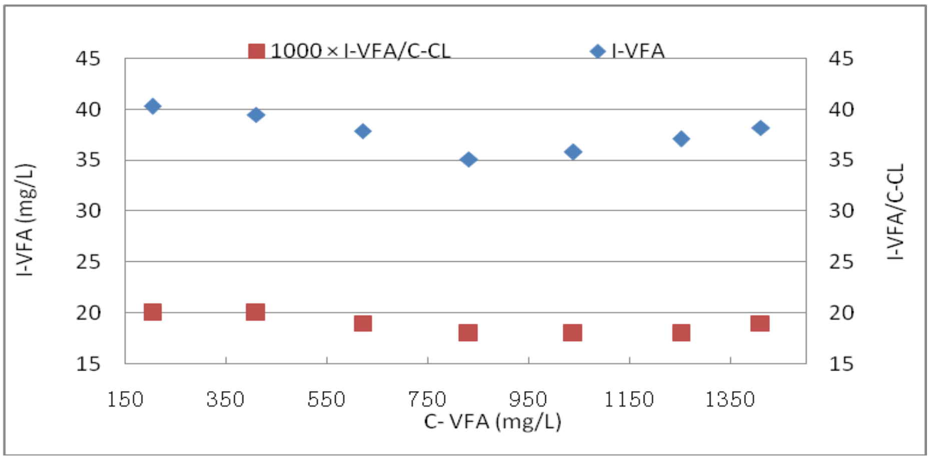

***Figure 2.** VFA increment of samples containing different amounts of acetic acid*

As shown in *Fig. 2*, after the standard water samples containing acetic acid of different concentrations of 205.0, 410.0, 620.0, 835.0, 1044.0, 1250.0 and 1410.0 mg/L were fed with sodium chloride of 2000 mg/L, the determined VFA values all increase, and the data range of I-VFA is 35.1～40.3 mg/L. It is noted that the data range of VFA increment is only 5.2 mg/L; meanwhile, the data range of I-VFA/C-CL is 0.002. Hence, it can be declared that the data range is small for all samples. In addition, *Fig. 2* also indicates that VFA increment is not linearly correlated with the acetic acid concentration.

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VFA detection of the samples fed with different amounts of sodium chloride

Standard samples containing acetic acid of 500 or 700 mg/L, with different amounts of sodium chloride standard solution, were tested for VFA determination, and the relationship between the VFA increment relative to the sample without sodium chloride and the chloride concentration can be seen in *Fig. 3*. For the standard samples containing acetic acid of 500 mg/L, when they were fed with sodium chloride standard solution of 200～2600 mg/L, the determined VFA values all increase relative to the sample without sodium chloride, and the VFA increment is 13.8～39.5 mg/L. For the standard samples containing acetic acid of 700 mg/L, when they were fed with identical amount of sodium chloride, the VFA increment is 10.2～35.8 mg/L. It can be seen that VFA increments determined in the samples with acetic acid of 500 mg/L are larger than those with acetic acid of 700 mg/L. Besides, with increasing concentration of sodium chloride, the VFA increment presents an increasing trend, and shows a linear correlation with the amount of additional sodium chloride. In *Fig. 3*, I-VFA/C-CL data are from the samples containing acetic acid of 500 mg/L. As can be seen, I-VFA/C-CL presents a decreasing trend when the sodium chloride concentration is increased.

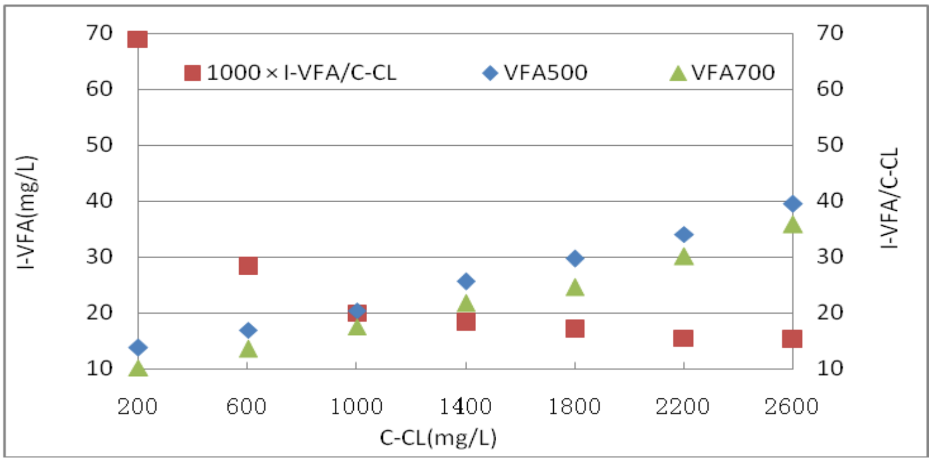

Figure 3. *VFA increment of samples fed with different amounts of sodium chloride*

Conclusions

When wastewater samples with VFA concentration of 160.0～293.5 mg/L were fed with sodium chloride standard solution, their determined VFA values showed an increasing trend, and the VFA increment was 12.9～38.4 mg/L. After addition of 0.5 g silver sulfate, the influences of chlorides on VFA determination could be partially shielded. Hence, it can be concluded that chlorides always have an influence on VFA detection of water samples using distillation method.

When the confected standard samples containing acetic acid were fed with sodium chloride standard solution of 2000 mg/L, chlorides only had a slight influence on VFA determination, with VFA increment of 35.1～40.3 mg/L. The increment range is small in comparison with the amount of sodium chloride, and the VFA increment is not linearly correlated with the acetic acid concentration.

Addition of sodium chloride between 200.0~2600.0 mg/L into the standard samples containing acetic acid of 500 or 700 mg/L could result in a VFA increment between

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13.8 ～ 39.5 mg/L or 10.2 ～ 35.8 mg/L. With the increase of sodium chloride concentration, the increment of VFA also increased, and the VFA increment is linearly correlated with the amount of additional sodium chloride.

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MICROBIAL COMMUNITY CHARACTERISTICS OF A HIGH SOLID CONTENT ANAEROBIC DIGESTER OF MUNICIPAL SOLID WASTE

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Abstract. The composition of municipal solid waste (MSW) was examined, including water content, cellulose content, organic ingredients, etc. Throughout the anaerobic digestion of the organic waste, the characteristics of anaerobic acidifying bacteria, ammonifying bacteria, cellulose-degrading bacteria and methane bacteria were analyzed with respect to time and spatial distribution. The function of the microbial community and the relationship between the metabolites were analyzed as well. The results showed that in the initial stage of anaerobic fermentation of municipal waste, large amounts of oxygen in the reactor enabled aerobic and facultative anaerobic bacteria to proliferate and transform most of the raw material into organic matter. With the formation of the anaerobic, nutrient-rich environment the number of anaerobic bacteria began to rise. Anaerobic acidifying bacteria reached a maximum number sooner and remained higher than ammonifying bacteria. Methane bacteria did not proliferate during the startup phase; the peak concentration of 3.36×10^9/mL occurred at the 25th day, then remained stable. The anaerobic cellulose-decomposing bacteria grew slowly. Within the reactor, the numbers of anaerobic acidifying bacteria, anaerobic ammonifying bacteria and methane bacteria were higher in the middle and bottom positions; the anaerobic cellulose-decomposing bacteria proliferated at the bottom. In addition, the change trends of volatile fatty acids and ammonia nitrogen concentration were consistent with the spatial distribution of the bacteria. The VFA composition of biogas slurry was primarily butyric acid, indicating that butyric acid fermentation was the dominant process.
Keywords: *anaerobic acidifying bacteria, ammonifying bacteria, cellulose-degrading bacteria, methane bacteria, time and spatial distribution, relationship of the metabolites*

Introduction

The quantity of municipal solid waste produced in China has increased by 8-10% per year over the past several decades (Shi et al., 2008). Of the 170.8 million tons created in 2012, 61.5% of the waste went to landfills, 21.0% was incinerated, and less than 2.3% was treated through anaerobic fermentation (China, 2012). Traditional landfills can cause problems such as the generation of heavily polluted leachates (Renou et al., 2008; Igoni et al., 2005) and the emission of volatile organic compounds and odors (Gonzáleza et al., 2013), which present a significant threat to public health and the environment. With food waste making up the largest fraction (50%) of MSW in China, and with moisture levels around 50% (Cheng and Hu, 2010; Hartmann and Ahring, 2006), the anaerobic fermentation process is a feasible way to reduce the

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consumption of fossil fuel. If the organic component of MSW is converted into energy through anaerobic digestion, it will reduce the adverse impact on the environment and contribute to the production of new energy. Anaerobic digestion is a process by which complex organic materials are first hydrolyzed and fermented by acidifying bacteria into volatile fatty acids (VFA) (Wan et al., 2013; Igoni, 2006). The VFA are then consumed by methanogenic bacteria and converted into methane gas. However, not all organic components of MSW can be digested easily (Macias-Corral et al., 2008; Nopharatana et al, 2007).

A number of anaerobic processes such as wet or dry fermentation have been adopted and developed for the treatment of MSW (Tyagi et al., 2014; De Baere and Mattheeuws, 2010). Because the traditional wet fermentation process requires the addition of water to the fermentation material and produces residual waste byproducts, building a wet fermentation biogas plant would require more equipment and investment than other types (Motte et al., 2013; Ghosh et al, 2000). Anaerobic fermentation of high solid content waste is becoming a research focus. Several factors affect the dry fermentation process for biogas production, such as pH, ammonia inhibition, and the microbial community (Yin et al., 2014). Controlling the microbial community in particular could affect the efficiency of biogas production (El-Mashad et al., 2008; Eikmeyer et al., 2013; Kovács et al., 2013). Previous research has suggested that adjusting parameters such as substrate concentration and initial solid waste composition could improve the environment for anaerobic digestion (Fernández et al., 2008), and that mixing different types of organic waste may also improve biogas production (Li et al., 2010). However, few studies to date have directly analyzed the microbial community in dry anaerobic fermentation to improve the digestion process.

In this study, microbial community composition was investigated during dry anaerobic fermentation in a vertical reactor designed by our research group. The presence of volatile fatty acid (VFA), ammonia concentration and biogas production were measured over time at different locations within the reactor. Observation of the changes in the microbial community leads to understanding of the relationship between the microbial community and organic conversion.

Material and methods

Feedstock selection

Raw materials were collected from a landfill in Yingkou, China. Nonbiodegradable substances such as metal, waste residue, glass and fabric were sorted out of the municipal garbage. The physical and chemical properties of fermentation materials were analyzed, as shown in *Table 1*. The inoculum was a biogas slurry using pig manure as fermentation feedstock (8.64% TS, 3.52% VS).

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Table 1. *Physical and chemical properties*

Month	1~3	4~6	7~9	10~12
TS %	48.4	36.4	31.9	36.8
VS %	42.88	30.35	27.68	31.39
TOC %	25.1	18.9	24.2	24.5
TN %	2.08	1.84	1.95	1.98
pH	6.72	5.91	5.90	6.12
Protein %	12.49	11.62	12.11	11.87
Fat %	8.81	5.55	7.31	7.24
Cellulose %	9.21	12.55	13.80	11.61
Hemicellulose %	2.95	3.23	3.45	3.24
Lignin %	1.78	2.24	2.25	1.87
Reducing sugar %	2.34	2.83	3.32	1.95
Lower heat value (kJ/kg)	9412	4734	4038	5541

Fermentation design

The test device was a batch reactor for mesothermal (37°C) anaerobic fermentation, as shown in *Figure 1*. The design incorporated six sampling positions: the top center, the top edge, the center, the middle edge, the bottom center and the bottom edge. The distance from the center to the edge was 15 cm, the distance from the bottom sampling port to the tank bottom was 8 cm, and the distance from the center to the top or bottom was 17 cm. The device featured an external water bath circulation heating system The effective volume was 30 L. The fermentation experiment used municipal solid waste that had been in the landfill for 4 to 6 months. Of the18 kg of material added to the fermentation tank, the inoculum accounted for 30%. The pH value was adjusted to 7.0. The gas produced by anaerobic fermentation in the reactor was recorded daily. Quanitities of ammonia nitrogen, acetic acid and propionic acid, as well as other parameters, were measured at each sampling port to assess the microbial community at different stages of fermentation. Each test was repeated three times and the results were averaged.

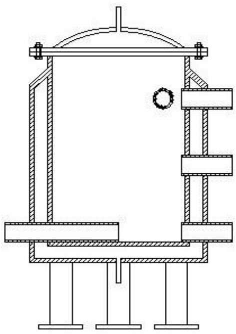

Figure 1. *Anaerobic fermentation test device*

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Analytical method

Physicochemical property

Gas production, methane content, ammonia nitrogen concentration, and volatile fatty acids were each analyzed according to the conventional index method (APHA, 1995).

Microbial quantity determination

The most probable number (MPN) method was followed to estimate a scope, or range, within a confidence interval, of the actual number of colonies based on the sample collected from the reactor. The MPN method was used for the following organisms:

Acidifying bacteria: MPN, training 2 days at 37°C, a bromocresol purple indicator turning yellow showed a positive result.

Ammonifying bacteria: MPN, training 7 days at 28°C, Nessler's reagent was added to the culture medium; generation of a precipitate indicated a positive result.

Cellulose-decomposing bacteria: MPN, training 30 days at 37°C, when the filter paper in the medium produced gray translucent or yellow spots, the test result was positive.

Methane bacteria: MPN, the medium was prepared according to the Hungate technique, training 7 days at 37°C, The presence of methane was detected by gas chromatography, then the MPN tables were used to determine the quantity of methane bacteria.

Experimental reagents and culture medium

Acidifying bacteria culture medium: The medium was composed of dipotassium phosphate (0.4 g), ammonium chloride (1.0 g), yeast cream (1.0g), magnesium chloride (1.0 g), glucose (8.0 g), sodium chloride (1.0 g), 1% bromocresol purple indicator (5.0 mL), and water (1,000 mL), with a pH value of 7.0-7.2.

Ammonifying bacteria culture medium: The medium contained peptone (5.0 g), dipotassium phosphate (0.5 g), monopotassium phosphate (0.5 g), magnesium sulfate (0.5 g), and water (1000 mL), with a pH value of 7.0-7.2.

Cellulose-decomposing bacteria medium: The medium was composed of sodium hydrogen phosphate ammonia (2.0 g), monopotassium phosphate (1.0 g), peptone (1.0 g), magnesium sulfate (0.5 g), calcium carbonate (5.0 g), and calcium chloride (3.0 g). Inserted the 1×10 cm filter into per tube.

Methane bacteria culture medium: The medium contained methyl alcohol (3.5 g), acetic acid (3.5 g), sodium acetate (5 g), ammonium chloride (1 g), monopotassium phosphate (0.4 g), magnesium chloride (0.1 g), dipotassium phosphate (0.4 g), yeast extract powder (2 g), and water (1,000 mL). For every 5.0 mL of medium, 1.0 mL of the sterile mixture solution of 5% $NaHCO_3$ and 1% Na_2S was added. After sterilization at 121°C, for every 5.0 mL of medium, 0.1 mL of sterile penicillin solution was added.

The anaerobic culture medium contained the same components as the aerobic and facultative anaerobic culture medium with the following additions: redox indicator (0.1%), resazurin (2 mL) and reducing agent L-cysteine (0.5 g).

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Nessler's reagent: To prepare this solution, potassium iodide (35.0 g) and mercuric chloride (1.3 g) were dissolved in 70 mL distilled water and added to 4 mol/L potassium hydroxide solution (30 mL), filtered when necessary, and stored in an airtight jar.

Results and discussion

Biogas production and methane

The process of dry anaerobic fermentation of MSW can be divided into a start-up period (1-15 d), rich phase (16-28 d), and continued gas phase (29-40 d). As *Figure 2* shows, in the 3-4 days after feeding there was a small peak in gas production during the start-up period, however, the CH_4 content was only 8.1%. Following this small peak, the gas production decreased rapidly, while CH_4 content increased gradually. By the 21st day after feeding, CH_4 content had reached 58.63%. The amount of gas production reached its maximum value of 26.8 L on the 25th day; the maximum methane content was 75.41% on the 27th day. As the fermentation progressed, a large amount of organic matter was consumed within the fermentation substrate. At the same time, the physicochemical properties of the fermented liquid changed accordingly, and some harmful substances accumulated in the fermentation system. Microbial activity began to decrease after 27 days, as evidenced by the gradual decline in gas production.

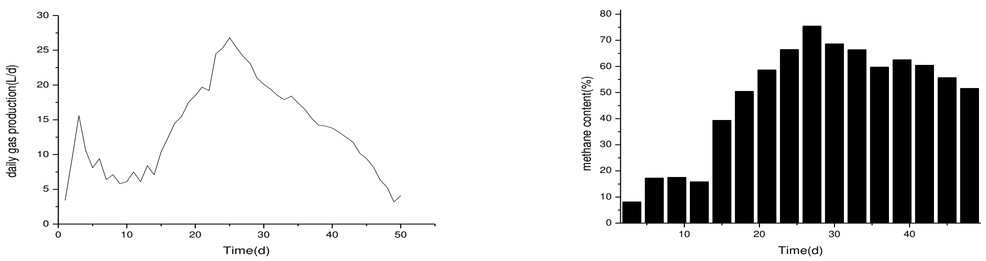

Figure 2. *Trend of daily gas production and methane content*

Temporal and spatial distribution of microbial communities

Aerobic and facultative anaerobic acidifying bacteria

As shown in *Figure 3*, y-axis representatived the logarithm bacteria number. The number of aerobic and facultative anaerobic acidifying bacteria rose rapidly to its peak value of 2.86×10^9/mL, then decreased rapidly and maintained a stable trend within the range of 10^6-10^8/mL. In terms of tank position, the fastest growth distribution of aerobic and facultative anaerobic acidifying bacteria was observed in the top center position, followed by the top edge (1.1×10^9/mL), the middle edge (1.32×10^8/mL), the center (7.02×10^7/mL), the bottom edge (1.21×10^7/mL) and the bottom center (6.11×10^6/mL), in that order. At the top center and top edge positions, these bacteria proliferated rapidly to the maximum quantity, but then fell sharply back to the initial value. The amplitude of the rise and fall at the two middle locations and the two bottom locations was not significant,

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indicating slow proliferation. At each level (top, middle, and bottom), there was no significant difference between the center and edge measurements.

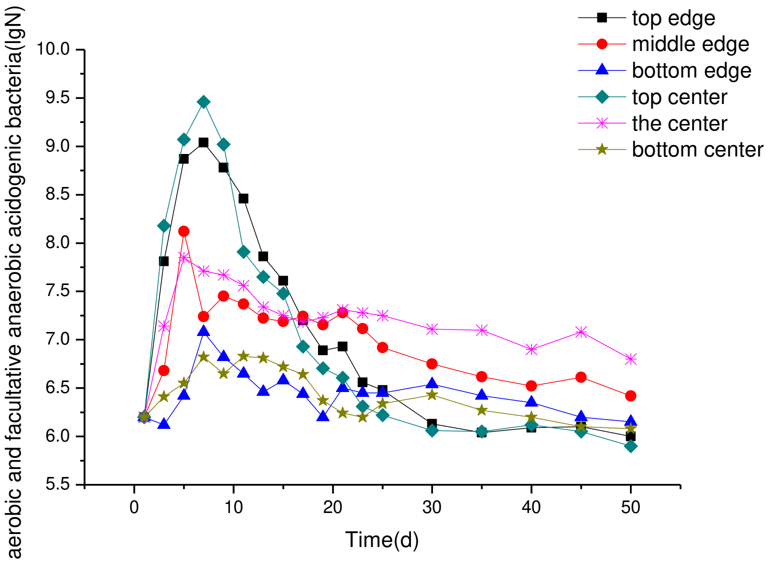

Figure 3. *Space and time differences of aerobic and facultative anaerobic acidifying bacteria*

In the top position, residual air provided the proliferation conditions for aerobic bacteria to produce acid. The conditions of the middle and bottom were relatively stable anaerobic environments. Therefore, acidifying bacteria proliferation was lower and mostly consisted of facultative anaerobes. Higher numbers at the middle positions compared to the bottom indicate that the anaerobic environment and nutrition conditions were more stable in the middle relative to the bottom.

Aerobic and facultative anaerobic ammonifying bacteria

As shown in *Figure 4*, the multiplication rate of aerobic and facultative anaerobic ammonifying bacteria was lower and reached its peak later than that of aerobic and facultative anaerobic acidifying bacteria. The maximum concentration of 2.11×10^{8}/mL was observed on the 15th day at the center location. Maximum values measured at the other positions are as follows: middle edge, 1.24×10^{8}/mL; top edge, 1.05×10^{8}/mL; bottom edge, 4.23×10^{7}/mL; top center, 2.21×10^{7}/mL; bottom center, 1.10×10^{7}/mL.。

For aerobic and facultative anaerobic ammonifiers, the extremum did not appear in the top position, indicating less dependency on oxygen than acidifying bacteria, which comprised most of the facultative anaerobic bacteria. The spatial change trend of aerobic and facultative anaerobic ammonifying bacteria showed similarities to acidifying bacteria, but its distribution was more uniform inside the reactor, with higher values at the center. At the middle height, edge values were slightly higher than the center. Analysis of the change trends for the different types of bacteria shows that in the early stage of dry anaerobic fermentation, aerobic and facultative anaerobic acidifying bacteria were

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predominant and played a role in the degradation of organic matter and the combination of the necessary precursor substances for methane production. In the next phase, the aerobic and facultative anaerobic ammonifying bacteria proliferated and dominated, effectively preventing pH decline, and providing the appropriate conditions for the next step of methane bacteria activity.

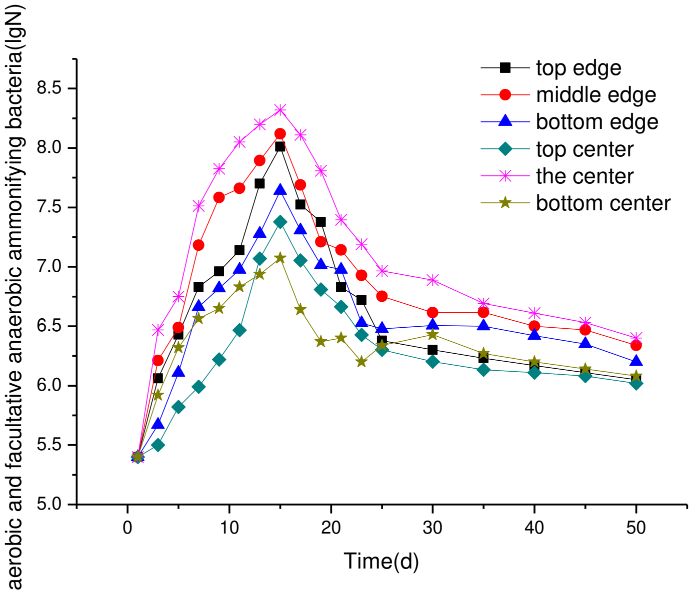

Figure 4. *Space and time differences of aerobic and facultative anaerobic ammonifying bacteria*

Anaerobic acidifying bacteria

At the start of the anaerobic fermentation process, the presence of oxygen in the reactor inhibited the growth of anaerobic bacteria (Hansen and Yu, 2005). With the formation of the anaerobic environment and the nutrient-rich phase, the number of anaerobic acidifying bacteria started rising rapidly in the 10th day after feeding, as shown in *Figure 5*. As oxidation-reduction potential decreased, environmental conditions became suitable for the growth of anaerobic bacteria. The maximum number of anaerobic acidifying bacteria was reached around the 15th day, then the number dropped steadily.

As shown in the spatial distribution of *Figure 5*, the number of anaerobic acidifying bacteria was largest at the bottom center, with a maximum of 3.82×10^{10}/mL. The maximum bacteria numbers at the other positions were as follows: bottom edge, 1.24×10^{10}/mL; middle edge, 8.17×10^{9}/mL; center, 7.71×10^{9} /mL; top center, 3.26×10^{9} /mL; top edge, 1.18×10^{9} /mL. An overall trend in the anaerobic acidifying bacteria numbers can be seen: the center positions contained more than the edge positions, and the bottom positions more than the top. Anaerobic acidifying bacteria belong to the bacterial flora that degrade organic matter in the later stages of dry anaerobic fermentation (Rincon et al., 2006). At this point, the number of aerobic and facultative anaerobic acidifying

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bacteria decreased, while anaerobic acidifying bacteria and aerobic and facultative anaerobic ammonifying bacteria reached the maximum. These two groups of bacteria are believed to form a dynamic balance within the space at this time. The number of anaerobic acidifying bacteria was lower at the top of the reactor than at any other position, because the residual air at the top during the initial stage was not conducive to the growth of anaerobic bacteria.

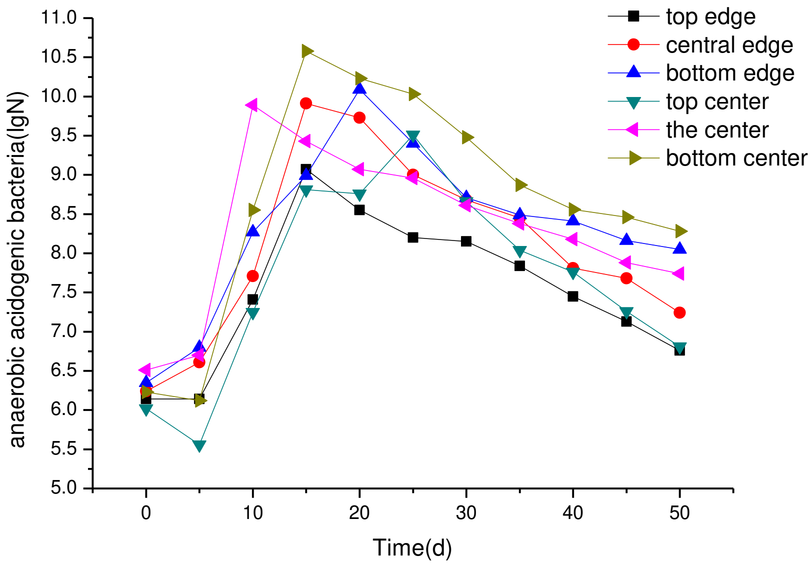

Figure 5. *Space and time differences of anaerobic acidifying bacteria*

Anaerobic ammonifying bacteria

Ammonifying bacteria can decompose protein to produce ammonia. The rapid hydrolysis of nitrogen material in the substrate by ammonifying bacteria provides the requisite material for methane bacteria growth, while adjusting the pH value to create a conducive environment for the growth of a mixed microbial community (Forster-Carneiro et al., 2008; Ahring et al., 2001). As shown in *Figure 6*, at the beginning of fermentation, the number of bacteria decreased slightly, possibly because the addition of oxygen and environmental pH change was not suitable for bacterial growth. Subsequently, through a variety of microbial synergies, the anaerobic environment emerged, and anaerobic ammonifying bacteria multiplied. The change trend of the anaerobic ammonifying bacteria was similar to that of anaerobic acidifying bacteria, but the ammonifying bacteria proliferation rate was lower than that of acid-producing bacteria in the initial fermentation period. Among the types of anaerobic bacteria present, acid-producing bacteria reached the maximum earlier than ammonifying bacteria and were dominant. As a result, the pH within the tank dropped during the initial fermentation period to the lowest point in the whole fermentation process.

In the spatial distribution, anaerobic ammonifying bacteria were most abundant at the middle edge and the center position, suggesting that proliferation requires relatively stable pH value and moisture content. Conversely, the residual air present at the top during the initial stage, the water shortage at the top in the later stage, and the accumulation of biogas slurry at the bottom all resulted in fewer ammonifiers at those positions. The maximum number of anaerobic ammonifiers at each test site was reached at around 20 days: middle edge, 1.23×10^{10}/mL; center, 9.41×10^{9}/mL; bottom edge, 6.57×10^{9} /mL; bottom center, 4.05×10^{9}/mL; top edge, 2.22×10^{9} /mL; top center, 1.17×10^{9}/mL.

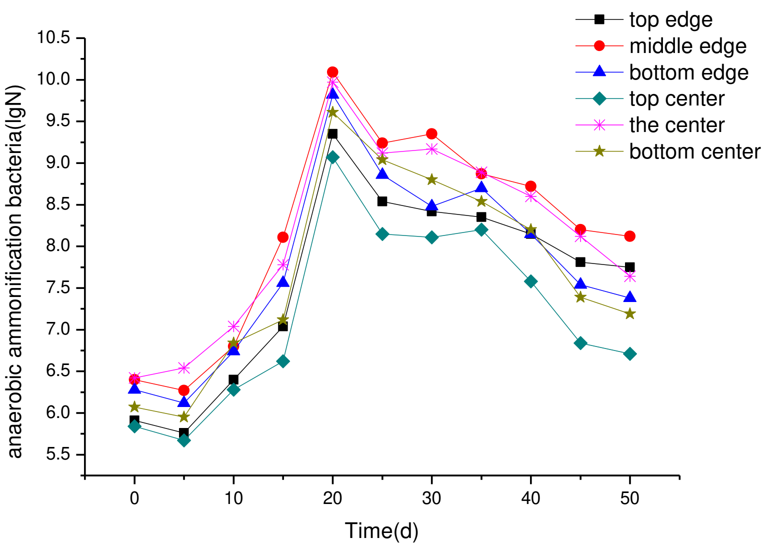

Figure 6. *Space and time differences of anaerobic ammonifying bacteria*

Anaerobic cellulose-degrading bacteria

As shown in *Figure 7*, the number of anaerobic cellulose-degrading bacteria showed a trend of slow growth, rising slowly at the early stages of the fermentation, and increasing to 10^{6}/mL at 45 days. The period of greatest gas production (around 25 days) did not coincide with the maximum number of anaerobic cellulose-degrading bacteria; the material used for the production of biogas was not from the decomposition of cellulose, but from the easily decomposed organic matter. In the continuous gas phase, the number of anaerobic ammonifying bacteria and anaerobic acidifying bacteria began to decline; the cellulose-degrading bacteria were the only microbial colony to continue rising during this period. The main precursor of the methane produced during this period was formed by the decomposition of cellulose (Klockea et al., 2007; Mladenovska et al., 2003).

In the spatial distribution of anaerobic cellulose-degrading bacteria, the most abundant position was the bottom of the fermentation tank. Liquefication of the bottom substrate provided the necessary liquidity, so anaerobic degrading bacteria first proliferated from the bottom edge. The maximum values at each position were as follows: bottom edge,

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1.16×10^7/mL; bottom center. 7.21×10^6/mL; middle edge, 3.31×10^6/mL; center, 1.05×10^6/mL; top edge, 2.25×10^5 /mL; top center, 1.17×10^4/mL.

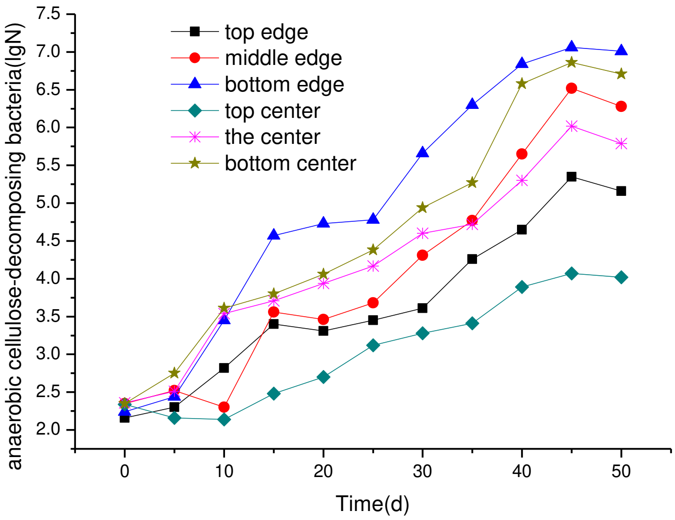

Figure 7. *Space and time differences of anaerobic cellulose-decomposing bacteria*

Methane bacteria

As shown in *Figure 8*, methanogens did not proliferate at the beginning of fermentation, but later in the initial stage (around 15 days), the methanogens grew in number from 10^5 to 10^6. During this period, although gas production rate was very low, CH_4 content increased from 8% to 40%, primarily due to the rapid proliferation of methanogens. The 15-day period of slow methanogen growth was followed by a fast-growth period, and the maximum of 3.36×10^9/mL was observed at around 25 days. At this time, the methane content reached more than 60%. The number of methanogens remained at the same order of magnitude throughout the gas-producing period, then declined to 10^7/mL until the end of fermentation. After the gas peak, because of the consumption of easily decomposable substrate, anaerobic ammonifying bacteria and anaerobic acidifying bacteria began to decline, while the methane bacteria count remained high. The decline in gas production despite the abundance of menthanogens shows that the decreasing anaerobic acidifying bacteria and anaerobic ammonifying bacteria resulted in a lower gas production rate. Therefore, coordination of the three types of microbial growth is necessary to maintain optimal gas production (Zhang et al., 2006; Schluter et al., 2008).

The spatial distribution of methane bacteria numbers in the fermentation tank show more in the center than at the edges, and more at the bottom than the top. This is due to the matrix condition being the most stable at the center of the reactor, the acidifying bacteria producing the most organic acid in the middle and bottom, and the higher liquidity of the bottom allowing a faster mass transfer process that is suitable for methane bacteria

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growth. The maximum numbers of methane bacteria were, in order, bottom center, 3.36×10^9/mL; center, 1.64×10^9/mL; middle edge, 8.75×10^8/mL: bottom edge, 4.91×10^8/mL; top center, 3.79×10^8/mL; top edge, 1.78×10^8/mL.

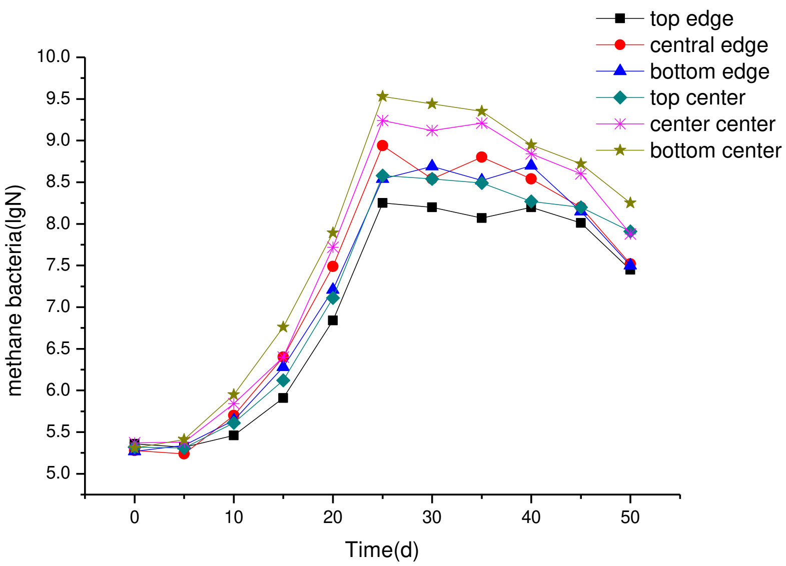

Figure 8. *Space and time differences of methane bacteria*

The relationship between metabolites

The function between acidifying bacteria, methane bacteria, and ammonifying bacteria

The acidogenic and methanogenic stages of anaerobic fermentation connect with and complement each other in the production of biogas (Funk et al., 2005). Anaerobic ammonifying bacteria played a regulatory role in the two stages (Hoj et al., 2008). As *Figure 9* shows, in the initial fermentation period, volatile fatty acid concentration increased rapidly due to the rapid proliferation of acidifying bacteria. VFA concentration reached the highest value of 8.62 g/L on the 15th day, then began to decline. The biogas production was 10.4 L/d at the 15th day, while the CH_4 content of biogas was only 40%. Gas production and methane content both increased rapidly from the 15th day until the 27th day, when gas production was 24.1 L/d and methane content reached a maximum of 75.41%. The results show that most of the acid was produced in the first 15 days after the feeding, which defined the acidogenic phase. The methane bacteria began using these organic acids after 15 days, when the fermentation process transitioned to the methane-producing phase. As the acidogenic phase gradually ended, the balance of acidifying bacteria and methane bacteria allowed fermentation to run proceed.

While acidifying bacteria were most active, ammonifying bacteria also played a crucial role. When the concentration of volatile fatty acid rose rapidly, the ammonia nitrogen concentration also increased sharply. As shown in *Figure 10*, the presence of ammonia was first observed about 10 days after feeding, and the concentration increased

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threefold to its peak value on the 20th day. As the concentration of ammonia nitrogen began to reach its peak, volatile fatty acid began to decline; this phenomenon suggests the role of ammonifying bacteria in adjusting pH.

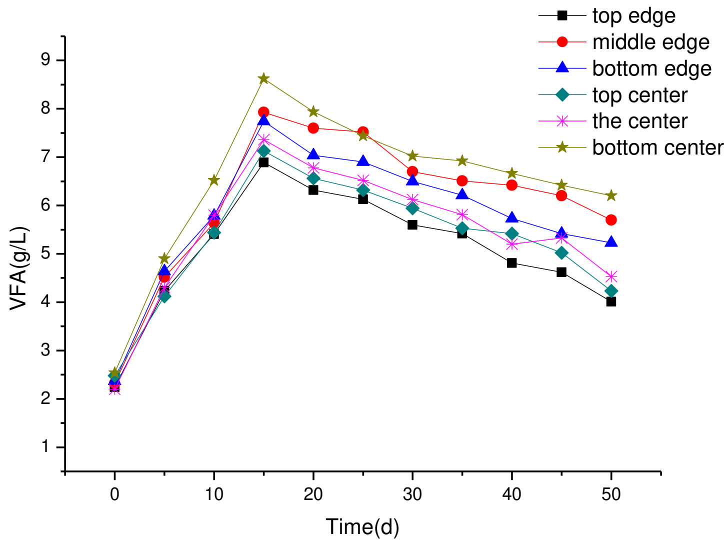

Figure 9. *Trend of VFA*

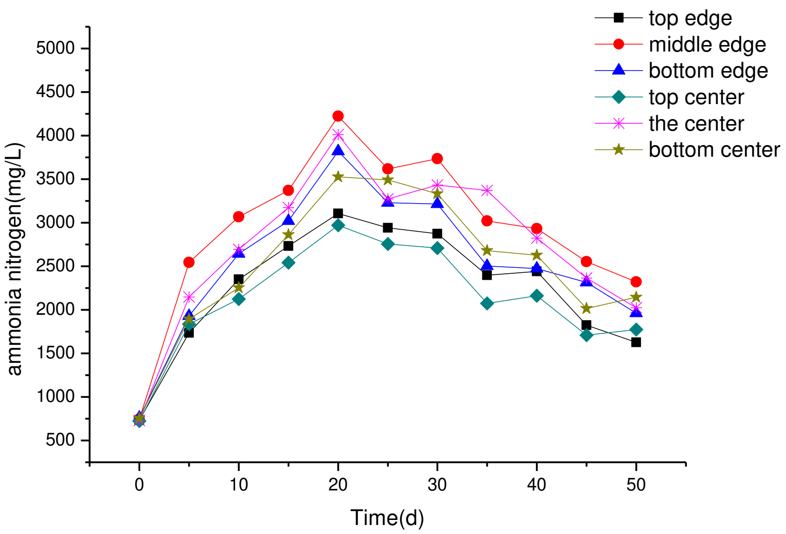

Figure 10. *Trend of ammonia nitrogen*

Due to the relatively static conditions at the center of the anaerobic dry fermentation reactor, there were differences in the spatial distribution of the various microbes. The highest numbers of acidifying bacteria were found at the middle and bottom positions, as were the most volatile fatty acids, and consequently more methane bacteria. There were

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more ammonifying bacteria at the middle positions, where the ammonia nitrogen concentration was higher.

These results indicate a dynamic balance of liquefying, acid-producing and methanogenic processes in the anaerobic fermentation of municipal solid waste. Initially, fermentation substrate liquefaction fueled acid-producing bacteria, and volatile fatty acid concentration and pH value rose rapidly. As ammonifying bacteria acted to lower the pH, the oxidation-reduction potential decreased, and the environment became suitable for the growth of methanogens, resulting in a rapid increase in the methane content (Lehtomaki et al., 2007). These three kinds of bacteria depended on each other and restricted each other, enabling fermentation to occur.

The relationship of acidifying bacteria

Within the mixed microbial community of the conventional reactor, niche complementarity and niche overlap exist between populations. These processes of collaboration and competition enable the formation of different acid products in the reactor (Cohen et al., 1979). The term ‘volatile fatty acids’ refers to a mixture of organic acids produced by the anaerobic fermentation of municipal solid waste, including acetic acid, propionic acid, butyric acid, valeric acid and caproic acid. The composition of VFA directly affects the efficiency of anaerobic fermentation, so analysis of the organic acids is important. Anaerobic acidification is classified according to the composition of the fermentation product, such as butyric acid fermentation, propionic acid fermentation, alcohol fermentation and mixed acid fermentation. Many researchers believe that propionate is unfavorable for methane production, while acetic acid, formic acid and hydrogen can be used directly by methane bacteria (Dinopoulou et al., 1988; Nane et al., 1996). Therefore, the major ingredients of volatile fatty acids were analyzed.

As shown in *Figure 11*, acetic acid and butyric acid concentrations were high at the beginning of fermentation, and increased with time until reaching a stable value. Butyric acid had the highest concentration in the fermented liquid; it reached a peak value of 3.86 g/L in the fourth day, accounting for 60% of the total VFA. Acetic acid content reached its highest value of 2.27 g/L in the 20th day, accounting for 35.7% of the total VFA. In the early stages of anaerobic hydrolysis-acidification, propionic acid content was low, and slowly rose with time. Due to the low hydrogen partial pressure, propionic acid produced in the initial fermentation period was easily converted into acetic acid. As the reaction proceeded, hydrogen partial pressure gradually increased, and the propionic acid was converted into acetic acid with more difficulty, leading to gradual accumulation and increased concentration of propionic acid. Valeric acid and caproic acid concentrations were lower throughout anaerobic fermentation, exhibited slow growth, and did not reach a steady state. Most of the valeric acid and caproic acid production occurred in the late fermentation period.

Among the acids created as bacteria break down organic matter, butyric acid is the most important to the anaerobic fermentation of municipal solid waste. Butyric acid was produced most readily in the early fermentation period, and is preferred over propionic

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acid fermentation. Analysis of the change trends showed that the increase in butyric acid content coincided with a decrease in acetic acid content, demonstrating that the acid-producing bacteria responsible for the decomposition of organic matter and the hydrogen-producing acetogenic bacteria responsible for breaking down fatty acids to produce acetic acid both belong to the advantageous flora of acid-producing bacteria. With the consumption of acetic acid by methanogens and the reduction in hydrogen-producing acetogenic bacteria, butyric acid began to accumulate again. As a result, the reduction of acetic acid content can be used as one indicator of the start of the methane-producing phase.

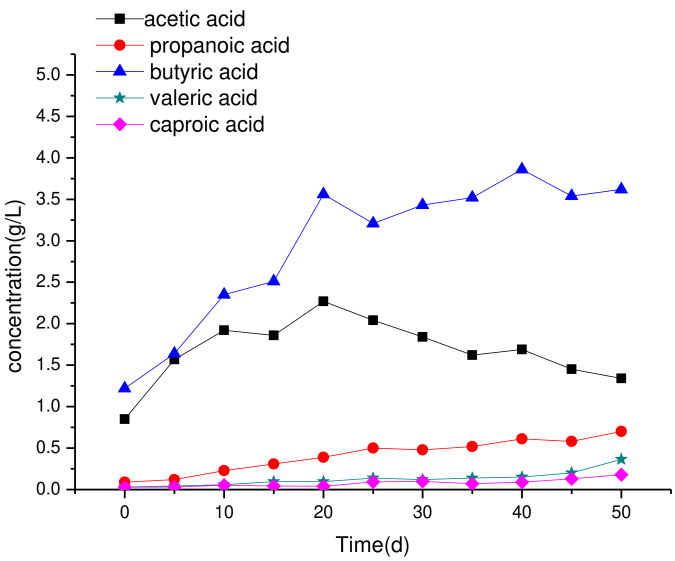

Figure 11. *Trend of VFA composition*

Conclusion

(1) During the initial stage of dry anaerobic fermentation, aerobic and facultative anaerobic bacteria proliferated, showing that this group is primarily responsible for transforming raw material into organic matter. Many precursors of methane come from this taxon, so research on the effect of aerobic and facultative anaerobic microbial flora in the early stage of dry fermentation can provide the theoretical basis for further improving the efficiency of biogas production. As anaerobic fermentation continued, the number of aerobes and facultative anaerobes decreased, while anaerobic bacteria reproduced at an increasing rate and became the dominant bacterium group. Anaerobic acidifying bacteria degraded organic matter to provide the precursors for methane synthesis. Among both aerobic and anaerobic bacteria, acidifiers dominated ammonifiers: acidifying bacteria multiplied at a higher rate, reached the maximum population size earlier, and maintained higher numbers than ammonifying bacteria. The number of anaerobic cellulose-degrading bacteria showed a trend of slow growth, increasing to 10^6/mL by 45 days after feeding. Methanogens essentially showed no sign of proliferation during the

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initial stage, then entered a period of fast growth, reaching a maximum of 3.36×10^9/mL at around 25 days. After the gas peak, ammonifying bacteria and anaerobic acidifying bacteria began to decline in number; the methane bacteria, however, maintained the same order of magnitude.

(2) Due to the relatively static condition of anaerobic dry fermentation inside the reactor, there were differences in the spatial distribution of the microbes. The aerobic and facultative anaerobic acidifying bacteria were more active at the top position, but the changing was not obvious in the same height. The matrix condition was stable at the middle position. The highest numbers of anaerobic ammonifying bacteria were seen at the middle edge and the center positions. Greater liquidity at the bottom position resulted in a higher number of acidifying bacteria to decompose organic acid, enabling the growth of methane bacteria. The anaerobic cellulose-degrading bacteria flourished in the more liquid environment at the bottom. At the top, the number of anaerobic bacteria was lower than in any other place, due to the initial presence of residual air that was not conducive to their growth. Future reactor designs could incorporate an intermediate feed, or supplemental feeding at the edge in order to make full use of the fermentation materials and improve the efficiency of the reactor.

(3) The function between acidifying bacteria, methane bacteria, and ammonifying bacteria was analyzed. During the early period of fermentation substrate liquefaction, acidifying bacteria used liquefied carbohydrates and fat to create large amounts of acid, raising the volatile fatty acid concentration. As the ammonifying bacteria produced more ammonia, the volatile fatty acid concentration decreased, and the oxidation-reduction potential was reduced. This environment, suitable for methanogen growth, allowed the methane content to rise rapidly. The change trend of volatile fatty acids and concentration of ammonia nitrogen was consistent with the spatial distribution of bacteria; where there were more bacteria, there were more products.

(4) The relationship between different types of acidifying bacteria. The most prevalent acid in the VFA composition of biogas slurry, and thus the main product of acidifying bacteria in the anaerobic fermentation of municipal solid waste, was butyric acid. Butyric acid fermentation was conducive to the methanogenic process. Acetic acid, the product of hydrogen-producing acetogenic bacteria, was the substrate directly utilized by methanogens in the next stage; its presence can be used as one of the indicators of the start of the methane-producing phase. In the early stages of anaerobic hydrolysis acidification, propionic acid content was low, and slowly increased with time. Valeric acid and caproic acid content remained low throughout the whole process of anaerobic fermentation; for these two acids, growth remained slow and did not reach a steady state.

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EVALUATION OF HEAVY METAL POLLUTION AND RISK ASSESSMENT OF DIETARY INTAKE OF POLLUTANTS IN CHINESE MITTEN CRAB (*ERIOCHEIR SINENSIS*) IN MAIN AQUACULTURE AREAS OF NORTHEAST CHINA

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Abstract. A systematic survey of heavy metal (Pb, Cd, Cr, As and Hg) concentrations in pond water, sediment and products of Chinese mitten crab (*Eriocheir sinensis*) aquaculture areas was carried out to assess their potential health risks to local people. The samples were all collected in the Liaoning Province, in the northeast of China. According to the results, the studied aquatic environment is not severely affected by heavy metal pollution at this time. As a result, local dietary exposure to these pollutants is still below the JECFA recommended values.

Keywords: *Crustacea, heavy metal, single factor pollution index method(SFPI), Nemero pollution index method (NPI), dietary exposure assessment*

Introduction

With the rapid development of industrialization and urbanization, the discharge of waste gas, waste water and waste residue containing heavy metals has greatly increased (Bhuiyan et al., 2011; Khillare et al., 2012; Rahman et al., 2014). Increasing attention has been paid to the human health risks caused by the release of these contaminants. Thus, it is important to assess the heavy metal pollution situation. The presence of heavy metal pollution in aquatic products has also raised more and more public health concerns in the last decades. In China, studies of the aquatic product heavy metal pollution situation have mainly focused on fish and shrimp (Yang, C.C., et al, 2013; Gu, 2012; Liu, et al., 2013) and shellfish (Tan et al., 2012; Sun et al., 2010; Wang et al., 2012; Conti et al., 2011; He, 1996). At this time, there is no research on toxic heavy metal pollution of Eriocheir sinensis crabs in this region. This study on the heavy metal pollution of crabs such as Eriocheir sinensis is the first such domestic study at present.

The Chinese mitten river crab (*Eriocheir sinensis* H. Milne-Edwards, 1853) is an arthropod crustacean, Order Decapoda of the Superfamily Grapsidae. It is an important aquaculture species in China. The natural breeding habitat of the river crabs is in Bohai bay, Liaoning Province, which is an appropriate habitat for breeding for large scale production. In 2015, crab farming in Bohai bay occupied 80000 hm^2 (2008), making it a major region for crab breeding in northeast China.

Based on its commercial importance, the evaluation of the river crab breeding environment, heavy metal pollution levels and product safety are vital. A better understanding of the crab breeding habitat will help the aquaculture industry to provide early warnings for supervision of food and health aspects. This research will provide

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knowledge for the establishment of quality control standards for the river crab commercial-scale-production, providing scientific guidance for the safety of river crab consumption as well as advice to the river crab breeding industry.

Materials and methods

Sample collection

The present study pilot is located in the main river crab aquaculture areas of Liaoning Province with a longitude range of 121°31'～ 122°28' and a latitude of 40°40'～ 41°27' (see *Figure 1*). Three categories of samples were collected: river water, sediment and adult crabs. These samples were collected from 9 breeding sites from September to October 2015. Each sample point was characterized by two parallel samples.

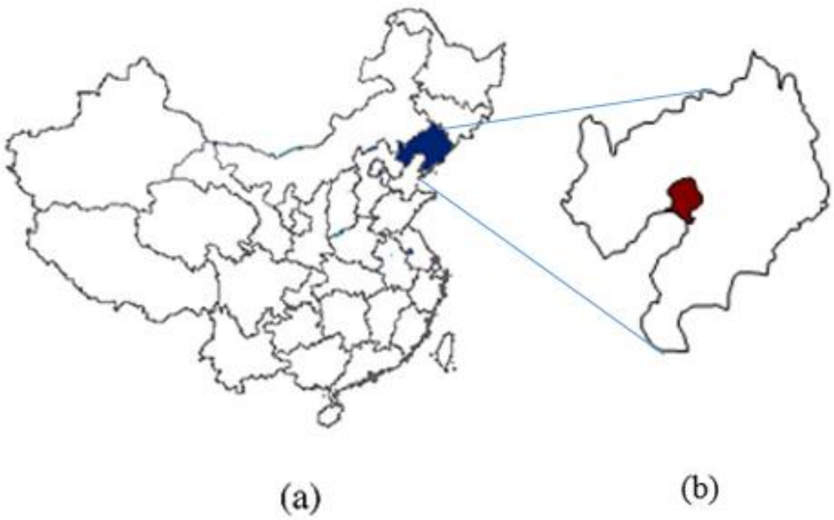

Figure 1. *(a) The mainland of China, and the location of Liaoning Province (blue area); (b)Sampling location of the study area in Liaoning Province (red area)*

Pond water

Clean polyethylene bottles where used to collect and store the water samples. Immediately after the samples were collected, 2 ml/liter nitric acid was added to guarantee the acidification of a XY molarity. The collection, storage, transportation and treatment of the water samples were performed according to the standards set forth in GB 12997-91 and GB 12998-91.

Sediment

Upper layer (0 ~ 10 cm) sediment samples were collected, stored and transported to the lab for analysis according to GB 17378.3-2007 standards. Gravel and plant debris were removed. The sediment samples were sealed and stored in a -18°C freezer. The samples were dried at 60°C, grinded, sieved through an 80 mesh sieve and stored in airtight bags after homogenization.

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Adult crab

The crab samples were washed and cleaned using a plastic knife and tweezers. The edible parts of the muscle tissues and ovaries were homogenized and stored in double layer polyethylene plastic bags in a -18℃ freezer.

Data determination

Concentrations of Pb, Cd, Cr, As and Hg were determined by graphite furnace atomic absorption spectrophotometer atomic fluorescence according to the National Standards NY 5361-2010 and GB 2762-2012. Pb, Cd and Cr concentrations were analyzed by graphite furnace atomic absorption spectrophotometry, while As and Hg concentrations were determined by atomic fluorescence spectrophotometry. The concentration of each element was expressed based on the quality control where the relative standard deviation was less than 10% of the concentration averages of 2 test samples. Statistical data analyses were performed using SPSS 16.0 software, and the standard of significant difference was set to P=0.05.

Criteria of evaluation and methods

Pollution degree

The single factor pollution index (SFPI) and Nemerow pollution index (NPI) were determined by the method set forth by Pang et al. (2015) (Zhu et al., 2014) to evaluate heavy metal pollution status and pollution risk. The SFPI was used to evaluate the pollution status of a single heavy metal element. It was calculated as follows:

$$p_i = \frac{C_i}{S_i} \quad \text{(Eq. 1)}$$

where P_i is the heavy metal element *i* pollution index, C_i is the heavy metal element *i* content in the specimen (pond water, sediment and crab tissues), and S_i is the heavy metal element *i* evaluation standard (*Table 1*). The Pi value indicates the degree of pollution; its size is directly proportional to the pollution degree. However, the NPI takes into account both the average pollution status of a single heavy metal element and the degree of heavy metal pollution. It was calculated as follows:

$$p = \sqrt{\frac{(P_{i\max})^2 + (P_{i\,ave})^2}{2}} \quad \text{(Eq. 2)}$$

where *P* is the comprehensive pollution index of Nemero, $P_{i\ max}$ is the maximum pollutant value of all investigated single factor pollution indices, and $P_{i\ ave}$ is the average pollutant value of all single factor pollution indices.

According to the pollution index calculations, the level of heavy metal pollution is divided into grades to evaluate the overall degree of pollution (*Table 2*).

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Table 1. Evaluation standard of heavy metals (NY 5361-2010; GB 2762-2012)

Sample species	Heavy metals				
	Pb	Cd	Cr	As	Hg
Pond water (mg L^{-1})	0.05	0.005	0.1	0.05	0.0001
Sediment (dry weight) (mg kg^{-1})	60	0.5	80	20	0.2
Crab Product (mg kg^{-1})	0.5	0.5	2.0	0.5[a]	0.5[b]

Note that (a) and (b) represent methyl mercury and inorganic arsenic, respectively; the same holds for Tables 3, 4, 5 and 6.

Table 2. Classification standard of heavy metal pollution index (Zhu et al., 2014)

Level	SFPI	Pollution level	NPI	Pollution level
1	$Pi \leq 1$	clean	$P \leq 0.7$	safety
2	$1 < Pi \leq 2$	slight	$0.7 < P \leq 1$	warning
3	$2 < Pi \leq 3$	moderate	$1 < P \leq 2$	slight
4	$Pi > 3$	heavy	$2 < P \leq 3$	moderate
5			$P > 3$	heavy

Dietary exposure assessment method

In this study, the evaluations complied with the declarations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The provisional tolerable weekly intake (PTWI) and provisional tolerable monthly intake (PTMI) were determined from the crab tissue (mg kg-1) to evaluate the safety of crab dietary intake for tenants of Liaoning province (Wang and Wang, 2014). Thus:

$$PTWI/PTMI = \frac{\bar{x} \cdot L \cdot D}{M} \quad \text{(Eq. 3)}$$

where *PTWI* is the provisional tolerable weekly intake (μg kg^{-1}), and D =7days; *PTMI* is the provisional tolerable monthly intake (μg kg^{-1}), and D=30days; $\bar{x}$ is the concentration (mg kg^{-1}); L is the daily intake of food (g/person/day); and M is the adult body weight (set M=60kg). According to the dietary survey data for the Liaoning province adults, the average per capita fish and shrimp consumption is 44.3 g/day (Zhu et al., 2014).

Result and discussion

Accuracy and precision

According to the standard methods (NY 5361-2010; GB 2762-2012), the spiked recoveries ranged from 108.9% ~ 91.3%, and the relative standard deviation (RSD, n=6) was between 7.4% and 4.2% (*Table 3*).

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Table 3. *Average recoveries and RSD (n=6)*

Sample species	Quality Control	Heavy metals				
		Pb	Cd	Cr	As	Hg
Pond water	Addition levels (mg L^{-1})	0.05	0.005	0.1	0.05	0.0001
	Recovery (%)	91.3	96.4	108.9	95.1	94.1
	RSD (%)	4.3	4.2	5.1	6.4	6.3
Sediment	Addition levels (dry weight) (mg kg^{-1})	2.0	0.5	5.0	3.0	0.2
	Recovery (%)	92.3	103.1	104.3	93.5	92.1
	RSD (%)	4.2	4.7	4.9	6.6	7.4
Crab Product	Addition levels (mg/ kg^{-1})	0.5	0.5	2.0	0.5^{a}	0.5^{c}
	Recovery (%)	96.3	98.2	96.1	94.2	93.1
	RSD (%)	4.3	4.2	4.7	6.4	5.3

Note: (c) represents total mercury; the same is true for Tables 4, 5 and 6.

Assessment of heavy metal pollution

Tables 4 and *5* show the average concentrations and pollution evaluations, respectively, of heavy metals in river water, sediment and crab products. According to these results, all of the samples were 100% suitable for human consumption (*Table 4*). There was significant variation of the heavy metal concentrations in all samples, even within the same kind. The highest heavy metal concentrations were for Cr, followed by Cd, Pb and As. The lowest concentrations for Hg were in the river water and sediment samples. In crabs, the concentration of As was the highest, followed by Cr, Pb, Cd and Hg. These findings are consistent with the general law of heavy metal content in fish and shellfish (Gu and Zhao, 2012). It can be deduced that bioaccumulation of As and Hg in crabs was relatively high, although their concentrations were not overly high in pond water and sediment.

Table 4. *Concentrations of heavy metal (* $\bar{x} \pm s$ *)*

Sample species	Sample size	Heavy metals				
		Pb	Cd	Cr	As	Hg
Pond water (mg L^{-1})	18	0.020±0.013	0.0030±0.0019	0.043±0.004	0.0115±0.0072	0.000021±0.000004
Sediment (dry weight) (mg kg^{-1})	18	22.2±2.1	0.21±0.06	27.2±3.1	6.2±0.5	0.044±0.021
Crab Product (mg kg^{-1})	36	0.06±0.01	0.05±0.01	0.12±0.03	$0.22±0.07^{a}$	$0.05±0.01^{c}$

Note that (*s*) represents standard deviation.

The values of the single factor pollution index (*Pi*) ranged between 0.06 ~ 0.60 in pond water, sediment and crabs (*Table 5*). The *Pi* values for the river water and sediment samples were the highest for Cd (0.60 and 0.42 respectively), while the *Pi* values for As and Hg in the river crabs were both 0.44. The values of the Nemero pollution index *P* ranged from 0.50 to 0.35 in pond water, sediment and crabs. These values were significantly lower (0.7) than the classification standard of the heavy metal pollution index (*Table 2*), which suggests that the crab breeding environment of the

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river is not subject to toxic heavy metal pollution. Therefore, this river is an excellent breeding area for river crabs.

Table 5. *Evaluation of pollution index of heavy metals*

Sample species	*Pi*					*P*	Pollution level
	Pb	Cd	Cr	As	Hg		
Pond water	0.40	0.60	0.43	0.23	0.21	0.50(n=5)	safety
Sediment	0.37	0.42	0.34	0.31	0.22	0.38(n=5)	safety
Crab Product	0.12	0.10	0.06	0.44a	0.44c	0.35(n=5)	safety

Edible safety evaluation

Compared with the PTWI/PTMI recommended by JECFA, the dietary intake of toxic heavy metal contaminated crabs was evaluated (*Table 6*). The *PTWI/PTMI* intakes of heavy metals in river crab were significantly below 10% of the values recommended by JECFA. The *PTWI/PTMI* intakes values recommended by JECFA were the highest for Cr, followed by As and Hg, and the lowest were for Pb and Cd.

Table 6. *Estimated weekly intakes of heavy metal (Li et al., 2010)*

Heavy metals	Concentrations (mg kg^{-1})	*PTWI/PTMI* (μg kg^{-1})	JECFA recommended values(μg kg^{-1})	Proportion in JECFA recommended value(%)
Pb	0.06	0.31	25	1.2
Cd	0.05	1.11	25*	4.4 *
Cr	0.12	0.62	6.7	9.3
As^a	0.22	1.14	15	7.6
Hg^c	0.05	0.26	5	5.2

Note: The * represents a tentative monthly tolerable intake of cadmium (PTMI) (WHO,2012)

Not all heavy metals are toxic. Some are essential for physiological purposes, but can have adverse health effects at higher concentrations. Other heavy metals are toxic even at relatively low concentrations. Most heavy metal pollution is due to anthropogenic and industrial activities (Al-Musharafi, 2016). Toxic heavy metals interact with protein and DNA, causing mutations and affecting physiological activities and metabolic processes (Al-Musharafi, 2016; Yao et al., 2014). Also, some heavy metal valences create different effects on metabolic activity. For example, Cr is an essential trace element in the human body, but is also a highly toxic metal element. The toxicity of Cr^{6+} is much higher than that of Cr^{3+}. Cr can cause skin reactions and even skin disease. It can also stimulate and/or corrode the respiratory tract, lead to cancer, or induce gene mutation (Teng et al., 2010). Lung cancer caused by chromium compounds is recognized worldwide as a chromium cancer (WHO, 1997). The most toxic metals include As, Hg, Pb and Cd, which are non-essential elements for the human body (Al-Musharafi, 2016). As and Hg take a variety of different forms. Inorganic arsenic is divided into pentavalent arsenic and trivalent arsenic, which are both more toxic than organic arsenic (Zhao et al., 2009).

Some organic forms of heavy metals are less toxic than the related inorganic forms. For example, the toxicity of methyl mercury is much higher than that of inorganic mercury (Jia et al., 2015). In the present study, the content of total mercury was

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significantly lower than the limit value of methyl mercury; therefore, it may not have a significant effect on humans.

In this study, bioaccumulation of toxic metals in crab shells was similar to that in tissue. However, accumulation in the viscera was significantly higher than that in the muscle tissue. Normally, only muscle tissue is consumed, which therefore reduces the risk of heavy metal toxicity from crabs. At present, the proportion of people's consumption of crab is very small (Li et al., 2010). Strict attention should be paid to the reduction of toxic heavy metal pollution. Public awareness is crucial to local residents to reduce crab dietary intake to avoid potential health risks, such as cancer risk.

Conclusion

All of the heavy metal values in the aquaculture water, sediment and river crab products were within the Chinese national standards. The results of this study indicate that the target aquatic environment is currently safe with respect to pollution by the investigated heavy metals (Pb, Cd, Cr, As and Hg). Therefore, the present-day dietary exposure to these pollutants is still well below the JECFA recommended values. However, people should work to reduce the pollution of industrial and domestic waste, which will protect human health and survival and ensure food safety.

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APPLICATION OF SBR TO TREAT DIFFERENT TYPES OF WASTEWATERS

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Abstract. The objective of this study was to investigate the pollutant removal efficiency of different types of wastewaters by a sequencing batch reactor (SBR). A laboratory-scale SBR was used to treat sewage, medical wastewater and industrial wastewater. The effects of different conditions of aeration, stirring and settling on the efficiency of pollutants removal were explored to optimize the performance, especially for the removal of organic matter (COD), ammonia nitrogen(NH_4^+-N), total nitrogen(TN) and total phosphorus(TP) of different types of polluted water. From the results it was showed that when aeration 6 hours, stirring 4 hours and settling 2 hours in SBR, the removal rates of COD, nitrogen and phosphorus of domestic sewage were 93%, 98% and 83%, respectively; when aeration 5 hours, stirring 5 hours and settling 1 hour in the SBR, the removal rates of COD and nitrogen of pharmaceutical wastewater were 83% and 90%, respectively; when aeration 4 hours, stirring 4 hours and settling 1 hour in the SBR, removal rates of COD, nitrogen and phosphorus of industrial wastewater were 97%, 93% and 97%, respectively.
Keywords: *sequencing batch reactor,activated sludge,biological treatment,COD*

Introduction

Biological treatment for wastewater mainly includes aerobic, anaerobic and anoxic processes. As a high efficiency and inexpensive method to treat wastewaters, SBR technology has been widely used through the years (Mace et al., 2002). It has been also successfully emplored to treat pollutants of various types of wastewaters (Lamine et al., 2007; Giordano et al., 2005; Obaja et al., 2003; Jin et al., 2015). SBR method has some unique characteristics that is distinguished from others activated-sludge process. For example, the processes of nitrogen removal and sludge sedimentation can be carried out in the same SBR reactor, while those need be separated in other biological treatment processes (Ng, 1987). The SBR was not only used efficiently for organic pollutants removal of municipal wastewater, but also successfully applied in the nitrogen removal of wastewater (Silverstein and Schroeder, 1983). Microbial reactions of nitrogen removal are performed within the same SBR tank (Ketchum et al., 1997; Gali et al., 2008). It can also be applied for pollutants removal of wastewater containing a high concentration of nitrogen and organic matters (Rodríguez et al., 2011). Both anaerobic and aerobic conditions can be used for there moval of biological phosphorus in cyclic processes in a single reactor (Sarioglu, 2005). The property of combination of organic matters, nutrients (nitrogen (N) and phosphorus(P)) removal in a SBR tank makes it economical prominently (Carucci et al., 1995).

The SBR biological processes are capable of achieving complete degradation of the biodegradable compounds contained in the olive mill wastewaters and therefore to significantly reduce the pollutant load. It showed high performances, achieving removal of the biodegradable organic content at all the investigated influent loadings, with average efficiencies constantly at about 60% and 90% for total polyphenols (TPPs) and COD, respectively (Chiavola et al., 2014).

A sequencing batch biofilm reactor (SBBR) was also used for removal of N and P from swinewastewater, resulting in excellent purification effect. The percentage removal of COD, NH_4^+-N, TN, and TP was 98.2%, 95.7%, 95.6%, and 96.2% (Hai et al., 2015). Combining the two treatment processes, microbial treatment and adsorption, in a SBR was applied for a simulated textile-dyeing wastewater. The results showed that removal efficiency reached to 53–79%, while color removal was rather low (10–18%)(Santos et al., 2015).

The research also showed that SBR was very effective for N removal in highly saline wastewaters by partial nitrification and denitrification (She et al., 2016). The performance of SBR was done in this work in the treatment of sewage, medical wastewater and industrial wastewater. Results showed that COD, nitrogen and phosphorus were effectively removed from the three types of wastewater under optimal conditions. This suggested that SBR method could be also applied in the treatment of medical and industrial wastewater.

Materials and methods

Activated-sludge system

Sludge cultivation

Activated sludge in this study was collected from the wastewater treatment plant (*Fig.1*).

Figure 1. *Sludge samples from the wastewater treatment plant*

The dry sludge from the wastewater treatment plant was in static culture. Nutrient solution was added to the activated sludge (C: N: P ratio of 100:5:1) and cultured with aeration, so that the growth of microorganisms in the activated sludge was promoted. The nutrient solution used for the cultivation of the sludge was composed of 2.814g glucose, 0.48g potassium dihydrogen phosphate, and 0.36g ammonium chloride, dissolving in 1 L of water. Maturity of the activated sludge was measured by chemical analysis.The monitoring indicators included settling velocity (SV), sludge volume index (SVI), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS). The microbial phase was determined by microscopic examination.

Sludge acclimation

After incubation, the sludge was taken into the SBR device (Subsequent description). Low-concentration wastewater (diluted sewage) was used for sludge cultivation. After 23 hours of aeration and 1 hour of sedimentation, the supernatantwas discarded, and the sludge was added with the same concentration of fresh sewage to continue aeration culture. The growth of

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activated sludge was observed under a microscope. The effluent increased gradually until the concentration reached that of the raw sewage. At the point that the activated sludge was stable, its maturity was determined by microscopy and chemical analysis.

Experimental design

Experimental model

The SBR was fabricated from plexiglass with a working volume of 128L, and the length×width×height was 80cm×40cm×50cm (*Fig. 2*). The reactant was mixed with a magnetic stirrer and aerated sufficiently with a compressor. Aeration, stirring and settling can be carried out in the same reactor.Operation time of reaction and precipitation was manually controlled.

Figure 2. *SBR reactor*

Wastewater sampling

Sewage and medical wastewater were taken from student dormitory and medical clinic of the university, respectively. High organic industrial wastewater was self-configured from three kinds of solution as follows: Solution A: 93.8 g/L glucose solution (equivalent to COD 100 g/L). Solution B: K_2HPO_4 320 g/L, KH_2PO_4 160 g/L, NH_4Cl 120 g/L. Solution C: $MgSO_4 \cdot 7H_2O$ 15.0 g/L, $FeSO_4 \cdot 7H_2O$ 0.5 g/L, $ZnSO_4 \cdot 7H_2O$ 0.5g/L, $CaCl_2$ 2.0 g/L, $MnSO_4 \cdot 3H_2O$ 0.5 g/L.10 mL of each solution was taken and mixed, diluted to 1000mL to form the industrial wastewater. Supernatants of the water samples from the SBR model at the beginning and the end of each cycle were taken and analyzed (*Table 1*). The suspended solids (SS) were so few that could be negligible, so the SS content changes were not considered in the experiment.

The wastewater samples were analyzed for COD, TP, TN, ammonia, MLSS, MLVSS, SV and SVI according to the Standard Methods (NEPA, 2002). All experiments were performed in triplicate.

Table 1. *Initial concentrations of various wastewaters*

	TP(mg/L)	NH_3-N(mg/L)	COD(mg/L)	TN(mg/L)	pH
Sewage	30.4	128	234	130	-
Medical wastewater	-	45.1	151	-	8.18
Industrial wastewater	14.4	36	1057	43	-

Experimental conditions

According to SBR biochemical mechanism, experimental conditions were designed as the following three stages: Aerobic aeration-Anoxic mixing-Settling.

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During the aerobic stage, large amounts of organic matter were assimilated by microorganisms for their life activities. The major consumed pollutant was COD. Phosphorus in sewage was also absorbed by phosphorus accumulating organisms (PAO). At anoxic mixing stage, residual free oxygen was consumed.

When organic materials and oxygen were completely consumed, the system entered into the anaerobic stage, and the release of carbon and denitrification by facultative denitrifying bacteria occurred. Phosphorus could be transformed into energy by PAOs for their biosynthesis or into phosphate by acid-producing bacteria to remove phosphorus in the anaerobic state.

Therefore, COD removal occurred in the aerobic stage, while phosphorus removal occurred in anaerobic phase. Different cycle conditions were selected to achieve the best treatment effect. The operating conditions of SBR for treating various wastewaters were shown in *Table 2*.

Table 2. *Design of operating conditions for various wastewaters.*

	Sewage			Medical wastewater			Industrial wastewater		
Cycle	Aeration hs	Anoxia hs	settling hs	Aeration hs	Anoxia hs	settling hs	Aeration hs	Anoxia hs	settling hs
1	4	4	1	3	2	1	4	4	1
2	5	5	4	2	3	1	5	5	2
3	4.5	4.5	2	4	4	1	4.5	4.5	1.5
4	3	3	1.5	5	5	1	3	3	2
5	6	3	5	6	6	1	3.5	3.5	1.5
6	7	5.5	2	-	-	-	-	-	-

Results and Discussion

Characterization of the activated sludge

This experiment was to determine the characteristics of the activated sludge throughout adaptation period, logarithmic growth, steady growth and decline phase. The parameters of the activated sludge were shown in *Table 3*. Early stage means the time after the start-up of sludge incubation in the SBR, while middle stage means the time after aeration for 23 hours and then settling for 1 hour.The MLSS in the early and middle stages of sludge incubation in SBR ranged from 5.82 mg/L to 12.89 mg/L and 7.98 mg/L to 12.98 mg/L, respectively. Corresponding SV ranged from 40% to 82% and 31% to 82%, respectively. The SVI ranged from 62 mL/g to 95 mL/g and 31 mL/g to 75 mL/g, respectively. The MLVSS in the early and middle stages of sludge incubation in SBR ranged from 4.77 mg/L to 7.84 mg/L and 7.98 mg/L to 12.98 mg/L, respectively. It was noteworthy that the MLVSS was higher in the middle stage than that in the early stage.

Table 3. *The parameters of the activated sludge.*

In the early stage of sludge incubation				In the middle stage of sludge incubation			
SV/%	MLSS /(mg/L)	MLVSS /(mg/L)	SVI /(mL/g)	SV/%	MLSS /(mg/L)	MLVSS /(mg/L)	SVI /(mL/g)
55	5.82	5.44	95	50	7.98	7.14	75
40	6.41	5.77	62	31	10.09	10.02	31
82	12.89	7.844	64	65	10.17	8.44	64
-	-	-	-	50	11.91	10.97	42
-	-	-	-	82	12.89	7.84	64

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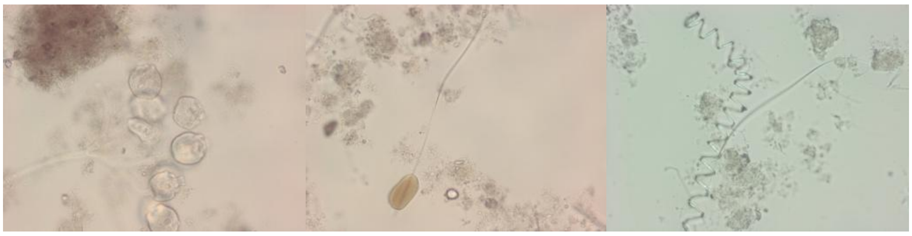
In the early stage of sludge incubation, DO concentration was about 2 mg/L. Organic matter was consumed rapidly and the amount of activated sludge increased. In the middle stage, the sludge concentrations significantly increased, and the demands for nutrients and DO also increased. The biological phase in the middle stage of sludge incubation was shown in *Fig. 3*. Activated sludge grew quickly, and the sludge cultivation was in good condition. Sludge bulking did not appear.



a. Euplot *b. Vorticella* *c. Spirillum*

Figure 3. *Biological phase in the middle stage of sludge incubation*

After 30 days of static culture stage, red nemato deappeared in the activated sludge (*Fig. 4*), which indicated that the activated sludge had been aging. The activated sludge bulking occurred obviously, and its characteristics greatly reduced. The treatment efficiency of pollutants was not high, so the activated sludge at the middle stage was selected to be used in the experiment.

Figure 4. *Red nematode in the final stage of sludge incubation.*

Pollutant removal efficiency of various wastewaters

Pollutant removal efficiency of sewage was shown in *Fig. 5*. When aeration time was 6 hours, the COD removal efficiency was the highest, 94%. After 6 hours, the COD treatment effect began to decline. Large fluctuations were observed in the nitrogen removal efficiency. The nitrogen removal rate was better under conditions1, 2 and 6 than that under others. Its treatment effect was high when stirring time was 4 hours and 5.5 hours. A 6 hour aeration period was estimated that it was sufficient for microorganisms in the endogenous respiration period, in order to minimize the amount of excess sludge. For the saving of economyand resource, 4 hours was selected as the best condition.

A pilot-scale sequencing batch reactor was also carried on with two phases, anaerobic and aerobic, to removal nitrogen and phosphorus simultaneously. Average removal of COD, TN and TP of sequencing batch reactor reached 95%, 94%, and 97%, respectively (Yin et al., 2015).

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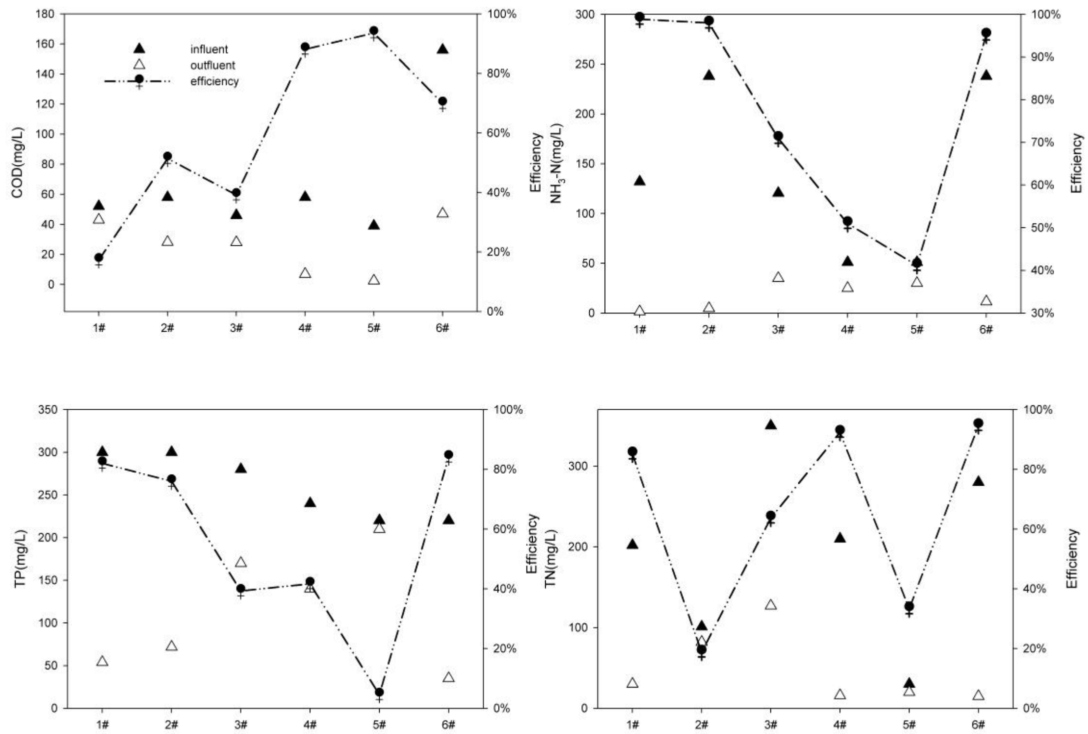

Figure 5. *Pollutant removal efficiency of sewage.*

The experimental results showed that phosphorus removal rate under the condition of settling for 5 hours was better than that under the other conditions. Considering the actual application, the best settling time was 2 hours.

The pollutant removal efficiency of medical wastewater was shown in *Fig. 6*. Ammonia and COD removal efficiencies of medical wastewater were high. The influent concentrations were different, but the ammonia removal efficiency did not have much difference among the five conditions. When aeration was 4 hours, anoxia was 4 hours and settling was 1 hour, the ammonia removal efficiency was 59.1%, which was lower than that under other conditions. Meanwhile, when aeration was 5 hours, anoxia was 5 hours and settling was 1 hour, the ammonia removal efficiency was 91.5%, which was the highest in all conditions. After aeration of 3 hours, the ammonia removal efficiency reached 69.7%. However, with continuous aeration, there was little difference in the purification effect. The COD removal efficiency was 85.8% under condition 4, which was the highest in all conditions. After aeration of 3 hours, the removal rate was 44.3%, which was the lowest in all conditions. The degradation of organic matter was not treated at this moment. SBR was also performed to remove organic matters and nitrogen compounds from medical wastewater. The experimental results elaborated that the removal efficiency of COD, NH_4^+-N and NO_3^--N were in the range of 88.7-89.3%, 77.2-96%, 35.7-92.5%, respectively (Hasan et al., 2016).

The pollutant removal efficiency of the industrial wastewater was shown in *Fig.7*. The removal efficiency of organic matter, ammonia, total nitrogen was 96.5％, 94％ and 91％, respectively after aeration for 4.5 hours, which was the highest in all conditions. The removal rate of total phosphorus was 90-95% in these working conditions. Notably, when SBR biological treatment process was used for pollutants removal of wastewater, large amounts of excess sludge were produced. This part of excess sludge was discharge into sludge treatment system by sludge pipe.

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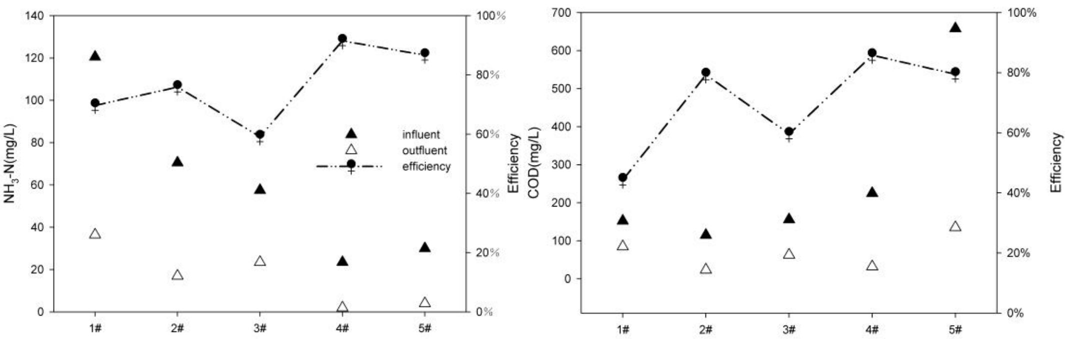

***Figure 6.** Pollutant removal efficiency of medical wastewater.*

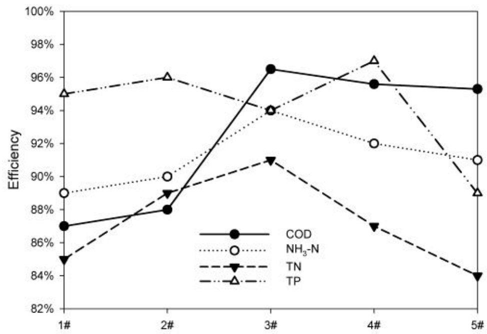

***Figure 7.** Pollutant removal efficiency of industrial wastewater.*

Conclusions

Activated sludge was cultivated and acclimatized before entering into the SBR. Three kinds of wastewater were selected to compare the pollutant removal efficiencies under different conditions.

The research data showed that, when aeration 7 hours, stirring 5.5 hours, and settling 2 hours in the SBR, purification rates of COD, nitrogen and phosphorus of domestic wastewater were 93%, 98% and 83%, respectively. When aeration 5 hours, stirring 5 hours, and settling 1 hour in the SBR, the purification rates of COD and nitrogen of pharmaceutical wastewater were 86% and 91%, respectively. When aeration 4 hours, stirring 4 hours, and settling 1 hour in the SBR, the purification rates of COD, nitrogen and phosphorus of simulated industrial wastewater were 97%, 93% and 97%, respectively. Therefore, the purification efficiencies of organic matters, nitrogen compounds and phosphorus by SBR bioreactor were all more than 80%.

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ANALYSIS OF NITROGEN REMOVAL PERFORMANCE OF CONSTRUCTED RAPID INFILTRATION SYSTEM (CRIS)

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Abstract. The Constructed Rapid Infiltration System (CRIS) is widely applied for wastewater treatment. In China, however, fundamental research on functionality of CRIS is lacking. We used a CRIS simulation column to treat domestic sewage under experimental conditions, allowing determination of nitrogen pollutants removal performance. The obtained results showed that CRIS can effectively remove ammonia nitrogen with the average removal rate of 82.2% but results in a relatively low removal of total nitrogen (TN) with the average removal rate of 31.9%. The reason is that the system is frequently subjected to alternation of drying and wetting, which allows effective aeration. This leads to aerobic conditions, which is incompatible with the anaerobic requirements of denitrifying bacteria. In addition, these bacteria likely lack a suitable carbon source, as organic matter is effectively removed in the upper (aerobic) layer of CRIS. Lastly, the filter material used is generally negatively charged, thus repels nitrate, which prevents its retention and results in its discharge with the outflow. As a net result, the TN removal rate is low.
Keywords: *ammonia nitrogen; TN; nitrate nitrogen; organic matter; removal performance and mechanism*

Introduction

The Constructed Rapid Infiltration System (CRIS) was developed for ecologically-friendly treatment of sewage and contaminated surface water, based on a rapid and simple infiltration system, by professor Zhong of China University of Geosciences (Xu et al., 2015; Xu et al., 2013). Since its development, it has been widely applied to wastewater treatment in domestic settings. The system is composed of a grille, a preliminary sedimentation basin, a high-permeability basin containing filter material and an outflow system. The high-permeability basin contains a fixed amount of artificial filter material, allowing to operate under alternating dry-wet conditions with a capacity of 1.0-1.5 m^3/m^2d or m/d; the system is typically subjected to frequent flooding with intermittent dry periods (Xie et al., 2010). Wastewater is purified through adsorption, interception and decomposition of aquatic pollutants by microorganisms that are naturally present in the filter material of CRIS. The unique structure and dry-wet alternate inflow mode of CRIS result in a diverse microbial growth on the surface of the percolation medium, so that the medium usually supports both aerobic, anaerobic, and facultative anaerobic bacteria, all of which contribute to effective treatment of wastewater (Xu et al., 2011b; Liu, 2006). CRIS is preferably applied to treat domestic sewage from small towns as well as contaminated surface water. Removal of COD is typically about 85-90%, ammonia nitrogen can be removed to over 90%, and SS and LAS removal is achieved above 95% (Jiang et al., 2011; Ma et al., 2008). With its convenient operation management and low investment and maintenance costs, CRIS is

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successfully promoted in many districts of China, with obvious benefits for the community, local economy and wildlife (Xu et al., 2011a; Liu, 2006). Nevertheless, at present most results on effectiveness are empiric, while basic and theoretical research is lacking. The research presented here was conducted to determine the efficiency and robustness of nitrogen removal by means of experiments performed using CRIS simulation columns processing domestic sewage. A theoretical interpretation of the biological processes at play is provided.

Experimental materials and method

Experimental device

A CRIS simulation column was constructed in the lab for research purposes. The main body of the reactor was composed of a hard PVC pipe of 200 cm high and an internal diameter of 20 cm, the filter material consisted of 85% natural sand, 5% ferrous powder, 5% marble sand and 5% zeolite sand, and the height of the filter layer was 150 cm. There was a sampling port every 0.25 m from top to bottom of the filter layer. Water was allowed to flow from top to bottom. A schematic of the experimental installation is shown in *Fig.1*.

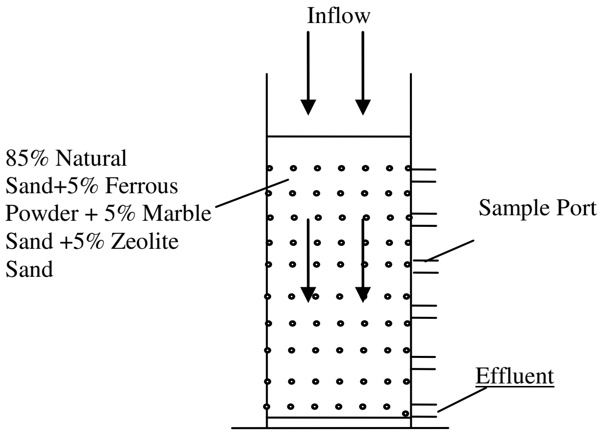

Figure 1. *Experimental installation*

Monitoring Variables

In order to resemble natural conditions, domestic wastewater was used as the experimental water sample, with quality indicators as shown in *Table 1*.

Table 1. *Raw water quality indicators*

WaterTemperature (^{0}C)	pH	COD (mg/L)	SS (mg/L)	TN (mg/L)	TP (mg/L)	NH_4^+-N (mg/L)
17-25	7.00-8.10	130-160	30-60	30-48	0.90-1.80	20-38

CRI was started up with raw wastewater. When the COD removal rate became stable, indicative of a successful and stable biofilm being formed, the CRIS analog column was fed with a hydraulic load of 1.00 m^3/m^2 per day. CRIS analog column was fed wastewater once every six hours and each feed lasted 20 minutes. To study nitrogen

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removal performance, the residual concentrations of nitrogen pollutants at various steps of the process were measured from water tapped from the sampling points and from the effluent once every two days. The experiment was performed from April 8, 2015 to June 30, 2015. The analytical methods used in the study are described in Methods of Water and Wastewater Monitoring and Analysis (edition 4) (Wang, 2002). The used tests determined COD_{Cr} levels by the Potassium dichromate method, NH_3-N by Nessler reagent colorimetry, NO_3-N by ultraviolet (UV) spectroscopy and total nitrogen (TN) by alkaline potassium sulfate digestion followed by UV spectrophotometry. Since nitrogen in the form of nitrite was relatively low in the inflow water and was previously shown to be difficult to accumulate by CRIS (Liu, 2006), this variable was not determined.

Results

Nitrogen Removal Principal of CRIS

The CRIS took 14 cycles before outflow was stable. Once stability was achieved, the concentrations of nitrogen pollutants in the inflow and effluent of the experimental CRIS were determined as shown in *Table 2*. The average removal of nitrogen present in the form of ammonia was 82.2%, while TN was removed with 31.9% efficiency on average. In contrast, concentrations of nitrogen in the form of nitrate increased from 2.4mg/L in the inflow to 12.5mg/L in the effluent (*Table 2*). These results were obtained with inflow water in which the ammonia concentration varied considerably, between 20.9mg/L and 35.8mg/L. This variation was approximately halved in the effluent, suggesting that the system is highly resistant to variation in ammonia nitrogen input. The amplitude of variation in TN removal was relatively large. As a result, the concentrations of nitrate nitrogen and TN in the effluent varied considerably (*Table 2*).

Table 2. *Inflow and effluent measurements*

No.	Ammonia Nitrogen			Total Nitrogen			Nitrate Nitrogen	
	inflow (mg/L)	effluent (mg/L)	removal (%)	inflow (mg/L)	effluent (mg/L)	removal (%)	inflow (mg/L)	effluent (mg/L)
14	31.2± 1.1	5.5± 0.2	82.5	43.6± 1.9	29.9± 1.1	31.4	2.3± 0.09	10.4± 0.3
15	27.8± 1.2	4.9± 0.2	82.4	40.5± 2.0	27.5± 1.2	32.2	1.9± 0.06	12.1± 0.5
16	32.5± 1.6	6.2± 0.1	80.9	42.9± 1.8	29.7± 1.3	30.9	2.4± 0.11	11.3± 0.3
17	31.8± 1.5	4.1± 0.2	87.1	40.5± 1.9	29.9± 1.4	33.7	2.3± 0.11	12.6± 0.4
18	33.6± 1.6	6.3± 0.3	81.3	43.8± 2.1	30.9± 1.4	29.4	2.4± 0.11	10.5± 0.2
19	28.1± 1.3	5.5± 0.2	80.6	38.6± 1.6	28.7± 1.1	33.5	1.8± 0.08	13.4± 0.3
20	23.9± 1.0	4.5± 0.1	81.1	34.2± 1.2	25.9± 1.2	30.2	2.3± 0.03	12.3± 0.4
21	21.7± 0.8	4.2± 0.2	80.5	30.9± 1.5	23.3± 1.0	27.8	2.6± 0.12	12.5± 0.1
22	34.8±	6.2±	82.3	42.5±	27.0±	36.4	1.6±	13.5±

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	1.2	0.1		2.1	1.2		0.05	0.1
23	27.6±1.2	4.6±0.2	83.2	39.4±1.8	27.1±1.3	31.2	2.9±0.13	12.4±0.5
24	20.9±0.6	3.8±0.1	81.7	32.6±1.4	23.3±0.9	28.5	2.5±0.10	11.6±0.1
25	26.9±1.1	4.7±0.2	82.4	37.8±1.6	26.3±1.2	30.5	3.1±0.15	14.4±0.2
26	27.7±1.3	5.2±0.2	81.1	37.6±1.5	27.7±1.3	34.4	2.4±0.10	13.7±0.2
27	35.8±1.5	6.7±0.3	81.2	45.5±2.1	30.6±1.4	34.9	2.6±0.11	13.3±0.4
28	33.1±1.6	5.8±0.2	82.4	41.5±2.0	28.6±0.8	31.1	1.7±0.04	12.8±0.1
29	26.9±0.9	4.8±0.1	82.1	37.8±1.8	26.2±1.1	30.6	2.5±0.10	12.2±0.1
30	29.8±1.3	5.9±0.2	80.1	41.5±1.9	28.4±1.1	31.7	3.3±0.15	13.8±0.3
31	31.7±1.5	5.6±0.2	82.3	43.8±1.8	28.4±1.2	35.2	2.8±0.13	11.9±0.1
32	32.1±1.2	4.6±0.1	85.7	44.3±2.1	29.5±1.3	33.4	2.3±0.09	13.0±0.5
Mean value	29.4	5.24	82.2	40.0	27.8	31.9	2.4	12.5

Liu (2006) concluded that the top 100cm of CRIS typically represent an aerobic zone, while the 30 cm below this would be an anoxic zone. As can be seen from *Table 3* and *Figure 2*, presenting findings from the individual sample points, the removal of ammonia nitrogen in the top section of CRIS (0-25 cm) was 9%, increasing to 78% accumulatively when the water had penetrated 100cm, after which the removal of ammonia nitrogen levelled off to 84%. The highest reduction increments were seen between 25cm, 50cm and 75cm. thus, most ammonia nitrogen is removed in the filtering between 25-75cm, not in the top 0-25cm. Possibly, the concentration of organic matter in the inflow promoted growth of heterotrophic bacteria, and this would inhibit the growth of nitrobacteria in the top layer of the filter (Hu et al., 2010). Related studies have shown that within a reactor there is competition for space to form biofilms as well as competition for dissolved oxygen among microorganisms, especially for the aerobic population (Hu et al., 2010). Nitrobacteria are chemo-autotrophs that, due to their lower growth rates, are generally outcompeted by heterotrophic bacteria. Moreover, dissolved oxygen will be abundant in the top layer of the column, where it is utilized by the surface biofilm consisting of heterotrophic bacteria residing there (Liu, 2006). By the time dissolved oxygen is diffused to where it can be utilized by nitrobacteria, its concentration would have decreased considerably, further limitating the reproduction of nitrobacteria (Xu et al., 2011a). Finally, in the early stage of inflow, most organic nitrogen was decomposed to ammonia nitrogen, which increased the concentration of ammonia nitrogen, further contributing to the low ammonia nitrogen removal in the top section of 0-25cm of CRIS (Xu et al., 2011b).

Table 4 and *Figure 2* lists the total nitrogen concentrations at individual sample points. As can be seen, TN removal in filtering layer 0-75cm was 13.4% on average, decreasing to 11.4%(24.8%-13.4%) in the section 75-100cm and to 6.5%(31.3%-24.8%) for the last 100-150cm. Nitrate nitrogen increased by 1.95mg/L in the section 0-25cm,

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and by 3.99mg/L in section 25-50cm, where it increased at the highest rate (*Table 5* and *Figure 2*). After this, the increment dropped to 3.11mg/L in layer 50-100cm, followed by marginal removal of 0.41mg/L in layer 100-150cm. Since ammonia nitrogen was the main form of nitrogen in the inflow, the nitrate nitrogen concentration is comparatively low, so the ammonia nitrogen was nearly completely nitrificated in layer 0-100cm, which increased the nitrate nitrogen concentration in this section (Ma et al., 2009). Removal of ammonia nitrogen was high in the top 100cm and low in the section 100-150cm, while nitrification occurring earlier had consumed most of the dissolved oxygen, resulting in inhibition of nitrification in layer 100-150cm. Instead, denitrification was promoted here by anaerobic bacteria. Taking together, nitrate nitrogen increased steadily in layer 100-150cm (Xu et al., 2011c).

Table 3. *Ammonia nitrate concentrations at individual sample points*

No.	Depth of CRIS Filtration Pool(cm)						
	0	25	50	75	100	125	150
43	30.5±1.2	27.4±1.1	19.3±0.9	9.6±0.4	6.1±0.3	5.0±0.2	4.3±0.2
44	31.0±1.5	27.9±1.2	19.7±0.7	10.2±0.5	6.3±0.3	5.5±0.1	4.9±0.2
45	31.7±1.4	28.2±1.1	20.0±0.7	10.7±0.5	6.7±0.2	5.8±0.2	5.2±0.1
46	29.3±0.9	27.4±1.2	18.4±0.6	9.2±0.3	6.8±0.1	5.4±0.1	4.6±0.2
47	32.6±1.2	29.3±0.8	19.8±0.5	11.3±0.4	7.3±0.3	5.8±0.2	5.2±0.1
Mean Concentration	31.0	28.0	19.4	10.2	6.6	5.5	4.8
Mean Removal(%)	0	9.6	37.3	67.1	78.6	82.3	84.4

Table 4. *Total nitrogen concentrations at individual sample points*

No.	Depth of CRIS Filtration Pool(cm)						
	0	25	50	75	100	125	150
43	43.5±2.1	41.9±1.1	39.7±1.9	37.6±1.8	32.3±1.6	30.5±1.5	29.3±1.4
44	42.0±2.0	40.8±0.7	38.8±1.8	36.8±1.8	32.5±1.6	30.2±1.2	29.6±1.4
45	40.1±1.1	38.5±1.9	37.5±1.8	35.7±1.6	31.0±1.5	29.8±1.4	29.4±1.3
46	45.2±2.0	43.3±1.7	42.0±2.0	38.9±1.7	33.4±1.6	31.7±1.2	28.5±1.2
47	42.3±1.8	40.7±2.0	38.5±1.6	35.5±1.6	31.1±1.5	29.9±1.4	29.6±1.3
Mean Concentration	42.6	41.0	39.3	36.9	32.0	30.4	29.2
Mean Removal(%)	0	3.7	7.7	13.4	24.8	28.6	31.3

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Table 5. *Nitrate nitrogen concentrations at individual sample points*

No.	Depth of CRIS Filtration Pool(cm)						
	0	25	50	75	100	125	150
43	2.50±0.12	5.74±0.26	8.58±0.42	10.07±0.39	11.10±0.55	11.68±0.43	11.72±0.57
44	2.20±0.09	5.60±0.25	8.82±0.41	9.89±0.47	11.95±0.57	12.06±0.60	12.12±0.60
45	2.71±0.13	5.93±0.29	7.95±0.39	9.62±0.44	11.88±0.56	12.09±0.48	12.11±0.59
46	3.15±0.28	6.75±0.33	8.89±0.27	10.19±0.48	12.38±0.55	12.54±0.52	12.97±0.61
47	2.40±0.10	6.12±0.29	8.45±0.37	9.95±0.42	11.24±0.45	11.66±0.54	11.71±0.52
Mean Concentration	2.59	6.03	8.54	9.94	11.71	12.01	12.13

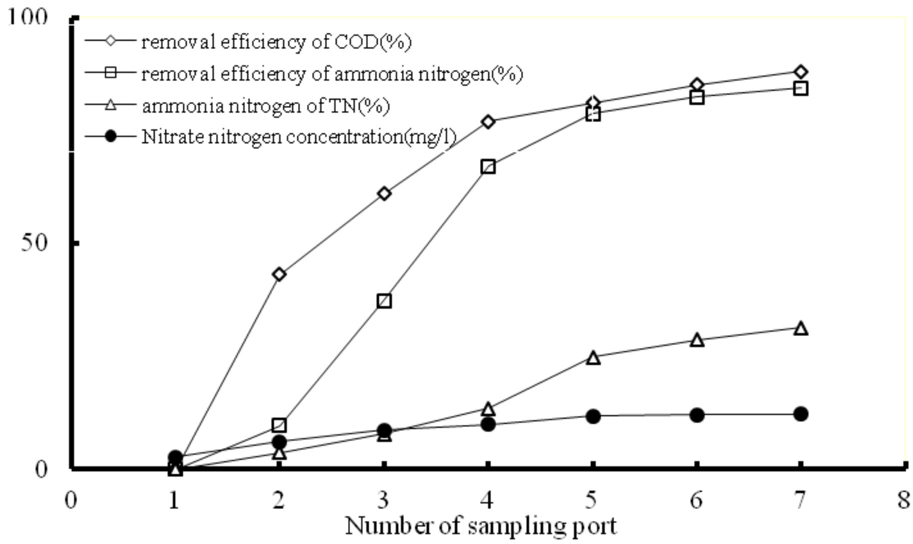

Figure 2. *Pollutant concentration and the removal rates at individual sample points*

Denitrification Mechanism Analysis of CRIS

Ammonia is mainly oxidized to nitrate by nitrosifying and nitrifying bacteria in CRIS (Zhao, 2010). It is first oxidized to nitrite by nitrosifying bacteria, which is then oxidized to nitrate nitrogen by nitrofying bacteria. The relative amounts and activities of nitrosifying and nitrifying bacteria affect the nitrofying process directly (Xu, 2011). Both populations depend on environmental factors for their survival such as pH, temperature, absence of toxic and other harmful substances such as heavy metals and dissolved oxygen (Xu, 2011). The experiments described here modelled treatment of domestic sewage, which contains very low concentrations of heavy metals. During the experiment the room temperature varied between 20-30°C, which is close to the optimal temperature for nitrification. The filtrate contained 10% marble sand that maintained the pH between 7.15 and 8.03, close to the optimal pH range for nitrification. We conclude that dissolved oxygen was the main factor affecting the nitrification in CRIS (Xu, 2011).

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The reason why TN removal was low in CRIS can be explained as follows. Ammonia nitrogen ions are positively charged and are absorbed by negatively-charged filter particles and microorganisms. The ions are transformed into nitrate by nitrifying bacteria under aerobic conditions, which can further be metabolized to gaseous nitrogen (N_2) under anaerobic conditions, resulting in nitrogen removal from the system (Xu, 2011). *Table 5* shows that in filter chamber, the oncentration of nitrate nitrogen was above 12mg/L in outflow, compared to approximately 2mg/L in the inflow, which suggested that nitrification dominated denitrification. Denitrifying bacteria use nitrate nitrogen as electron acceptor and organic matter as donor to conduct anaerobic respiration, with the net result that nitrate nitrogen is reduced to gaseous nitrogen. Nitrate nitrogen denitrification can only be achieved under strict anaerobic conditions, provided there is enough organic carbon present (Xu, 2011; Yao, 2006).

CRIS is used under conditions where it is frequently flooded with intermittent dry periods, which results in efficient aeration. Since nitrate nitrogen concentration increased in CRIS (*Table 5* and *Figure 2*), it can be concluded that aerobic conditions is applied, since denitrifying bacteria are strictly anaerobic. Nitrate nitrogen is not easily converted to nitrogen gas via denitrification. In deeper layers (100-150cm) of the filter chamber, the concentration of dissolved oxygen was low, but organic matter had most likely been decomposed and removed in the layers above, since CRIS is effective for organic matter removal (*Table 6* and *Figure 2*). As such, denitrification was most probably inhibited by lack of a suitable carbon source, and nitrate nitrogen couldn't be transformed into nitrogen gas to be removed. Nitrate nitrogen is negatively charged and dissolves well in water; therefore, it cannot be absorbed or retained by filter material or microorganisms. Instead, it maintains dissolved in the water and is removed with the outflow, resulting in a relatively high concentration of TN in the outflow of CRIS.

Table 6. *COD concentrations at individual sample points*

	Depth of CRIS Filtration Pool(cm)						
	0	25	50	75	100	125	150
Mean Concentration	160.10	90.87	61.67	36.23	30.82	23.33	19.69
Mean Removal(%)	0	43	61	77	81	85	88

Conclusions

There are three reasons why removal of ammonia nitrogen but not of total nitrogen is effective in CRIS:

(1) The lack of anaerobic conditions: frequent dry periods intermitting flooding result in effective aeration, and that leads to operation under aerobic conditions, which cannot support denitrifying bacteria.

(2) The lack of organic matter: organic matter is almost completely removed in the upper layer of CRIS, further contributing to low denitrification efficiency as the denitrifying bacteria lack a carbon source.

(3) Filter material has the same charge as nitrate: both are negatively charged and nitrate nitrogen is highly soluble in water, which makes it difficult to retain nitrate in the filter material; instead it is discharged with the outflow resulting in a net low TN removal rate.

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LIGNINOLYTIC ENZYME SYSTEM IN ECOLOGICAL ADAPTATION OF LIGNICOLOUS MACROFUNGI

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Abstract. Lignicolous macrofungi are the most important group of wood decomposers. Among the enzymes involved in wood decomposition, ligninases play an important role in this process and species that produce those enzymes degrade both cellulose and lignin. In this study we hypothesized that ligninases are influencing the ecological success of lignicolous macrofungi. Three hypotheses have been tested: h1 – the species producing several types of ligninases have a broader spectrum of hosts / substrates; h2 – the obligate saprotrophic species have a greater potential in degrading lignin and related compounds than the other groups; and h3 – the frequencies of lignicolous species is related to the production of highly active enzymes. Scientific data compiled from literature and completed with our own experimental results have been statistically interpreted using XLStat and MaxLite Software. Our results confirm the first two hypotheses, proving that ligninases play a direct role in colonizing a wide range of wood types, with chemical particularities. The third hypothesis should be rejected as no correlation has been observed. The present study offers new insights into ecological meanings of ligninases, and is the first attempt to connect the ligninolytic enzyme system to host range.
Keywords: *ligninases, wood decomposers, lignin degradation, white-rot fungi, laccases*

Introduction

Lignicolous macrofungi form a large group of fungi involved in the degradation of wood, with remarkable adaptations to different ecological niches.

Saprotrophic, parasitic or sapro-parasitic lignicolous macrofungi possess different strategies for colonizing wood and using it as a substrate and a source of nutrients. Depending on their ecology and type of nutrition, these species decay the wood, decomposing cellulose, hemicelluloses and sometimes lignin, which is one of the most abundant biomacromolecule on Earth (Knežević et al., 2013). Degradation of lignin might offer fungi advantages such as eliminating the barrier for the degradation of cellulose and increasing the availability of nitrogen in a substrate with a very high C : N ratio, through degradation of other wood constituents (Deacon, 2006).

This study is aimed at testing if the number of ligninases produced is influencing the spectrum of substrate / wood type / hosts, thus having ecological meanings. For this purpose three hypothesis have been formulated: h1 – the species producing several types of ligninases are able to colonize more types of wood compared to the species that produce a smaller number of ligninases; h2 – the obligate saprotrophic species (OS) have a greater potential in degrading wood (by degrading lignin) than sapro-parasitic (SP) and obligate parasitic species (OP); and h3 – competitive species produce highly active enzymes. To test these hypothesis data concerning the types of substrata, ligninase production and enzyme activity, for 69 lignicolous basidiomycetes, have been collected from literature and completed with personal experimental results.

Our interpretation suggests that there is a positive correlation between the number of ligninases and the number of hosts and OS are better wood decomposers, while the third hypothesis should be rejected. These findings offer new insights over the biochemical adaptation for colonizing a substratum with particular ecological characteristics such as wood. From our knowledge, this is the first study trying to search possible directly connections between the ligninolytic enzyme system and host range of lignicolous macrofungi.

Review of Literature

Wood decay takes place trough different biochemical mechanisms, and three types of rot can occur: a – soft rot, when fungi produce cellulases and H_2O_2; b – brown rot, when a cellulolytic enzyme system is involved and the cellulose / hemicelluloses are degraded while the lignin persists in the wood giving it a characteristic brown color; and c- white rot, in this case cellulose / hemicelluloses and lignin are degraded by particular enzymes. The concept of classifying wood rot is currently in revision, as some species “mimic” the white rot production (Nagy et al., 2015).

Ligninases are enzymes that produce the breakdown of lignin during wood decay. These enzymes play an important role in the nutrition of lignicolous fungi, frequently associated and acting synergistically.

There are several types of ligninases, and a particular species of macrofungi might produce one, few or all types. Many species produce two or three isomorphs of the same enzyme. The most common type of ligninases is represented by laccases, described for a large number of lignicolous macrofungi, including the ones that produce brown rot although the synthesis of laccases have been recorded in this cases as traces.

The occurrence of laccases (EC 1.10.3.2) and their role in the ecology of lignicolous fungi have been reviewed by several authors (Baldrian, 2006; Valderrama et al., 2003). Other common ligninases are Lignin Peroxidase-LiP (E.C. 1.11.1.14), Manganese dependent Peroxidase-MnP (E.C. 1.11.1.13), Manganese Independent Peroxidase-MIP (EC 1.11.1.16), while less frequent are Aryl Alcohol Oxidase-AAO, Versatile Peroxidase-VP, Dye-degrading Peroxidase-DyP (Anastasi et al., 2010; Grąz and Jarosz-Wilkołazka, 2011; Palmieri et al., 2005). In an extensive study involving the screening of genes encoding ligninases in a broad sense, Nagy and collaborators (2015) suggest that in the degradation of lignin, a much larger group of ligninases is involved, although some classes of enzymes play a secondary role. The production of a particular ligninase vary largely from one species to another in terms of amounts and the enzyme activity and the required conditions as well.

Materials and Methods

The available literature has been reviewed in order to collect information regarding the host’s spectrum, number of ligninases produced, frequency of species, type of nutrition and the efficiency of ligninolytic enzyme system for 69 species of lignicolous basidiomycetes. Most of the species listed in *Table 1* have also been isolated and stored

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in our laboratory collection. When data were missing from literature for particular species, the information has been completed with experimental data.

Table 1. *List of investigated species of macrofungi, their enzymatic properties, ecological features and data source (NE – number of enzymes, EA – enzyme activity, DE – dye degradation efficiency, CH – common host genera – TH, F – frequency, NT – nutrition type)*

Species	NE	EA	DE	CH	TH	F	NT	References
Abortiporus biennis	3	3	4	18	26	1	OS	Aggelis et al., 2002; ARS; Bernicchia, 2005; Casieri et al., 2010; Ryvarden and Gilbertson, 1993
Armillaria mellea	3	3	1	9	82	2	OP	Balaeş et al., 2013; Casieri et al., 2010; Diamandis and Perlerou, 2001; Matsushita and Suzuki, 2005; Stoytchev and Nerud, 2000; Otieno et al., 2003; PKB; Qin et al., 2007; Szczepkowsk, 2007
Auricularia auricula-judae	4	4	1	2	24	2	OS	ARS; Balaeş et al., 2013; Liers et al., 2010; Negrean and Anastasiu, 2004
Bjerkandera adusta	6	7	5	25	32	2	OS	Anastasi et al., 2010; Balaeş et al., 2013; Balaeş and Tănase, 2013; Bernicchia, 2005; Eichlerova et al., 2006a; Erkkilâ and Niemelâ, 1986; Guillén et al., 2011; Robinson and Nigam, 2008; Ryvarden and Gilbertson, 1993; Tinoco et al., 2007
Bjerkandera fumosa	1	1	3	15	20	1	OS	ARS; Balaeş et al., 2013; Bernicchia, 2005; Graz and Jarosz-Wilkolazka 2011; Ryvarden and Gilbertson, 1993
Cerrena unicolor	4	4	4	17	21	2	OS	Bernicchia, 2005; Elisashvili et al., 2010; Erkkilâ and Niemelâ, 1986; Hattori, 2005; Ranadive et al., 2011; Souza-Ticlo et al., 2009
Coriolopsis gallica	2	2	4	2	20	2	SP	ARS; Balaeş et al., 2013; Bernicchia, 2005; Hansent and Knudsen, 1997; Robinson et al., 2001; Ryvarden and Gilbertson, 1993
Cyathus striatus	1	1	2	1	3	2	OS	ARS; Balaeş et al., 2013; Casieri et al., 2010
Daedalea quercina	2	2	1	1	15	2	OS	Balaeş et al., 2013; Baldrian 2004; Bernicchia, 2005; Ranadive et al., 2011; Ryvarden and Gilbertson, 1993
Daedaleopsis confragosa	1	1	1	1	19	2	SP	Balaeş et al., 2013; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Orth et al., 1993; Ranadive et al., 2011; Ryvarden and Gilbertson, 1993
Datronia caperata	1	1	3	1	3	1	SP	Abrahao et al., 2008; ARS; Gilbert and Sousa, 2002; Minter et al., 2001
Dichomitus squalens	6	5	5	2	7	1	OS	Aggelis et al., 2002; ARS; Bernicchia, 2005; Eichlerova et al., 2006c; Novotný et al., 2012; Orth et al., 1993; Ryvarden and Gilbertson, 1993; Šušla et al., 2007
Flammulina velutipes	1	1	2	2	16	2	OS	ARS; Balaeş et al., 2013; Eichlerova et al., 2006a; Gerault, 2005; Petersen et al., 1999; Szczepkowski, 2007

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Fomes fomentarius	2	2	2	3	18	2	SP	Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Elisashvili et al., 2009; Erkkilâ and Niemelâ, 1986; Ryvarden and Gilbertson, 1993
Fomitopsis pinicola	1	1	1	5	23	2	SP	Balaeş et al., 2013; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Hattori, 2005; Ryvarden and Gilbertson, 1993
Ganoderma adspersum	1	1	3	1	9	2	OP	ARS; Balaeş et al., 2013; De Simone and Annesi, 2012; Elisashvili and Kachlishvili 2009
Ganoderma applanatum	2	2	2	9	53	2	SP	Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Elisashvili and Kachlishvili 2009; Elisashvili et al., 2009; Erkkilâ and Niemelâ, 1986; Levin et al., 2004; Ryvarden and Gilbertson, 1993; Sankaran et al., 2005; SIPMP; Szczepkowski, 2007
Ganoderma lucidum	4	4	2	13	45	2	OS	Asgher et al., 2010; Balaeş et al., 2013; Bernicchia, 2005; Elisashvili and Kachlishvili 2009; Erkkilâ and Niemelâ, 1986; Orth et al., 1993; Ranadive et al., 2011; Ryvarden and Gilbertson, 1993; Sankaran et al., 2005; SIPMP
Ganoderma resinaceum	1	1	3	1	16	2	SP	Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Ranadive et al., 2011; Ryvarden and Gilbertson, 1993
Gloeophyllum odoratum	3	3	3	1	4	2	SP	Anastasi et al., 2010, Casieri et al., 2010; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Ryvarden and Gilbertson, 1993
*Grifola frondosa**	2	1	1	1	7	1	SP	Bernicchia, 2005; Hansent and Knudsen, 1997; Orth et al., 1993; Ryvarden and Gilbertson, 1993
Gymnopilus junonius	2	2	2	4	14	1	OS	ARS; Balaeş et al., 2013; Rees and Strid, 2001; Valentin et al., 2009
Hemipholiota populnea	0	0	1	1	1	1	OP	ARS; Balaeş et al., 2013; Szczepkowski, 2007
*Hymenopelis radicata**	1	1	2	1	4	2	OS	ARS; Balaraju et al., 2010
Hypholoma fasciculare	2	2	2	2	7	2	OS	Abrahao et al., 2008; Angelini et al., 2012; Balaeş et al., 2013; Casieri et al., 2010; Gramss et al., 1999
Inonotus hispidus	3	3	1	3	23	2	OP	Aggelis et al., 2002; ARS; Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Gerault, 2006; Nerud and Mišurcova 1996; Ryvarden and Gilbertson, 1993
Irpex lacteus	6	6	5	14	20	2	OS	Aggelis et al., 2002; Balaeş et al., 2013; Bernicchia and Gorjón, 2010; Bernicchia, 2005; Hattori, 2005; Levin et al., 2004; Novotný et al., 2009; Ryvarden and Gilbertson, 1993
Kuehneromyces mutabilis	1	1	1	1	3	2	OS	Balaeş et al., 2013; Hansent and Knudsen, 1992; Steffen et al., 2007
*Laetiporus sulphureus**	1	1	2	3	23	2	SP	ARS; Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Hansent and Knudsen, 1997; Hattori, 2005; Ryvarden and Gilbertson, 1993

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Lentinula edodes	3	3	4	3	12	2	OS	ARS; Bisen et al., 2010; Boer et al., 2004; Kalmiş et al., 2008; Orth et al., 1993
*Lentinus neostrigosus**	2	2	3	3	20	2	OS	ARS; Hansent and Knudsen, 1992; Vaithanomsat et al., 2012
*Lentinus tigrinus**	3	3	4	3	12	2	OS	Aggelis et al., 2002; ARS; Hansent and Knudsen, 1992; Moreira et al., 2000
Lenzites betulina	5	5	5	8	21	2	OS	Anastasi et al., 2010; Balaeş et al., 2013; Balaeş et al., 2014; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Guillén et al., 2011; Hansent and Knudsen, 1997; Hattori, 2005; Moturi et al., 2009; Ryvarden and Gilbertson, 1993
Lycoperdon pyriforme	1	1	1	1	10	2	OS	Angelini et al., 2012; ARS; Balaeş et al., 2013; Casieri et al., 2010; Pegler et al., 1995
*Megacollybia platyphylla**	1	1	1	1	2	2	OS	ARS; Casieri et al., 2010
*Meripilus giganteus**	2	2	1	2	24	2	SP	ARS; Bernicchia, 2005; Kalmiş et al., 2008; Ryvarden and Gilbertson, 1994
Merulius tremellosus	2	2	1	1	18	2	OS	ARS; Balaeş et al., 2013; Bernicchia and Gorjón, 2010; Kum et al., 2011; Szczepkowski, 2007
*Oudemansiella mucida**	2	1	1	2	5	2	OS	ARS; Daniel et al., 1994; Gerault, 2005
*Panellus stypticus**	3	3	3	4	17	2	OS	Aggelis et al., 2002; ARS; Bernicchia and Gorjón, 2010; Casieri et al., 2010; Hansent and Knudsen, 1997; Nerud and Mišurcova 1996
*Peniophora quercina**	1	1	2	2	14	2	OS	ARS; Balaeş et al., 2013; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Hansent and Knudsen, 1997Ryvarden and Gilbertson, 1994
Phellinus igniarius	1	1	2	3	39	2	SP	ARS; Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Hansent and Knudsen, 1997; Ryvarden and Gilbertson, 1994
Phellinus pomaceus	1	1	2	1	24	2	OP	ARS; Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Ranadive et al., 2011; Ryvarden and Gilbertson, 1994
Phellinus torulosus	1	1	4	28	64	2	OP	Casieri et al., 2010; Nakasone and Burdsall, 1995
*Phlebia floridensis**	3	3	4	2	6	2	OS	Arora and Gill, 2005; ARS; Bernicchia and Gorjón, 2010; Szczepkowski, 2007
*Phlebia radiata**	6	7	5	2	15	2	OS	Anastasi et al., 2010; ARS; Casieri et al., 2010; Hilden et al., 2005; Mäkelä et al., 2006; Smith and Hesler, 1968
Pholiota aurivella	2	2	1	3	13	2	OP	Balaeş et al., 2013; Eichlerova et al., 2006a
*Piptoporus betulinus**	1	1	1	1	1	2	SP	Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Gerault, 2006; Hansent and Knudsen, 1997; Ryvarden and Gilbertson, 1994
Pleurotus florida	1	1	1	1	1	2	OS	ARS; Gbolagade et al., 2006; Pant and Adholeya, 2009
Pleurotus dryinus	2	2	2	10	18	1	OS	ARS; Eichlerova et al., 2006b; EOL
Pleurotus	7	7	2	11	43	2	OS	Aggelis et al., 2002; Anastasi et al., 2010;

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ostreatus								Balaeş et al., 2013; Casieri et al., 2008; Eichlerova et al., 2006a; Hansent and Knudsen, 1997; Palmieri et al., 2005; Pozdnyakova et al., 2010
Pleurotus pulmonarius	3	3	3	5	7	2	OS	ARS; Bernicchia, 2005; Eichlerova et al., 2006c; Hattori, 2005; Orth et al., 1993; Rigas et Dritsa, 2006; Ryvarden and Gilbertson, 1994
*Polyporus alveolaris**	2	2	5	9	20	1	OS	Barassa et al., 2009; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Lee et al., 2010; Ryvarden and Gilbertson, 1994
Polyporus brumalis	3	3	2	22	27	2	OS	ARS; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Kum et al., 2011; Rigas si Dritsa, 2006; Ryu et al., 2008; Ryvarden and Gilbertson, 1994
Polyporus squamosus	2	2	1	25	28	2	SP	ARS; Casieri et al., 2010; Eichlerova et al., 2006a
Postia caesia	1	1	1	7	30	2	OS	ARS; Balaeş et al., 2013; Eichlerova et al., 2006a; Gerault, 2006
Postia stiptica	1	1	1	3	22	2	OS	ARS; Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Hansent and Knudsen, 1997; Ryvarden and Gilbertson, 1994
Pycnoporus cinnabarinus	2	2	4	12	28	2	OS	ARS; Bernicchia, 2005; Casieri et al., 2010; Fedrova et al., 2013; Hansent and Knudsen, 1997; Orth et al., 1993; Ryvarden and Gilbertson, 1994
Trametes gibbosa	3	3	5	24	34	2	OS	ARS; Balaeş et al., 2013; Balaeş et al., 2014; Bernicchia, 2005; Elisashvili and Kachlishvili 2009; Elisashvili et al., 2009; Fedrova et al., 2013; Hansent and Knudsen, 1997; Orth et al., 1993; Ryvarden and Gilbertson, 1994; Szczepkowski, 2007
Trametes hirsuta	5	5	5	38	52	2	OS	Aggelis et al., 2002; Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Haibo et al., 2009; Hansent and Knudsen, 1997; Hattori, 2005; Orth et al., 1993; Ryvarden and Gilbertson, 1994; Szczepkowski, 2007; Tomšovsky and Homolka 2003
Trametes ochracea	3	3	5	19	23	2	OS	ARS; Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Elisashvili and Kachlishvili 2009; Gerault, 2006; Ryvarden and Gilbertson, 1994; Tomšovsky and Homolka 2003
Trametes pubescens	6	7	5	15	20	2	OS	Anastasi et al., 2010; ARS; Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2008, 2010; Erkkilâ and Niemelâ, 1986; Nikitina et al., 2005; Ryvarden and Gilbertson, 1994
Trametes suaveolens	2	2	4	2	9	2	SP	ARS; Balaeş et al., 2013; Bernicchia, 2005; Erkkilâ and Niemelâ, 1986; Hansent and Knudsen, 1997; Knežević et al., 2013; Ryvarden and Gilbertson, 1994
*Trametes trogii**	6	6	5	2	9	2	OS	ARS; Bernicchia, 2005; Dhouib et al., 2005; Levin et al., 2002, 2004; Ryvarden

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								and Gilbertson, 1993
Trametes versicolor	8	9	4	40	61	2	OS	Anastasi et al., 2010; Balaeş et al., 2013; Bernicchia, 2005; Casieri et al., 2010; Erkkilâ and Niemelâ, 1986; Guillén et al., 2011; Hattori, 2005; Levin et al., 2004; Liu et al., 2004; Lorenzo et al., 2006; Moturi et al., 2009; Orth et al., 1993; Ryvarden and Gilbertson, 1994; Tomšovsky and Homolka 2003; Ungureanu et al., 2015
Trichaptum abietinum	2	2	2	5	22	2	OS	ARS; Bernicchia, 2005; Eichlerova et al., 2006a; Erkkilâ and Niemelâ, 1986; Ryvarden and Gilbertson, 1994
*Trichaptum biforme**	2	2	2	7	18	2	OS	ARS; Bernicchia, 2005; Elisashvili et al., 2009; Hansent and Knudsen, 1997
Schizophyllum commune	4	3	2	6	42	2	SP	ARS; Balaeş et al., 2013 ; Bhatti et al., 2008; Hansent and Knudsen, 1997; Levin et al., 2004; Li et al., 2009; Szczepkowski, 2007
Stereum hirsutum	1	1	1	2	11	2	OS	Balaeş et al., 2013; Guillén et al., 2011; Orth et al., 1993; Szczepkowski, 2007
Xylobolus frustulatus	1	1	1	1	3	1	OS	Balaeş et al., 2013 ; Cookson, 1995; Hansent and Knudsen, 1997; Ranadive et al., 2011

*experimental results from the present research are included

Analytical procedures

For assessing the efficiency of dye degradation, the protocol described in Balaeş and collaborators (2013) has been used. Pure fungal cultures were grown on solid and liquid media and used as sources of inoculum. The presence / absence of laccase, and the enzyme activity has been highlighted through a modified protocol (Guo et al., 2011), using liquid medium supplemented with wheat bran (per L^{-1}: 6 g glucose, 3 g $(NH_4)_2SO_4$, 6 g wheat bran, 0.01 g $CuSO_4$), and inoculated with pelletized mycelium (10 mL inoculum L^{-1}). As a specific substrate, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) has been used. The reaction mixture contained 0.25 mL 50mM ABTS, 0.5 mL 0.1M acetate buffer, 0.25 mL enzyme extract. Substrate oxidation was monitored by measuring the increase of absorbance at 420 nm (ε420, 3.6•104 M^{-1} cm^{-1}), after 1 min at 25º C. A unit of laccase activity was defined as the amount of enzyme oxidizing 1 µmoL of ABTS per minute in the considered analysis conditions (Bourbonnais et al., 1995).

Data processing

Compiled dataset included 6 variables (*Table 1*): number of ligninases, enzyme activity (expressed as classes from 1– poor enzyme activity / traces, to 7 – very strong activity), dye degradation efficiency (expressed as classes from 1 – very weak degradation, few dyes or no degradation at all, to 5 – very efficient degradation of different dyes), number of common host genera, number of total host genera and frequency (expressed as classes, 1 – less frequent, 2 – frequent or very frequent). A seventh variable refers to the type of nutrition – obligate saprotrophic (OS), sapro-

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parasitic (SP) and obligate parasitic species (OP). For particular tests, values from previous datasets were grouped into three categories corresponding to the nutrition type.

Statistical interpretations

For all statistical calculations and analysis XLStat (trial version) and MaxLite (freeware) softwares have been used. As data do not follow normal distribution, non-parametric tests were used. For assessing the correlations between different variables, Spearman correlation coefficients were used, at a confidence interval of 95%. Mann-Whitney test has been used to compare variables related to the three types of fungi according to their nutrition. A Principal Component Analysis was run for a better view of correlations between all variables, considering the nutrition type as a qualitative supplementary variable.

Results and Discussions

The present investigation is an attempt to elucidate the role of ligninolytic enzyme system in ecological adaptation of lignicolous macrofungi in terms of diversity of substrata (hosts colonization). For this purpose, three hypotheses have been formulated.

Hypothesis number 1: *the species producing several types of ligninases are able to colonize many types of wood compared to the species that produce a smaller number of ligninases*.

One first step of our investigation was to analyze the correlation between the number of ligninolytic enzymes and the number of hosts. In the table of correlation (*Table 2*) it can be seen that there is a positive correlation (r=0.5529, p <0.0001) between the number of enzymes and the number of common host genera for all 69 species studied, while the correlation is weaker when considering the total host genera (r=0.3738, p<0.0001). In the latter case there are also included species of trees / lignicolous substrate that are accidentally colonized by fungi and not as a normal behavior of a particular fungal species (e.g. *Stereum hirsutum* usually grows on deciduous trees, but it has been reported also on coniferous wood, although one may not expect to find *S. hirsutum* on this substrate in the field). Hence, using the number of common hosts for assessing the correlation between the number of ligninolytic enzymes and host diversity gives a better understanding of how the ligninolytic enzymes system is influencing the ecology of these fungi.

Table 2. *Correlations between ligninolytic enzyme system and number of hosts – Spearman correlation coefficients (r values in upper, and p values, two-tailed, in lower matrix; confidence interval 95%; with bold are marked the relevant results)*

	Number of enzymes	**Enzyme activity**	**Dye degradation efficiency**	**Common host genera**	**Total host genera**
Number of enzymes		0.9898	0.5712	**0.5529**	0.3738
Enzyme activity	< 0.0001		0.5902	**0.5604**	0.3883
Dye degradation efficiency	< 0.0001	< 0.0001		**0.4559**	0.182
Common host genera	**< 0.0001**	**< 0.0001**	**< 0.0001**		0.759
Total host genera	0.0016	0.001	0.1344	< 0.0001	

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We have included in our tests one other variable: the general enzyme activity, expressed as classes of efficiency (data collected from literature and completed with our own results). These data have the disadvantage of being organized as classes: the quantitative interpretation is thus affected by subjectivity to some degree. Raw quantitative data could not be used due to very different experimental conditions of various researchers. Including of this variable had the role of verifying if there is still a positive correlation between enzymes and hosts number. The correlation in this case was slightly stronger (r=0.5604, $p < 0.0001$), re-confirming h1.

A second attempt to verify this hypothesis consisted in using a new variable, derived from the first one – the efficiency of dye degradation as a measure of enzyme versatility. Ligninolytic enzymes are very versatile and attack a large spectrum of chemical compounds with structure similar to lignin. This property confers the ability to degrade different human made aromatic compounds, hydrocarbons, dyes with heterocycles (Balaeş et al., 2014), while in natural conditions these fungi degrade lignin and also other chemical compounds from wood (Deacon, 2006). Considering these facts, we hypothesize that the versatility of ligninolytic enzymes could also be a factor that influence the ecological adaptation in terms of colonizing very different types of wood (varied chemical composition). However, we found weaker correlation compared to previous variables (r=0.4559, $p < 0.0001$).

The Principal Component Analysis (PCA) is an explanatory statistical tool used for visualization of data and to a lesser extent for testing hypotheses. Running PCA lead to confirm the results from previous tests, offering, in the same time, a largely view over the connections between variable. The procedure is based on the projection of all variables (six + one in our case) onto two axes, considering the first two factors that are contributing to the variability. We have considered the first six variable as principal quantitative variables and the seventh one (*Nutrition type*) as a supplementary qualitative variable.

In *Fig. 1* we can observe on the correlation circle that the first two components explain 73% of the data variability, making the projection a trusting one. A very strong positive correlation is found between the *Number of enzymes* and *Enzyme activity*, as it would have been expected. A good positive correlation is seen between these two variables and *Common host genera* on a side and *Dye degradation efficiency* on the other side. The position of projection points at a particular distance from the circle prove that the projection should be considered with caution for these two variables.

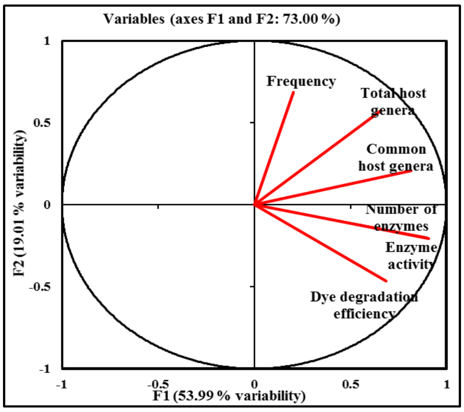

***Figure 1.** Circle of correlation between factors and variables after Principal Component Analysis (numbers on axes represent the correlation coefficients)*

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As seen in *Table 3*, factor loadings for these variables have the highest values on F1 dimension and second values (close to the first ones) on F3 and F4 dimensions respectively. The *Total host genera* variable is better projected on the chart (*Table 3*) and it can be observed that it is not correlated with *Number of enzymes*. The highest values of factor loadings for *Frequency* are on the F2 and F3 dimensions and are almost equal, and therefore no explanations can be assumed based on the projection.

Table 3. *Factor loadings for the six quantitative variables in Principal Component Analysis (with bold are marked the highest values for each variable)*

Variables\Factors	F1	F2	F3	F4	F5	F6
Number of enzymes	**0.900**	-0.199	0.263	-0.273	-0.015	-0.085
Common host genera	**0.815**	0.206	**-0.443**	0.067	0.303	0.000
Frequency	0.205	**0.687**	**0.664**	0.205	0.060	0.000
Enzyme activity	**0.907**	-0.209	0.253	-0.250	-0.022	0.087
Total host genera	**0.657**	**0.574**	-0.415	-0.007	-0.259	-0.001
Dye degradation efficiency	**0.685**	-0.463	0.046	**0.554**	-0.080	-0.003

Hypothesis number 2 – OS species have a greater potential in degrading wood (by degrading lignin) than SP and OP species.

OS macrofungi use products derived from wood decomposition as sources of nutrients: cellulose and hemicelluloses are primary sources of C and energy, while lignin is degraded especially for making cellulose and other constituents of wood more accessible (Deacon, 2006). Since OS are dependent on the dead wood constituents, it was assumed that these species tend to have a highly versatile and active enzymes system compared to SP and OP macrofungi, which are using additional or mainly other strategies for nutrition. In other words, the second hypothesis assumed that OS have more active enzyme and degrade a broader spectrum of synthetic dyes (different chemical structure), being more versatile.

When applying the Mann-Whitney test for two by two datasets (*Table 4*) we observed that there is a significant superiority of dye degradation efficiency of OS compared to SP (one tailed *p* value 0.047) and to OP (one tailed *p* value 0.048), while between SP and OP there are no significant differences for this property. Concerning enzyme activity, OS produce more highly active enzymes than SP (one tailed *p* 0.008) and OP (one tailed *p* 0.046). These results are confirming our hypothesis.

Table 4. *Mann-Whitney test (right-tailed test) for comparing the enzyme activity and dye degradation efficiency for the three type of macrofungi (the Mann-Whitney's U is normalized and tested against the normal distribution)*

	Dye degradation efficiency			Enzyme activity		
	OS-SP	OS-OP	SP-OP	OS-SP	OS-OP	SP-OP
U	469.000	222.500	64.000	514.000	223.500	57.000
U (expected value)	368.000	161.000	56.000	368.000	161.000	56.000
U (variance)	3651.782	1369.084	200.316	3627.943	1381.644	193.233
Z (observed value)	1.671	1.662	0.565	2.424	1.681	0.072
Z (critical value)	1.645	1.645	1.645	1.645	1.645	1.645
One-tailed p-value	0.047	0.048	0.286	0.008	0.046	0.471
Alpha	0.05	0.05	0.05	0.05	0.05	0.05

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Lignin is considered a barrier for wood decomposition, but woody species also produce tannins, alkaloids, resins and other types of chemical compounds with defensive and/or structural role. Lignin is formed from three different monomers: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, and their varied ratio give spatial different structures for each species. Broad leaves trees (dicotiledons) have lignine made predominantly from *p*-coumaryl, while coniferous trees contain lignin composed mainly from coniferyl alcohol. Ligninases are versatile enzymes, able to breakdown the lignin with its different structure from a species to another, as well as oxidize other compounds polyphenols and heterocyclic compounds (Elisashvili et al., 2010). For OS macrofungi ligninases play important roles in their nutrition.

The Principal Component Analysis also offered information for a better view of ecological features of the three groups of macrofungi separated based on their nutrition type. *Fig. 2* shows the projection of each observation (species characteristics) on the chart, grouped according to the nutrition type (three colors for OS, SP and OP). There is a distinct projection of the three groups, with only OS sharing some features with other two groups and with a higher dispersal on the chart, meaning there is a higher variability inside group, in terms of ligninases synthesis and host spectrum. OP group is more homogenous, but three species have particular characteristics, with *Armillaria mellea* (AM) and *Phellinus torulosus* (PT) having a very large host spectrum and producing ligninases to some degree, while *Hemipholiota populnea* (HP) has a completely distinct projection with a reduced host range (only *Populus*) and does not produce known ligninases.

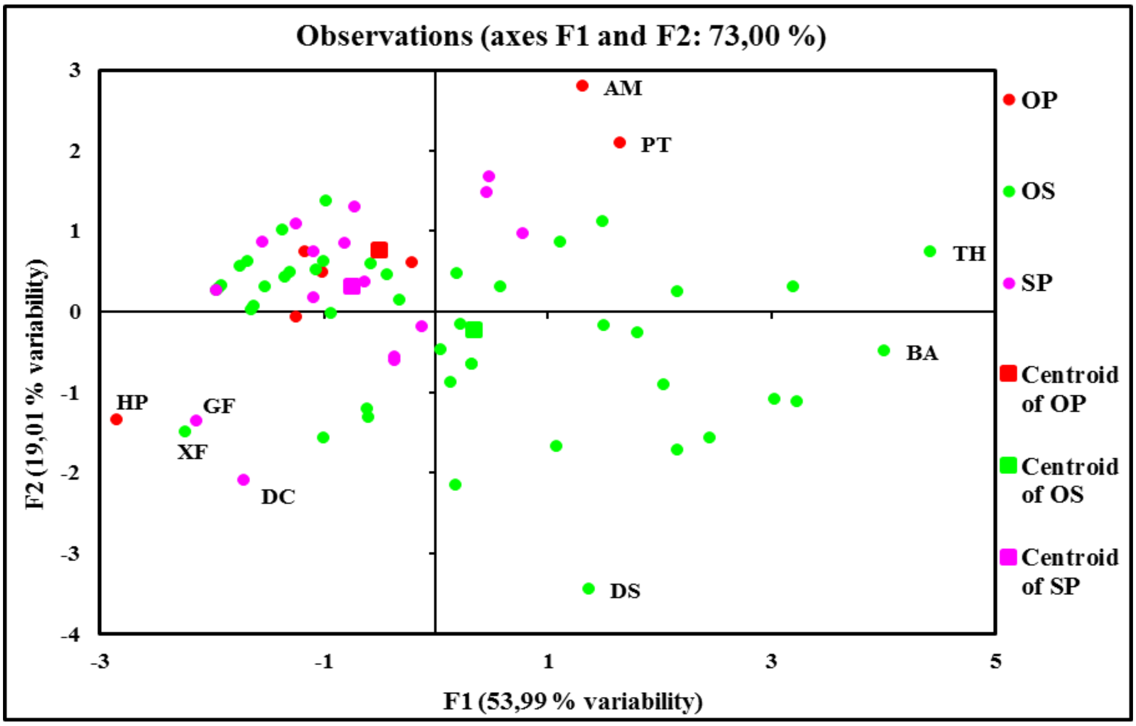

Figure 2. *The projections of observations (species characteristics) on the first two factors axes (AM – A. mellea, BA – B. adusta, DC – D. caperata, DS – D. squalens, GF – G. frondosa, HP – H. populnea, PT – P. torulosus, TH – Trametes hirsute, XF – Xylobolus frustulatus; legend: OP – obligate parasitic, OS – obligate saprotrophic, SP – sapro-parasitic macrofungi)*

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Among the SP species, *Grifola frondosa* (GF) and *Datronia caperata* (DC) possess features distinct from the other species in the group, with reduced host range, less frequent and producing only laccase in small amounts. Four OS species have characteristics different from other species in the group: *Bjerkandera adusta* (BA) and *Trametes hirsuta* (TH) produce many enzymes with strong activity and have a large host spectrum, while *Xylobolus frustulatus* (XF) produces few enzymes and has a reduced host spectrum (only *Quercus*) and *Dichomitus squalens* (DS) with strong enzyme activity but reduced host spectrum.

The projection of the species based on the nutrition type offers an interesting view of their ecological behaviors. The higher variability for OS fungi can be explained through their very different strategies for survival and development, some species growing on wood not affected by decay, while other grow on rotten wood; some species manifest antagonism against competitors, while other expand their mycelium in soil and litter, being more opportunistic. In contrast, SP and OP appear to be more specialized on their ecological niche, probably their strategies being oriented toward "fighting" their hosts.

Hypothesis number 3 – competitive species (frequent species) produce highly active enzymes.

To test this assumption, we verified if there is a positive correlation between frequency and enzyme activity / dye degradation efficiency. As it can be observed in *Table 5*, there is no correlation, at least in the case of our limited data, and we might assume that other factors are being involved in the process (hosts number, host frequency, other ecological factors). The third hypothesis has been rejected.

Table 5. *Correlations between frequencies and enzyme activity / dye degradation efficiency – Spearman correlation coefficients (r values in upper, and p values, two-tailed, in lower matrix; confidence interval 95%)*

	Enzyme activity	Dye degradation efficiency	Frequency
Enzyme activity		0.5902	0.1650
Dye degradation efficiency	< 0.0001		-0.0383
Frequency	0.1755	0.7544	

An attempt to include data for distribution of fungal species has been abandoned since the distribution is strongly dependent on their hosts and production of ligninases is irrelevant in this case. The limitations of our study consist in the reduced availability of data concerning ligninase production and activity for many species of macrofungi, forcing us to take into consideration only 69 species. Different macrofungi might produce a particular ligninase in different amounts or with a different activity, and a reasonable assumption is that the quantity and quality of ligninases also play roles in wood degradation efficiency. In these circumstances is difficult to compare the ligninolytic activity of certain species as different authors are using varied protocols and particular conditions to test the enzyme activity. However, using classes of efficiency, we overcome this limitation and tested the formulated hypotheses.

In summary, we have investigated the influence of ligninolytic enzyme system over ecological success of lignicolous fungi in terms of hosts' spectrum. We assumed that fungal species producing several types of ligninases are cosmopolitan and colonize a broader spectrum of tree hosts and we found positive correlation that sustain this

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hypothesis (r=0.5529, p <0.0001). Our analysis suggests that obligate saprotrophic fungi tend to degrade several types of wood and more efficiently due to higher enzyme activity and versatility. It appears that this group of macrofungi is more heterogeneous in terms of ligninases production and host range, probably due to other ecological factors involved in their adaptation strategies than in the case of parasitic species.

In was assumed that fungal species with a larger host spectrum produce highly active and versatile ligninases, but this hypothesis has been rejected.

Future studies might contribute further to the understanding of the ecological importance of ligninases.

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PRESENTATION OF THE HUSEED^{WILD} – A SEED WEIGHT AND GERMINATION DATABASE OF THE PANNONIAN FLORA – THROUGH ANALYSING LIFE FORMS AND SOCIAL BEHAVIOUR TYPES

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Abstract. In this paper, we present the HUSEED^{wild}, which is an online seed weight and germination database relying on our investigations concerning the regional seed collection (Pannon Seed Bank) of the Pannonian flora (available at: http://huseed.nodik.hu:8243/en/wild). Our data of 806 taxa (*sensu* species and subspecies) represent nearly 40% of the Pannonian flora. With our database, we contribute to former Hungarian databases with seed weight data of 50 taxa, and data of 20 taxa are first to be published in Europe. Our database is the first national germination database, and it contributes to European databases with germination data of 228 taxa. Additionally, we exemplify the ecological applicability of the dataset through analysing two plant strategies: (i) life forms and (ii) social behaviour types. Correlations between seed traits and plant strategies are investigated. Our findings are the following: (i) woody plants have significantly higher seed weight and significantly lower germination capacity than herbs (incl. forbs and graminoids); (ii) within herbs, perennials have significantly lower germination capacity than annuals and within forbs, they have significantly higher seed weight as well; (iii) herbaceous ruderals have significantly lower seed weight and significantly higher germination capacity than herbaceous stress tolerants. Our online database is being extended continuously, and its standardised data make it suitable for integration into larger European online databases.
Keywords: *seed mass, seed dormancy, plant growth forms, ruderals, Pannon Seed Bank*

Abbreviations: GP – germination percentage; SBT – social behaviour type; TSW – thousand-seed weight
Nomenclature: scientific plant names and authors follow the nomenclature of Flora Europaea (RBGE, 1998)

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Introduction

By definition, “plant traits” are morphological, anatomical, biochemical, physiological and phenological characteristics, which are measurable at individual level (Violle et al., 2007). Easy-to-measure “soft traits” are suitable for describing difficult-to-measure “hard traits” that correlate with them (Weiher et al., 1999). Seed weight is one of the most used soft traits (e.g. Csecserits et al., 2009; Török et al., 2013), while germination, due to dormancy, is a trait that is more difficult-to-measure and harder to interpret. However, both attract special interest because as “seed traits”, they are essential plant traits that primarily affect the early phases of a plant’s life cycle and thus the success of reproduction.

The application of plant trait databases (e.g. Csecserits et al., 2009; Kattge et al., 2011), including seed trait datasets (e.g. Csontos, 1998; Csontos et al., 2013; Török et al., 2013) is widespread in plant ecological researches. They are particularly effective in the case of analyses in community, comparative, evolutionary, conservation or invasion ecology for instance (Kattge et al., 2011). Thus, several complex European online plant trait databases including seed weight [e.g. Ecoflora (Fitter and Peat, 1994), BiolFlor (Klotz et al., 2002), LEDA (Kleyer et al., 2008), TRY (Kattge et al., 2011), D^3 (Hintze et al., 2013), SID (RBGK, 2016)] and germination [e.g. Ecoflora (Fitter and Peat, 1994), SID (RBGK, 2016)] datasets have been established recently. These databases contain widely accessible records of thousands of indigenous and non-indigenous species of Europe.

However, characteristics of species may differ depending on their geographical distribution (e.g. Csecserits et al., 2009; Valkó et al., 2009), thus regional databases possess great value. As a consequence, attribute databases in Hungary related to vascular plant species of the Pannonian Biogeographical Region (EEA, 2016) have been established. Such seed trait databases in Hungary are the SEED (including slenderness, flatness and weight records of seeds often based on literature data) by Csontos (1998), soil seed bank type database by Csontos (2001), and seed dispersal database by Csontos et al. (2002). Seed weight data of the Pannonian flora based on direct, original measurements are in the database of Török, P. et al. (2013, 2016). With former relevant data collections (Schermann, 1967; Csontos et al., 2003, 2007), it publishes records of approximately 70% of the Pannonian flora (Török, P. et al., 2016). On the other hand, an extensive germination database has not been established in Hungary so far.

In consequence of the above, we aimed at establishing an extendable, online database, which serves the extension of the seed weight dataset and provides basis for developing the germination dataset of the Pannonian flora. In this paper, we present the HUSEED^{wild} database relying on our direct, original measurements concerning the regional (Pannonian Biogeographical Region) propagule collection established for *ex situ* conservation within the frame of the Pannon Seed Bank project (LIFE08 NAT/H/000288). In addition, we exemplify the ecological applicability of our seed weight and germination datasets through analysing two selected plant strategies: (i) life forms (Soó, 1964-1980) and (ii) social behaviour types (SBTs; Borhidi, 1995). Scientifically proven and presumed correlations between seed traits and plant strategies are explored as well.

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Materials and Methods

The HUSEED^{wild} is based on our investigations concerning the wild plant seed collection maintained by the Center for Plant Diversity genebank. We established this collection within the frame of the Pannon Seed Bank project (LIFE08 NAT/H/000288) for *ex situ* conservation of wild native vascular plants of the Pannonian Biogeographical Region (see Peti et al., 2015; Török, K. et al., 2016).

Seed collection program

The collection of wild plant seeds on the territory of the Pannonian Biogeographical Region was started within the frame of the project in 2011, and with the contribution of the Center for Plant Diversity, it is continuous even after finishing the project in 2014. Here we refer to the activities and outputs of the period between 2011 and 2014.

The list of target species of the collections encompassed plant groups recommended for conservation by ENSCONET (2009a), for instance protected and strictly protected species, endemisms, character species, indicator species and species threatened by climate change. Additionally, several ruderal and some invasive species were included. The latter may be of importance in the case of seed biological and ecological investigations.

During seed collections, ENSCONET (2009a) specifications for sampling, qualities and quantities were followed. Seeds were harvested from spontaneous populations in ripening phase. Our goal was to get samples which represent the population's genetic diversity, thus we gathered pooled seed samples from several mother plants of the population (further on, we refer to seed samples that represent individual populations as "accessions"). We aimed at sampling as many populations in the region as possible, thus providing the most complete data of the regional genetic material of each taxon typical in the flora. Metadata of the collections [e.g. location, date, sampling method, habitat type according to Bölöni et al. (2011), etc.] were thoroughly documented. For further details of seed collecting methodology see our former publications by Peti et al. (2015) and by Török, K. et al. (2016).

Seed weight measurement protocol

Seed weight measurements were carried out within three months after collecting. Until measurement, accessions were stored in paper bags, in a dry place using silica gel.

Before seed weight measurements, the accessions were cleared of non-propagule fractions. In the case of fleshy fruits, cleaning occurred by washing, while dry fruits were cleaned by hand sorting, by sieves of various wire mesh sizes and by seed blower according to recommendations of methodological literature (Smith et al., 2003; Rao et al., 2006; ENSCONET, 2009b). For the investigations propagules which seemed visibly healthy (i.e. presumably not unfertile) based on identification guides of Bojňanský and Fargašová (2007) and Cappers et al. (2006) were used. Corresponding with standards of international databases (Kleyer et al., 2008; Hintze et al., 2013), air-dry weights of propagules (germinules or dispersules, including appendages) were measured with 0.0001 g accuracy using analytical balance.

Four replicates of samples with 100 propagules in each (400 propagules in total) per accession were counted to measure seed weight, and the results were averaged for each (i) accession and (ii) taxon, *sensu* species and subspecies (for further analyses, we used means (ii) as raw data). Seed weight was expressed as thousand-seed weight (TSW) [g].

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Germination protocol

Germination testing was carried out under laboratory circumstances, using freshly-matured visibly healthy seeds, within three months after collecting. In the case of taxa where germination tests were not recommended, tetrazolium test was used (results of these tests are not shown here).

Germination tests were carried out either on the Jacobsen table with 20-30 °C operating temperature, or in Petri-dishes in germination cabinets with 15-25 °C operating temperature depending on the species. Germination substrate was wetted filter paper, and the tests took 30 days (considering the clear discharge in germination dynamics). Species-specific germination methods – proper combination of pretreatment, temperature setting and light/dark setting – were applied, which allowed the investigation of "organic dormancy" [after Nikolaeva (1977), further on, we refer to it as "dormancy"] instead of "enforced dormancy" [after Baskin and Baskin (2004)] caused by inadequate environmental factors. We used an online database of RBG Kew, named SID (RBGK, 2016) and in some cases of crop-wild relatives, standards of ISTA (2013) as a basis for selecting the species-specific germination methods. We tested two to five recommended germination methods per species, and adjusted them, if needed, to the specifics of the local flora. Further on, the most effective method was applied. Depending on the species, scarification, soaking or warm and/or cold stratification were used as a pretreatment to break non-deep dormancy. When germination of a taxon was unsuccesful in spite of several attempts, tetrazolium test was applied.

Since, we focused on *ex situ* conservation purposes during determining the proper sample size and the repeat count, we took the ENSCONET (2009b) germination recommendations for wild plant species into account. Based on this, two replicates of samples with 50 seeds in each (100 seeds in total) per accession were germinated, and the results were averaged for each (i) accession and (ii) taxon, *sensu* species and subspecies (for further analyses, we used the means (ii) as raw data). Germination capacity was expressed as germination percentage (GP) [%].

After seed weight measurements and germination tests, moisture content of the seeds were reduced to 3-7%, and were banked in cold rooms of the Center for Plant Diversity and associated institutions, namely Aggtelek National Park and Institute of Ecology and Botany of the MTA Centre for Ecological Research. The cold rooms operate on 0 °C (active store) and on -20 °C (base store) according to the FAO (2013) international genebank standard based on the FAO/IPGRI (1994) standard, which have been successfully applied by Center for Plant Diversity for decades (see Peti et al., 2015).

Data processing

We used the dataset of HUSEED^{wild} accessed on 1 June 2015, which includes records for 1257 accessions collected between 2011 and 2014. All further reported quantifications are based on this access.

The taxon set of our database was compared on presence-absence level to taxon sets of national paper format databases of Török, P. et al. (2013, 2016), Csontos et al. (2003, 2007) and Schermann (1967), and to taxon sets of international online databases, namely LEDA (Kleyer et al., 2008) and SID (RBGK, 2016).

The raw dataset of our further analyses consisted of the single TSW means [g] and GP means [%] of all taxa available in HUSEED^{wild} (the mean calculated for the taxon

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from accessions belonging to it). We took taxa with germination result of 0% into consideration, since the lack of germination can be a species-specific strategy.

We analysed (i) life forms and (ii) SBTs in terms of seed weight and germination capacity, respectively. For gathering the Raunkier's life form (Soó, 1964-1980) and Borhidi's SBT (Borhidi, 1995) categories of species concerning the Pannonian flora, we used the FLORA database (Horváth et al., 1995). Borhidi's SBT classification is the adapted version of Grime's CSR plant functional type system (Grime, 1979) to the Pannonian flora, by supplementing the "competitor", "stress tolerant" and "ruderal" original categories with several sub-categories.

We set up different (i) life form and (ii) SBT groups by merging certain categories of the original life forms and SBTs. We compared these groups with each other based on the TSW means and GP means of taxa belonging to the given groups, respectively. In the case of life forms, the following simplified morphological life form groups were compared to each other: "woody plants", "forbs" and "graminoids". Trees and shrubs were classified as woody plants, herbaceous dicots and non-graminoid herbaceous monocots were classified as forbs, and herbaceous members of the *Cyperaceae*, *Juncaceae* and *Poaceae* families were classified as graminoids (further on, "herbs" means forbs and graminoids together). In addition, we treated "perennial", "biennial" and "annual" groups separately. In the case of SBTs, "competitor", "stress tolerant" and "ruderal" groups were compared to each other, and these groups were analysed within groups specified by life forms as well. The analyses of SBT groups within woody plants was omitted due to small number of samples.

The above mentioned groups were compared using Kruskal-Wallis test (with Tukey-Kramer significant difference criterion), and differences between the two means of two groups were tested by two-sample t-test (Zar, 2010). The rejection of the null hypothesis meant the differences in the expected values of the groups. Transformed data were used because the distribution of the original data violated the two-sample t-test's assumption of normality. In the case of TSW, the logarithms of the data showed normal distribution. In the case of GP, we normalised the data by using Box-Cox transformation (Sakia, 1992). Normality of data in each case was tested by Lillefors test (Conover, 1999). Confidence interval concerning mean of each group were determined by Bootstrap method due to the non-normal distribution of data (Devore and Berk, 2012). For statistical analyses, Statistics and Machine Learning Toolbox of Matlab R2015a software (MathWorks, 2015) was used.

Results

The HUSEED^{wild}

We established the online HUSEED^{wild}, which is available on the http://huseed.nodik.hu:8243/en/wild website. The database includes the following information: TSW mean with standard error calculated for taxon [g], TSW range (minimum–maximum) determined for taxon [g], the weighed morphological unit, GP mean with standard error calculated for taxon [%], GP range (minimum–maximum) determined for taxon [%], the applied germination method, all the above on accession-level, collection metadata (location, date) of accession. The content of the database can be searched by submitting terms (family, genus, species, subspecies, author) in the search field.

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Electronic Appendix 1 shows the extract of the online HUSEED^{wild} concerning the single TSW data, GP data and the applied germination methods of all taxa available in it (without detailing the data of accessions). As a result of our seed weight measurements, we got TSW data of 806 taxa (*sensu* species and subspecies) from 83 different families including 32 taxa which are strictly protected and 158 taxa which are protected by Hungarian law (Környezetvédelmi Minisztérium, 2001). As a result of the germination tests, we got GP data and germination methods applicable to the local flora of 744 taxa (*sensu* species and subspecies) from 73 families including 25 taxa which are strictly protected and 143 taxa which are protected by Hungarian law. In the case of the remaining 62 taxa where germination methods were not applicable tetrazolium test was applied. Out of the 744 taxa, 613 taxa from 63 families showed GP different from zero, while 131 taxa from 10 families did not germinate at all. However, tetrazolium tests proved these latter taxa were viable as well (members of *Orobanchaceae* were not investigated since their seed sizes were not suitable for tetrazolium test). Thus, failure of their germination was interpreted as germination strategy and their data were not excluded from the analyses.

Out of the 806 taxa where seed weights were measured, 199 taxa were not listed in LEDA (Kleyer et al., 2008), 101 taxa were not listed in SID (RBGK, 2016), and 50 taxa were not listed in national databases of Török, P. et al. (2013, 2016), Csontos et al. (2003, 2007) and Schermann (1967). TSW data of 20 taxa are novel in Europe (*Electronic Appendix 1*). Out of the 20 taxa, nine taxa are protected and four taxa are strictly protected by Hungarian law. The strictly protected **Serratula lycopifolia* (Vill.) A. Kern. is also listed in Habitats Directive Annex II. (European Commission, 1992).

Out of the 613 taxa which had GP different from zero, GP data and germination method recommendation of 170 taxa were not listed in SID (RBGK, 2016) which is the most complete European database containing germination records we know of (*Electronic Appendix 1*). Out of these, 61 taxa are protected and 10 taxa are strictly protected by Hungarian law. Five taxa of them, the protected *Cirsium brachycephalum* Jur. and *Iris humilis* Georgi, and the strictly protected **Onosma tornensis* Jáv., **Serratula lycopifolia* (Vill.) A. Kern. and *Seseli leucospermum* Waldst. et Kit. are listed in Habitats Directive Annex II.

The 131 taxa where GP was zero came from the *Aceraceae*, *Apocynaceae*, *Balsaminaceae*, *Celastraceae*, *Cornaceae*, *Ephedraceae*, *Fagaceae*, *Orobanchaceae*, *Oxalidaceae* and *Violaceae* families. Out of these, GP data of 58 taxa were not listed in SID (*Electronic Appendix 1*). Out of the 58 taxa, 19 are protected and five are strictly protected by Hungarian law. The protected *Echium russicum* J.F. Gmel., the strictly protected *Colchicum arenarium* Waldst. et Kit., *Iris aphylla* L. and **Pulsatilla pratensis* (L.) Mill. subsp. *hungarica* Soó are also listed in Habitats Directive Annex II.

The most TSW and GP data in the HUSEED^{wild} are reported for the *Compositae* (TSW: 101 taxa, GP: 96 taxa), *Poaceae* (TSW: 88 taxa, GP: 80 taxa), *Leguminosae* (TSW: 60 taxa, GP: 58 taxa), *Cruciferae* (TSW: 46 taxa, GP: 40 taxa), *Charyophyllaceae* (TSW: 45 taxa, GP: 38 taxa), *Umbelliferae* (TSW: 43 taxa, GP: 28 taxa) and *Labiatae* (TSW: 40 taxa, GP: 29 taxa) families. Additionally, the HUSEED^{wild} includes TSW and GP data of taxa where seed collecting is difficult due to either narrow distribution area [e.g. *Angelica palustris* (Besser) Hoffm., *Onosma tornensis* Jáv. and *Teucrium sorodonia* L.], or methodological problems (e.g. *Balsaminaceae*, *Geraniaceae* and *Oxalidaceae*) including ephemeral species [e.g. *Corydalis* and *Helleborus* (for latter, only TSW data is available)].

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Application of TSW and GP data in analysing plant strategies

Analysis of life forms

We analysed the different life form groups in terms of seed weight and germination capacity, respectively. Based on the Kruskal-Wallis test, the woody plant group differed significantly from other groups regarding both seed weight ($P<0.001$, where P is the significance level) and germination ($P<0.001$), while significant differences between the forb and graminoid groups were not detectable (*Table 1*).

The groups showed the following decreasing order of magnitude regarding their TSW means: woody plants (56.9 g), forbs (3.5 g) and graminoids (2.6 g) (*Table 1*). The two-sample t-test confirmed that the TSW mean of the woody plant group was significantly higher than those of the forb ($P<0.001$) and graminoid groups ($P<0.001$). Regarding germination, the forb group showed the highest average value (39.9%), and the average value of graminoid group was only moderately lower (38.5%) (*Table 1*). Significantly the lowest GP mean (12.5%) belonged to the woody plant group (two-sample t-test, $P<0.001$).

Table 1. *Thousand-seed weight means [g] and germination percentage means [%] of the simplified morphological life form groups. Notation: N_t – number of samples (where sample means taxon, sensu species and subspecies), CI95% – 95% confidence interval. Letters "a" and "b" written as superscript indicate significantly different ($P<0.001$) groups based on the Kruskal-Wallis test (with Tukey-Kramer significant difference criterion).*

Life form group	Thousand-seed weight			Germination percentage		
	N_t	Mean [g]	CI95%	N_t	Mean [%]	CI95%
woody	39	56.9 ^{a}	37.9 - 88.2	28	12.5 ^{a}	6.6 - 25.8
forb	635	3.5 ^{b}	3.0 - 4.3	597	39.9 ^{b}	37.3 - 42.6
graminoid	132	2.6 ^{b}	1.9 - 4.0	119	38.5 ^{b}	32.1 - 45.5

Further investigating herbs regarding plant life span – perennial, biennial and annual groups – we found the GP mean of the annual group (48.9%, $N_t=159$, where N_t is the number of taxa used as sample) to be significantly higher ($P<0.001$) than that of the perennial group (36.6%, $N_t=503$), but the GP mean of either group above did not differ significantly from that of the biennial group (41.3%, $N_t=54$) based on the two-sample t-test. However, the groups did not differ significantly regarding their TSW means (annual: 2.7 g, $N_t=162$; biennial: 3.3 g, $N_t=54$; perennial: 3.6 g, $N_t=551$) based on the two-sample t-test.

The woody plant, forb and graminoid morphological life form groups were investigated further by dividing them into perennial, biennial and annual groups (*Table 2*). The groups showed the following decreasing order of magnitude regarding their TSW means: woody perennials, annual graminoids (5.4 g), perennial forbs (4.0 g), biennial forbs (3.3 g), annual forbs (2.4 g) and perennial graminoids (2.1 g). The two-sample t-test confirmed that the TSW mean of the perennial forb group was significantly higher than those of the annual forb ($P<0.01$) and perennial graminoid groups ($P<0.01$) (*Table 2*). Regarding the GP means of the different groups, we found the following decreasing order of magnitude: annual graminoids (59.4%), annual forbs (47.5%), biennial forbs (41.3%), perennial forbs (37.1%),

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perennial graminoids (34.6%) and woody perennials. The two-sample t-test confirmed that the GP mean of the annual graminoid group was significantly higher than those of the perennial graminoid (P<0.05) and perennial forb groups (P<0.05). Furthermore, the GP mean of the annual forb group was significantly higher than those of the perennial forb (P<0.005) and perennial graminoid groups (P<0.005) based on the two-sample t-test (*Table 2*).

Table 2. *Thousand-seed weight means [g] and germination percentage means [%] of the simplified life form groups, and differences between means based on the two-sample t-test. Notation: wp – woody perennial, pf – perennial forb, bf – biennial forb, af – annual forb, pg – perennial graminoid, ag – annual graminoid, N_t – number of samples (where sample means taxon, sensu species and subspecies), P – significance level, ns – not significant difference. Abbreviation "tsw" written as subscript refer to thousand-seed weight, abbreviation "gp" written as subscript refer to germination percentage.*

Life form group				**wp**	**pf**	**bf**	**af**	**pg**	**ag**
				Thousand-seed weight					
		N_t		39	438	54	143	113	19
			Mean [g] [%]	56.9	4.0	3.3	2.4	2.1	5.4
wp	**Germination percentage**	28	12.5	-	P_{tsw}<0.001	P_{tsw}<0.001	P_{tsw}<0.001	P_{tsw}<0.001	P_{tsw}<0.001
pf		403	37.1	P_{gp}<0.001	-	ns_{tsw}	P_{tsw}<0.01	P_{tsw}<0.01	ns_{tsw}
bf		54	41.3	P_{gp}<0.001	ns_{gp}	-	ns_{tsw}	ns_{tsw}	ns_{tsw}
af		140	47.5	P_{gp}<0.001	P_{gp}<0.005	ns_{gp}	-	ns_{tsw}	ns_{tsw}
pg		100	34.6	P_{gp}<0.001	ns_{gp}	ns_{gp}	P_{gp}<0.005	-	ns_{tsw}
ag		19	59.4	P_{gp}<0.001	P_{gp}<0.05	ns_{gp}	ns_{gp}	P_{gp}<0.05	-

Analysis of SBTs

We analysed the different SBT groups in terms of seed weight and germination capacity, respectively. Since the TSW mean and GP mean of the woody plant group differed significantly from those of the other groups (see above), they could have hidden the relations among the SBT groups, so woody plants were excluded from the analyses first. Based on the Kruskal-Wallis test, the ruderal group differed significantly from the stress tolerant group (P<0.05), but it did not differ significantly from the competitor group, furthermore, there was no significant difference between the stress tolerant and competitor groups regarding their seed weights (*Table 3*). Regarding germination, the ruderal group differed significantly from both the stress tolerant (P<0.01) and competitor groups (P<0.01), while significant difference between the two last groups was not detectable based on the Kruskal-Wallis test (*Table 3*).

The groups showed the following decreasing order of magnitude regarding their TSW means: stress tolerants (3.9 g), ruderals (2.8 g) and competitors (2.6 g) (*Table 3*). The two-sample t-test confirmed that the TSW mean of the ruderal group was

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significantly lower than that of the stress tolerant group (P<0.05). Regarding germination, the ruderal group had the highest average value (45.3%), which was followed by the stress tolerant (36.6%) and competitor groups (31.1%) (*Table 3*). The two-sample t-test confirmed that the GP mean of the ruderal group differed significantly from those of the other two groups (P<0.005). Furthermore, the histogram based on GP values showed that germinations closer to 100% were more frequent for taxa of the ruderal group, than for taxa of the competitor and stress tolerant groups.

Table 3. *Thousand-seed weight means [g] and germination percentage means [%] of the social behaviour type groups based on the herbs belonging to them. Notation: N_t – number of samples (where sample means taxon, sensu species and subspecies), CI95% – 95% confidence interval. Letters "a" and "b" written as superscript indicate significantly different (thousand-seed weight: P<0.05, germination percentage: P<0.01) groups based on the Kruskal-Wallis test (with Tukey-Kramer significant difference criterion).*

Social behaviour type group	Thousand-seed weight			Germination percentage		
	N_t	Mean [g]	CI95%	N_t	Mean [%]	CI95%
competitor	72	2.6 ^{ab}	1.8 - 4.2	66	31.1 ^{a}	24.0 - 40.3
stress tolerant	394	3.9 ^{a}	3.2 - 5.1	357	36.6 ^{a}	33.0 - 40.0
ruderal	301	2.8 ^{b}	2.3 - 3.6	293	45.3 ^{b}	41.6 - 49.2

Table 4 and *5* show the detailed analyses of TSWs and GPs of the SBT groups within groups specified by life forms such as perennial, biennial and annual forbs, and perennial and annual graminoids (analysis of woody perennials by SBT groups was omitted due to small number of samples). The relative magnitude of the TSW means and GP means of competitors, stress tolerants and ruderals within the different life form groups showed different order compared to our former findings in many cases (*Table 4*). However, if groups with reliable number of samples ($N_t \geq 20$) were only considered, significant differences were detected mainly among the SBT groups mentioned above (*Table 5*). Within the group of perennial forbs, the TSW mean of stress tolerants (4.4 g) was significantly higher (two-sample t-test, P<0.005) than that of ruderals (2.6 g). Within the group of perennial graminoids, the TSW mean of stress tolerants (2.7 g) was also significantly higher (two-sample t-test, P<0.05) than that of ruderals (1.0 g). Within the group of annual forbs, such relation regarding TSW means was not detectable between ruderals and stress tolerants. Within the group of perennial forbs, the GP mean of ruderals (42.8%) was significantly higher (two-sample t-test, P<0.05) than that of stress tolerants (35.4%). Within the group of annual forbs, the GP mean of ruderals (48.2%) was also higher than that of stress tolerants (44.3%), although this difference was not significant based on the two-sample t-test. The differences between competitors and stress tolerants and between competitors and ruderals either regarding TSW means or GP means were not significant within any life form groups.

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Table 4. *Thousand-seed weight means [g] and germination percentage means [%] of the combined groups of simplified life form and social behaviour type groups. Notation: N_t – number of samples (where sample means taxon, sensu species and subspecies), CI95% – 95% confidence interval, pf – perennial forb, bf – biennial forb, af – annual forb, pg – perennial graminoid, ag – annual graminoid.*

Combined group	Thousand-seed weight			Germination percentage		
	N_t	Mean [g]	CI95%	N_t	Mean [%]	CI95%
pf competitor	12	5.2	3.2 - 9.0	11	25.7	8.1 - 52.9
pf stress tolerant	317	4.4	3.4 - 5.9	284	35.4	31.4 - 39.1
pf ruderal	109	2.6	1.8 - 3.8	108	42.8	36.7 - 49.1
bf stress tolerant	19	1.8	0.5 - 6.3	19	46.4	33.1 - 59.7
bf ruderal	35	4.1	2.7 - 7.3	35	38.4	28.7 - 48.4
af stress tolerant	24	1.6	0.9 - 2.9	24	44.3	31.2 - 59.1
af ruderal	119	2.5	1.7 - 3.7	116	48.2	42.0 - 54.7
pg competitor	59	2.2	1.3 - 3.9	54	32.6	24.0 - 42.0
pg stress tolerant	34	2.7	1.5 - 5.2	29	35.9	25.2 - 52.1
pg ruderal	20	1.0	0.6 - 1.5	17	38.4	23.5 - 55.1
ag competitor	1	0.2	- - -	1	13.0	- - -
ag ruderal	18	5.7	2.6 - 12.2	18	61.9	44.4 - 79.8

Table 5. *Comparison of the combined groups of simplified life form and social behaviour type groups by the two-sample t-test regarding respectively their thousand-seed weight means [g] and germination percentage means [%]. Notation: pf – perennial forb, bf – biennial forb, af – annual forb, pg – perennial graminoid, ag – annual graminoid, ns – not significant, nt – not tested (due to small number of samples).*

Combined group	Significance level	
	Thousand-seed weight	Germination percentage
pf competitor vs. pf stress tolerant	nt	nt
pf competitor vs. pf ruderal	nt	nt
pf stress tolerant vs. pf ruderal	P<0.005	P<0.05
bf stress tolerant vs. bf ruderal	nt	nt
af stress tolerant vs. af ruderal	ns	ns
pg competitor vs. pg stress tolerant	ns	ns
pg competitor vs. pg ruderal	ns	nt
pg stress tolerant vs. pg ruderal	P<0.05	nt
ag competitor vs. ag ruderal	nt	nt

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Discussion

Genebank seed collections have multiple benefits: they store genetic material for conservation and for research, and their data can provide valuable scientific information. In this paper the HUSEED^{wild}, a database relying on investigations of seed collection established within the frame of Pannon Seed Bank project (LIFE08 NAT/H/000288), and its ecological applicability were presented through analytical examples.

The HUSEED^{wild}

The HUSEED^{wild}, which is available on the http://huseed.nodik.hu:8243/en/wild website, reports regional data on TSWs of 806 taxa and on GPs of 744 taxa based on direct, original measurements (*Electronic Appendix 1*). Thus, our data cover nearly 40% of the Pannonian flora. The presented data, as original and regional data, are valuable on their own. At the same time, the value of the database is increased by the fact that we published several TSW (20 taxa) and GP (228 taxa) data of the European flora for the first time (*Electronic Appendix 1*). Our database contains mainly native species of Hungary, within which the easy-to-collect *Compositae*, *Poaceae* and *Leguminosae* families dominate, but it contains several hard-to-collect taxa as well.

In accordance with our expectations, seed weight data were in the 10^{-6}-10^{4} g range which was predicted by Harper (1977). In some cases, we found diverse seed weights within the same species. This phenomenon can be found in other databases as well. The reason for this phenomenon might be for example weather fluctuations or in the case of species with wide ecological tolerance the adaptation to different environmental conditions.

During germination evaluation, we took into account that most wild plant species are characterised by some form of dormancy (e.g. ENSCONET, 2009b; Baskin and Baskin, 2014). Matured seeds of most wild plant species do not reach their ability to germinate at the same time in order to avoid competition for resources (Grubb, 1988). Given this knowledge, we considered the germination of most species successful, although considerable proportion of their seeds did not show germination willingness. Successful germination results also meant that we properly adapted the applied germination methods to the species of the local flora.

Seeds, which did not complete germination, were most likely deep dormant, or less likely enforced dormant (insofar the selected germination method was not optimal despite our efforts).

Germination percentages often differed widely between different accessions of the same species. The differences may be caused by variability in proportion of dormant seeds and seeds able to germinate among different populations of species or rather among different individuals of populations (Milberg et al., 1996; Baloch et al., 2001; ENSCONET 2009b; Baskin and Baskin, 2014). Furthermore, the well-known seasonal changes in dormancy/non-dormancy cycle (Baskin et al., 2003; Baskin and Baskin, 2014, Garcia et al., 2014) can also cause the above mentioned phenomenon.

Some of the investigated taxa did not show willingness to germinate at all. Since these taxa proved to be viable by tetrazolium test, failure of germination was interpreted as a reproductive strategy. Presumably, these taxa were characterised by deeper dormancy level, which we could not break by scarification, soaking and warm/cold stratification either.

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Application of TSW and GP data in analysing plant strategies

Here, we considered a difference significant with a significance level of $P<0.05$ (for the exact P-values, see the "Results" section).

Analysis of life forms

We found correlations between the seed weights and the life forms of species. This is in accordance with literature (e.g. Salisbury, 1942; Fenner, 1985; Westoby et al., 1996; Csontos, 1998; Csontos et al., 2007; Moles et al., 2007; Tautenhahn et al., 2008; Török et al., 2013). According to the expectations (e.g. Salisbury, 1942; Fenner, 1985; Westoby et al., 1996; Csontos et al., 2007) the TSW mean of the woody plant group was significantly higher than those of the groups of herbs (*Table 1*). The higher seed weight of woody plants has ecological importance in providing advantages during emergence and establishment of seedlings under canopy light conditions. The larger (heavier) seeds are less dependent on light for germination than smaller (lighter) ones and there is a greater chance of establishment due to their reserved resources (e.g. Leishman and Westoby, 1994; Saverimuttu and Westoby, 1996; Milberg et al., 2000; Burmeier et al., 2010; Pivatto et al., 2014). The heavier seeds of species which develop in shady environment were supported by Csontos (1998) based on comparing relative light demand of 193 Pannonian flora elements. First, we did not detect significant differences among the herbaceous perennial, biennial and annual groups, which corresponded to the results of Csontos et al. (2007). However, the detailed analyses showed more complex differences. Corresponding to Török et al. (2013), we found the following decreasing order of the groups regarding their TSW means: annual graminoids, perennial forbs, biennial forbs, annual forbs and perennial graminoids (*Table 2*). Within the forb group, the TSW mean of perennials compared to that of annuals proved to be significantly higher, which corresponded to our expectations based on r/K selection theory (Grime, 1977). Within the graminoid group, we found the opposite of it, which contradicted our expectations based on r/K selection (Grime, 1977). Although, we could not confirm the significance of the difference statistically, TSW of the annual graminoids was higher than that of the perennial graminoids on average, and this relation was in accordance with the results of Török et al. (2013) and furthermore with the results of Csontos and Kalapos (2012). We presume, that the lighter seeds of perennial graminoids compared to annual graminoids are a result of the fact that most species in this group produce both seeds and clonal offspring (Kleyer et al., 2008), thus the energy budget they allocate for reproduction has to be shared between two sorts, and this may lead to smaller seeds.

We found correlations between the germination attributes and the life forms of species, which confirm, among others, the assumptions of Csontos and Kalapos (2006). Corresponding to the results of Grime et al. (1981), we found the woody plant group to have the significantly lowest GP mean (*Table 1*). Tendency for dormancy of woody plants is well-known from literature (Baskin and Baskin, 2014), which explains their low willingness to germinate. For the herbs, we found that GP mean of the annual group was significantly higher than that of the perennial group, however germination failure was not negligible even in the case of the annual group (on average ca 50%). The significantly higher GP mean of the annuals compared to that of the perennials was confirmed within both the graminoid and forb groups (*Table 2*). Corresponding with our

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expectations based on r/K selection (Grime, 1977), our results lead to the conclusion that a relatively faster response to the environmental conditions that are optimal for germination is in the interest of annuals which have faster life cycle compared to perennials. This is because early germination means competitive advantage in utilising limited nutrients of the environment in contrast to delayed germination, and this plays a more important role in the survival of annuals than that of perennials (Abraham et al., 2009). This is supported by the results of Grime et al. (1981), that germination rate of annuals is higher than that of perennials.

Analysis of SBTs

We found correlations between the seed weights and the SBTs of species. Based on the maximised reproductive rates of ruderals and the limited reproductive rates of stress tolerants (Grime, 1977, 1979), and based on the negative correlation between the seed weight and seed number (e.g. Shipley and Dion, 1992; Moles et al., 2004), we presumed a huge number of small seeds for ruderals, and a smaller number of larger seeds for stress tolerants. Our results confirmed the expectations: the TSW mean of the herbaceous ruderal group proved to be significantly lower than that of the herbaceous stress tolerant group (*Table 3*). Such relation regarding TSW means was detected between the ruderals and stress tolerants within both the perennial forb and perennial graminoid groups (*Table 4-5*). Our results are in accordance with the proven fact that ruderal species usually have long-lived (persistent) seed bank in the soil (e.g. Harper, 1977; Thompson and Grime, 1979; Fenner, 1985; Thompson, 1992; Bakker et al., 1996; Thompson et al., 1997; Bekker et al., 1998; Matus et al., 2005; Bossuyt and Honnay, 2008; Török et al., 2009) and this persistent seed bank assumes small seed size. Latter correlation between soil seed bank persistence and seed weight (persistent seed bank – small seed size) is proved in most flora types (e.g. Thompson and Grime, 1979; Thompson et al., 1993, Bakker et al., 1996; Bekker et al., 1998; Hodkinson et al., 1998; Funes et al., 1999; Thompson et al., 2001; Cerabolini et al., 2003; Peco et al., 2003; Zhao et al., 2011), including in the Pannonian flora (Csontos, 1998, 2001), however, it was not detected for the flora of Australia (Leishman and Westoby, 1998) and New Zealand (Moles et al., 2000).

Regarding germination, the herbaceous ruderal group showed significantly higher GP mean than the herbaceous stress tolerant and herbaceous competitor groups (*Table 3*). The significant difference between the ruderals and stress tolerants also was confirmed within the group of perennial forbs (*Table 5*). Although the ruderal group showed greater germination willingness compared to the other SBT groups, it is important that even its germination did not exceed the 45% average value. Presumed cause of low average GP may be the high frequency of “risk-spreading” survival strategy (Grubb, 1988) within the group. Species characterised by such strategy maintain persistent seed bank in the soil with the help of numerous dormant seeds, and even under optimal conditions their germination is partially delayed. However, germinations of species in the ruderal group came closer to 100% more often than germinations of species in the other groups. The presumed cause of their great germination willingness may be the “disturbance-broken” strategy, which is another typical strategy for ruderals (Grubb, 1988). Species with this strategy build up their persistent soil seed bank by their seeds becoming enforced dormant due to fast burial, and they start to germinate from this explosively under favourable environmental conditions caused by disturbance, which makes their fast colonisation possible.

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Ecological uses of our data

The knowledge of seed weight and germination data, and optimal conditions for germination can be directly used in applied and theoretical fields of plant ecology. This information has particular importance, even by species respectively, for example during restoration works (Török, P. et al., 2016): in view of seed weight and germination capacity of species, necessary amount of seeds in case of sowing can be designed in order to achieve the expected number of individuals. Knowledge of optimal germination conditions of species is also necessary for *ex situ* propagations during restoration works. Seed weight and germination capacity of species can be used in invasion and migration ecology as well. In the light of seed weights, dispersal capacity of seeds (Bekker and Bakker, 2003) and seed longevity in soil (e.g. Thompson and Grime, 1979; Thompson et al., 1993, Bakker et al., 1996; Bekker et al., 1998; Hodkinson et al., 1998; Funes et al., 1999; Thompson et al., 2001; Cerabolini et al., 2003; Peco et al., 2003; Zhao et al., 2011) can be estimated and this information can help to predict the probability of spontaneous regeneration of native species.

The data are useful not only on their own, but can be used as a dataset. Our seed trait dataset, which includes data of 806 taxa, can provide input data to large scale ecological analyses as well. It can be used for revealing correlations between the seed traits and other plant traits or environmental factors, and in the light of these correlations, hard traits can be estimated. In this paper, the applicability of our seed trait dataset was exemplified through analysing correlations between (i) seed traits and life forms and (ii) seed traits and SBTs. The results of these analyses can be utilised among others in community, conservation and restoration ecology.

The HUSEED^{wild}, which is available online, is being extended and updated continuously, and due to its standardised data it can be suitable for integration into other larger European online databases (e.g. SID), which allows even more widespread accessibility and applicability.

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ELECTRONIC APPENDIX

Electronic Appendix 1. *The extract of the online HUSEED^{wild} (available at: http://huseed.nodik.hu:8243/en/wild) concerning the single thousand-seed weight data [g], germination percentage data [%] and the applied germination methods of the 806 taxa (sensu species and subspecies) accessible in it on 1 June 2015.*

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FLORISTIC COMPOSITION AND PLANT COMMUNITY TYPES IN MAZE NATIONAL PARK, SOUTHWEST ETHIOPIA

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Abstract. Ethiopia has established protected areas throughout the country and designated many areas of land in order to conserve the natural resources and biodiversity. Hence, identification of the diversity in these protected areas is essential for conservation and management purposes. The study was conducted with the objective to investigate and document the floristic composition and plant community types of Maze national park. A preferential sampling technique was used as a sampling design and a total of 65 plots with a size of 20 m x 20 m (400 m^2) were laid out for the woody species; a sub-plot of 5 m x 5 m was used at the center of each quadrat to assess the herbaceous species. A total of 144 species of vascular plants belonging to 109 genera and representing 37 families with, 40%, 37%, 17%, and 6% herbs, trees, shrubs and climbers respectively were recorded and identified. The most diverse family was Fabaceae followed by Poaceae, Euphorbiaceae, and Asteraceae. *Combretum adenogonium*, *Rhus natalensis, Panicum maximum*, are the most frequently occurring tree layer, shrub layer and herb layer plant species respectively. The output of TWINSPAN (Two-Way Indicator Species Analysis) has shown a cluster of 6 plant community types. The Analysis of Variance (ANOVA) has shown that there was statistically significant difference among plant communities in species richness, altitude, diversity and evenness at 95% confidence interval with a P value <0.05. Anthropogenic activities in Maze National Park were identified to exert high pressure on the vegetation and wildlife in the area resulting in the loss of biodiversity underpinned by troubled natural habitat. The most feasible recommendation to reverse the undesirable condition in the national park is to actively participate the local people in the conservation activities of the national park via ecotourism which is one form of sustainable tourism.
Keywords: *biodiversity, Ethiopia, Maze National Parks, plant diversity; protected areas*

Introduction

Ethiopia is a country located in the Horn of Africa and has great topographical diversity with flat-topped plateaus, high mountains, river valleys, deep gorges, rolling plains, and with great variation of altitude from 116 meters below sea level, in some areas of Kobar Sink to 4620 meters above sea level at Ras Dashen (IBC, 2012). These diverse physiographic features have contributed to the formation of different ecosystems characterized by variations in biodiversity. The country is endowed with rich diversity of flora and fauna as well as rich in endemic species. The flora of Ethiopia encompasses about 6,027 vascular plant species with 10% endemic (Kelbessa and Demissew, 2014); this distribution puts the country in the fifth largest floral composition in tropical Africa (Didita et al., 2010).The fauna

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includes 860 avian species 16 of which are endemic and two genera are endemic too; Mammals 279 species, of which 35 are endemic and six genera are endemic too. The country is also home of a number of charismatic and endemic flagship species. The protected areas such as national parks, sanctuaries, controlled hunting, open hunting, wildlife reserves and community conservation areas coverage share only about 15% of the total land area of the country (BIDNTF, 2010; Chanie and Tesfaye, 2015). The rich biodiversity of the country is under serious threat from deforestation and land degradation, overexploitation, overgrazing, habitat loss, invasive species and some water pollution (IBC, 2009).

IUCN defines a protected area as an area of land and/or sea especially dedicated to the protection and maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means. Protected areas represent a defined and delimited geographical space which aims at the conservation of nature with associated ecosystem services and cultural values (Dudley, 2008). Protected areas play a key role in safeguarding biodiversity and sustaining the crucial services provided by the ecosystems (Chanie and Tesfaye, 2015). Protected areas are playing an important role in scientific research; wilderness protection; preservation of species and genetic diversity; maintenance of environmental services; protection of specific natural and cultural features; tourism and recreation; education; sustainable use of resources from natural ecosystems; maintenance of cultural and traditional attributes (Ahmet and Meric, 2000). National parks are the most extensive type of protected areas in Africa and globally (Muhumuza and Balkwill, 2013). National parks are important in biodiversity conservation and maintaining natural ecosystems functioning, as refuges for species and maintaining ecological processes. However, many of these biological sanctuaries are currently affected by a series of social, political, industrial, and institutional challenges (Khadka, 2010).

The primary mechanism used by Ethiopia to protect biodiversity, ecosystems and ecological processes has been through a network of wildlife conservation areas and priority forest areas (FDRE, 2007). Ethiopia has established protected areas throughout the country and designated many protected areas of land as national parks, wildlife ranches, wildlife sanctuaries, wildlife reserves, and biosphere reserves and national forest priority areas for biodiversity, wildlife and forest conservation (Kelboro and Stellmacher, 2012). Protected areas play an important role in the sustainable development of national economy by providing environmental services such as offering ecotourism and safari experiences to the recently blooming tourism sector (Vreugdenhil et al., 2011). National parks are among widely used means of nature conservation in Ethiopia (Stellmacher, 2007). National parks enable a country to protect biodiversity in its representative ecosystems within their natural habitats (Kelboro and Stellmacher, 2012). Ethiopian national parks are characterized by exclusive conservation approaches, and this may not be effective because of conflicts between local people living in or around the parks and state authorities (Debelo, 2012; Kelboro and Stellmacher, 2012; Stellmacher and Nolten, 2010), leading to the loss of biodiversity. Ethiopian protected areas face significant challenges in meeting the needs local community and state conservation dilemma. The loss of forest cover and biodiversity due to human-induced activities is a growing concern in many parts of the world (Senbeta and Teketay, 2003) and protecting these areas is important to curb the loss of

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habitat and biodiversity. The major threats to the biodiversity of the country are over-harvesting, deforestation, conversion of natural vegetation to farmland, forest fires, land degradation, habitat loss and fragmentation, invasive species, illegal trafficking of domestic and wild animals, poaching, wetland destruction and climate change (IBC, 2009) whereas protected areas frequently serve to insulate biodiversity from the impacts of human development (Rai and Sundriyal, 1997; Shackleton, 2000)

In order to sustain the ecological equilibrium of the environment and meet the forest resource requirements of the population, collection of scientific information on the composition, structure and distribution of the species becomes a primary activity in areas where it has not been achieved (Ayalew et al., 2006). Maze National Park is one of the national parks in Ethiopia where such study has not been conducted before on plant species diversity, distribution patterns and plant community composition. Systematic field survey of flora and fauna is a prerequisite for developing effective conservation programs and its implementation. These collected information on vegetation may be required to solve an ecological problem, for biological conservation and management purposes; as an input to environmental impact statements; to monitor management practices or to provide the basis for prediction of possible future changes (Kent and Coker, 1992). Hence, identification of the diversity in these areas is essential for conservation and management purposes.

The role of protected areas in conserving natural resources is well established; while, the role of humans in these areas continues to be debated (Katherine, 2013). Conventional protected areas approaches have tended to see people and nature as separate entities, often requiring the exclusion of human communities from areas of interest, prohibiting their use of natural resources and seeing their concerns as incompatible with conservation, this exclusionary approaches have engendered profound social costs (IUCN, 2004). This further caused an illegal and unsustainable natural resource utilization (Siraj et al, 2016). The importance of protected areas and their nexus to environment and development issues has got worldwide recognition and reflected on different international conference such as, the UN Conference on Environment and Development in 1992 and other international and regional environmental agreements (Chape et al., 2003). In Ethiopia the importance of biodiversity conservation and benefits of ecosystem services are not well-understood by a large number of people at all levels, particularly by policy makers (IBC, 2009). Understanding plant species distribution patterns and plant community composition along environmental gradients gives key information for effective management of forest ecosystems (Lovett et al., 2000; Noss, 1999; Naveh and Whittaker, 1980). Thus, it is important to identify plant species diversity and community composition along environmental gradients. Hence, the objectives of this study was to investigate and document the floristic composition of the Maze National Park, to classify the vegetation of Maze National Park into plant community types, and to assess the status of the vegetation and to make some recommendations on the management and conservation of the park.

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Materials and Methods

Study area description

The study was conducted in Maze National Park at Gamo Gofa Zone, Southern Nations, Nationalities and Peoples' Regional State (SNNPRS), southwest Ethiopia as shown *(Fig. 1)*. The park is surrounded by five districts namely Qucha on the northern part, Gofa on the northwest, Deramalo on the south and southeast, Zala on the southwest, and Kemba on south. The study area Maze National Park lies between (06° 3'to 060 30' N latitude and 37°25' to 037°40'E longitude (Refera and Bekele, 2006) and altitude ranges from 900 m to 1200 m above sea level. The park is located 460 km and south west of Addis Ababa in Gamo-Gofa Zone on the road to Sawula city. Maze National Park was established in 2005 and serves as one of the last remaining sites for the conservation of the Swayne's Hartebeest second to Senkelle Hartebeest Sanctuary (Tekalign and Bekele, 2011). The park is mainly in Combretum-Terminalia woodland type of vegetation and covers an area of 204 Km^2 and lies near the middle of Omo river valley. Maze National Park was named after the Maze river which rises from southern parts of the surrounding highland and passes through the park from south to north direction and eventually drain in to Omo river.

Maze National Park is one of the national parks designated for wildlife conservation and known for its good population of the critically endangered endemic Swayne's Hartebeests population in Ethiopia. Maze National Park is an important biological area and has great potential for the conservation of the endangered endemic subspecies of Swayne's hartebeest population and other wildlife (Refera and Bekele, 2006).

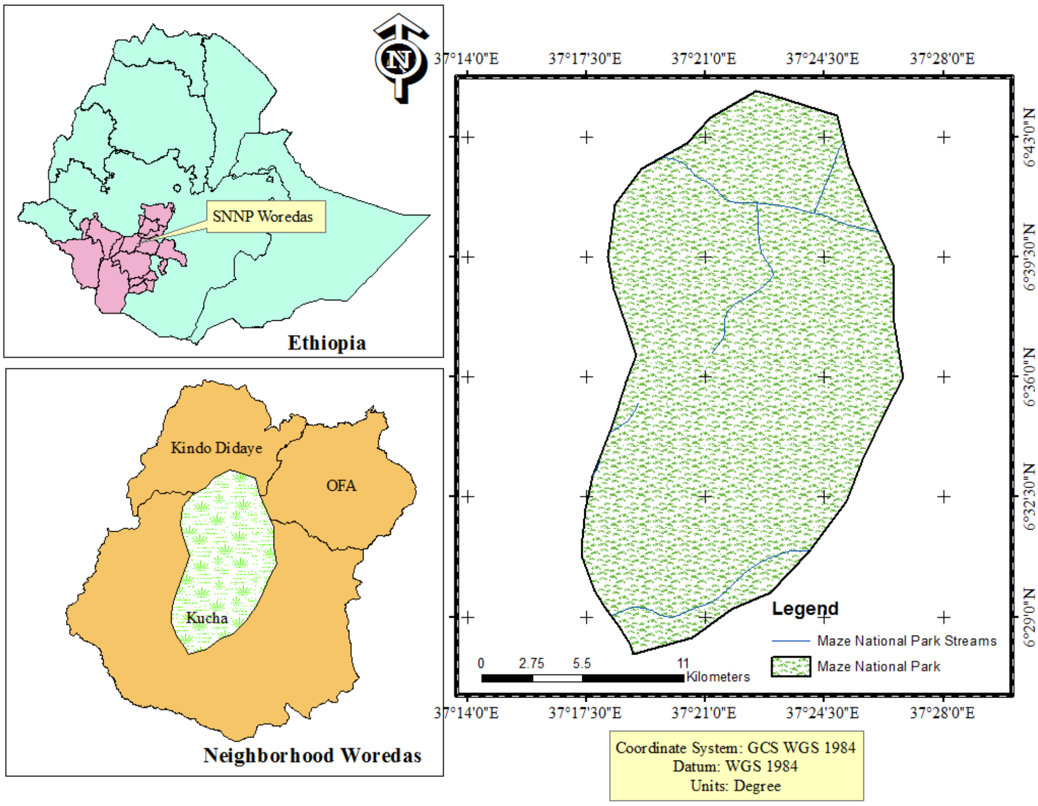

***Figure 1.** Location Map of the Maze National Park*

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Sampling design

Following a reconnaissance survey, actual sampling of vegetation was done between November 8 and December 5, 2007 focusing on homogeneity via preferential means in some parts of the park (Tela Ayisera, Molisha, Maze river and Lemase river) areas. The data collection was done following the methodology used in studying the vegetation of Ethiopia by (Ayalew et al., 2006; Demissew, 1980; Andargie, 2001; Bekele, 1994; Woldu, 1985). A preferential sampling technique was used as a sampling design and a total of 65 plots with a size of 20 m x 20 m (400 m^2) were laid out for the woody species; a sub-plot of 5 m x 5 m was used at the center of each quadrat to assess the herbaceous species. All the vascular plant species in the plots were recorded and their cover abundance in each plots was estimated and rated according to modified Braun Blanquet approach (Van der Maarel, 1979). The collected plant specimens were pressed properly and were brought to the National Herbarium (ETH), Addis Ababa University for identification. The specimens were dried and identified by using authenticated specimens, consulting experts and referring to the Flora of Ethiopia and Eritrea.

Climate

The rainfall and temperature data for 9 years between1997-2005 was taken from Ethiopian National Meteorological Agency (ENMA) which is recorded at Morka Peasant Association situated near Maze National Park, that lies almost at the same altitudes. Based on the 9 years rainfall summarized data, the rainfall in the study area is bimodal (having two rain seasons). The total amount of annual rainfall in the area varies between 2841.6 and 3299.8 mm. The longest rainy season in the area extends from April up to October month in which high amount of rainfall is recorded between April and June *(Fig. 2)*.

There is no consecutive and regularly recorded temperature data of the area; instead a summarized temperature data of the area was taken. The hottest months of the area include December, January, February and March with mean maximum temperature 32.88°C. The cooler months in the area include June, July and August with mean minimum temperature of 17.5 °C *(Fig. 2)*.

Data analysis

Plant community

A plant community is characterized by its essential homogenous physiognomy and ecological structure and by its floristic composition, at least with regard to dominance (Tothill, 1978). The description of plant community involves the analyses of species diversity, evenness and similarity (Whittaker, 1975). All vascular plant species present in sampling unit were recorded and percentage aerial cover/abundance of each species was estimated based on 1-9 scale of Braun-Blanquet (as modified by Van der Maarel, 1979): 1 = rare generally only one individual; 2 = sporadic (few) which are less than 5% cover of the total area; 3 = abundant with less than 5% cover of the total area; 4 = very abundant and less than 5% cover of the total area; 5 = 5-12% cover of the total area; 6 = 12.5-25% cover of the total area; 7 = 25-50% cover of the total area; 8 = 50-75% cover of the total area; 9 = 75-100% cover of the total area. TWINSPAN was used to classify plant communities in the study area and MINITAB 14 computer program was used to

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analyses the variation among the communities in species richness, altitude, evenness and species diversity.

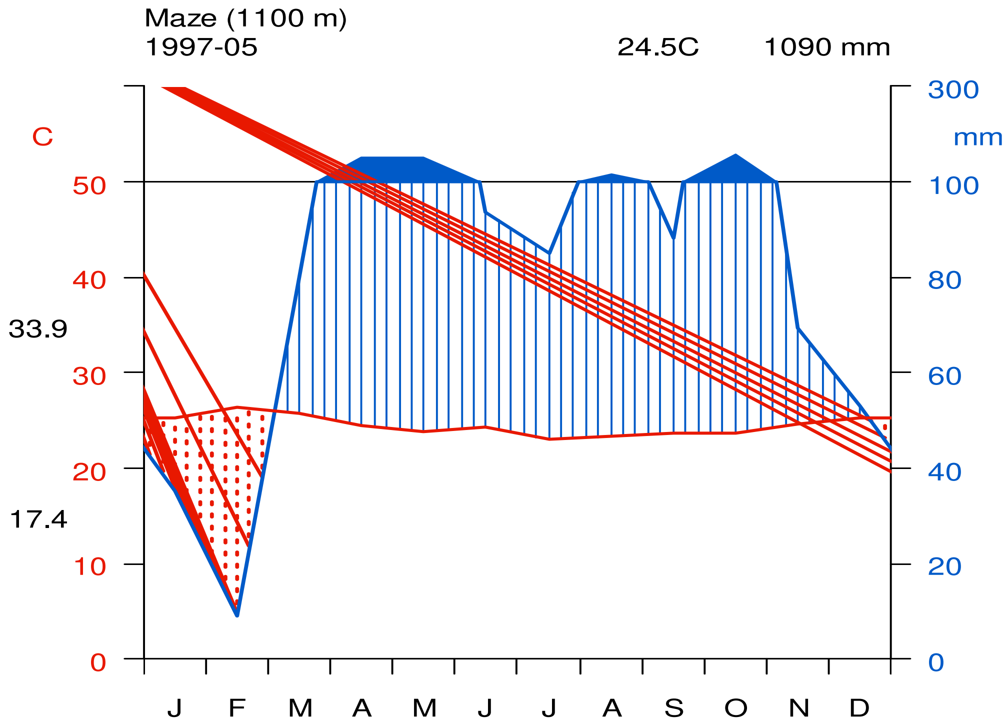

Figure 2. *Average maximum, minimum temperature and average rainfall, Source: - ENMA (2007)*

Plant diversity analysis

The evenness and diversity of plant species was analyzed using the Shannon Evenness Index (E) and Shannon-Wiener Diversity Index (H) (Jayarman, 2000). Shannon and Wiener (1949) index of species diversity was applied to quantify species diversity and richness. This method is one of the most widely used approaches in measuring the diversity of species. The diversity of each cluster was calculated using Shannon-Weiner diversity index, based on cover/abundance value of the species as input source. The relative equitability (evenness) of the species in each cluster was also calculated.

Frequency

Frequency is the number of times a species occurs in a given number of repeatedly placed small sample plots or sample points. The frequencies of the vascular plant species in all the 65 plots were computed. The higher the frequency, the more important the plant is in the community. The importance of a species with frequency was obtained by comparing the frequency of occurrences of all of the plant species present. Relative frequency was calculated for the most frequent tree species in the study area following Martin (1995) and Jha (1997) (*Eq. 1 and 2).*

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$$\text{Relative Frequency} = \frac{\text{Frequency.of.a.species} \times 100}{\text{Total.frequency.of.all.species}} \quad \text{(Eq.1)}$$

$$\text{Frequency\%} = \frac{\text{Number.of.plots.with.individual.species} \times 100}{\text{Total.number.of.plots.studied}} \quad \text{(Eq.2)}$$

Results and Discussion

Floristic composition and diversity

The analysis of floristic data on Maze National Park vegetation occurring between altitudinal gradients of 937 m and 1076 m a.s.l. indicates the presence of higher species diversity. A total of 144 species (a complete species list can be provided upon request) belonging to 109 genera and 37 families were recorded. The most species-diverse families are Fabaceae/Leguminosae represented by 29 species (20.14 %) followed by, Poaceae 23 species (15.97%), Euphorbiaceae 9 (6.25) species; Asteraceae 7 (4.86) species; Lamiaceae 6 (4.17) species; Acanthaceae, Combretaceae and Anacardiaceae each by 5 (3.47) species as shown in *Figure 3*. These 8 families constituted 61.8 % of the species in the study area while the rest of the families contributes 38.2%. Out of the identified species, 40% were herbs, 37 % trees, 17 % shrubs, and 6% were climbers (*Fig. 4*).

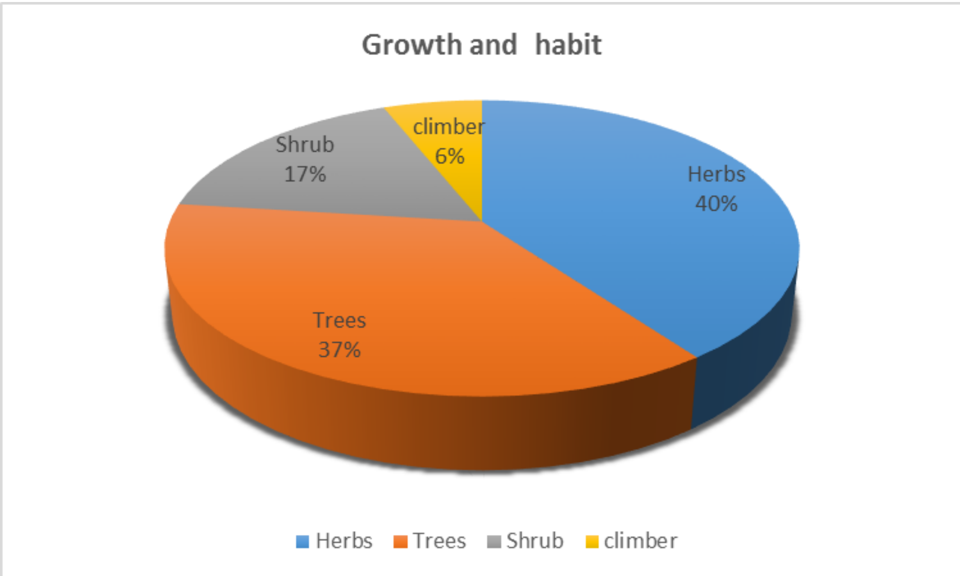

Figure 3. *Growth and habit of plants in Maze national park*

When compared with other national parks in Ethiopia such as Nechisar National Park and Omo National Park in species richness, the result is more or less similar in species composition. For example, Schloder (1999) in Omo National Park recorded 121 plant species among which grass species were 27. In Nechisar National Park 199 species belong to 44 families (Andargie, 2001) and 131 plant species representing 44 different

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families (Hasan, 2008). In all these studies the family Poaceae and Fabaceae were the dominant.

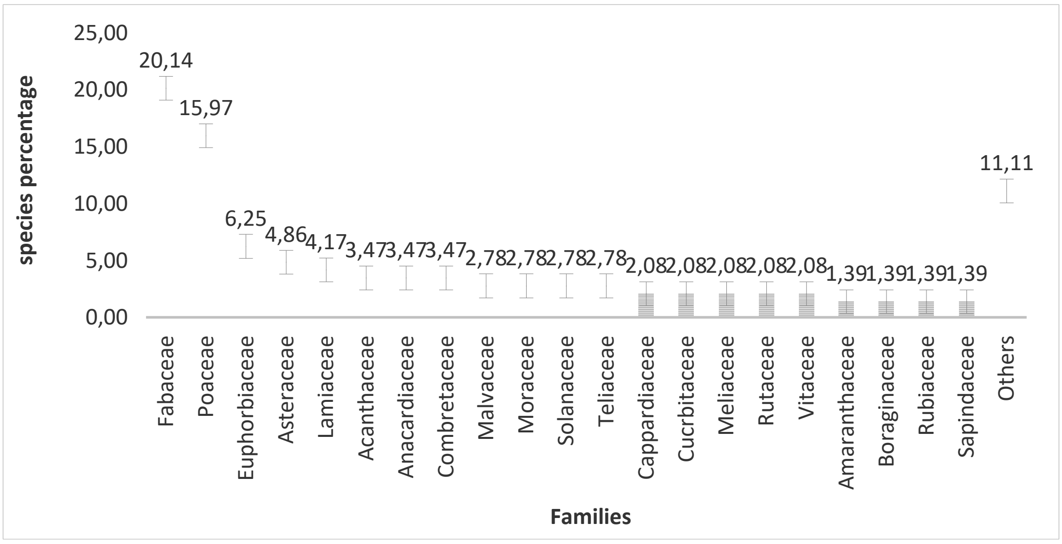

Figure 4. *Families of plants with percentage of their species*

The most diverse genus was Acacia represented by 8 species, Ficus and Panicum each represented by 4 species. Combretum, Hyparrhenia, Solanum, Grewia and Vernonia are the genera represented by 3 species while other genera were represented by one or two species as shown in the *Figure 5*.

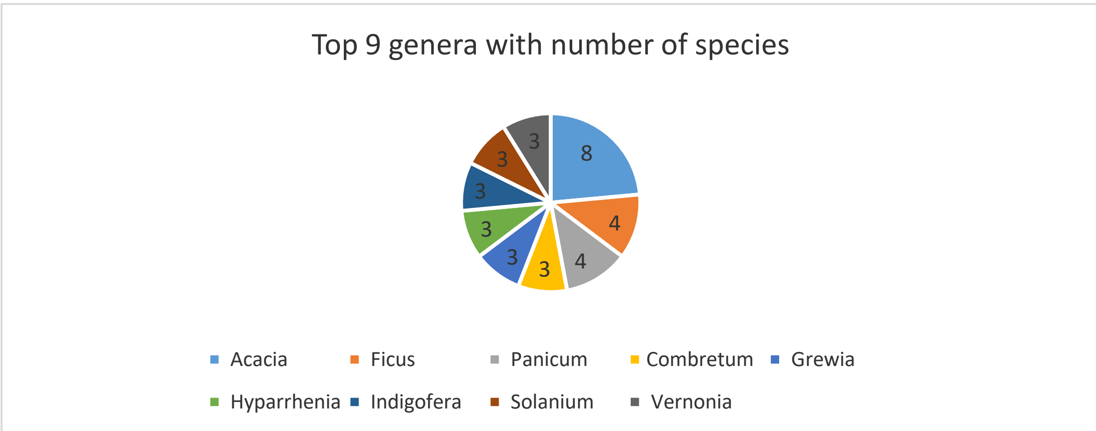

Figure 5. *Genera with the number of species*

Vegetation is said to have high diversity if it has many species and their abundance is fairly even, conversely, diversity is low when the species are few and their abundance is unevenly distributed. In the present study, the highest species richness was found in community type IV, which is from the plain grassland with an open Combretum-Terminalia woodland vegetation. An arrangement of the community types in decreasing order of species richness is IV, VI, V, II, I and III. The high species richness is probably

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attributed to the optimum environment that supports the Savanna Wooded land species or attributed to the minimum level of disturbances.

The community, which is relatively lower in species richness (community III), appears to have high evenness. An arrangement of the groups in decreasing evenness gives the order III, I, IV, VI, II, V. This arrangement does not agree with the arrangement of the community type in decreasing species richness. In some community type such as community type III, where the constituent species appear to be distributed evenly; there is less species richness as compared to others.

This study does not follow the diversity trends observed in species richness (Whittaker, 1975). It is highest in species rich and lowest in species poor community. Nevertheless, maximum diversity is achieved when each species is represented by equal numbers of individuals (Pielou, 1969). Therefore, from the present information at hand, maximum diversity could not be achieved, because communities with species rich in general did not appear to have an evenly distributed species. It is known that, the more number of species in an ecosystem and or community, the greater the stability. In other words, high environmental stability leads to higher community stability which in turn permits high diversity of species (Bormann and Kellert, 1991). Hence, community type II and V are considered to be in less stable environments and therefore have less species richness. Community type I and III also show lower species richness compared to the other community types, because they represent species from two and three sample plots respectively.

According to Kent and Coker (1992), The Shannon index is the most frequently used for the combination of species richness and relative abundance and the index normally varies between 1.5 and 3.5 and rarely exceeds 4.5. In the present study, the index is between 2.11 and 2.66, showing more or less even representation of individuals of all species in the sampled quadrats. Since it is known that the increase in the number samples will increase the species encountered (McNaughton and Wolf, 1973), the less species richness encountered in community types I and III could be the few sample stands (plots) representing the community. Moreover, the area covered by these two (2) community types is also small, thus represented by few sample stands (plots). Riparian Plant Communities are rich in species and highly variable in species composition in both temperate and tropical systems (Pollock, 1998). However in this study the species richness is low in community VI which is from riparian vegetation and even the smallest species richness is recorded in plots which are under this community such as plot 50, 51 and 52 each with 6 species. The diversity of each cluster was calculated using Shannon diversity index based on cover abundance value of the species as input source. The Shannon Diversity computed for six different communities (*Table 1*) shows that community type I is the most diverse whereas community type V is the least diverse of plant species.

Table 1. *Shannon Diversity Index of Species*

Diversity Index	Community type					
	I	II	III	IV	V	VI
Species richness	26	46	25	96	47	56
Species diversity	2.66	2.29	2.45	2.45	2.03	2.41
Species evenness	0.97	0.94	0.98	0.96	0.91	0.96

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Plant communities

The 65 plots studied were grouped into clusters with the aid of computer program TWINSPAN (Hill, 1979). Two-way TWINSPAN output resulted in a cluster of six plant community types at altitudes between 937 m and 1076 m a.s.l. including type I (*Achyranthyus aspera- Combretum molle*), type II (*Rhus natalensis – A. drepanolobium*), type III (*Sorghum versicolor- Heteropogon contortus*, type IV (*Combretum adenogonum - Loudetia arundinacea*), type V (*A. polyacantha – A. senegal*), type VI (*A. polyacantha - Saccharum spontaneum*) in Maze National Park.

The synoptic abundance cover value (*Table 2*) was used to clearly determine the dominant species in the community. The average cover/abundance value of each species in each cluster identified was computed and then characteristic species and local plant community types of the cluster were recognized. To know about the variation among plant communities ANOVA has been done using MINTAB 14 computer program (MINITAB, 1995). The following plant communities have been named by two dominant species based on the highest mean cover/abundance value that appears within a cluster (*Table 2*).

Table 2. *Synoptic cover abundance values for species reaching ≥ 2% in at least one community*

Botanical Name	**CI**	**CII**	**CIII**	**IV**	**V**	**VI**
Achyranthyes aspera	**32.5**	7.86	0	0	2.6	3.2
Combretum molle	**27.5**	0	0	4.6	0	0
Acacia seyal	**25**	0	0	0	8.3	0
Rhynchosia minima var prostrata	**22.5**	0	0	0	0	0
Asparagus flagellaris	**10**	0	0	0	0	0
Ocimum urticifolia	**10**	0	0	0	0	0
Harrisonia abyssinica	**7.5**	0	10.3	0	0	0
Rhus natalensis	**7.5**	**31.57**	6.7	2	5.1	0
Acacia drepanolobium.	0	**20.71**	**12**	4.9	0	0
Acacia seyal	0	**20**	0	0	4.4	0
Sorghum versicolor	0	0	**30**	16.3	0	4.6
Heteropogon contortus	0	0	**15**	8.2	0	0
Capparis tomentosa	0	0	**15**	0	0	0
Grewia bicolor	0	0	**13.3**	0	0	0
Euphorbia schimperiana	0	0	**13.3**	0	0	0
Combretum adenogonium	0	0	0	**25.2**	0	2.1
Loudetia arundinacea	0	0	6.7	**24.2**	0	0
Acacia hockii	0	0	0	**4.3**	0	0
Acacia senegal	17.5	20.7	0	2.7	**20**	0
Panicum maximum	0	5	0	4.1	**18.3**	19.8
Panicum porphyrrhizos	0	17.1	0	13	**16.4**	2.8
Acacia polyacantha	2.5	0	0	0	**21.3**	**40.8**
Ficus sycomorus	0	0	0	0	5.8	**15.8**
Saccharum spontaneum	0	0	0	0	0	**31.5**

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The following plant communities were identified from the output of TWINSPAN

Type I. Achyranthyes aspera - Combretum molle community

This community type is found between altitudinal ranges of 1024 and 1050 m a.s.l and consists of 2 plots with 26 species. The dominating tree layer species in this community are *Combretum molle, A. senegal, A. seyal, Bridelia scleroneura*. *Harrisonia abyssinica, Ocimum urticifolia, Lantana viburnoides, Asparagus flagellaris* and *Rhus natalensis* are the shrub layer species in the community. The herb layer species belonging to this community are *Achyranthes aspera, Justicia ladanoides* and *Sporobouls pyramidalis*. This community is seen in the Tela Ayisera localities at the northern part of the park. This community is from disturbed area of the park and dominated by *Achyranthes aspera*.

Type II. Rhus natalensis - Acacia drepanolobium community

This community type is distributed between altitudinal ranges of 941 m and 1076 m a.s.l. and consists of 7 plots with 46 species. The dominant tree species are *Acacia drepanolobium, A. seyal* and *A. senegal*. *Rhus natalensis* and *Clerodendrum myricoides* are among the shrub and *Panicum porphyrrhizos* is the dominant grass in the community.

Type III. Sorghum versicolor - Heteropogon contortus *community*

This community consists of 3 plots and 25 species distributed between altitudinal ranges of 1010 m –1016 m a.s.l. The community is dominated by the cover abundance of grasses such as *Sorghum versicolor*, *Heteropogon contortus* and *Hyparrhenia anthistirioides*. *Capparis tomentosa*, *Combretum aculeatum*, *Euphorbia schimperiana* and *Grewia bicolor* are the common shrubs in the community while *Acacia drepanolobium* is the tree species found scattered in the community.

Type IV. Combretum adenogonum - Loudetia arundinacea community

This community consists of 3 plots and 25 species distributed between altitudinal ranges of 1010 m –1016 m a.s.l. The community is dominated by the cover abundance of grasses such as *Sorghum versicolor*, *Heteropogon contortus* and *Hyparrhenia anthistirioides*. *Capparis tomentosa*, *Combretum aculeatum, Euphorbia schimperiana* and *Grewia bicolor* are the common shrubs in the community while *Acacia drepanolobium* is the tree species found scattered in the community.

Type V. Acacia polyacantha - Acacia senegal community

This community type consists of 8 quadrats in which 46 species distributed between altitudinal ranges of 956 m and 1027 m a.s.l. This community consists of plots from riparian vegetation. In this community *Acacia polyacantha* and *A. senegal* are the most dominating tree layer species. *A. albida, A. seyal, Ficus sycomorus*, and *Ziziphus spina-christi* are the other common tree layer. *Rhus natalensis* and *Panicum maximum* are the dominant shrub and herb layer respectively.

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Type VI. Acacia polyacantha - Saccharum spontaneum Community

This community type consists of 13 quadrats with 56 species distributed between altitudinal ranges of 937m and 1025 m a.s.l. This community also consists of plots from riparian vegetation and it is the second community in species richness next to community type IV. The most dominating tree species in this community are *Acacia polyacantha, Ficus sycomorus, Lepidotrchilia volkensii, Terminalia brownii* and *Sclerocarya birrea, Setaria barbata, Saccharum spontaneum, Panicum maximum* and *Sorghum versicolor* are the most dominating herb species

In order to know whether there is variation among the plant communities in species richness, evenness, diversity and altitude, one way ANOVA was done using MINTAB 14 computer program. The result of ANOVA showed that there was statistically significant difference among plant communities in species richness, altitude, evenness, and species diversity at 95% confidence interval (*Table 3*).

Table 3. *Summary of ANOVA among the 6 communities*

Source	DF	SS	MS	F	P	Remark
Species richness	5	180.16	36.03	3.61	0.006	Significant
Altitude	5	25868	5174	3.35	0.01	Significant
Species evenness	5	0.0235	0.0047	7.18	0	Significant
Species diversity	5	2.0409	0.4082	5.75	0	Significant

A 2 sample t-test was done in order to know the existence of variation within a community using species richness, evenness, species diversity and altitude. The result of 2-sample t-test shows that there is a significant difference at a 95% confidence interval within community types (*Table 4*).

Table 4. *Summary of t-test within 6 plant communities*

	Community type					
	I	II	III	IV	V	VI
Species richness	15.50 a	11.86 aa	13.33 aaa	13.00 aaab	9.50 aaaca	9.62 aaada
Species Evenness	0.973 a	0.944 aa	0.976 aaa	0.964 aaaa	0.908 bbbba	0.957 aaaab
Species diversity	2.657a	2.290 aa	2.452 aaa	2.446 aaa	2.030 aaaba	2.109 aaaca
Altitude(m)	1037.0 a	988.3 aa	1013.0 aaa	1013.2 aaaa	992.4aaa aa	965.2 aabba

The comparison of plant communities based on the mean of species richness showed that plant community type IV, is significantly different from community type V and VI.

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Because community type IV is found on the upland plain while community type V and VI are found along riparian area. There is a similarity in species composition between community V and VI. This is due to their habitat that they are riparian vegetation along the Lemase and Maze rivers. In both community types *Acacia polyacantha* is the dominant species while *Ficus sycomorus* and *Ehretia cymosa* are commonly found. Plant communities also vary in their mean of species evenness. Community type V is significantly different from all other community types in species evenness. There is also a significant difference with in a community types in species diversity. Community type IV is significantly different from Community types V and VI. The reason for this is community type IV is found on the wooded grassland and wooded land part of the park while community type V and VI are from the riparian area. There is also significant variation in altitude with in the plant community types. Community type VI is significantly different from community type III and IV. The result of ANOVA showed that there is a significant difference among plant communities with respect to species diversity, richness, evenness and altitude at $P< 0.05$.

Frequency

Frequency denotes the number of plots in which a given species occurred in the study area. The four most frequently occurring tree species in Maze National Park are *Combretum adenogonium* 29 (44.6 %), *Acacia polyacantha* 26 (40.00%), *Grewia mollis* 23 (35.38 %) and *Maytenus senegalensis* 22 (33.85%), *Acacia drepanolobium* 20 (30.77), where as *Panicum maximum* 28 (43.08), *Panicum porphyrrhizos* 26 (40.00%), *Hyparrhenia filipendula* 23 (35.38 %), *Loudetia arundinacea* 23 (35.38 %), and *Sorghum versicolor* 22 (33.85%) are the frequently occurring grasses in the study area. *Rhus natalensis* 20 (30.77%) is the shrub with highest frequency. *Combretum adenogonium* and *Panicum maximum* are widely found in the wooded grassland while *Acacia polyacantha* is found along riverine vegetation and swamp areas. The frequently occurrence of *Combretum adenogonium* in the woodland area may be due to fire resistant nature of the bark.

Threats to the biodiversity of the area

Ecosystems are being affected and destroyed by a wide variety of human activities which results in an irreversible loss of biodiversity. There are several threats that can cause reduction of biodiversity in Maze National Park, such as poaching, uncontrolled fire, and honey production (smoke), deforestation by the local communities which have been observed during the study time.

The armed groups of people around and inside the park have been causing problems on the park, and, hence it is very dangerous to enter the park. These illegal hunters and other people set fire to burn the vegetation in order to attract wild games for hunting, to make open foot tracks for better visibility, to avoid or protect themselves from dangerous harmful animals and to obtain young grass shoot for their livestock. Burning of the vegetation is commonly seen in almost all parts of the park during the dry season.

Seasonal burning is largely determined by the availability of ignition source and seasonal variation inflammability (Waring and Schlesinger, 1985). Besides seasonal burning in Maze National Park, the local people set fire deliberately during the dry season and just before the rainy season of the area to burn the vegetation in order to stimulate new and fresh leaves for grazing purpose. Burning of the vegetation results in

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altering the natural soil balance considerably by removing the humus from the surface of the topsoil, destroying microorganisms, and increasing the concentration of salts (Vickery, 1984). It also affects successions and disrupts the natural stability of the original climax community (Abrahmson, 1984). Fire can also leads to the extinction of unique community of plants and animals. On the other hand controlled fire management is necessary to the range lands of the park; to remove unpalatable grass, to avoid coarse forage and to initiate the microbial activity in the soil.

In the present study area, the cause of fire appears to be anthropogenic aiming at certain goals such as to attract wild games for hunting, to make open foot tracks for better visibility, to avoid or protect themselves from wild animals and to obtain new grass growth for their livestock. This may result in loss of biodiversity in the area in the future. Fire, clearing forest for construction, hunting for bush meat, overgrazing and over browsing by domestic stocks are the factors responsible for the change in vegetation composition of Maze National Park, while hunting is so dangerously affecting the wildlife fauna in the area, such conclusion is in line with Daubenmire (1974). Another threat to the biodiversity of the area is that the local community around the park cut trees inside the park for the purpose of building material and firewood (*Figure 6*) which results in deforestation of the area. Some of the wild animals in Maze National Park such as lion and leopard are feared by the local population and hence want to protect themselves by burning their habitat.

Figure 6. *Title of the given figure, graph or image included in the document*

In Ethiopia within 6 years a total of more than 148 fires covering an area of 100966 hectares has been reported (*Table 5*). The figure was noted in 2000 where forest areas of 95000 hectares have been burned.

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Table 5. *Forest fire statistics of Ethiopia*

Year	**Total no. of fires on forests and other wooded lands**	**Area of forest burned (ha)**
1990	4	1072
1991	2	153
1992	1	32
1993	20	3159
1994	1	1550
2000	>120	95000

Conclusion and Recommendations

Maze National Park is one of the national parks of Ethiopia with rich species' diversity representing the Combretum-Terminalia woodland vegetation. The study resulted in documentation of 144 vascular plant species representing 109 genera and 37 families.The five most species-diverse families tree layer in Maze National Park were Fabaceae/Leguminosae, Poaceae, Euphorbiaceae; Asteraceae and Lamiaceae. The most diverse genus was Acacia followed by Ficus and Panicum. Six plant communities were identified and described with varying degree of species richness, evenness and diversity. Plant community IV exhibited the highest species richness (96) while the highest diversity was observed for community type II. Community type III was known with the least species richness but highest species evenness. The result of ANOVA showed that there is statistically significant difference among plant communities in species richness, altitude, evenness, and species diversity at 95% confidence interval.

Anthropogenic activities in Maze National Park were identified to exert high pressure on the vegetation and wildlife in the area resulting in the loss of biodiversity underpinned by troubled natural habitat. The major anthropogenic activities in the study area with the aforementioned detrimental effects were: poaching, fire, cutting of trees for fuel wood purpose and construction. The most feasible recommendation to reverse the undesirable condition in the national park is to actively participate the local people in the conservation activities of the national park via ecotourism which is one form of sustainable tourism. For example landscape, wild animals, the hot springs and associated medicinal values, and many other cultural packages in the national park could be sustainably utilized for ecotourism in line with the sustainable development goals.

Since this study was limited to certain parts of the park, there is a need for more comprehensive studies in different parts of the park if proper and long-term management plan of the park is desired. In a nutshell, analysis of soil and other components of the natural environment should be some of the future research areas of the national park to enable sustainable management of the national park and other protected areas in Ethiopia.

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SEASONAL PERFORMANCE AND CHARACTERISTIC OF ABR FOR LOW STRENGTH WASTEWATER

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Abstract. The performance and the characteristics of a laboratory-scale ABR (anaerobic baffled reactor) were investigated during different seasons (summer, spring, autumn and winter). ABR successfully achieved COD removal efficiencies 74% during summer, 68% during autumn/spring and 62% during winter. Compartment I, II and III showed high removal rate of COD during whole study period. At lower OLR (organic loading rate), COD removal rate was high. The analysis of biogas production during all seasons showed downward trend with increase of HRTs (hydraulic retention time). Compartment I showed high VFA (volatile fatty acid) synthesis compared to others compartments. The ABR has the potential to provide a greater efficiency and be applicable for all type of seasons and temperature conditions.
Keywords: *anaerobic baffled reactor (ABR); chemical oxygen demand; hydraulic retention time; volatile fatty acids; organic loading rate*

Introduction

Because of rapid urbanization and economic growth, world water resources are becoming constantly polluted and deficient in most of the region (Paraskevas et al., 2002). Worldwide, adequate sanitation and access to safe water is a big problem for billions of peoples (Moe and Rheingans, 2006). The demand is increasing for efficient, reliable and low cost wastewater treatment systems, particularly in scattered regions and where insufficient wastewater treatment systems were existing. Therefore, there is a need to implement sustainable and appropriate sanitation and wastewater management practices (Katukiza et al., 2012).

Conventional wastewater treatment systems are costly for small localities and housing societies (Nath and Sengupta, 2016). The wastewater treatment method is constrained by considerations of local regulation, population and topography, lead to challenging performance and design. Because of environmentally friendly and low energy requirement, ABR was found as an attractive method (Boonsawang et al., 2015), and suitable wastewater treatment solution for low income areas (Kamali et al., 2016). The biological advantages of the ABR are well documented, and from last decade anaerobic processes has proven to be a better alternative of wastewater treatment (Zhu et al., 2016). Over the last few decades, several papers have been published on ABR performance. Grobicki and Stuckey (1991) studied the hydraulic loading rates on mass transfer and reaction rate limitations. Nachaiyasit and Stuckey (1997) investigated the effect of shock loads on the performance of an ABR. Liu et al. (2007) studied

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hydrodynamic characteristics of ABR. Design simplicity, high treatment efficiency, non-sophisticated equipment, low operational and capital costs are main advantages of ABR (Zwain et al., 2014).

The ABR is a powerful anaerobic digester which internally comprises by a series of hanging and standing baffles, wastewater flow from one chamber to next under and over the baffles as flow from inlet to outlet (Ayaz et al., 2012). Treatment is achieved naturally selected anaerobic biota in the form of anaerobic digestion without application of oxygen or mechanical mixing. In addition, anaerobic digestion could be achieved by separation between HRT (hydraulic retention time) and SRT (solid retention time), that allow anaerobic microbes to remain within the reactor independently from the wastewater flow (Plósz, 2007). Although organic material and suspended solids are efficiently removed by ABR, the process has no or very little effect on nutrients (nitrogen and phosphorus) and pathogens removal (Nasr et al., 2009). Therefore, post-treamtent is needed in removing, residual COD and total suspended solids as well as reducing concentration of nutrients and pathogens. The main purpose of this research was to investigate seasonal performance of ABR for domestic wastewater. The reactor performance was evaluated under different HRTs and seasonal condition. A five chamber PVC made reactor selected for this study to provide a simple and low-cost treatment system for seasonal wastewater treatment. HRT, OLR and seasonal effect on COD removal, gas production and VFAs synthesis were studied.

Material and methods

Reactor setup

A lab-scale anaerobic baffled reactor was constructed from PVC material with dimension 1m long, 0.2 m wide and 0.75 m high with 100 L effective volume (*Fig. 1*). The reactor contained five compartments and each compartment subdivided into down-flow and up-flow units by using high/low vertical baffles. These baffles regulated the flow of wastewater in ABR. Each chamber was filled with 5 cm wide and 35 m long non-woven cloth to prevent biomass washout. Outlet of each compartment had DN10 sampling port and bottom equipped with mud tubes and valves. A peristaltic pump was used to adjusted flow rate.

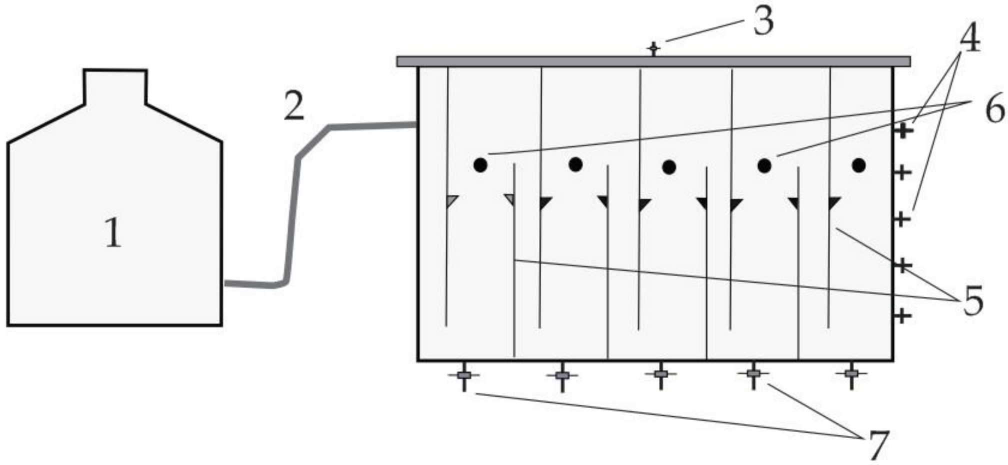

Figure 1. *Schematic diagram of anaerobic baffled reactor; 1. Wastewater Storage Tank; 2. Inflow; 3. Gas outlet; 4. Outflow; 5. Baffles; 6. Sampling points; 7. Valves.*

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Reactor setup

The raw wastewater was obtained from the campus of the Southeast University at Wuxi. The wastewater generated from dormitories, restaurants and laboratories of Universities campus. The quality of sewage fluctuated because of dilution by rainwater, and behaves similar as a decentralized wastewater. The sewage quality is presented in *Table 1*.

Table 1. *Main characteristics of the influent water*

Parameters	pH	COD[1]	TSS[1]	Temperature (˚C)		
		(mg l^{-1})		Summer	Autumn	Winter
Range	6.89 - 7.23	183.0 - 324.5	172 - 364	25 - 35	17 - 22	5 -15
Mean	7.06	258.4	276	30	20	9

[1]*In this table COD stand for chemical oxygen demand, whereas TSS is total suspended solids.*

Analytical methods

Standard methods (American Public Health Association, 2005) were used for analytical determination. Chemical oxygen demand (COD) was measured by closed reflux colorimetric method (Method 5220 D). DO and pH analyzed by DO200 and PH100 probes (YSI) respectively. VFA was measured by modified distillation method.

Experimental procedure

The experimental reactor had been running for all season from the system start-up. Air temperature during the summer, autumn and winter seasons was 25 – 35 ˚C, 15 – 20 ˚C and 3 – 12 ˚C respectively with ± 4 ˚C wastewater temperature. HRTs was adjusted 24 h, 48 h, 72 h and 96 h for summer, 48 h, 72 h, 96 h and 120 h for autumn/spring and 72 h, 96 h, 120 h and 144 h for winter season. During autumn and spring season temperature range was similar hence considered single season.

Data analysis

SPSS version-18.0 (SPSS incorporation Chicago, Illinois, USA) and MS-excel programs were used for data analysis and presentation.

System start-up

An ABR start-up is a complex process, slow growing anaerobic biomass first needs to be established in the reactor and reactor requires period of several months to reach full treatment capacity (Barber and Stuckey, 1999). Reactor was inoculated with anaerobic bacteria by activated sludge, which obtained from local wastewater treatment plant (WWTP), Wuxi, China. These added bacteria multiply and adapted to wastewater. Many factors can affect start-up of ABR such as concentration and composition of wastewater, pH, temperature, HRT, reactor size and structure etc. (Hassan et al., 2015). Potential problem can arise during start-up because of plug flow, low pH and accumulation of Volatile Fatty Acid (VFA) (Liu et al., 2010). Some approaches such as feed dilution, organic loading rate (OLR), periodic feeding and effluent recycling, can help to overcome these difficulties. Low loading start-up,

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reducing the concentration of organic matter promotes granular sludge growth, allowing the bacteria enough time to multiply before suspended solids are washed out (Sallis and Uyanik, 2003). For start-up, the reactor operated for 50 days with HRT 72 h and gradually reduced to 48 h and then 24 h until the COD removal efficiency stabilized at 60%, and PH stabilized between 7.03 - 7.23 (*Table 2*).

Table 2. *ABR start-up operational condition*

Phase	Time (days)	Temperature (˚C)	HRT[1] (Hours)	Volume Load (kg/m$_3$•d)	COD[2] Removal (%)
I	20	16 - 23	72	0.67 - 1.06	51
II	15	18 - 28	48	0.92 - 1.61	60
III	15	20 - 31	24	1.76 - 3.24	61

[1]*HRT = hydraulic retention time;* [2]*COD = chemical oxygen demand*

Results and discussion

COD removal

HRT is one of the most important factors affecting the COD removal in the anaerobic reactor (Ozgun et al., 2013). At higher HRT, contact time of sewage was increased in reactor which results in the improvement of COD removal rate (Chelliapan et al., 2014). But too long HRT decrease flow rate which unable to stirred anaerobic sludge. *Fig. 2* shows HRT relation with the COD removal rate during different seasons. HRTs were "24 h, 48 h, 72 h, 96 h"; "48 h, 72 h, 96 h, 120 h" and "72 h, 96 h, 120 h, 144 h" during summer, autumn and winter respectively. In general the trend is increase COD removal efficiency with the increase of HRT. The average COD removal efficiencies were 60%, 65%, 72%, and 74% during summer, 59%, 64%, 67% and 68% during autumn and 51%, 55% , 61% and 62% during winter. The increase of the HRT from 24 h to 72 h during summer, 48 h to 96 h during autumn and 72 h to 120 h during winter rises in the COD removal efficiency significantly but when HRTs increased from stated HRTs no significant changes in COD removal efficiency occurred. As compared to summer, during autumn and winter temperature was low. In those seasons microbial activities and metabolic rate was lower. Seasonal prolonging of HRTs helps to increase the contact time of microorganisms and the substrate to improve the microbial activity and thus COD removal rate. While too long increase in HRT effect reactor feeding and reduces the mass transfer between the sludge and the substrate (Bayo et al., 2016). Therefore no significant changes occurred in COD removal rate.

Fig. 3 demonstrate COD removal rate at different compartment of ABR at different HRTs during summer, autumn and winter seasons. First compartment showing higher removal efficiency compared to all other compartments and this followed by second, third, fourth and fifth compartment. As HRT increases the removal rate also increase. This was possibly the result of elevated substrate concentration which also increased substrate flux into the bioaggregates resulting increased of microbial growth (Pirsaheb et al., 2015). Removal rate in compartment I, II and III show high removal rate of COD in all seasons while compartment IV and V showing low removal rate probably because most of the COD has been removed in first three compartments and anaerobic microbes have low nutrients (Zhu et al., 2008).

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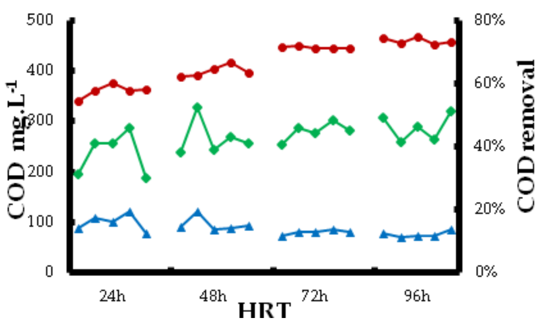
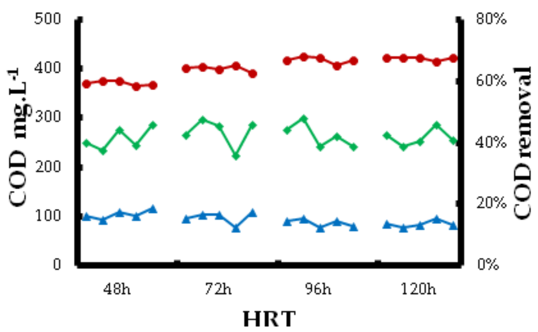
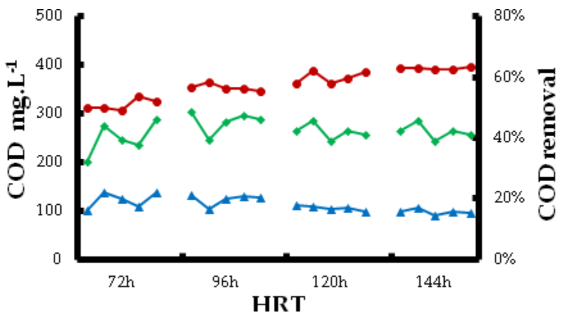




a. Summer

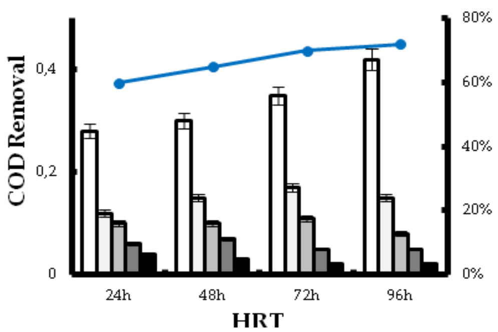
b. Autumn

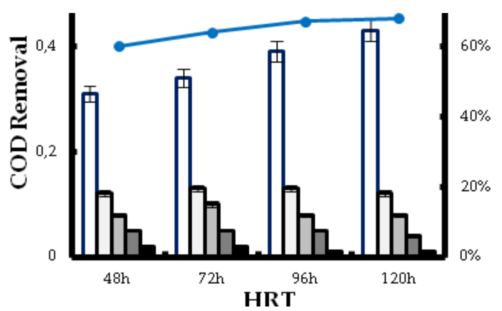


c. Winter

***Figure 2.** COD removal efficiency of ABR under different HRTs*

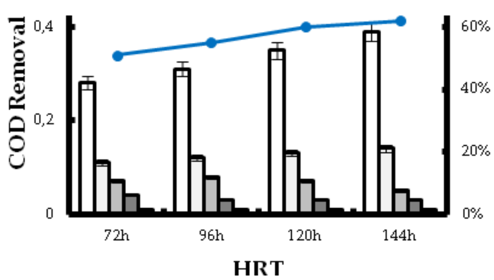
Influent water *Effluent water* *COD removal efficiency*





a. Summer

b. Autumn



c. Winter

***Figure 3.** COD removal efficiency of ABR under different HRTs*

Compartment I *Compartment II* *Compartment III*
Compartment IV *Compartment V*

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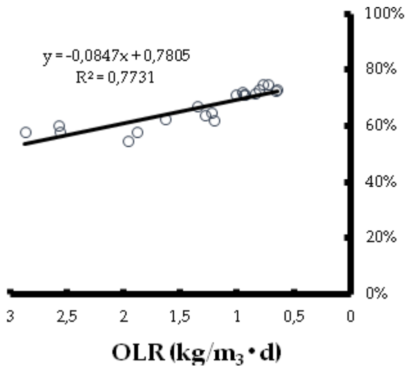
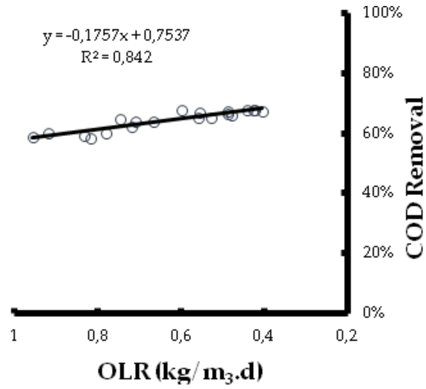
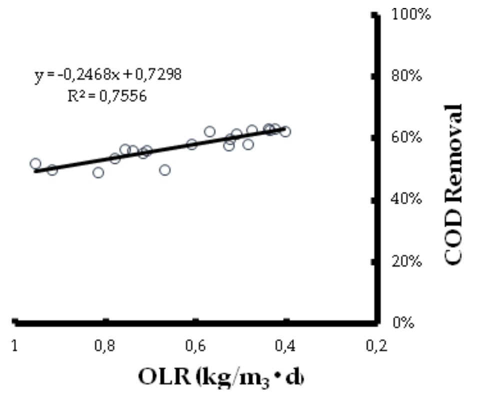
OLR is chief factor which indicates the amount of volatile solids to be fed into the reactor every day (Dhar et al., 2015). OLR control the growth of sludge, microbial activity and degradation efficiency. It's directly linked to supply and demand relationship between substrate and microbes in the reactor (Boonsawang et al., 2015). *Fig. 4* shows the relationship of organic loading rate (OLR) with COD removal efficiency. With the change of OLR removal efficiency also affected, at the lower OLR removal efficiency was high. Increasing OLR gave an increased substrate concentration and elevated microbial growth resulting high COD removal rate (Kanimozhi and Vasudevan, 2014). However, further increased in the OLR dropped the removal efficiency. This might be due to the fact that high organic loadings brought a decrease in volatile suspended solids (VSS) and accumulation of inorganics subsequent destabilization of the reactor and process, which affects the reactor performance (Demirer and Chen, 2005).



a. Summer



b. Autumn



c. Winter

Figure 4. *Showing the correlation between OLR and COD removal*

Biogas production

Reduction of organic matter in the reactor is directly related to the gas production. The final product of anaerobic biological degradation is biogas. The main composition of biogas produced in ABR is methane and carbon dioxide (Pereira et al., 2013). Methane and carbon dioxide production decrease from compartment I to V during all seasons. *Figure 5* illustrating the gas production during different seasons at different HRTs. Biogas production showed a wide fluctuating during entire study period. During

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all seasons, biogas production showed downward trend with increase of HRTs. This maybe at low HRT up-flow velocity in each compartment was high, which produce uniform mixing in the reactor and make the nutrients available resulting promotion of biogas production. While at higher HRTs although wastewater and sludge contact time was sufficient but because of low flow rate of wastewater in the reactor no mixing was taken place. During summer at 24 h of HRT the maximum average gas production was 1.53 L/ d and when HRT was 96 h, the lowest gas production was 0.43 L/ d. During autumn and winter when HRT increases from 48 h to 120 h and 72 h to 144 h gas production was decreased significantly from 0.55 to 0.14 L/ d and from 0.23 to 0.02 L/ d respectively. As compared to summer and autumn, during winter season temperature was very low, the gas production dropped significantly. Due to the increased solubility of gases at low temperatures, a large amount of biogas dissolved in water (Cadena-Pereda et al., 2012). Meantime, low temperature also impact on methanogen bacterial activities and suppressed biomethanation processes.

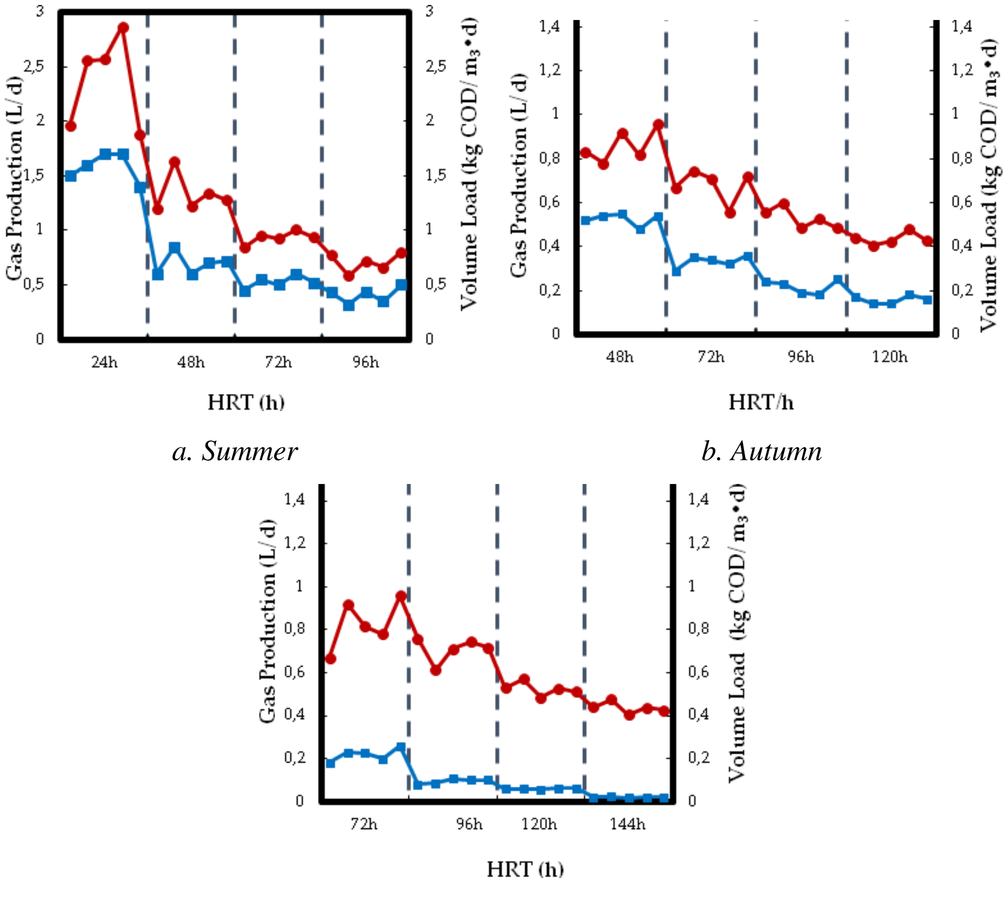

Figure 5. *Biogas production in ABR under different HRTs during different seasons*
Gas production ■ *Volume load* ●

Volatile fatty acids (VFA)

High concentration of VFA during anaerobic process can inhibit methanogenesis process. Under overloading conditions, methanogenic activity cannot remove volatile

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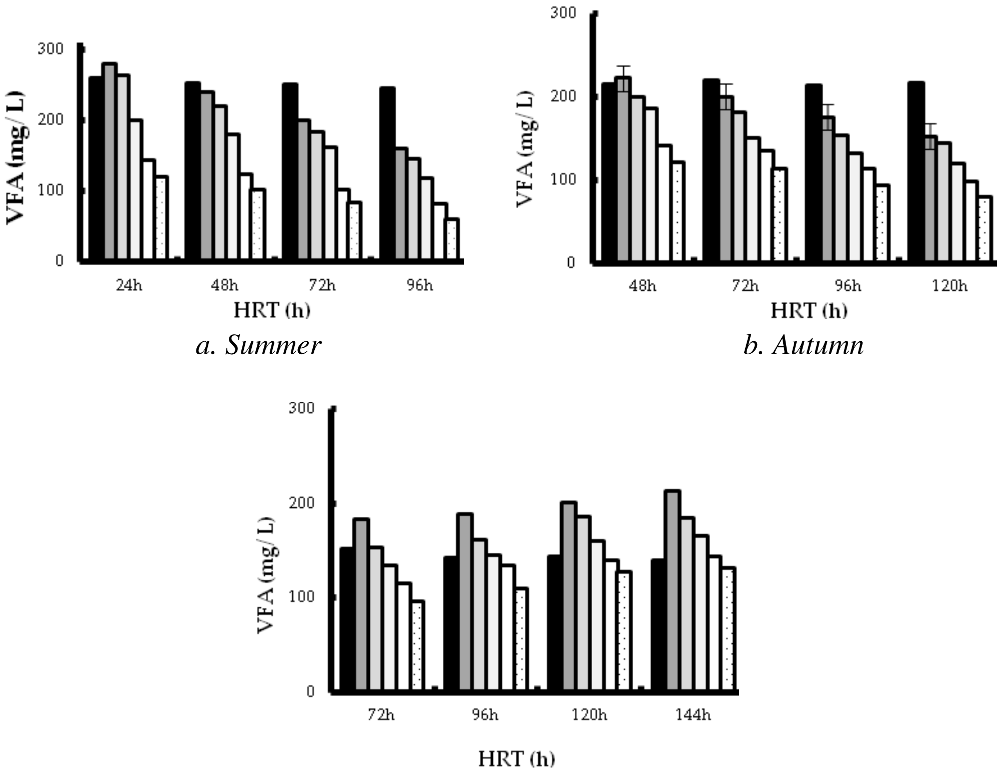
organic solids as result acids accumulate in the reactor and depress the pH at levels that inhibit acidogenisis or hydrolysis phase. It also shown even at optimum pH volatile fatty acids may contribute to reduced rate of hydrolysis. The pH is chief element to control anaerobic process. The optimum pH for methanogen organism is 6.6 - 7.6. At higher pH free ammonia can inhibit anaerobic metabolism, in addition, if pH not held fairly constant accumulation of excess volatile acids occur (Yirong, 2014).

Figure 6 shows the VFA concentration during summer, autumn and winter seasons at different HRTs. When HRT prolonged, VFA concentration in reactor decreased. At all phases of HRTs, the first compartment had higher VFA concentration and this followed by II, III, IV and V compartment. This is mainly because the compartment I received the maximum organic load, thus anaerobic microbes produced higher concentration of VFA and descending order in other compartments. During summer and autumn seasons, compartment I had higher concentration of VFA than influent concentration at lower HRTs, but as HRTs increase compartment I shows decrease concentration compare to influent VFA. At lower HRT high flow rate cause acidification and supress the methanogen degradation of VFA (Chelliapan et al., 2011), however at higher HRTs when organic flow rate was low, most of the VFA was consumed. Throughout winter seasons mostly compartments showing higher VFA values than influent VFA. During winter seasons, low temperature limited the activities of methanogen bacteria, resulting accumulation of VFA.



a. Summer *b. Autumn*

c. Winter

Figure 6. *VFA removal efficiency of ABR under different HRTs*

Inflow *Compartment I* *Compartment II*
Compartment III *Compartment IV* *Compartment V*

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Conclusions

Following are the main conclusion of the study:

- COD removal efficiency enhanced with increasing HRTs. During summer, autumn and winter season when HRTs were 72 h, 96 h and 120 h COD removal efficiency were 72%, 67% and 60% respectively.
- Most of the organic matters were degraded in first four compartments.
- The change of OLR would affect the organic matter removal efficiency and it is found that at low OLR removal efficiency was high.
- Biogas production decrease at low temperature, average gas production during summer, autumn and winter was 1.53, 0.58 and 0.23 L / d respectively.
- VFA concentration decrease with increase of higher HRT, and compartment I had higher production of VFA.
- The ABR has the potential to provide a greater efficiency and be applicable for all type of seasons and temperature conditions for organic loading, however post treatment is required for nutrients and pathogens removal.

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THE COMPLEX INVESTIGATION OF THE COLONIZATION POTENTIAL OF *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE) IN THE SOUTH PANNONIAN ECOREGION

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Abstract. *Aedes albopictus* is the potential vector of several pathogens. Recently, the South Pannonian ecoregion has been the border of the east Mediterranean established range of the species. We aimed to determine the current range limiting factors, to predict the potential seasonality and to model the future distribution of the mosquito in the Carpathian Basin. Mosquito trapping was performed in the border region. Larvae were collected from local waters. Based on the new and the previously performed collections, we analysed the limiting climatic factors of the occurrence. The calculation of the full ontogeny was based on previously published experimental results. The analysis of the climate of the successful and non-successful trapping sites showed the complex conditionality of the habitat preference of *Aedes albopictus*. We confirmed the presence of *Phlebotomus neglectus* in South-western Hungary and of *Aedes albopictus* in Zagreb. We found that *Aedes albopictus* can tolerate the climate of the mountainous regions of the Mediterranean where there are relatively cold winters due to high annual precipitation. Both the climate envelope model results and the gained range limiting factors support the hypothesis that the recent climate of Hungary allows the expansion of the mosquito in the Carpathian Basin.
Keywords: *climate envelope modeling, established occurrence, Köppen-Geiger climate classification, potential generation number, mosquito trapping, Phlebotomus neglectus*

Introduction

Aedes (Ae.) (*Stegomyia*) *albopictus* Skuse (1894), also known as the Asian tiger mosquito is a competent vector for at least 22 arboviruses including Dengue virus, Chikungunya virus, Zika-virus, West Nile virus, St. Louis encephalitis and Japanese encephalitis (Bonialuri et al., 2008; Grard et al., 2007; Gratz 2004; Ibáñez-Bernal et al., 1997; Knudsen et al., 1996; Moore and Mitchell, 1997; Sardelis et al., 2002; Wong et al., 2013) as well as several species of filarial nematodes e.g. *Dirofilaria* species (Hochedez et al., 2006; Cancrini et al., 2003). The rapid spread of *Ae. albopictus* and the transmitted pathogens were observed in the last few decades (Knudsen et al., 1996; Mitchell, 1995; Urbanelli et al., 2000). Since the first European appearance in Albania, the Asian tiger mosquito demonstrated a remarkable invasive potential in the Mediterranean region (Urbanelli et al., 2000). The mosquito recently occurs in each of the countries from Portugal to Greece (Benedict et al., 2007; Kalan et al., 2011; Klobučar et al., 2006; Merdić, 2011; Petrić et al., 2001, 2006; Scholte and Schaffner, 2007). Due to Yugoslav war and the political redistributions of the past few decades in the Balkan there are relatively sparse data about the climatic limitations of *Ae. albopictus* populations in the South Pannonian ecoregion and the Northern Balkan which is the doorstep of the Central European region. What is an alarming phenomenon for Central Europe that introduced occurrences of the mosquito were observed in Slovakia and the Czech Republic more recently (Bocková et al., 2013; Šebesta et al., 2012).

Ae. albopictus is abundant in such areas where the mean annual temperature is more than 11°C (the threshold of adult activity and survival), mean winter temperature more than 0°C (the threshold of egg overwintering) and the mean annual precipitation is at least 500 mm (Medlock et al., 2006; Mitchell, 1995). For comparison, the mean annual temperature is 10-11 °C, the annual precipitation is 500-750 mm and the mean January temperature is between -0.3 and -2 °C in most of the areas of Hungary according to the 1971-2000 reference period (OMSZ). It was pointed out that *Ae. albopictus* may not survive through winter if the diapause period exceeds six months (Medlock, 2006). It means that in certain parts of Hungary, the climatic threshold already approximates the climatic requirements of *Ae. albopictus* in the last three decades of the 20th century. Based on the recent dispersal rate and climate suitability of the species, the predicted effects of climate change, the future spread of the mosquito into the recent temperate climate areas of Europe is very likely (Knudsen et al., 1996; Mitchell, 1995). *Ae. albopictus* prefers the peridomestic environment, breeding in both natural and artificial small waters colonizing even barrels, drinking troughs, rainwater gulley catch basins, tires and several other type of technotelmata (Gatt et al., 2009). According to their breeding habitat preference, the Asian tiger mosquito can inhabit the urban and suburban habitats (Juliano and Lounibos, 2005). The length of the activity and the reproductive season and egg diapause is controlled predominantly by the ambient temperature conditions. The photoperiodic threshold of the production of diapausing eggs occurs below 13 or 14 hours of daylight in many locations; however in some northern areas it can occurs at 11 or 12 hours (Medlock, 2006). Air temperature has also important influence on the population dynamics (Alto and Juliano, 2001) and the ontogeny of *Ae. albopictus* (Delatte et al., 2009; Calado and Silva, 2002). Higher temperature conditions increase the speed of the development, the number size and the rates of the winter survive of diapausing eggs (Medlock et al., 2006). The minimal threshold of each ontogenic stage is at 10.4°C, the optimum is at 29.7°C based on the experimental investigations of Delatte et al. (2009). The populations of the Asian tiger mosquito living in the tropical and subtropical areas have no winter diapause. Under cooler climatic conditions, the populations of *Ae. albopictus* can overwinter by producing eggs which have winter diapause (Medlock et al., 2006). The production of

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overwintering eggs is triggered by the decreasing tendency of daylight hours and occurs in late summer and early autumn.

The adaptation of *Ae. albopictus* to the cooler European conditions were observed due to the increasing hatching success and cold tolerance of diapausing eggs of the European *Ae. albopictus* populations (Thomas et al., 2012). In addition, in the Mediterranean, as the coastal and lowland areas of the Apennine Peninsula where the more severe freeze is rarely occur, the populations of the Asian tiger mosquito shows the signs of cold-acclimation as adults and remain active throughout the entire winter season (Romi et al., 2006). Larval development including each of the instars takes 3-8 weeks, while the mean lifespan of the adults is about 3 weeks, although in case of the overwintering individuals of female mosquitoes in the Mediterranean area it can takes some months (Gatt et al., 2009). The length of activity varies according to the annual temperature patterns and the peak season also changes according to the availability of blood meal and water in a certain area. Giatropoulos et al. (2012) found that the numbers of the produced eggs are the highest in mid-July to November.

Romi et al. (2006) proved that *Ae. albopictus* can adapt for the local environment which means that natural selection results strains with different climatic requirements and overwintering potential. According to this fact, the special investigation of the environment requirements of the North Balkan climatic strains is justifiable. While the potential future established range of the species falls within the Carpathian Basin, it remained a somewhat under-represented area in the more recent distribution modeling studies. We studied the range-limiting climatic factors of *Ae. albopictus* supplementing the previously published occurrence data with the result of our collecting survey in the transition of the south part of the Pannonian ecoregion and the North Balkan. We focused on the established occurrences of the species. Our aim was also to model the potential near future distribution and generation numbers of the Asian tiger mosquito according to the temperature patterns of the last 10 years.

Materials and Methods

The three employed approaches

The course of the study was the following: according to the results of our survey and completed with the data and results of other trapping surveys (respecting the mapped data of the European Centre for Disease Control and Prevention's V-BORNET program, 2015) we aimed to analyze the climatic factors of the distribution in the studied area and to correlate the found suitable and non-suitable climatic conditions with the climate categories of the Köppen-Geiger climate classification system. In this study, the analysis of the climatic factors of the present occurrence of *Ae. albopictu*s also was based on the established habitats of the mosquito in the studied area. Occurrences based on a single observation were not taken into consideration since only one milder can lead to the survival of the species in a given site.

The near future spread of the Asian tiger mosquito was studied along three main ideas:

1) the analysis of the limiting factors of the current distribution that may reflect rather the climate patterns of the near past than the present conditions,

2) the calculation of the potential annual generation numbers which approach also show the climatic suitability of the mosquito in a given area

3) and the prediction of the recent and the near future potential distribution of the mosquito using a climate envelope model.

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Climate data sources

The climatic database of the Climate Explorer of the European Climate Assessment and Data was used at the Climate Explorer homepage of the Koninklijk Nederland Meteorologisch Institut (KNMI, 2014). The half-gridded climate data of the E-OBS climate model was used with 0.25° resolution. Mean minimum January temperature (°C), January mean temperature (°C), sum of the autumn and winter precipitation (mm) and mean July precipitation (mm) were invoked for the period of 1950-2014. Since *Ae. albopictus* appeared in Croatia and Slovenia in the 2000's, the most recent climatic data from the 1st of January 2000 to the 1st of October 2014 were used and the data was averaged for this period in each of the 49 involved trapping sites. The 49 sites represent 20 control non-successful and 29 successful trapping sites which consist of 16 sites from our own collector survey representing the surveyed area in climatic aspect (7 sites from Hungary, 7 from Croatia and 2 from Slovenia). For the calculation of the potential generation number in Hungary, the daily temperature values of the last ten years were averaged according to the following covering grid: 45.75-48.0N and 16.0-23.0 E.

For modeling the recent and future distributions of *Ae. albopictus*, we used the REMO climate model, which is nested into the ECHAM5 global climate model and is based on the IPCC SRES A1B scenario. 1961-1990 is the reference period and the periods of 2011-2040 and 2041-2070 are the selected prediction periods. REMO has 25 km horizontal resolution and the entire Europe is within its domain. The A1 scenario family describes an economically rapidly developing world with a similar rapid technological evolution and unimodal global population run with a peak in the mid-21th century (IPCC reports; Working Group I: The Scientific Basis). The A1 is based on the idea of the increasing volume of the socio-cultural and innovative interactions what is a clear trend in the age of the recent electronic social network revolution and in the light of the volume of the multinational cooperation. The decrease of the regional differences is also an important basic idea of the A1 which trend recently is visible in the relation of the decreasing difference between the economy of the Western and the BRICs countries (Jain, 2006). Since the model studied the climate requirements only of the European populations – the North African distribution segments were excluded –, it was able to project the shift of this part only.

Mosquito field survey

The study area covers selected parts of four major river valleys, which fall in this area: the valley of Sava, Drava, Tisza (at Szeged city, Southeastern Hungary) and the Danube (at the Mohács town, South Hungary). Most of the mapping points were performed on the settlements which are adjacent to the floodplain of the Sava and Drava rivers. Drava is a river at the Hungarian-Croatian and Slovenian border, which was preserved in its natural conditions with oxbows, branches and a meandering mainstream with islands. We considered these areas optimal for natural expansion of *Ae. albopictus*, because river valleys are often regarded as natural conveyance routes in the temperate climate zone. *Fig. 1* shows the sampling points.

Two CDC Miniature Light Traps (John W. Hock Company, Florida State, USA) were operated at five sites in or near to three villages in Hungary and in a recreational part of Zagreb, Croatia. Light traps were not baited with attractants such as carbon dioxide. Twenty eight, mostly small scale water bodies were sampled, 24 of which contained mosquito larvae. The larvae were collected with a fine scale sieve and

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reloaded into the filtered water of the respective sampled water body in individual two-liter open PET containers with openings covered by nets. About 10 to 20 dips with a fine scale sieve were taken per aquatic systems and 1-3 aquatic systems were sampled at each site. The containers were incubated at room temperature (20-25°C) and protected from direct sunlight for 2 weeks. Later emerged adults were released to PVC bags and immediately killed by dry cold shock (-20 °C) lasting for one hour, which left characteristic morphological features intact.

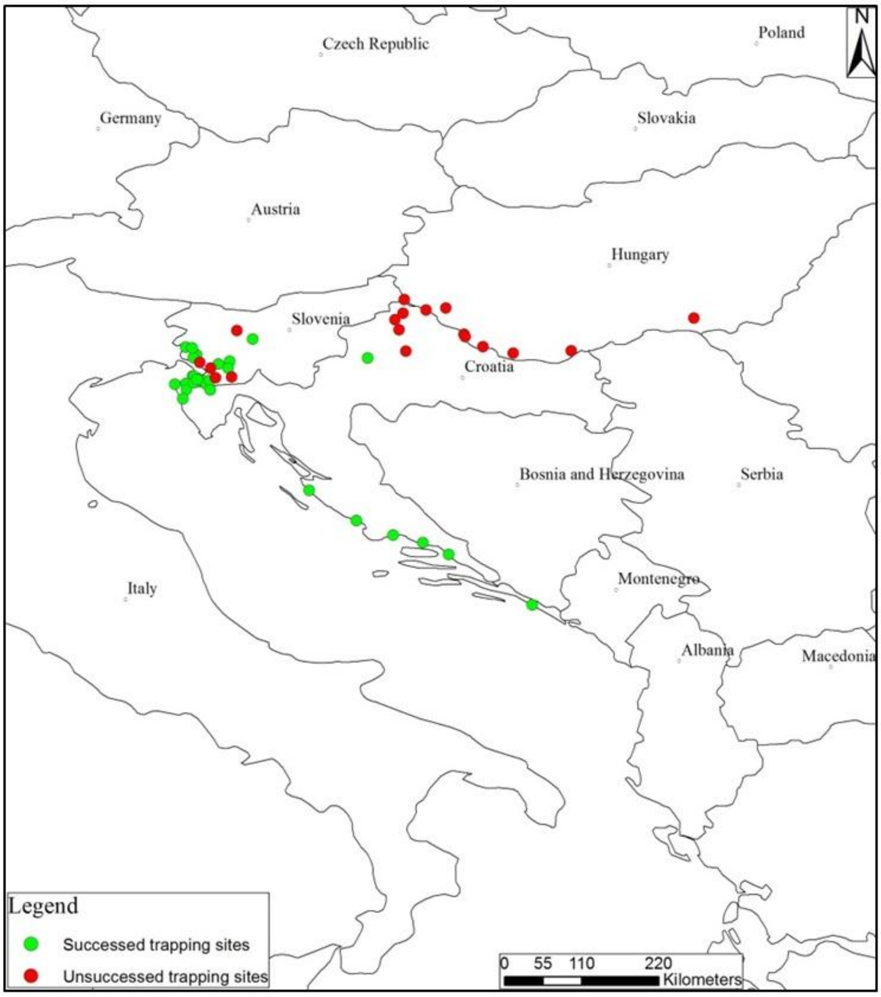

Figure 1. *The sites of the trapping points used in the climatic analyses. Our trapping points were completed with the established (green) and non-established (red) occurrences of Ae. albopictus according to the trapping monitoring of Kalan et al. (2011) and Merdić et al. (2011).*

Additional trapping data

In total, 49 trapping sites were added to the analysis, of which 8, 22 and 19 represented Southern Hungary, Slovenia and Croatia, respectively. The latter two sets of tiger mosquito trapping sites (Slovenia: Kalan et al., 2011; Croatia: Merdić et al., 2011) were introduced into our model to gain a wider climatic context. Positive and negative trapping points from every sub-region in the three studied countries were selected. In case of some Croatian data the 25° grid of the trapping point were identifiable after the

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published maps, although the exact settlements are not in some north coastal area. In this cases a reference settlement were indicated in the subtitles of the figures. It is important to note that this circumstance does not affect the results of the study since only the climatic factors were used in the analyses, which are the same in a given 25° grid according to the averaged values. For CEM, the distribution data of the mosquito was gained from the VBORNET database (VBORNET, 2015). Only the established occurrences of the mosquito were involved to the model.

The calculations of the development times

To create the temperature based development by ontogeny stages model of *Ae. albopictus* mosquito we used the observations of Calado and Silva (2002) who examined the development of the Asian tiger mosquito in each ontogeny stages under permanent temperature regimes (15°C, 20°C, 25°C and 30°C). Since the minimal threshold temperature of the development of *Ae. albopictus* is 10.4°C (Delatte et al., 2009), under this threshold the development was not interpreted. We fit exponential regression model to the mean monthly temperature-period of development pairs of the data of Calado and Silva (2002) to gain correlations between temperature and the mean time of the steps of the metamorphosis according to the minimum time of the ovule-instar 1° metamorphosis (Eq.1,), the minimum cumulative time of the instar 1° to the instar 4° metamorphosis (Eq.2) and the mean time of the pupa-adult metamorphosis (Eq.3) under different temperature conditions. The basic equations can be seen in the *Fig.6* according to the experimental results of Calado and Silva (2002):

$$t_{ov-1°} = 82.171e^{-0.101T} \text{ if } 30°C \geq T \geq 10.4°C \quad \text{(Eq.1)}$$

$$t_{1°-4°} = 155.57e^{-0.114T} \text{ if } 30°C \geq T \geq 10.4°C \quad \text{(Eq.2)}$$

$$t_{p-a} = 34.051e^{-0.098T} \text{ if } 30°C \geq T \geq 10.4°C \quad \text{(Eq.3)}$$

where T is the daily mean temperature in °C, tov-1° is the minimum cumulative time of the ovule-instar 1° metamorphosis, t1°-4° is the minimum cumulative time of the instar 1° to the instar 4° metamorphosis and tp-a is the time of hatching (*Fig.2*).

The total (minimum) development time an individual is the sum of the (minimum) time of the each ontogeny times (Eq.4):

$$t_{total} = t_{ov-1°} + t_{1°-4°} + t_{p-a} \quad \text{(Eq.4)}$$

An ontogeny stage is completed, when the sums of the daily development proportions reach the value of 1. Numbers of above 1 were considered and depicted as 1 since in biological sense a value above 1 is uninterpretable. The daily development proportion of the total development is the reciprocal of the daily value. The numbers of the reciprocal amounts that provide the value of 1 give the days of the full ontogeny time which is the function of the changing temperature. For example in case of the larval development the number of days can be determine according to the following formula (Eq. 5):

$$n \gg \sum_{i=1}^{n} \frac{1}{82.171\, e^{-0.101\, T_1}} + \ldots + \frac{1}{82.171\, e^{-0.101\, T_n}} \leq 1 \quad \text{(Eq.5)}$$

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where n is the number of days of full larval development; $T1$ is the mean daily temperature of the first day of the week, when the temperature exceeds the 10.4°C value and no longer fall under this value until autumn, Tn is the temperature of the n^{th} day when the sums of the daily development values reach first or exceed the value of 1.

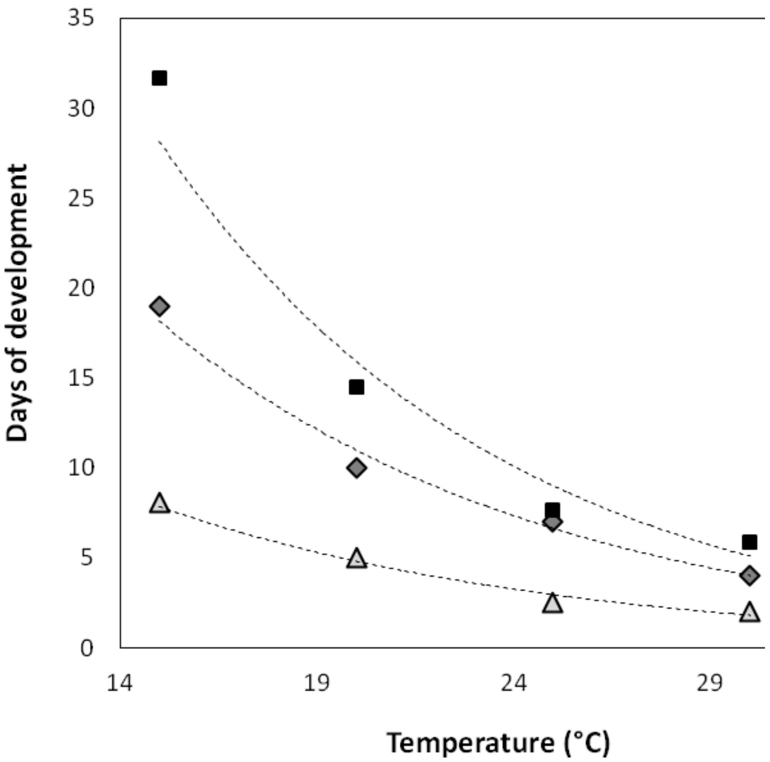

Figure 2. *The mean time of the ovule-instar 1° metamorphosis, the mean cumulative time of the instar 1°- instar 4° metamorphosis and the mean time of the pupa-adult metamorphosis under different temperature conditions according to Calado and Silva (2002).*

According to the development, the logical order of the calculation line is the following: larval development time, the time of pupation and finally, the time of hatching. It means that a day after that the sums of the daily reciprocal larval development values reach first or exceed the value of 1, starts the calculation of the development time of pupation, when a day after that the sums of the daily reciprocal pupal development values reach first or exceed the value of 1 starts the calculation of the hatching time and when the sums of the daily reciprocal hatching time values reach first or exceed the value of 1, the ontogeny ended and theoretically the individual is replication competent. Since the aim is to calculate the maximal annual generation number, it was considered to start the calculating of the development a next generation a day after the ending of the previous. The calculation of the sequence become terminated when the daily mean temperature drop below the threshold 10.4°C in autumn.

The developments of the generations throughout the year were depicted in one figure. The first part of the curve represents the larval ontogeny, the second the pupal and the third shows the hatching time. The proportion values above 1 were depicted as 1

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in the figures. The starting value is not 0, since in the activity season of the mosquito each day has a correspondent development value.

Data processing and statistics

Mood's median test and two sample t-tests were performed using R statistical tool27 to compare the distribution explanatory climatic conditions according to the control (non-successful) and case (successful) trapping sites. We encoded the successful trapping (presence) sites as 1, the non-successful (absence) sites as 0. The tests were evaluated at 5% significance level. Successful (case) and control (unsuccessful) trapping points were marked and used in each of the studied countries. Receiver Operating Characteristic (ROC) curve was drawn and Area under the ROC curve (AUC) value was calculated by pROC module28 of statistical software R. Principal Component Analysis and Hierarchical Clustering Analysis were performed by XLSTAT 10.029 (Adinsoft, 2010)

The geographical data were based on the satellite images of Google Earth™ 2013 application. We used the site appointing function of Google Earth imagery to mark the trapping points. To gain a higher resolution map further (non-trapping) points were involved into the model. Each marked points were named after its status (trapping, non-trapping or other points). We converted the designated spatial data into keyhole markup language (*kml*) file format type (altitude or gamma intensity data). After opening the *kml* data we converted them into shape file format in the ArcGIS 10.1 software. To create the potential annual generation number map of Croatia, Slovenia and Hungary we linked the points with the calculated generation numbers. The different values were assigned into the referred points and were sorted into attribute table. We interpolated the values of the spatial data by the IDW interpolation function of the Spatial Analyst Tool in the ArcGIS.

Climate envelope modeling

Climate has the greatest influence on the geographical distribution of the species in Europe (Thuiller et al., 2004). To project the current potential range and possible impact of climate change on the distribution of *Ae. albopictus*, we used the climate envelope modeling (CEM) method (Hijmans and Graham, 2006), which was applied with success earlier by Fischer et al. (2011) to predict the potential expansion of the same species. This modelling approach is based on statistical correlations between the observed ranges of species and environmental variables to define the limiting ecological factors, the climatic requirements (e.g. temperature and precipitation patterns) of the species (Hijmans and Graham, 2006; Guisan and Zimmermann, 2000; Elith and Leathwick, 2009). Using a climate scenario the climate envelope model can characterize the present and predict the potential future distributions of vector species. Important to note, that in the case of some vector-borne infectious diseases long-distance transport of vectors and migration of infected individuals might play a hardly predictable but crucial role as the modifier of the geographical occurrence (Walther et al., 2009). We used three physical (climate) factors averaged in the 30-years periods: the monthly mean temperature (T_{mean}, °C), monthly minimum temperature (T_{min}, °C), and monthly precipitation (P, mm) of the 12 months. Cumulative distribution functions were calculated by PAST statistic analyzer (Hammer et al., 2001) for the selected 3×12 climatic parameters (T_{mean}, T_{min}, P). A 10-10% from the extrema, except the precipitation (we neglected 0%

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only) in the case of *Ae. albopictus* were neglected from the climatic values found within the observed distribution (*Fig. 3*).

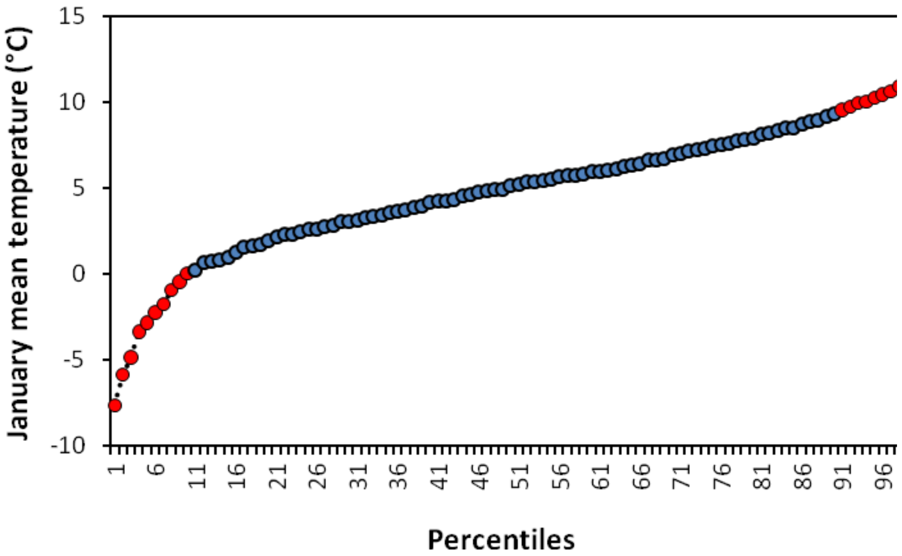

Figure 3. *The distribution of the percentiles of the mean January temperature. The 10-10% from the extrema were neglected (red points).*

The selection of the amount of percentiles to be left from the climatic values was based on our former results and elaborations. The aim was to reduce the false positive error of the model result in a reasonable degree. We refined the climatic data by Inverse Distance Weighted interpolation method of ESRI ArcGIS 10 software (ESRI, 2006). The modeling steps were the follows: first, the grid points within the distribution were queried; second, the percentile points of the climatic parameters were calculated; third, the suitable percentiles of the climatic parameters were chosen; fourth, modeling phrases (3 strings) were created by string functions of Microsoft Excel 2007 for the three modeling periods; fifth, the ranges were selected where all the climatic values of the certain period were between the extrema selected in step 3.

Results

Number of trapped biting mosquitoes (Culicidae) - A total of 2015 biting mosquito (Culicidae) specimens were collected and identified from the trapped and the incubated material. The major part (1644/2015; 81.6%) of the biting mosquitoes was gained through incubation, and 371 adults (18.4% of the total) were trapped at the four trapping sites (Nagyharsány: 33 and 5 Letenye, South-Southwestern Hungary; Zagreb: 12). The incubation was unsuccessful in 4 cases and in 3 cases incubation resulted in less than three specimens. In contrast, in 14 cases the number of the identified biting mosquitoes was more than 25 individuals, while 7 incubations resulted in over 100 adults. Four *Ae. albopictus* individuals were collected during this survey: 1 was incubated (*Fig. 4A, B*), 1 was trapped with a CDC trap, while 2 specimens were collected directly from our skin. Successful collections were restricted to the already known occurrence at the Prečko district of Zabreb, Croatia. Four non-culicid dipterans with vector importance

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(*Leishmania infantum*), namely *Phlebotomus neglectus* Tonnoir specimens were trapped in Nagyharsány (*Fig. 4C*). The identified single *Ae. albopictus* larva was found in a 60x40 cm rainwater collector. The dissolved oxygen-content was 6.89 mg l-1 at 22.5 °C water temperature (80.6% oxygen saturation) with 7.89 pH, 335 mS cm-1. The water collector stood in a moderately shaded environment close to the edge of the floodplain forest of the river Sava. A female mosquito was captured by hand at the same site (*Fig. 4D*).

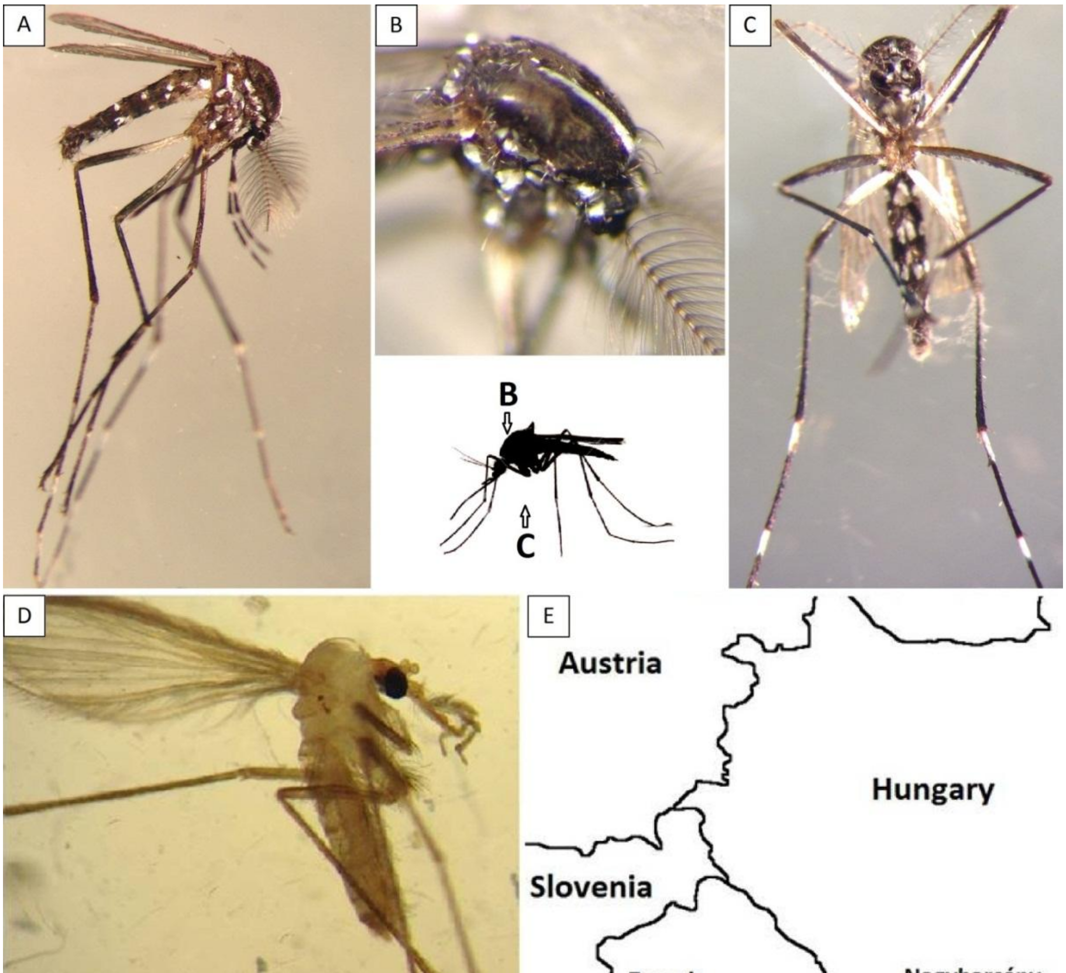

Figure 4A. *The right lateral view of a male Ae. albopictus mosquito individual from Zagreb (district Prečko), Croatia.* ***4B.*** *The single white line on the doro-lateral view of the thorax is characteristic of the Asian tiger mosquito.* ***4C.*** *the abdominal view of the specimen.* ***4D.*** *a male Phlebotomus neglectus individual from Nagyharsány, Hungary.* ***4E.*** *collecting sites of the individuals.*

Climatic factors potentially influencing the occurrence -We found a non-significant difference (p=0.3541) between the habited and non-habited trapping sites according to the sum of the July precipitation. The number of the successful trapping sites (presence) was 29; the number of the non-successful trapping sites (absence) was 20 (*Fig. 5A*). We found

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significant difference (p<0.001) between the successful and non-successful trapping sites according to the difference of the sum of the July precipitation where the mean minimum January temperatures were under the mean (*Fig. 5B*). To test the equality of the successful and the non-successful trapping sites according to the January mean temperature, the Mood's median test were used due to the non-normal distribution of the successful (presence) group. The test found a strongly significant difference (p<0.0001) between the two groups (*Fig. 5C*). To test the equality of the successful and the non-successful trapping sites according to the sum of the autumn and winter precipitation, the Mood's median test were used due to the non-normal distribution of the successful (presence) group. The test found significant difference (p<0.001) between the two groups (*Fig. 5D*). We found significant difference (p<0.001) between the successful and non- successful sites according to the mean of the minimum temperature in January. The number of the successful trapping sites (cases) was 29; the number of the non-successful trapping sites (controls) was 20. The mean of the successful (positive) trapping sites is -0.11 °C (SD: 1.7047), in case of the unsuccessful (negative) sites is -1.88 °C (SD: 1.110; *Fig. 5E*). We found significant (p=0.0185) difference between the habited and non-habited sites according to the difference of the mean of the January minimum temperature under mean of the minimum July sum of precipitation (*Fig. 5F*).

The results of the ROC analysis of the sum of the July precipitation where the following: true negative: 12, false negative: 10, true positive: 19, false positive: 8; n_{cases}=29, $n_{controls}$=20, auc=0.5594, power=0.1079. The best cut point is at 66.14 mm July precipitation according to the positive and negative condition values (*Fig. 6A*). The results of the ROC analysis of the mean minimum temperature in January where the following: true negative: 17, false negative: 7, true positive: 22, false positive: 3; nc_{ases}=29, $n_{controls}$=20, auc=0.7629, power=0.9194. The best cut point is at -1.2 °C January mean minimum temperature according to the positive and negative condition values (*Fig. 6B*).

Climate related habitat characteristics of *Ae. albopictus* - The difference from the average of the mean minimum January temperature (abscissa) and the difference from the average of the mean July precipitation (ordinate) of the studied sites for the period of 2000-2014 were depicted in a Cartesian-like coordinate system. Although the successful trapping sites overlap the humid subtropical climate (Cfa), the (Csa) warm Mediterranean climate, the cool oceanic climate (Dfc) and the (Cfb) temperate oceanic climate, as well as the (Dfb) temperate continental climate/humid continental climate. Most of the unsuccessful trapping sites are positioned in the lower left quadrant of the diagram (where the sites with colder winters and dryer summers of the temperate continental climate/humid continental climate (Dfb) according to the Köppen-Geiger climatic categories (*Fig. 7A*) are represented. The difference from the average of the mean minimum January temperature (abscissa) and the difference from the average of the sum of the autumn and winter precipitation (ordinate) of the studied sites for the period of 2000-2014 show a greater separation of the successful and non-successful sites (*Fig. 7*B). The studied trappings were performed in 3 major climate ranges of the North Balkan Peninsula according to the Köppen-Geiger climatic classification: in the Mediterranean coastline category which consists of the Cfa and the Csa climates, the Mediterranean Mountains which includes Dfc and the Cfb climates, as well as the (Dfb) continental climate. *Ae. albopictus* can be found in the Mediterranean Mountains and the Mountains of the Balkans (19 of the studied sites) and the Mediterranean coastline (15 of the studied sites) and only one habitat of the Asian Tiger mosquito established under continental climatic conditions (Zagreb; *Fig. 7C*). The difference from the average of the mean minimum January temperature (abscissa) and the difference from the mean of the sum of the autumn

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and winter precipitation (ordinate) of the studied sites for the period of 2000-2014 show a greater separation of the different climate points (*Fig. 7D*).

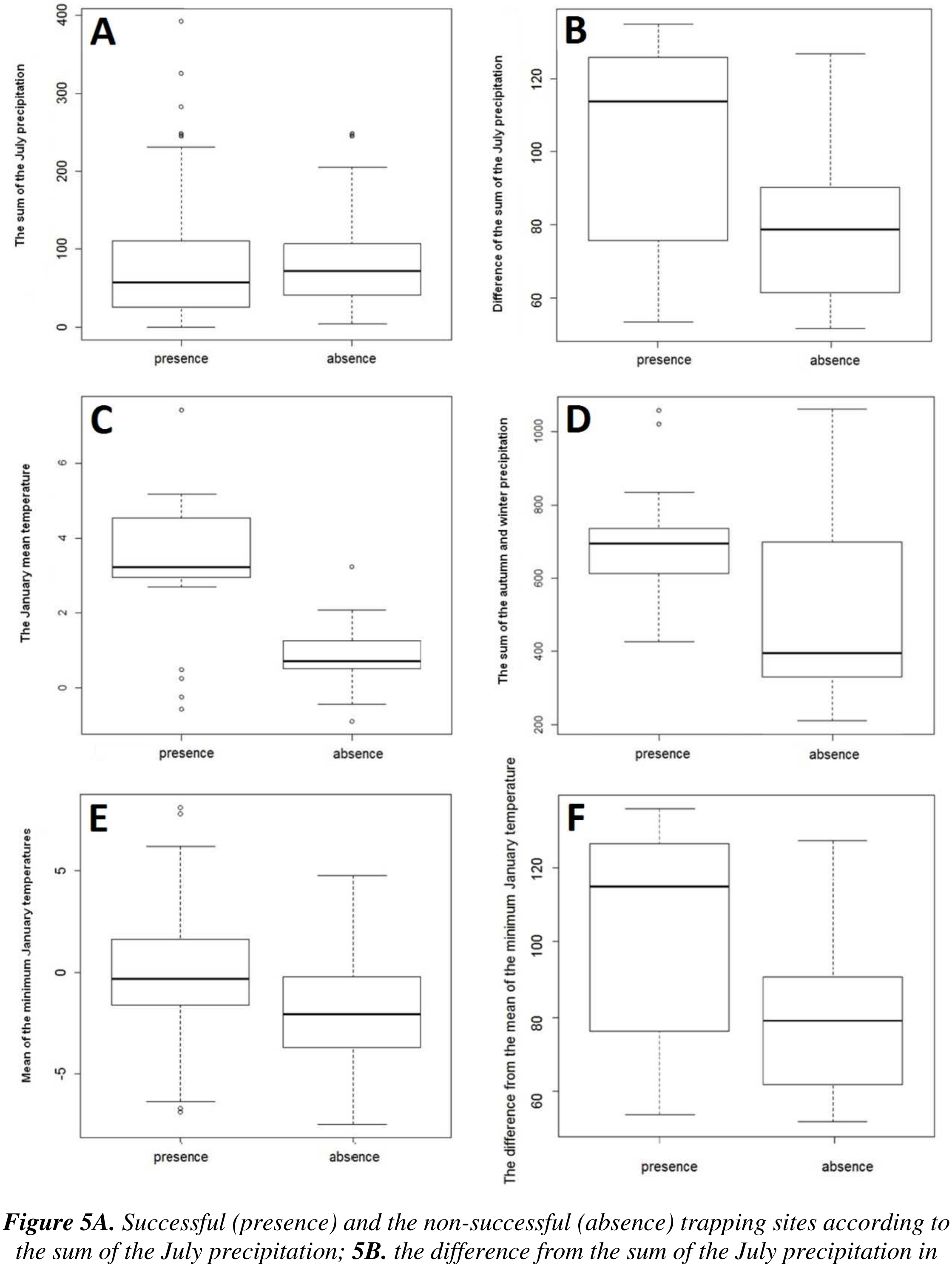

***Figure 5A.** Successful (presence) and the non-successful (absence) trapping sites according to the sum of the July precipitation; **5B.** the difference from the sum of the July precipitation in sites where the mean January temperature were under the mean; **5C.** the January mean temperature; **5D.** the sum of the autumn and winter precipitation; **5E.** the mean of the minimum January temperatures; **5F.** the difference from the sum of the mean minimum January temperatures in sites where the sum of July temperatures were under the mean.*

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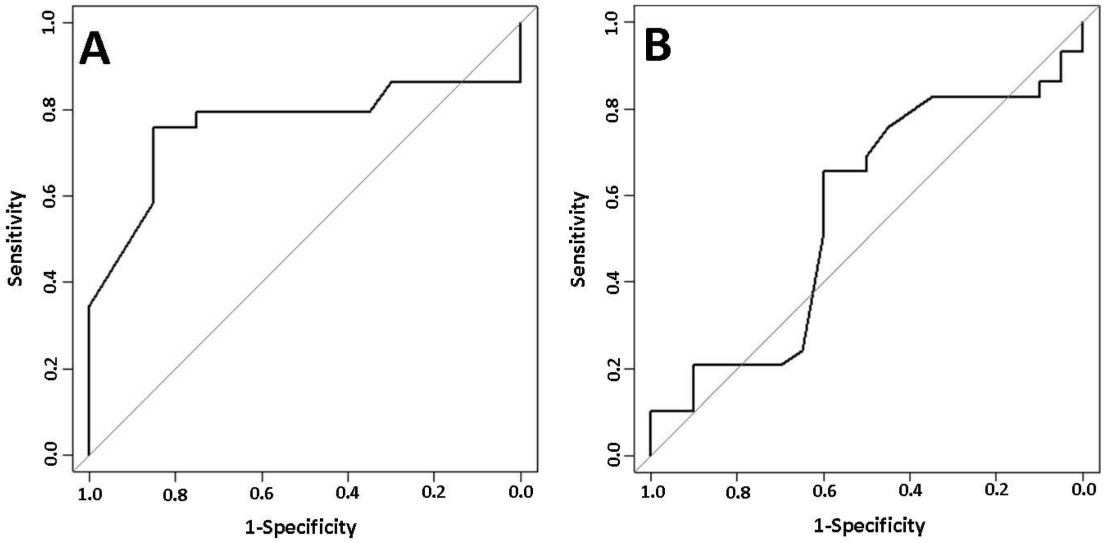

Figure 6A. *The receiver operator characteristic curves of the mean minimum temperature in January and **6B**. the sum of the July precipitation.*

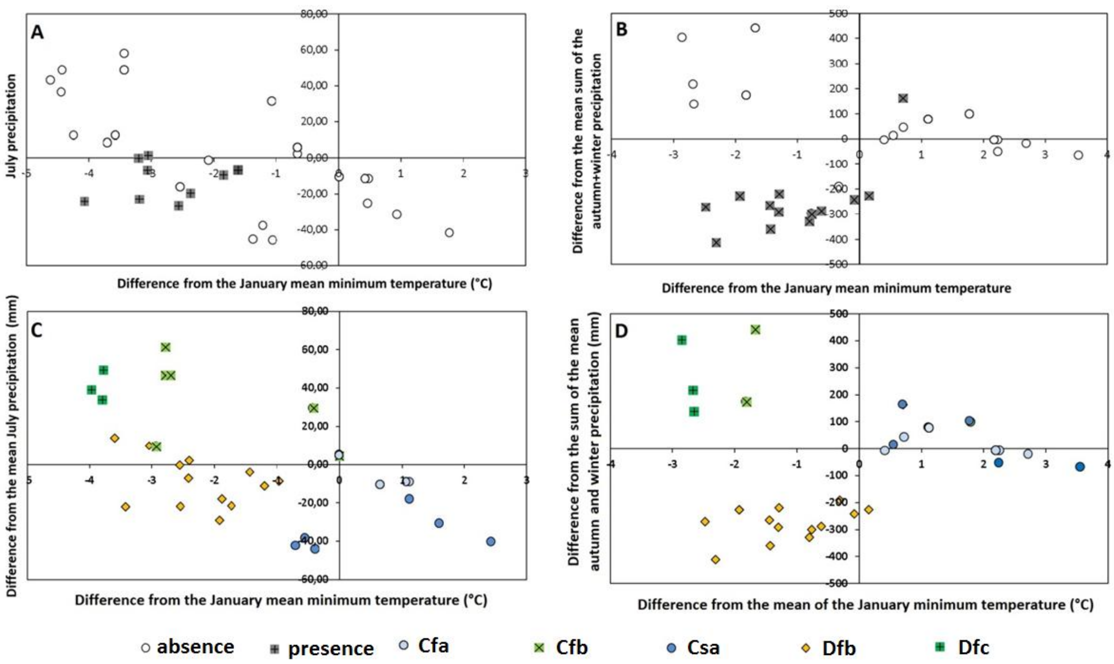

Figure 7A*. The successful and the abortive trapping sites according to the scales of July precipitation and the difference from the mean January minimum temperature, **7B**. sum of the difference from the sum of the mean autumn and winter precipitation and the difference from the January mean minimum temperature, **7C**: The Köppen-Geiger climates of the sites according to the difference from the mean July precipitation and the January mean minimum temperature or **7D**: the sum of the mean autumn and winter precipitation and the difference from the mean of the January minimum temperature.*

Cluster Analysis was used to group the positive and negative collecting/trapping sites according to the following climatic factors: January mean temperature (°C), mean

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minimum January temperature (°C), mean July precipitation (mm) and sum of the autumn and winter precipitation. According to the Cluster Analysis there are three significant distinctive climatic groups in the studied area: 1) continental temperate climate including most of the inland sites in Croatia and Hungary with only one successful trapping site, furthermore the 2) Mountainous Mediterranean climate including some sites in the valleys of the Julian Alps in Slovenia and 3) coastal Mediterranean climate sites in Slovenia and Croatia with several successful trapping sites (*Fig. 8A*). The 2[nd] climate group is the sister group of the 3rd and except ones sites positive sites belong to these climatic groups. The 3[rd] climate groups have two subgroups: a colder and warmer coastal Mediterranean one. The Cluster Analysis put the climate of the coastal Mediterranean group next to the group of Mountainous Mediterranean. In the Principal Component Analysis the above found four major distinguishing variables were used. The results are confirmed, since the planes stretched by the first and second components are responsible for the 51.85% (sum of the mean precipitation) and 36.43% (mean January minimum temperature), in all 88.28% of the variance. The result of PCA proved the former statistical conclusions (*Fig. 8B*).

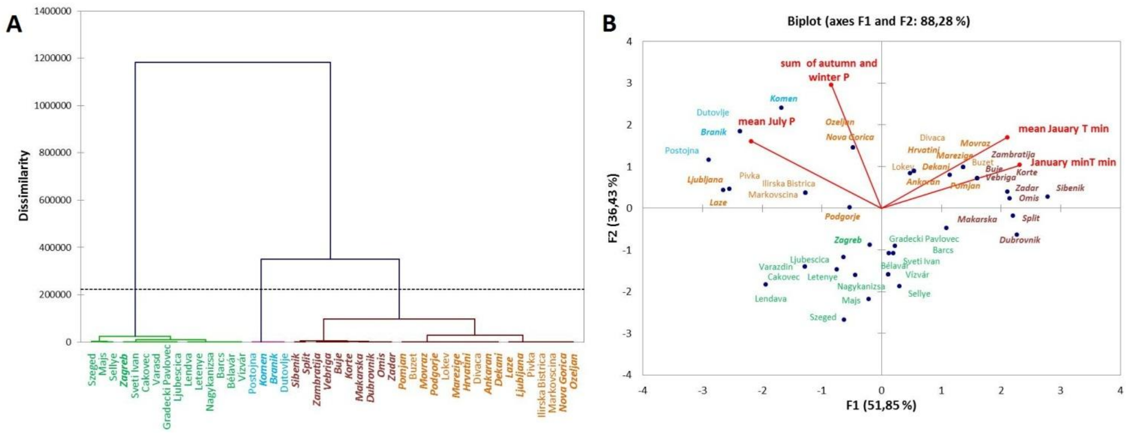

Figure 8. *The results of HCA (8**A**) and PCA (8**B**) based on the winter and autumn precipitation sums, the mean annual precipitation sums, the January mean minimum temperature and the January minimum temperature values. Trapping and reference settlements of the grids were colored according to the color of the corresponding climatic strains. Positive trapping or larva collecting sites were marked with bold, italic letters. Orange: Cfa, blue: Cfb, brown: Csa, green: Dfb climates according to the Köppen-Geiger climate classification system.*

According to the biplot displaying the four major variables it can be stated that 1) the cluster of the Mediterranean climate types are segregated from the continental type mainly both by cold winters and dry summers; 2) the positive difference of the variables are reliable for the presence of *Ae. albopictus* in the given sites and the negative differences for the absent of the mosquito in most of the cases and 3) a temperature or a precipitation-type variable itself cannot explain the presence/absence status of the mosquito in a given area. The variables of the successful trapping and/or collecting sites are bound mainly to the coastal Mediterranean territories of the northern Adriatic area. In the continental climate Zagreb was the only positive site for the occurrence of *Ae. albopictus*, although the winters in Zagreb are milder than in case of the continental areas of Slovenia and Hungary. The outputs of cluster and principal component analysis

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are confirmed the results of the analysis of 1 to 1 climatic factors characterized by the areas according to the Köppen-Geiger climate zones.

The potential annual generation numbers

The mean number of the potential generations in the successful trapping sites is 4.33 per year in the coastal areas, 3.61 per year in the mountainous areas and 3.52 per year in the solo continental site, Zagreb. The difference between the generations of the successful and non-successful sites were no significant (p=0.2902). According to the one-way variance analysis there are significant (p<0.05) differences between the potential generations of studied coastal (n=14) and mountainous (n=19), respectively the coastal and the continental (n=15) trapping sites and there are non-significant (p>0.05) difference between the mountainous and the continental trapping sites, whether the given trapping site was successful or not. We found significant difference (p<0.05) between the potential generations of the successful coastal (n=14) and the mountainous (n=12) trapping sites. The single successful continental trapping site of Zagreb was not involved to the analysis. The interpolated map of the potential number of generations predict 4-5 generations per year in the southern Mediterranean coastline of Croatia and Istria, similar number was also calculated for the continental parts of Croatia and the main part of Hungary (*Fig. 9*).

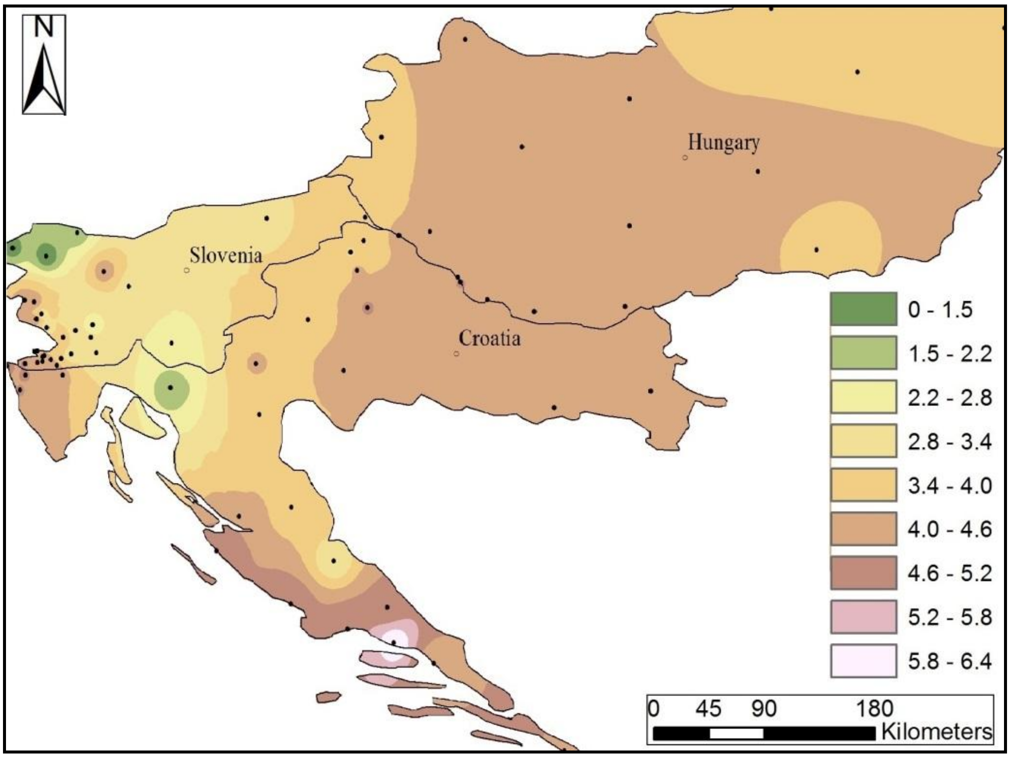

Figure 9. *The potential number of the annual generations of Aedes albopictus according to the mean monthly temperatures of the trapping and the additional points in 2000-2014.*

It was found that recently five completed generation is possible in Hungary. The mean diapausing time between the generations of two years is 161 days (about 5.3 months). The potential season of the mosquito could takes 204 days (about 6.7 months)

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in the area. The mean times of the larval developmental times are 26, 14, 9, 9, 10 and 20 days in the order of the annual generations. The mean time of the pupal ontogeny stage is 26, 14, 12, 12, 14 days and the sixth cannot be completed. The mean hatching times are 6, 5, 4, 4, 7 and the sixth one cannot be achieved in the same year. The time of the full developments are take 58, 33, 25, 25 and 36 days in the order of the annual generations (*Fig. 10*).

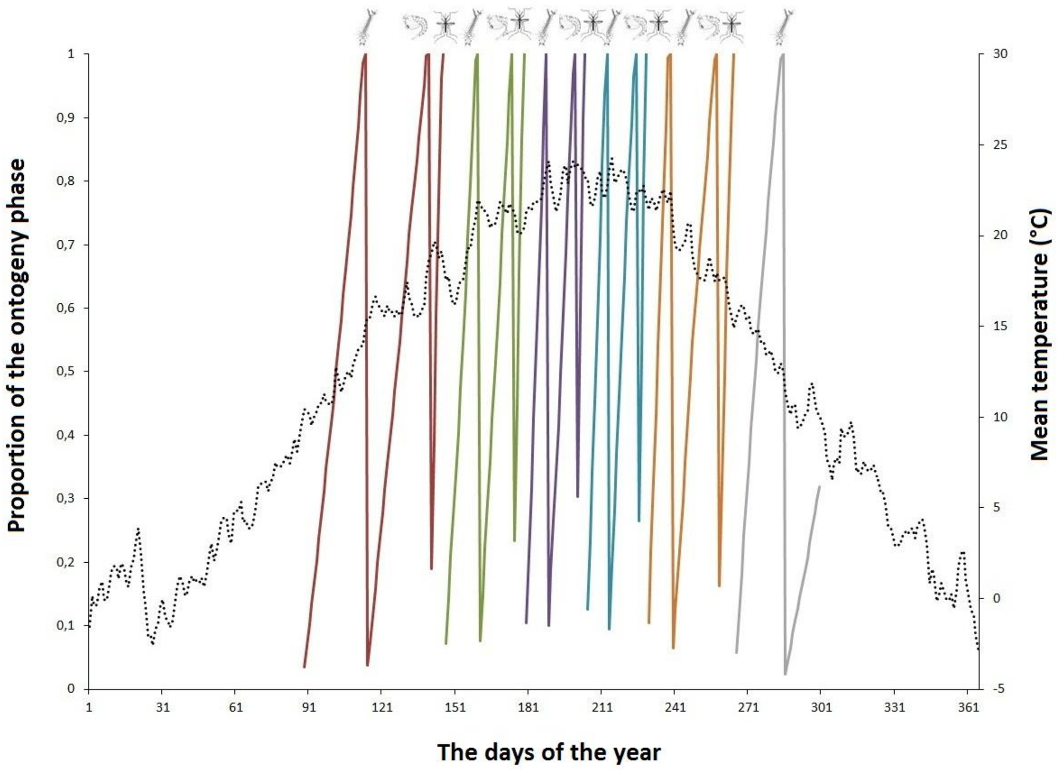

Figure 10. *The modeled generations of Aedes albopictus in Hungary. The pictograms show the current ontogeny stages.*

Climate envelope modelling

The modeled recent potential occurrence of *Ae. albopictus* includes the observed range of the species and contains a large part of the Carpathian Basin and the whole Northern Balkan Peninsula. It was found, that the January mean temperature and the July precipitation are determining the northern expansion of the mosquito in the eastern Mediterranean Basin. The model predicts the expansion near the northern borders of the distribution in the studied region for the period of 2011-2040 and 2041-2070. The modelled potential occurrence of the mosquito shows that the environmental conditions are permissive for the mosquito in the entire Northern Balkan and the south-western parts of Hungary even in the present period. However the model properly outlined the line of the Alps as the boundary of the northern expansion in the north-western edge of the area, no limiting effect of the Mediterranean mountain region was represented. The model suggests that the potential further invasion to the Carpathian Basin of the species can occur from Slovenia, Croatia or Serbia. Geographically the known living habitat in Zagreb is the nearest initial point of the mosquito to the Hungarian border (*Fig. 11*).

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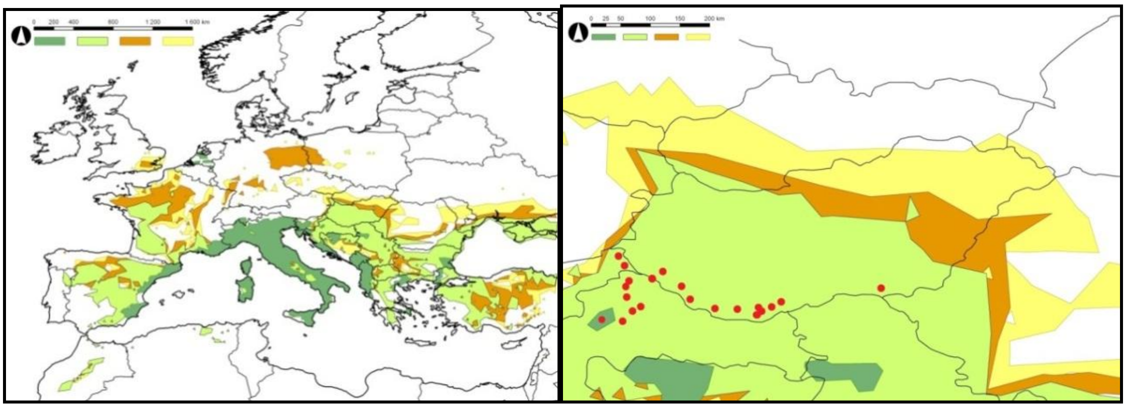

Figure 11. *The recent (2012) distribution of Ae. albopictus mosquito according to the European occurrence of the species survey (modified model results of Trájer et al. 2013; dark green according to the VBORNET database, 2013), the potential distribution area for the reference period (1961-1990, light green), the projected future distribution for the period of 2011-2040 (orange) and 2041-2070 (yellow) in Europe and the Carpathian Basin. Red points mark the sampling sites of ours.*

Discussion

According to the previous results of a multi-criteria decision based analysis, the limiting lower thermal suitability thresholds of the distribution of *Ae. albopictus* in the winter season are the -1 °C January mean temperature (ECDC, 2015). We found that the January mean minimum is a better differentiator between successful and non-successful trapping sites than the January mean temperature. It was found that -1.2 °C January minimum temperature is the cut point of the presence and the absence of the mosquito. However, in Slovenia, the Asian tiger mosquito is present in localities where the winter minimum temperatures can approximate -4°C in January. Our findings could be consistent with the statements of the previous studies which found that daily mean 0°C January isotherm is the limit of the overwintering potential of the species and the limit of -5°C isotherm is the limit of summer expansion range (Hawley et al., 1989; Nawrocki and Hawley, 1987). It is difficult to determine the lowest survivable January temperature for this mosquito, since in higher latitudes, *Ae. albopictus* spends the winter month's diapausing in the egg stage and only if the temperature goes below a certain level, will the first stage larvae in these eggs die. Hawley et al. (1989) described how prolonged cold and photoperiod induced diapause can increase the overwintering ability of *Ae. albopictus* eggs. It is easier to explain the effect of the July drought on the occurrence. It is known that *Ae. albopictus* is much more resistant to severe desiccation than e.g. forest *Aedes* species the high temperatures and dry conditions of this summer month can cause high levels of mortality even in non-diapausing eggs (Sota and Mogi, 1992). An important result of our analysis is that the January minimum temperature cannot explain the observed distribution since in mountainous areas *Ae. albopictus* can only persists at sites where the sum of precipitation is more than about 60-70 mm in July. Neither the July precipitation, nor the sums of the autumn and winter precipitation alone is the differentiator of the presence and the absence of the mosquito. July precipitation over 66 mm has a positive effect on the presence of the Asian tiger mosquito.

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The Varaždin Mountains (North Croatia) due to its topographically low altitudes does not constitute an affective barrier against the expansion of the species. Sporadic and occasional occurrences are possible anywhere due to cargo transport, but a sporadic, single occurrence does not mean that the mosquito is established in the area. Either a single occurrence does not assume even the climatic suitability of an area, because the species can reproduce during one summer. It is possible to find the individuals of a species in a causal occasion since the field collector trips are typically organized during the summer season. It is no coincidence that in the vector maps the following categories of the occurrences and observations were distinguished: established, introduced, absent, no data. A good example for this case that in the 2015th year of the Asian tiger mosquito maps of V-BORNET mosquito maps a single introduced occurrence of *Ae. albopictus* can be seen in the southern part of the Hungarian Great Plain (ECDC, 2015). Since this causal occurrence does not mean the overwintering ability of the species, this data was neglected from the climatic analysis.

The fluctuating phenomena of the observation is visible in the trapping data of the mosquito e.g. in Belgium where local environmental conditions made the establishment possible (Schaffner et al., 2004) or in the Netherlands where the Asian tiger mosquito resides in horticultural companies and it was most likely introduced from China by a Dracaena plant (Asparagaceae) cargo (Scholte et al., 2007). In Croatia, even in the coastal areas of the Adriatic Sea, where the presence of the Asian tiger mosquito was demonstrated to be continuous, effective catching sites show fluctuation (which also depends on the trapping activity). It is similar to that found in Zagreb, where the species was first observed in 2004, later in 2005, but not in 2006, while it was collected again in 2007 (Merdić, 2011). Nevertheless, even the remarkable occurrence of the species in Zagreb is quite curious since the nearest distribution area is the Adriatic coastal part of Croatia. These observations support the idea that the absence of *Ae. albopictus* during our collecting expedition provides an indicative rather than strictly conclusive result with regard to the absence of the established populations of the species in the studied area. According to Knudsen et al. (1996) the 0°C mean winter temperature is one of the most important criteria of the overwintering of *Ae. albopictus* in Europe. This might be consistent with the fact that Zagreb lies on/within the 0°C January isotherm. An important result of our study that results of climatic analysis may esteem only the established occurrences of the species, although causal, single introduced occurrences cannot be predicted.

According to the PCA and HCA analyses the climate of the continental areas form a markedly different climatic group both from the coastal and the mountainous Mediterranean sites. The only positive, but isolated, but established continental habitat Zagreb belongs to a same group with the typical continental points of Hungary, Croatia and Slovenia. In order to evaluate this information, we need to mention that climatic values were averaged for 0.25° grids which cover a relatively extended area. Since Zagreb itself is surrounded north by Mèdvednica mountain, it is clear that both the climate of the Sava valley, the mountainous climate of the Mèdvednica (the highest peak is 1,035 meters above sea level) and the climate of the city were averaged in its grid. In addition, it is likely that the urban heat effect of Zagreb and the warmer climate of the south slopes make the humid continental climate of the capital of Croatia warmer than the surrounding areas due to anthropogenic heat emission. Urban heat island effect can explain similarly the presence of *Ae. albopictus* in Zagreb to the case of the also

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isolated occurrence of *Ph. neglectus* in the agglomeration of the Hugarian Capital due to the urban heat effect (Trájer et al., 2014).

It is particularly important to mention the finding (which was also indicated by our results), that since its first record in Zagreb the mosquito has not become a very common element of the mosquito fauna in the neighboring eastern and northern Northern Balkan areas. Köppen-Geiger type biozone characteristics support the presence of *Ae. albopictus* in the studied area. In general, warm oceanic or humid subtropical, temperate oceanic and warm Mediterranean climate might be permissive for the Asian tiger mosquito, while the temperate continental climate is not. Based on our results, the Asian tiger mosquito has not yet spread further from the site of its first observation in Zagreb, but became a constant contributor to the local mosquito fauna. However, the recent situation in the region of the northern Balkans is very similar to the peripheral occurrence of the species in the United States, where beyond the continuous distribution border; the sporadic occurrence of the mosquito is mainly associated with large cities and extends far into the non-permissive inland areas of the country (Moore and Mitchell, 1997). *Ae. albopictus* invaded the state of Florida in only 6 years in 1987-1993 executing a cca. 600 km linear spread (O'Meara et al., 1995), meaning a remarkable 100 km linear expansion capability annually. Although the climate of Florida is milder than that of the northern Balkan's, the cca. 90 km distance between the stable occurrence of the mosquito in Zagreb and the wet floodplain of Hungarian border is a justified reason for future vigilance and monitoring. Several other authors also predict the potential continuous occurrence or the presence in Hungary of the Asian tiger mosquito e.g. Fischer et al. (2014) predicted about 0.41-0.6 climatic suitability in the period of 2011-2040 for the larger part of Hungary. Concerning the effect of the combination of heavy cargo transport and climate suitability, Thomas et al. (2014) found the North Balkan Peninsula especially vulnerable. Our trapping results and climatic analysis did not contradict the results of climatic models. It is also likely that *Ae. albopictus* as a typical invasive species has not yet filled the entire suitable area of the North Balkans and Carpathian Basin.

We also need to mention the limitations of our study. Since *Ae. albopictus* is an invasive, non-native species in the studied area it is likely that the mosquito does not fill the entire suitable area in climatic sense. Modeling results suggest that the climate of Transdanubia, Hungary could be suitable for the species (Fischer et al., 2014, Trájer et al., 2014). In contrast to the model results there are only few known established occurrences of *Ae. albopictus* in the continental areas of Europe. Based on the model results of Fischer et al. (2014), *Ae. albopictus* mainly live in areas where the climatic suitability value for the mosquito is more than 0.41. The climatic suitability of Transdanubia for *Ae. albopictus* was about 0.21-0.40 according to the climatic conditions in the end of the 20th century. Although, the used mosquito distribution data of this study were based on collecting and trapping activities which were performed in the first and the second decades of the 21st century, the current observed established distribution of the mosquito corresponds to the modeled one of Fischer et al. (2014) based on the climate of the end of the 20th's.

Due to the fact that each site was sampled on only one date and the overall period for this intensive survey was limited to 4 days (8.17.14 to 8.20.14). We acknowledge that the conclusion that the mosquito is really absent in the case of a given location, would require more systematic sampling. Even so, the probability of abundance for a wider studied area is also low if the species was not collected from any sampling site. In

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contrast, the mosquito was immediately found in the known occurrence of Zagreb by different collecting techniques. In some cases only one container or water body was sampled per site. At six of the sites sampling was conducted on groundwater pools, which are microhabitats that seldom harbor *Ae. albopictus* larvae. Since the mosquitoes were reared to the adult stage for species identification, some individuals surely died before reaching the adult stage.

Some sites or areas with *Ae. albopictus* occurrences of the additional data may be listed as negative due to inadequate or improper sampling activities. It cannot be excluded either that the additional trapping data may be plagued with some of the same type of limitations as the present collecting survey, this kind of limitation may be valid for any mosquito occurrence record in the world. On the other hand, sites designated positive for *Ae. albopictus*, as a result of sampling endeavours, may represent very temporary or permanent populations.

For a correct estimation of the potential spread of *Ae. albopictus* into new regions, it would be important to be able to distinguish these two population types. Unfortunately data for accomplishing this task is often unavailable. To avoid this pitfall we mixed the occurrence data of *Ae. albopictus* in Croatia of 4 different years and aimed to reach a relatively frequent sampling around the Croatian-Hungarian border area. Naturally, the selected incidental collections from neighbouring countries do not give a complete picture of the "ecological plasticity" of this species which is why we know our findings for the studied area to be valid. Last, but not least our trapping results confirm the previously observed occurrence (Farkas et al., 2011) the important vector of *Leishmania infantum*, the sandfly *Phlebotomus neglectus* in Nagyharsány, South-western Hungary.

Interpretation and conclusion

Trappings provided important additional information for the clarification of the climatic requirements of the Asian tiger mosquito by representing the relatively dry and cold winters of the North Balkan and South Pannonian continental region. Our results provide a strong indication that the mean of the minimum January temperature was an important determinant of the northern expansion of *Ae. albopictus* in the reference period. Dry summers and cold winters in the continental parts of the area recently provided relatively unfavorable conditions for *Ae. albopictus* in the past that has changed due to climate change. It was found that *Ae. albopictus* can tolerate the relatively low absolute temperature values under wet winter conditions of the Mediterranean mountainous regions. Temperature pattern cannot limit the current spread of *Ae. albopictus* in the South Pannonian ecoregion. Our climate envelope model result predicts a more extensive current potential distribution for *Ae. albopictus* compared with our observations; however the model predicted correctly the suitability of the climate of the Mediterranean mountains for the mosquito. The analysis of the climate of the successful and non-successful trapping sites showed a complex conditionality of the habitat preference of *Ae. albopictus*, however the coexistence of dry summers and cold winters under continental climatic conditions does not seem to be preferable for the mosquito. *Ae. albopictus* can tolerate the climate of the Mediterranean mountainous region where the relatively cold winters are associated with high annual precipitation. The calculation of the potential number of the annual generations of *Ae. albopictus* showed that climate would allow the similar high speed spread of the mosquito in the Carpathian Basin as in the northern Adriatic coasts due to the relatively

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long and warm summers. The number of the generations between the successful and non-successful trapping sites cannot explain the presence of the mosquito in the area.

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ENVIRONMENTAL CONDITIONS ARE IMPORTANT INFLUENCES ON THE RECRUITMENT OF NORTH PACIFIC ALBACORE TUNA, *THUNNUS ALALUNGA*

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Abstract. The effects of changing climate on numerous commercially and ecologically important fish species including the South Pacific albacore tuna, *Thunnus alalunga* have been documented over the past decades. The objective of this study was to explore and elucidate the relationship of environmental variables with the stock parameters of albacore tuna. The relationship of the North Pacific albacore tuna recruitment (R), female spawning stock biomass (SSB) and recruits per spawning biomass (RPS) from 1970 to 2012 with the environmental factors of sea surface temperature (SST), Pacific decadal oscillation (PDO) and El Niño southern oscillation (ENSO) was construed. SST and PDO were used as independent variables with SSB to construct stock reproduction models for R and RPS as they showed most significant relationship with the dependent variables. Model selections were based on Akaike Information Criterion (AIC) with the condition of significant parameter estimates at $p<0.05$. Models with single independent variables of SST, PDO and ENSO were also constructed to illuminate their individual effect on albacore R and RPS. From the results it can be stated that SST and PDO resulted in the most significant models for reproducing North Pacific albacore tuna R and RPS time series.
Keywords: *spawning stock biomass, sea surface temperature, El Niño southern oscillation, Pacific Decadal Oscillation*

Introduction

Albacore tuna is a commercially important species of tuna in the Pacific Ocean. The economy and livelihood of various Pacific Island countries and territories (PICTs) depend heavily on commercial tuna fisheries (SPC, 2012; Bell et al., 2009; Gillett, 2009). It is therefore important that fishery managers take measures to ensure the economic and biological sustainability of albacore tuna in the Pacific Ocean. Albacore tuna fishery has a long history in the Pacific Ocean; however its ecological characteristics are still not sufficiently understood. The North Pacific albacore and the South Pacific albacore are recognized as two reproductively isolated distinct stocks in the Pacific Ocean with negligible mixing (Takagi et al., 2001; Ramon and Bailey, 1996; Ueyanagi, 1969). Albacore reaches sexual maturity at around 5 years and they are mostly harvested using troll, pole-and-line and longline gears in the North Pacific where most of the fish caught range

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between 2-5 year olds (Zhang et al., 2014; Farley et al., 2014; ISC, 2014; Wells et al., 2013; Chen et al., 2012; ISC, 2011; Ueyanagi, 1957).

The Albacore Working Group of the International Scientific Committee for Tuna and Tuna-like Species (ISC) carries out stock assessments for the North Pacific albacore tuna (ISC, 2014). These assessments do not extensively analyze the influence of climatic conditions on the stock patterns and time-series trajectory. The importance of alterations in climatic conditions and their effects in structuring the ecological conditions supporting commercially important tuna species have been documented.

Changes in SST have been linked to primary productivity and albacore tuna stock dynamics in New Caledonia's EEZ (Briand, 2011). The early life stages, spatial distribution and spawning favorability of the South Pacific, North Pacific and North Atlantic albacore tuna have been shown to be related to the varying gradients of oceanic surface temperatures (Nieto et al., 2015; Lehodey et al., 2015; Dragon et al., 2015; Pearcy, 1973). Laurs et. al. (1984) studied the catch activities of the North Pacific albacore tuna off California in the summer of 1981 and established significant links between the satellite images of the SST and albacore aggregation pattern. The spawning oceanic temperature preferences for the South Pacific albacore tuna tends to be warmer surface water which occurs near the equatorial zone at the warm pool (Lehodey et al., 2008; Lehodey et al., 2015; Dragon et al., 2015) and albacore population has been projected to relocate in following the geographical movement of the preferable warm pool waters (Lehodey et al., 2011; SPC, 2012; Le Borgne et al., 2011; Ganachaud et al., 2013; Bell et al., 2013; Ganachaud et al., 2011). The effect of the warm pool SST index on the South Pacific albacore tuna stock has been demonstrated previously by Singh et al., (2015).

The negative and positive phase of the climatic variable of the Pacific decadal oscillation (PDO) has been shown to affect the recruitment stage of albacore tuna in the South Pacific (Lehodey et al., 2003). Links between the albacore tuna catch per unit effort (CPUE) and the PDO has also been established by Singh et al. (2015). Phillips et al. (2014) determined the presence of significant relationship between the PDO and SST variability and the spatial distribution of the juvenile North Pacific albacore tuna catch per unit effort (CPUE) off the United States West coast. In the South Pacific, albacore recruitment has been shown to be correlated to the El Niño Southern Oscillation (ENSO) climatic index and the related El Niño and La Niña events (Lehodey et al., 2003; Zainuddin et al., 2004). The North Pacific albacore productivity has been shown to have significant link with the dynamics of ENSO (Zhang et al., 2014).

It is evident that oceanic temperature indices and climatic conditions have substantial affect the stock parameters of albacore tuna and it needs to be elucidated in order for albacore fishery managers to be able to incorporate such effects into their management plans for the conservation and sustainability of albacore tuna stock in the Pacific. This study was undertaken to determine the effect of the SST and the environmental conditions of PDO and ENSO on the recruitment (R), spawning stock biomass (SSB) and recruits per spawning biomass of the North Pacific albacore tuna and use those independent variables exhibiting significant correlation with the albacore tuna stock parameters to construct suitable

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models that can significantly reproduce the R and RPS trajectory of the North Pacific albacore tuna.

Materials and Methods

The data

Stock data

The albacore working group of the ISC compiles the report on the stock assessment of the North Pacific albacore tuna (*Thunnus alalunga*) in the North Pacific Ocean (ISC, 2014). The assessments are based on data from longline, troll, pole and line, gillnet, purse seine fishing gears as well as recreational gear, hand lines and harpoons. ISC collected catch and size composition data from its member countries of Canada, Chinese Taipei, Japan, Korea and USA including some member countries of the Western and Central Pacific Fisheries Commission (WCPFC) and Inter-American Tropical Tuna Commission (IATTC) including China. These data were used by the ISC to estimate the annual recruitment (R) and female spawning stock biomass (SSB) using the base case assessment model. The 2011 stock assessment estimated of the ISC (ISC, 2011) were independently reviewed (Chen, 2011; Cordue, 2011) and shortfalls were Identified. In addition, different studies that were conduct post-assessment (Brodziak et al., 2011; Iwata et al., 2011; Childers et al., 2011; Chen et al., 2012; Wells et al., 2013; Cosgrove et al., 2014) provided improvement on the understanding of albacore biology. These information and findings were used to conduct improved assessment of the North Pacific albacore stock by the ISC (ISC, 2014). The annual R and SSB data for albacore tuna from 1970 to 2012 was obtained from the 2014 stock assessment report, stock synthesis 3 results of the ISC (ISC, 2014).

The study area and distribution of the North Pacific albacore tuna stock falls within the geographical coordinates of 50ºN - 120ºE, 10ºN - 120ºE, 50ºN - 120ºW, 10ºN - 120ºW as shown in *Fig. 1*. For this study the stock of albacore tuna within the study area in the North Pacific (*Fig. 1*) was treated as a distinct and reproductively isolated stock as previous work (Ichinokawa et al., 2008; Takagi et al., 2001; Ramon and Bailey, 1996; Chow and Ushiama, 1995; Suzuki et al., 1977; Ueyanagi, 1969) have shown the discreteness of this stock in comparison to the albacore tuna stock in the Southern Pacific Ocean.

The annual recruits per spawning biomass (RPS) from 1970 to 2012 was calculated from the R and SSB data. The trajectory of R, SSB and RPS are shown in *Fig. 2*. Also shown is the stock recruitment plot for albacore. Recruitment for each year is defined as the number fish at age-0 for that particular year. Annually, a single recruitment and single spawning period occurs based on assessments by Chen et al. (2010). The maturity ogive used for the calculation of the SSB was based on the estimation by Ueyanagi (1957) where it was determined that 50% of albacore tuna maturity occurs at age-5 and 100% at age 6. This estimation has been used in previous assessments of albacore tuna (ISC, 2011) and also supported by recent work by Farley et al. (2014). The annual SSB for this study was the total weight of sexually mature female fish for that year. Spawning of albacore tuna occurs between the longitudes of 155ºW and 120ºE and between the latitudes of 10ºN and 25ºN (*Fig. 1*) between Hawaii, Taiwan and Philippine waters (Chen et al., 2010; Yoshida, 1968; Otsu and Uchida, 1959; Ueyanagi, 1957). The SSB trajectory shows a decreasing pattern from 1970 to 1993 followed by some

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recovery up to 1999 which is followed by a gradual declining pattern (*Fig. 2*). R has chaotic behavior throughout the years with a general declining pattern from 1971 to 1987 and a stable pattern on average up to 2012. For the RPS the trajectory exhibits a lower average from 1970 to 1988 and a distinctively higher average from 1989 to 2012.

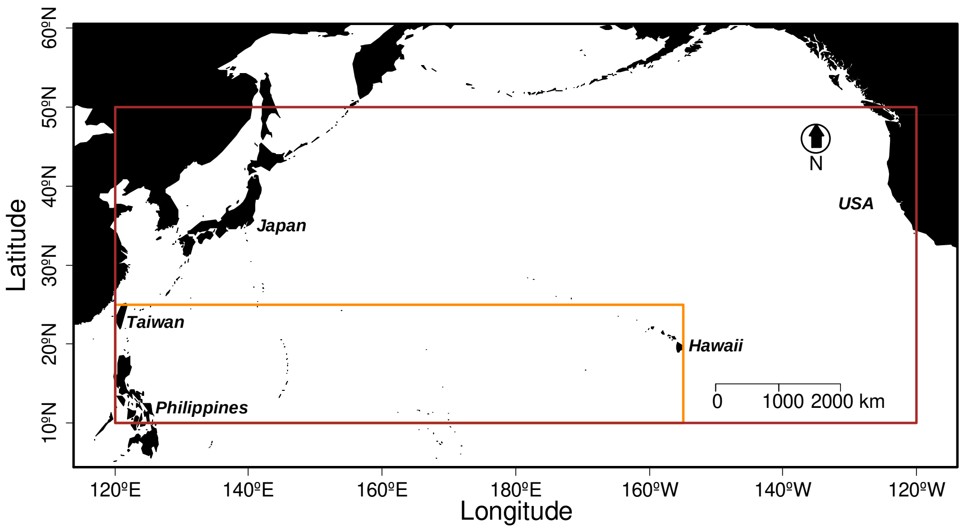

Figure 1. *Map showing the recruitment and female spawning stock biomass data distribution (represented by the red enclosure box) of the albacore tuna (T. alalunga) in the North Pacific Ocean between 10ºN to 50ºN and 120ºE to 120ºW for the years 1970 to 2012. The spawning area is also shown by the orange enclosure box as determined by Ueyanagi, (1957), Otsu and Uchida (1959), Yoshida, (1968) and Chen et. al., (2010).*

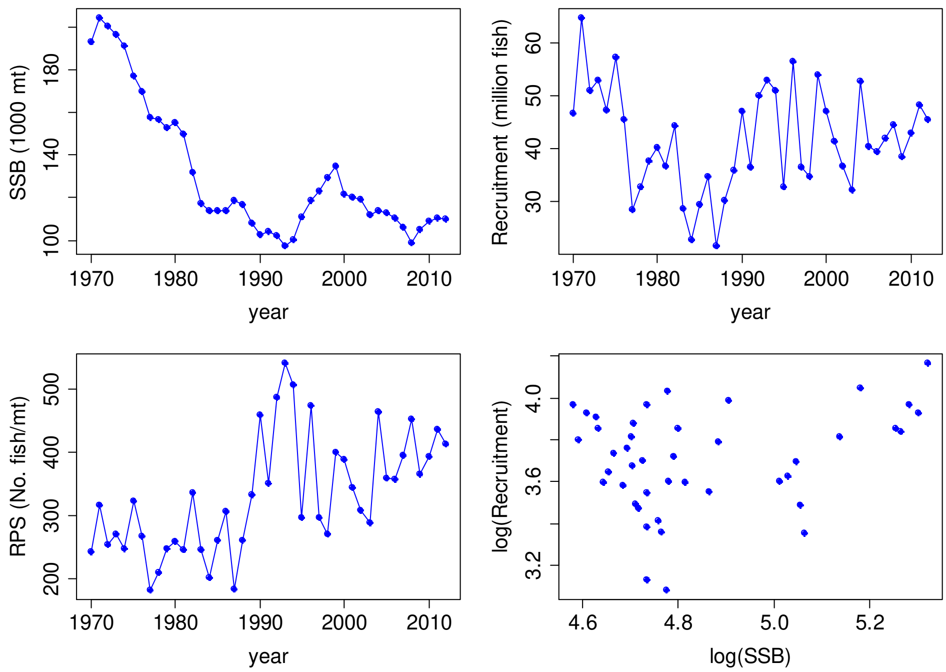

Figure 2. *The recruitment (R), female spawning stock biomass (SSB) and recruits per spawning biomass (RPS) time series trajectory of the albacore tuna (T. alalunga) in the North Pacific Ocean for the years ranging from 1970-2012. Also shown is the stock recruitment scatterplot plot for albacore tuna.*

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Environmental data

The mean SST observation data for the North Pacific Oceanic study area (*Fig. 1*) on a 1° by 1° resolution (Rayner et al., 2003) from 1970 to 2012 on a monthly scale from January to December was obtained from the monthly compiled Hadley Centre Sea Ice and Sea Surface Temperature data set (HadISST), complied by the Meteorological Office Hadley Center, UK. Monthly and annual SST anomalies were retrieved from this data set and the means calculated for the coordinates between 10ºN to 50ºN and 120ºE to 120ºW for the standard period of 1965 to 2012. The public domain data is available from the following link http://www.metoffice.gov.uk/hadobs/hadisst/. The detailed description of the SST dataset and its production process is decribed in Rayner et al. (2003).

The PDO is a dominant mode of climatic variability of the North Pacific oceanic surface temperature over decadal timescales (Linsley et al., 2015; Chhak et al., 2009; Deser et al., 2004; Mantua et al., 1997; Zhang et al., 1997). The Multivariate El Niño Southern Oscillation Index (ENSO) is an important climatic variability index occurring over sub-decadal time scales and affecting most part of the tropics and sub-tropics. Its calculation is based on multiples variables of SST, sea-level pressure, surface air temperature, atmospheric conditions and surface wind indexes (Wolter and Timlin, 1998). The monthly climatic data from January to December from 1965 to 2012 on the PDO and ENSO was obtained from the National Oceanic and Atmospheric Administration (NOAA), Earth System Research Laboratory (ESRL), Physical Sciences Division.

Exploration and comparison of variables

Relationships between the dependent variables of R, SSB and RPS and the independent variables of SST, PDO and ENSO were determined using absolute correlations and linear regression. Correlations with $R>0.500$ at $p<0.05$ were considered as significant. R, SSB and RPS from 1970 to 2012 were compared with monthly and annual mean SST (from the raw 1° by 1° resolution HadISST data), PDO and ENSO from 1965 to 2012 for lag periods of $t-n$ years where $n=(0,1,\ldots,5)$. The lag periods were based on the age group of most of the harvested stock in the Pacific Ocean which ranges between 2-5 years (ISC, 2014; Wells et al., 2013; Chen et al., 2012; ISC, 2011) and albacore tuna becomes reproductively active at around 4-5 years of age (Farley et al., 2014; ISC, 2011; Ueyanagi, 1957). Data exploration for the independent and dependent variables were carried out using scatterplots, boxplots, histograms and kernel density overlays. This was used to assess data distribution and identify collinearity effects among independent variables and possible outliers which can affect the outcomes of statistical analysis and contribute to assessment bias. The data exploration protocol as outlined by Zuur et al. (2010) was followed to avoid violations of assumptions from the statistical methods utilized.

Time series data normally have two kinds of trends, stationary and stochastic. When significant changes or shocks have transitory effect, the time series is classified as having a stationary process. Trends where shocks permanently alter the trajectory are classified as stochastic trend or having a unit root. Stochastic trends can results in false correlations among variables and unit roots tests are designed to detect such trends (Kwiatkowski et al., 1992; Dickey and Fuller, 1979). The dependent variables and independent variables where subjected to MacKinnon's (MacKinnon, 1996) and the Augmented Dickey-Fuller (Dickey and Fuller, 1979) unit root tests.

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Model formulation for R and RPS

Independent variables which exhibit significant correlations with the dependent variables of R and RPS with reference to *R* values at $p<0.05$ were used to develop suitable stock reproduction models which can significantly reproduce the R and RPS trajectory for the North Pacific albacore tuna from 1970 to 2012. We attempted to explain the trajectory of the R and RPS by fitting of a stock-recruitment model to the albacore data and obtaining the Akaike Information Criterion (AIC) for each model. Similar modeling techniques have been used in previous work (Sakuramoto, 2016; Singh et al., 2014; Sakuramoto, 2013; Cahuin et al., 2009). To ease the comprehensiveness and complexity of the models, only two environmental variables were incorporated in the final models for R and RPS. The Generalized Linear Model (GLM) used for R (Equation 1) and RPS (Equation 2) are shown below

$$\log(R_t) = \log(\alpha_{0,n}) \times \log(SSB_{t-n}) + \alpha_{1,n} \times c_{1,t-n} + \alpha_{2,n} \times c_{2,t-n} + \alpha_{3,n} \times (c_{1,t-n} \times c_{2,t-n}) + \varepsilon_t \quad \text{(Eq.1)}$$

$$\log(R_t / SSB_t) = \log(\beta_{0,n}) + \beta_{1,n} \times c_{1,t-n} + \beta_{2,n} \times c_{2,t-n} + \beta_{3,n} \times (c_{1,t-n} \times c_{2,t-n}) + \varepsilon_t \quad \text{(Eq.2)}$$

where *R* is the albacore recruitment for year *t*, α_0 and β_0 are the intercept parameters, α_1, α_2, α_3 and β_1, β_2, β_3 are parameter estimates, *SSB* is the albacore tuna spawning stock biomass, c_1, c_2 are the independent environmental variables, *R/SSB* is the recruits per spawning biomass and ε is an unsolved normally distributed random variable.

To test the non-linear responses of the predictor variables on the dependent variables the generalized additive models (GAMs) (Hastie and Tibshirani, 1986) were used. When using environmental condition as predictor variables, GAMs have frequently shown to perform better in comparison to other types of predictive modeling techniques (Drexler and Ainsworth, 2013; Moisen and Frescino, 2002; Guisan et al., 2002; Walsh and Kleiber, 2001). Second and third order polynomial functions were inserted into Equation 1 and Equation 2 to investigate if these models (GAMs) performed better. The resulting Equation 3 and Equation 4 are shown below

$$\log(R_t) = \log(\gamma_{0,x,n}) \times \log(SSB_{t-n}) + \gamma_{1,x,n} \times c_{1,x,t-n}^{x} + \gamma_{2,x,n} \times c_{2,x,t-n}^{x} + \gamma_{3,n} \times (c_{1,t-n} \times c_{2,t-n}) + \varepsilon_{x,t} \quad \text{(Eq.3)}$$

$$\log(R_t / SSB_t) = \log(\delta_{0,x,n}) + \delta_{1,x,n} \times c_{1,x,t-n}^{x} + \delta_{2,x,n} \times c_{2,x,t-n}^{x} + \delta_{3,n} \times (c_{1,t-n} \times c_{2,t-n}) + \varepsilon_{x,t} \quad \text{(Eq.4)}$$

where γ_0 and δ_0 represent the intercept parameters, γ_1, γ_2, γ_3 and δ_1, δ_2, δ_3 are parameter estimates and $x= (1, 2, 3)$. The dependent variables and intercept parameter were log transformed in order to reduce the effect of possible outliers and skewness. Equations 3 and Equation 4 were used and independent environmental variables were successively incorporated and various combinations were investigated by successive elimination. Model selection was based on the AIC (Akaike, 1981) values.

All residuals for the selected models were subjected to tests for homogeneity of variance. The residual variance in all cases should be <4.00 or there will be significant degradation of the least square estimators (Fox, 2008) which may result in the selection of false models. The referred and estimated trajectory for albacore tuna R and RPS were plotted and compared. All statistical analysis for this study was carried out using the statistical software "R", version 3.0.1(R Core Team., 2014).

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Results

Exploration and comparison of variables

For comparison of predictor and response variables, the scatterplot matrix showing the histograms, kernel density overlays, absolute correlations and significance asterisks (p<0.05*, p<0.01**, p<0.001***) are shown in *Fig. 3*. The relationships of the North Pacific albacore tuna R, RPS and SSB against the monthly and annual independent environmental variables of SST, PDO and ENSO with lag periods of t-n years are presented. Only selected independent variables and lag periods are shown based on their significance and correlations with the response variables. Months, years and lag periods not showing significant correlations with the response variables are excluded from further analysis. All three stock parameters of R, RPS and SSB exhibited significant correlation with SST, PDO and ENSO. From *Fig. 3* it can be seen that R has highest correlation with $SST_{a,t\text{-}2}$ for the month of April (a) and the annual (u) average $PDO_{u,t}$. RPS had highly significant correlation with $SST_{m,t\text{-}2}$ for the month of March (m) and $PDO_{v,t\text{-}5}$ for January (v). SSB exhibited highly significant relationship with $SST_{o,t}$ for October (o) and $PDO_{w,t\text{-}5}$ for May (w). ENSO for September (s) and May had significant relationship with R and RPS but exhibited lower correlation for albacore stock parameters of R, RPS and SSB in comparison to PDO and SST (*Fig. 3*).To instill simplicity into the stock reproduction models only two independent environmental variables were selected for modeling.

The relationships between R and RPS and SSB and RPS are highly significant and the correlation between R and SSB is quite low in significance (*Fig. 3*). This is an indication that a signficant fraction of the R is not determined by SSB but other ecological factors. *Fig. 4* shows the boxplots for the spread of the dependent and independent variables. All variables have quite uniform distribution except for $ENSO_s$ and $ENSO_w$ where possible outliers are detected; however scatterplots for $ENSO_s$ and $ENSO_w$ do not show any points that are abnormally large or small. Based on this argument, we accept that the likelihood of observation or process errors in the dependent and independent variables are prominently minimized.

When statistical techniques such as simple linear regression are used, there is a tendency for spurious correlations to arise due to the presence of a unit root in time series data. Unit roots tests are techniques to detect such cases (MacKinnon, 1996; Kwiatkowski et al., 1992; Dickey and Fuller, 1979). The results of MacKinnon's (M-test) and the Augmented Dickey-Fuller (ADF-test) unit root test for the dependent and independent variables are shown in *Table 1*. All the time series data should have a t-test value (t-value) of <0 at p<0.05 to have a stationary process and the correlation results to be authentic. M-test and ADF-test showed that all variables exhibited stationarity (*Table 1*) and did not have unit root processes.

Model formulation for R and RPS

The most significant stock reproduction models for the albacore tuna R and RPS are shown in *Table 2*. Model significance was taken with reference to the AIC resulting from the incorporation of the independent variable of SST and PDO into Equation 3 and Equation 4. Modeling results from single independent variable using Equation 3 and Equation 4 are also shown in *Table 2*. Model (i) is the most significant for albacore tuna R and incorporates the independent variables of $SST_{a,t\text{-}2}$ and $PDO_{u,t}$. For RPS model (v) the most significant and has the independent variables of $SST_{m,t\text{-}2}$ and $PDO_{v,t\text{-}5}$. For the

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models with single independent variables for R, all models are significant with the model incorporating $SST_{a,t-2}$ being the most significant followed by $PDO_{u,t}$ and $ENSO_{s,t-1}$. For the RPS all models with single independent variables are significant with the most significant model incorporating $SST_{m,t-2}$ followed by $PDO_{v,t-5}$ and $ENSO_{w,t-2}$.

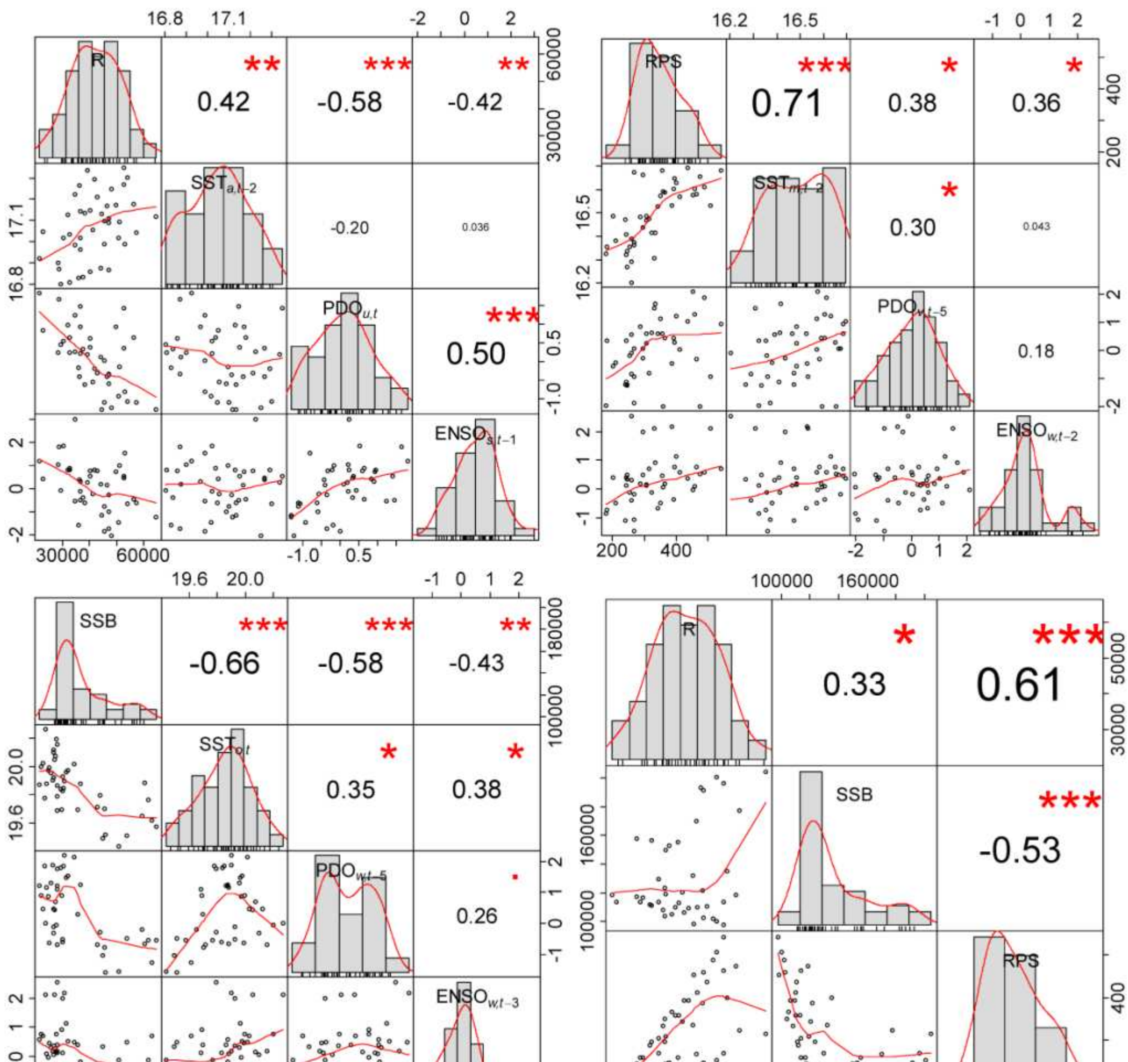

Figure 3. *Scatterplot matrix showing histograms, kernel density overlays, absolute correlations and significance asterisks (p<0.05*, p<0.01**, p<0.001***) for the relationship of North Pacific albacore tuna (T. alalunga) recruitment (R), recruits per spawning biomass (RPS) and female spawning stock biomass (SSB) against the environmental variables of sea surface temperature (SST), Pacific decadal oscillation (PDO) and El Niño southern oscillation (ENSO). The relationship between R, RPS and SSB can also be observed. Correlations are significant at p<0.05.*

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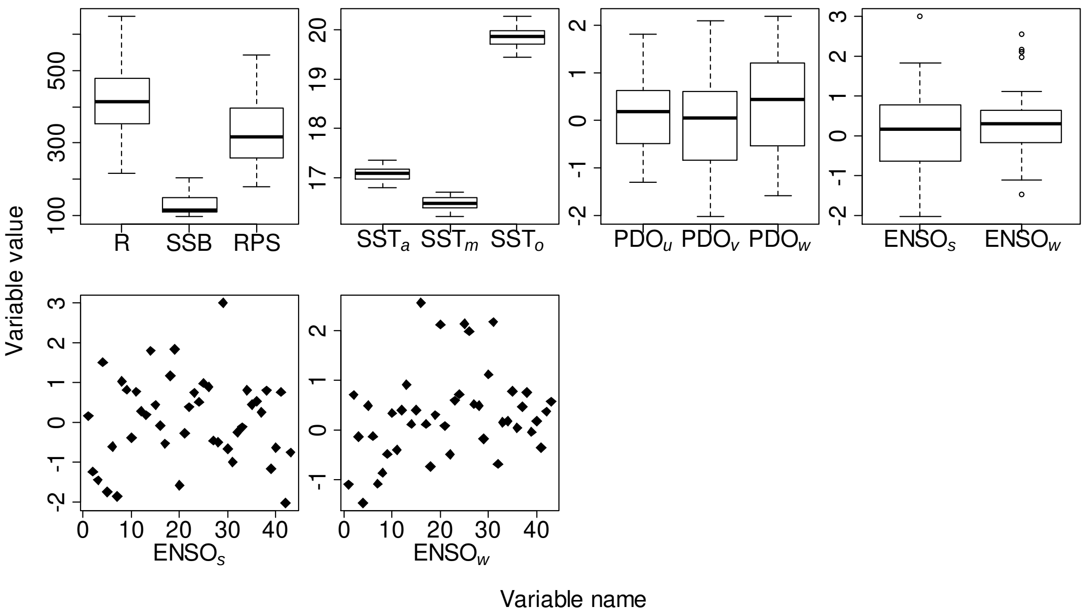

__Figure 4.__ Boxplots for the North Pacific albacore tuna (T. alalunga) stock variables and environmental variables showing the spread of the data. The median is represented by the line in the middle of the boxes. Scatter plots further show the distribution of El Niño southern oscillation (ENSO) data to detect for the presence of outliers. The subscripts refer to the months and annual averages with: v – January, m – March, a – April, w – May, s – September, o – October, u – annual.

__Table 1.__ Unit root test results for dependent and independent variables time series used in correlation analysis from Fig. 3. The subscripts refer to the months, annual averages and lag periods with: v – January, m – March, a – April, w – May, s – September, o – October, u – annual.

Time series	M-test		ADF-test	
	t-value	*p*-value	t-value	*p*-value
R_t	-9.440	1.28×10^{-11}	-9.439	$<1.00\times10^{-2}$
SSB_t	-4.329	1.01×10^{-4}	-4.329	$<1.00\times10^{-2}$
RPS_t	-9.243	2.27×10^{-11}	-9.243	$<1.00\times10^{-2}$
$SST_{a,t-2}$	-9.213	2.47×10^{-11}	-9.213	$<1.00\times10^{-2}$
$SST_{m,t-2}$	-10.561	5.34×10^{-13}	-10.560	$<1.00\times10^{-2}$
$SST_{o,t}$	-8.081	7.38×10^{-10}	-8.081	$<1.00\times10^{-2}$
$PDO_{u,t}$	-7.803	1.74×10^{-9}	-7.803	$<1.00\times10^{-2}$
$PDO_{v,t-5}$	-10.035	2.32×10^{-12}	-10.035	$<1.00\times10^{-2}$
$PDO_{w,t-5}$	-7.927	1.18×10^{-9}	-7.928	$<1.00\times10^{-2}$
$ENSO_{s,t-1}$	-10.569	5.22×10^{-13}	-10.569	$<1.00\times10^{-2}$
$ENSO_{w,t-2}$	-8.653	1.30×10^{-10}	-8.653	$<1.00\times10^{-2}$
$ENSO_{w,t-3}$	-8.589	1.58×10^{-10}	-8.589	$<1.00\times10^{-2}$

Fig. 5 shows the plot of the predicted R from model (i) (*Table 2*) and the referred R for the North Pacific albacore tuna. The referred RPS and predicted RPS from model (v) can also be observed. In both cases the predicted and referred trajectories seem to fit well. *Fig. 6* shows the trajectories of predicted R using single independent variables from models (ii), (iii) and (iv) (*Table 2*) and the referred R. Model (ii) with SST seems

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to be the best fit followed by model (iii) with PDO and model iv with ENSO. The trajectories for the predicted RPS from models (vi), (vii) and (viii) (*Table 2*) using single variables with the referred RPS are shown in *Fig. 7*. Model (vi) with SST is the best fit followed by model (vii) with PDO and model (viii) with ENSO.

The linear correlation between the predicted R from models (i)-(iv) and referred R and predicted RPS from models (v)-(viii) against referred RPS is shown in *Fig. 8*. The correlation between R predicted and R referred and RPS predicted and RPS referred is significantly higher for models incorporating two environmental variables (models (i) and (iv)) compared to using single variables. For models with single variables, SST and PDO result in models with higher significance compared to ENSO. This justifies the incorporation of the SST and PDO in the final models (models (i) and (iv)).

Table 2. *Stock reproduction models and some parameters using the female spawning stock biomass (SSB) and the independent environmental variables from Fig. 3 for the albacore tuna (T. alalunga) recruitment (R) and recruits per spawning biomass (RPS) in the North Pacific Ocean. Models using single variables are also shown. Only Models with highest significance are shown in each case with reference to the Akaike information criterion (AIC) values. The subscripts refer to the months, annual averages and lag periods with: v – January, m – March, a – April, w – May, s – September, o – October, u – annual.*

	Recruitment				
	Model with multiple variables				
i.)	$\log(R_t) = 1.284 \times \log(SSB_t) + 0.450 \times SST_{a,t-2} - 11.423 \times PDO_{u,t} + 0.661 \times (SST_{a,t-2} \times PDO_{u,t})$	t-value	7.945	*p*-value	1.41×10^{-10}
		F statistic	63.12	R^2	0.606
		DF	41	AIC	875
	Models with single variables				
ii.)	$\log(R_t) = 1.610 \times \log(SSB_t) + 1.01 \times 10^{-3} \times SST^3_{a,t-2}$	t-value	5.298	*p*-value	4.28×10^{-6}
		F statistic	28.07	R^2	0.406
		DF	41	AIC	893
iii.)	$\log(R_t) = 2.467 \times \log(SSB_t) - 0.106 \times PDO_{u,t}$	t-value	3.556	*p*-value	9.65×10^{-4}
		F statistic	12.65	R^2	0.236
		DF	41	AIC	904
iv.)	$\log(R_t) = 2.466 \times \log(SSB_t) - 6.25 \times 10^{-2} \times ENSO_{s,t-1}$	t-value	2.935	*p*-value	5.44×10^{-3}
		F statistic	8.616	R^2	0.174
		DF	41	AIC	907
	Recruits per spawning biomass (RPS)				
	Model with multiple variables				
v.)	$\log(R_t/S_t) = 0.127 + 1.74 \times 10^{-2} \times SST^2_{m,t-2} + 4.21 \times 10^{-2} \times PDO^2_{v,t-5} + 2.19 \times 10^{-2} \times PDO^3_{v,t-5}$	t-value	7.620	*p*-value	2.23×10^{-9}
		F statistic	58.06	R^2	0.586
		DF	41	AIC	478
	Models with single variables				
vi.)	$\log(R_t/S_t) = 5.64 \times 10^{-9} + 1.50 \times SST_{m,t-2}$	t-value	6.481	*p*-value	8.94×10^{-8}
		F statistic	42.01	R^2	0.506
		DF	41	AIC	486
vii.)	$\log(R_t/S_t) = 301.21 + 0.115 \times PDO_{v,t-5} + 5.01 \times 10^{-2} \times PDO^2_{v,t-5}$	t-value	3.293	*p*-value	2.05×10^{-3}
		F statistic	10.84	R^2	0.209
		DF	41	AIC	506
viii.)	$\log(R_t/S_t) = 307.96 + 0.280 \times ENSO_{w,t-2} - 4.67 \times 10^{-2} \times ENSO^3_{w,t-2}$	t-value	3.055	*p*-value	3.94×10^{-3}
		F statistic	9.34	R^2	0.186
		DF	41	AIC	507

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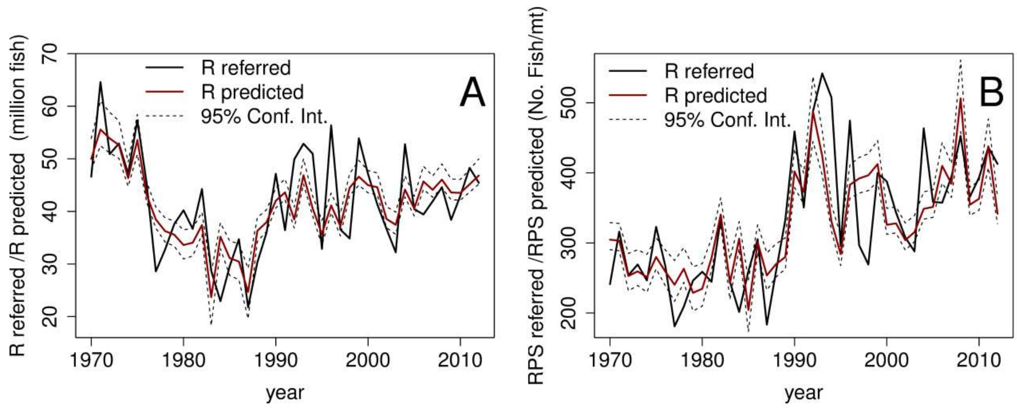

Figure 5. *Key model plots showing; (A) the referred and the predicted recruitment (R) from model (i) (Table 2) and (B) the referred and predicted recruits per spawning biomass (RPS) from model (v) (Table 2) time series trajectory of the North Pacific albacore tuna (T. alalunga) stock from 1970-2012. The 95% confidence interval for the estimated R and RPS are shown.*

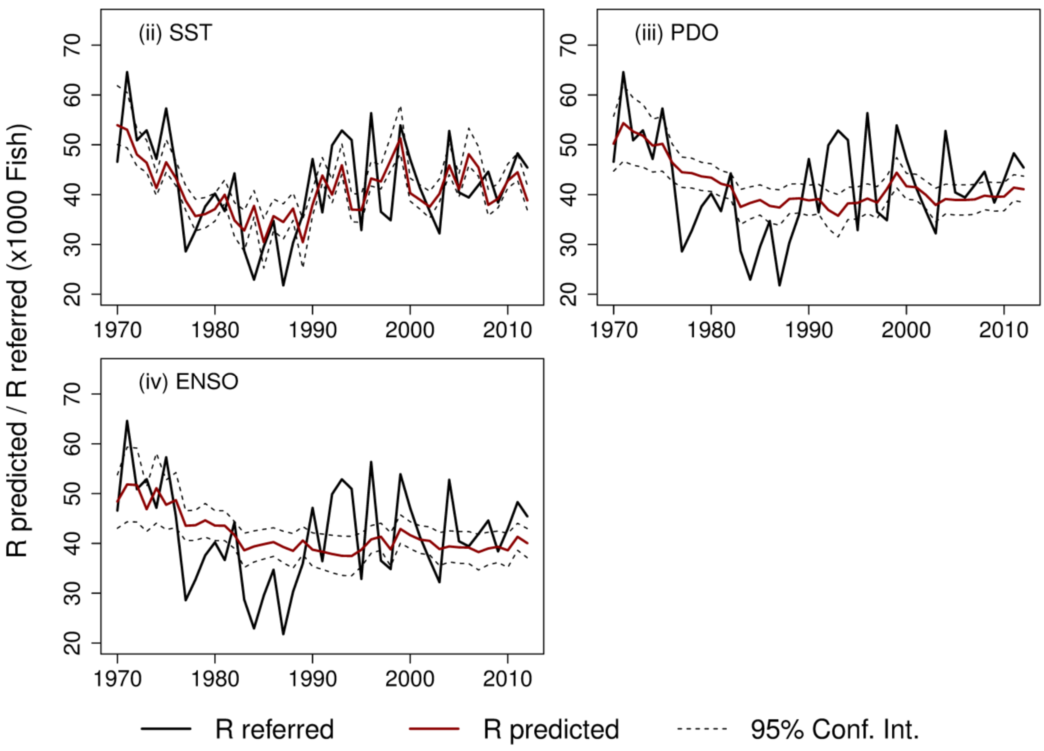

Figure 6. *Graph showing the referred recruitment (R) time series trajectory of the North Pacific albacore tuna (T. alalunga) stock and the trajectory which resulted from models with single variables of sea surface temperature (SST), Pacific decadal oscillation (PDO) and El Niño southern oscillation (ENSO) for the years 1970-2012. The 95% confidence interval for the estimated R and RPS are also shown. The roman numerals refer to the models from Table 2.*

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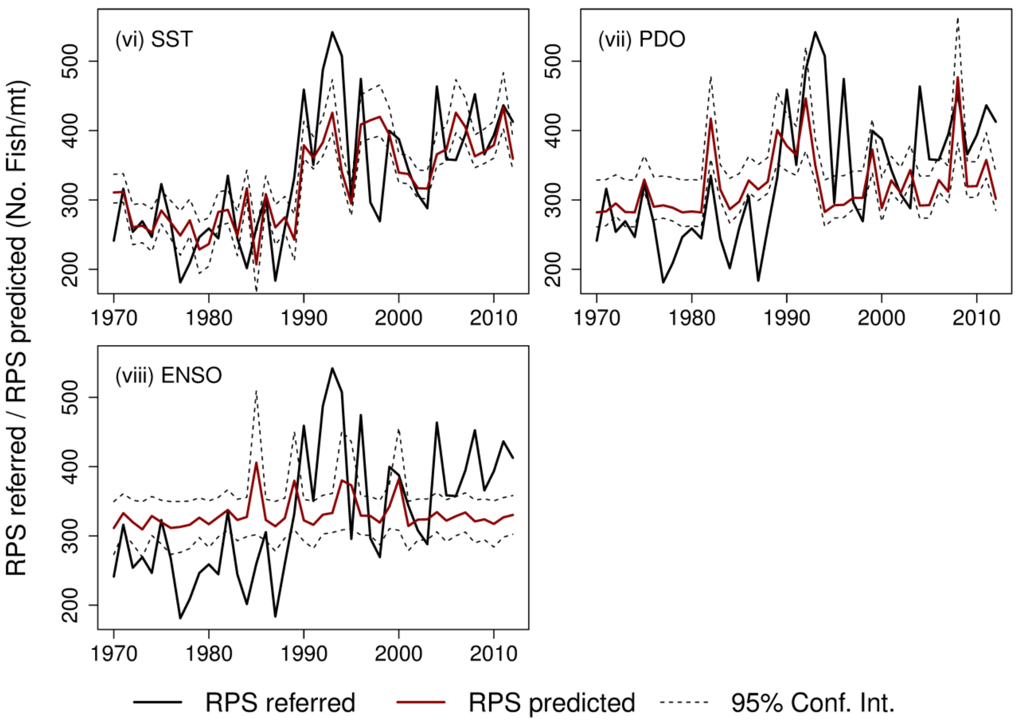

Figure 7. *Graph showing the referred recruits per spawning biomass (RPS) time series trajectory of the North Pacific albacore tuna (T. alalunga) stock and the trajectory which resulted from models with single variables of sea surface temperature (SST), Pacific decadal oscillation (PDO) and El Niño southern oscillation (ENSO) in red for the years 1970-2012. The 95% confidence interval for the estimated R and RPS are also shown. The roman numerals refer to the models from Table 2.*

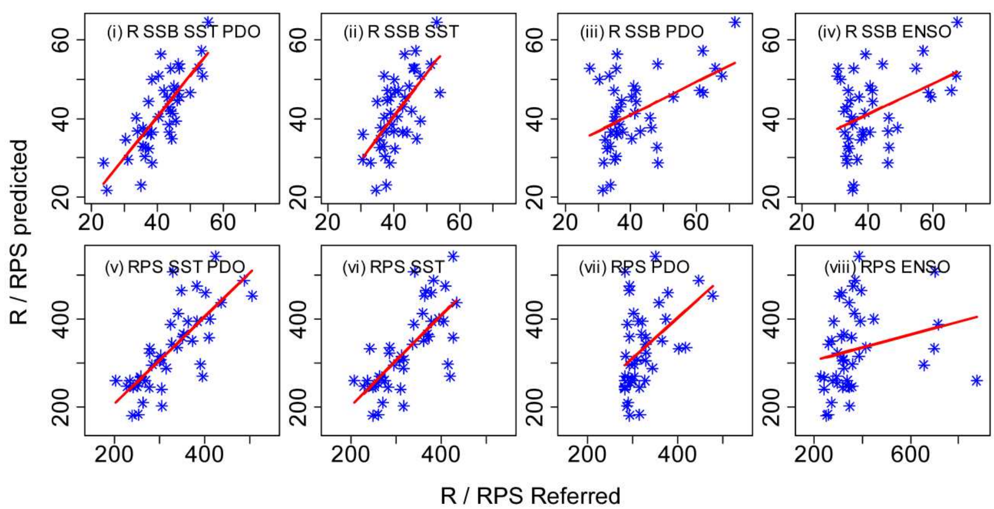

Figure 8. *The linear relationship between the predicted recruitment (R predicted) and referred recruitment (R referred) (i, ii, iii, iv) and recruits per spawning biomass predicted (RPS predicted) and recruits per spawning biomass referred (RPS referred) (v, vi, vii, viii) for the albacore tuna (T. alalunga) stock in the North Pacific Ocean. The roman numerals refer to the model numbers presented in Table 2. Axis values are same for all figures for R and RPS except for (viii) where some extreme values can be observed.*

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The histograms for the residuals of models (i)-(viii) from *Table 2* are shown in *Fig. 9* to test for the homogeneity of variance. If the model residual variance is >4.00 then the least square estimators will be considerably degraded and this can lead to the selection of erroneous models (Fox, 2008). Models (i)-(iv) for R had residual variances of <0.9 and for RPS the models (v)-(viii) had residual variance values of <1.2 in all cases which fulfilled the requirements for the homogeneity of variance.

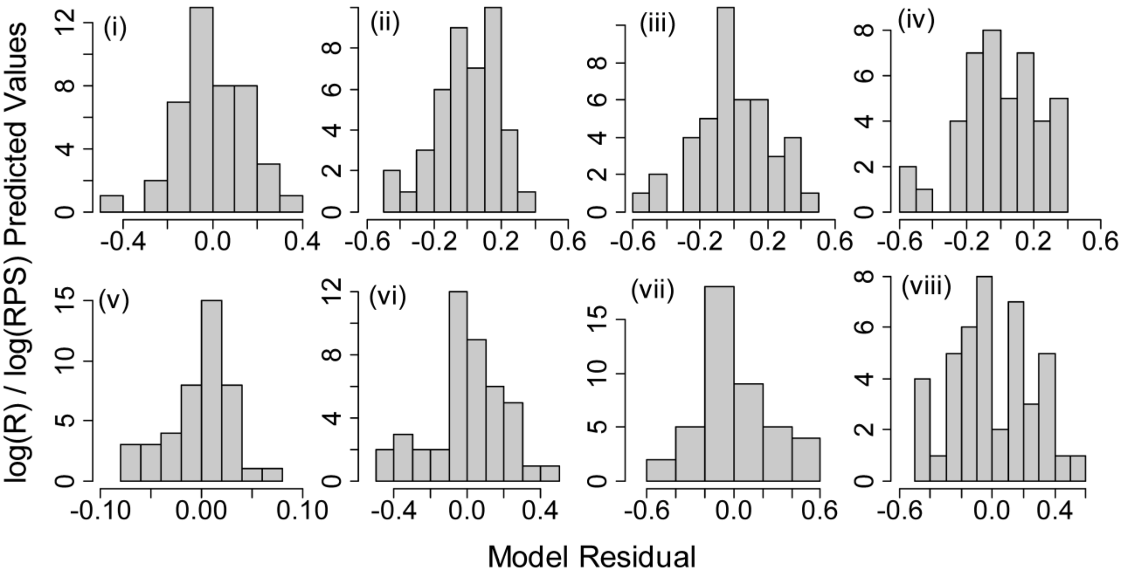

Figure 9. *Histograms of model residuals from Table 2 against the predicted values. The roman numerals refer to the model numbers in Table 2. The residual variance model (i) for recruitment (R) and model (v) for recruits per spawning biomass (RPS) are <0.9 and <1.2 respectively. The residual variance for models incorporating multiple environmental variables (model i and v) are smaller compared to models with single environmental variable.*

According to *Fig. 3* and *Table 2* the principle independent variable that has the most significant effect on the North Pacific albacore tuna R and RPS trajectory is the SST. *Fig. 10* shows the heat maps for the spatial distribution of the annual SST from the year 1970 to 2012. Visual changes in annual spatial SST can be observed including part of the warm pool (represented in purple and white) which can be observed to change annually.

It should be noted that the standard deviation (± SD) for albacore SSB and R from 2014 ISC report was 97,569 ± 30,203 mt for 1993 and 204,401 ± 73,551 mt for the year 1971 for SSB. For R the SD was 21.8 ± 7.4 million fish for 1987 and 64.6 ± 18.8 million fish for the year 1971 (ISC, 2014). Most annual deviations were quite significant, however this was the best available estimate for the South Pacific albacore tuna from 1970 to 2012 and the models presented are based on these estimates.

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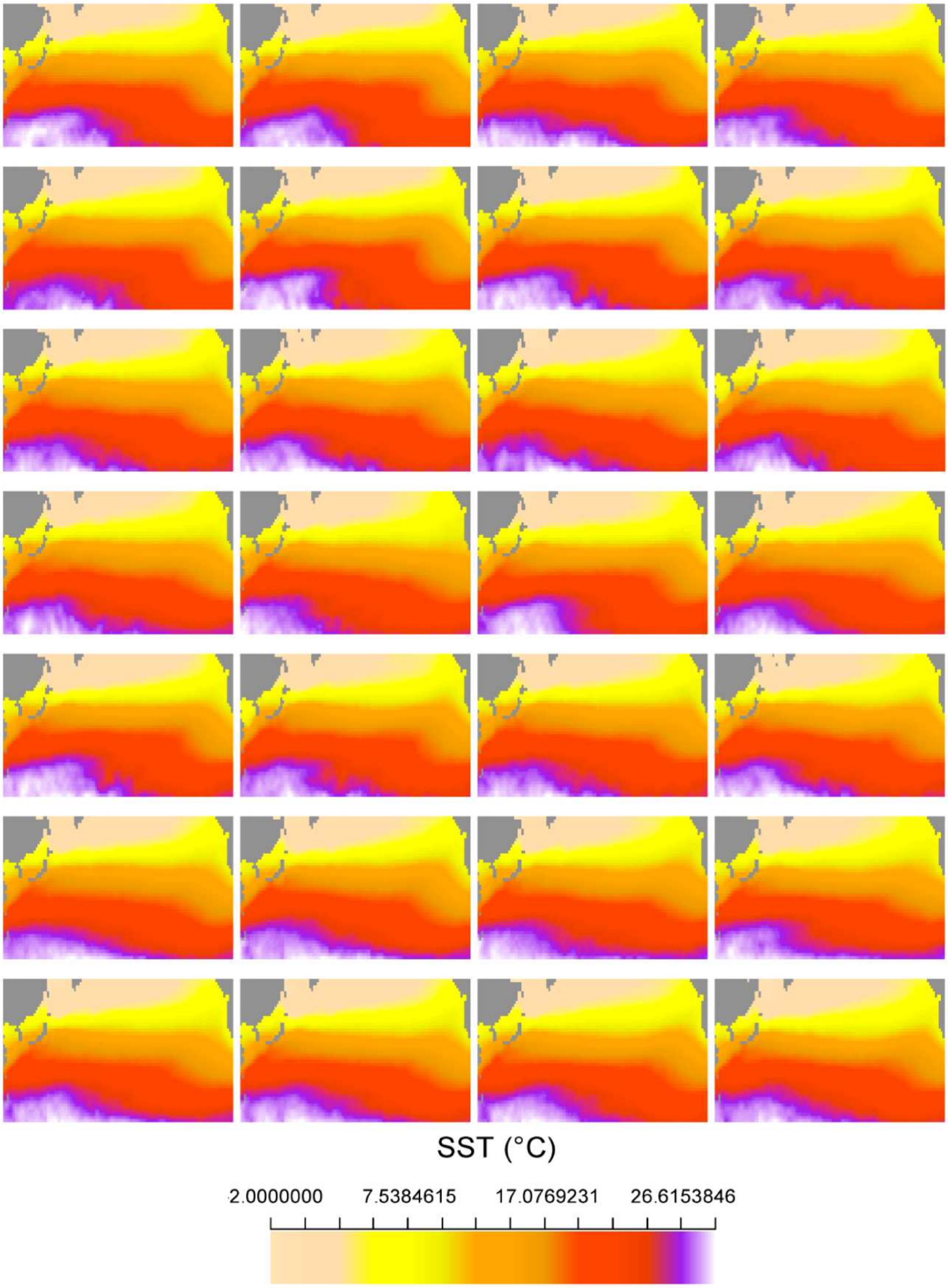

Figure 10. *Sea surface temperature (SST) of study area in the North Pacific from 1970 to 2012 from top left to right. The region in purple and white belong to the warm pool where the sea surface temperature is higher than the rest of the geographical oceanic area and seems to fluctuate through the years. Note that the warm pool area coincides with the spawning area for North Pacific albacore shown in Figure 1. The axis labels for latitude, longitude and scales have been removed for enhanced visual representation. Refer to Fig. 1 for study area details.*

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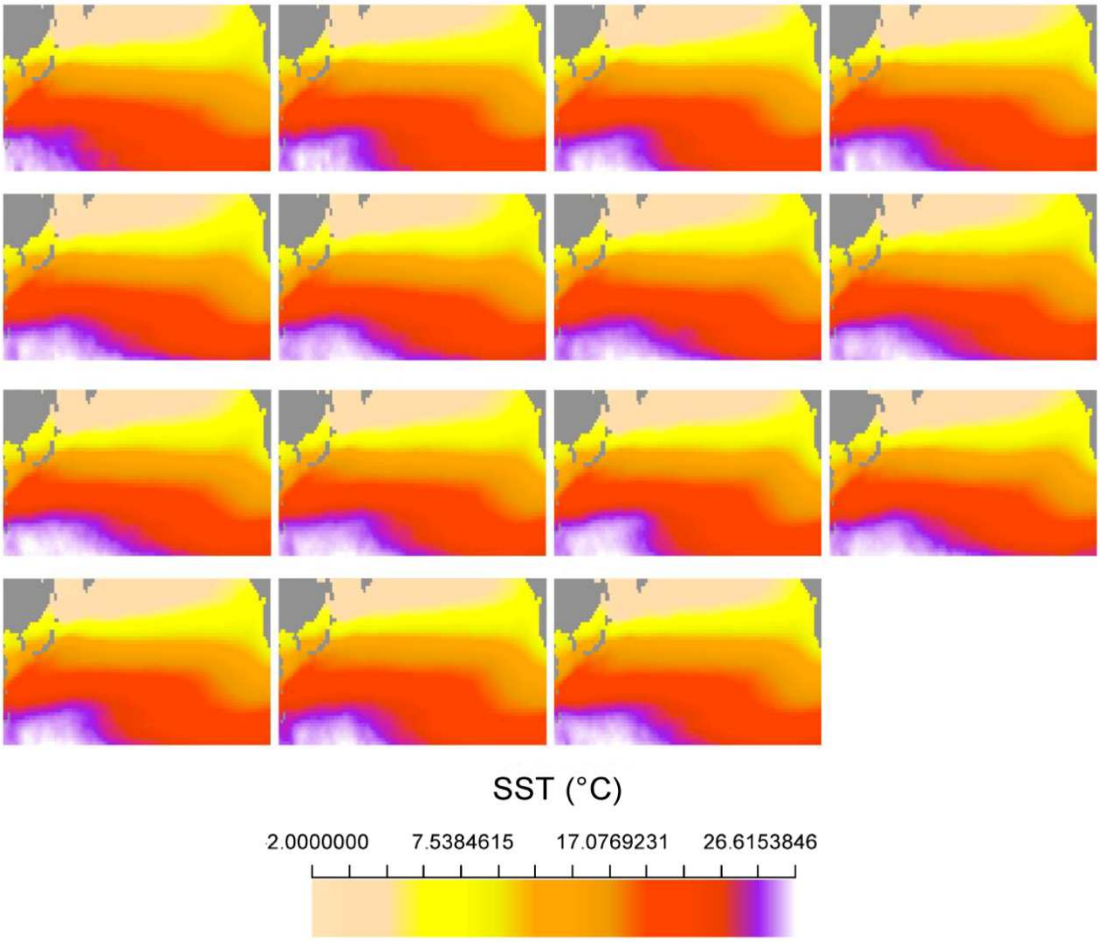

Figure 10. *(Cont.)*

Discussion

This work was undertaken with one of the objectives to identify whether alterations in environmental and climatic conditions affected the stock dynamics of the North Pacific albacore tuna. Significant relationships were detected between the albacore tuna R, RPS and SSB with the environmental variables of SST, PDO and ENSO. GAM modeling (Equation 3 and Equation 4) resulted in better fitting of models compared to GLM models (Equation 1 and Equation 2) and the selected models (model (i) and (v), *Table 2*) for most significantly explaining R and RPS trend were GAM models. This is consistent with the findings that GAM performs better than other types of population modeling (Drexler and Ainsworth, 2013; Moisen and Frescino, 2002; Guisan et al., 2002; Walsh and Kleiber, 2001).

The incorporation of SST and PDO resulted in stock reproduction models for the albacore tuna R and RPS which fit quite well with the referred R and RPS stock trajectory (*Table 2, Fig. 5*). R exhibited highest correlation with PDO and RPS had most significant correlation with SST. However, when modeling was performed using single variables (*Table 2, Fig. 6, Fig. 7*), SST resulted in the stock reproduction models with highest fitness for both R and RPS. With reference to the combined and individual effects of the predictor environmental variables (*Table 2*) it is evident that SST is the principle factor affecting the R and RPS time-series of albacore and the North Pacific

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SST and PDO act together to result in the stock dynamics pattern for the North Pacific albacore tuna R and RPS.

The importance of SST in structuring the stock characteristics of albacore tuna has been shown in previous work. Briand et al. (2011) used the GLM to investigate the links between environmental conditions and albacore tuna in New Caledonia EEZ. Findings showed that albacore CPUE was significantly linked to the variability in SST and primary productivity. Lehodey et al. (2015) investigated the impacts of environmental conditions on the spatial dynamics of albacore tuna in the South Pacific. Findings indicated possibly large impact of changes in oceanic temperature on larval survival and early life stages of albacore. The link between SST affecting dissolved oxygen levels and primary productivity was also established. This gives some indication on the possible mechanisms on how oceanic temperature affects albacore tuna stock abundance and distribution. Dragon et al. (2015) used the Spatial Ecosystem and Population Dynamics Model (SEAPODYM) and the maximum likelihood approach to understand the spatial distribution, ecological characteristics and migratory behavior of the North Atlantic albacore tuna in relation to alterations in the environmental patterns. Spawning favorability and foraging behavior of adult fish were shown to be correlated to different oceanic temperature gradients. From the positive fit of SST with R and RPS (models (i), (ii), (v), (vi), *Table 2*) it can be inferred that warmer temperatures are favorable and cooler temperature are unfavorable for the reproductive success and recruitment of albacore.

The incorporation of PDO results in significant improvement of the models for both R and RPS as can be seen from *Table 2*. Singh et al. (2015) investigated the relationship between albacore CPUE and climatic time series using GLM. Significant links were established between PDO and albacore time series trajectory from 1957 to 2008 in the South Pacific Ocean. Phillips et al. (2014) studied the effect of alterations in the SST and large–scale variability in the climatic conditions of PDO and ENSO on the spatial distribution of the juvenile North Pacific albacore tuna CPUE off the US West coast. Significant relationships were determined between the CPUE and the environmental variables. The incorporation of the SST and PDO as independent variables in the threshold Generalized Additive Mixed Models resulted in statistically significant models with the highest R^2 value of 0.290. Lehodey et al. (2003) attempted to deduce the mechanisms by which alterations in environmental variables affects the stock of important tuna species in the Pacific. Results indicated that albacore tuna recruitment was significantly affected by the negative and positive phases of PDO. PDO had highly significant correlation with R in the same year and with SSB having a lag period of 5 years. This indicates that variability in the PDO pattern influences the early life stages of the North Pacific albacore tuna. With reference to *Figure 3*, the relationship of PDO with R and SSB is negative. This means that the negative PDO phase is favorable and positive PDO phase is unfavorable for the larvae and juvenile stages of albacore. Similar explanations have been derived for the South Pacific albacore stock by Lehodey et al. (2003) and Singh et al. (2015).

Although ENSO were excluded from model formulation, it did exhibit significant relationship with albacore tuna R, RPS and SSB according to *Fig. 3*. Models with single variables incorporating ENSO are significant for both R and RPS (*Table 2*). Zhang et al. (2014) used a logistic production model to study the link between changes in climatic indices and albacore productivity and determined significant relationship of the North Pacific albacore tuna productivity with ENSO.

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Briand et al. (2011) elucidated that extreme ENSO events are favorable for local albacore tuna fishery in New Caledonia EEZ.

The warm pool SST area (*Fig. 10*) extends from the North to the South Pacific and has been shown to also affect the stock trajectory of the South Pacific albacore tuna (Singh et al., 2015). The warm pool geographical area coincides with the spawning ground for the North Pacific albacore tuna (*Fig. 1, Fig. 10*) as determined by Chen et al. (2010), Yoshida (1968), Otsu and Uchida (1959), Ueyanagi (1957). This makes it ecologically viable for the SSB and RPS to correlate with annual changes in the SST of this area and affect the stock dynamics of albacore tuna. Albacore tuna prefer warmer water temperatures for spawning and larvae habitat (Lehodey et al., 2008; Lehodey et al., 2015) which occurs at the warm pool zone (*Fig. 10*). Dragon et al., (2015) identified that the maximum spawning for the North Atlantic albacore tuna occurs at SST of 25.77ºC with a standard error of 1.74ºC. With reference to *Fig. 10*, this temperature falls within the temperature range at the warm pool which is further evidence of variability in the SST affecting the spawning and other stock characteristics of albacore tuna. Further work on the specific warmpool area and its influence on the South Pacific albacore tuna is needed to quantify its effect.

Lehodey et al., (2015) showed that both fishing and environmental conditions significantly affect the abundance of albacore tuna in the South Pacific. Albacore tuna fishery managers and policy makers need to take into account the effects of environmental conditions when formulating their stock harvest plans and management strategies in order for the biological and economical sustainability of this commercially important but limited resource.

The GAM models developed in this study (model (i) and (v), *Table 2*) were highly significant with reference to the *p*-values. These models were able to explain a substantial component of the y-axis variation for R and RPS trend from 1970 to 2012. With reference to the R^2 values, there are still considerable portion of the variation that is not explained. It is dangerous to assume that environmental and climatic variables are the only factors affecting the North Pacific albacore stock trajectory. Other variables, such as fishing intensity (effort) and various biotic and abiotic factors are also responsible for stock variation. For fishery management it is also dangerous to assume that fishing related activities are the only factors affecting a fish stock. Fish stock levels are determined by a combination of complex interactions between, fishing related activities, environment change, climatic variation and various biotic and abiotic factors. Further research is needed in order to elucidate the underlying mechanism by which environmental conditions interact with other factors to influence albacore tuna stock abundance and distribution in order to develop coherent policies for fisheries management.

The models presented here and the arguments are based on the estimates of the 2014 ISC report where significant deviation was noted for R and SSB data. Better estimates in the future will enable further improvement of the models and analysis.

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MAIN ENVIRONMENTAL FACTORS AFFECTING CONCENTRATIONS OF CULTURABLE AIRBORNE BACTERIA IN INDOOR LABORATORIES OVER A PERIOD OF ONE YEAR

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Abstract. This study aimed to assess temporal changes in the concentration of culturable airborne bacteria (CAB) in three microbiology laboratories to determine the environmental factors that affects CAB concentration. The CAB concentration was determined once per month from March 2011 to February 2012 in the three laboratories. An Andersen one-stage sampler was used to collect CAB for 5 min, three times per day. CAB concentrations demonstrated an increasing tendency in summer and fall, but it was difficult to detect consistent seasonal patterns. Temperature, relative humidity (RH), number of people, and activity of people were associated with CAB concentrations. The overall CAB concentrations were significantly greater in the study rooms than that in the laboratory. CAB concentrations in indoor microbiology laboratories varied greatly depending on the number of people and whether or not a humidifier was used.
Keywords: *airborne bacteria, temperature, relative humidity, humidifier, seasonality*

Introduction

Air quality in closed environments is an important factor for human health because people tend to spend most of their time in various indoor environments, such as the home, the workplace, or other microenvironments (Klepeis et al., 2001). Exposure to indoor microbial airborne particles, especially fine (<1 mm) and ultrafine (<0.1 mm) particles, has been identified as an important factor affecting human health, and is known to cause adverse pulmonary effects, headache, and allergies (Douwes et al., 2003; Dockery, 2009). Airborne bacteria are known to cause infectious diseases, hypersensitivity pneumonitis, and lung functions (Pastuszka et al., 2000).

People who work with microbes or in the field of biotechnology have greater potential to be directly exposed to microorganisms than those in other occupations. Most currently operating clinical microbiology laboratories are busy and crowded. They were often designed several decades ago, before molecular diagnostic methods or bioterrorism preparedness became commonplace. Their air-handling systems have become overloaded or unstable as instruments and personnel have multiplied (Baron and Miller, 2008). Characterization and measurement of the concentration of airborne microorganisms in a laboratory is difficult because of the diversity of infectious microorganisms handled, variation in the efficiencies of air

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sampling equipment, differences in the viability of infectious microorganisms, and lack of a standardized method for measuring individual microorganisms in the air (Hwang et al., 2014a). Many positive changes have been made in standard safety practices, and most laboratory workers feel that they are safer than they were earlier, but evidence-based research is lacking to confirm that supposition. Therefore, maintenance of a sanitary environment is crucial for laboratories dealing with microorganisms.

The purpose of this study was to assess temporal changes in CAB concentrations in three microbiology laboratories to determine which environmental factors, such as temperature, relative humidity (RH), number of people, and activity of people, are associated with CAB concentrations.

Materials and methods

Characteristics of the laboratories and environmental factors

This study was conducted for a 12-month period from March 2011 to February 2012. A total of 212 samples were collected once a month in the three microbial laboratories. *Table 1* summarizes the characteristics of the laboratories. The environmental factors such as temperature, RH, Number of people, and activity of people were measured in the three microbial laboratories. Temperature and RH were recorded by VelociCalc Air Velocity Meter (Model 9555, TSI Inc., USA). Number of people was counted only during CAB sampling times and activity of people was measured by counting of people who were passing by the sampling site within one meter during CAB sampling times.

Sampling and analysis

CAB was collected three times a day (9:00- 10:00, 13:00 - 14:00, and 16:00- 17:00), for once a month in each laboratory. Every sampling was conducted at the same location: the center of the each room. A single-stage viable cascade impactor (SKC Inc., USA) connected with a pump (Quick Take 30, SKC Inc., USA) was operated at a flow rate of 28.3 l min^{-1} for 5 min. Before the sampling operation, the sampling equipment was sterilized with a 70 % ethyl alcohol swab, and nutrient media in Petri dishes were placed on the one-stage impactor. The CAB samples were collected on trypicase soy agar (TSA) plates. The sampled media were sealed with laboratory film to prevent contamination and desiccation during the incubation. The samples were transferred to the laboratory in a sterilized ice box with refrigerant packs to keep the samples below 4°C. TSA plates were incubated at 35 °C for up to 2 days. The positive hole correction table was used to adjust colony counts (ACGIH, 1999). The concentrations of CAB was calculated by dividing the number of colonies by air volume and written as colony-forming unit per cubic meter of air ($CFUm^{-3}$).

Statistical analysis

Kolmogorov-Smirnov test was used to determine whether the data were normally or log-normally distributed. Arithmetic means (AM) of CAB concentrations and their standard deviation (SD) were calculated. Independent *t*-test was used to compare the differences between environmental factors such as divided room by structure of laboratory, use of humidifier, and use of air-conditioner. CAB concentrations in the three microbial laboratories, and p value less than 0.05 was considered to be statistically

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significant. Correlation analysis was used to identify the association between CAB concentrations and environmental factors. All statistical analyses were performed by SPSS (version 23.0; IBM Inc., USA).

Results

Table 2 summarizes the CAB concentrations in the three microbiology laboratories each month. The AM of CAB concentrations over the year ranged from 7–5823 CFUm^{-3} in the three microbiology laboratories. The highest AM concentration of CAB was found in August for laboratories A (945 CFUm^{-3}) and B (1162 CFUm^{-3}) and in October in laboratory C (2396 CFUm^{-3}), whereas the lowest AM concentration of CAB was observed in February in laboratory A (60 CFUm^{-3}), in March in laboratory B (91 CFUm^{-3}), and April in laboratory C (319 CFUm^{-3}). The AM of CAB concentrations differed significantly between laboratories A and B and C ($p < 0.05$).

To show seasonal changes in CAB concentrations, the 12 months were grouped into four seasons: March to May were grouped as spring, June to August as summer, September to November as fall, and December to February as winter. *Figure 1* presents seasonal changes in CAB concentrations. Laboratories A and B showed similar seasonal changes in CAB concentrations, gradually increasing from summer to fall and then decreasing from fall to winter. However, the CAB concentrations in laboratory C increased from fall to winter and decreased from spring to summer.

Spearman correlation analyses were performed to identify relationships between CAB concentrations, temperature, RH, number of people, and activity (*Table 3*). A significant positive correlation was observed between CAB concentration and temperature ($r = 0.269$, $p < 0.001$), RH ($r = 0.451$, $p < 0.001$), number of people ($r = 0.328$, $p < 0.001$), and activity of people (0.321, $p < 0.001$).

Table 4 shows the CAB concentrations by location and by factors within laboratories. Two groups were defined for each factor, as follows: laboratory rooms and study rooms, off and on for the humidifier, laboratory room and study room among rooms with the humidifier off, off and on for the air conditioner. The CAB concentrations were significantly higher in study rooms than in laboratory rooms; they were significantly higher in the laboratories in which the humidifier was on than in laboratories in which the humidifier was off; when the humidifier was on, they were higher in study rooms than in laboratories; they were higher in the laboratories in which the air conditioner was on than in the laboratories in which it was off. However, no significant difference in CAB concentrations was observed between groups when the humidifier was off or when the air-conditioner was on ($p > 0.05$).

Fig. 2 shows associations between CAB concentrations and number of people in each study room. CAB concentrations in laboratory C ($r = 0.451$), which contained more people, were significantly more strongly associated with number of people than those of laboratories A or B, although CAB concentrations in laboratories A ($r = 0.137$) and B ($r = 0.331$) were also associated with number of people.

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Table 1. *The characteristics of environmental factors in the three microbial laboratories*

	Laboratory A	Laboratory B	Laboratory C
Room volume (m^{-3})	380	393	390
(Area × Height)	(146 × 2.6)	(151 × 2.6)	61.4 (151 × 2.6)
Temperature (°C) (Mean ± SD)	23.6 ± 2.4	24.2 ± 2.5	24.5 ± 1.4
RH (%) (Mean ± SD)	37.7 ± 20.5	34.3 ± 18.3	35.7 ± 18.2
No. of people (Mean ± SD)	0.98 ± 1.38	1.10 ± 1.56	2.90 ± 3.40
Laboratory room	0.4 ± 0.6	0.4 ± 0.6	0.8 ± 0.70
Study room	1.6 ± 1.7	1.8 ± 1.9	5.0 ± 1.25
Activity of people (Mean ± SD)	1.00 ± 1.30	0.84 ± 1.31	1.46 ± 1.67
Experiment	Production of amino acid with microorganism	Production of amino acid with microorganism	Waste purification with microorganism

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Table 2. *Monthly concentrations of CAB in three microbial laboratories*

Months	CAB ($CFUm^{-3}$)								
	Lab. A			Lab. B			Lab. C		
	N	Mean(SD)	Range	N	Mean(SD)	Range	N	Mean(SD)	Range
Jan.	6	107 (55.6)	50 – 213	6	120 (112.1)	28 – 329	6	1780 (2434.0)	108 – 5823
Feb.	6	126 (117.2)	7 – 337	6	122 (112.5)	35 – 346	6	594 (383.2)	190 – 1175
Mar.	5	60 (32.4)	21 – 101	6	91 (44.6)	35 – 130	5	1191 (1158.2)	290 – 2572
Apr.	6	335 (165.4)	138 – 543	6	110 (94.1)	28 – 251	6	319 (159.2)	86 – 587
May	6	273 (235.3)	115 – 729	6	241 (167.2)	64 – 435	6	375 (238.4)	175 – 842
Jun.	6	576 (293.4)	213 – 988	6	485 (183.4)	314 – 823	6	767 (490.5)	167 – 1317
Jul.	6	387 (187.5)	220 – 693	6	507 (163.6)	329 – 712	6	623 (360.2)	220 – 1273
Aug.	6	945 (1117.7)	190 – 3177	6	1162 (896.1)	451 – 2888	6	641 (373.1)	71 – 1196
Sept.	4	510 (59.7)	451 – 570	6	290 (199.6)	93 – 596	6	706 (211.1)	329 – 968
Oct.	6	748 (1210.5)	64 – 3177	6	206 (73.3)	86 – 314	6	2396 (2013.6)	306 – 5180
Nov.	6	630 (661.0)	130 – 1931	6	210 (68.0)	123 – 298	6	517 (166.1)	306 – 739
Dec.	6	91 (44.2)	42 – 153	6	290 (151.4)	115 – 493	6	1133 (1369.2)	259 – 3754
Total	69	401 (571.3)	7 – 3177	72	320 (391.5)	28 – 2888	71	916 (1157.6)	71 – 5823

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Table 3. *Spearman correlation analysis between CAB and environmental factors in the microbial laboratories*

	Conc. (CFUm^{-3})	Temperature (°C)	RH (%)	No. of people	Activity of people
Conc. (CFUm^{-3})	1.000				
Temperature (°C)	0.269**	1.000			
RH (°C)	0.451**	0.301**	1.0000		
No. of people (%)	0.328**	0.120	-0.038	1.000	
Activity of people	0.321**	0.232**	0.218**	0.232**	1.000

*: $p < 0.05$, **: $p < 0.001$, N = 204

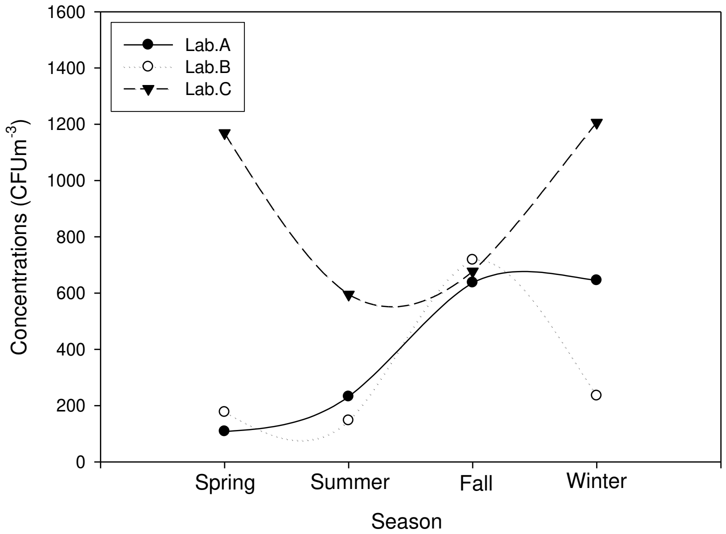

Figure 1. *Seasonal changes in concentrations of CAB in microbial laboratories*

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Table 4. *Comparisons of CAB concentrations between the categorized groups by the environmental factors of the three microbial laboratories*

	Categorized groups	No. of sample	CAB concentrations ($CFUm^{-3}$) Mean ± SD [b]	Min	Median	Max	*p*-value
[a] Separation	Laboratory room	106	384 ± 382	21	298	3177	< 0.01
	Study room	105	712 ± 1077	7	329	5823	
	Total	211	547 ± 821	7	314	5823	
Humidifier	Turn off	198	423 ± 508	7	298	3754	< 0.001
	Turn on	13	2432 ± 1861	123	2337	5823	
	Total	211	2432 ± 1861	7	314	5823	
Turn off humidifier	Laboratory room	106	384 ± 382	7	298	3177	> 0.05
	Study room	92	469 ± 621	7	314	3754	
	Total	198	2432 ± 1861	7	298	3754	
Air-conditioner	Turn off	77	426 ± 622	21	290	4191	> 0.05
	Turn on	132	621 ± 917	7	329	5823	
	Total	209	549 ± 825	7	314	5823	

[a] These laboratories were made up of one space divided by two spaces into laboratory room and study room with a partition wall [b]; Standard deviation

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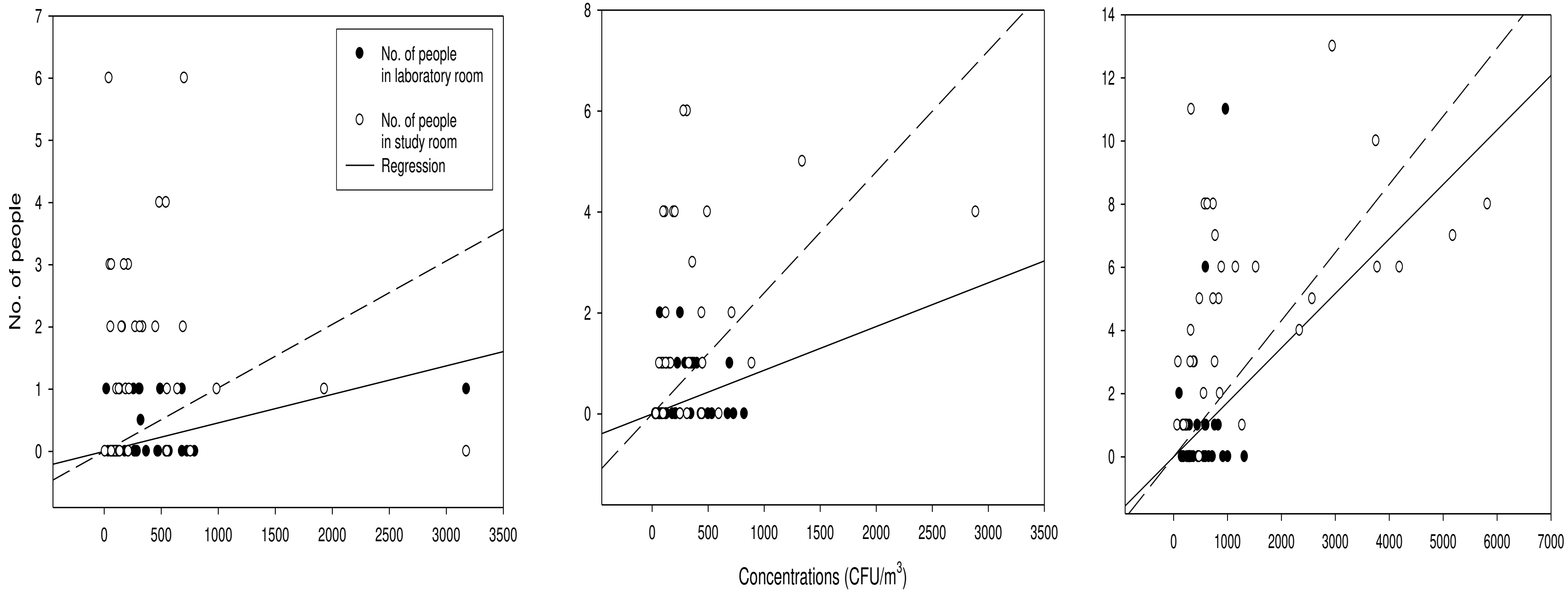

(a) Laboratory A (r =0.137) (b) Laboratory B (r =0.331) (c) Laboratory C (r =0.451)

Figure 2. *Association between CAB concentrations and the number of people both for laboratory room and study room of laboratory A, B, and C*

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Discussion

CAB concentrations were measured in three microbiology laboratories to assess monthly and seasonal changes and to investigate the effects of several environmental factors (temperature, RH, number of people, and activity of people) to determine whether there are any associations between these factors and CAB concentrations.

In 29 of a total of 212 samples (13.7%) from the three microbiology laboratories, CAB concentrations exceeded 800 CFUm^{-3}, according to Korean guidelines (Ministry of Environment of Korea, 2014). Among the 29 samples that exceeded 800 CFUm^{-3}, 21 were from laboratory C. The overall mean concentration (916 CFUm^{-3}) of CAB in laboratory C was threefold higher than the Indoor Air Quqlity Association recommendation. A previous study showed that the mean concentration of total airborne bacteria in indoor environments, including occupational environments, was 308 CFUm^{-3} in subway stations (Hwang et al., 2015), 684 CFUm^{-3} (median) in homes, 222 CFU/m^{-3} (median) in elderly car centres (Madureira et al., 2015), 105 CFU/m^{-3} in swine confinement buildings (Douwes et al., 2003), 113 CFUm^{-3} in a feedstock manufacturing factory (Kim et al., 2007), 198 CFUm^{-3} during a pelleting and powdering process, and 281 CFUm^{-3} (maximum level) in 100 U.S. office buildings (Tsai and Macher, 2005). These concentrations of total airborne bacteria were relatively low compared to that measured in laboratory C in this study. Airborne bacteria levels exceeding 600 CFUm^{-3} can be associated with insufficient ventilation or abnormal sources of microorganisms (Salonen et al., 2007).

The CAB concentrations in laboratories A and B presented similar seasonal patterns, whereas the CAB concentrations in laboratory C showed a contrasting pattern (*Fig. 1*). In previous studies of indoor air quality conducted in Chicago homes, culturable bacteria were highest in summer and fall (Moschandreas et al., 2003), whereas in Finland, only a slight yet significant difference was observed between summer and winter bacterial levels (Reponen et al., 1992). However, other studies in homes have shown a large decline from spring to summer, an increase in fall, followed by a decrease toward winter (Frankel et al., 2012). These discrepancies might be caused by other factors, which can influence CAB concentrations, rather than by seasonal changes themselves. Sources of bacteria in outdoor air can change over short periods of time, in relation to climatic conditions (Rintala et al., 2008; Womack et al., 2010); however, indoor air bacterial concentrations are less strongly related to climatic conditions than those of outdoor air.

To identify factors influencing CAB concentrations in microbiology laboratories, Spearman's correlation analyses were used to identify correlations between CAB concentrations, temperature, RH, number of people, and activity of people (*Table 3*). Positive correlations were observed between CAB concentrations, temperature ($r = 0.269$), RH ($r = 0.451$), number of people ($r = 0.328$), and activity of people ($r = 0.321$). Temperature is an environmental factor that typically influences biological agents (WHO, 2009). In our study, higher CAB concentrations were significantly related to higher temperature. This result is consistent with previous studies (Guo et al., 2004; Jo et al., 2005; Hwang et al., 2011a; Hwang et al., 2015). However, other studies did not show associations, positive or negative, between CAB concentrations and temperature (Frankel et al., 2012; Madureira et al., 2015). The authors hypothesized that the

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discrepancy might be caused by small variations in indoor parameters, which exclude any association with biological pollutants. RH was significantly associated with CAB concentrations ($p < 0.05$) and was the environmental factor most strongly associated with the CAB concentration ($r = 0.451$). RH is known to be crucial for microorganism growth, even at low temperatures (Tsai et al., 2009). However, no relationship was observed between comparatively low RH (< 60%) and CAB concentration (Hwang et al., 2011a). The number of people ($r = 0.328$) and activity of people ($r = 0.321$) were associated with CAB concentrations. Other studies have found that the number of people is positively associated with the concentration of CABs in subway station environments, consistent with airborne microorganisms being dispersed into the air from subway passengers' clothing and hair (Boudia et al., 2006; Cho et al., 2006; Bogomolova and Kirtsideli, 2009; Hwang et al., 2014b). Moreover, changes in microbial communities between peak and nonpeak commuting hours can largely be attributed to increases in skin-associated genera (Leung et al., 2014). Sources of airborne bacteria in built environments include humans, pets, soils, and plants (Jo and Seo, 2005). Indoor human occupancy was found to be closely related to indoor microbial levels (Scheff et al., 2000), and settled spores were resuspended in indoor air by air movement caused by human activities, such as walking and running (Buttner and Stetzenbach, 1993). In classrooms, sampling time supports the effect of activity, as indoor bioaerosol ratios were higher during break times, when childrens' activity was higher than during class time (Jo and Seo, 2005).

CAB concentrations were higher in study rooms than in laboratory rooms, even in which the humidifier was turned off (*Table 4*). These results may be influenced by peoples' activity levels, which could contribute to increased CAB concentrations, as mentioned earlier. *Fig. 2* demonstrates that the CAB concentration increased as the number of people increased in three study rooms. Laboratories using a humidifier showed significantly higher concentrations of CAB than laboratories not using a humidifier. Humidifiers can introduce bacteria into the air, as many spray water, especially those that use recirculated water or water from stagnant indoor reservoirs; *Legionella* spp. can colonize warm to hot water systems, living in biofilms that develop on surfaces in contact with water (ACGIH, 1999). Higher concentrations of CAB were observed in laboratories with the air conditioner on than in laboratories with the air conditioner off. Ventilation systems affect indoor bioaerosol concentrations because they prevent outdoor microorganisms from being transported inside buildings (Wu et al., 2005). Good ventilation and hygiene decrease the concentrations of airborne contaminants. That is, a higher ventilation rate may lead to decreased exposure to inflammatory microbial components, as measured in a granulocyte assay (Frankel et al., 2012). However, it is well known that indoor facilities such as rooms, hallways, and underground parking lots show poor indoor air quality, and poor ventilation is known to be one of the most important causes of poor air quality (Fisk et al., 2009). In addition, condensation on filters and surfaces within heating, ventilating, and air conditioning systems can be a source of biological contamination (ACGIH, 1999).

Although we identified environmental factors that can affect CAB concentrations throughout our one-year assessment cycle, this study has several limitations. First, the incubation temperature was high. The optimum temperature range for growth of CAB is known to be 25–30°C, but in this study, CAB were incubated at 35°C. However, the optimum temperature differs depending on the species (Ayersi, 1969; Pitt et al., 1983).

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Second, because of issues of accessibility, resources, and permits, we were unable to conduct the tests at the three laboratories at the same time. Finally, we were unable to identify the isolated CAB due to a funding shortage. However, we were able to predict the species of some CAB based on previous studies conducted in similar indoor microbiology laboratories (Hwang et al., 2011b; 2013).

Conclusion

We assessed the monthly and seasonal changes in CAB concentrations in three microbiology laboratories and determined which environmental factors, such as temperature, RH, number of people, and activity of people, were associated with CAB concentrations. CAB concentrations did not show consistent patterns of seasonal variation between laboratories. Temperature, RH, number of people, and activity of people were associated CAB concentrations. The overall CAB concentrations were significantly greater in the study rooms than in the laboratory rooms. CAB concentrations varied greatly depending on the number of people and use of a humidifier. Therefore, it is important to clean humidifiers regularly to prevent overgrowth of CAB in indoor environments.

Acknowledgements. This research was supported by Basic Science Research Program through the National Research Foundation of Korean (NRF) funded by the Ministry of Science, ICT & Future Planning (2015R1C1A1A02037363).

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IMPACT OF CLIMATE CHANGE ON GROUNDWATER RECHARGE IN A SEMI-ARID REGION OF NORTHERN INDIA

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Abstract. A study was undertaken to assess the impact of climate change on groundwater recharge under various climate change scenarios including the scenarios which influence the vadose zone processes and groundwater recharge for an agriculturally dominant semi-arid region. Climate change scenarios based on the predictions by Inter-Governmental Panel for Climate Change (IPCC), Indian Network for Climate Change Assessment (INCCA) and Auto Regressive Integrated Moving Average (ARIMA) model were used in this study. Ground water recharge under different scenarios was simulated using HYDRUS-1D and MODFLOW models. Results indicated that the average groundwater recharge during 2030s may increase by 0.03m compared to 2005 if simulations were based on ARIMA predicted average annual temperature, relative humidity, wind speed, and sunshine hours. It was observed that the cumulative groundwater recharge in the study area would increase if all the climatic parameters are considered for climate change impact assessment. This is in contrary to common perception that the groundwater recharge would decrease in semi-arid region as a result of climate change. Average groundwater recharge would decrease by 0.09 to 0.21 under IPCC and 0.11 m under INCCA projected temperature during 2100s and 2030s, respectively.
Keywords: *evapotranspiration, CROPWAT-8.0, Vadoze zone, HYDRUS-1D, MODFLOW*

Introduction

Groundwater is one of the major sources of irrigation in India and has played a significant role in increasing agricultural production and food security in the country. The contribution of groundwater in ultimate irrigation potential of India is about 48.19 % (CGWB, 2009). Importance of groundwater can be realized by the facts that about 61 % of the net irrigated area of the country is irrigated by ground water (CWC, 2010). However, large scale development and utilization of ground water in various parts of India has caused depletion of ground water resources. In several areas of Delhi, Punjab, Haryana, Rajasthan and Uttar Pradesh, the annual ground water pumping is more than the annual ground water recharge. With expected change in climate, it is anticipated that availability of ground water resources will further be affected in several regions.

Recharge from the rainfall is the major source of groundwater. Groundwater recharge primarily depends on rainfall and its intensity, evapotranspiration, infiltration, soil moisture storage in the vadose zone, hydraulic property of the aquifer and the depth of the water table. Vadose zone processes *viz.* the evapotranspiration, infiltration rate and soil moisture storage and depletion determine the availability of water for groundwater recharge. Evapotranspiration and soil moisture depletion depend on

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principal climatic parameters *viz.* temperature, relative humidity, wind speed and duration of sun shine hours. In the event of climate change, some of the climatic parameters are expected to increase, whereas a few are expected to decrease. Due to the compensating effect of different parameters, evapotranspiration and soil moisture depletion pattern may not be described solely by the rise in temperature, which is considered as the main parameter for describing the climate change. Therefore, for assessing the impact of climate change on groundwater recharge, it is essential to consider all important climatic parameters for estimating the evapotranspiration and soil moisture depletion pattern. A plethora of studies relating to ground water recharge under changing climate scenarios are available with consideration of only rise in temperature. However, limited studies have been reported to assess the impact of climate change on groundwater recharge by taking into consideration of vadose zone processes. It is reported that the strategic importance of ground water for global water and food security will probably intensify under climate change due to occurrence of more frequent and intense climate extremes (droughts and floods) besides, pronounced variability in precipitation, soil moisture and surface water (Taylor et al., 2012). It is also reported that there will be major change in rainfall pattern due to climate change. High intensity and short duration rainfall events will become more common in future (IPCC, 2007). Taylor et al. (2013) analysed 55-year record of groundwater level observations in an aquifer of central Tanzania and observed occurrence of episodic recharge resulting from high intense seasonal rainfall. They also observed that such episodic recharge would interrupt multiannual recessions in groundwater levels and would maintain the water security of the groundwater dependent communities in this region. Olago et al. (2009) studied the impact of climate change on ground water in the lake basins of Central Kenya Rift. They observed that the IPCC projected rainfall increase of 10–15% might not necessarily result in a proportional increase in groundwater recharge. Loáiciga et al. (2000) assessed the likely impacts of aquifer pumping on the water resources of the Edwards Balcones Fault Zone (EBFZ) aquifer, Texas in the United States and reported that the ground water resources appeared to be threatened under $2\times CO_2$ climate scenarios under predicted growth and water demand. They also reported that without proper consideration to variations in aquifer recharge and sound pumping strategies, the water resources of the EBFZ aquifer could be severely impacted under a warmer climate. Nyenja and Batelaan (2009) investigated the effects of climate change on groundwater recharge and base flow in the upper Ssezibwa catchment of Uganda and reported intensification in the hydrological cycle resulting in increase in groundwater recharge from 20 to 100% from the prevailing recharge of 245mm/year. Water resources would come under increasing pressure in Indian subcontinent due to the changing climate (Mall et al., 2007). Mizyed (2009) reported that the increase of temperature alone could reduce the natural recharge of groundwater aquifers by 7 to 21 % in the West Bank of Jordan Rift Valley. Ranjan et al. (2006) observed the reduction in fresh groundwater resources in Central America, the Mediterranean region, South Asia, and South Africa under both high and low emission scenarios. The trend in increasing temperatures may reduce the net recharge in the Southern Manitoba, Canada (Chen et al., 2004). Gokhale and Sohoni (2015) developed a quantitative groundwater assessment protocol to use the data available at different scales with government agencies in Maharashtra State to predict the groundwater level fluctuations under varying rainfall depths. It was reported that there existed an uncertainty in the prediction of groundwater table depth both within and across years

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and rainfall alone was a poor predictor of groundwater depths. It was suggested to consider the land use and irrigation requirement besides the hydro-climatic parameters while predicting the groundwater table fluctuations at regional scales.

As the ground water recharge depends upon its dynamics and pathways within the vadose zone, so variably saturated model such as HYDRUS-1D can be used to simulate the moisture movement in the vadose zone and recharge flux joining the water table. Ficklin et al. (2010) used HYDRUS-1D model to simulate the impact of climate change on vadose zone hydrologic processes and groundwater recharge for three different crops *viz.* Alfalfa, almonds and tomatoes in the San Joaquin watershed in California. They reported that the increase in the daily temperature by 1.1 and 6.4 ^{0}C would decrease the cumulative groundwater recharge due to reduced evapotranspiration and irrigation water use by crops. Leterme and Mallants (2011) evaluated the impact of climate and land use change on groundwater recharge in the Dessels region of North-Eastern Belgium. They used HYDRUS-1D for simulation of groundwater recharge under various climate change and land use scenarios and reported that the transition to a warmer climate may decrease the groundwater recharge. Groundwater flow model combined with the vadose zone model would provide a more realistic assessment of groundwater recharge. The recharge flux at the bottom of vadose zone obtained from HYDRUS-1D can be used to estimate the groundwater recharge using MODFLOW. Twarakavi et al. (2008) evaluated the performance of HYDRUS package developed for MODFLOW in the Las Cruces trench infiltration experiment. They reported that HYDRUS could simulate the vadose zone processes realistically. Anilkumar (2011) investigated the groundwater recharge processes in a semi-arid region using HYDRUS-1D and MODFLOW. The recharge flux upto the water table depth was simulated using HYDRUS-1D under various scenarios and subsequently the MODFLOW was used to simulate the groundwater recharge. MODFLOW was used by several researchers to simulate groundwater behavior and estimate the groundwater recharge (Ravi et al., 2001; Senthilkumar and Elango, 2001; Leslie et al., 2002; Boronina et al., 2003; Mali, 2004; Mane et al., 2007; Biswas et al., 2008; Anilkumar, 2011, Shahid, 2011 and Tesfagiorgis et al., 2011). In these studies, MODFLOW was used to estimate the groundwater recharge without considering the vadose zone processes. Use of HYDRUS-1D and MODFLOW not only facilitate the accounting of vadose zone processes, but also take into account the effect of climatic parameters in simulation of ground water recharge.

This study was undertaken to assess the impact of climate change on groundwater recharge considering all the climatic parameters which influence groundwater recharge for an agriculturally dominant semi-arid region under National Capital Territory (NCT) of Delhi, India. The main objective of the study was to evaluate the impact of vadose zone processes on groundwater recharge which depends on all climatic parameters and not only on the temperature. Crop evapotranspiration estimated from ARIMA forecasted climatic parameters were used in the variably saturated flow model to simulate the recharge flux at the water table. Recharge flux at water table was given as input to groundwater model MODFLOW for prediction of groundwater recharge. Groundwater recharge was also estimated from the IPCC and INCCA predicted rise in temperature data to highlight the importance of other climatic parameters in assessing the impact of climate change on groundwater recharge.

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Materials and Methods

Study area

The study was undertaken for an agriculturally dominant Najafgarh Block under South West District of National Capital Territory (NCT), Delhi, India. The area is enclosed between latitude 28° 30’ 10” N to 28° 39’ 30” N and longitude 76°51’ 45”E to 77° 6’ 15”E (*Fig.1*) and falls in semi-arid regions of Northern India. The study area is a part of National Capital Region (NCR) which comprises mainly of Haryana, Utter Pradesh, Rajasthan and entire NCT of Delhi. These surrounding States of the study region are the major food grain producers of the Country. Major crops grown in NCR are rice, wheat, barley, sorghum, pearl millet and maize. Groundwater is being used to irrigate about 90% of the cultivated area.

Major part of the study area falls in the Trans-Gangetic plain region of India under Agro Climatic Region (ACR) - VI. Average altitude is 214.5 m above mean sea level (amsl). The total geographical area is about 200.64 km^2. Out of this, 112.67 km^2 area was under cultivation (*Fig.1*).

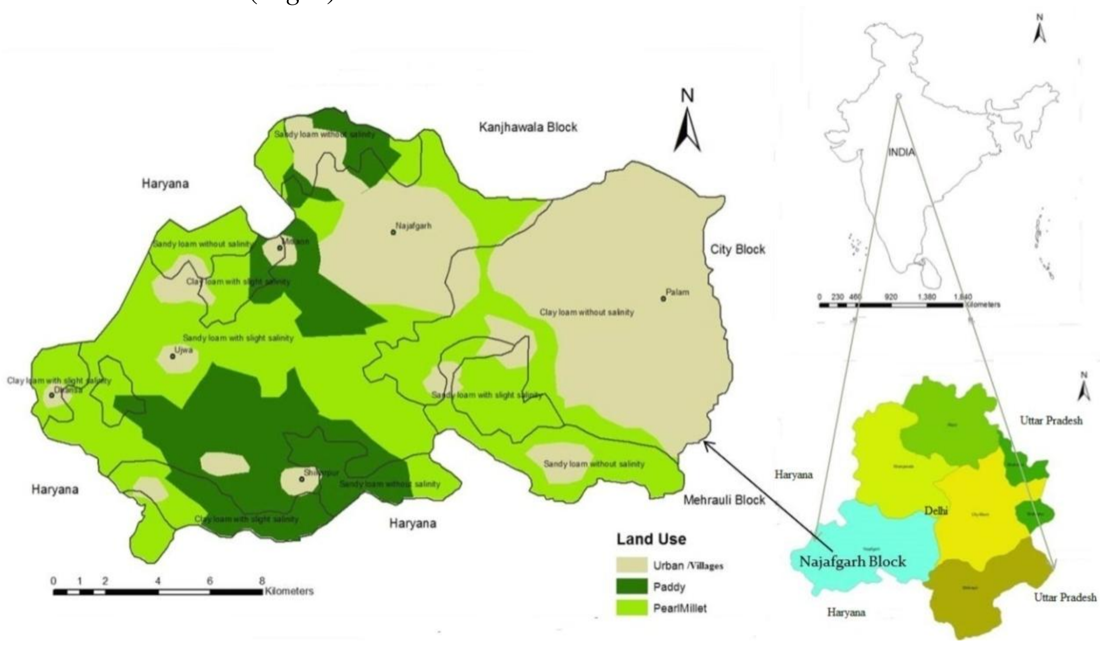

Figure 1. *Location and land use map of the study area*

The climate of the area is characterized by subtropical and semi-arid climate. Summers are very hot and dry with very cold winter season. The mean annual temperature is 24 ºC. May and June are the hottest months and the maximum temperature goes up to 47 ºC. The normal daily maximum and minimum temperatures of 30 years are 31ºC and 17 ºC, respectively. The minimum temperature dips to even 0 ºC and the mean annual rainfall observed in the study area is 730 mm. The major share of rainfall is received during monsoon season. The soil texture of the study region is of sandy loam and clay loam type with some patches having slight salinity (*Fig. 2*)

Climatic parameters of two weather stations *viz.* Palam and ICAR-Indian Agricultural Research Institute (IARI) located in South West District of NCT were collected from concerned agencies. The soil maps and related information were collected from Regional Station of National Bureau of Soil Survey and Land Use

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Planning (NBSS&LUP), New Delhi, India. Data pertaining to groundwater resources were acquired from Central Ground Water Board (CGWB), New Delhi. Data on land use and cropping pattern were collected from Department of Agriculture, NCT of Delhi, India.

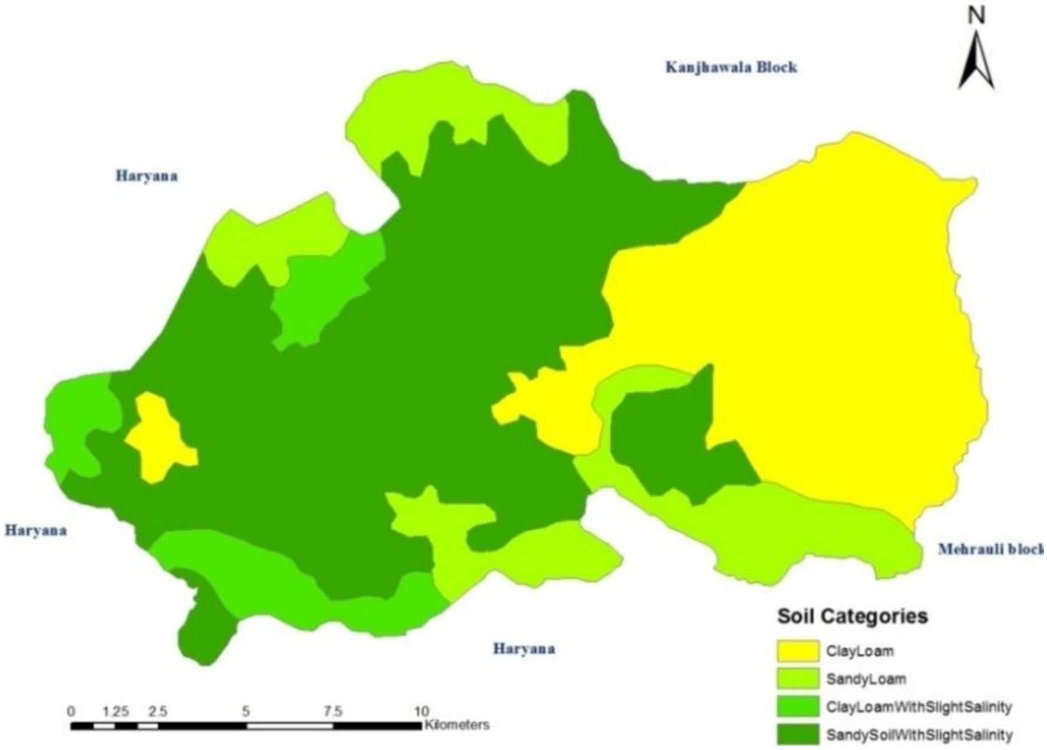

Figure 2. *Soil texture map of the study area*

Modelling of groundwater recharge

Variably saturated flow model, HYDRUS-1D was used to simulate the daily water flux at the bottom boundary of vadose zone (unsaturated zone) which coincided with the groundwater table (*i.e.* Upper most boundaries of the saturated zone). Water flux obtained as output from HYDRUS-1D was taken as the recharge rate at the water table surface. The net bottom flux was obtained by subtracting the pumping rate. The net bottom flux was given as recharge rates at the water table during simulation with MODLFOW for estimation of groundwater recharge. A conceptual representation of modelling of recharge flux and groundwater recharge using both HYDRUS-1D and MODFLOW models is shown in *Figs. 3* and *4*.

Description of models used for ground water recharge
HYDRUS-1D model

The vadose zone model HYDRUS-1D *ver.* 4.08 (Šimůnek et al., 2009) was used to simulate the vertical water movement, root water uptake, soil moisture storage, surface runoff and evaporation from the soil surface in one-dimensional variably-saturated media. The basic assumption of the model was that the air phase does not affect liquid flow processes and the water flow due to thermal gradients became negligible.

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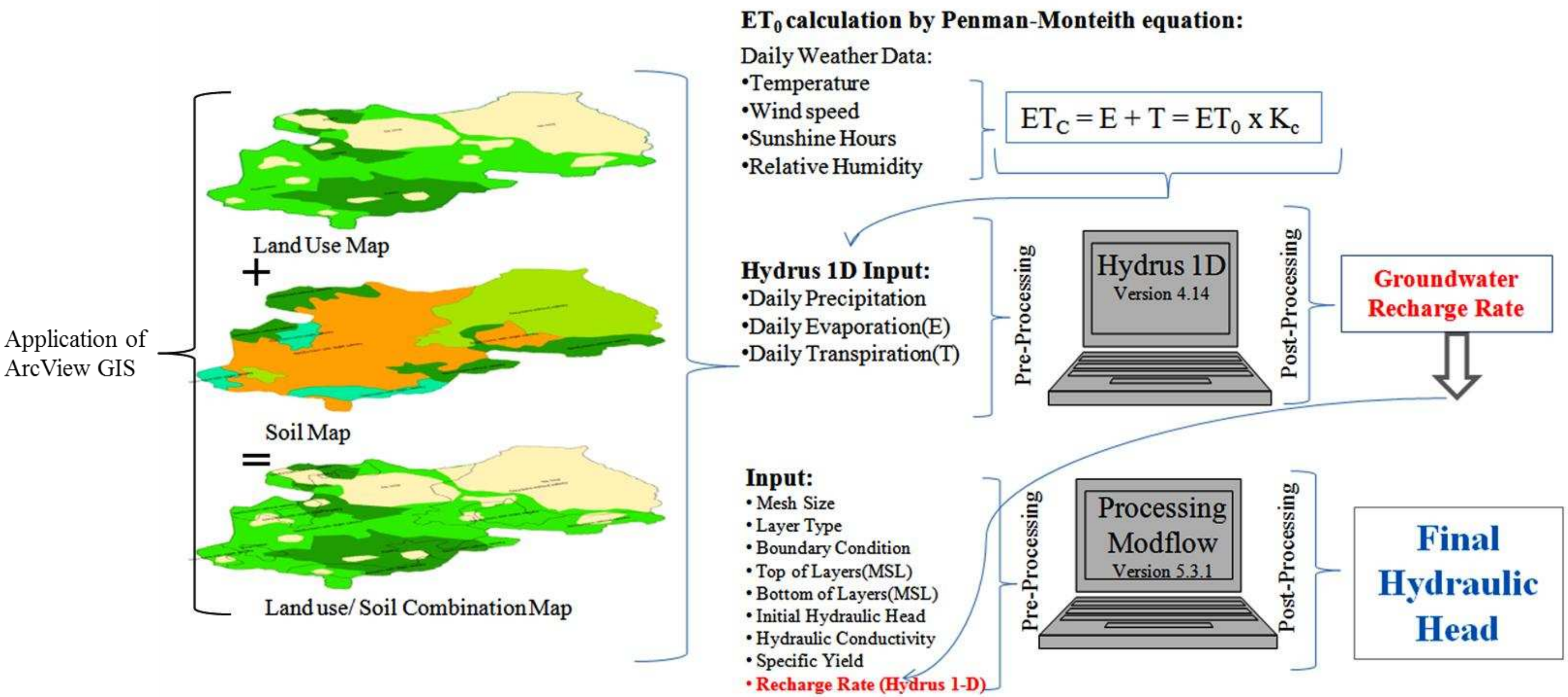

Figure 3. *Conceptual frameworks for simulation of groundwater recharge using HYDRUS-1D and MODFLOW models*

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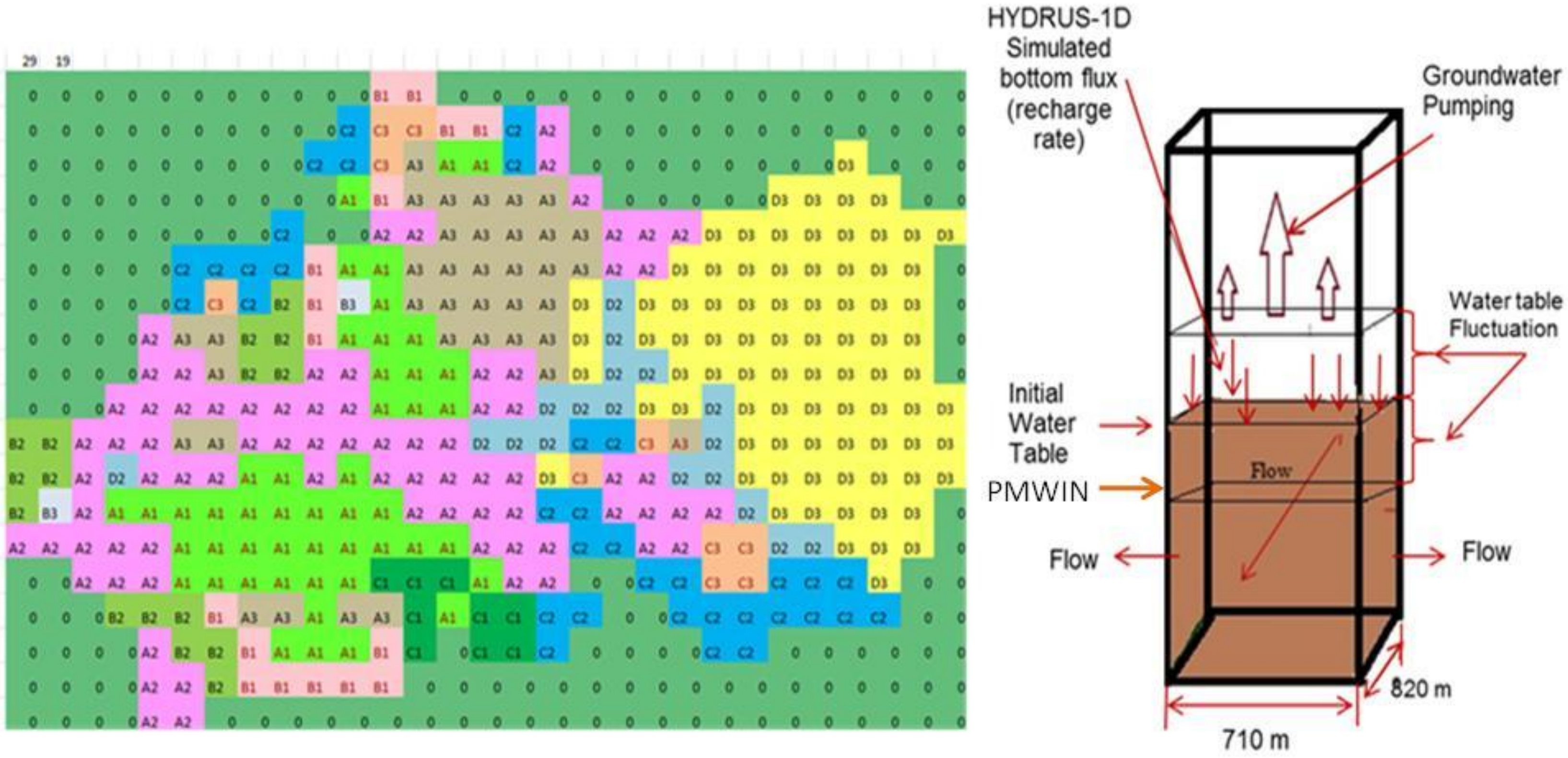

(a) (b)

Figure 4. *Conceptual framework for simulation with MODFLOW and gridded map with cell values of the study region*

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HYDRUS-1D is a finite element model which simulates these processes in vadoze zone by solving the Richards equation (Richards, 1931). The governing equation for water flow in porous medium and root water uptake used in HYDRUS-1D is:

$$\frac{\partial\theta(h)}{\partial t} = \frac{\partial}{\partial z}\left[K(h)\left(\frac{\partial h}{\partial z} - 1\right)\right] - S(h) \qquad \text{(Eq.1)}$$

Where h is the water pressure head (L), θ is the volumetric water content ($L^3\ L^{-3}$), t is the time (T), z is the spatial coordinate (L), K is the unsaturated hydraulic conductivity function (LT^{-1}), and S is the sink term in the flow equation ($L^3\ L^{-3}\ T^{-1}$) which is the volume of water removed from a unit volume of soil per unit time due to plant water uptake.

Saturated and unsaturated hydraulic conductivity are related by

$$K(h,z) = K_s(z)K_r(h,z) \qquad \text{(Eq.2)}$$

Where K_r is the relative hydraulic conductivity [dimensionless] and K_s is the saturated hydraulic conductivity [LT^{-1}].

Soil-hydraulic functions of van Genuchten (1980) with the statistical pore-size distribution model of Mualem (1976) are used in HYDRUS-1D to obtain a predictive equation for the unsaturated hydraulic conductivity in terms of soil water retention parameters (equations 3, 4, 5, 6)

$$\theta(h) = \begin{cases} \theta_r + \frac{\theta_s - \theta_r}{[1 + |\alpha h|^n]^m} & h < \\ \theta_s & h \geq \end{cases} \qquad \text{(Eq.3)}$$

$$K(h) = K_s S_e^l \left[1 - \left(1 - S_e^{l/m}\right)^m\right]^2 \qquad \text{(Eq.4)}$$

Where

$$m = 1 - \frac{1}{n}\ , n : \qquad \text{(Eq.5)}$$

and

$$S_e = \frac{\theta - \theta_r}{\theta_s - \theta_r} \qquad \text{(Eq.6)}$$

Where α is inverse of the air-entry value (or bubbling pressure), n is pore-size distribution index, and *l* is pore-connectivity parameter, *n* is a pore-size distribution index, Se is effective saturation, θr and θs are residual and saturated water contents. The parameters α, n and *l* in HYDRUS are empirical coefficients affecting the shape of the hydraulic functions. The details of modeling procedures with HYDRUS-1D are described in HYDRUS manual (Simunek et al., 2008).

Processing MODFLOW for Windows (PMWIN)

Processing MODFLOW for Windows (PMWIN) was used to estimate the groundwater recharge in terms of rise of the water table in different land parcel units.

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PMWIN *ver.* 5.3.1 (Processing MODFLOW for Windows) is widely used for modelling groundwater flow and transport processes with the modular three-dimensional finite-difference groundwater model MODFLOW (McDonald and Harbaugh, 1988). Effects of well, river, drains, head dependent boundaries, recharge and evapotranspiration on groundwater behaviour under steady and unsteady flow conditions can be simulated efficiently using PMWIN. The principle of conservation of mass and Darcy's law are the basic concepts used in the model to describe the groundwater flow behaviour. The three-dimensional partial differential equation used to describe the groundwater flow in the unconfined aquifer under specified initial and boundary conditions is given by:

$$\frac{\partial}{\partial x}\left(Kxh\frac{\partial h}{\partial x}\right)+\frac{\partial}{\partial y}\left(Kyh\frac{\partial h}{\partial y}\right)=-Sy\frac{\partial h}{\partial t}-R \qquad \text{(Eq.7)}$$

Where Kx, Ky = Directional components of hydraulic conductivity (L T^{-1})
h = Total head (L)
Sy= Specific yield
R= General sink/source term
t = Time (T)

The detailed description of governing flow equations and solution techniques has been given in MODFLOW manual (McDonald and Harbauch, 1988).

Simulation of recharge flux using HYDRUS-1D

The major source of groundwater recharge in the study area was due to the monsoon rainfall and return flow from irrigation. Normal non-monsoon rainfall amounting 179.5 mm was distributed in 8 months from November to June. Therefore, the possibility of recharge from rainfall in non-monsoon months was assumed to be negligible. This assumption doesn't deviate from reality as the area falls in the semi-arid region. The major factors which control the groundwater recharge in the study area are infiltration, hydraulic conductivity, soil moisture storage, evapotranspiration, surface runoff and depth of the water table below ground surface. The HYDRUS-1D model was used to simulate the recharge flux in the vadose zone and the MODFLOW was used to simulate the ground water recharge in terms of rise in the water table. The conceptual framework for simulation under varying climatic conditions is shown in *Fig. 3*.

The study area was divided into 11 land parcel units (LPUs) based on land use, soil type and salinity levels using ArcGIS 9.3.1. The delineated areas of LPUs are presented in *Table 1*. HYDRUS-1D was used to simulate the recharge flux from each LPU. Soil profile of 3 m depth in each LPU was considered for the simulation of recharge flux. The flux estimated at the bottom of 3 m soil profile was considered as maximum possible recharge under given field and climatic situations. Simulations were carried out on a daily basis for one crop growing season starting from 1st July of the year 2005, which was the date of onset of monsoon in the study region. The total simulation period was 122 days. This was considered to match the duration of crops grown in the study area. The simulation period also coincided with the normal duration of monsoon in this region.

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Table 1. *Area under different land parcel units (LPUs)*

Soil types	Land use/ crops	LPUs	Area (ha)
	Paddy	A1	2736
Sandy loam with low salinity (A)	Pearl millet	A2	4446
	Urban	A3	2109
	Paddy	B1	912
Clay loam with low salinity (B)	Pearl millet	B2	912
	Urban	B3	114
	Paddy	C1	513
Sandy loam (C)	Pearl millet	C2	1995
	Urban	C3	571
Clay loam (D)	Pearl millet	D2	1026
	Urban	D3	4731

Input parameters

Evaporation and transpiration

The HYDRUS-1D model requires evaporation and transpiration as separate input parameters in each time step for simulating the influence of soil water on root water uptake. Reference evapotranspiration (ET_o) and crop evapotranspiration (ET_c) were estimated from CROPWAT 8.0 (FAO, 2009) using climatic, crop, and soil parameters besides the crop coefficients for local conditions. Reference evapotranspiration is the potential evapotranspiration from reference crop under adequate moisture conditions, whereas the crop evapotranspiration is the potential evapotranspiration from the crops considered in the study *viz.* Rice and pearl millet. Reference evapotranspiration was converted to crop evapotranspiration by multiplying it with the crop coefficients of respective crops. As required by HYDRUS-1D, crop evapotranspiration was bifurcated into evaporation from soil (E) and transpiration from plants (Tp) using equation given by Belmans et al. (1983):

$$E = ET_c \times e^{-K_{gr} \times} \quad \text{(Eq.8)}$$

Where

K_{gr}: Extension coefficient for global solar radiation {0.3 for rice (Phogat et al., 2010) and 0.56 for pearl millet (Oosterom et al., 2001)}.

LAI: Leaf Area Index {for rice: 0.24, 1.25, 3.86, 0.45 (Tyagi et al., 2000) and for pearl millet: 0.5, 2.5, 2.8, 1.0 (Oosterom et al., 2001). Leaf area index depends on the area of leaf at different growth stages. Rice and pearl millet have different growth pattern and the area of leaves are different at different growth stages.

Soil hydraulic parameters

Different hydraulic parameter used in HYDRUS-1D are saturated water content (θ_s; L^3L^{-3}), residual water content (θ_r: L^3L^{-3}), saturated hydraulic conductivity (Ks; LT^{-1}), the inverse of the air entry value (α), pore size distribution index (n) and pore connectivity parameter (l). These hydraulic parameters were estimated based on sand,

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silt and clay content and bulk density of soils of the study region using pedo transfer function model *Rosetta Lite V 1.1* available as a module in HYDRUS-1D.Values of these parameters for the study area are presented in *Table 2*. Hydraulic conductivity of LPUs under residential land uses (*i.e.* Urban/villages/building) was reduced to 10 % to account for the lower infiltration rate and higher surface runoff. The pore connectivity parameter l was taken as 0.5 as suggested by Mualem (1976) for major soils.

Table 2. *Soil hydraulic parameters of different soil textures in the study area*

Sub area	Soil Type	θ_r(cm^3cm^{-3})	θ_s(cm^3cm^{-3})	α (cm^{-1})	n	K_s (cm day^{-1})
A	Sandy Loam with low salinity	0.0551	0.3836	0.0301	1.4549	33.642
B	Clay Loam with low salinity	0.0753	0.4113	0.0193	1.3079	8.487
C	Sandy Loam	0.0551	0.3836	0.0301	1.4549	37.38
D	Clay Loam	0.0753	0.4113	0.0193	1.3079	9.43

Root water uptake

Estimation of root water uptake was undertaken by HYDRUS-1D in which the method proposed by Feddes et al. (1974) was used. Root penetration depth for rice and pearl millet was taken as 45 cm and 90 cm, respectively. The function proposed by Feddes et al. (1978) and Homaee et al. (2002) were used to parameterize the inherent water stress reduction coefficient. The values of Feddes parameters were adopted from Phogat et al. (2010) and Wesseling (1991) and presented in *Table 3*.

Table 3. *Feddes parameters for root water uptake used in HYDRUS-1D*

Feddes Parameter	Crops	
	Pearl Millet	Rice
PO	-15	55
POpt	-30	100
P2H	-325	-160
P2L	-600	-250
P3	-8000	-15000
r2H	0.5	0.5
r2L	0.1	0.1

Initial and boundary conditions

Initial and boundary conditions were assigned for the partitioned soil profile depth of 3 m for each LPU. There were 11 such simulated flow domains having one in each LPU. Initial conditions were defined in terms of moisture content in the soil profile on the starting day of simulation. In the study area, rice is transplanted and grown under puddled method of rice cultivation. Therefore, initial moisture content in the soil profile for rice was taken equal to saturated moisture content. In case of pearl millet, initial

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moisture content varied uniformly from 10 % in the top layer to 30 % in the bottom layer. Upper boundary was defined as an atmospheric boundary condition with surface runoff. Bottom boundary was set to free drainage boundary as water table in each case was under the simulated domain (Ŝimunek, 2009). The bottom boundary flux was considered as the recharge rate. Rainfall, evaporation and transpiration were specified on the surface of upper boundary on a daily basis for the entire simulation period. In case of rice, daily percolation rate of 4 mm day^{-1} was specified to account for the return flow from irrigation (Khepar et al., 1999; Dash et al., 2015). For pearl millet, the irrigation amount of 0.09 m was accounted by specifying this at the surface as per the irrigation schedule.

Simulation of groundwater recharge using PMWIN

Groundwater recharge in each land parcel unit was estimated using MODFLOW. A conceptual framework for simulation of groundwater recharge with Processing Modflow for Windows (PMWIN) is shown in *Fig. 4 (a, b)*. The study area was discretized into 19 rows and 29 columns with square grids of 710 m x 820 m size (*Fig 4a*). The conceptual diagram indicating governing equations to simulate the water table behavior in a cell is shown in *Fig. 4b*. The net recharge flux obtained as the output of HYDRUS-1D simulations for each land parcel unit were given as an input recharge rate at the water table for simulation with MODFLOW.

Initial and boundary conditions

Initial hydraulic heads (*i.e.* pre-monsoon water table above mean sea level, AMSL) in the study area were used as initial conditions. The observed water table below ground surface in the pre-monsoon season and corresponding surface elevation values were used as input to generate water table contours with respect to mean sea level. Further, the water table contours were used to select the elevations of initial hydraulic heads in different cells. The pre-monsoon water table in different land parcel units (initial hydraulic head) was considered as the top boundary of the saturated zone, which coincided with the bottom boundary of the vadose zone, whereas the aquifer bottom was considered as the bottom boundary. The top boundary was set to time dependent flow boundary condition to account for the variable net recharge flux obtained from HYDRUS-1D. Elevations of the top and bottom boundaries, and initial water table depths are shown in *Figs. 5a, b,* and *c*, respectively.

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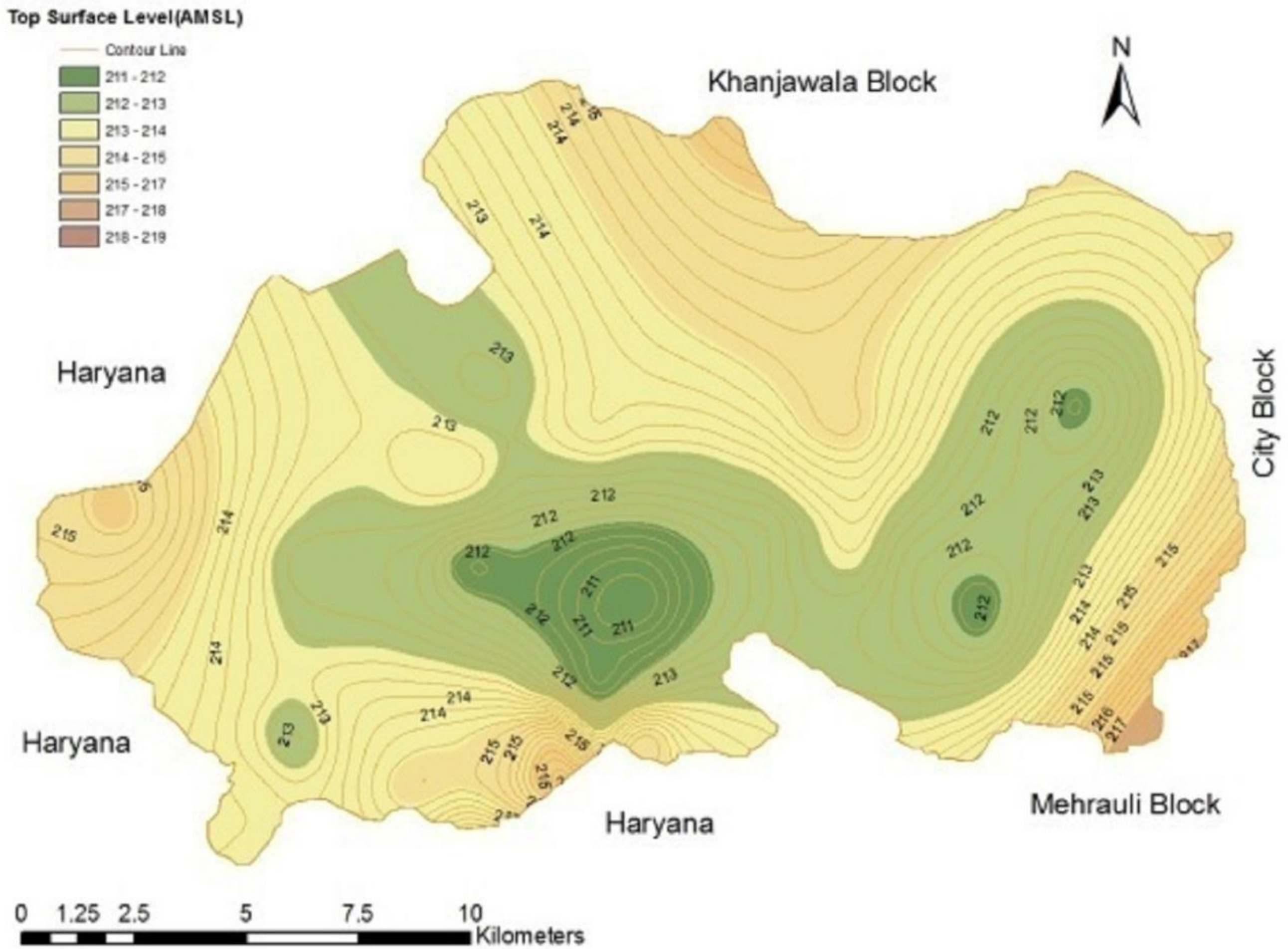

Figure 5a. *Surface elevations of all land parcel units (LPUs) above mean sea level (amsl)*

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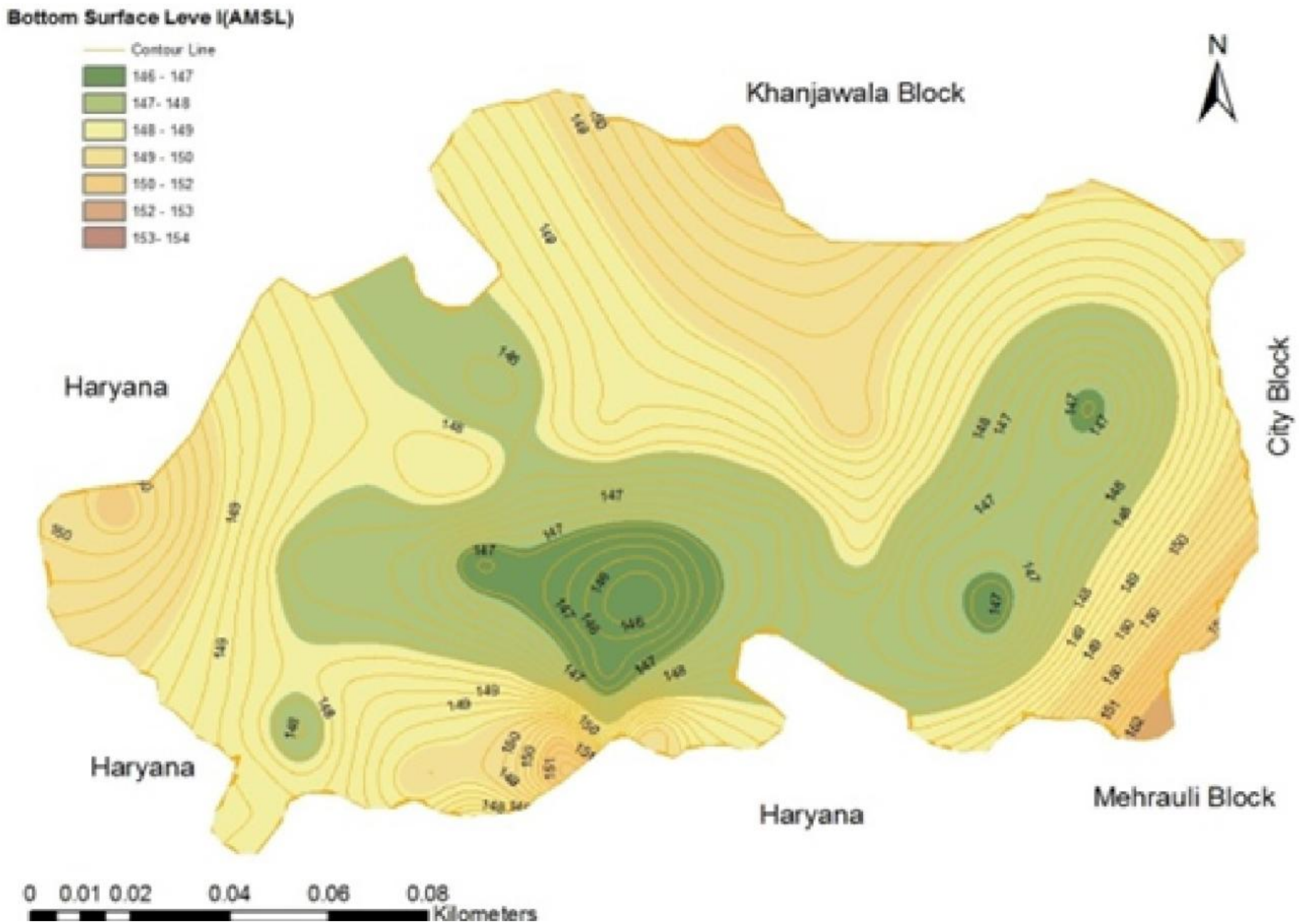

Figure 5b. *Bottom elevation of all LPUs above mean sea level (amsl)*

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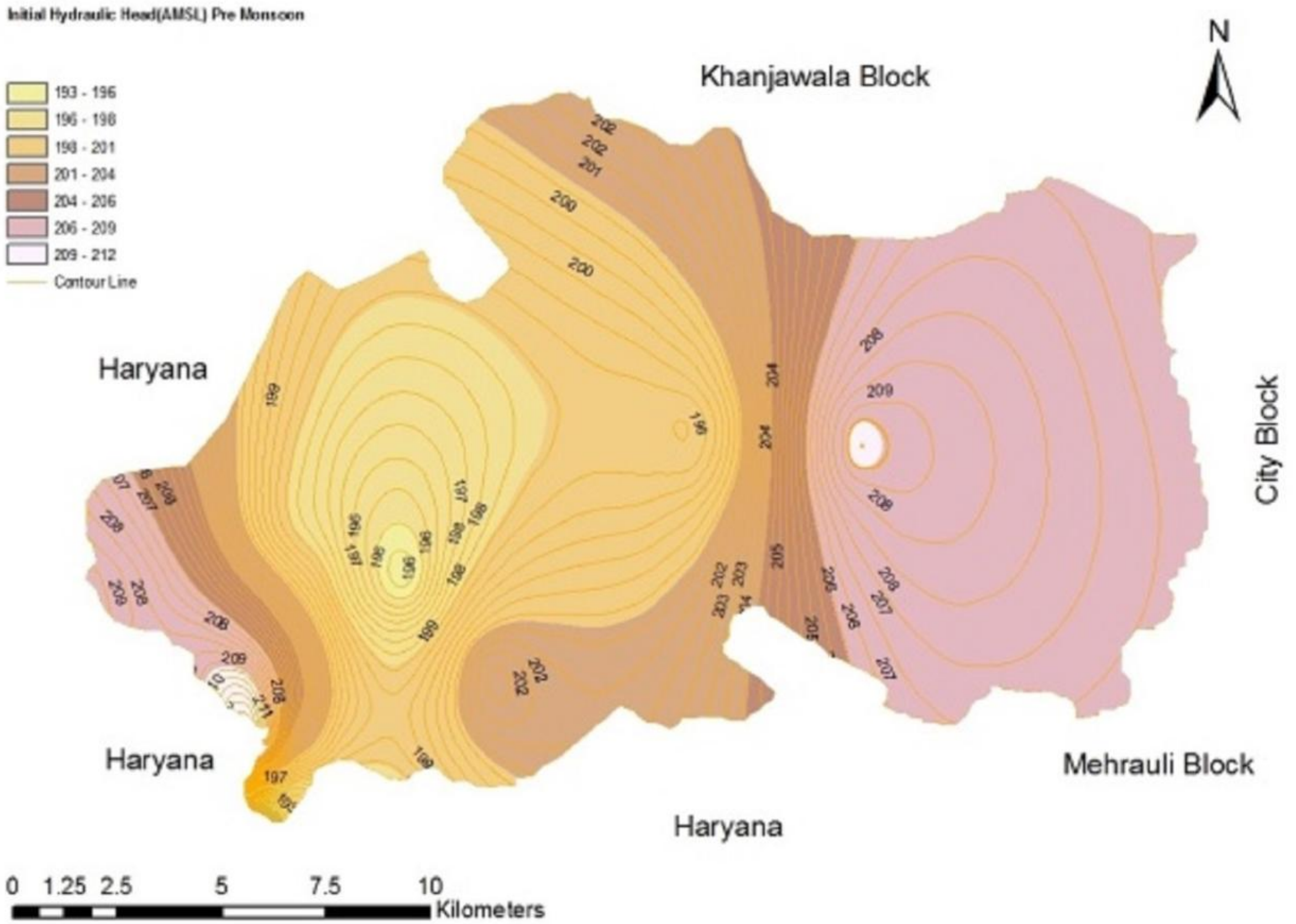

***Figure 5c.** Initial hydraulic heads for all LPUs above mean sea level (amsl)*

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Calibration and validation of models

The calibration of MODFLOW was undertaken by comparing the observed and predicted water table rise during pre and post monsoon seasons. Nash-Sutcliffe Efficiency (NSE), Coefficient of determination (R^2) and Root Mean Square Error (RMSE) prediction error statistics were used to describe the model performance. Simulation was done for the monsoon period of the year 2005. The total simulation period was 122 days (*i.e.* From 1st July 2005 to 31st October, 2005). Four stress periods with time step of one day were considered for the simulation of water table fluctuations. The time dependent boundary condition was imposed at the beginning of each stress period. The input at the beginning of each stress period was the net recharge flux and the hydraulic conductivity and specific yield were kept constant during the simulation period. A similar procedure was adopted for estimation of net recharge flux for other climate change scenarios.

The groundwater pumping in the study area was from the unconfined aquifer. The depth of the aquifer was 65 m below ground level in the unconsolidated alluvium zone. The specific yield was taken as 0.16 (CGWB, 2009). Total groundwater pumping in the year was uniformly distributed over the entire study area. The total groundwater pumping per unit area for various uses was 0.4946 m y^{-1}. The daily pumping rate was estimated from the total pumping value which was termed as prevailing pumping rate. This was estimated to be 0.001355 m day^{-1}. The net daily recharge flux was estimated by subtracting the pumping rate from daily recharge flux obtained from the HYDRUS-1D simulated output.

Climate change scenarios considered for simulations

Calibrated and validated models were used to predict the groundwater recharge in terms of water table fluctuations under various climate change and groundwater pumping scenarios. Climate change scenarios considered for estimation of groundwater recharge were adopted from predictions by the Intergovernmental Panel for Climate Change (IPCC), Indian Network for Climate Change Assessment (INCCA), and Auto Regressive Integrated Moving Average (ARIMA) model. ARIMA model is one of the most popular models for analysis of time series data. It contains autoregressive (AR), integrated (I) and moving average (MA) estimation components which are expressed as ARIMA (p, d, q). Where, p is autoregressive component, d is integrated component and q is moving average component. ARIMA model was used for forecasting the variation in climatic parameters using the data for 35 years (1975 to 2010). The time series data were used to forecast the variation during the same period and co-relate it with the observed data. Thereafter, it was used to forecast the climatic parameters up to 2030s. The climatic data obtained from IARI, New Delhi for the period from 1975 to 2010 was used to forecast the changes in climatic parameters *viz.* Average temperature, relative humidity, wind speed and sunshine hours. ARIMA forecast for average temperature, wind speed, annual rainfall, sunshine hours and relative humidity are presented in *Fig. 6 (a-e)*. The performance of ARIMA forecast was determined through the coefficient of determination between observed and predicted values during the period of 1975-2010. Higher coefficient of determination between observed and predicted values indicated that the ARIMA forecast were realistic and can be used for forecasting the future scenarios (*Table 4*). The average changes in climatic parameters in the 2030s, with respect to the 1995 are presented in *Table 4*. The values of the climatic parameters

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forecasted by ARIMA for 2030s are given in *Table 5* as the climate change scenario 1. In case of IPCC predictions, two climate change scenarios (Scenarios 2 and 3) under low and high emission of CO_2 leading to increase average daily temperature of 1.1 ^{0}C and 6.4^{0}C, respectively, by the year 2100 were considered (Ficklin *et al.*, 2010) (*Table 5*). Indian Network for Climate Change Assessment (INCCA) had generated climate change scenarios for major climate sensitive regions of India using regional climate change model Providing Regional Climates for Impacts Studies PRECIS (INCCA, 2010). PRECIS simulations indicated an all-round warming over the Indian subcontinent. It is reported that the annual mean surface air temperature would rise by 1.7 ^{0}C and 2 ^{0}C (Scenarios 3 and 4) in 2030s. In case of scenarios based on IPCC and INCCA predictions, only rise in temperature was considered for assessing the impact of climate change on groundwater recharge whereas in case of ARIMA based forecast, all climatic parameters were considered. The climate change scenarios presented in *Table 5* were used to estimate crop evapotranspiration and was given as input in HYDRUS-1D after separating it into evaporation and transpiration for simulating the recharge flux under various climate change scenarios.

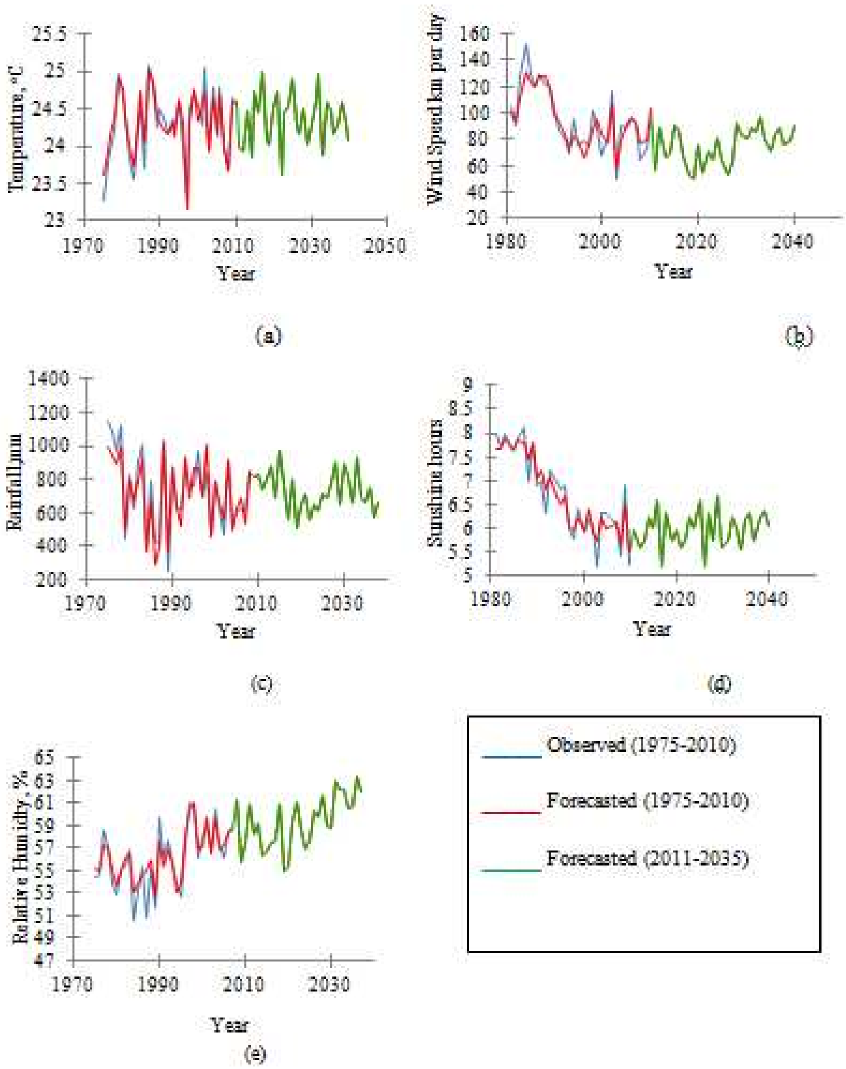

Figure 6. *ARIMA forecasted (a) average annual temperature (b) wind speed (c) total rainfall (d) sunshine hours and (e) relative humidity.*

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Table 4. *Performance indicator of ARIMA model and climate forecast for 2030s*

Meteorological Parameters	Coefficient of determination (R^2) between observed and forecasted values during 1975-2010	Forecasted variation in climatic parameters for 2030s with respect to 1995
Total rainfall (mm)	0.86	+0.50
Sunshine hours (hour)	0.89	-0.26
Relative humidity (%)	0.77	+4.00
Average wind speed (km day^{-1})	0.78	-5.15
Average temperature (^{0}C)	0.84	+0.26

Table 5. *Climate change scenarios considered for model simulations*

Scenarios	Average temperature (^{0}C)	Relative humidity (%)	Wind speed (km day-^{1}	Sunshine hours	Rainfall (mm)	Number of rainy days
	Predictions for 2030s with local weather data using ARIMA					
Scenario 1	+0.26	+4	-5.15	-0.26	+0.5	-
	IPCC Predictions for 2100s					
Scenario 2	+1.1	-	-	-	-	-
Scenario 3	+6.4	-	-	-	-	-
	INCCA Predictions for 2030s					
Scenario 4	+1.7	-	-	-	-	-
Scenario 5	+2.0	-	-	-	-	-

Results and Discussions

Results of calibration are presented in *Fig 7 (a, b).* The *Fig. 7a* shows the comparison between observed and predicted average water table elevations (hydraulic heads) in different land parcel units. The *Fig. 7b* compares the water table elevations in different cells located in the study area. These figures showed that the observed and predicted water tables are in close agreement with each other. Predictability of models was also evaluated in terms of performance indicators such as Nash-Sutcliffe Efficiency (NSE), Coefficient of determination (R^2), Root Mean Square Error (RMSE) and are presented in *Table 6.* It can be observed from *Table. 6* that R^2 and NSE approaching one and RMSE approaching zero corroborated that the observed and model predicted values were in line with each other. Therefore, integrated use of the HYDRUS-1D model for the vadose zone moisture movement and MODFLOW for ground water flow could predict the groundwater recharge with acceptable accuracy in any region.

Cumulative recharge flux

Simulations were undertaken to predict the bottom flux, cumulative bottom flux, cumulative evaporation, cumulative root water uptake, actual root water uptake, soil water storage, cumulative surface runoff and surface runoff under different climate change scenarios from 11 land parcel units (*Table 5*). The simulations were carried out under the prevailing conditions during 2005, which is referred as the reference scenario.

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The moisture migration (boundary fluxes) simulated by HYDRUS-1D for land parcel, unit A1 is presented in *Fig.8 (a to f).* Similar output was also obtained from other land parcel units delineated in the study area. It was observed from *Fig. 8* that the cumulative recharge flux from rice cultivated area (A1) having sandy loam soil and low salinity was 71.6 cm. Besides this, the cumulative root water uptake, cumulative evaporation and cumulative surface runoff for A1 were 24.7 cm, 29.2 cm and 0.1 cm, respectively. Cumulative recharge flux from rice, pearl millet and urban land use under the various scenarios is shown in *Fig. 9, Fig. 10*, and *Fig. 11*, respectively. Comparison of cumulative recharge flux, cumulative root water uptake, cumulative evaporation and cumulative surface runoff from various LPUs for a baseline and other climate change scenarios are presented in *Table 7*. In case of baseline scenario, minimum and maximum cumulative recharge flux of 0.2 cm and 72.5 cm, respectively, were obtained from B3 (clay loam with low salinity) and C1 (sandy loam) LPUs, respectively. The cumulative recharge flux from LPUs *viz.* A1 (sandy loam with low salinity), B1 (clay loam with low salinity) and C1 (sandy loam) under rice crop varied from 42.6 cm to 72.5 cm. The cumulative recharge flux from different land parcel units under pearl millet *viz.* A2 (sandy loam with low salinity), B2 (clay loam with low salinity), C2 (sandy loam) and D2 (clay loam) varied from 1.2 cm to 8.6 cm. The cumulative recharge flux from A3 (sandy loam with low salinity), B3 (clay loam with low salinity), C3 (sandy loam) and D3 (clay loam) LPUs under urban land use varied from 0.2 cm to 1.3 cm. The variances in the cumulative recharge flux from these land parcel units (LPUs) were primarily due to differences in soil type and salinity levels. Simulation results showed that the maximum cumulative recharge flux was from rice fields. The cumulative root water uptake from cultivated land, cumulative evaporation, and cumulative surface runoff from various land units ranged from 18.1 to 24.7 cm, 13.72 to 29.45 cm and 0.01 to 24.15 cm, respectively. The highest and lowest cumulative root water uptakes were 24.7 cm (from A1, B1, and C1) and 18.15 (from D2) (*Table 6*). Average recharge (*i.e.* Average cumulative recharge flux) from rice fields was 38.4 % and from the pearl millet field was 8.3 % of total rainfall depth during the crop growth period. Average recharge from other land units was 1.3 % and total rainfall depth during the period of simulation was 504 mm.

Table 6. *Model performance indicators in prediction of average hydraulic head in different LPUs and gridded cells of the study region*

Observed and predicted head of average hydraulic head	RMSE	NSE	R^2
Land Units	0.653	0.89	0.83
Cells	0.826	0.95	0.94
Acceptable limits*	≥0	0.75 to 1	0.5 to 1

*Moriasi et al. (2007)

Cumulative recharge under various climate change scenarios from rice fields varied from a minimum of 36.6 cm to a maximum of 75.9 cm. The highest and lowest cumulative recharge under various scenarios were 66.6 cm to 75.9 cm and 36.6 cm to 45.6 cm from LPUs C1 (Sandy loam) and B1 (clay loam with low salinity), respectively

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in rice fields. Differences in cumulative recharge can be attributable to different soil types and saline conditions of the study region. Cumulative recharge under various climate change scenarios from pearl millet fields varied from a minimum of 1.2 cm to a maximum of 10.8 cm. Highest and lowest cumulative recharge from the pearl millet field were 7.4 cm to 10.8 cm and 1.2 cm in LPUs B2 (clay loam with low salinity) and C2 (sandy loam) respectively. From the land parcel units under urban and village land uses, the cumulative recharge varied from 0.2 cm to 1.3 cm under various climate change scenarios. The highest and lowest cumulative recharge was observed to be 1.3 cm and 0.2 cm in C3 (sandy loam) and B3 (Clay loam with low salinity) and D3 (Clay loam) LPUs, respectively. The comparison of cumulative recharge flux, cumulative root water uptake and cumulative evaporation from various land units obtained under different climate change scenarios are presented in *Table 7*. Moreover, for rice, the cumulative recharge flux under all climate change scenarios decreased with the rise in temperature in all land parcel units except the scenario 1. Cumulative groundwater recharge from land parcel units under pearl millet except B2 and D2 scenarios increased in all climate change scenarios except scenario 3. There was no change in cumulative recharge flux in land units B2 and D2 under pearl millet. Effect of climate change in cumulative recharge flux was not observed in another six LPUs *viz.* A3, B2, B3, C3, D2 and D3. Results obtained from scenario 1 suggested that the cumulative groundwater recharge increased in all land parcel units compared to baseline scenario and varied from 0.2 cm to 75.9 cm. This indicates that the cumulative groundwater recharge would increase if all the climatic parameters are considered for assessment of the impact of climate change. This is in contrary to common perception that groundwater recharge would decrease in semi - arid regions as a result of climate change. This can be attributable to the decreasing trend of certain climatic parameters such as wind speed and sunshine hours and increasing trend of relative humidity as observed in the study region under semi-arid environment.

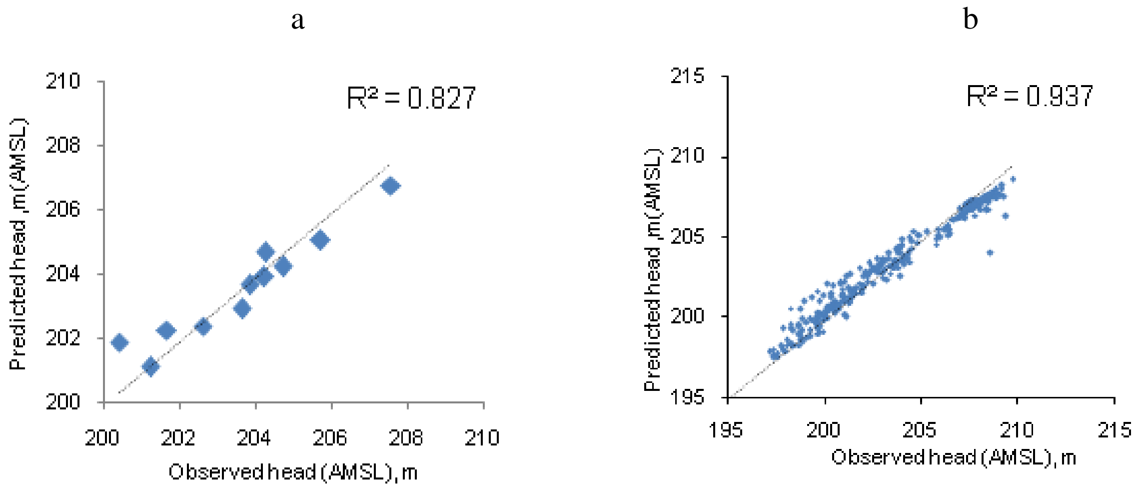

Figure 7. ***(a)*** *Observed and predicted average hydraulic heads in different land parcel units,* ***(b)*** *observed and predicted hydraulic heads in all grided cells*

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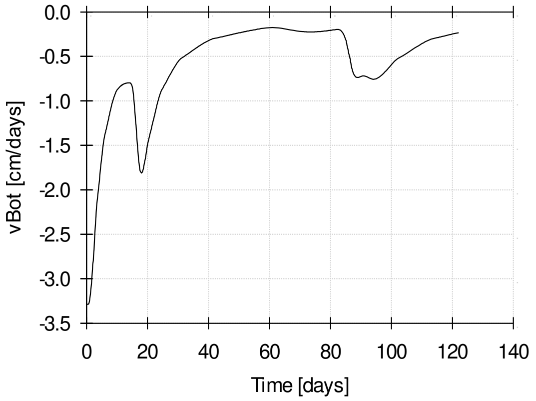
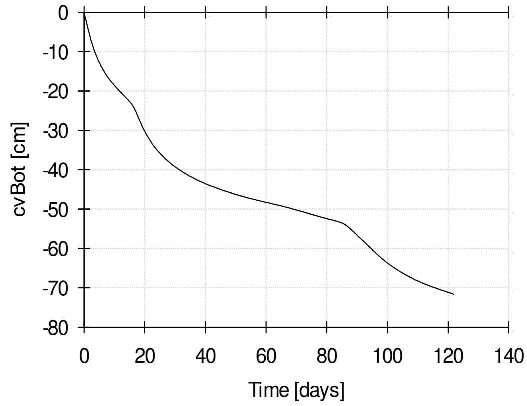
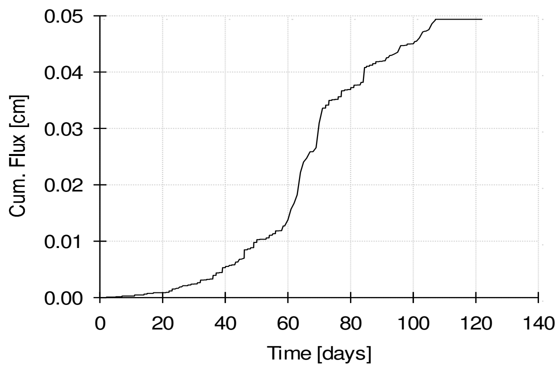


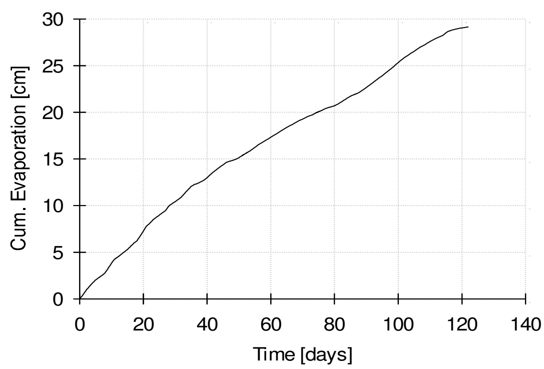
a. Bottom flux



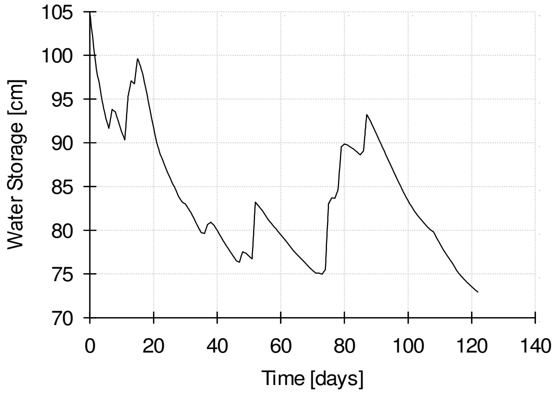
b. Cumulative bottom flux



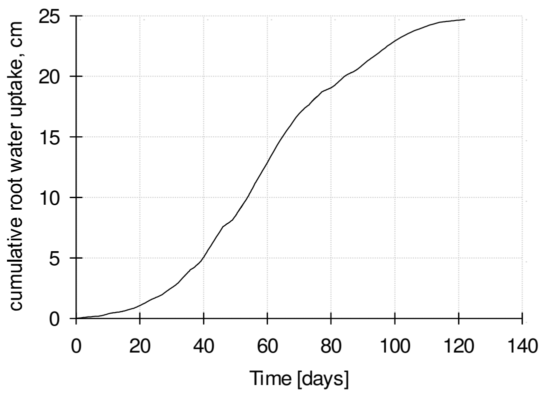
c. Cumulative surface runoff



d. Cumulative evaporation



e. Soil water storage



f. Cumulative root water uptake

Figure 8. (a to f) *Simulated boundary fluxes and heads in land parcel unit A1 under rice crop for the year 2005*

Results presented in this study indicated that the effect of climate change on cumulative groundwater recharge was more pronounced in permeable soils with higher hydraulic conductivity. It was also observed that evaporation and transpiration rates during the growing period of rice increased with the rise in temperature compared to reference scenarios resulting in lower cumulative recharge under various climate change scenarios. However, in case of pearl millet, the transpiration rate increased marginally and evaporation decreased considerably in case of scenario 2, 4 and 5 as compared to

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the reference scenario. Such variation can be attributed to the increase of net cumulative groundwater recharge during the growing period of pearl millet.

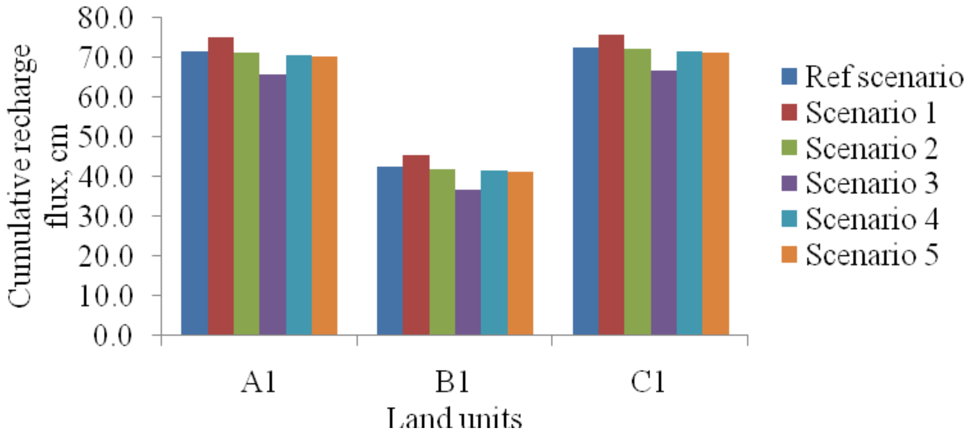

Figure 9. *Comparison of cumulative groundwater recharge fluxes from land parcel units under rice*

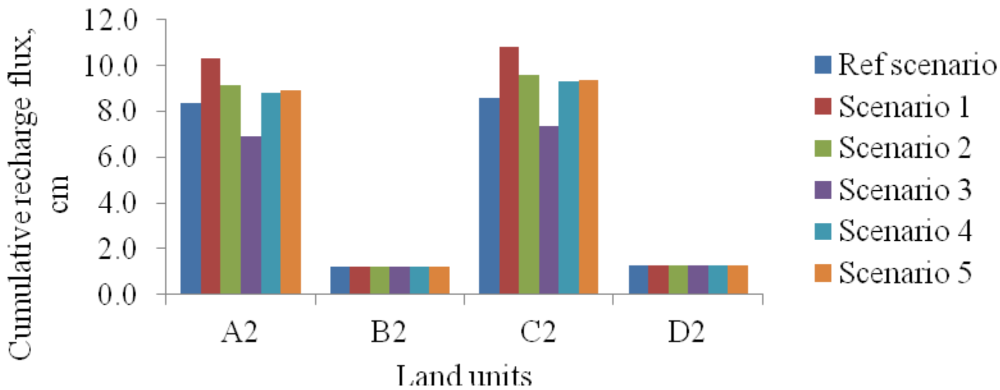

Figure 10. *Comparison of cumulative groundwater recharge fluxes from land parcel units under pearl millet*

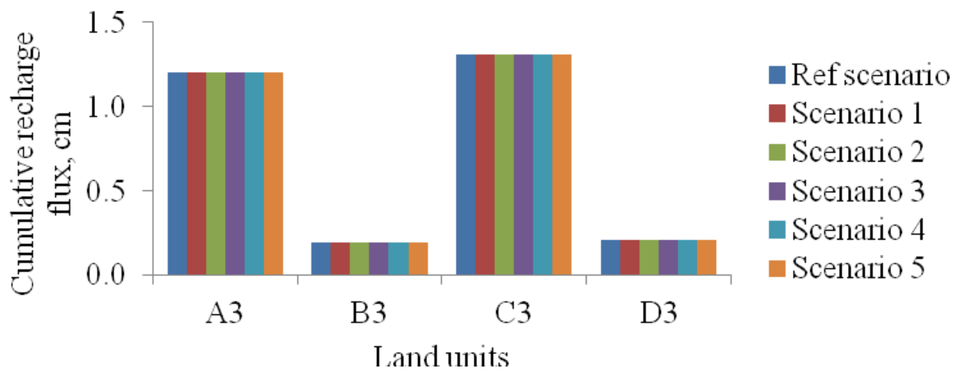

Figure 11. *Comparison of cumulative groundwater recharge fluxes from land parcel units under urban land use*

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Table 7. *Cumulative recharge flux, cumulative root water uptake, cumulative evaporation and cumulative surface runoff from various land units under different climate change scenarios.*

	Cumulative recharge flux (cm)						Cumulative root water uptake (cm)						Cumulative evaporation (cm)						Cumulative surface runoff (cm)					
	Scenarios						Scenarios						Scenarios						Scenarios					
LPUs	**Ref**	**1**	**2**	**3**	**4**	**5**	**Ref**	**1**	**2**	**3**	**4**	**5**	**Ref**	**1**	**2**	**3**	**4**	**5**	**Ref**	**1**	**2**	**3**	**4**	**5**
A1	71.6	75.0	71.3	65.9	70.6	70.3	24.7	23.5	24.8	28.0	25.2	25.3	29.2	28.0	29.4	32.6	29.7	29.9	0.0	0.0	0.1	0.0	0.1	0.0
A2	8.3	10.3	9.1	6.9	8.8	8.9	21.6	20.4	21.6	24.3	21.9	22.0	13.8	12.2	12.5	13.1	12.6	12.8	0.0	0.1	0.1	0.1	0.1	0.0
A3	1.2	1.2	1.2	1.2	1.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0	25.0	25.1	25.1	26.1	25.2	25.3	8.5	9.0	8.5	8.1	8.4	8.4
B1	42.6	45.6	41.8	36.6	41.5	41.3	24.7	23.5	24.8	28.0	25.2	25.3	29.5	28.3	29.7	32.9	30.0	30.2	0.9	1.0	1.0	0.9	0.9	1.0
B2	1.2	1.2	1.2	1.2	1.2	1.2	20.6	19.5	20.7	23.4	21.0	21.1	13.9	12.6	12.9	13.5	12.9	13.0	0.5	0.6	0.5	0.5	0.6	0.6
B3	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	21.7	20.0	21.0	21.8	20.8	20.8	24.1	23.8	24.4	23.9	24.1	24.2
C1	72.5	75.9	72.1	66.7	71.5	71.2	24.7	23.5	24.8	28.0	25.2	25.3	29.2	28.0	29.4	32.6	29.7	29.9	0.0	0.0	0.1	0.0	0.1	0.0
C2	8.6	10.8	9.6	7.4	9.3	9.4	21.6	20.4	21.6	24.3	21.9	22.1	13.7	12.3	12.6	13.2	12.7	12.8	0.1	0.1	0.1	0.1	0.1	0.0
C3	1.3	1.3	1.3	1.3	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	25.2	25.3	25.3	26.3	25.4	25.5	7.0	7.4	7.0	6.7	7.0	6.9
D2	1.3	1.3	1.3	1.3	1.3	1.3	18.1	19.6	20.7	19.9	21.0	21.2	12.3	11.6	13.4	17.1	13.5	13.5	1.1	1.3	1.1	1.6	1.1	1.1
D3	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	21.2	21.5	22.0	21.9	21.1	21.2	23.9	25.1	24.0	23.5	23.7	24.9

A1, B1, C1: Land units under rice **A2, B2, C2, D2**: Land units under pearl millet **A3, B3, C3, D3**: Land units under urban/villages **Ref:** Reference scenario (Data of year 2005)

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Therefore, further investigation is required to evaluate the effect of evaporation and transpiration separately on groundwater recharge under different cropping systems. Moreover, in general, the higher surface runoff in various land parcel units resulted in lesser cumulative groundwater recharge under different climate change scenarios (*Table 7*).

Water table fluctuations under different climate change scenarios

Water table fluctuations under different climate change and pumping scenarios were simulated using PMWIN. Simulation results are presented in *Fig.12* and *13*. Average hydraulic heads (water table elevations in the post monsoon period) above mean sea level (amsl) for different scenarios are presented on Y-axis (*Fig. 12*). The comparison of water table fluctuation under different climate change scenarios revealed that the post monsoon water table depth increased marginally (*i.e.* By 0.03 m) in scenario 1 compared to the reference scenario. This may be due to the higher cumulative recharge flux under scenario 1. Moreover, the impact of climate change on groundwater recharge investigations can be more realistic with consideration of all principal climatic parameters instead of considering only the temperature for estimation of cumulative recharge flux and groundwater recharge in any study region. It is worth mentioning that scenario 1 represented the prediction of climate change for 2030s using long climatic data collected from the study area. Groundwater elevations in the post monsoon period (hydraulic heads) under scenario 2 and 3 (*i.e.* Based on IPCC predictions) and scenario 4 and 5 (*i.e.* based on INCCA predictions) were lower than that of the reference scenario. This suggested that the groundwater recharge would decrease if climate change is described only in terms of rise in temperature. It may be mentioned that scenarios 2, 3, 4 and 5 represented the effect of climate change on groundwater recharge by considering the temperature rise only.

Water table fluctuations between pre and post monsoon period and change in hydraulic heads under different climate change with respect to reference scenario are presented in *Table 8*. It was observed from *Table 8* that the water table in the post monsoon period increased from 0.02 m to 0.15 m in scenarios 1, 2, 4, 5 and it decreased by 0.08 m under scenarios 3. The decrease in the post monsoon water level in various scenarios was mainly due to increase in temperature and increase in groundwater pumping in the study region. Change in groundwater levels under climate change scenarios with respect to reference scenario is shown in *Fig. 13*. It was observed that the annual groundwater recharge would decrease by 0.09 m to 0.21 m under scenarios 2 to 5 under IPCC temperature predictions as compared to the reference scenario. Moreover, under scenario 1, the annual groundwater recharge would increase by 0.03 m compared to the reference scenario.

Table 8. *Water table fluctuations under different scenarios*

Scenarios	**Pre-monsoon water table elevation (AMSL, m)**	**Post-monsoon water table elevations (AMSL, m)**	**Water table fluctuations between pre and post monsoon (m)**
Reference	203.53	203.65	0.12
Scenario 1	203.53	203.68	0.15
Scenario 2	203.53	203.56	0.03
Scenario 3	203.53	203.45	-0.08
Scenario 4	203.53	203.55	0.02
Scenario 5	203.53	203.55	0.02

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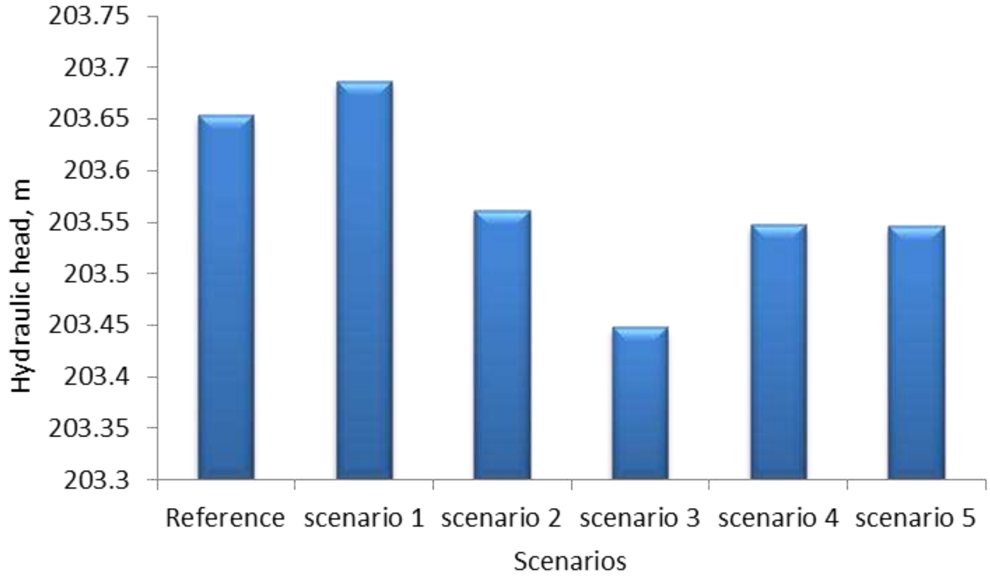

Figure 12. *Simulated groundwater elevations (hydraulic heads) under different climate change scenarios during post monsoon period*

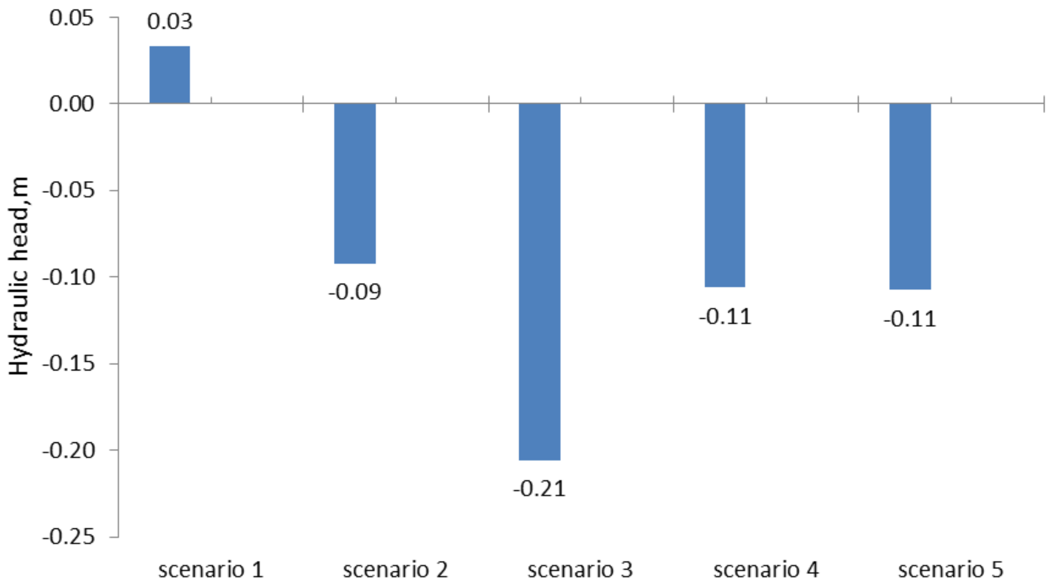

Figure 13. *Change in groundwater levels with respect to the reference scenario*

Conclusions

Assessment of impact of various climate change scenarios on groundwater recharge indicated that the groundwater recharge in the study area will not change much by the 2030s as compared to the reference year 2005, if it is estimated using all principal weather parameters. However, using the rise in temperature based on the IPCC and INCCA predictions, the groundwater recharge would decrease by 0.09 m to 0.21 m and 0.11 m, respectively, during 2030 and 2100 as compared to the reference year 2005 for the study area. Therefore, the difference in the estimated ground water recharge depth in

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the study region using all climatic parameters and only the temperature data revealed that for realistic assessment of the impact of climate change on groundwater recharge, the effect of changes in all important climate parameters should be considered rather than considering only the effect of temperature. It was also observed that the effect of climate change on groundwater recharge was more pronounced in permeable soils with higher hydraulic conductivity. Moreover, the effect of temperature rise on groundwater recharge was not observed in less permeable soils with low salinity. In the scenarios based on IPCC and INCCA predictions, evaporation and transpiration during the growing period, particularly for rice increased considerably with rise in temperature resulting in lower cumulative ground water recharge. An integrated modelling protocol using HYDRUS and PMWIN models was conceptualized and validated in this study for simulating the moisture dynamics in the vadose zone and its subsequent movement to ground water under different climate change scenarios. Nonetheless, this study revealed that for accurate estimation of ground water recharge at regional scales, all climatic parameters should be considered besides the cropping system of the region.

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REGENERATION, GROWTH AND NUTRIENT PARTITIONING OF THREE WOODY SPECIES ON DEGRADED TROPICAL RAINFOREST LAND

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Abstract. Forestry provides the conditions for regeneration of forest species, shortened vegetation succession and duration to reach colonization climax. The impact of inorganic fertilizers [urea (46% N), single superphosphate (33.5% P) and muriate of potash (49.8% K)] single or in combination on growth, regeneration rate and nutrient partitioning in woody species (*Millettia laurentii*, *Microberlinia bisulcata* and *Lophira alata*) was investigated. Some growth parameters [number of leaves (NL), shoot length (SL), stems diameter (SD) and number of branches (NB)], regeneration rate and nutrient uptake (N, P, K, Ca and Mg) were determined. *M. laurentii* and *L. alata* showed higher regeneration rates (82.40 and 84.80% respectively) than those of *M. Bisulcata* (46.40%) 123 DAP. The NL, SL, SD, NB and N, P, K, Ca and Mg uptake in *M. Laurentii* plants were positively influenced by inorganic-N, P, K and NPK fertilizers compared to those of *L. alata* and *M. Bisulcata* 331 DAP. The highest accumulation of N, P, K, Ca and Mg concentrations was found in leaves of all the species. These results suggest that *M. laurentii* could adapt to soil nutrient amendment and can be used as catalyst for reforestation of degraded tropical rainforest land. The specific combination (NPK) was found as efficient fertilizer to enhance the woody species regeneration.
Keywords: *biodiversity, deteriorated soil, forestry, mineral nutrition, restoration*

Introduction

Biodiversity is the occurrence of different types of ecosystems, different species of organisms with the whole range of their variants and genes adapted to different climates, environments along with their interactions and processes (Shmida and Wilson, 1985). According to Dajoz (1985), the ecosystem diversity is due to diversity of niches, trophic levels and ecological processes like nutrient cycling, food webs, energy flow, role of dominant species and various related biotic interactions. Such type of diversity can generate more productive and stable ecosystems or communities able to tolerate various types of stresses such as drought (Bergonzini, 2004). Biodiversity is very

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essential for natural pest control, maintenance of population of various species, pollination by insects and birds, nutrient cycling, conservation and purification of water, and soil formation (Dajoz, 1985). Tropical rain forest is a luxuriant forest composed of broad-leaved trees that form a dense upper canopy and contain a diverse array of vegetation (Gay, 1993). It is found in wet tropical uplands and lowlands around the Equator and usually characterized by a combination of high species diversity, density and productivity (Whitmore, 1984; Choula et al., 2013).

The implementation of development projects such as agriculture, infrastructure construction, mining and forestry exploration leads to land degradation, nutrient load loss and destruction of forest cover (Zapfack et al., 2013; Bonansoa et al., 2016; Ndema Nsombo et al., 2016). These anthropogenic factors are at the beginning of vegetation succession which leads to the creation of a new forest in balance or climax state. One aspect of forest degradation is excavation carried out during the implementation of various projects (i.e. mines and parks) or logging. These areas, once abandoned by man, can be re-colonized by natural succession of vegetation. But for some, there are barriers to the establishment of these successions, such as the insufficient amount of recruitment of vegetative propagules (roots and branches), seeds entering the site, seeds and seedling predation, the absence of a micro-habitat for seed germination and seedling establishment, lack of nutrients in the soil, periodic droughts and competition among herbs (Duncan and Chapman, 1999; Holl, 1999; Guariguata and Ostertag, 2000; Slocum et al., 2004). It has been demonstrated that some successions result in formation of forests in which a particular species is relatively abundant (Bourland et al., 2015). Factors that might influence succession and species diversity include rainfall, temperature and soil chemical properties (Fonge et al., 2011). Succession also depends on the substrate such as on excavated land, sand and lava deposits. The duration of forest succession which takes over a hundred years could be reduced by forestry.

Planting trees is recognized as an effective way to counteract barriers that prevent the vegetation succession process. It changes the physical and biological conditions of the site such as light, temperature and humidity at the soil surface to promote germination; creating shelter to attract wildlife that brings seeds; fertilizes the soil by falling leaves and protects against erosion (Parrota et al., 1997; Aide et al., 2000; Chazdon, 2003). Bailly et al. (1979) describes the forest as the most efficient plant arrangement for the protection of land, with its action exerted through several factors such as the development of the root system that increases soil porosity, the presence of canopies that form an evaporation surface at a certain height, the litter which acts as a sponge that increases retention capacity and promotes the infiltration of water. In addition to the challenges of rapidly restoring forest cover, the choice of planted woody species takes into account several criteria such as the conservation of endangered forest species like *Millettia laurentii* which due to over-exploitation, is currently under conservation measures taken by the state of Cameroon. Despite the fact that it is not in its natural environment, it could adapt to this type of forestry just like a local (*Microberlinia bisulcata*) or pioneer (*Lophira alata*) tree species (Newbery et al., 1998; Lemmens et al., 2008). A variety of soil nutrients must be present in available form for seedlings to be successful. Elements such as carbon, nitrogen (N) and hydrogen usually cycle through the organic material present in the forest, while potassium (K) and phosphorus (P) come from the mineral portion of the soil (Ward and Worthley, 2003). Seedlings also require a variety of minor nutrients such as copper, iron and zinc (Nouck et al., 2016). Moreover, inorganic fertilization can influence soil fertility, regeneration and

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growth of these species on degraded sites in tropical rainforests. Developing soil fertility management options for increased productivity of woody species must be a challenge in most parts of sub-Saharan Africa, where soils are constrained by N and P deficiencies (Abdel-Motagally et al., 2009; Jemo et al., 2010). Adequate supply of inorganic-N fertilizer is beneficial for carbohydrates and protein metabolism, promoting cell division and cell enlargement (Shehu et al., 2010). Similarly, good supply of inorganic-P fertilizer is usually associated with increased root density and proliferation which aid in extensive exploration and supply of nutrients and water to the growing plant parts, resulting in increased growth and yield traits (Maiti and Jana, 1985). Due to the vital role that K plays in plant growth and metabolism, K-deficient plants show a very general phenotype, which is characterized by reduced growth, photosynthesis and impaired osmoregulation and transpiration (Amtmann et al., 2006). Nutrients exported from the soil through harvested biomass or loss from soil by gaseous loss, leaching, or erosion must be replaced with nutrients from external sources. The judicious use of chemical fertilizer is also essential to maintain soil fertility (Hossner and Juo, 1999). There is little knowledge available on the impact of the chemical fertilizers on the growth characteristics and regeneration rate of the woody species on degraded soil of the tropical rainforest for efficient utilization.

Therefore, this study was undertaken to evaluate the impact of inorganic fertilization sources on growth, nutrient uptake and regeneration rate of *M. laurentii*, *M. bisulcata* and *L. alata* plants on degraded tropical rainforest land. We hypothesized that inorganic fertilization sources (N, P and K), single or in combination, can act as efficient fertilizers that will lead to increased plant growth, nutrient uptake and regeneration rate of woody species on degraded tropical rainforest land. A comparative study of the use of these three woody species as catalysts for reforestation in degraded tropical rainforest was also discussed.

Materials and methods

Study area and soil sampling

The SIPO I site located at the South West region of Cameroon covers approximately 30000 km^2 and lies between 9°00′09″ - 9°00′13″E long. and 4°24′40″ - 4°24′45″N lat. (*Fig. 1*). This site was completely cleared of forest cover during oil exploration operations and the soil was excavated about ten meters deep. The climate of SIPO I is characterized by abundant rainfall with average annual rainfall of 3470 mm and average temperature of 26.1 °C. Soils are from sedimentary rocks and are highly acid and eroded (*Fig. 2, Table 1*). The horizons of the basement are usually red or yellow, indicating accumulation of free iron oxides. These ultisols are formed on old land surfaces covered by forest vegetation. The physical and chemical characteristics of the soil taken from the excavated land and unaltered land (control sample) are shown in *Table 1.*

Table 1. *Mean values of soil sample constituents from the SIPO I site*

Soil properties	Experimental site		Control	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm
Particles				
Clay %	20.6d	21.9d	19.0d	18.7d
Limon %	9.3e	10.0e	3.9ef	4.3ef
Sand %	70.1b	68.1b	77.1a	77.0a

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Organic carbon %	0.8g	0.6g	2.4f	1.9f
Total N	0.01i	0.01i	0.3h	0.1h
C/N	80a	60c	80e	19.0d
Available P, mg/kg	14.0de	12.70de	6.5e	5.0e
Al + H (cmol/kg)	4.7ef	4.8ef	1.9f	2.5f
Acidity				
pH (H_2O) 1:2.5	4.5f	4.6f	4.1f	4.1f
pH (KCl) 1:2.5	3.8f	3.8f	3.1f	3.3f
Exchangeable bases (mg/kg)				
Ca^{2+}	4.2f	5.1f	2.9f	4.2f
Mg^{2+}	3.2f	3.1f	3.0f	3.1f
K^+	0.2h	0.2h	0.2h	0.1h
Na^+	0.1h	0.1h	0.1h	0h
Total bases	7.7e	8.5e	6.2e	7.4e
CEC	7.8e	6.9e	15.4d	11.7de

Data represent mean±SD (n = 5); within rows, means followed by the same letter are not significantly different ($p < 0.05$) by Fisher LSD test. CEC: Cation Exchange Capacity

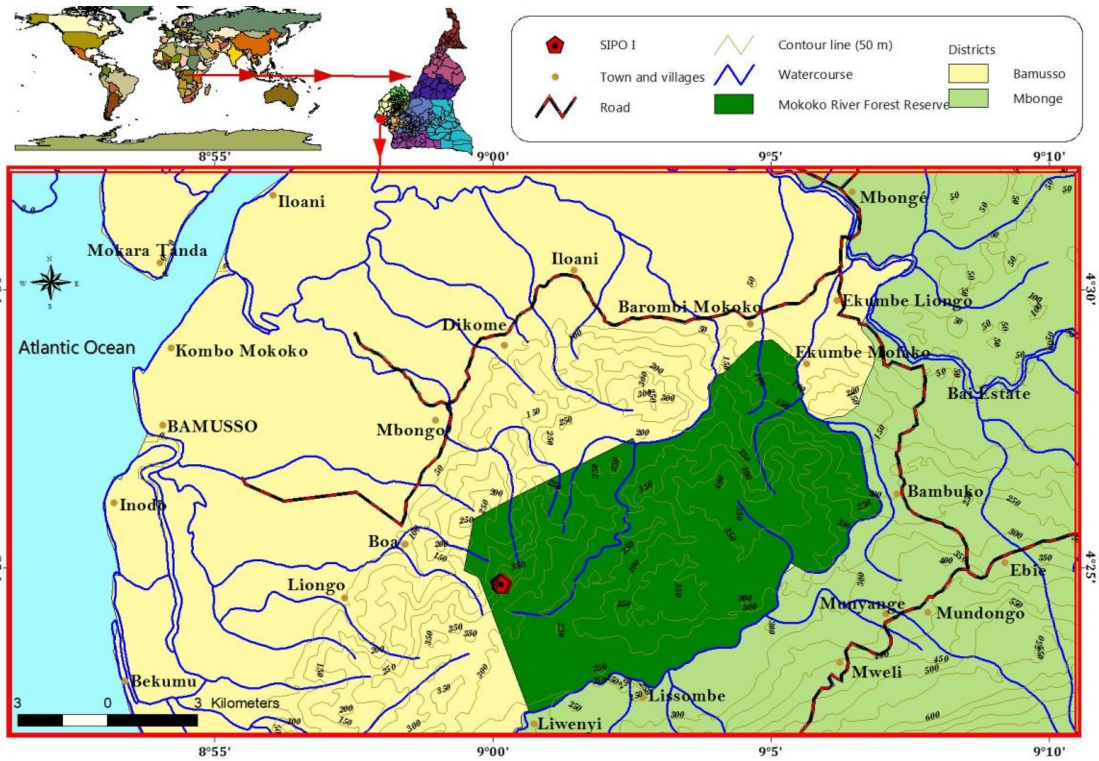

Figure 1. *Location of the SIPO I site in the Mokoko River forest reserve*

Plant material

Millettia laurentii De Wild is a leguminous tree from Africa and native to the Democratic Republic of Congo, Cameroon, Gabon and Equatorial Guinea. It is listed as endangered in the International Union for Conservation of Nature (IUCN) Red List, principally due to destruction of its habitat and over-exploitation for timber. *Microberlinia bisulcata* A. Chev. is also a leguminous species found only in southwestern Cameroon and its natural habitat is subtropical or tropical dry

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forests (Cheek and Cable, 2000). It is also listed as endangered in the IUCN Red List of Threatened Species. This valuable timber species occurs in lowland rainforest areas, usualy on sandy soils in flat areas. Large-scale habitat decline due to clearance for agriculture and exploitation have caused population declines. *Lophira alata* Banks ex Gaertn. is a species of plant in the Ochnaceae family. Its natural habitat is tropical moist lowland forests. The timber is extremely hard and used for railroad ties, electric fences and bridge planking. *L. Alata* needs full sunlight to grow, seedlings can persist for some time in the shady undergrowth and resume growth if breaks in the canopy occur (Biwolé et al., 2012). The seeds of *M. Laurentii* were collected from Mvengue and Mvangan in South Cameroon while those of *L. alata* were originating from the South Bakundo forest. The seedlings of *M. bisulcata* were harvested in the nearby forest.

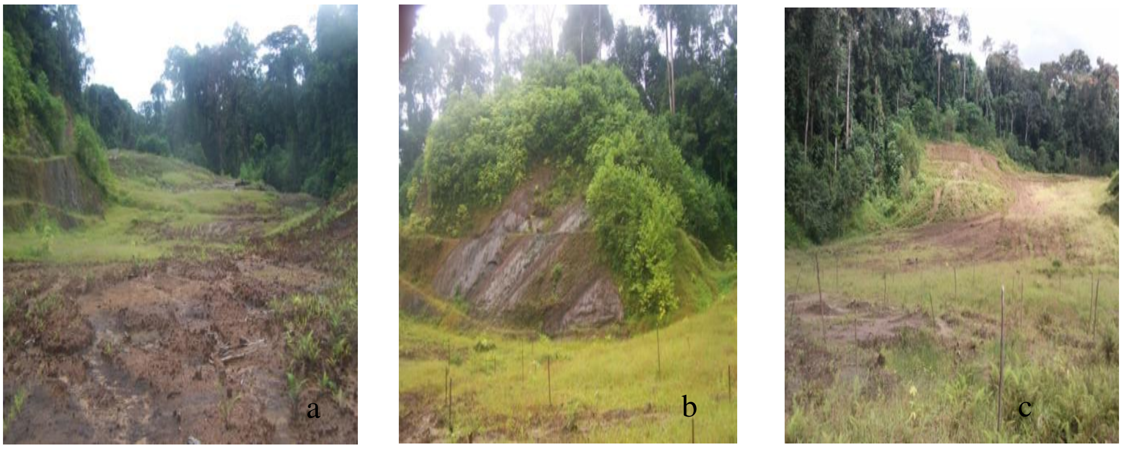

Figure 2. *View of SIPO I showing traces of erosion on the cleared area (a), cleared/ excavated hill (b) and platform (c)*

Methods

Experimental design

The experiment was performed in a randomized complete block design with six replicates. Three fertilizer applications (urea, single superphosphate and muriate of potash) were used on plots each measuring 30 x 30 m. A plot with no fertilizer additions was taken as control. Thirty plots were established to cover the entire area. Each plot consisted of 5 plant rows and 5 columns spaced 4 x 4 m. Each plant was identified with a code (*Fig. 3*) consisting of the name of the plot, the line number and column number. One of the three species was planted on the first three columns of each plot and all three species were planted on the last two. The different fertilizers selected for the experiment were applied in each case around the plant (3 g/plant) 123 days after planting (DAP). Inorganic-N fertilizer was applied as urea (46% N), Inorganic-P as single superphosphate (33.5% P) and Inorganic-K as muriate of potash (49.8% K), singly or in combination (Wamba et al., 2012).

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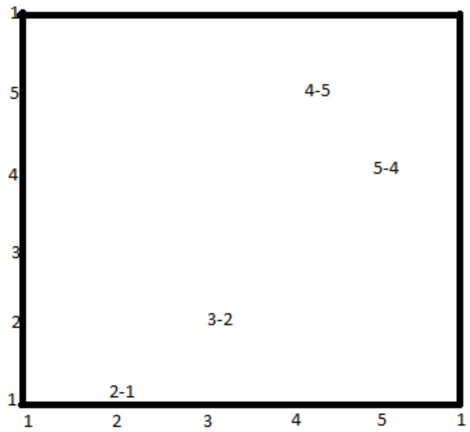

Figure 3. *Codification of plants on the experimental plots.*

Field and laboratory data collection

Stem diameter (SD), shoot length (SL), number of leaves (NL) and number of branches (NB) were recorded at 123, and 331 DAP. Leaves, stems and roots were separately dried at 62 °C for 72 h and their dry weights determined (Taffouo et al., 2010). Leaf Powders were analyzed for total phosphorus (P), total nitrogen (N), calcium (Ca), potassium (K) and magnesium (Mg) in laboratory according to the methods described by Taffouo et al. (2010).

Regeneration rate of plants

The regeneration rate (μ) of plants was calculated as in the following equation:

$$\mu = NL/NT \times 100 \qquad \text{(Eq. 1)}$$

where NL represents the number of live plants and NT the total number of plants in plots.

Soil analysis

Five composite samples prepared from 0-30 and 30-60 cm depths within plots were analyzed in the laboratory of the Institute of Agricultural Research for Development (IRAD), Cameroon. The parameters analyzed included (i) particle size distribution by the method of Davidson (1955), (ii) organic carbon measured by the procedure of Walkley and Black (1934), (iii) exchangeable Ca and Mg of the soil using the procedure of Jackson (1958), (iv) total N content by the method of Kjeldahl (AOAC, 1980), (v) available P by the method of proportioning colorimetric starting from the nitrochlorhydric solution of ashes (Stuffins, 1967), (vi) soil pH measured by the procedure of Nanganoa et al. (2013), and (vii) K content determined using flame photometer (Jenway) (Prevel et al., 1984).

Statistical analyses

All data were statistically analyzed using Statistica (version 9, Tulsa, OK, USA). They were first subjected to analyses of variance (ANOVA). Statistical differences between treatment means were established using the Fisher LSD test at $p<0.05$. Two-

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way ANOVA was used to estimate whether species, nutrient fertilization sources, singly or in interaction had a significant influence on the measured parameters.

Results

Regeneration of seedlings

The regeneration rate of seedlings was estimated by evaluating the number of live plants 123 and 331 days after planting (DAP) (*Figs. 4 and 5*). A significant difference between species was observed for the behavior of seedlings 123 DAP (*Fig. 4*). *M. laurentii* and *L. alata* showed significantly ($p<0.05$) higher number of live plants (82.40 and 84.80% respectively) than those of *M. Bisulcata* (46.40%).

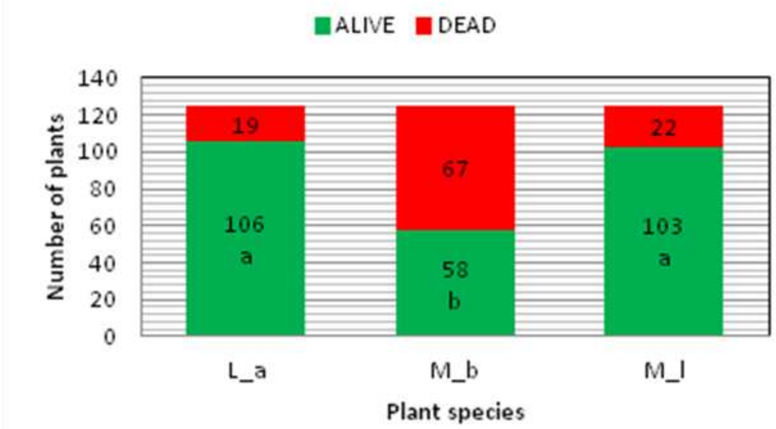

Figure 4. *Number of live plants 123 days after planting (M_b : Microberlinia bisulcata A. Chev. ; L_a : Lophira alata Banks ex Gaertn. ; M_l : Millettia laurentii De Wild). The letters (a, b) indicate significant differences between plant species using Fisher test (p< 0.05).*

Under field conditions, inorganic-N, P or K fertilizer sources supplied singly or in combination had significant effects on regeneration rate (*Fig. 5*). Application of N, P or K fertilizers in combination led to a significant ($p<0.05$) increase of number of live plants in all woody species compared to untreated plants (*Fig. 5A, B and C*). Number of live plants was negatively affected by P supply in all plant species (*Fig. 5A, B and C*). *M. bisulcata* had relatively higher number of live plants under NPK fertilization than *L. alata* and *M. laurentii* as compared to the plants fed with K, N or P singly and untreated controls (*Fig. 5A, B and C*). However, *L. alata* and *M. laurentii* showed higher number of live plants than *M. bisulcata* when plants were enriched only with N fertilizer (*Fig. 5A, B and C*).

Growth characteristics

Plant growth was estimated by measuring the stem diameter (SD), shoot length (SL), number of leaves (NL) and branches (NB) 331 DAP (*Table 2*). This study showed a significant increase ($p<0.05$) of SD, SL and NL in *M. laurentii* under inorganic fertilizer application compared to untreated control (*Table 2*). No significant differences were noted for SD, SL and NL in *L. alata* and *M. bisulcata* when inorganic-N, P or K fertilizers was applied singly or in combination, but only N fertilization had a negative influence in SL and NL of *M. bisulcata* (*Table 2*). N, P or K fertilizers applied singly

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and in combination did not influence NB in *M. bisulcata* and *M. laurentii* plants except for K fertilizer with a significant decrease observed compared to untreated plants (*Table 2*). A significant two-way interaction between the factors ‘nutrient fertilization sources’ and species was observed for SL and NL (*Table 2*). In *M. laurentii*, the plants supplied with N, P or K fertilizers singly or in combination showed significantly ($p<0.05$) higher SD, SL, NL and NB compared to *L. alata* and *M. bisulcata* plants 331DAP (*Table 2*).

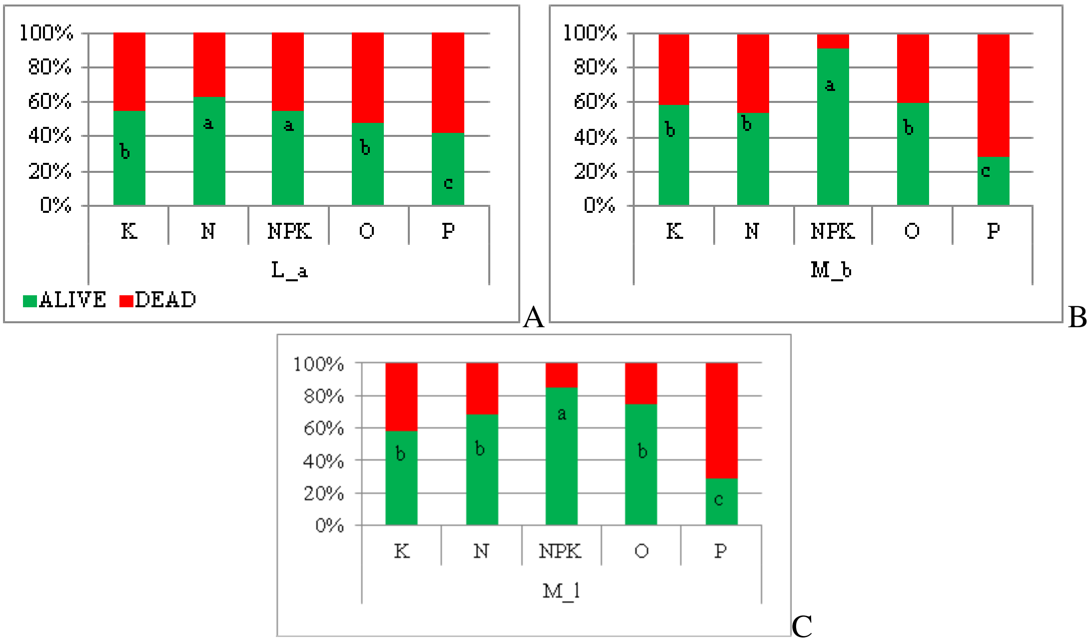

Figure 5. *Number of live and dead plants (%) 331days after planting under different fertilizer treatments. A : L_a : Lophira alata Banks ex Gaertn. ; B : M_b : Microberlinia bisulcata A. Chev. ; C : M_l : Millettia laurentii De Wild N : N 46% ; K : K_2O 49.8% ; NPK : NPK 20- 10- 10 % ; P : P_2O_5 33.5% and O : Control treatment. The letters (a, b) indicate significant differences between treatments using Fisher test ($p < 0.05$).*

Nutrient partitioning

The nutrient contents in leaves, stems and roots of *L. Alata, M. bisulcata and M. laurentii* were affected by different inorganic fertilizer sources (*Table 3*). Application of inorganic-N, P or K fertilizers singly or in combination had a positive effect on leaf N concentrations in all three woody species (*Table 3*). Leaf K content was positively influenced by different inorganic fertilizer sources in *M. Laurentii* (*Table 3*). *M. Laurentii* plants supplied with N, P, and NPK fertilizers showed significantly ($p<0.05$) higher leaf N, P, Ca and K concentrations, respectively than those of *L. Alata and M. bisulcata*. Application of inorganic-N, P or K fertilizers singly or in combination on the contrary, decreased leaf Mg concentration in *L. Alata*, stem N concentration in *M. Bisulcata* and root Ca concentrations in *M. Laurentii* plants (*Table 3*). Application of inorganic-P fertilizer enhanced leaf Mg concentration significantly ($p<0.05$) in *M. Laurentii* plants compared to untreated control (*Table 3*). Under inorganic fertilizer application, the leaves of all three species showed significantly ($p<0.05$) higher amount of N, P, K, Ca and Mg than those of stems and roots except for stem Ca and Mg in *L.*

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Alata plants. A significant two-way interaction between the factors 'species' and 'inorganic fertilizer sources' was observed for N and Mg concentrations (*Table 3*).

Table 2. *Mean values of growth parameters in woody species at the vegetative stage (331 DAP) under inorganic fertilizer applications*

Species	Inorganic fertilizer (g/plant)	Plant growth parameters			
		Stem diameter (mm)	Shoot length (cm)	No. of Leaves	No. of branches
L. alata	control	4.29±1.16c	22.07±3.15f	5.43±0.80e	-
	NPK	5.31±0.98c	21.00±2.55f	7.19±0.91d	-
	N	4.50±0.91c	21.29±3.26f	5.50±0.50e	-
	P	4.25±1.16c	19.50±5.32f	4.25±0.42e	-
	K	4.10±0.83c	18.00±3.85f	4.30±0.68e	-
M. bisulcata	control	10.63±2.62a	43.67±3.00d	8.87±1.11d	2.33±0.47b
	NPK	7.14±1.67b	45.76±3.46d	6.82±0.37de	2.00±0.00b
	N	8.18±1.89ab	36.00±5.26e	4.86±0.12e	2.00±0.00b
	P	10.57±3.89a	53.05±3.41c	9.89±1.40d	-
	K	9.07±2.05a	49.57±2.05c	8.43±1.96d	1.00±0.00c
M. laurentii	control	7.38±1.50b	44.23±2.28d	15.85±1.77c	3.67±0.93a
	NPK	8.93±2.43ab	71.86±7.30a	19.36±1.95b	3.67±0.63a
	N	9.17±1.71a	60.74±6.31b	21.39±1.96a	4.04±0.16a
	P	9.85±2.93a	74,85±8.64a	18.23±1.54b	2.92±0.54ab
	K	9.29±3.77a	71,86±7.86a	18.86±1.59b	2.29±0.45b
Two-way ANOVA results					
Species (S)		**	**	**	ns
Fertilization sources (F)		*	*	*	ns
Interactions S X F		ns	*	*	ns

Data represent mean±SD (n =12); within columns, means followed by the same letter are not significantly different ($p<0.05$) by Fisher LSD test. The result of the two-way ANOVA analysis showing effects of inorganic fertilizer sources, species, and their interaction (S×F) on the different plant growth parameters. ns not significant, *Significant at $p<0.05$, **Significant at $p<0.01$

Table 3. *N, P, Ca, K and Mg partitioning (mg/plant) of woody species grown under inorganic fertilization sources*

Species		Inorganic fertilization (g/plant)	N	P	Ca	K	Mg
L. alata	Leaf	Control	13.3±0.5c	1.6±0.4b	9.0±0.2d	10.6±0.1bc	9.7±0.4b
		NPK	13.9±0.2c	1.5±0.5b	4.4±0.1f	9.3±0.2bc	1.5±0.2c
		N	19.0±1.2b	1.9±0.2b	5.4±0.1ef	11.0±0.3b	2.8±0.2c
		P	12.7±1.0c	1.5±0.2b	4.9±0.1ef	7.4±0.2c	3.2±0.2c
		K	19.0±1.4b	1.9±0.4b	4.6±0.1f	8.5±0.2c	1.3±0.3c
	Stem	Control	3.0±0.2e	0.6±0.0cd	3.4±0.4f	5.5±0.4cd	1.1±0.0c
		NPK	3.6±0.2e	0.4±0.0de	13.2±0.2c	3.2±0.1d	12.5±1.1a
		N	3.0±0.1e	0.9±0.1c	9.4±0.3d	8.2±0.7c	11.4±1.2a
		P	2.3±0.1ef	1.0±0.2c	17.3±1.7b	1.9±0.1e	9.7±1.1b
		K	9.0±0.1d	1.4±0.1bc	13.4±1.3c	4.7±0.1d	11.8±1.2a
	Root	Control	4.0±0.1e	0.3±0.0de	12.5±0.4c	2.1±0.2g	2.3±0.2c
		NPK	2.7±0.2ef	0.2±0.0e	4.2±0.1f	6.6±0.5c	1.4±0.4c
		N	4.0±0.4e	0.3±0.0de	6.2±0.3e	6.8±0.6c	1.3±0.2c
		P	2.7±0.1ef	0.4±0.0de	10.5±0.7c	6.7±0.5c	1.9±0.8c
		K	5.6±0.5de	1.0±0.1c	8.9±0.5de	6.1±0.6c	1.8±0.1c

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Species		Inorganic fertilization (g/plant)	N	P	Ca	K	Mg
M. bisulcata	Leaf	Control	1.7±0.1f	0.3±0.0de	10.4±0.1c	6.4±0.1c	2.6±0.1c
		NPK	5.9±0.2de	0.5±0.0d	8.1±0.5de	5.6±0.9cd	2.0±01c
		N	7.3±0.7d	0.7±0.0cd	1.3±0.8g	6.5±0.1c	3.3±0.1c
		P	4.2±0.6e	1.2±0.2c	16.9±2.1b	5.7±0.2cd	0.2±0.0f
		K	5.1±0.3de	0.8±0.1c	11.4±0.9c	4.4±0.1d	1.3±0.0c
	Stem	Control	5.6±0.2de	1.0±0.0c	2.3±0.2fg	3.8±0.1d	1.3±0.0c
		NPK	0.8±0.1f	1.9±0.1b	5.9±0.3ef	8.1±0.5c	2.1±0.1c
		N	1.1±0.1f	1.2±0.3c	3.7±0.7f	5.6±0.1cd	1.0±0.0c
		P	0.7±0.0f	1.4±0.1bc	4.4±0.3f	6.6±0.2c	1.4±0.1c
		K	2.2±0.1ef	1.6±0.1b	4.0±0.7f	6.7±0.3c	1.2±0.1c
	Root	Control	3.8±0.5e	0.8±0.1c	3.4±0.5f	3.5±0.7d	1.3±0.1c
		NPK	7.0±0.6d	0.6±0.1cd	7.4±0.5e	4.1±0.1d	1.5±0.3c
		N	3.9±0.2e	0.3±0.0de	3.1±0.3f	4.4±0.3d	1.6±07c
		P	5.7±0.2de	0.7±0.1cd	2.8±0.4f	4.2±0.1d	1.3±0.4c
		K	11.3±1.2c	0.7±0.1cd	2.6±0.5f	4.9±0.2d	1.1±0.3c
M. laurentii	Leaf	Control	14.0±1.8c	1.2±0.1c	11.7±0.6c	13.8±0.2b	2.3±0.4c
		NPK	20.6±1.2b	1.4±0.0bc	18.4±0.4a	17.1±0.3a	3.8±0.8c
		N	26.6±0.8a	1.7±0.1b	10.2±0.5c	16.3±0.6a	2.9±0.5c
		P	24.8±0.7a	2.9±0.1a	16.0±0.7ab	15.9±0.6a	8.9±0.9b
		K	26.8±1.9a	1.5±0.0c	12.9±0.2c	16.0±0.7a	3.2±0.5c
	Stem	Control	1.0±0.0g	0.5±0.0d	15.2±0.4b	6.6±0.1c	1.9±0.1c
		NPK	11.5±0.2c	0.6±0.1d	14.6±0.6bc	11.0±0.6b	1.9±0.1c
		N	6.2±0.2d	0.3±0.0de	15.4±0.7b	11.3±0.5b	2.7±0.0c
		P	7.1±0.3e	0.3±0.0de	17.5±0.8a	13.5±0.4b	2.3±0.0c
		K	10.1±0.1cd	0.9±0.1c	17.6±0.9a	9.1±0.6bc	2.6±0.1c
	Root	Control	1.5±0.3f	0.3±0.0de	13.5±0.5c	5.0±0.1cd	1.1±0.0c
		NPK	1.4±0.1f	0.3±0.0de	6.3±0.5d	7.8±0.3c	1.9±0.0c
		N	1.4±0.1f	0.2±0.0e	8.5±0.4cd	6.5±0.1c	2.3±0.1c
		P	3.2±0.3e	0.2±0.0e	5.6±0.4e	4.0±0.2d	0.7±0.0d
		K	1.5±0.0f	0.4±0.0de	7.8±0.7d	5.5±0.2cd	1.9±0.1c
Two way ANOVA results							
Species (S)			*	ns	*	ns	*
Fertilization sources (F)			**	ns	*	ns	*
Interaction S x F			*	ns	ns	ns	*

Data represent mean±SD (n = 5); within columns, means followed by the same letter are not significantly different (p<0.05) by Fisher LSD test. The result of the two-way ANOVA analysis showing effects of species, soil nutrient fertilization, and their interaction (S x F) on plant nutrient status ns not significant,*significant at p<0.05, **significant at p<0.01

Discussion

The restoration of degraded land in the SIPO I site of Boa forest can be catalyzed by forestry techniques which in addition, provide the conditions for regeneration of forest species, shortened vegetation succession and duration to reach colonization climax (Dajoz, 1985). *M. laurentii* and *L. alata* showed higher number of live plants than those of *M. Bisulcata* 123 DAP. These results suggested the adaptation of *M. laurentii* and *L. alata* to distinct combinations of light, moisture, and soil amendments of SIPO I site. Similar results were also observed by Ward and Worthley (2003). The lower performance presented by *M. Bisulcata* at this vegetative stage (123 DAP) compared to those of pioneer species (*M. laurentii* and *L. Alata*) could be

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explained by some limiting factors: (1) top soils of forests are covered with litter which endows them with particular nutritive characteristics (Ibrahim et al., 2010); (2) degraded zones lost some soil characteristics during implementation of industrial and mining projects. In fact, in the SIPO I site where excavation was done, the structure ranges from sandy loam to loamy-sandy-clay with low values of CEC and total N at all depths, rendering it vulnerable to erosion. Erosion is accentuated by aggressiveness of rainfall on naked land (Roose and Sarrailh, 1990; Graf et al., 2003). The carbon content, total N and CEC are low compared to reference soil and values obtained by Taffouo et al. (2010), Sharma and Raghubanshi (2011), Wamba et al. (2012) and Fokom et al. (2013). The low mineral content of this soil is due to degradation of forest cover (Bonansea et al., 2016; Ndema Nsombo et al., 2016) and the top soil horizons which were absent after excavation (Duryea, 2000). On the contrary, *M. bisulcata* had relatively higher number of live plants under inorganic-NPK fertilizer than *L. alata* and *M. laurentii* as compared to the plants fed with K, N or P fertilizers singly or in combination 331 DAP. Ouédraogo et al. (2014) and Fayolle et al. (2015), studying the regeneration of a pioneer species (*Milicia excelsa*) and a non-pioneer species (*Pericopsis elata*) found that a pioneer species presented significantly lower performance than a non-pioneer species when planted on forest clearings. These results could be explained by the fact that pioneer species might not require optimum environmental conditions to thrive. According to Duryea (2000), the main root of these plants was reduced during planting to avoid folding of the root system in the form of “L” or “J” which is one of the drawbacks of using seedlings for regeneration. Efficient sylvicultural operations on such soil requires selection of plant species that are capable of adapting to it. Otherwise, it is important to consider modification of the site’s soil structure and fertility (Lamd, 1994; Ndema Nsombo et al., 2010).

Soil nutrients play a role in the life cycle of the tree and must be present for survival and successful growth. In short supply, one or more nutrients can be the limiting factor to the growth and development of trees or stands (Ward and Worthley, 2003). In the present study, application of inorganic-N, P or K singly or in combination led to a significant increase in SL, SD, NL and NB in *M. laurentii* plants. Adequate supply of inorganic-N is beneficial for carbohydrates and protein metabolism, promoting cell division and cell enlargement (Shehu et al., 2010; Debere et al., 2014). Similarly, good supply of inorganic-P is usually associated with increased root density, soil porosity and proliferation which aid in extensive exploration and supply of nutrients and water to the growing plant parts, resulting in increased growth and yield traits (Bailly et al., 1979; Maiti and Jana, 1985). Due to the vital role that K plays in plant growth and metabolism, K-deficient plants show a very general phenotype, which is characterized by reduced growth, photosynthesis and impaired osmoregulation and transpiration (Amtmann et al., 2006). In this study, no significant differences were noted for SD, SL and NL in *L. alata* and *M. bisulcata* when inorganic-N, P or K was applied singly or in combination. The minimum amount of light required for optimum growth and development varies dramatically among tree species. According to Ward and Worthley (2003), species that compete best in full sunlight have the capacity for rapid height growth and are often found in the upper layers of the forest canopy while those that are capable to compete in the shade of other trees can occupy lower layers in the canopy, and each canopy layer will intercept additional sunlight. In *M. laurentii*, the plants supplied with inorganic-N, P or K fertilizers singly or in combination showed higher SD, SL, NL and NB compared to *L. alata* and *M. bisulcata* 331 DAP. Similar results

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have previously been documented by Ashton et al. (2001) who studied the rain forest in SouthWest Sri Lanka and suggested that the early formation of the branches and the large number of leaves allow trees (catalysts) to cover the site and to create a favorable microclimate for the growth of other forest species. The branches and leaves falling constitute litter which is important for the formation of the humus layer and prevention against erosion (Ibrahim et al., 2010).

The results of this study highlighted the importance of nutrients uptake and their distribution in plant parts of the three woody species. Application of inorganic-N, P or K fertilizers singly or in combination had a positive effect on leaf N concentrations in all the three woody species. Mineral uptake is largely influenced by the availability of soil mineral nutrients which in turn affects the chemical composition of the plants (Juma and Van Averbeke, 2005). Taffouo et al. (2014) reported that N is directly transferred from the roots towards the leaves of plants where the N compounds are used for protein biosynthesis. In this study, *M. Laurentii* (N fixing species) plants supplied with inorganic-N, P or K fertilizers singly or in combination showed higher leaf N, P, Ca and K concentrations than those of *L. Alata and M. bisulcata*. Ojiem et al. (2000) demonstrated that legumes have the potential to improve soil nutrients status through biological N fixation and incorporation of biomass into the soil as green manure. In cowpea, the N requirements for developing pods are not only covered by root uptake or biological N fixation, but also by mobilization of N in vegetative tissues (Douglas and Weaver, 1993). Some food and fodder legumes are known for N fixing ability; however their establishment with P fertilization enhances nodulation and hence fixation of atmospheric N (Masinde and Omolo, 2007). According to Jemo et al. (2010) and Taffouo et al. (2014), the process of foliar N mobilization is dependent on the amount of P uptake by plants. Under inorganic fertilization, the leaves of all the three woody species showed higher amounts of N, P, K, Ca and Mg than those of stems and roots except for stem Ca and Mg in *L. Alata* plants. According to Amtmann et al. (2006), leaves are important to plants and trees because they convert sunlight energy to food through the process of photosynthesis. In this study, the highest accumulation of nutrients was recorded in *M. Laurentii*. In fact this species has been successfully tested for forestry by macro-cuttings (Nsielolo Kitoko et al., 2015). In the analysis combining regeneration rate, growth and nutrient partitioning of the parameters measured, the results revealed the best adaptation by *M. laurentii* to soil nutrient amendment. This species could be used as a catalyst for reforestation in degraded tropical rainforest land.

Conclusions

Restoration of degraded tropical rainforest land in the SIPO I site can be catalyzed by intervention of forestry and agronomic management techniques which provide the conditions for regeneration of forest species, shortened vegetation succession and duration to reach a state of balance of the forest (climax). *M. laurentii* and *L. alata* showed higher number of live plants (82.40 and 84.80% respectively) than those of *M. Bisulcata* (46.40%) 123 DAP. The specific combination (NPK) was found as efficient fertilizer to enhance the woody species regeneration. The highest accumulation of N, P, K, Ca and Mg concentrations was found in leaves compared to stems and roots of all the species.

In *M. Laurentii* plants, SD, SL, NL, NB and N, P, Ca and K uptake were positively influenced by inorganic-N, P, K or NPK fertilizer treatments compared to *L. alata* and *M. Bisulcata* 331 DAP. These results revealed the best adaptation by *M. laurentii* to soil

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nutrient amendment. Therefore, this woody species can be considered as catalyst for reforestation of degraded tropical rainforest land such as that encountered in the SIPO I site of Boa forest in Cameroon.

Based on these attributes *M. laurentii* which is subjected to over-exploitation is strongly recommend for reforestation in degraded tropical rainforest land.

Developing soil fertility management options for increasing productivity of woody species must be a challenge in most parts of sub-Saharan Africa, where soils are constrained by N and P deficiencies.

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LANDSCAPE-ECOLOGICAL OPTIMIZATION OF HYDRIC POTENTIAL IN FOOTHILLS REGION WITH DISPERSED SETTLEMENTS – A CASE STUDY OF NOVÁ BOŠÁCA, SLOVAKIA

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Abstract. The article shows how to implement scientific results from a methodology of landscape ecological planning (LANDEP) into integrated river basin management. This methodology creates a framework of integrating research of land use structure with modelling hydric potential by using runoff curve number into the assessment and subsequent optimization of hydric potential in a foothills region of the Biele Karpaty Mts. Analysis and hydric evaluation of the study area shows that foothills region has a relatively high hydric potential. The research question is: could landscape ecological optimization reduce runoff significantly and improve hydric regime in the landscape. The proposed measures are linked to existing land use structure, where they increase the quality of existing hydric elements. They have character of non-technical solutions with maximum exploitation of the potential of ecosystem services, therefore they are economically undemanding. On the basis of our results, the proposed changes in land use will lead to the capture of water in landscape and an overall better use of water in landscape.
Keywords: *land use structure, CN curves, retention, river basin management, Biele Karpaty Mts.*

Introduction

In recent years, there is a higher frequency of torrential rains and related flash floods which is often thought as a result or an attribute of climate change. With the appearance of extreme flood situations a new space for research work has been created. Nevertheless, researchers should also study land use changes (Kozma et al., 2014) which can contribute to the occurrence and severity of these extreme weather events. Due to complexity, fast, dynamic and non-linear development, torrential rains belong to the challenging phenomena for the accurate weather forecasting. If we take into considerations a location of their occurrence and quantification of expressions, accurate

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forecasting is currently not possible for a period exceeding several tens of minutes. Consequences of these torrential rains are expressed by flash floods and subsequent soil erosion. These two types of events belong among the most dangerous weather events for human society in Central Europe, where their devastating effects are often reflected.

The most vulnerable regions are foothills of the Carpathians Mountains, where dominant land use is agriculture. Strengthening ecological stability of these foothills is important (Pavličková et al., 2004), as flash floods can cause damage especially in small basins with inappropriate spatial structure of land use. Land uses different from the primary landscape structure lead to disruption of functional ecosystems. This is reflected in changes not only in structure of landscapes, but also in their functions. Therefore a detailed knowledge of the structure and functioning of ecosystems is necessary (Kozová and Pauditšová, 2001).

The environment, local and global climate are affected by the ecosystems through the climate-ecosystem feedbacks (Drégelyi-Kiss et al., 2008). Correspondingly, structures, functions and services of ecosystems may be influenced by climate change. On local, regional and global scales, the most significant human impacts on the hydrologic system are caused by land-use change (Bhaduri et al., 2000; Izakovičová, 2000; Liekovský, Bezák, Izakovičová, 2010). Landscape structures as integral part of ecosystems integrated within landscape ecology was the subject of several authors (e.g. Naveh and Liebermann, 1990; Forman and Godron, 1993; Bastian, 2001; Estrada-Carmona et al. 2014; Muchová et al. 2016).

Scientific team led by Ružička and Miklós (1982) developed a detailed methodology of landscape-ecological planning (LANDEP), which is efficient in the process of territorial planning. A goal oriented application of the traditional landscape-ecological theory and methodology brings a new process for landscape management (Miklós, 1996; Hrnčiarová, 2003, Miklós and Špinarová, 2011). LANDEP represents an approach to the management of natural resources in different landscape ecological units by integrating environmental, economic and social issues. It is focused on sustainable benefits for future generations, while protecting natural resources, especially water, and minimizing negative environmental, economic and social impacts (Walmsley, 2002). As such it can be considered as a predecessor of ecosystem assessment focusing on ecosystem services (see e.g. Haines-Young and Potschin, 2008).

In case of river basins results from LANDEP can be applied in integrated river basin management. This term was used for the first time in proposal for management plan of Atchafalaya River basin in North America (Van Beek, 1981). It is a holistic approach and can be defined as a process of coordinating conservation, management and development of water, land and related resources across sectors within a given river basin, in order to maximise the economic and social benefits derived from water resources in an equitable manner while preserving and, where necessary, restoring freshwater ecosystems (GWP, 2000).

An essential step for dealing with integrated river basin management is adoption of Directive 2000/60/EC. Its purpose is to establish integrated framework of water for the EU policy, in order to protect physical and biological integrity of water systems and to reduce negative pressure on drinking water sources (Directive 2000/60/EC, 2000).

So far, application part of integrated river basin management and flood protection measures has been significantly behind scientific progress. As a crucial step we perceive application and integration of scientific research into the actual land use planning in the form of its implementation into Forest management plans, Water management plans and

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completed Territorial systems of ecological stability and Land consolidations. Quality of landscape documentation has great importance for their successful implementation.

One of the scientific methods that are used in integrated river basin management are modelling runoff with the help of runoff curve number. Methodology of runoff curve number (so called CN curves), using an empirical parameter for predicting direct runoff or infiltration from rainfall excess, was developed by the United States Department of Agriculture Natural Resources Conservation Service and is still popular and effective tool for runoff modelling (USDA, 1986; Ward and Trimble, 2004). An important part of the model is detail information about the spatial structure of landscape – the current land use. In the post-socialist countries there were significant changes in spatial structure of landscape due to several stages of transformation processes, which affected runoff, especially in areas with a dominant agricultural use. Changes were taking place also in foothills regions with typical historical structures, which are nowadays in many areas disappearing and are considered very vulnerable.

Our research is based on the LANDEP methodology by using methodology of CN curves and large-scale data processing for flood risks in a local area.

The aim of the paper is the proposal of possibility to harmonize the use of methodology LANDEP and integrated landscape management methodology for landscape ecological optimization of hydric potential of landscape in source and headwater areas. The method was applied in rural landscape with the historical dispersed settlement on the example of upper part of Bošáčka basin in the central part of the Biele Karpaty Mts.

Materials and Methods

Study area

Study area (*Fig. 1*) is situated in the Slovak Republic, Trenčín region, Nové Mesto nad Váhom district. Majority of the area is located in cadastre of Nová Bošáca and partly in cadastre of Zemianske Podhradie. It borders with Czech Republic in the north, where part of study area of micro-basin is situated. Due to the subject of research, the study area is determined based on micro-basins and consists of three units.

Landscape of the Biele Karpaty Mts. and Bošáčka valley is characterized by a rich diversity of meadow and pasture habitats, supplemented with orchards and hedgerows, which is result of dispersed rural settlements and landscape management.

Detailed characteristics of the study area are part of the result section, namely chapter Landscape ecological analysis.

Data

Outputs from landscape-ecological optimization of potential hydric potential (i.e. fourth step in LANDEP) are an essential input into integrated management of basin. Within the evaluation of landscape-ecological significance in the assessment of integrated river basin management, emphasis is placed on positive functioning of ecological processes in the landscape, and natural resources management, especially water. It is necessary that soil receives as much rainwater as possible. Therefore significant environmental characteristics enter the evaluation process. These are in particular:

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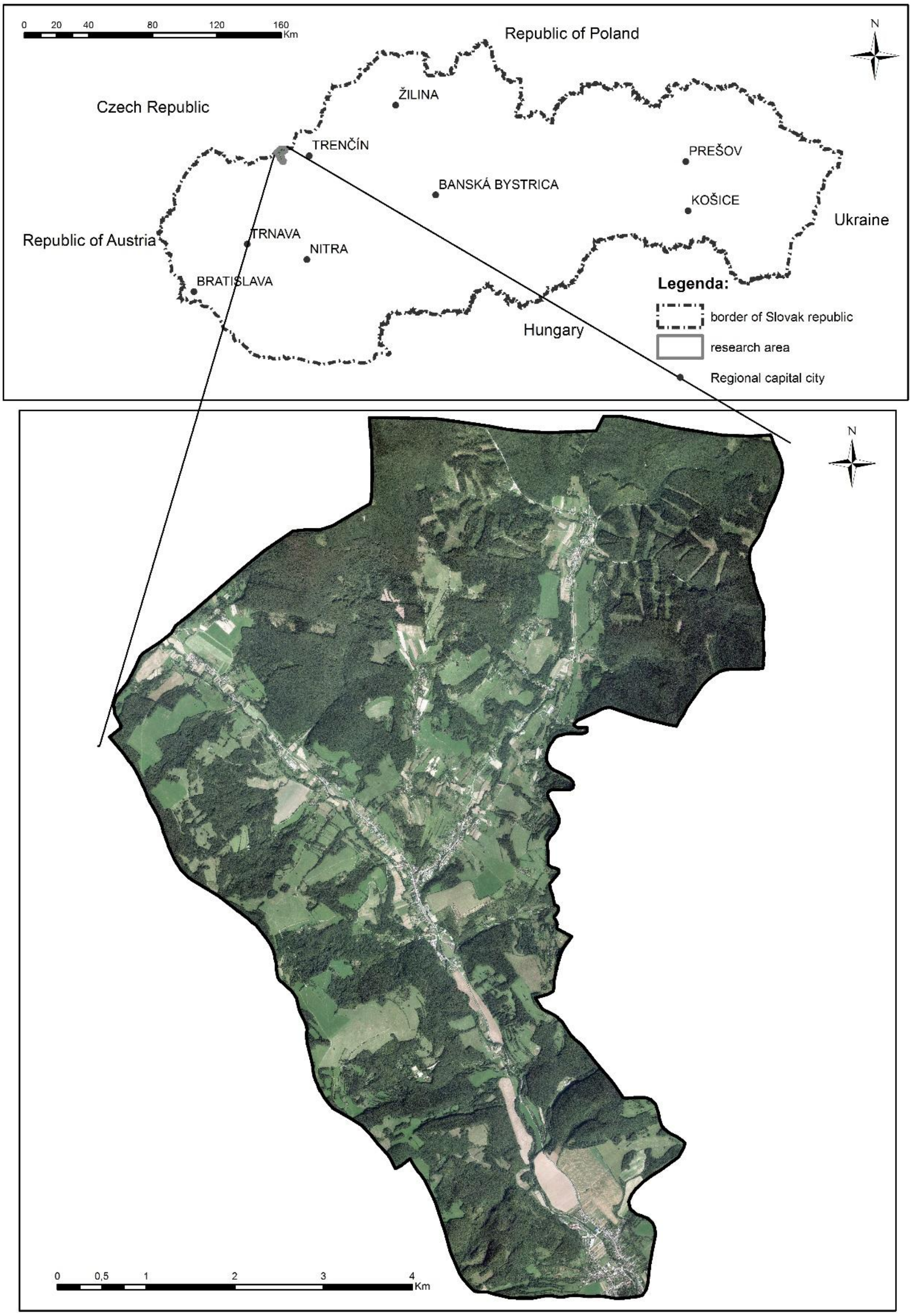

Figure 1. *Location of study area*

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- Hydrogeological characteristics of the basin (flow rate trough bedrocks)
- Meteorological conditions (average annual precipitation, significant precipitation events)
- Geomorphological characteristics of the basin (slope)
- Characteristics of soil (soil type, hydrologic soil group)
- Characteristics of forests (forest area, water balance, proportion of forest area with good water management of vegetation, etc.)
- Land use
- Interests of nature conservation and natural resources protection which are included in the legislation of landscape protection or other significant conventions (ecological priority of landscape)

Data from geological, pedological and geomorphological maps, The Slovak Hydrometeorological Institute, The National Forest Centre and Projects of Ecological Network were inputs for landscape-ecological analyses of the study area, which were verified and supplemented in field research. Terrain mapping of current landscape structure were according to universal legend of landscape elements was also part of analyses (Petrovič et al., 2009). It was adjusted to the aim of the research and to create current landscape structure map. Analyses were focused on selected landscape features which enter evaluation of potential retention and infiltration of study area.

Based on data from evaluated soil-ecological units and forest database, hydrologic soil groups (HSG) have been identified in the study area as a synthesis of hydric characteristics of sedimentary rocks and soil types, which are basis for CN curves methodology. Hydrologic soil groups for CN curves were determined according to the table of Hydrological categorization of soils (*Table 1*) (Antal and Igaz, 2006).

Table 1. *Hydrologic soil groups (HSG) for CN method (modifed by Antal and Igaz., 2006; Janeček et al., 2002)*

HSG	Infiltration and drainage soil properties	Typical soil types	Representation of grains of I. category (% hm.)	Consistent infiltration intensity (mm.min^{-1})
A	Soils with high infiltration ability even when fully saturated with water	Deep sands and gravel	< 10	High speed > 0,12
B	Soils with medium infiltration ability, even in the their fully saturated with water and good drainage	Medium deep to deep sand and silt-sand soils	10 - 45	Medium speed 0,06 – 0,12
C	Soils with low infiltration ability as well as in their fully saturated with low drainage	Clay-silt till clay shallow soil	45 - 75	Low speed 0,02 – 0,06
D	Soils with very low infiltration capacity and without drainage	Clay or soils with limited drainage and infiltration ability	> 75	Very low speed < 0,02

Methods

Theory and methods of LANDEP distinguishes two basic parts. The scientific content is included in the first part of the system as landscape-ecological analysis of a

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studied area. The second part includes landscape-ecological optimization of land use, which synthesises data and results from the first part.

The aim of the landscape-ecological synthesis in LANDEP is to create useable complex set of information about landscape characteristic for each landscape unit of a study area. In particular, LANDEP distinguishes four steps: the first step is represented by landscape ecological analysis that gives detailed information about all components of a study area. This is followed by landscape ecological synthesis which results in establishment of landscape ecological complexes; in case of integrated river management these complexes would be represented by units with different hydric potential. Third step, so called evaluation, confronts landscape features that are reflected in landscape ecological complexes with society requirements for the development of an area. The final step, proposition, proposes optimal localization of socio-economic activities in the landscape. The aim of this step is to harmonize its current environmental performance of the landscape with the proposed use.

In our case, the proposition aims at creating ecologically optimal landscape structure where the proposed use, including alternatives, is most suitable for every type of landscape ecological complexes. Proposition of optimal landscape structure is based on the potential possibilities of landscape structure and requirements of its current state.

Methodology of integrated river basin management (IMP) (Lepeška, 2005, 2013) was used to specify attributes entering evaluation of hydric potential of the landscape. The methodology is partly derived from methodology of evaluation of landscape carrying capacity (LCC) (Hrnčiarová et. al., 1997), which is based on basic procedures of LANDEP (Ružička and Miklós, 1982). A CN curve was used in the synthesis method. It was introduced on the basis of many annual observations of drainage on agricultural land. Therefore, the input characteristics for calculating the surface runoff according to CN method characterize in detail not only hydrological conditions but also land use. The value of CN is between 0 and 100. A value 100 means that all rainwater that falls on the catchment area or entire river basin flows away as surface runoff. In case that CN value is 0 all rainwater is infiltrated. CN value is synthesis of: Hydrologic soil group, Current landscape structure, Hydrologic characteristic of the various soil cultivation methods and Hydrologic characteristic of soil moisture (index determination of previous rainfall). CN values are included into the calculation of potential retention A (mm), which is the basis for the calculation of direct runoff H_0 (mm) and runoff volume O (mm^3) (Gajdošík et al., 2005). Following calculation of potential retention was used (Gajdošík et al., 2005):

$$A = 25{,}4 * (1000/CN - 10) \qquad \text{(Eq.1)}$$

Where A – potential retention (mm)

Based on previous data (runoff CN curve numbers and potential retention A) with the addition of the proposed precipitation volume (in the calculations, we use the sum of torrential rains 06/07/1997 – 120.02 mm) amount of direct runoff H_o (mm) was calculated. Subsequently, from H_o values the amount of surface runoff at the level of unit was calculated (pixel 5m x 5m) – O_{bunka} (m^3), which using Arc Hydro – Flow accumulation toll entering into the calculation of the surface runoff volume in the river basin $O_{O,P}$ (m^3).

The total volume of basin surface runoff O_{op} (m^3) was calculated through the function Flow Accumulation. This function as input requires a calculated function Flow

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Direction, which we obtained from hydrologically correct digital elevation model (DMR), thereby relief shape (topography) entered the calculation.

Runoff curves method is based on the assumption that the ratio of the runoff volume to total torrential rain is equal to the ratio of the water volume collected during runoff to the potential volume that can be collected. Runoff does not start immediately, but after some initial loss, which is the sum of interception, infiltration and surface accumulation, which was estimated based on experimental measurements at 20% of the potential retention (I_a = 0,2 A).The equation to determine the amount of direct runoff:

$$Ho = (Hs - 0.2A)2 / (Hs + 0{,}8) \qquad \text{(Eq.2)}$$

$$Ho = (Hs - 0.2A)^2 / (Hs + 0.8A)$$

Where Hs ≥ 0,2A
H_o – amount of direct runoff [mm]
H_s – total proposed precipitation [mm]
A – potential retention [mm]

For proposing measures of landscape-ecological optimization we partly used catalogue of non-technical flood control measures which was implemented into the GIS environment during the modelling process by using modelling tool Arc Hydro. This procedure was implemented by Rozsívalová (2007) in her work for the Ministry of the Environment of the Czech Republic. By applying proposed measures of landscape-ecological optimization into modelling tool Arc Hydro, we were able to receive final calculation of the optimized surface runoff volume in the study area.

Results

Landscape ecological analysis

Study area is geologically part of the Western Carpathians, which are part of Central European Alpide. From geological point of view they are mostly covered with sedimentary rocks. South-western part is covered with flysch where claystone is dominant, the northern and north-western part is mostly covered with sandstone with a dominance of flysch and central part is mostly covered by flysch. Regular appearance of slope deformation is typical for this area. Relief of the Biele Karpaty Mts. largely reflects the different resilience of various flysch layers to weathering. Geomorphologic factors, mostly slope, significantly affect runoff conditions and ability of landscape to infiltrate rainfall (*Fig. 2*).

Atmospheric precipitation is crucial resource of the water in the area. In the lower part of the territory there is 330 – 430 mm of precipitation during vegetation period and 250 – 300 mm of precipitation during the winter. In the highest part of the territory there is 500 – 600 mm of precipitation during vegetation period and 350 – 400 mm of precipitation during the winter. The area is susceptible to flash floods; Kravarčík et al. (2000) recorded the most devastating floods in the cadastral area of Nová Bošáca which were results after short intensive rainfall, in April 1994 and 1999, in July 1972 and 1997 and in August 1972.

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Study area is situated in Váh River basin. Ridge of Váh River and Morava River basins mostly follows the main ridge of the Biele Karpaty Mts.. Considerable flow volatility is typical for the streams of the Biele Karpaty Mts., which is caused particularly due to small retention capacity of the flysch zone with the terrain morphology and climate factors. Altitude, slope conditions and characteristic of the substrate cause rapid rainwater runoff. Therefore only small amount of groundwater resources are accumulated. Besides natural factors insensitive ways of landscape management are also reflected, such as extensive deforestation, removing of natural boundaries, changes in natural structure of forest, transformation of grasslands into arable land even on high slopes, growing unsuitable crops, using heavy machinery, stream regulation, melioration and other factors.

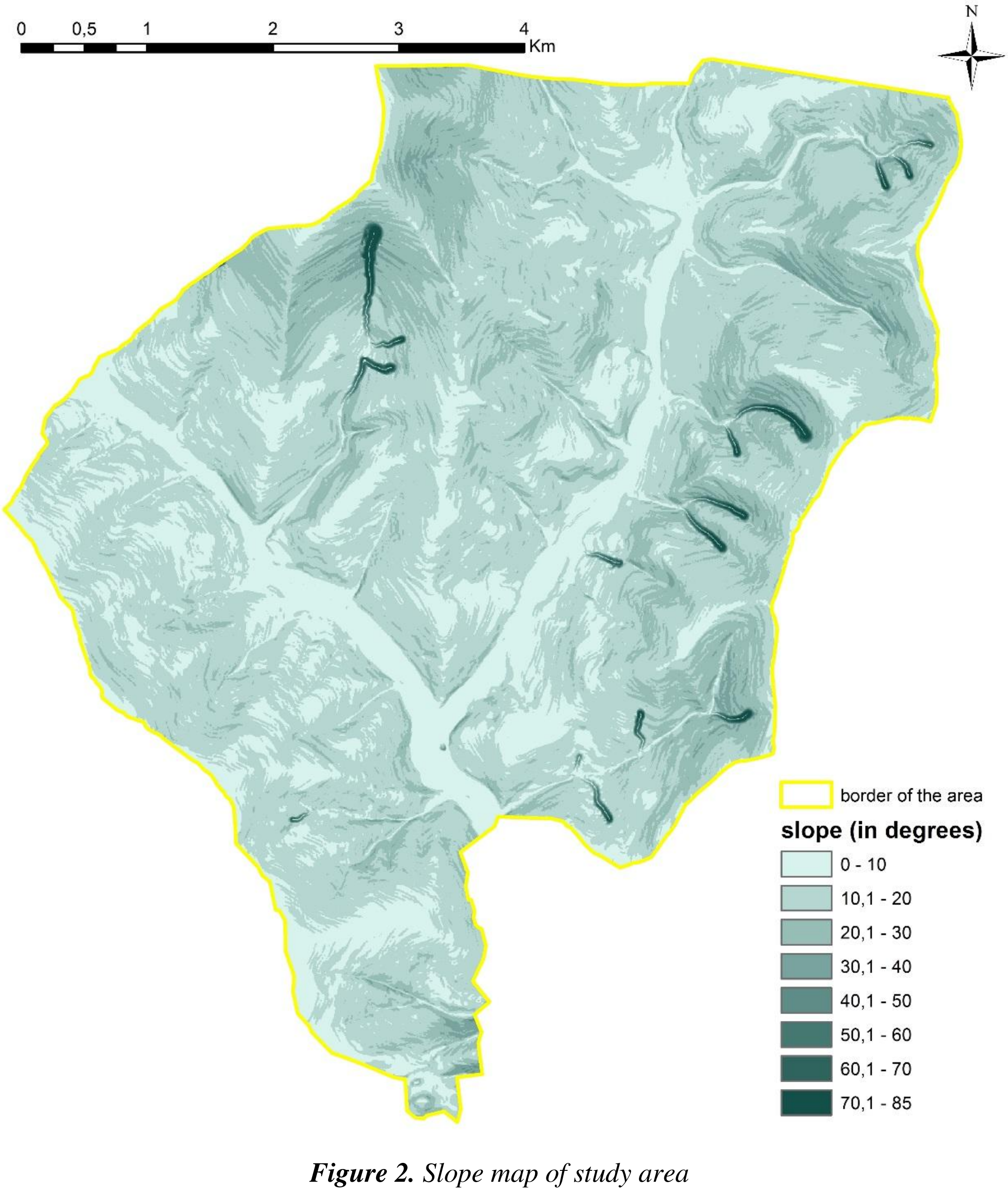

Figure 2. *Slope map of study area*

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Predominant soil type of the Biele Karpaty Mts. is represented by less fertile 'brown soils' (cambisols), whose fertility mainly depends on the substrate and the water regime. On the hilly parts of Biele Karpaty Mts. ridge, oligotrophic cambisols on acid substrates are typical. River valleys are covered by alluvial soils (VÚPOP, 2013). Based on data from evaluated soil-ecological units and forest database, four hydrologic soil groups (HSG) have been identified (*Table 1*) with only three being present in the study area (*Fig. 3*).

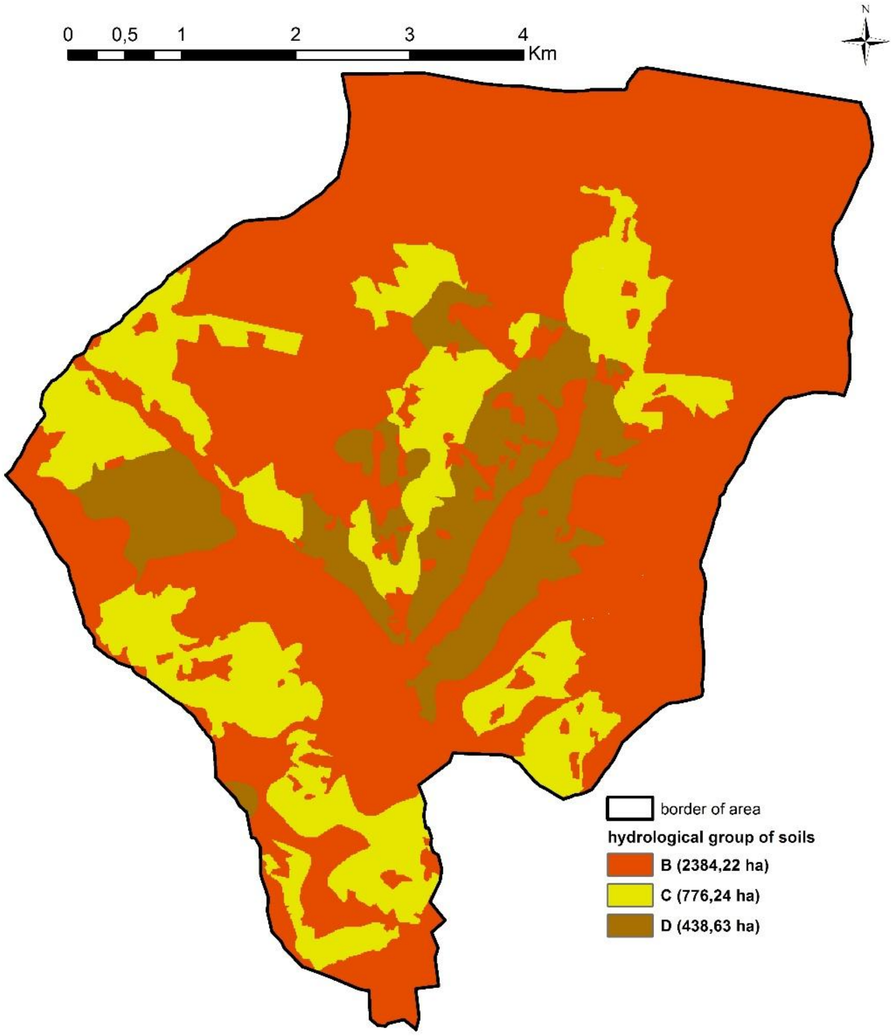

Figure 3. *Map of hydrological soil groups(HSG)*

Secondary landscape structure

Secondary landscape structure is a result of human impact on natural landscape. It reflects lifestyle of people, their relationships, values and environmental needs that change over time. In total, 35 elements of the secondary landscape structure were identified on the area of 3611.73 ha (*Fig. 4*). They were subsequently merged into 18 elements of secondary landscape structure according to the CN curves methodology (*Table 2*). Dominant groups of landscape elements are forests (61.53 %), permanent

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grasslands (22.7 %), agricultural lands (10 %), group of built-up areas and communications (3.13 %), linear vegetations (1.81 %) and group of streams and wetlands (0.83 %).

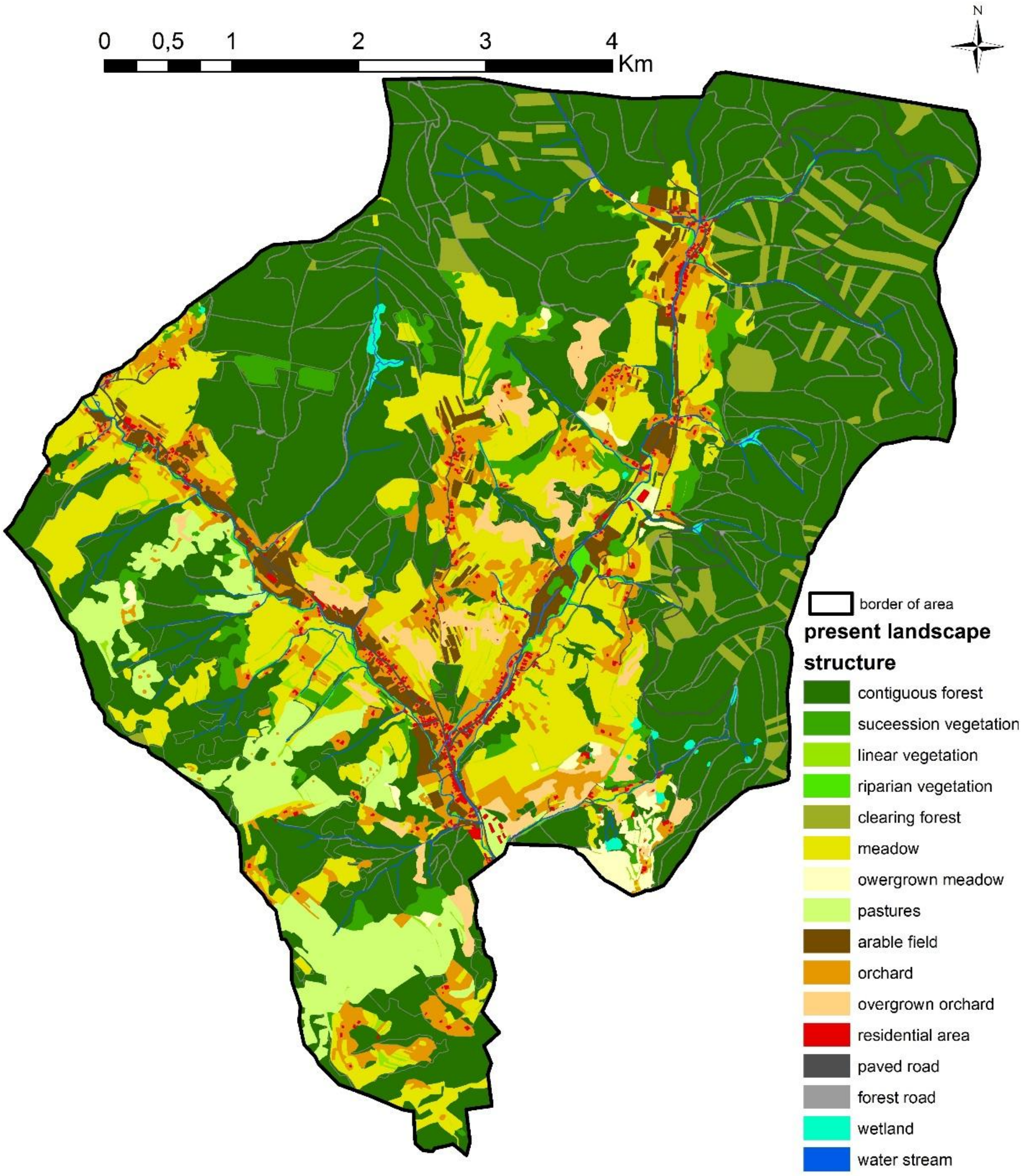

Figure 4. *Map of current landscape structure for study area*

Continuous forests (1980.79 ha) naturally have the highest hydric potential among all secondary landscape structure elements and provide optimal system for protection and formation of soil. Forest species include many native plants (e.g. European beech, European hornbeam, European ash, wild cherry, European silver) as well as non-native plants (e.g. Norway spruce and European larch). Selective and shelter-wood system is performed for economic logging while windfall logging is preformed in the form of area-wide clear-cuts (effect on the soil is similar to the large-scale clear-cut). From precipitation that falls on the forest, treetops catch approximately 20 %, which is called interception loss. Tužinský (2002) reports average interception value for the period

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1981 – 1984 in the range from 20.6 to 26.9 % for area of the Little Carpathians in an adult beech forest (90 years), belonging to the group of forest types *Querceto Fagetum* in the altitude of 450 m.

Table 2. *CN curves values for elements of secondary landscape structure*

Elements of current landscape structure	CN curve value HSG B	Area (ha)	Area (%)	Hydric potential
Contiguous forest	30	1980.79	61.53	very high
Riparian vegetation	36	42.43	1.17	very high
Linear vegetation	48	23.17	0.64	high
Overgrown orchards	50	75.53	2.09	high
Orchards	53	199.89	5.53	high
Succession vegetation	56	90.77	2.51	high
Meadows	58	579.13	16.03	medium
Pastures	61	187.67	5.19	medium
Overgrown meadows	62	28.95	0.80	medium
Small fields	75	85.41	2.36	medium low
Forests clearings	77	103.29	2.85	medium low
Wetlands	84	8.79	0.24	low
Slightly eroded forest roads	82	3.99	0.11	low
Medium eroded forest roads	87	43.41	1.20	low
Strong eroded forest road	89	20.93	0.57	very low
Water streams	98	15.44	0.42	very low
Residential area	98	21.79	0.60	very low
Paved roads	98	24.42	0.67	very low

In the western part of the study area field mapping discovered proportional increase of forest with autochthonous tree species such as silver birch (*Betula pendula*) and European aspen (*Populus tremula*). Riparian vegetation covers area of 42.43 hectares. It is formed by linear vegetation and woodlots of common alder (*Alnus glutinosa*), European ash (*Fraxinus excelsior*) and white willow (*Salix alba*). Root system of this vegetation stabilizes shorelines from erosion and slows down the runoff. It is an element with significant hydric and ecological stabilisation value.

Orchards on total of 199.89 hectares are typical feature of the study area. Old and extensively used orchards with grassland represent landscape elements with most appropriate combination of production, hydric and ecostabilizing characteristics within all identified agricultural cultures. Permaculture edible forest that combines production and non-production properties (food, wood) of trees, shrubs and herbs represents even better combination of above mentioned characteristics. There is typical production of apples, plums, peers (*Pyrus*) and service trees (*Sorbus domestica*), which are often approximately one hundred and in some cases two or three hundred years old (*Fig. 5*). The orchards are degrading and are gradually overgrown which is similar also for meadows and pastures. According to mapping growing orchards covered 75.53 hectares. Successional transitions between orchards and forests have been documented.

Meadows and pastures are elements of the agricultural landscape with medium hydric potential. Meadows and pastures cover 579.13 hectares and they are known for their high rate of biodiversity with significant locations of orchid plants. On warmer hillsides, so called "stoklasové" (*"hundred ears" – author's free translation*) meadows are located. There are large numbers of grasses and flowering herbs such as clustered bellflower

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(*Campanula glomerata*), European columbine (*Aquilegia vulgaris*), green-winged orchid (*Orchis morio*), Hollub's ophrys (*Ophrys hollubyana*) and many others. Besides meadows, tufa springs on slopes are typical for this area. They are results of landslides, which are common in flysch sequences. These areas, with water-loving vegetation, can be distinguished from the surrounding meadows by cottongrass (*Eriophorum*) or red flowering thistle (*Cirsium rivulare*); furthermore we can find here marsch helleborine (*Epipactis palustris*) and western marsch orchid (*Dactylorhiza majalis*).

Figure 5. *Orchard on the Špaňom, which is managed by organic farm Pangaea since 2013.*

Elements with low and very low hydric potential are represented by unpaved forest roads (68.23 hectares), mapped in three categories: heavily eroded (20.93 hectares), medium eroded (43.31 hectares) and slightly eroded (3.19 hectares). With an average width of 4 m, total roads length in the study area is 170 km.

Wetland communities are important ecological components of the landscape. They are represented by forest and meadow springs, representing ecologically significant localities that can be found at 8.79 hectares of the study area. They act as important water collectors since they accumulate during the period of precipitation and gradually release a large amount of water during the period without precipitation (*Fig. 6*).

Figure 6. *Meadow spring of Blažejová and forests spring in Hubotica*

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The whole area is abundant on many animal species, from amphibians, through shellfish and birds to mammals.

Straightened and regulated streams are mainly in urban areas, where the intentions are to divert the water as quickly as possible (*Fig. 7*). Thus diverted water have the potential to slow down and partly to accumulate in natural streams, with wetlands and floodplain forests situated below settlements, therefore their qualitative and quantitative support can increase the hydric potential of the landscape.

Figure 7. *Elements of current landscape structure with very low (1 – forest road with high erosion devastation, 2 – regulated stream) and higher hydric potential (3 – natural stream)*

Agricultural activity in the study area is represented by grazing, mainly of cattle, less sheep, goats and horses. Other areas are covered by various mosaics of hay meadows and orchards. There is no exception to encounter more than a hundred years bearing fruit trees of rare old varieties.

The study area is a part of large protected area of bilateral Protected Landscape Area White Carpathian Mountains, where the second level of environmental protection is present. Ecologically valuable sites have been declared as small protected areas. A large part of the study area is included in the European nature protection sites of NATURA 2000, specifically in the Special Area of Conservation Hollubyho kopanice.

Potential retention

On the basis of the CN curve values, values for potential retention of the study area were calculated (*Figure 8*). According to the CN curves in the study area, very high and high hydric potential dominates (58.5 %), mainly in forests; medium and medium-low hydric potential covers 28.3 % of the territory, and low and very low hydric potential covers 13.3 % of the study area. The average value of CN curve is 78.

Areas with very low potential retention are landscape elements with impermeable surfaces (settlements, paved roads, waterways) and with elements of naturally very low retention (forest roads, clearings, arable lands) in combination with unfavourable soil properties (soil with very low infiltration capacity and without drainage). According to the calculation, potential retention on soils with very small infiltration capacity is in the forest 169.33 mm, in the orchard 71.44 mm, in the meadow 58.59 mm, and in the arable land 34.64 mm. In the forest road with medium erosion the potential retention is 22.09 mm.

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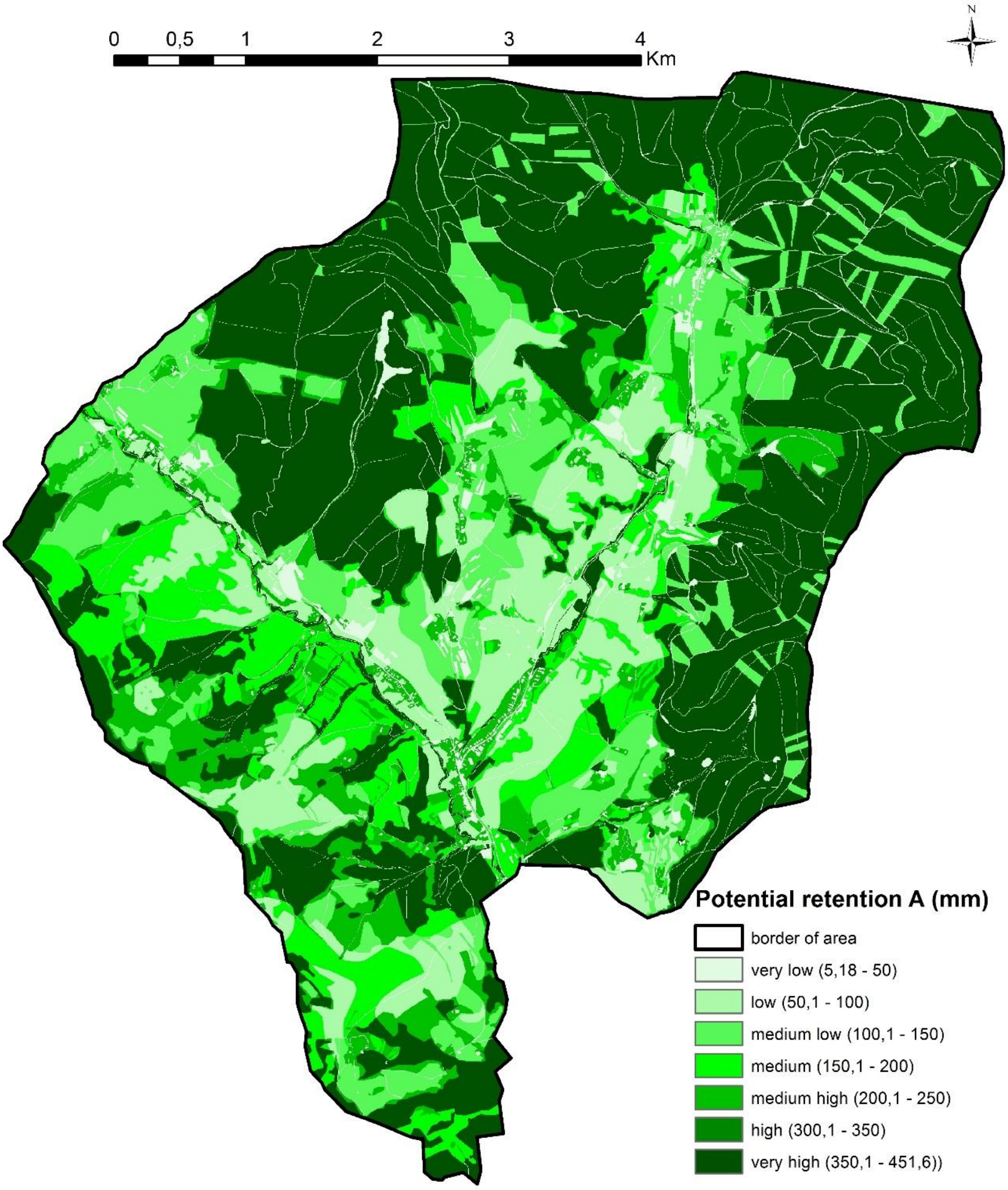

Figure 8. *Potential retention of study area*

Figure 9 shows the volume of surface runoff for the current landscape structure. The highest volume of surface runoff is naturally concentrated in the stream beds. From the confluence of Bošácka stream and Španie stream, which represent outflow of the study area, the model calculated surface runoff of 787 105.8 m^3 at proposed 120.2 mm precipitation. Significant increase of surface runoff is recorded in the clearings on steep slopes. The increased outflow was also recorded in localities with hydrologic soil group D (soils with very low infiltration capacity and without drainage) and hydrologic soil group C (soils with low infiltration ability with low drainage). These soils in the lower parts of slopes are used as arable land, pastures and meadows.

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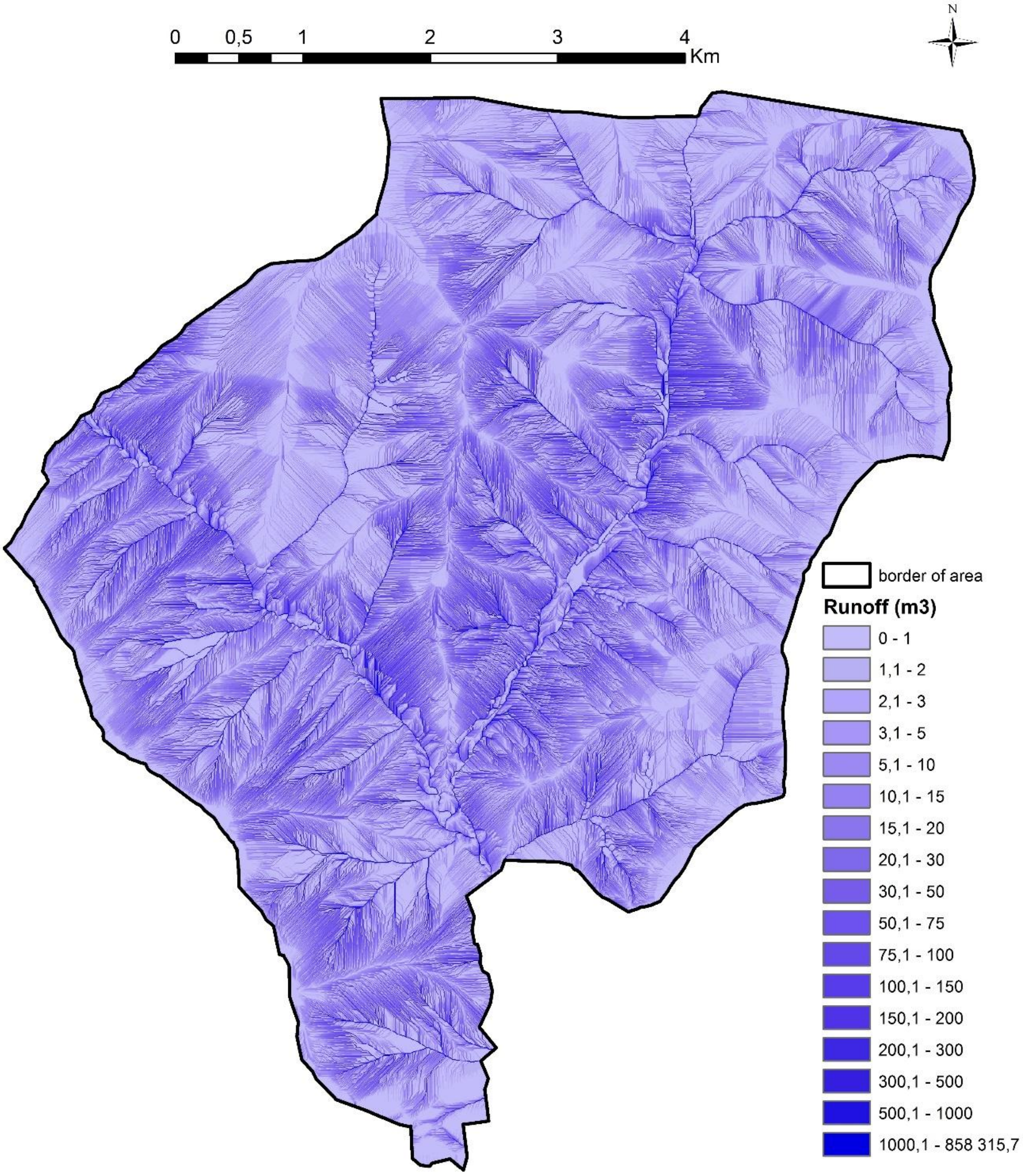

Figure 9. *Volume map of surface runoff in study area for current landscape structure*

Proposition of optimized landscape management

Based on both analyses and synthesis of here presented results, optimized landscape management in the study area should include following propositions: Economic activities should be excluded on localities with forest springs mapped during field works. Localities with non-forest springs should be excluded from grazing. It is recommended to exclude forestry activities also in a 10 m buffer zone around water courses and to create protecting buffers around them. To protect natural processes of the major hydrological basin with ecologically stable old beech forest, a natural reserve with area of 240 ha should be declared.

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Considering forest road network, at least 50% of present roads should be destroyed by disrupting compact surface with excavator and left to successional processes. The selection should be undertaken by professional forest managers. Remaining roads should be maintained in terms of technical standards (STN 73 6108) and should be accompanied by drainage channels which enable water to flow into surrounding vegetation. Tow of logged wood should be carried out in winter month when the ground is frozen and covered with snow. On steep slopes use of traditional forms of forest work with workhorses is recommended.

In terms of hydric functions, wetland Močarina in Predpolomská valley on Predpolomský stream is significant due to its location and size. As part of the intensification of agriculture the system of drainage channels was built and ash-alder alluvial forests were changed to arable land. In terms of increasing landscape retention and accumulation function, this wetland should be revitalized on the area of 22 hectares (*Fig. 10*). The recommendations are to abolish drainage channels system by burying pipes and leaving smaller ponds, and changing agricultural land use to flood protection use by restoring functional forest ecosystem instead of arable land and meadows.

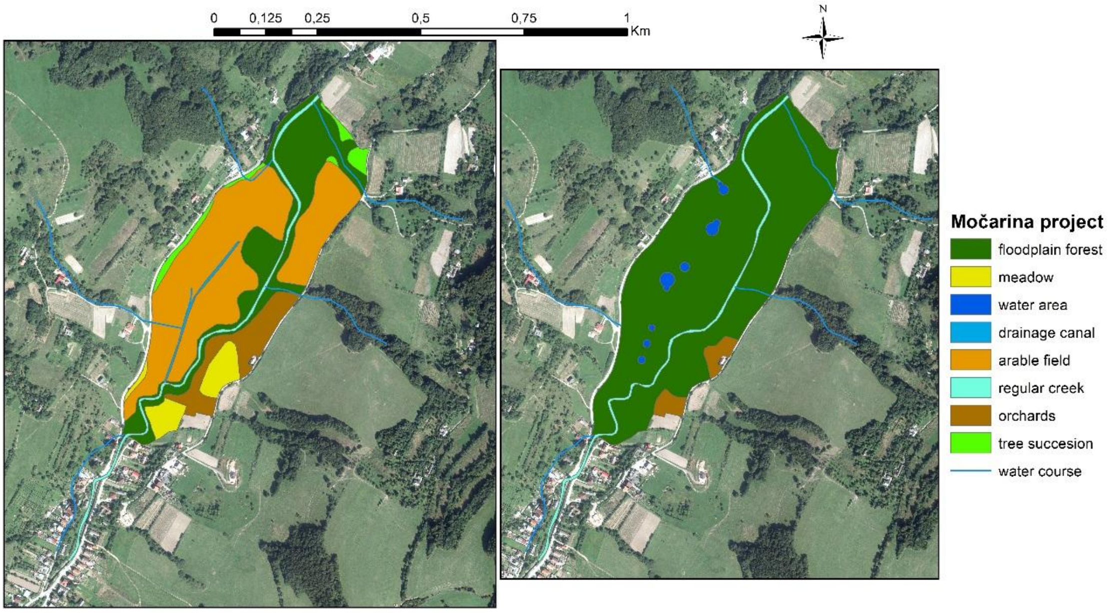

Figure 10. *Proposal for Močarina wetland restoration, changing agricultural land use to flood protection, by supporting viable ecosystem of floodplain forest*

The study area has quite well-preserved functional landscape structures on agricultural land, created by generations, which include hedgerows of fruit trees and shrubs planted along the contour lines. They divide meadows and pastures on the long slopes, creating a barrier for runoff and wind, and thus protecting agricultural land from water and wind erosion. Also existing network of riparian vegetation is in relatively good condition. To increase hydric functions of the landscape the existing structure of hedges as well as riparian vegetation should be supplemented with further landscape elements (for instance in the form of shrubs and fruit trees in a 6-10 wide strip along contour lines) which will divide long slopes in some localities where these slopes occur without existing landscape structures. Another measure for increasing hydrological

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conditions of arable land consists of planting a 2-3 m wide strip of riparian vegetation in the form of fast-growing energy crops (willow, hazel), which will be coppiced and will bring the economic effect, either in the form of biomass for energy production, or as rods used in basketry.

In localities on soils with very low infiltration capacity, the recommendations are to adjust their use in order to improve quality of soil by converting them into an edible forest. The concept of edible forest respects the climax stage of a locality and may lead to optimal spatial and age structure. Species suitable for this type of forest are sweet chestnut (*Castanea sativa*), common walnut (*Juglans regia*), common hazel (*Corylus avellana*), edible species of rowan (*Sorbus*) and many others. Successful example of functionality of this concept in the Central European conditions is sweet chestnut forest in the mountains of Tríbeč, where main trees form forest vegetation with optimal age and spatial structure.

From proposed measures, ecologically sustainable forest management has the most significant effect as it concerns a large part of the study area. All proposed measures have ecosystem character, thus achieving optimized landscape structure. The results of hydrological modelling show volume values of surface runoff in study basin (for the proposed 120 mm precipitation), which have been established for potential landscape structure representing the highest hydric potential (159 318 m^3), current landscape structure (858 315 m^3) and the proposed landscape structure (733 856 m^3). The fundamental difference between current landscape structure and proposed landscape structure is reflected in the increasing size of settlement with very low volume of surface runoff by 13.1 % (473 ha). By applying proposed measures, according to calculations of modelling software, the volume of surface runoff in study area would be reduced by 124 459 m^3 in the proposed 120 mm precipitation (*Fig. 11*).

Discussion and conclusions

Smiraglia et al. (2015) underline the importance of integrated methodologies based on mixed quantitative and narrative approaches to achieve a thorough comprehension of landscape changes. Application of LANDEP methodology provides among others possibilities for landscape-ecological optimization of hydric potential. Using the universal legend of landscape elements (Petrovič et al., 2009) and method of CN curves to calculate the volume of runoff (Gajdošík et al., 2005) landscape ecological optimization was implemented in the upper part Bošáčka basin in the central part of the White Carpathians. Typical feature of the study area is represented by dispersed settlements, which form mosaic of forests, orchards, meadows, pastures and wetlands. Košťál (2010) indicates that colourful meadows with rich diversity, which is typical for the Biele Karpaty Mts., suffered mainly after World War II, during the socialist collectivization. Elements with low hydric potential are typical for disturbed soil cover with sparse or even no vegetation.

Conversion of land to agriculture, mining, industrial, or residential uses significantly alters the hydrologic characteristics of the land surface and modifies pathways and rates of water flow (Bhaduri et al., 2000). Other negative factors for hydric potential include massive logging. According to Kantor and Šach (2002) erosion processes in clearings are not only the result of felled trees, but they are also an expression of poorly organized deployment and movement of heavy machinery and another human activities. An extreme negative impact of such activities on soil quality and thus hydric potential of

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the landscape can be seen especially on flysch bedrock, which was confirmed by various authors (Simon and Sucharda, 2004; Baláž et al., 2008). A suitable alternative is selective logging, taking into account the soil protection.

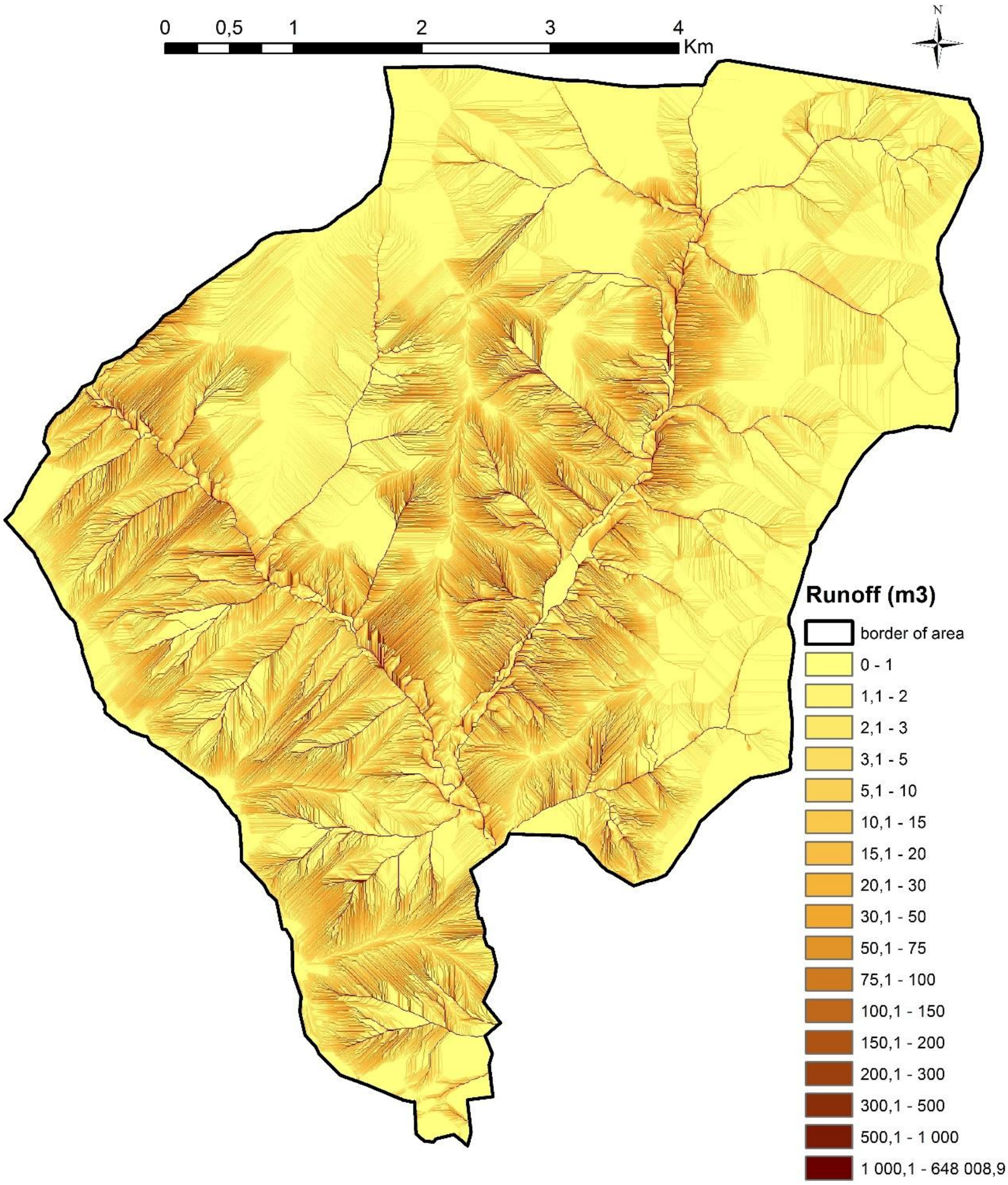

Figure 11. *Volume map of surface runoff for proposed (optimized) landscape structure*

Forest stands are an important factor in terms of their impact on the quantity and quality of water resources (Abildtrup, Garcia and Stenger, 2013; Erol and Randhir, 2013; Fiquepron, Garcia and Stenger, 2013; Lima et al., 2016). A significant problem during torrential rain in forested land is a dense network of logging roads. Paved surfaces in forest ecosystems concentrate surface runoff, increasing drainage density of

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basin and are source of 30 – 80 % sediment transported in the rivers (Hagans et al., 1986). Surface runoff on logging roads can reach up to 1300 time the surface runoff in the adult forest which means that almost all water that falls on logging roads flows away. 95 % of this water gets in streams (Midriak, 2004). Up to 99 % of soil loss in forest comes from unpaved roads and only small amount from the surface of undisturbed land in forests. Compacted linear surfaces are often directly constructed or eroded as deep cuts into the slope terrain, resulting into slope drainage and transformation of subsurface to surface runoff which consequently leads to speeding up outflow from the basin in the form of flash floods after torrential rains. Therefore, revitalization of forests roads and supporting the non-forest vegetation is necessary. Important are mainly riparian vegetation restoration near communication. Riparian ecosystems play a vital role in providing ecosystem services that include habitat support and protection of water quality (Randhir and Erol, 2013; Munteanu et al., 2014; Chen et al., 2016; Schroeder-Georgi et al., 2016).

Final step in LANDEP is proposing environmentally optimal localization of socio-economic activities in the landscape which leads towards harmonization of landscape ecological characteristics with its current and proposed use (Ružička, 2000). In the studied basin the volume of surface runoff as a representative of hydric function of the landscape is the main object of decision-making process in this final step. Proposed objectives are the elements of the current landscape structure, reflecting the society activities. The aim is therefore to harmonize land use with its hydric potential, leading to increase of the retention and accumulation functions which represent ecological quality of the landscape. Based on the analysis of localities with large volume of surface runoff for current landscape structure, optimized landscape structure was proposed. The main proposed measure is ecological forest management since forests represent optimal ecosystems of retention and accumulation of rainwater in the study area.

Other recommendations for landscape optimization in order to achieve increase in hydric functions of the study area include establishment of a protected area, restoration of a wetland, restoration of forest road network or planting edible forests in localities with soils with very little infiltration capacity and without drainage. Proposed optimization measures were implemented in the model of proposed landscape structure. This resulted in the change of land use layers entering calculation of CN curves and consequently in the change of the CB curves.

Analysis and hydric evaluation of the study area shows that foothills region has a relatively high hydric potential. The proposed measures are linked to existing landscape structure, where they increase the quality of existing hydric elements. They have character of non-technical solutions with maximum exploitation of the potential of ecosystem services, therefore they are economically undemanding. With their application, according to software modelling calculations, volume of surface runoff in study area can be significantly reduced. This represents an effective instrument of flood protection with ecosystem approach. It is essential, however, to implement them into integrated river basin management by all entities using the landscape.

Landscapes and watersheds are complex cultural biogeoclimatic systems that are not easily bounded, measured or understood by a single body of expertise. This makes it very challenging to locate and synthesize the best available science to identify what decision-makers need to know about landscape and watershed impacts of hydraulic fracturing. 'Landscape' is not a physical object as much as it is a spatial context for multiple natural processes and human activities (Quinn et al., 2015).

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In our paper we used methods as LANDEP and integrated landscape management, by means of which we tried to show the possibility of improving the water management. It is possible in areas where is enough water but its use can be rationalized by changes in land use. On the basis of our results, the synthesis of both methods confirms its suitability for such proposals to deal with the problems in landscape.

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YIELD AND QUALITY OF MYCORRHIZED PROCESSING TOMATO UNDER WATER SCARCITY

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Abstract. An open field trial was established to evaluate production, carotenoids, ascorbic acid, and soluble solids of processing tomato inoculated with arbuscular mycorrhizal fungi either at sowing, and re-inoculated at transplanting compared to non-treated plants under different soil moisture conditions. Depending on plant water requirement, all treatments induced to three levels of water supply: Full water supply, half water supply, and no water supply by adjusting the water amount. Regardless of mycorrhizal inoculation time and dosage, plants under no water supply conditions faced severe stress and showed no enhancement in growth, yield, and water use efficiency. Mycorrhizal re-inoculation significantly increased the marketable fruit yield, the total biomass, the water use efficiency, and leaf water potential under water shortage conditions in half water supply compared to at sowing inoculated mycorrhizal plants, and non-treated plants as well, suggesting that field mycorrhizal re-inoculation enhances colonized plant water stress avoidance. A strong negative correlation was observed between yield and soluble solid content. Concisely, mycorrhizal re-inoculation was more effective than inoculation at sowing.
Keywords: *Solanum lycopersicum* M., *biofertilizer, carotenoids, water use efficiency, different water regimes*

Introduction

Processing tomato is one of the most important vegetable crops, its production reached 41,374 Mt worldwide, 17,424 Mt in Mediterranean area, and 10,759 Mt in EU (WPTC, 2016). Its high content of carotenes, made tomato an important component in human diet. Humans should take carotenes, since they are ubiquitous organic molecules, which cannot be produces by human body. These group of pigments are accumulate during the ripening process (Helyes et al., 2006; Pék et al., 2010). In Hungary Helyes and coworkers (2002) measured total carotenes in range of 39 to 171 mg/ kg^{-1} in 16 different cultivars of tomatoes. Lycopene and β-carotene are the most significant compounds belonging to carotenoids. Lycopene forms about 83% of all carotenoids according to (Lopez et al., 2001), while the later gives 1-3% of the total carotenoids including the orange tone. β-carotene is the main source of vitamin A, therefor it is very important for the human health (Francis, 2002).

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In processing tomato high soluble solid content is very important, not only because it results in higher tomato processed into paste, but also needs lower energy cost during the processing (Barrios-Masias and Jackson, 2014). Refractive index is considered the most common tool to estimate the soluble solid content, and its values are reported as percentage or °Brix (Johnstone et al., 2005).

Processing tomato consumes large amounts of water (Patanè et al., 2011), thus demanding irrigation throughout the growing season ranging between 400 to 600 mm depending on climatic conditions (Rana et al., 2000), and this restricts the production in most parts of Europe.

Mycorrhizal fungi are the most vital components within the microbial community, interfacing between the soil and photosynthetic aerial plant parts through their association with plant roots. They are able to form symbiotic relationship with more than 80% of terrestrial plant families (Smith and Read, 2008) including many land and industrial crops. The mutual symbiosis relationship leads to enhanced plant capability to overcome many abiotic and biotic stressors through enhancing plant nutrient uptake, while in turn the fungus gains plant photosynthates and an ecological niche (Daei et al., 2009; Zhang et al., 2014).

Among the environmental factors, drought stress is considered one of the most fateful abiotic factors that limits agricultural production worldwide (Farooq et al., 2009). Many studies concluded that this symbiosis association protects host plants against the negative impact of drought (Augé, 2001; Smith and Read, 2008; Ruiz-Sánchez et al., 2010). This symbiosis relationship enhances growth and yield in processing tomato (Candido et al., 2015), and may affect tomato quality as well (Ordookhani et al., 2010).

The main mechanisms of arbuscular mycorrhizal symbiosis to alleviate drought stress are the direct and indirect hyphal contribution to the total water uptake which was estimated to be about 20% (Ruth et al., 2011). According to Endresz et al. (2015), the presence of endogenous arbuscular mycorrhizae fungi (AMF) in the soil can ameliorate the influence of water deficit for resident plant species, but not for new invasive species. Despite the natural presence of AMF in most of the agricultural lands, where members of the Glomeraceae family are dominant (Magurno et al., 2015), field studies illustrated that further inoculation with AMF can enhance both crop productivity and colonization rate (Lekberg and Koide, 2005).

The improvement of stress tolerance is often related to enhanced contents of antioxidant compounds in plants (Hasanuzzaman et al., 2012). Mycorrhized tomatoes increased lycopene and β-carotene in fruits (Ulrichs et al., 2008); according to Pék and coworkers (2014) abiotic factors such as temperature, light, and water supply affect natural antioxidants composition.

This study is to investigate effects of AMF commercial bio-inoculants on processing tomato UNO ROSSO F1, grown under three different water supplies. Field effectiveness of both inoculation at sowing and re-inoculation during transplantation of mycorrhizae on total biomass, yield, fruit nutritional content, agronomical water use efficiency, and leaf water potential were evaluated.

Materials and Methods

Experimental conditions

This experiment was conducted in processing tomato UNO ROSSO F1 (United Genetics Seeds Co. CA, USA) in 2015 on the Institute of Horticulture experimental farm, Szent Istvan University (SIU) in Gödöllő, Hungary (47°59′ N, 19°35′ E). The experimental soil was brown forest soil, sandy loam in texture consists of 69% sand, 22% silt, and 9% clay,

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1.57 g cm^{-3} bulk density, 19% field capacity, neutral in pH, free from salinity (0.16 dS m^{-1}) and low in organic carbon, NO_3^- -N (5 g kg^{-1}), P_2O_2 (15 g kg^{-1}), K_2O (35 g kg^{-1}); the water table was below 5 m, which could not influence the water turnover.

Tomato seedlings have been propagated on 13^{th} of April in a greenhouse using special horticulture substrate (Klasmann TS3) and inoculated with mycorrhizae (AM+) or not (Control). The mycorrhizal fungi corresponding to a commercial product Symbivit® (mixture of *G. mosseae*, *G. etunicatum*, *G. claroideum*, *G. microaggregatum*, *G. geosporum, and R. irregularis*) produced by Symbiom Ltd. (Czech Republic, www.symbiom.cz) was applied at a dosage of 25 g/ L^{-1} substrate. After 4 weeks of growth the AM+ and Control- seedlings were bedded out on 11^{th} of May. During transplantation to the field one-half of the inoculated seedlings were re-inoculated (AM++) by adding (20 g/ $plant^{-1}$) of Symbivit inoculum into the planting hole and seedlings were planted immediately.

The experimental design was randomized block with four replications per treatment. Seedlings were arranged in double (twin) rows with 1.2 m and 0.4 m inter rows distance and 0.2 m between plants. Three watering regimes: Full water supply (WS_{100}), half water supply (WS_{50}), and no water supply (WS_0), were implemented through a drip irrigation system. Water amount was controlled according Pék et al. (2014) depending on air temperature (daily water demand = daily average temperature x 0.2). Weather forecasts from the National Metrological institute were used to calculate the plants daily water demand depending on the daily average air temperature and precipitation. Plant nutrition requirements and plant protection were regulated according to Helyes and Varga (1994) throughout the growing season. Fruits and total biomass were harvested within two weeks first the WS_0 tomatoes on 11^{th} of August, followed by both WS_{50}, and WS_{100} tomatoes on 25^{th} of August.

Soil water content assessment

Volumetric soil water content was taken by digital soil moisture meter PT1 (Kapacitiv Kkt., Budapest, Hungary) at six different soil depths (5, 10, 15, 20, 25, and 30 cm) just prior to watering.

Root colonization estimation

Samples for estimating root colonization five plants were dug out (with a soil core of 25 × 25 × 25 cm) randomly chosen from the repetitive plots of the same treatment. A representative subsample of the roots regarding different treatments was cut to 10 mm pieces and five randomly selected pieces from each sample were subjected to Trypan Blue staining (Phillips and Hayman, 1970). Internal fungal structures (hyphae, arbuscules) were examined under a stereomicroscope at × 100 magnification and the percentage of root length colonized was calculated using the gridline intersect method (Giovannetti and Mosse, 1980). Mycorrhizal colonization was measured only at harvesting therefore the interpretation of colonization level should be put on more guard. Moreover the applied staining method was not able to make differences between active and non-active mycorrhizal part.

Water use efficiency and mycorrhizal dependency calculation

Water use efficiency (WUE) was calculated depending on total fresh biomass (WUE = kg biomass per hectare/ m^{-3} water consumed per hectare), and mycorrhizal

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dependency (MD %) was estimated after Planchette et al. (1983) depending on marketable yield by [MD % = marketable yield (AM+) – marketable yield (Control)/ marketable yield (AM+)].

Leaf water potential measurement

Pressure bomb (PMS Instruments Co., Corvallis, OR, USA) was used to determine leaf water potential (ψ_{leaf}) at midday by cutting a newly mature leaf from each plant, four replication per treatment and for three consecutive weeks.

Analysis of carotenoid components and vitamin C

Extraction of carotenoids and ascorbic acid

Carotenoids extraction was done according to the method of Daood et al. (2014). Five grams of well homogenized tomato has been taken in triplicate followed by disintegrated in a crucible mortar in the presence of quartz sand. The water was then removed by adding 25 ml of methanol along with the repeat disintegration of the aggregating bulk. After the addition of 70 ml of a 6:1 dichloroethanemethanol solution, the mixture was transferred quantitatively into 100 ml conical flask. Moreover, the mixture was shaken up to 15 min by mechanical shaker. Few drops of double distilled water were added to separate the two phases. The pigment containing lower layer was separated in a separating funnel, dried over anhydrous sodium sulphate and passed into a round-bottom flask. The organic solvent was evaporated with vacuum by rotary evaporator (IKA® RV10, Sigma-Aldrich Ltd., Budapest, Hungary) at maximum 40 °C and the residues were re-dissolved in 5 ml of HPLC grade acetone.

Ascorbic acid was extracted from 5 grams of well homogenized tomato by crushing in a crucible mortar and shaking for 15 min with 3% metaphosphoric acid solution. The mixture was then filtered through a filter paper and purified by a 45um nylon syringe filter before injection on to the HPLC column.

HPLC equipment and conditions

A Chromaster liquid chromatograph in (Hitachi, japan) consisting of a Model 5110 Gradient pump, a Model 5210 auto sampler and a Model 5430 photodiode array detector was used. Operation and data processing were performed by EZChroma Elite software.

The separation of carotenoids was done on cross-linked C-18, 3µm, 150 x 4.6 mm column using gradient elution of water in acetone as described by Daood et al. (2014). The column effluents were detected at their maximum absorption wavelength for identification and quantification. The retention properties and spectral characteristics of the detected peaks were compared with some available standard materials like lycopene, β-carotene and zeaxanthin (Sigma-Aldrich Ltd., Budapest, Hungary). In case of absence of standards, the tentative identification was done based on comparison of retention times and spectral characteristics with literature data. Additionally, the compounds were quantified as either lycopene- or β- carotene-equivalent based on their spectral characteristics.

As for ascorbic acid, separation was performed on C-18, 240 x 4.6 mm, 5um column under ion-pair chromatographic conditions optimized and validated by Daood et al.,

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(1994). Ascorbic acid was identified using standard material (Sigma-Alrrich, Budapest), from which stock and working solutions were prepared for getting the calibration curve.

Brix determination

Digital Refractometer Krüss DR201-95 (Küss Optronic, Hamburg, Germany) was used to estimate the ^{o}Brix.

Statistical analysis

Statistical analyses were performed using the software IBM SPSS Statistics for Windows, Version 22.0. (IBM Hungary, Budapest, Hungary). Analysis of variances was done by two way ANOVA to separate the mycorrhizal effect, water supply effect, and their interaction. Tukey's tests were performed for every possible pairwise comparison.

Results

Precipitation occasions and their amounts were relatively well distributed during the first two months of the growing period, and plants received the first rain directly after the transplantation, thus the first watering was started in the first week of June.

Irrigation supply resulted in 426.3 mm and 306.3 mm totally for both WS_{100}- and WS_{50}-regimes respectively including 186.3 mm of rain, while WS_0 plants received only 186.3 mm of rainfall (*Fig. 1*). Volumetric soil water contents was ranging between 0.14-0.17, 0.11-0.14, and 0.07-0.10 during the growing season, corresponding to 73-89%, 58-73%, and 37-52% of field capacity in WS_{100}, WS_{50}, and WS_0 blocks respectively.

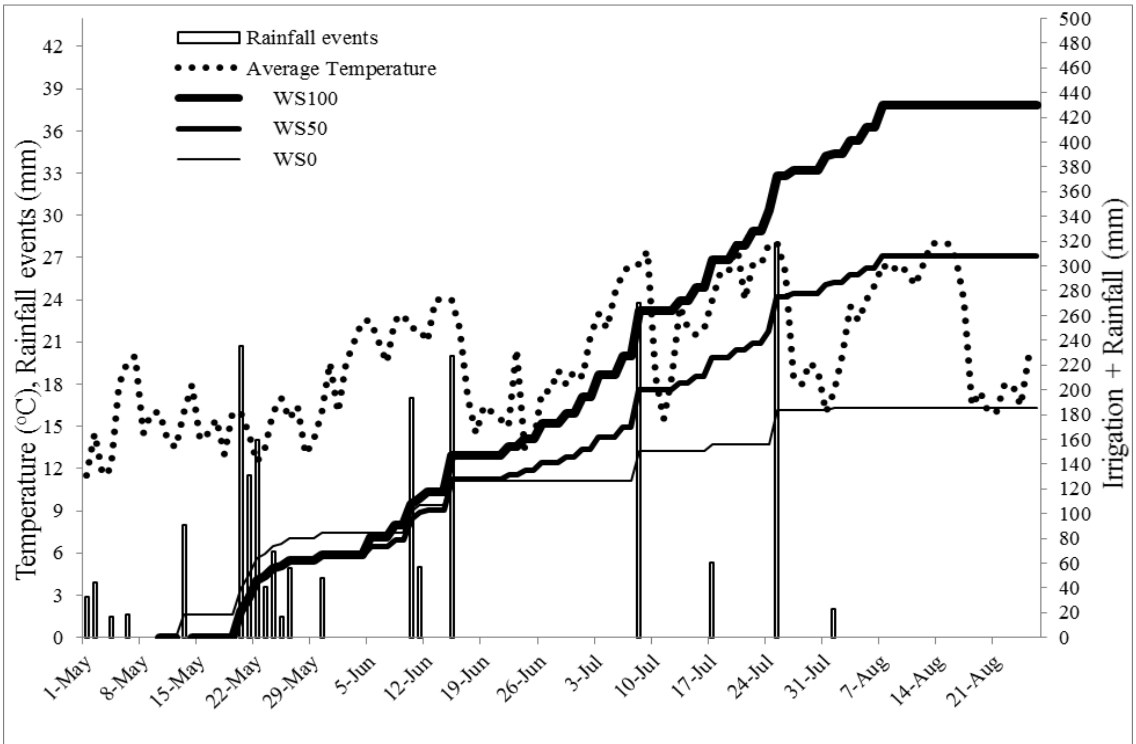

Figure 1. *Average daily temperature, precipitation, and accumulative irrigation amount in 2015.*

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The last 3 weeks of no rain caused severe stress to all mycorrhized and non-inoculated plants in unirrigated WS_0 treatments (*Fig. 1*), therefore no enhancements in total biomass and water use efficiency were observed due to mycorrhizal inoculation, although root colonization rates in both AM+ and AM++ significantly increased (*Table 1*). AM++ plants improved the total biomass and WUE by 73% and 42% compared to Control and AM+ plants under WS_{50}, while mycorrhizal effect lessened under full water supply WS_{100}. The same trend was also observed in mycorrhizal dependency MD %, which was pronounced clearly in AM++ treatment especially under half water supply WS_{50} regime (*Table 1).*

Table 1. *Water Use Efficiency (WUE), Mycorrhizal Dependency (MD %). Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Capital letters represent mycorrhizal inoculation effect, small letters represent water supply effect.*

Water Supply	Treatments	Total Biomass t ha^{-1}	WUE kg m^{-3}	Root Colonization %	MD %
No water supply	Control	$33.5^{Aa} \pm 2.2$	$18.0^{Aa} \pm 2.4$	$54^{Aa} \pm 6$	
	AM+	$32.6^{Aa} \pm 0.4$	$17.5^{Aa} \pm 0.4$	$67^{Ba} \pm 8$	3.14
	AM++	$34.6^{Aa} \pm 1.4$	$18.6^{Aa} \pm 0.4$	$70^{Ba} \pm 6$	1.57
Half water supply	Control	$74.3^{Ab} \pm 1.4$	$24.3^{Ab} \pm 0.9$	$49^{Aa} \pm 6$	
	AM+	$91.2^{Bb} \pm 1.8$	$29.8^{Bc} \pm 1.2$	$64^{Ba} \pm 9$	11.66
	AM++	$128.9^{Cc} \pm 1.5$	$42.1^{Cc} \pm 1.0$	$63^{Ba} \pm 8$	41.49
Full water supply	Control	$92.6^{Bc} \pm 1.2$	$21.7^{Bc} \pm 0.6$	$55^{Aa} \pm 6$	
	AM+	$74.3^{Ac} \pm 1.5$	$17.4^{Ab} \pm 0.7$	$73^{Ba} \pm 6$	-18.68
	AM++	$100.0^{Cb} \pm 2.6$	$23.5^{Cb} \pm 1.2$	$71^{Ba} \pm 6$	9.24
Significant of Source of variation		(ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)			
Mycorrhizae (M)		***	***	***	
Water Supply (WS)		***	***	ns	
M * WS		***	***	ns	

Re-inoculated plants (AM++) at transplantation gave higher yield compared to those inoculated at sowing (AM+) or non-inoculated (Control) . AM++ plants under WS_{50} level gave the highest marketable yield reaching 96.47 t ha^{-1} exceeding both control and AM+ treatments by 71% and 51% respectively, while mycorrhization effect was slowed down under WS_{100} regime and AM++ plant gave higher marketable fruits only by 10% and 31% compared to control and AM+ plants with no remarkable differences in WS_0 (*Table 2*).

Table 2. *Fruit and antioxidant production per unit area. Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4). Capital letters represent mycorrhizal inoculation effect, small letters represent water supply effect.*

Water Supply	Treatments	Marketable t ha^{-1}	Total Carotene kg ha^{-1}	Lycopene kg ha^{-1}	β-Carotene g ha^{-1}	Ascorbic Acid kg ha^{-1}
No water supply	Control	$14.69^{Aa} \pm 2.6$	$2.00^{Ba} \pm 0.3$	$1.47^{Ba} \pm 0.24$	$38.5^{Ba} \pm 6.5$	$4.90^{Aa} \pm 1.3$
	AM+	$15.16^{Aa} \pm 1.4$	$1.15^{Aa} \pm 0.1$	$0.87^{Aa} \pm 0.08$	$22.2^{Aa} \pm 3.5$	$4.73^{Aa} \pm 0.8$
	AM++	$14.92^{Aa} \pm 2.0$	$2.18^{Ba} \pm 0.3$	$1.68^{Ba} \pm 0.22$	$48.7^{Ba} \pm 9.9$	$5.41^{Aa} \pm 0.9$
Half water supply	Control	$56.45^{Ab} \pm 2.3$	$6.01^{Ab} \pm 0.7$	$4.07^{Ab} \pm 0.49$	$126.0^{Ab} \pm 25$	$16.1^{Ab} \pm 1.6$
	AM+	$63.91^{Ab} \pm 6.1$	$7.64^{Ac} \pm 1.4$	$5.84^{Bc} \pm 1.14$	$161.2^{Ab} \pm 31$	$18.5^{Ab} \pm 2.4$
	AM++	$96.47^{Bc} \pm 6.0$	$8.71^{Bc} \pm 0.6$	$6.48^{Bc} \pm 0.50$	$182.2^{Bb} \pm 19$	$29.4^{Bc} \pm 1.9$

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Full water supply	Control	$68.41^{Bc} \pm 3.7$	$6.46^{Ab} \pm 1.4$	$4.53^{Ab} \pm 0.62$	$165.3^{Ab} \pm 28$	$18.7^{Ab} \pm 1.6$
	AM+	$57.64^{Ab} \pm$	$5.40^{Ab} \pm 0.8$	$3.89^{Ab} \pm 0.40$	$172.3^{Ab} \pm 28$	$16.7^{Ab} \pm 1.9$
	AM++	$75.38^{Bb} \pm 3.4$	$6.05^{Ab} \pm 0.4$	$3.70^{Ab} \pm 0.24$	$223.0^{Ab} \pm 52$	$20.5^{Bb} \pm 1.5$
Significant of Source of variation		(ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)				
Mycorrhizae (M)		***	*	*	**	***
Water Supply (WS)		***	***	***	***	***
M * WS		***	**	***	ns	***

Re-inoculated plants (AM++) gave not only the highest yield under WS_{50}, but also accumulated higher ascorbic acid, while irrigation influenced negatively the carotenoids concentration (*Table 3*), but higher yield overcame the concentration loss by higher production of antioxidants per unit area (*Table 2*).

Table 3. *Antioxidants concentrations (mg kg^{-1}). Means with same letters are not significantly different at (P<0.05) as determined by Tukey's HSD test (Mean ± SD, n=4).Capital letters represent mycorrhizal inoculstion effect, small letters represent water supply effect.*

Water Supply	Treatments	Total Carotene	Lycopene	β-Carotene	Ascorbic Acid
No water supply	Control	$136.3^{Bb} \pm 1.3$	$100.1^{Bb} \pm 2$	$2.63^{Ba} \pm .22$	$330^{Ab} \pm 30$
	AM+	$76.01^{Aa} \pm 5.2$	$57.4^{Aa} \pm 5$	$1.46^{Aa} \pm .14$	$311^{Aa} \pm 32$
	AM++	$146.5^{Bb} \pm 15$	$113.4^{Bc} \pm 12$	$3.24^{Cb} \pm .36$	$361^{Ab} \pm 12$
Half water supply	Control	$106.3^{Ba} \pm 7.7$	$72.0^{Ba} \pm 6$	$2.23^{Aa} \pm .42$	$286^{Aa} \pm 26$
	AM+	$119.5^{Bb} \pm 19$	$91.3^{Bb} \pm 15$	$2.50^{Bb} \pm .26$	$293^{Aa} \pm 58$
	AM++	$90.75^{Aa} \pm 11$	$67.5^{Ab} \pm 9$	$1.89^{Aa} \pm .88$	$304^{Aa} \pm 07$
Full water supply	Control	$94.27^{Aa} \pm 19$	$66.1^{Ba} \pm 7$	$2.42^{Aa} \pm .30$	$273^{Aa} \pm 14$
	AM+	$93.83^{Aa} \pm 6.3$	$67.7^{Ba} \pm 7$	$2.32^{Ab} \pm .50$	$289^{Aa} \pm 10$
	AM++	$80.29^{Aa} \pm 4.6$	$49.0^{Aa} \pm 1$	$2.70^{Ab} \pm .76$	$273^{Aa} \pm 31$
Significant of Source of variation		(ns= not significant, * P≤0.05, ** P≤0.01, *** P≤0.001)			
Mycorrhizae (M)		**	Ns	ns	ns
Water Supply (WS)		***	***	***	***
M * WS		***	***	***	ns

Despite differences between treatments within the same water supply regime, but total carotenoids and lycopene concentrations were decreased as much as water supply was increased. In Control plants, total carotene decreased by 22 and 31% in both WS_{50} and WS_{100} compared to WS_0, and similar losses was observed for lycopene. Decreases in both total carotene and lycopene contents a long water supply increase were even more in AM+ and AM++ (*Table 3*). Remarkable increase in β-Carotene content was measured in AM++ fruits under both WS_0 and W_{100} but not under WS_{50}. Fruits contained more ascorbic acid only in unirrigated WS_0 plants, while a slight improve was found in AM++ in half water supply without reaching significant levels (*Table 3*).

The soluble solid content (ºBrix) showed a strong adverse relationship with the yield.plants with no water supply WS_0 were able to support only about 20% of the potential yield, thus the highest ºBrix was obtained about 8, regardless of mycorrhizal inoculation. Under both WS_{50} and WS_{100}, a reduction of one unit of ºBrix was observed in the range of 50 to 100 t ha^{-1} of marketable fruit. Although the concentration of soluble solids decreased, yield increase compensated ºBrix loses as a result of the mass quantity of the soluble solids per area (t h^{-1}). Soluble solid production illustrated a strong positive linear correlation with the marketable yield (*Fig. 2*).

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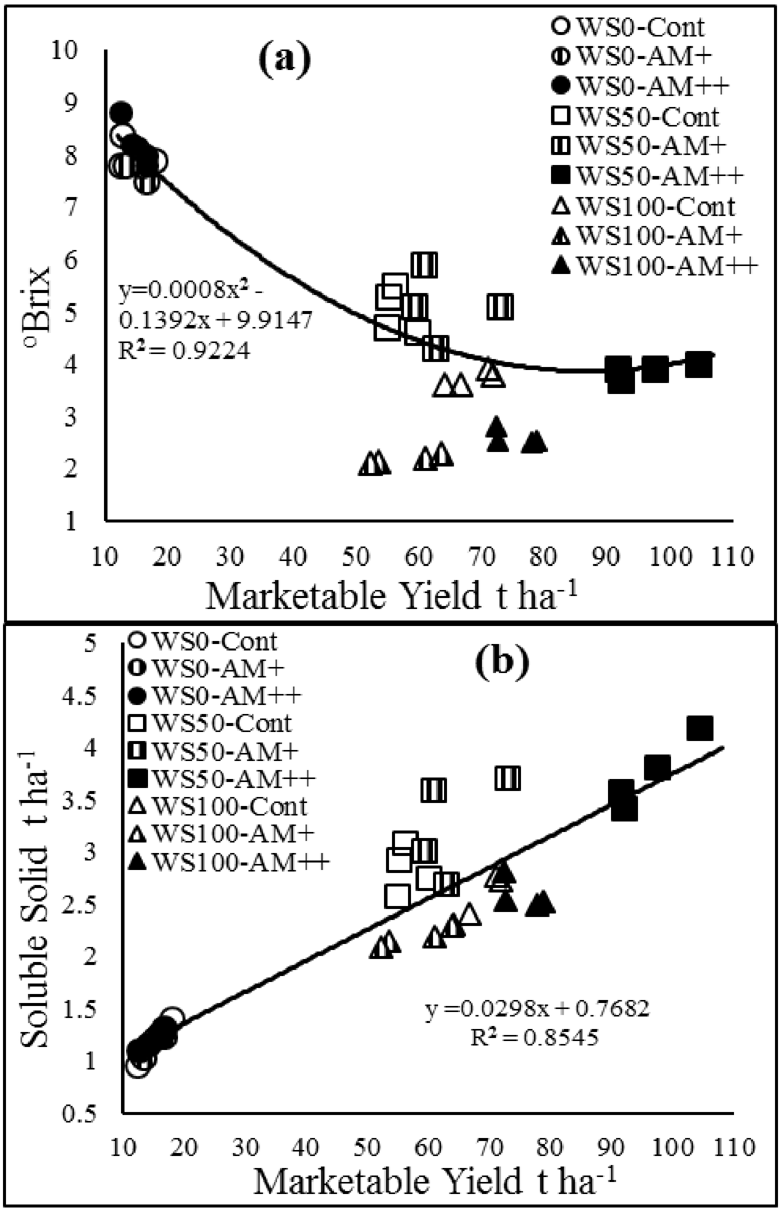

Figure 2. *Yield impact on (a) soluble solid (ºBrix) content, and (b) soluble solid production.*

Along gradients of decreasing water supply, the leaf water potential (ψ_{leaf}) decreased too (the more negative the ψ value, the drier or more water-stressed is the plant), from (-0.91 MPa) in WS_{100} to (-1.06 MPa) in WS_{50} and (-1.55 MPa) in WS_0 in control plants, while re-inoculated AM++ treatments enhanced and increased the ψ_{leaf} significantly under all water supply intensities with the best interaction under WS_{50} (-0.87 MPa). This trend was less pronounced in AM+ treatments. Moreover a strong positive linear correlation (R^2 = 0.73) was observed between the ψ_{leaf} and total biomass (*Fig. 3*).

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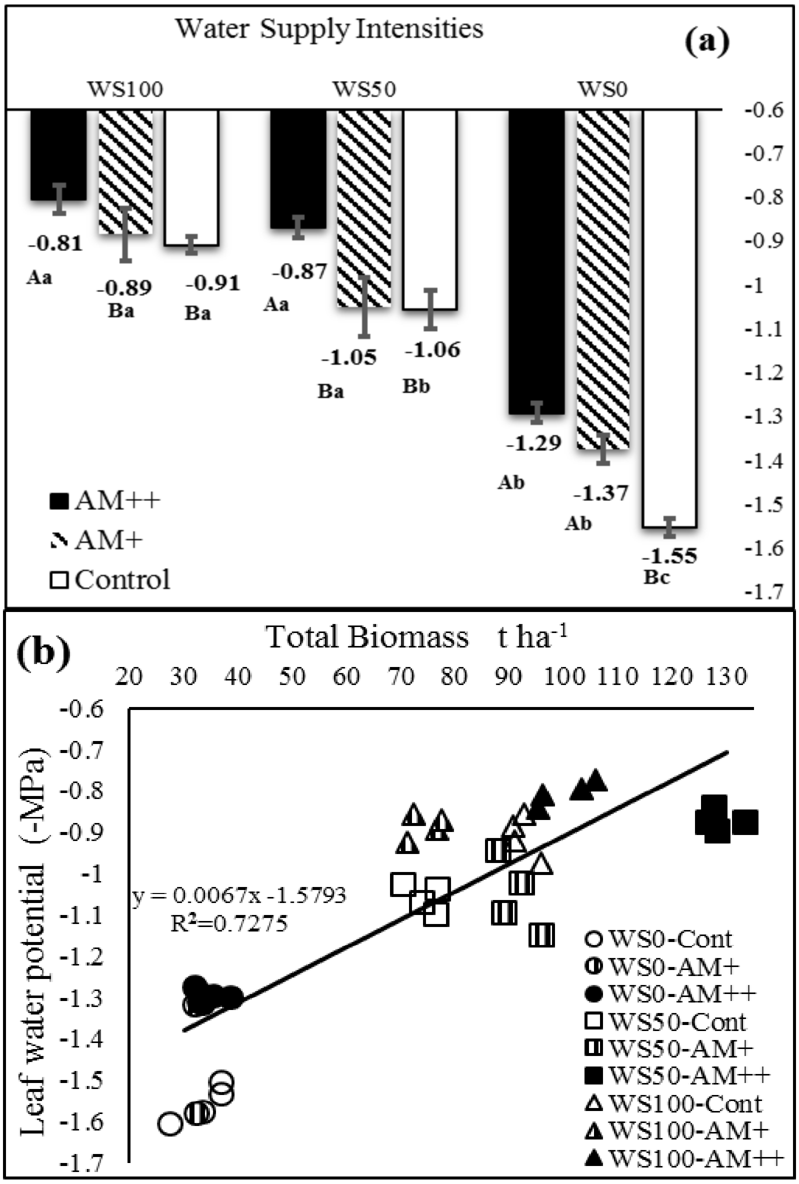

Figure 3. *(a) Leaf water potential, (b) Total biomass and leaf water potential relationship. Vertical bars represent standard deviation, capital letters represent mycorrhizal inoculation effect, and small letters represent water supply effect.*

Discussion

Worldwide, field crop production faces water stress that limits crops productivity, and mycorrhizal symbiosis is considered as a key component backing up host plants to overcome water lack stress as it is addressed in numerous of studies (Augé, 2001; Smith and Read, 2008; Ruiz-Sanchez et al., 2010; Candido et al., 2015). In this field-based trial, mycorrhizal re-inoculation, boosted yield, and enhanced growth and water use efficiency under both half- and full-water supply regimes compared to non-inoculated and at sowing inoculated plants as it was also found by others (Di Cesare et al., 2012).

Despite, the relatively high colonization rate by the endogenous mycorrhizal fungi, control tomato plants showed no enhancement neither in growth nor physiologically compared to inoculated plants (*Table 1*). This may related to mycorrhizal effectivity,

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which affects by the genotype combinations of host plant and the mycorrhizae, and the fact of functional differences of AMF: different fungal species are different in their effectiveness to enhance plant water uptake from the soil, as it was reviewed from several previous studies (Baum et al., 2015). Regardless of water supply levels, both inoculation at sowing and re-inoculation with the commercial Symbivit significantly increased the rate of root colonization, moreover the colonization rate was not affected by water stress (*Table 1*), which also found in previous studies as summarized by Augé (2001). Thus resulted in higher total biomass under half water supply condition and this was in agreement with other reports that additional mycorrhizal inoculations can improve growth, and colonization of field crops (Lekberg et al., 2005; Ortas et al., 2013).

The great increase in water use efficiency in inoculated plants compared to non-inoculated under half water supply (*Table 1*) is a definite prove, that mycorrhizal inoculation increased the ability of the root to increase water uptake from the soil (Ruth et al., 2011). Enhancing the water use efficiency by AM inoculation was also found in sweet pepper (Jezdinsky et al., 2012), and in tomato (Bowles et al., 2016). Moreover the higher leaf water potential in inoculated plants (*Fig. 3*) also supports this assumption.

Leaf water potential is the most important index of plant water status showing its potential to resist drought through better water uptake or better hydration. We recorded higher (less negative) leaf water potential in mycorrhizae colonized plants under half water supply (*Fig. 3*), supporting a study, illustrated higher leaf water potential in host plants colonized by arbuscular mycorrhizae fungi (Porcel and Ruiz-Lozano, 2004), and contradicting many previous studies surveyed by Augé (2001).

Volumetric soil water content has been in rage of 0.11-0.14 corresponding to 37-52% of the field capacity throughout the growing season, resulted in partial soil drying in half water supply zone. Moreover the enhancement in growth, and water use efficiency due mycorrhizal inoculation was more pronounced under half water supply, providing field-based evidence that AMF was more effective under moderate dried soil conditions in half water supply compared to both severe dried soil in none irrigated and optimum soil moisture conditions under full water supply. More effective phosphorus absorption by mycorrhized roots from dry soil was also evidenced by Neumann and George (2005) in a compartmented pot system.

In this field trial, AM re-inoculation was the most potential in enhancing marketable fruit yield in WS_{50}, with increases of about 71% compared to non-inoculated, and about 51% compared to inoculation at sowing (*Table 2*), exceeding that obtained by Candido et al. (2015), who used a single inoculum *G. mosseae* GP11, and recorded an increase of about 11%, and also the 25% yield increase by Bowles et at. (2016) under field conditions.

In general WS_0 fruits contained relatively higher antioxidants and ascorbic acid concentrations compared to WS_{50} and WS_{100} (*Table 3*); this could be because of the cooler period (*Fig. 1*) at the end of July prior to the harvest, since the rain-fed tomatoes were harvested two weeks earlier compared to $WS_{\mathbf{50}}$- and $WS_{\mathbf{100}}$-tomatoes and this is in agreement with the explanation of Dumas et al. (2003), and the fact that abiotic factors such as temperature, light, and water supply affect natural antioxidants composition in tomatoes (Pék, et al., 2014) or in plants in general (Hasanuzzaman et al., 2012).

During the ripening stage high temperature affected lycopene formation negatively, this also affected the total carotenoids, since lycopene forms most of carotenoids. No changes in total carotene, lycopene, β-Carotene and ascorbic acid contents were found in full water supply due to mycorrhization, expect a slight loos for lycopene in AM++,

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while higher total carotene, lycopene, and β-Carotene levels was observed in AM+ fruits under half water supply (*Table 3*). This increase in antioxidant compound in fruit of mycorrhized plants could be due to enhanced growth and nutrient acquisition (Ulrichs et al., 2008), but we could not meet both high yield accompanied with high nutrient content.

Conclusion

Results of this experiment support field-based evidence that arbuscular mycorrhizae fungal inoculation with the commercial product Symbivit can be an integrated application of processing tomato production alleviating water deficit stress as well as improving processing tomato cultivation by increasing its yield with respect to the quality. AM re-inoculation at transplanting is more effective than inoculation at sowing only, but economical aspect should be considered, since more inoculum is required. Under actual agroecosystem conditions many biological and environmental factors are interacting, therefore optimizing AM fungi application is required to reach promising results.

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THE FLORISTIC COMPOSITION AND BIOLOGICAL SPECTRUM OF VEGETATION IN THE MEYMEH REGION OF NORTHERN ISFAHAN PROVINCE, IRAN

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Abstract. This research details the flora of Meymeh, a region situated in the province of Isfahan in the central plateau of Iran and entirely within the Irano-Turanian phytogeographical region. In total, 164 species of flora belonging to 32 families and 108 genera were surveyed and identified in several field trips during the study period from 2013 to 2014. Based on the number of species, Asteraceae (30 species, 18.3%), Poaceae (20 species, 12.2%), and Papilionaceae (19 species, 11.6%) were the most important families. The largest genera were *Astragalus* (15 species), *Cousinia* (6 species), *Acanthophyllum*, and *Centaurea* (each with 5 species). In this study, the life-form spectra were classified on the basis of Raunkiaer's system. Then, the χ^2 test and correlation analysis were used to compare the biological spectrum with Raunkiaer's normal spectrum and with those in other floristic studies conducted in the Irano-Turanian growth zone. The results showed that the life-form spectrum in the present study was characteristic of a cool steppe climate region and dominated by hemicryptophytes (50% of the recorded species), followed by chamaephytes and therophytes. Findings also indicated that the effects of climate, altitude, and human activities such as overgrazing caused a reduction in phanerophytes from around 46 to 4%, increases in chamaephytes from about 9 to 26% and hemicryptophytes from 26 to 50% in comparison with Raunkiaer's normal spectrum. It can be concluded that hemicryptophytes and phanerophytes usually comprise the highest and lowest percentages of life forms in studies conducted in the Irano-Turanian growth zone.
Keywords: *flora; life form; endangered species; Irano-Turanian; biodiversity; ecology*

Introduction

Sustainable development requires the acquisition of basic information on terrestrial ecosystems. A major section of an ecosystem is vegetation where plant communities and species spread based on environmental factors (Nimais, 1985). Humans and most other animals directly and indirectly depend almost totally upon plants. Mismanagement and overexploitation have resulted in critical conditions and the degradation of vegetation. Therefore, understanding the distribution of plant species (floristic studies) is essential to the management and conservation of these ecosystems. In addition, flora studies of each region, including the list of species, the life-form spectrum, geographical

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distribution, and the identification of threatened species is useful for ecological issues like biodiversity and determining growth capacities and potentials of a region. A life form is a group of plants which have the same general morphological features (Cain, 1950). Generally, plants are understood to be a growth form that displays an obvious relationship with key environmental factors (Mueller-Dombois et al., 1974). Some techniques for categorizing plant life form have been developed, among which Raunkiaer‘s system is still the simplest and, in many ways, the most satisfactory classifier of plant life forms (Asri, 2003). Raunkiaer (1934) provided this classification system based on the position and degree of protection of the renewing buds which are responsible for the renewal of the aerial plant body after an unfavorable season. Accordingly, plant species can be classified into five main classes: phanerophytes, chamaephytes, hemicryptophytes, cryptophytes and therophytes.

Identification of plant species, especially rare and threatened ones, is very important to the management, protection, reclamation, and development of natural ecosystems throughout the world. The International Union for Conservation of Nature and Natural Resources (IUCN) was formed to identify plant species and take the necessary measures to prevent their extinction (IUCN, 1981). In Iran, Jalili and Jamzad (1999) attempted to introduce species based on the IUCN criteria in the “Red Data Book of Iran." Accordingly, the threatened species of Iran include four categories: endangered, vulnerable, low risk, and data deficient.

Iran has one of the richest floras with a large number of endemic species (Zohary, 1963). A significant proportion of the species are belonging to the Irano-Turanian vegetation zone. The Irano-Turanian flora and vegetation is known by the following features (Djamali et al., 2012): (i) high species richness, (ii) high diversity, (iii) scarcity of forest vegetation, (iv) high endemism (exceeding 25%), (v) dominance of chamaephytes (mainly dwarf shrubs) and hemicryptophytes (mainly Poaceae), forming steppe vegetation, and (vi) development of several specific taxonomic groups, including the genera *Astragalus* (Fabaceae), *Cousinia* and *Centaurea* (Asteraceae), and *Acantholimon* (Plumbaginaceae).

Iran’s rich and diverse flora have been studied by foreign (Zohary, 1973; Rechinger, 1963-2010) and Iranian botanists (Mobayen, 1975-1996; Ghahraman, 1975-2005) for many years. Sources report that the number of plant species in Iran is around 9500-8500 which, in comparison, is near the total number of species in Europe (Akhani, 2006). The Meymeh region in the central plateau of Iran is an example of a cold steppe region with typical steppe plants. Part of the area is located in the Karkas Mountains, and species diversity there is high because of topographic and climatic conditions. The particular climate condition, drought, over-grazing, human activity, and the reduction in valuable species in the area provide enough reasons to study the flora of the region. There are no published studies regarding the vegetation of the area. Therefore, the main objectives of the present study were to enrich the knowledge of vegetation in the area by identifying total plant species and their life-form spectrum, life span, growth habits, and geographical distribution and too identify threatened species. In addition, the study can be very useful for bridging botanical gaps in Iran. Since the climate of the area is classified as Cold Mountain, it could be expected to find a high proportion of Hemicryptophytes. Also, because this area is an indicator of the cold steppe region in central Iran, it can be expected that a high percentage of species will be related to the Irano-Turanian growth zone. In this research, the following questions were asked: (i) What is the floristic composition and life-form spectrum of the study area? (ii) Which

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life-form classes are significantly different from Raunkiaer's normal spectrum? (iii) Are the identified floristic spectra the same as those in other studies conducted in the Irano-Turanian growth zone?

Materials and methods

Study area

This study was conducted in the Meymeh region, located north of Isfahan in the central plateau of Iran (*Fig. 1*). The study area occupying approximately 83.4 km^2 is located between latitudes 33° 20' to 33° 41' N and longitudes 51° 6' to 51° 33' E and the elevation ranges between 2004 and 3157 m and the average slope is 18%. The annual precipitation of the study area influenced mainly by the Mediterranean atmospheric system is about 177 mm with a mean annual temperature of about 12 °C. Most rainfall (73%) was concentrated between November and May. The climate of the study area was, following Emberger method, cold-arid and following Köppen–Geiger climate classification system was cold steppe (cold semi-arid) climate with average annual temperature below 18°C 'Bsk'.

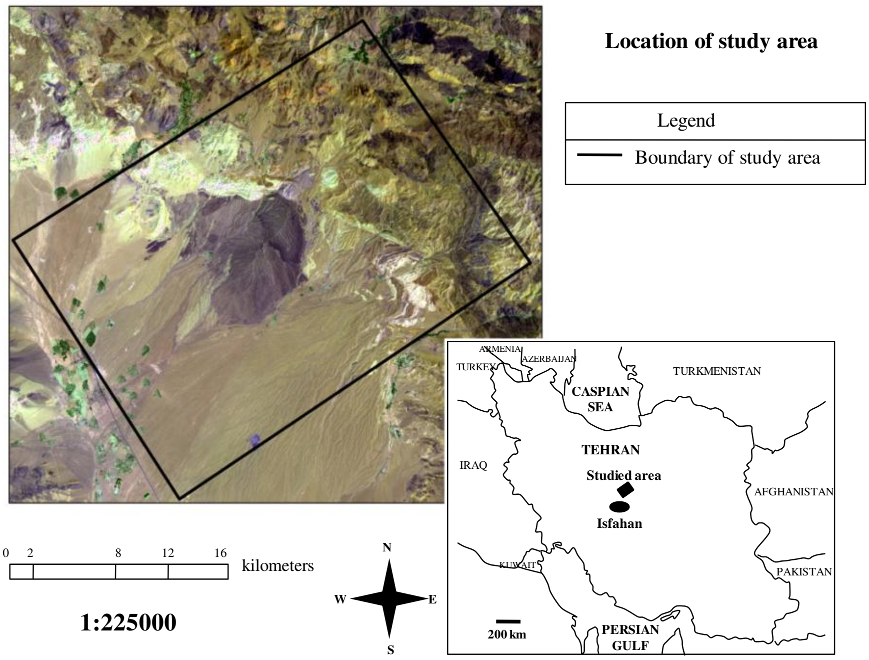

Figure 1. *Location of the study area.*

Data collection and statistical analysis

The flora was surveyed and identified in different seasons on several field trips taken during the period 2014 to 2015. The specimens collected were prepared according to standard herbarium techniques and recognized according to the Flora Iranica (Rechinger, 1963–2010), Flora of Turkey (Davis, 1965–1988), Flora of Iran (Mobayen, 1975-1996), *Astragalus* communities of Iran (Ramak Masomi, 1986-2000) Colored Flora of Iran (Ghahraman, 1975-2005), Flora of Iran (Assadi et al., 1988–2011), and the number of the available papers (Abbasi et al., 2012; Khajeddin et al., 2012). The

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number of genera in each family and the number of species in each genus were determined using "The Plant Book" (Mabberley, 2008). All species were classified into annuals and perennials according to life spans and into herbs, shrubs, and trees according to their growth habits. The life forms of the identified species were assigned on the basis of definitions by Raunkiaer (1934). The geographical distribution and endemism of plant species were also determined according to mentioned Flora and "Biodiversity of Plant Species in Iran" (Ghahreman and Attar, 1998). The proportion of species in each life-form class (biological spectrum) was compared with Raunkiaer's normal spectrum using a χ^2 test (Moradi et al., 2010). Then, a pairwise comparison was made of the floristic biological spectra of the study and those in other floristic studies conducted in the Irano-Turanian growth zone in the vicinity of the area by using χ^2 tests and correlation analyses. The conservation status of the various species was evaluated using a series of criteria such as life span, geographical distribution, life form, population size, and the reproduction of plants in their natural habitats as well as the exploitation of the plant by humans, livestock, and wildlife. Finally, the threatened species in the area were classified on the basis of the method described by Jalili and Jamzad (1999).

Results

Floristic composition

In total, 164 species out of 108 genera and 32 families were collected and identified *(Table 1)*. In this plant collection, the families Asteraceae, Poaceae, and Papilionaceae were treated as the largest plant families respectively by allocating 18.3% (30 species), 12.2% (20 species), and 11.6% (19 species) of all the available species in the region *(Fig. 2)*. The genera with the highest species richness were *Astragalus* (with 15 species), *Cousinia* (with 6 species), *Acanthophyllum* and *Centaurea* (each with 5 species). Annuals and perennials accounted for 17% (28 species) and 83% (136 species) of total number of plant species, respectively. The evaluation of growth habits showed that 124 species (83%) made up a high proportion of the herb form, 20.7% (34 species) belonged to the shrub group, and 3.7% (6 species) belonged to tree species.

Table 1. *List of species, families and their life-form, growth habit, life span, and geographical distribution in Meymeh region, Abbreviations used in this article: He= Hemicryptophyte, Ch= Chamaephyte, Cr= Cryptophyte, Th= Therophyte, Ph= Phanerophyte (Raunkiaer, 1934), IT= Irano-Turanian, M= Mediterranean, ES= Euro-Siberian, Cosm= Cosmopolitan, SA= Saharo-Arabian, SS= Sahara-Sendiananan, Plur= Pluriregional, End= Endemic (Rechinger, 1963-2010), Vu= Vulnerable, LR= Lower risk (Jalili and Jamzad, 1999), A= Annual, P= Perennial, H= Herb, S= Shrub, T= Tree.*

	SCIENTIFIC NAME	Life form	Chorotype	Threatened species	Growth habit	Life Span
	Apiaceae					
1	*Echinophora platyloba* DC.	He	IT (End.)	LR	H	P
2	*Eryngium billardieri* F. Delaroche	He	IT, ES	-	H	P
3	*Eryngium bungei* Boiss.	He	IT (End.)	-	H	P
4	*Ferula ovina* (Boiss.) Boiss.	He	IT	Vu	H	P
	Asteraceae					
5	*Achillea wilhelmsii* C. Koch	He	IT, ES	-	H	P
6	*Artemisia aucheri* Boiss.	Ch	IT	-	S	P
7	*Artemisia sieberi* Besser.	Ch	IT, ES	-	S	P

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8	*Centaurea albonitens* TURRILL	He	IT, ES	LR	H	P
9	*Centaurea gaubae* (Bornm.) Wagenitz	He	IT (End.)	LR	H	P
10	*Centaurea lurestanica*	He	IT (End.)	-	H	P
11	*Centaurea ispahanica* Boiss.	He	IT (End.)	-	H	P
12	*Centaurea virgata* Lam.	He	IT, ES	-	H	P
13	*Cirsium congestum* Fisch. & C.Amey. *Ex*	He	IT, M	-	H	P
14	*Cousinia bachtiarica* Boiss & Hausskn.	He	IT (End.)	-	H	P
15	*Cousinia cungesta*	He	IT (End.)	-	H	P
16	*Cousinia lactiflora* Rech. f.	He	IT (End.)	-	H	P
17	*Cousinia lasiolepis* Boiss	He	IT	-	H	P
18	*Cousinia multiloba* DC.	He	IT (End.)	-	H	P
19	*Cousinia piptocephala* Bunge	He	IT (End.)	Vu	H	P
20	*Echinops caphaletes*	He	IT	-	H	P
21	*Echinops elymaticus* Born	He	IT (End.)	-	H	P
22	*Echinops robustus* Bunge.	He	IT (End.)	-	H	P
23	*Gundelia tournefortii* L.	Ch	IT, M	-	H	P
24	*Hertia angustifolia* (DC.) O. Kuntze	Ch	IT	LR	S	P
25	*Lactuca glauciifolia* Boiss.	Th	IT	-	H	A
26	*Lunea spinosa*	He	IT	-	H	P
27	*Onopordon heteracanthum* C. A. Mey.	He	IT	-	S	P
28	*Outreya carduiformis* Jaub. & Spach	He	IT	-	S	P
29	*Scariola orientalis* (Boiss.) Sojak	Ch	IT	-	H	P
30	*Scorzonera mucida* Rech.f., Aellen &	He	IT (End.)	LR	H	P
31	*Scorzonera tortuosissima* Boiss.	He	IT (End.)	-	H	P
32	*Tanacetum polycephalum* Schultz-Bip.	He	IT, ES	LR	H	P
33	*Taraxacum bessarabicum* Fisch	He	IT	-	H	P
34	*Trapopogon caricifolius* Boiss	He	IT (End.)	-	H	P
	Boraginaceae					
35	*Lappula microcarpa* (Ledeb.) Gurke	Th	IT, ES	-	H	A
36	*Onosma elwendicum* Wettst.	He	IT	-	H	P
37	*Rochelia macrocalyx* Bunge	Th	IT	-	H	A
	Brassicaceae					
37	*Alyssum heterotrichum* Boiss	Th	IT, ES	-	H	A
39	*Alyssum linifolium* Stephan ex Willd.	Th	IT, M	-	H	A
40	*Alyssum bracteatum* Boiss. & Buhse	He	IT (End.)	-	H	A
41	*Alyssum szovitsianum* Fisch. & C.A.Mey	Th	IT	-	H	A
42	*Barbarea vulgaris-Beloris vulgaris*	Ph	-	-	T	P
43	*Clypeola dichotoma* Boiss.	Th	IT	-	H	A
44	*Descurainia sophia (*L.) Schur.	Th	Cosm	-	H	A
45	*Isatis cappodocica* Desv.	He	IT	Vu	H	P
46	*Lepidium persicum* Boiss.	He	IT, M	-	H	P
47	*Matthiola alyssifolia* (DC.) Bornm.	He	-	-	H	P
48	*Matthiola ovatifolia* (Boiss.) Boiss.	He	IT (End.)	LR	H	P
49	*Moriera spinosa* Boiss.	He	IT (End.)	-	H	P
50	*Sisymbrium irio* L.	Th	IT	-	H	A
	Capparidaceae					
51	*Capparis spinosa* L.	Ch	IT,M,SS	-	H	P
52	*Cleome coluteoides* Boiss.	He	-	-	H	P
	Caprifoliaceae					
53	*Lonicera nummulariifolia* Jaub. & Spach	Ph	IT, M	-	T	P
	Caryophyllaceae					
54	*Acanthophyllum bracteatum* Boiss.	Ch	IT (End.)	-	S	P
55	*Acanthophyllum crassifolium* Boiss	Ch	IT	LR	S	P
56	*Acanthophyllum microcephalum* Boiss.	Ch	IT	-	S	P
57	*Acanthophyllum squarrosum* Bioss	Ch	IT (End.)	-	S	P
58	*Acanthophyllu spinosum*	Ch	IT	-	S	P
59	*Buffonia macrocarpa* Ser.	He	IT (End.)	LR	H	P
60	*Dianthus orientalis* Adams	Ch	IT	-	S	P
61	*Dianthus crinitus* Sm.	He	IT	-	H	P

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62	*Gypsophila acantholimoides* Bornm.	Ch	IT (End.)	LR	S	P
63	*Gypsophila virgata*	Ch	IT	LR	H	P
64	*Silene commelinifolia* Boiss.	He	IT	-	H	P
	Chenopodiaceae					
65	*Anabasis aphylla* L.	He	IT	-	H	P
66	*Eurotia ceratoides* (L.) C. A. Mey.	Ch	IT	-	S	P
67	*Kochia prostrate* Roth.	Ch	IT	-	S	P
68	*Kochia stellaris* Moq.	Th	IT	-	H	A
69	*Noaea mucronata* (Forsk.) Aschers. Et	Ch	IT, M	-	S	A
70	*Salsola tomentosa (*Moq.) Spach	He	IT	-	H	P
	Convolvulaceae					
71	*Convolvulus fruticosus* Pall.	Ch	IT	-	H	P
	Cyperaceae					
72	*Carex stenophylla* L.	Cr	IT	-	H	P
	Euphorbiaceae					
73	*Euphorbia cheiradenia*	He	IT	-	H	P
74	*Euphorbia decipiens* Boiss. & Buhse	He	IT (End.)	LR	H	P
75	*Euphorbia heteradena* Jaub. & Spach	He	IT	-	H	P
	Iridaceae					
76	*Iris songarica* Schrenk	Cr	IT	-	H	P
77	*Ixillarion tataricum*	He	IT	-	H	P
	Lamiaceae					
78	*Eremostachys macrophylla* Montbret & Aucher	He	IT	-	H	P
79	*Marrubium crassidens* Boiss.	He	IT	-	H	-
80	*Mentha longifolia* (L.) Huds.	He	IT, SS,	LR	H	P
81	*Nepeta oxyodonta* Boiss	He	IT (End.)	LR	H	P
82	*Nepeta persica* Boiss.	He	IT (End.)	-	H	P
83	*Phlomis olivieri* Benth.	He	IT	-	H	P
84	*Salvia macrosiphon* Boiss.	He	IT	-	H	P
85	*Salvia eremophila* Boiss.	He	IT (End.)	Vu	H	P
86	*Scutellaria multicaulis* Boiss. subsp.	He	IT (End.)	LR	H	P
87	*Stachys inflata* Benth.	He	IT, ES	-	H	P
88	*Teucrium orientale* L.	He	IT	-	H	P
89	*Thymus daenensis* Celak	Ch	IT (End.)	LR	H	P
90	*Zataria multiflora* Boiss.	Ch	IT (End.)	Vu	H	P
91	*Ziziphora tenuior* L.	Th	IT, ES	-	H	A
	Liliaceae					
92	*Eremurus spectabilis*	Cr	IT	-	H	P
93	*Tulipa biflora* Pall	Cr	IT	-	H	P
	Malvaceae					
94	*Alcea aucheri* (Boiss.) Alef.	He	-	-	H	P
95	*Malva silvestris*	He	IT	-	H	P
	Orobanchaceae					
96	*Orobanche alba* Stephan	Cr	-	-	H	P
	papaveraceae					
97	*Glusium oxylobum*	Cr	-	-	H	P
98	*Hypecoum pendulum* L.	Th	IT, ES	-	H	A
99	*Papaver macrostomum*	Th	IT (End.)	-	H	A
100	*Roemeria hybrida* (L.) DC.	Th	IT	-	H	A
	Papilionaceae					
101	*Alhagi camelorum* Fisch.	He	IT	-	H	P
102	*Astragalus callistachys* Boiss. et Buhse	Ch	IT	LR	S	P
103	*Astragalus campylathus* Boiss.	Ch	IT	LR	S	P
104	*Astragalus fisheri*	Ch	IT	-	S	P
105	*Astragalus globiflorus* Bioiss	Ch	IT	-	S	P
106	*Astragalus glucocanthus* Fisch.	Ch	IT	LR	S	P
107	*Astragalus glumaceus*	Ch	IT	-	S	P
108	*Astragalus gossypinus* Fisch	Ch	IT	-	S	P
109	*Astragalus hamosus*	Ch	IT	-	S	P

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110	*Astragalus microphysa* Boiss.	Ch	IT	-	S	P
111	*Astragalus piptocephalus* Boiss	Ch	IT	-	S	P
112	*Astragalus podolobus* Bioiss. & Hohen	Ch	IT	-	S	P
113	*Astragalus pycnocephalus* Fischer	Ch	IT	-	S	P
114	*Astragalus schistosus* Boiss.&Hohen	He	IT	Vu	H	P
115	*Astragalus scleroclodus*	Ch	IT	Vu	S	P
116	*Astragalus verus* Boiss & Hausskn.	Ch	IT	LR	S	P
117	*Onobrychis cornuta* (L.) Desv.	Ch	IT	-	S	P
118	*Onobrychis melanotricuma* Boiss	He	IT	-	H	P
119	*Trigonella monantha* C.A. Mey.	Th	IT	-	H	A
	Phyllanthaceae					
120	*Andrachne telephioides* L.	He	IT, M	-	H	A
	Plantaginaceae					
121	*Plantago major* L.	He	-	-	H	P
	Plumbaginaceae					
122	*Acantholimon aspadanum* Bunge	Ch	IT (End.)	-	S	P
123	*Acantholimon festocaceum* (Jaub. at Sp.)	Ch	IT	-	S	P
124	*Acantholimon scorpius* (Jaub. & Spach)	Ch	IT (End.)	LR	S	P
125	*Acantholimon oliganthum* Boiss	Ch	IT	-	S	P
	Poaceae					
126	*Agropyrom intermedium* (Host) P.	He	-	-	H	P
127	*Agropyrom tauri* Boiss. & Bal.	He	-	-	H	P
128	*Boissiera squarrosa* Hochst. ex Steud	Th	IT, ES, M	-	H	A
129	*Bromus danthoniae* Trin. ex C.A.Mey.	Th	IT	-	H	A
130	*Bromus tectorum* L.	Th	IT, ES, M	-	H	A
131	*Bromus tomentellus* Boiss.	He	IT, ES	Vu	H	P
132	*Cynodon dactylon* (L.) Pers.	Cr	Cosm	-	H	P
133	*Dactylis glomerata* L.	He	IT, ES, M	-	H	P
134	*Eremopoa persica* (Trin.) Roshev.	Th	IT, M	-	H	A
135	*Eremopyrum bonaepartis* (Spreng.)	Th	IT	-	H	A
136	*Eremopyrum distans* (K.Koch) Nevski	Th	IT, ES	-	H	A
137	*Hordeum bulbosum* L.	Cr	IT	-	H	P
138	*Hordeum vulgare* L.	Th	IT	-	H	A
139	*Lolium rigidum* Gaudin	He	IT	-	H	P
140	*Melica persica* Kunth	He	IT (End.)	LR	H	P
141	*Oryzopsis holciformis* (M.B.) Hack	He	IT	-	H	P
142	*Poa bulbosa* L.	Cr	IT, ES	-	H	P
143	*Poa sinaica* Steud.	He	IT, SA	-	H	P
144	*Stipa barbata* Defs	He	IT, ES	-	H	P
145	*Stipagrostis plumosa* Munro ex	He	IT	-	H	P
	Polygonaceae					
146	*Atraphaxis spinosa* L.	Ph	IT	-	T	P
	Ranunculaceae					
147	*Adonis aestivalis* L.	Th	IT	-	H	A
148	*Thalictrum minus* L.	Cr	IT	-	H	A
	Resedaceae					
149	*Reseda buhseana* Mull.Arg	He	IT (End.)	-	H	P
150	*Reseda lutea* L.	He	IT (End.)	-	H	P
	Rosaceae					
151	*Amygdalus scoparia* Spach	Ph	IT	Vu	T	P
152	*Poterium sanguisorba* L.	He	IT (End.)	Vu	H	P
	Rubiaceae					
153	*Gaillonia bruguieri* A.Rich. ex DC.	Th	IT,SS	-	H	A
154	*Rubia florida* Boiss.	He	IT (End.)	LR	H	P
	Scrophulariaceae					
155	*Scrophularia leucoclada* Bunge	Ch	IT	-	H	P
156	*Scrophularia striata* Boiss.	Ch	IT	LR	H	P
157	*Verbascum songaricum* Schrenk	He	IT,ES	-	H	P
158	*Verbascum speciosum* Schrad.	He	IT, ES	-	H	P

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	Solanacea					
159	*Hyoscyamus nigrum*	He	IT	LR	H	P
160	*Lycium depressum* Stocks.	Ph	IT	-	T	P
	Tamaricaceae					
161	*Tamarix ramosissima* Ledeb.	Ph	IT, ES	-	T	P
	Thymelaeaceae					
162	*Dendrostellera lessertii* (Wikstr.) Van Tigeh	Ch	IT	-	H	P
	Zygophyllaceae					
163	*Peganum harmala* L	He	IT, ES, M	-	H	P
164	*Zygophyllum fabago* L.	He	IT	LR	H	P

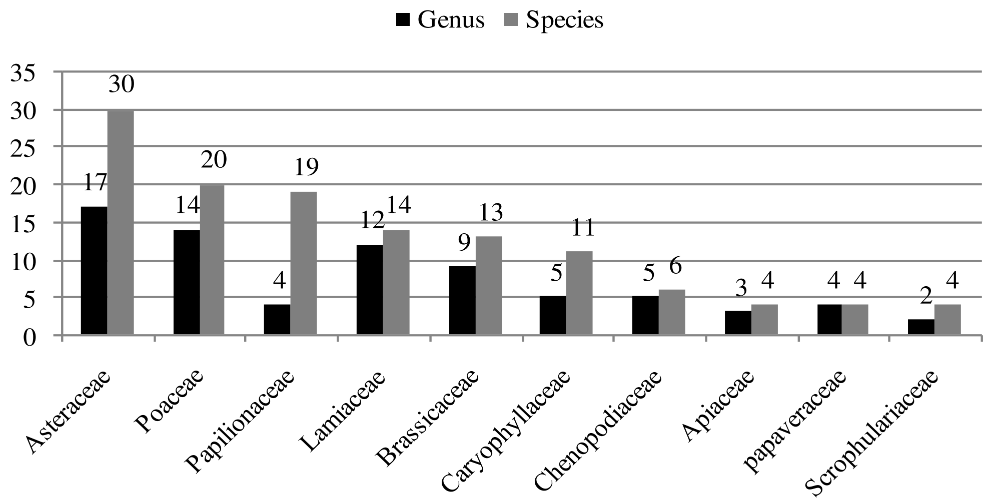

Figure 2. *The large ten families in terms of the number of genera and species in the area*

Biological spectrum

Hemicryptophytes were the dominant life forms accounting for 50% (82 species) of all species in the area, followed by chamaephytes (25.6%), therophytes (14.6%), cryptophytes (6.1%), and phanerophytes (3.7%). The comparison of the floristic life-form spectrum and Raunkiaer's normal spectrum pointed out a statistically significant difference between them ($p<0.05$). The observed proportions were higher than expected for the chamaephytes, hemicryptophytes, and therophytes, and they were lower for the phanerophytes. Phanerophytes, followed by chamaephytes and hemicryptophytes, had the highest individual values determined from the χ^2 test *(Table 2)*.

Table 2. *Comparison among life-form spectra and Raunkiaer's normal spectra in the Meymeh region (For Abbreviation, see Table 1).*

Life- form	Ph	Ch	He	Cr	Th	Total
Number of species	6	42	82	10	24	164
Percentage of plant species	4	26	50	6	14	100
Raunkiaer's normal spectrum (% of species)	46	9	26	6	13	100
Percentage Deviation	-42	17	24	0	1	-
χ^2	38.35	32.11	22.15	0.00	0.08	92.69

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The comparison of the life form spectrum of the case study with different studies in the Irano-Turanian growth zone is presented in Table 3. There were some differences and likenesses between the studies. The results of the χ^2 test and correlation analysis demonstrated a significant correlation between the life-form spectrum in the area and those of other studies conducted in the vicinity of the area, particularly the Karkas hunting prohibited region ($\chi^2 = 8, P = 0.001$) and Yahya Abad region ($\chi^2 = 20.3, P = 0.01$).

Table 3. *Comparison of different items in the study area and other studies conducted in Irano-Turanian Region (For Abbreviation, see Table 1).*

References	**This study**	**Batooli *et al.*, 2003**	**Abbasi *et al.*, 2012**	**Khajeddin *et al.*, 2012**	**Yousofi *et al.*, 2011**	**Rahchamani *et al.*, 2014**
Location	Meymeh	Qazaan	Yahya Abad	karkas	Chadegan	Sarigol
Elevation	2004-3157	1600-3550	2000-2720	1389-3880	1950-3915	1400-2840
Annual rainfall (mm)	177	181.5	147.22	240	324.3	273
Annual temperature (°C)	12	6.8	15.41	2.1	9.8	14
Ph	4	10.8	1.6	7.9	5	7.4
Ch	26	8.2	18.4	16.9	11	10.5
H	50	35.4	44.7	51.8	44	33.9
Cr	6	8.5	6.3	5.7	13	13.3
Th	14	36.9	29	17.7	27	34.9
Total species	164	398	190	278	339	498
χ^2 with this study	0.0	66.5	20.3	8.0	29.9	57.4
Pearson Correlation	1.00	0.53	0.88*	0.96**	0.81	0.56

** Correlation is significant at the 0.01 level (*p<0:001*); *Correlation is significant at the 0.05 level (*p<0:01*).

Chorological affinities

About 74.4% of total plant species in the area belonged to the Irano-Turanian chorotype, whereas Irano Turanian-Euro Siberian, Irano Turanian-Mediterranean, and Irano Turanian-Euro Siberian-Mediterranean plant species contained 13.4%, 5.5%, and 2.4%, respectively, of all plant species. Less than 4.3% of the total plant species belonged to other chorotypes *(Table 4)*. The number of endemic species was 37, and the endemism rate was 22.6%.

Table 4. *The Chorological affinities of plant species in the Meymeh region (For Abbreviation, see Table 1).*

Chorotypes	**Number of species**	**Percentage of species**
Monoregional		
IT	85	51.8
Biregional		
IT, ES	22	13.4
IT, M	9	5.5
IT, SA	1	0.6
IT,SS	1	0.6
Pluriregional		
IT, ES, M	4	2.4
IT, SS, ES, M	1	0.6
IT,M,SS	1	0.6
Cosmopolitan	3	1.8
Endemic	37	22.6
Total	164	100

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IUCN categories

The threatened species of the Meymeh region were evaluated on the basis of the criteria given by Jalili and Jamzad (1999) for assessing threat levels. Accordingly, 36 species (22%) were situated in the different stages of the method. Ten and 26 species were ranked as vulnerable and lower risk, respectively. The herbaceous perennial species accounted for 72% (26 species) of the total threatened species, and 47.2% (17 species) of them belonged to Irano-Turanian endemic species. Moreover, the families Asteraceae, Papilionaceae, and Poaceae were treated as the most important families as 16.7% (6 species) of their total species were allocated to the categories of threatened species *(Fig. 3)*.

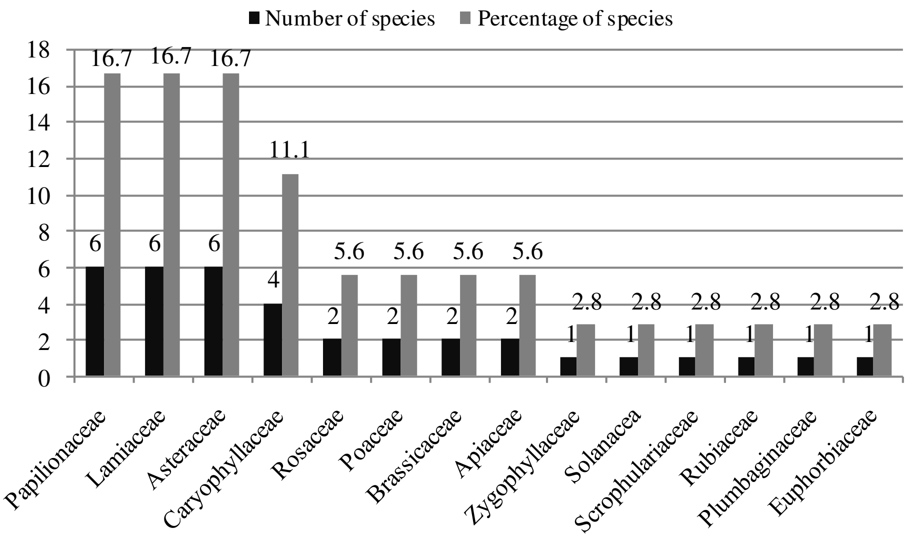

Figure 3. *The most important families in terms of having the largest number of species in the categories of threatened species.*

Discussion

The presence of 164 plant species belonging to 108 genera and 32 families indicates a considerable level of plant diversity in the study area. These species have formed diverse communities tailored to their ecological needs and the management imposed by humans over the past years. It seems that high plant diversity in the area is due to edaphic, topographic, and physiographic conditions. Of course, the climatic factor is also effective in this regard, but variations in climatic conditions of the area are less than the other factors (Khajeddin et al., 2012).

Asteraceae, Poaceae, and Papilionaceae were among the richest families in the area which is due to the high compatibility of these families with the arid and semiarid climate conditions. They are common compared to other plant families in the Irano-Turanian growth zone (Aghaei et al., 2013; Jafari et al., 2016), especially in the central region of Iran (Yousofi et al., 2011; Abbasi et al., 2012; Khajeddin et al., 2012). In addition, the high frequency of Asteraceae may be related to the high percentage of vegetation destruction in the area (Archibold, 1995). Compared with the nearby Karkas hunting-prohibited region (Khajeddin et al., 2012), there are fewer

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species of flora in the Meymeh region. This is probably due to the higher habitat diversity along the Karkas Mountains.

In this study, the main fraction of species composition was *Artemisia aucheri*/*Artemisia sieberi* gradient. Distribution of these two species was contrasting, as *Artemisia sieberi* was the most dominant species at low altitude and that was gradually replaced by *Artemisia aucheri* at higher altitude. This finding is in agreement with the results of study conducted by Mousaei Sanjerehei et al., (2013), in which it was shown that distribution of these two species is associated to the complex environmental variations along the altitudinal gradient.

According to Raunkiaer's system, the life-form composition in the Meymeh region was dominated by hemicryptophytes followed by chamaephytes and therophytes in succession. The low percentages of cryptophytes and phanerophytes show that they are not adapted to climatic conditions in the region. A comparison of the life-form spectra of the area with those in the Karkas hunting-prohibited region (Khajeddin et al., 2012) and pastural region of Yahya Abad (Abbasi et al., 2012) showed the same results. In all the studies conducted in the Irano-Turanian growth zone, hemicryptophytes and phanerophytes usually comprise the highest and lowest percentages of life forms, respectively *(Table 3)*. Hemicryptophytes are a large and complex life-form group with the resting bud at or near the soil surface. The frequency of the life form in the flora of the area is characteristic of the cool steppe region (Cain, 1950) and also indicates the dominance of cold and mountainous climates (Arcihold, 1995). Furthermore, the high percentage of chamaephytes characterized the colder climate and high altitude (Braun-Blanquet, 1932). Chamaephytes are often protected in the unfavorable season by fallen leaves or by the dense growth of the plant itself. In this life form, the species that provide the greatest protection of the buds are undoubtedly the very compact cushion plants such as *Astragalus spp*, *Acanthophyllum spp*, and *Acantholimonsp spp* in certain dry steppes (Cain, 1950). This type of plants was fairly common in the area, comprising about 15% (24 species) of all the available species. The remarkably high percentages of hemicryptophytes and chamaephytes (76% for both) were also emphasized. Hemicryptophytes are always abundant in areas where chamaephytes are abundant, but they show no consistent trend (Cain, 1950).

Next in abundance were the therophytes, which indicate the existence of a heavy biotic pressure due to overgrazing and human interference. Low rainfall levels, high temperatures, consecutive droughts, a short growing season, and various factors of destruction such as overgrazing and agriculture were the most important factors for the increase of therophytes; this result was consistent with the results of studies conducted by Raunkiaer (1934) and Cain (1950), in which it was shown that the increase of therophytes reflects an effective strategy for avoiding water loss. Although therophytes occur abundantly in desert areas (Archibold, 1995), more or less, the high occurrence of the life form indicates there are some anthropogenic and overgrazing effects in the study areas (Grime, 2001; Ravanbakhsh et al., 2014).

Results of the χ^2 test showed that Raunkiaer’s normal spectrum was significantly different from the amount of phanerophytes, hemicryptophytes, and chamaephytes, while the differences between the amounts of cryptophytes and therophytes are not significant. The phanerophytes showed a maximum divergence from the normal spectrum. There was less of them than that of Raunkiaer’s normal spectrum, which may be related to dryness of the region. This change seems much more significant

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and true to the climatic differences than those in other life forms, because the phanerophytes occur in the most favorable climates with high temperatures and humidity (Cain, 1950). The total number of chamaephytes was higher than that of Raunkiaer's normal spectrum. This finding can be related to the cold and mountainous conditions of the region and is in agreement with the results reported by Cain (1950) that demonstrated the positive correlation of this class' percentage with increasingly high altitudes, especially in areas with such widely diverse vegetation types as certain steppes. The hemicryptophytes reached a maximum in the area, where they were at about double the normal spectrum. This finding shows that they are adapted to the cold steppe climate of the area. Many species belonging to the Irano-Turanian phytogeographic region were found in this study, indicating that the study area belongs to this growth zone. In the flora of adjoining areas and even neighboring countries such as Turkey (Vural, 2005), Pakistan and Afghanistan (Rechinger, 1963–2010), Irano-Turanian elements compose a large proportion of the total species (Akhani, 2006), which represents the homogeneity of this growth zone (Abbasi et al., 2012).

The results of the assessment of threatened species showed that most belonged to herbaceous perennial species, which is consistent with the results reported in Jalili and Jamzad (1999) that demonstrated that herbaceous perennial species accounted for 71% of the total threatened species in Iran. Also, the same results were also obtained in Karkas hunting prohibited region (Khajeddin et al., 2012). Various factors caused the increase in numbers of vulnerable species in the area. Overgrazing was a major cause which led to the destruction of *Astragalus schistosus*, *Poterium sanguisorba*, *Ferula ovina* and *Bromus tomentellus* habitats. In contrast, finite population and low natural reproduction were determined to be the factors most effective on the vulnerability of *Astragalus scleroclodus* and *Salvia eremophila*. *Cousinia piptocephala* and *Zataria multiflora* the endemic species of the Irano-Turanian growth zone that established in individual habitats in terms of edaphic and biological characteristics. The most important factor that caused the deterioration of these species was human activity, such as overexploitation of the plant and land use change.

Conclusion

This study supports the general hypotheses; tests demonstrated that plant communities in areas that have a cold steppe climate with dry summers and relatively wet winters in the central plateau of Iran would have a high proportion of hemicryptophyte species belonging to the Irano-Turanian growth zone.

Besides the variations in species composition, the composition of life forms reflects the response of vegetation to variations in certain environmental factors. In this study, the dominance of hemicryptophytes, chamaephytes, and therophytes over other life forms seems to be a response to the cold steppe climate, high altitude, and human activities. Regardless of the type of ecosystem, it was noted that overgrazing in some parts of the area, especially in the lowlands, had led to the occupation of the area by invasive therophytes, indicating hyper-degradation. Therefore, overgrazing is viewed as a major cause of the deterioration of vegetation in the area. The best solution for sustainable management of the area would be to comply with the proper principles of range management aimed at reducing the intensity of grazing and prolonging exclosure.

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This study provides fundamental data about the flora of the area by means of a thorough botanical inventory. These findings will have special significance for further ecological research and for recommendations of proper guidance for the management, reclamation, and development of the area and other similar regions.

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WINDOWED LEAST-SQUARES SPECTRAL ANALYSIS OF GRACE K-BAND RANGE RATE MEASUREMENTS

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Abstract. The objective of this manuscript is to utilize windowed least-squares spectral analysis for extracting the frequency contents of GRACE K-band range rate time series related to Iran's main catchments. The spectral behavior of the unequally spaced time series affected by satellites and Earth rotations should be studied using the Least-Squares Spectral Analysis due to the inherent limitations in the traditional Fourier and Wavelet Transformations techniques. We explain the principles of windowed least- squares spectral analysis as an alternative method of the Least-Squares Spectral Analysis in order to access a time-frequency representation of irregularly sampled GRACE range rate time series related to Iran's main catchments. The results are in good agreement with the spectral behavior of the total water storage changes modeled in the catchments, as well as with the previous research findings.
Keywords: *Iran's main catchments; hydrology; twin-satellite mission; least squares approximation; time series analysis*

Introduction

The classical Least-Squares Spectral Analysis (LSSA) method was first introduced by Vanicek (1969) as an alternative to Fourier analysis, the most commonly used spectral approach in science. This analysis was developed in order to overcome some of the limitations of the Fourier technique; most importantly the requirement that the data is equally spaced and equally weighted with no gaps and datum shifts (Hui and Pagiatakis, 2004).

Both Fourier and classical LSSA use trigonometric functions in different frequencies as the basis for analyzing signals. This choice provides a very good frequency resolution and determines the frequency contents of the signal without any information about the corresponding time of occurrence of these frequencies. Currently, the most popular and useful method of time- frequency analysis is the wavelet transformation in which the base functions will be the waveforms of limited duration, instead of sinusoids extending from minus to plus infinity. Similar to Fourier analysis, wavelet transformation also requires equally spaced and equally weighted data. Fortunately, this shortcoming has been addressed in the second generation wavelets, which are capable of applying the unevenly data without any spectral interpretation (Sweldens, 1998). In this paper, by formulating a windowed transformation scheme, the weighted least-squares approximation is introduced. The experimental studies assessing this method are conducted using the time series obtained from GRACE level 1B observations related to Iran's main catchments with the related gaps and weights.

The Gravity Recovery and Climate Experiment (GRACE) mission, launched in March 2002, has globally mapped the temporal variations of the Earth's gravity. The

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spatio-temporal alteration of the precisely measured distance between two satellites is affected by the mass changes in the Earth system (Han et al., 2005). Many studies have used different types of GRACE data, individually or together with other remote sensing or land surface observations, in order to monitor water storage pattern globally and regionally (see for example, Fatolazadeh et al., 2015; Lemoine et al., 2007; Rowlands et al., 2010; Tourian et al., 2015 and Voss et al., 2013).

In all of the above mentioned approaches, the desired hydrological signals extracted from GRACE measurements have been converted to different formats such as spherical harmonic coefficients and equivalent water thicknesses of juxtaposed tiles, resulting in missing information during the smoothing process. Since all of the mentioned products have been obtained from the GRACE K-band range rate measurements, we decided to use them directly in the present research.

In this manuscript we first present the mathematical foundations of Windowed Least-Squares Spectral Analysis (WLSSA). Then, we discuss its application for extracting the spectral behavior of the range rates time series related to Iran's main catchments. Lastly, we compare the results of the analysis of the time series with those of the monthly modeled Total Water Storage (TWS) changes related to the catchments extracted from WaterGAP (Water – a Global Assessment and Prognosis) hydrological model (Döll et al., 2003).

Mathematical foundations

The proposed method, as an application of Least-Squares Approximation (LSA) (Vaníček and Wells, 1972) is similar to LSSA, which is connected to the linear least squares parametric adjustment (Wells et al., 1985).

Given a vector of observations *f*, sampled at uniform or non-uniform time instants, we can set up a parametric model as follows:

$$f(t) = \sum_i c_i\,\varphi_i(t) = \boldsymbol{\Phi} * \boldsymbol{c} \qquad \text{(Eq.1)}$$

Where, Φ is a matrix consisting of several column vectors, as base functions. Each function is of the same dimension as $\boldsymbol{f}$, and $\boldsymbol{c}$ is the vector of unknown coefficients.

In the classical LSSA, the form of the base functions is selected to be sines and cosines of different frequencies ω_j, j=1,2,…,m, and for each frequency, the best fitting approximant $\boldsymbol{p}$ to $\boldsymbol{f}$ will be obtained by minimizing the residual vector $\hat{\boldsymbol{v}} = \boldsymbol{f} - \boldsymbol{p}$ in the least-squares sense as:

$$\hat{\boldsymbol{c}} = [\hat{c}_1\ \hat{c}_2]^T = (\boldsymbol{\Phi}^T\boldsymbol{\Phi})^{-1}(\boldsymbol{\Phi}^T\boldsymbol{f}) \Longrightarrow\ \boldsymbol{p}(\omega_j) = \hat{c}_1 \cos\omega_j t + \hat{c}_2 \sin\omega_j t \qquad \text{(Eq.2)}$$

Then the fractional content $s(\omega_j)$ of $\boldsymbol{f}$ represented by $\boldsymbol{p}(\omega_j)$ can be measured by:

$$s(\omega_j) = \frac{\boldsymbol{f}^{\mathrm{T}}\,\boldsymbol{p}(\omega_j)}{\boldsymbol{f}^T\boldsymbol{f}} \qquad \text{(Eq.3)}$$

In the following subsection, the windowed least-squares spectral analysis is developed by the use of truncated or weighted sines and cosines as the base functions in the parametric model represented by *Eq.1*.

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Windowed least-squares spectral analysis

In order to estimate the frequency content of local sections of a signal sampled at uniform or non-uniform instants, the sinusoidal base functions can be multiplied by a window function, which is nonzero for only a short period of time, exactly similar to the Short-Time Fourier Transform (STFT) (Okamura, 2011). Therefore, the parametric model (Eq.1) can be rewritten as:

$$\forall\, j;\ \boldsymbol{f} = \begin{bmatrix} f_1 \\ \vdots \\ f_n \end{bmatrix} = \begin{bmatrix} \mathrm{w}(t_1-\tau)\cos\omega_j t_1 & \mathrm{w}(t_1-\tau)\sin\omega_j t_1 \\ & \vdots \\ \mathrm{w}(t_n-\tau)\cos\omega_j t_n & \mathrm{w}(t_n-\tau)\sin\omega_j t_n \end{bmatrix} \begin{bmatrix} c_1 \\ c_2 \end{bmatrix} = \boldsymbol{\Phi} * \boldsymbol{c} \quad \text{(Eq.4)}$$

With the Gaussian window function:

$$\mathrm{w}(t) = \sqrt{\alpha/\pi}\,\mathrm{e}^{-\alpha t^2} \quad \text{(Eq.5)}$$

Where, α determines the length of the window. The least-squares estimation of the coefficients c_1 and c_2 and consequently, the best fitting approximant $\boldsymbol{p}(\tau,\omega_j)$ to $\boldsymbol{f}$ for each selected frequency ω_j and translation parameter τ will be computed as follows:

$$\begin{bmatrix} \hat{c}_1 \\ \hat{c}_2 \end{bmatrix} = (\Phi^T\Phi)^{-1}(\Phi^T\boldsymbol{f}) \Rightarrow \boldsymbol{p}(\tau,\omega_j) = \hat{c}_1\,\mathrm{w}(\mathrm{t}-\tau)\cos\omega_j t + \hat{c}_2\,\mathrm{w}(\mathrm{t}-\tau)\sin\omega_j t \quad \text{(Eq.6)}$$

Finally, the spectral value corresponding to the selected frequency and translation parameter is obtained as:

$$s(\tau,\omega_j) = \frac{\boldsymbol{f}^T\boldsymbol{p}(\tau,\omega_j)}{\boldsymbol{f}^T\boldsymbol{f}} \quad \text{(Eq.7)}$$

Unlike the classical LSSA (Eq.3), spectrum calculated by this method (Eq.7) not only contains the information about the frequency contents of the signal but also the time of occurrence of these frequencies.

Multivariate analysis

The proposed WLSSA can be used to detect common components in multivariate time series with the ability to simultaneously focus on the time and frequency behavior of the signals. If several (r) time series with identical design matrix Φ exist in a linear model, the model will be referred to a multivariate linear model (Amiri-Simkooei and Asgari, 2011). In this case, the generalized form of the parametric model (Eq.1) will be:

$$vec(\boldsymbol{f}) = (I_r \otimes \Phi) * vec(\boldsymbol{c}) \quad \text{(Eq.8)}$$

Where, *vec* is the vector operator and ⊗ is the Kronecker product. If the series are uncorrelated with covariance matrix $\Sigma = \mathrm{diag}(\sigma_{11}, \sigma_{22}, \ldots, \sigma_{rr})$, the power spectrum is:

$$s(\tau,\omega_j) = \textstyle\sum_{i=1}^{r} s_i\,(\tau,\omega_j)/\sigma_{ii} \quad \text{(Eq.9)}$$

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That is, the weighted sum of the individual spectral values.

Time series creation and analysis

Presence of numerous processing methods for GRACE data has led to different types of released data products at several levels (Chen, 2007). In this study, the inter-satellite K-band range rate (KBRR) measurements with an accuracy of 0.1 μm/s and 5 seconds sampling, included in the products labeled L1B, in the period of January 2003 to December 2011 are used (GRACE LEVEL 1B JPL RELEASE 2.0. Ver. 2. PO.DAAC, CA, USA) (Case et al., 2002). Since the spatio-temporal variation of the GRACE K-band range rate measurements shows the mass changes at the surface of and within the Earth, the range rate time series corresponding to a selected area contains regional information about temporal variations of the gravity field, caused by fluctuations in total water storage, provided that the contributions to range rates from tide and non-gravitational accelerations measured by the GRACE onboard accelerometers are reduced. In this study, the tides are modeled as variation to the spherical harmonic coefficients according to IERS Conventions, 2010 (Petit and B. Luzum, 2010), then the simulated perturbed orbits of the two GRACE satellites are generated by adding these variations to the static gravity field of the Earth, EGM (Earth Gravitational Model) 2008, and the tidal corrections are estimated by comparing the synthesized range rates derived from perturbed and unperturbed orbits. In the case of non-gravitational accelerations, a similar procedure is used, with the difference that these accelerations have to be calibrated before use in the perturbed orbit simulation. We estimate the accelerometer parameters by comparison with Precise Orbit Determination (POD) based non gravitational accelerations; for details, see Bezděk (2010).

As a case study, in the present paper, the above mentioned time series are produced by monthly averaging of the reduced observations related to the Iran's main catchments: Caspian Sea, Oromieh (Urmia) Lake, Serakhs (Ghareghoum), Central, Hamoon and Persian gulf (*Fig. 1*).

Figure 1. *Map of the main catchments of Iran.*

Based on the degree of relevance of each observation to a catchment, a weight that is proportional to the percentage of the catchment area covered by the instantaneous

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relative position vector between two satellites is assigned to that observation and the monthly quantities are computed as a weighted mean. The estimated monthly range rates are carrying various weights because of the different observational weights and also the difference in numbers of measurements therein a catchment in a month. At the same time, there are some months without any observed quantity in some catchments. This leads to unevenly spaced time series in these cases which cannot be analyzed using Fourier transformation or STFT. The extracted range rates are shown in *Fig. 2*.

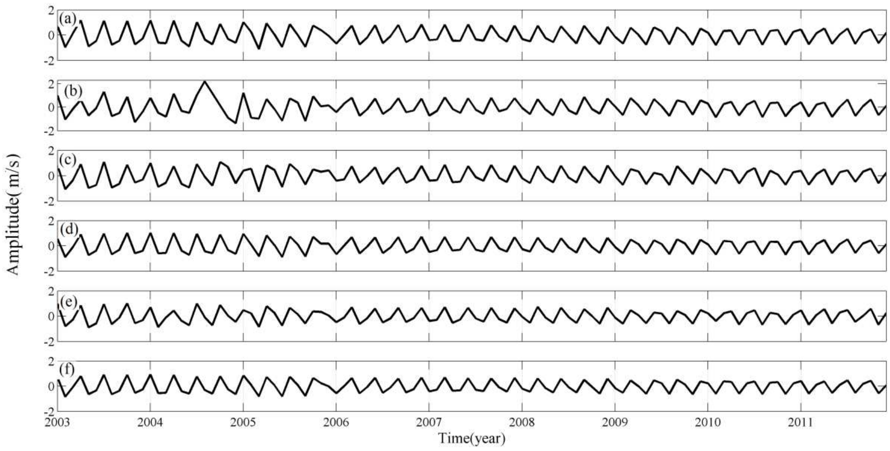

Figure 2. *The range rate time series related to the main catchments of Iran: (a) Caspian Sea, (b) Oromieh Lake, (c) Serakhs, (d) Central, (e) Hamoon and (f) Persian gulf.*

After removing the previously known periods in the range rate signals associated with the GRACE orbital configuration (Visser, 2005), the classical LSSA and the proposed WLSSA are applied to the filtered time series to extract the time-frequency contents affected by the regional hydrology and the results are depicted in *Fig 3*.

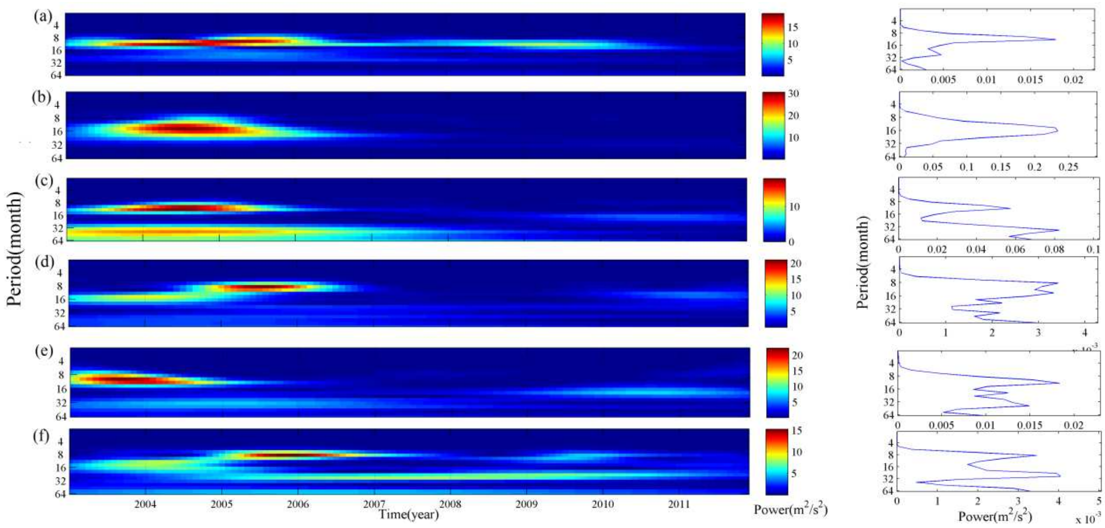

Figure 3. *The estimated periodograms of the range rate time series related to the main catchments of Iran: (a) Caspian Sea, (b) Oromieh Lake, (c) Serakhs, (d) Central, (e) Hamoon and (f) Persian gulf, as the results of applying the classical LSSA (right) and WLSSA (left).*

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As it can be seen in the above periodograms, a main constituent with the period of about 12 months exists in all signals corresponding to the main hydrological cycle in the catchments rooted in the annual circulation of the atmosphere.

The considerable expected time-frequency analyzing of the time series, as the specific result of using the WLSSA instead of the classical LSSA, implies that the annual constituent is not stationary. This explains the intensity of some of the spectral values in each shown periodogram. The identifiable decreases in the spectral values can be explained as the effect of the reported drought in the region containing Iran started around 2007 and 2008 (Joodaki et al., 2014; Voss et al., 2013). This result has been reported for Urmia catchment in Tourian et al. (2015), too.

In order to validate the results of the analysis, the monthly total water storage changes between 2003 and 2011 related to each catchment were obtained from WaterGAP hydrological model (*Fig. 4*) and were analyzed using the two above-mentioned methods (*Fig. 5*).

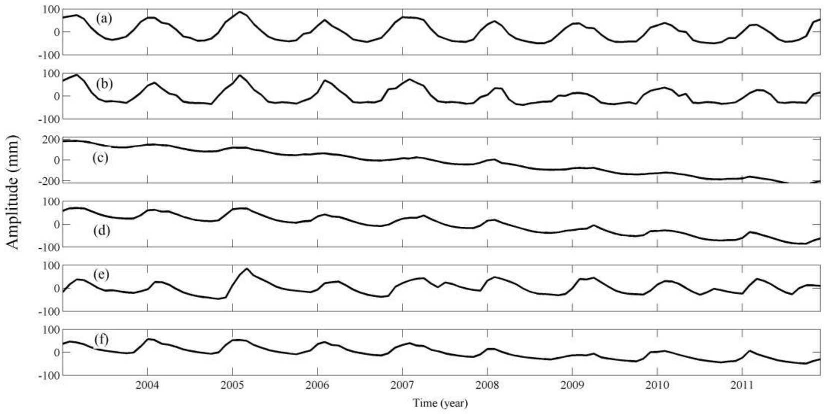

Figure 4. *The modeled TWS changes related to the main catchments of Iran: (a) Caspian Sea, (b) Oromieh Lake, (c) Serakhs, (d) Central, (e) Hamoon and (f) Persian gulf.*

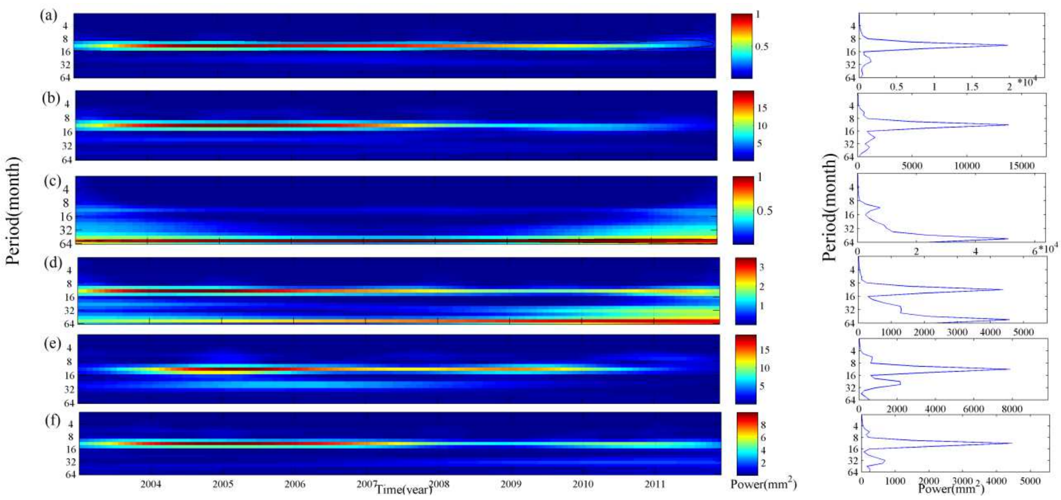

Figure 5. *The estimated periodograms of the modeled TWS changes related to the main catchments of Iran: (a) Caspian Sea, (b) Oromieh Lake, (c) Serakhs, (d) Central, (e) Hamoon and (f) Persian gulf, as the results of applying the classical LSSA (right) and WLSSA (left).*

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We expected that the one-year alternation occur in each TWS change signal again. However, we noted that all signals have been weakened after 2007 in all catchment areas except Serakhs catchment, in which the annual spectral contents started to decrease with a 2-year delay. This difference can also be seen in a similar fashion in the corresponding periodogram for the range rates.

In addition to the individual analysis, the multivariate analyzing of the both range rates and TWS change time series using the LSSA and WLSSA resulted in the multivariate periodograms shown in *Fig. 6*. The results are almost similar to those of individual signals, implying the weakness of annual constituent after 2007, as the main common component of the time series.

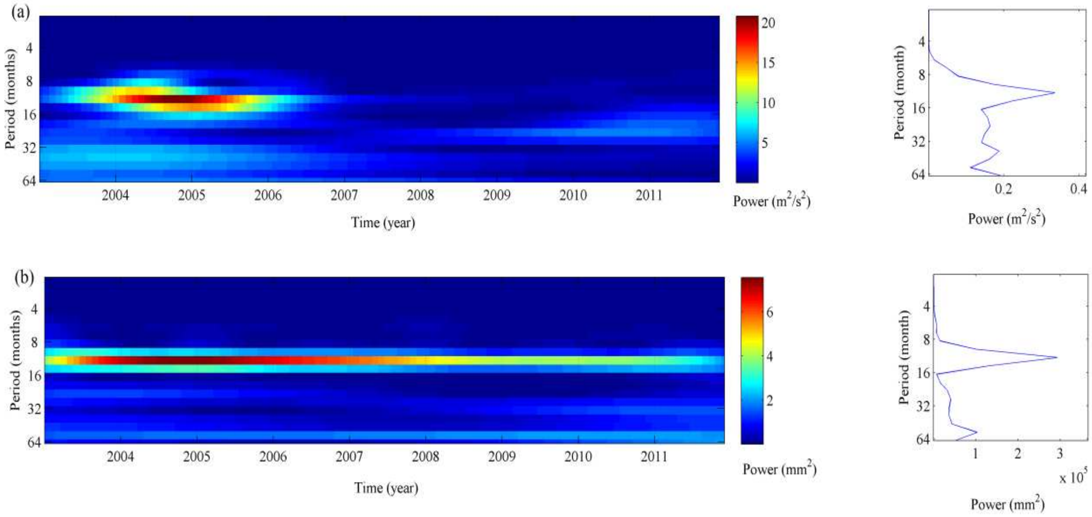

Figure 6. *The estimated multivariate periodograms of (a) the range rates and (b) the modeled TWS changes related to the main catchments of Iran, as the results of applying the classical LSSA (right) and WLSSA (left).*

Conclusions

In this study, the windowed least-squares spectral analysis was discussed as an alternative method to the classical LSSA for studying the time-frequency behavior of GRACE K-band range rate time series.

As a case study, the monthly averaged range rates related to the main catchments of Iran were analyzed. Each catchment time series had a main element with the period of about 12 months. This annual component is affected by the total water storage variations in the region and the significant decrease of its corresponding spectral values is most likely the result of the desiccation that started after 2007.

Monthly modeled TWS changes over each selected catchment were analyzed using the same analyzing tools. This led to extracting the spectral behavior of the annual component which has weakened after 2007.

Multivariate analyzing of both monthly range rates and TWS changes confirms the above mentioned time-frequency behavior as common contents of the signals related to the selected catchments.

The results of GRACE level 1B measurements related to the regions under study are in good agreement with similar information sources and those of previous researches.

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This indicates that these measurements could directly be used as an alternative to the conventional schemes.

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DETERMINATION OF LEAD AND CADMIUM IN THE WATER OF THE DAMAVAND RIVER, IRAN

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Abstract. Heavy metals contamination of surface waters has become an important issue in the last decades. The present study aimed to investigate the levels of lead and cadmium in the water of the Damavand River, Iran. Sampling was carried out in 10 stations during summer and autumn 2015. Metal analysis was performed by atomic absorption spectrophotometer. Surface water at each sampling point was measured for selected physicochemical parameters with a multiparameter meter. Statistical analysis was carried out by SPSS and Excel software. The results showed that the mean concentrations (mg/L) of lead in the samples were higher than cadmium over the studied months. The highest levels of lead (2.13±0.80) and cadmium (1.03±0.50) were found in station 9 while the lowest levels of lead (0.09±0.00) and cadmium (0.03±0.00) were in station 1 (before entering the city of Damavand). Significant differences ($P<0.05$) were observed between the stations, however, there were no significant variations between the levels of metals in the months. Significant differences were found between the levels of physicochemical parameters among different months at 95% confidence level. Present values revealed that the concentrations of metals in the stations were higher than the maximum permitted levels for drinking water set by different organizations.
Keywords: *anthropogenic activities, heavy metals, river pollution, surface waters, water quality*

Introduction

The pollution of surface water ecosystems as a result of anthropogenic and natural sources has become a growing global concern (Aktar et al., 2010). Over the past half century, human activities have altered the natural balance of aquatic environment. This can lead to destroy the natural environment and natural resources of future generations (Salati and Moore, 2009). The contamination of surface waters by heavy metals is one of the most serious environmental problems due to their bioaccumulation capabilities (Diagomanolin et al., 2004). In order to develop and implement an effective environmental management, knowledge of the changing levels of heavy metals and their possible source in the environment is necessary (Owamah, 2013).

Heavy metals are found in natural aquatic systems in low levels; however, the excess load of these metals in water bodies as a result of anthropogenic inputs could be a great concern (Abubakar et al., 2015). Although some metals like iron and zinc are needed as micronutrients for plants and animals in low levels, other metals like cadmium (Cd) and lead (Pb) are known to be extremely toxic to living organisms even at low concentrations (Kar et al., 2008). Industrial activities and discharges of untreated effluents, waste and agricultural runoff are the major human sources of heavy metals pollution (Varol and Sen, 2012). Heavy metals can accumulate in aquatic organisms, persist in water and find their way to human body through drinking water or via the food chain (Reyhani et al., 2013; Varol and Sen, 2012). The levels of metals in aquatic environment are generally monitored by determining their levels in water, sediment and biota (Alex et al., 2013).

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Water quality in urban river systems is under increasing stress because of industrial and domestic wastewater effluents and agricultural runoff (Al Obaidy et al., 2014). Many rural citizens throughout the world tend to use river water for household needs and thus anthropogenic activities have threatened their water supply (Sibanda et al., 2014). The River Damavand running by the side of the Damavand City in Iran, receives municipal and industrial loads from its surrounding environment. Many industrial units have set up in the downstream of the river and the number of industries in this area is continuously increasing. This river plays an important role in supplying water for municipal and agricultural uses, and thus there is an urgent need to measure and monitor metals of the river.

Some studies in the literature have focused on heavy metal pollution of water resources in Iran (Salati and Moore, 2010; Diagomanolin et al., 2004); however, no studies have been conducted examining the levels of metals in the Damavand River. Cd and Pb are among the most important pollutants in the study area, as a result of surface runoff discharges of industries like steel and cement factories. The objective of this study was, therefore, to determine the concentration of Pb and Cd in the water of Damavand River and to compare the results with the acceptable metal limits given by international organizations.

Materials and Methods

The Damavand River situated between 35° 70′ 13″ N and 52° 05′ 86″ E and originates from the Damavand Mountains in the northeast of Tehran province in Iran. There are various industries like electroplating, chemicals, wood preservations, iron and steel, cement, plastic and rubber industries which are in an industrial area, located in downstream of the river (Khoramdasht region). There are also villages, and agricultural lands built on both sides of the river. The river is used by the local residents for various activities such as swimming and drinking.

Water samples were collected once monthly from July to December 2015. Samples were chosen from 10 sampling stations along the Damavand River (*Table 1*) at a depth of approximately 20 cm below the surface, poured into sterilized bottles, kept in an ice pack and transported to the laboratory for analysis. No sample was collected at station 9 in September and October due to very low flow at this site. On each sampling station, selected water physicochemical parameters (temperature, pH and electrical conductivity) were monitored using a multiparameter meter from Hach (model senION156). The levels of Cd and Pb in the water samples were determined using method 3111 of the standard methods for the examination of water and wastewater by Air-acetylene flame atomic absorption spectrophotometry (APHA et al. 1998).

The analytical data quality was guaranteed through accomplishment of laboratory quality assurance and quality control methods, including calibration with standards, use of standard operating procedures and analysis of both reagent blanks and replicates. Statistical analysis was performed using SPSS v.22 and Excel 2010 software. The analysis of variance (ANOVA) followed by Duncan test (α= 0.05) was applied to determine the differences among the groups.

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Results

The geographical characteristics of the sampling stations and the concentrations of Pb and Cd in water samples from different stations are presented in *Table 1*. Analysis of variance revealed significant differences ($P < 0.05$) in Cd and Pb levels between sampling stations. The abundance of Pb and Cd in the stations followed the order: S1<S2=S3=S4<S5=S6<S7=S8=S10<S9 and S1<S2=S3=S4<S5=S6=S7<S8=S10<S9, respectively, according to Duncan test. Temperature, pH, and electrical conductivity levels of the Damavand River surface water during sampling months are given in *Table 2*. The Duncan test revealed that there was a significant variation between the levels of these three physicochemical parameters among different months at 95% confidence level. *Fig 1.* provides information about monthly variations of Cd and Pb concentrations in the samples during six-month sampling period. Levels of both metals fluctuated slightly, but the trends were downward. The results from the present study were also compared with standards established by different organizations.

Table 1. *Geographic locations of the stations and mean levels (Mean±SD) of heavy metals in the stations during sampling months*

Stations	Location	Altitude above sea level	Pb (mg/lit)	Cd (mg/lit)
1	Rouh-afza region (Before entering the city of Damavand)	2033	0.09±0.00	0.03±0.00
2	Entrance to the city of Damavand	1994	0.10±0.00	0.04±0.02
3	Farameh region	1924	0.10±0.01	0.04±0.02
4	Valiran region	1895	0.13±0.04	0.05±0.02
5	Under the Shalambeh Bridge	1826	0.21±0.14	0.18±0.12
6	Hesar paein region	1786	0.35±0.27	0.24±0.19
7	Mara region	1735	0.67±0.35	0.45±0.41
8	Khoramdasht region	1695	1.17±0.65	0.65±0.36
9	Zaredareh region	1421	2.13±0.80	1.03±0.50
10	Entrance to the Mamlo Dam	1309	1.52±0.93	0.78±0.47

Table 2. *The mean and standard deviations of physicochemical parameters of water in the studied stations during the 6-months studied period*

Parameters	Months					
	July	August	September	October	November	December
Temperature (°C)	22.82±0.55	24.89±0.51	24.64±0.33	14.47±8.54	6.27±0.19	6.07±0.35
pH	7.65±0.34	7.46±0.32	7.95±0.25	7.72±0.30	7.70±0.11	7.69±0.17
Electrical conductivity (µmhos/cm)	764.30±80.70	826.33±11.88	805.78±104.87	701.56±96.60	730.00±65.78	791.40±187.63

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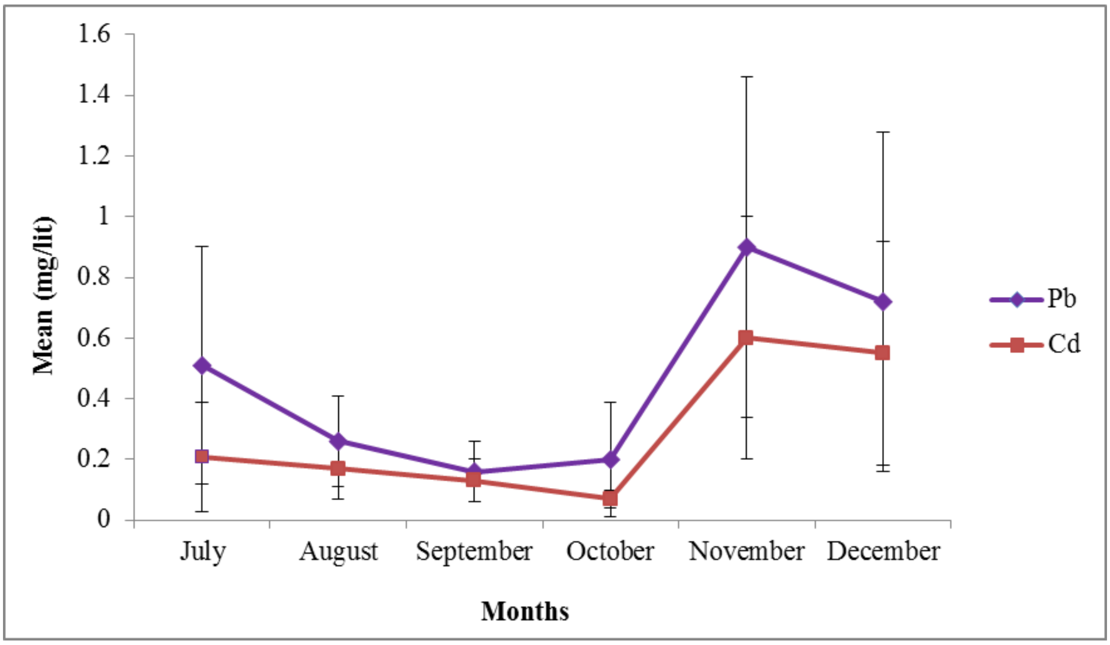

Figure 1. *Monthly variation in mean levels of heavy metals (mg/lit) in the studied months*

Discussion

Regarding *Table 1*, station 1 showed significantly lowest levels of Pb (0.09±0.00) and Cd (0.03±0.00) in water samples at 95% confidence level while station 9 had significantly highest levels of Pb (2.13±0.80) and Cd (1.03±0.50). An obvious explanation for this result is that the Damavand River at upstream points receives lower amounts of wastewater effluents, due to location of the industrial unit in the downstream part of the river. An earlier study on Tigris River, Turkey also reported that heavy metal concentrations in water samples at sites situated downstream of the copper mine plant were higher than the other sites (Varol and Sen, 2012). A decrease in metal levels at station 10 is probably due to a dilution process. The nonsignificant differences between levels of Cd and Pb in some stations indicate that these metals originate from the same source of pollution. The concentrations of heavy metals in this study were higher than those reported by Salati and Moore (2010) in the Khoshk River at Shiraz, Iran and those found in Sava River in Serbia (Vukovic et al., 2011).

The comparison of metal levels in the presents study with international standards revealed that Cd and Pb concentrations in water samples from all the stations were above standard values of 0.003 mg/lit Cd and 0.01 Pb mg/lit in drinking water set by World Health Organization (WHO) and 0.005 mg/lit Cd and 0.015 Pb mg/lit suggested by Environmental Protection Agency (EPA) (WHO 2008; USEPA, 2009). The same condition was reported by Owamah (2013) in a petroleum impacted river in the Niger Delta region. In a study from Sardabrud River in north of Iran, the levels of Pb and Cd were recorded lower than the maximum permitted concentration for drinking water quality set by WHO and EPA (Reyhani et al., 2013).

According to *Table 2*, there was a marked decrease in water temperature (from 24.89±0.51˚C to 6.07±0.35 ˚C) from August to December. The mean levels of temperature across the sampling months followed the order: August= September= July> October> November=December in accordance with Duncan test results. Generally,

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warmer water temperatures in aquatic ecosystems (more than 25 ˚C) may affect the toxicity of some chemicals in the water (Sibanda et al., 2014); however, the temperature range in this study was lower than 25 ˚C. Significantly ($P<0.05$) lower mean level of pH (7.46±0.32) was recorded in August while significantly ($P<0.05$) higher levels (7.95±0.25) were observed in September. pH values in this study were within the permissible pH ranges for drinking water recommended by WHO (WHO, 2008). A study on river waters in western Tamil Nadu, India indicated that pH levels of the waters were within the acceptable ranges for aquatic life (Rajiv et al., 2012). Data also shows a significant ($P<0.05$) lower level of electrical conductivity (701.56±96.60) in October and significant ($P<0.05$) higher mean level of this parameter (826.33±11.88) in August. The levels of electrical conductivity in this research were higher than that in Densu River of Ghana (Karikari and Ansa-Asare, 2006).

As we can see in this graph, the mean concentrations (mg/lit) of Pb in the samples were higher than Cd over the studied months. The highest mean levels of Pb (0.90±0.56) and Cd (0.60±0.40) were found in November. The lowest values of Pb (0.16±0.10) were recorded in September while lowest levels of Cd (0.07±0.03) were in October; however, ANOVA showed that there were no significant variations between the levels of both metals in the months at 95% confidence level. This result could be explained by the fact that there was heavy rainfall in both summer and autumn seasons in the study area that resulted in distribution of metals within the river water .In a previous research study on evaluating heavy metals in Karoon River, Iran levels of heavy metals in water samples were reported higher during rainy months (Diagomanolin et al., 2004). A report from Ganga River in India showed significant seasonal changes in the levels of Cd and nonsignificant changes in the concentration of Pb during the studied period (Kar et al., 2008).

In conclusion, Damavand River has been seriously polluted by heavy metals due to industrial and municipal activities in the surrounding area. High levels of heavy metals (Pb, Cd) in water samples compared to WHO and EPA guidelines for water quality signifies potential health risks to consumers. Untreated discharges of urban sewage and industrial wastes are the main causes of poor water quality in this region. To reduce the existing quantity of metal contamination in the Damavand River, solid and liquid waste management practices are highly required.

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REINTRODUCTION OF *DACTYLORHIZA INCARNATA* (L.) SOÓ INTO THE NATURAL HABITATS OF THE EUROPEAN RUSSIA

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Abstract. Reintroduction of rare plant species into the natural habitats is one of the important trends of biodiversity conservation. The article presents the results of experiments on the reintroduction into the natural habitat of rare plant species *Dactylorhiza incarnata* (L.) Soó (*Orchidaceae*). During the experiment on the reintroduction of the species in 2012 an artificial population was grown in the Nizhny Novgorod region (Russia). According to the results of three years of monitoring, the success of reintroduction was evaluated. The main morphometric parameters, the ontogenetic structure of artificial population and carpological productivity were analyzed. Recommendations for cultivating the artificial populations of *Dactylorhiza incarnata* in natural habitats were developed.
Keywords: *orchids, rare species, reproduction in vitro, ontogenetic stages, reintroduction*

Introduction

Orchids is one of the largest families among flowering plants. Specific biological and ecological features, an active impact on the environment and the ruthless harvesting from the nature has led most species of this family to the edge of extinction. As a consequence, many orchids are recognized as endangered and vulnerable species. There are 30 species of orchids growing in the Nizhny Novgorod region (Biryukova et al., 2014), 8 of them are listed in the Red Book of the Russian Federation (Krasnaja kniga, 2005) and 21 in the Red Book of the Nizhny Novgorod region (Krasnaja kniga, 2008). One such species is *Dactylorhiza incarnata* (L.) Soó, which is also listed as «endangered» in many regional red lists of Central Europe and Scandinavia (GBIF, 2009).

Human impact on habitats of the most plant species causes degradation and impoverishment of vegetation, as a result, the problem of biodiversity conservation and reintroduction of rare and lost species is particularly relevant. Experimental research concerning the reintroduction of rare and endangered species is of great importance as components of a row of measures for the conservation of biological diversity. At the same time, it is very effectively to enrich phytocenoses with the species that need of protection within areas already protected (Gorbunov et al., 2008; Shmaraeva and Ruzaeva, 2009; Guerrant, 2012; Akeroyd and Jackson, 1995; Guerrant, 2013).

The aim of this study is the development and testing techniques of reintroduction and restoration of dying orchid populations on the example of *D. incanata* in the Pustynsky Reserve the Nizhny Novgorod region (European part of Russia). Under investigation the following targets were faced:

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-Germinating *D. incarnata* seeds in vitro, cultivation of plants and their preparation for experiments in reintroduction.

-Carrying out experiments on reintroduction of *D. incarnata* into natural habitat.

-Monitoring the transplanted population of *D. incarnata*.

-Developing recommendations basing on studies on the reintroduction of orchids in natural habitats.

Material and methods

Study species

The early-marsh orchid (*Dactylorhiza incarnata*) is a perennial herb with tubers, dissected by 2-5 (6) of the lobes. Stem is 25-55 (70) cm tall, hollow with diameter of 10 mm at the base, slightly thickened up to the top, slightly striated, and leafy top to bottom; 4-6 leaves are upward directed, pressed to the stem, narrowly lanceolate without spots. Inflorescence is a dense and many-flowered spike (of 17-74 flowers), 4-12 cm long and 2. 5-3 cm wide. Bracts are long, lanceolate, pointed, exceeding the flower. Flowers are small, reddish-purple. The outer tepals are oblong-ovate, obtuse with three veins. Two petals of inner whorl are oblique, ovate-lanceolate, obtuse. Labellum has purple-violet pattern of dots and dashes on the top, broadly rhombic, one-piece or three-lobe, obtuse (the length of labellum-4. 2-5. 5 mm, width-5-7 mm). Spur is paler than tepals slightly bent 5-6 mm long and about 1. 5-2 mm thick, slightly longer than the labellum plate. Fruit is a capsule with numerous seeds (Vakhrameeva, 2011). The high degree of species variability is noted by many researchers (Füller, 1972; Averyanov, 1983; Hedrén and Nordström, 2009; Vakhrameeva et al., 2008). There are many varying characteristics: height of the plant, shape, size and color of the leaves, bracts; color of flowers. *D. incarnata* combines many forms, varieties, ecological types and hybrids.

D. incanata distributed throughout Europe and is characterized as Eurasian (palearctic) species (Flora Europaea, 1980). In Asia, the area encompasses the territory, including Asia Minor, Iran, North Caucasus, Central Asia, Siberia, Mongolia and northwest China. In Russia, it found by the European part of Karelia to the Volga-Don region, the Lower Volga region, east of the Volga and the Pre-Caucasus, Western and Eastern Siberia to Yakutia (Vakhrameeva, 2011). *D. incarnata* is the species of wet grasslands. It grows mainly in wet and flooded meadows, on the banks of waterbodies, fens and marshs (Schrautzer at al., 2011; Zheleznaya, 2009; Barlybaeva, 2012; Barlybaeva and Ishbirdin, 2013). It may be founded in the forest-steppe regions, in the mountain meadows, coastal dunes, sometimes even in saline soils.

Study area

Nizhny Novgorod region is located in the central eastern part of the European part of Russia, in the center of the Russian Plain, covering the territory of 76. 9 thousand km^2. The territory stretches in the meridian direction of more than 400 km, reaching the north N 58°06' the south N 54°27'. From west to east the area extends for about 300 km from the E 41°48' to E 47°46' (Haritonychev, 1978).

The climate of the Nizhny Novgorod region is temperate continental with a warm and short summer, relatively cold winter, the prevailing western transfer of air masses and moderate moisture. According to climatic zoning Nizhny Novgorod region is a part

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of the eastern half of the largest in the Russian Plain climatic region-Atlantic-continental European temperate climate zone (Trent'ev and Kolkutin, 2004). Nizhny Novgorod region is located in zones of the southern taiga, mixed and broad-leaved forests and forest-steppe. South taiga and subtaiga (mixed) zones encompasses the left side of the Volga River, while broad-leaved forests and forest-steppe covers the right side (Gribova at al., 1980).

The State Natural Biological Reserve "Pustynsky" is located in the north-western part of the Arzamas district of the Nizhny Novgorod region. Its total area is 6200 hectares. The reserve is located on the border of the coniferous-deciduous and broad-leaved forests close to the islets of meadow steppes. The territory includes the river floodplains and large lakes. In the reserve there are over 700 species of plants, of the 1, 200 found in the region. More than 60 species are rare and relict or being at the border of its range (Bakka and Kiseleva, 2009). Coordinates of artificial population of *D. incarnata* are N 55° 39'56" E 43° 35'45".

There are small populations of *D. incarnata* being found on the territory of the Pustynsky Reserve, the population number varies from 1-3 to 5 individuals and more. Many factors impact on the species population: disperse distribution of individuals and populations, poor resistance to increased competition with neighbor species, excessive grazing and other human activities in the areas of its growing, collection of plants by people.

Methods

Seeds of *D. incarnata* were sown in the biotechnology laboratory of the Botanical Garden of UNN in vitro. For sowing unripe capsules (seeds inside them are sterile and do not require immediate sterilization) were used. Alcohol is used to sterilize capsules. Sowing was conducted out on sterile medium proposed by Malmgren (Malmgren, 1996), which does not contain inorganic nitrogen and consists of 16 amino acids. After this sowing flasks are kept in the dark in an air-conditioned chamber at 18°C. After the germination flasks are transferred to a refrigerator (4°C) for 3 months. Temperature conditions of cultivation alternate every 3 months (cool – cold) to simulate the change of conditions for vegetation. As using up the medium and developing seedlings they are transferred to other culture vessels as well. The duration of cultivation was 1. 5-2 years. As the seedlings develop up to immature stage (that is characterized with developed tubers) plants are transferred into wooden boxes (the size of 40 x 25 x 15 cm) filled with a mixture of soil, for adaptation and growing. It was made at the end of May-beginning of June (with the onset of summer temperatures being not less than 15°C) immediately after keeping in the refrigerator to stimulate growth processes. The soil substrate used is follows: 1 part crushed pumice (fraction 0. 5 cm); 1 part of crushed limestone (fraction 0. 5 cm); 1 part of perlite; 2 parts semi-humificated conifer litter (sifted through a large sieve). Boxes are dig into a seedbed. Seedlings were put into the shade and regular watered. In those conditions the plants had grown for 2 years. Such period is usually enough for plants to grow up to the adult vegetative (virginal) stage. Thus the plants grown are viable to transfer in natural habitats.

Experiments on transferring *D. incarnata* in the natural habitat were conducted in the territory of Pustynsky Reserve in 2012. Habitats were selected carefully, as well as sites for planting were prepared. In the selected sites seedbeds were made. There were boxes made of planks 1. 5 x 1. 5 x 0. 2 m, which dig in the trenches of the same size (their upper edges were placed at ground level). After that, seedbeds were filled with a soil

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substrate consisting of 3 parts of peat, one part of dolomite aggregate (crushed limestone fraction of up to 1 cm), 3 parts of the local soil. Then the specimens of *D. incarnata* were transferred in seedbeds. Each plant was separate by not less than 10 and not more than 20 cm from an adjacent (for higher density to reduce the competitive effect by "weeds"). After transferring, the plants watered. Seedlings were inspected to control and weed every month (3-4 times per the growing season). Next monitoring of artificial populations was carried out. The basic morphometric parameters of plants were measured: the number of leaves, length and width of the leaf blade for vegetative individuals and-the length of the peduncle, the length of inflorescence, the length of bracts, the number of flowers, and the number of formed capsules for generative individuals. Seed production was also calculated. All the values were compared with similar ones in natural populations. Also ontogenetic structure of artificial populations were analyzed. According to periodization of the species ontogenenesis several stages of development are distinguished. Protocorm – the initial stage of seedling. Plantlet (pl) – the stage is characterized by developing the bud with leaf primordiums on top of the protocorm. Juvenile (j) stage is characterized by developing the first green leaf and the first adventitious roots. Immature (im) individuals have 2-3 leaves with veins. The underground part is oblong tuber 0. 5-0. 7 cm long, sometimes bifid. Immature stage usually continues 3-4 years. Virginal stage (v) lasts 2-3 years, and is a transitional period to the generative phase of the life cycle. The plant has 3-4 leaves with the length of up to 18 cm with 10 veins. Tuber is with 3-4 lobes. Generative (g) individuals have 5-6 leaves with the length of 14-17 cm, the number of veins increases to 12. Tuber has 4-6 lobes of 1-1. 2 cm long. Generative individuals can be divided into three groups-young, middle-aged and old, differing in the number of flowers per stem (Vakhrameeva, 2000). Monitoring artificial populations the ontogenetic status of each individual was determined and ontogenetic spectrum was designed every year.

Results

Experiment on horticulture cultivation and reintroduction of *D. incarnata* in natural habitats consists of several stages:

- -sowing seeds on sterile culture medium in vitro;
- -growing seedlings;
- -the adaptation and growing the seedlings to adults;
- -transferring individuals in natural habitats;
- -monitoring of artificial populations and estimation of success of the species reintroduction in the natural habitats.

Seeds of *D. incarnata* germinate well in a culture medium; their germination rate is 60%. When cultivated in vitro, protocorms are able to vegetative propagation under the impact of hormones, as a result the number of cultivated individuals can be significantly increased (Kryukov et al., 2010, 2011). After transferring into containers the adaptation and growing the seedlings to adults were conducted in the Botanical Garden of UNN during 2 years.

Experiments on the reintroduction of the species were started in 2012: 81 individuals were transferred into natural habitats – the survival rate was 100%. All the plants have got acclimatized and were successful in the adult vegetative (virginal) ontogenetic stage. In the second year (2013) 12 species flowered and developed viable seeds. In

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2013, the second site was set up, where another 81 plants were transferred, and the survival rate in the first year was 100% as well. Thus, in 2013, the artificial population consisted of 162 plants. The analysis of population trend has shown the death of six individuals at two sites in 2014, and another 36 individuals – in 2015. Thus, the artificial population consists of 120 individuals that are 74% of the original number. It should be noted that growing next to each other individuals were usually dying. Death can be caused by infection, dry soil, and competition for resources. But there is a possibility that after the first flowering plants have gone into a state of secondary dormancy (Gribova, 1980).

To evaluate the introduction success, the following morphometric parameters are used: for the virginal individuals – the length and width of the leaf blade, for the generative individuals-stem height, inflorescence length, number of flowers per stem, number of developed capsules and number of seeds per capsule. These figures were compared with plants from natural populations (*Fig. 1*).

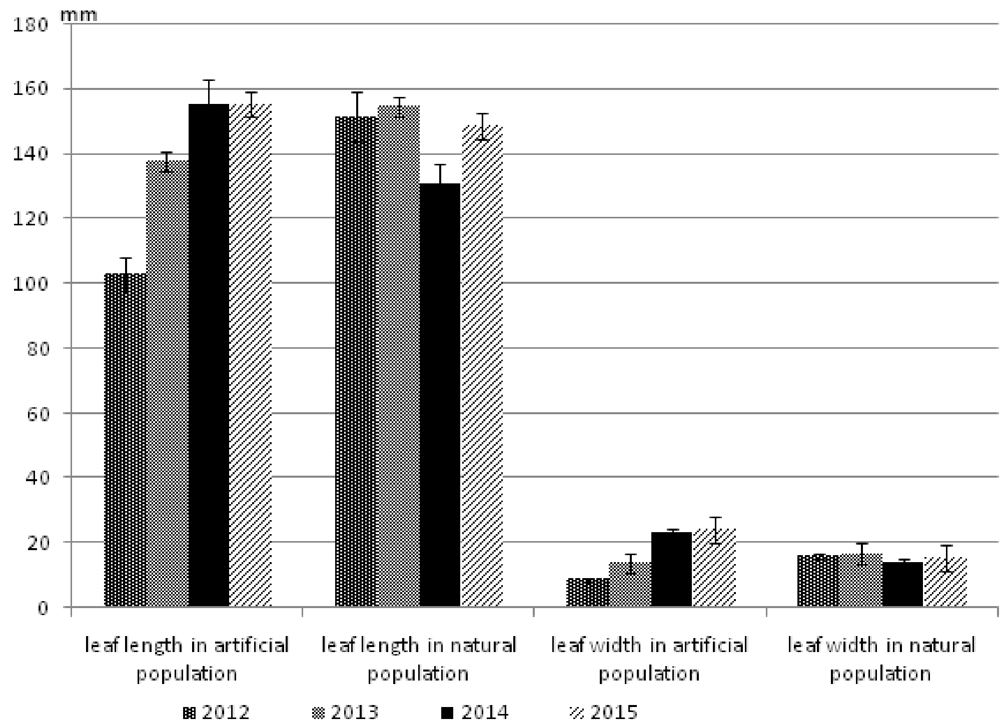

Figure 1. *General morphometric parameters (length and width of the leaf) in the artificial and the natural populations for 2012-2015*

Data analysis showed the average length of the leaf blade being 102. 7 ± 3. 5 mm and an average width of the leaf blade – 8. 8 ± 0. 9 mm in 2012, 123. 1 ± 4. 2 mm and 17. 6 ± 1. 4 mm in 2013 accordingly. In natural populations, those figures were 154. 6 ± 4. 4 mm and 16. 8 ± 2. 5 mm. In 2014 and 2015, the length and width of the leaf of reintroduced individuals exceeded similar parameters of individuals from natural populations (*Fig. 1*). The data obtained indicate active and successful growth processes of transferred plants. In generative individuals most variational character was the height of generative stem – it varied from 198 to 445 mm, averaging 385. 3 ± 2. 5 mm. This

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range of values may indicate the heterogeneity of the artificial population and the successful use of a particular microsite caused by their adaptation to the natural conditions. In natural populations that value was some higher and averaged 583 ± 1. 7 mm. However, the measured individuals of natural populations could be in the generative old ontogenetic state, whilst the reintroduced individuals were generative young. The same reason can cause a decrease in numbers of flowers in the inflorescences – this value averaged 30 ± 1. 9 in the artificial population and 57 ± 1. 4 in natural ones. The number of capsules was also less in artificial populations, averaging 14 ± 0. 9, than in natural ones (27 ± 1. 3), as well as the number of seeds in capsules: 6775 ± 147. 3 in the artificial population and 8305 ± 201 in natural ones. However the artificial population was recorded fruiting for the first time.

The *Table 1* shows the number of flowers and capsules in individuals of the natural population being higher than in the artificial. But it should be kept in mind that the intensity of orchids flowering and fruiting depends largely on age. Comparing these values have been obtained we can confirm the success of flowering individuals of the artificial population. They had flowered the next year after transferring into natural habitats. Five individuals of 12 had more than 30 flowers per stem; the maximum number of flowers was 44. The average number of fruits was 22. 9 ± 5. 3.

Table 1. *Morphometric characters of studied populations*

Characters	**Artificial population**	**Natural population**
Generative stem stature, M±m (mm)	385. 3±2. 5	583±1. 7
Length of inflorescence, M±m (mm)	115. 5±1. 8	130±2. 2
Length of bracts, M±m (mm)	22. 6±0. 7	23±0. 3
Wight of bracts, M±m (mm)	5. 5±0. 03	4. 7±0. 06
Number of flowers, M±m (ind.)	30±1. 9	57±1. 4
Number of unfertilized flowers, M±m (ind.)	8±1. 3	15±0. 9

According to the analysis of morphometric characters of seeds and their comparison between the artificial and the natural populations it can be said that much of a difference in the size of seeds was not observed. There is a significant difference in the number of seeds per capsule and, as a result, the difference in the seed production. Seed production was 149614. 6 ± 123. 4 seeds per individual in the artificial population and 348810 ± 257. 2 seeds per individual in the natural one. The share of developed capsules was similar in both populations (73. 6% for the artificial and 73. 7%, for the natural population). The share of full-grown seeds with embryo in the artificial population was 91% and 89% in the natural one.

The analysis of fruit and seed production for artificial and natural populations shows the effectiveness of pollination, full development of flowering individuals, high rates of fruit and seed production, high quality of seeds as well.

The analysis of ontogenetic structure showed the artificial population predominating by virginal individuals against the background of a little number of immature and generative plants in 2013 (*Fig. 2*).

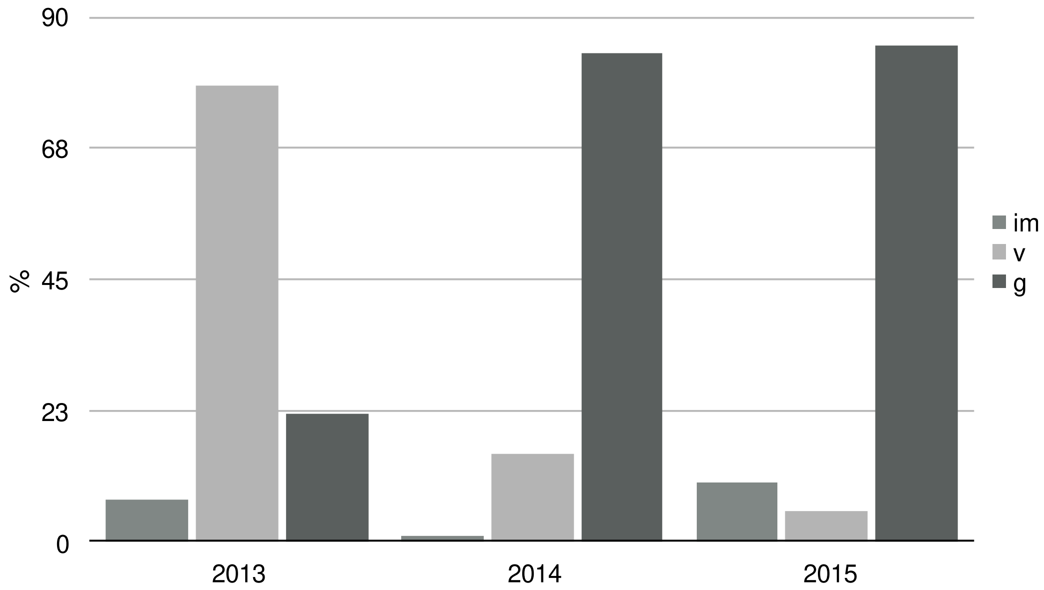

Figure 2. *Ontogenetic structure of the artificial population in 2013-2015*

In 2014, the structure had changed: the generative individuals began to dominate. In 2015, the ontogenetic spectrum was also characterized by the predominance of generative individuals and a high percentage of immature plants. The origin of these individuals is unclear. Perhaps they have sprouted from seeds or secondary protocorms, which had developed, when were cultured in vitro, and rested on tubers, or the generative individuals after their first flowering and a period of dormancy have turned into the immature stage. So at the moment, the population has consisted of individuals of different ages and been sustainable.

Discussion

The criteria for assessing the reintroduction success divides into of the short-term and long-term ones (Valee at al., 2004). The short-term criteria are follows: more than 70% of transferred plants survive, providing the genetic diversity of the population; the artificial population has similar characteristics to the wild populations; transferred plants survive to reproductive stage, develop flowers and fruits; the level of reproductive yields and seed viability is close to that of wild populations. Long-term criteria: the seedlings developing; the number of individuals within a population stabilizes or increases; an adequate level of biological diversity, particularly genotypic, is preserved, when generations changes (Gorbunov at al., 2008).

We have created an artificial population of *D. incarnata* meets all the short-term criteria of success. The artificial population number in the third year of monitoring was 74% of the original. The morphometric analysis showed the positive dynamics of growth processes for individuals have transferred for three years. The number of leaves,

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their length and width were increasing. These figures are similar or in some cases higher than the same in natural populations. In the developed population a significant number of individuals became generative (i. e. flowered and fruited) in 2014-2015. According to the published data (Vallius at al., 2007), the success of pollination depends on the number of flowers and density of individuals. In this case, the number of flower was lower than in the natural populations, however the density was higher, which contributed to attract insect pollinators. The number of seeds produced was similar to figures in nature population, while the share of seeds with developed embryo exceeded these figures in natural populations.

In many ways, the reintroduction success is determined by careful selection of the right conditions for the artificial population growing (Sprunger, Prendergast, 2010). *D. incarnata* like most orchids of moderate cold climate is sensitive to changes in edaphic conditions and characterized by weak competitiveness. Therefore, in addition to the selection of typical habitats for this species (it is optimal if several wild individuals have already been in the site selected) before transferring the sites were carefully preparing.

Concerning the environmental conditions *D. incarnata* belongs to the species of wet meadows and marshes (Vakhrameeva, 2000; Schrautzer at al., 2011; Zheleznaya, 2009; Barlybaeva, Ishbirdin, 2013). In the Pustynsky reserve it distributes in wet and floodplain meadows along the banks of waterbodies, fens and marshes. Therefore, the hydrological regime is one of the species limiting factors. Populations of *D. incarnata* we have found near the Staraya Pustyn village is located are in the meadows, on the territory of the groundwater egress. In such areas the soil is waterlogged and, as a rule, this species grows on the border of the drained and wet areas. Choosing a site for artificial population all these features of the natural habitat of *D. incarnata* were taken into account. As a result, selected site was as close as possible to the actual habitat with waterlogged soil. The site is located in the karst depression with the groundwater egress on the protected area of water well, belonged to the biological station of UNN. The angle of slope is 5°, the exposure is southwest. The dominants of herbage are follows: *Scirpus sylvaticus* L., *Deschampsia caespitosa* (L.) Beauv. P., *Filipendula ulmaria* (L.) Maxim., *Equisetum sylvaticum* L., *Athyrium filix-femina* (L.) Roth.

According to the results obtained, we have developed recommendations to horticulture *D. incarnata* under conditions of moderate cold climate:

1. Plants for transferring in the natural habitats should be well developed and healthy. The best results are obtained when plants have been pre-grown for 2 years after their transferring from the nutrient medium. The planting should be carried out in the spring period. For the best pollination and fruit set in the artificial population is advisable to use the seedlings developed from germinated seeds of different populations.
2. Habitats should be selected carefully. D. incarnata grows in wet meadows and marshs often near the groundwater egress. Usually associated species are follows *Equisetum sylvaticum* L., *Scirpus sylvaticus* L., *Eupatorium cannabinum* L., *Filipendula ulmaria* (L.) Maxim, *Geum rivale* L. et al. It is desirable that the selected site has been inhabited by single wild individuals of *D. incarnata* (dying population) or at least the species had been registered in that site earlier.
3. To prevent competitive effects on cultivated plants the site should be prepared-a trench width of 60-70 cm, 20 cm deep is made with sides reinforced by planks. The trench is filled with substrate-peat (50%) and the local ground, purified

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from plants (50%), as well as the addition of dolomite aggregate 3. 0 liters per $1m^2$ of seedbed.

4. The transferred plant is separate by not less than 10-15 cm from an adjacent. In the process of horticulture it is recommended to loosen the upper layer of the soil and remove weeds carefully. During the dry season (if it is necessary) periodic watering is also recccomended, especially in the first year after planting.
5. To obtain a stable artificial population the seedlings should be planted repeatedly. The annual planting of seedlings should be done at least during 3 years. Thus, in a short period the population consisting of individuals of different ages has developed.

Conclusion

As a result of this study an artificial population of rare orchid *D. incarnata* was created in the natural habitat in the territory of the Pustynsky Reserve, which included 162 specimens of plants grown from seeds. Three years later, the population size was 120 individuals. The percentage of survival specimens was 74%.

According to analyzes of seed production and morphometry in the artificial populations of *D. incarnata* the significant variation in values was discovered, but at the same time it indicates a positive trend and successful adaptation of transferred plants in their natural habitat.

The analysis of the ontogenetic structure shows a steady spectrum and predicts a positive trend of the artificial population developing in the future.

To maintain the artificial population further transferring of seedlings and monitoring are needed.

Basing on the study results recommendations for the reintroduction of tuber orchids in natural habitats were developed.

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EFFECTS OF SPORT TOURISM ON TEMPERATE GRASSLAND COMMUNITIES (DUNA-IPOLY NATIONAL PARK, HUNGARY)

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Abstract. Effects of sport tourism were examined in dry grassland stands with fix sample plots in four sites of strictly protected areas of Buda Mountains and Pilis in Duna-Ipoly National Park in the vicinity of Budapest (Hungary). The size of all sample plots was 2×2 m in all sites, we designated 4×10 sampling quadrats and 4×10 control quadrats. The date of surveying was in May and July in 2013. The sampling sites were exposed to different type of illegal sport activities. The questions we tried to answer were which changes were caused by different sport activities, is the nature protection work in the surveyed areas effective enough. Data of sample and control plots were analyzed by multivariate analyses. The species composition of parallel plots of samples and controls were compared with their ecological parameters. According to the results of cluster analysis and redundancy analysis, the composition of species of the sample plots deviated from the control plots and the difference between them were at Pilis site the most considerable. In the sample plots the rate of uncovered soil surface was meaningful and the stony surface and the leaf litter cover were higher there than in the control plots. At the sample plots the distribution of social behaviour categories showed higher ratio in the categories of generalists, ruderal competitors and weeds, while the ratio in the categories of natural competitors and specialists decreased there. At the sample plots the life forms categories showed higher ratio in the categories of therophytes and chamaephytes than at the control plots.
Keywords: *degradation, sport tourism, grassland, nature conservation, coenology*

Introduction

According to researchers the main topic in leisure science is the sport tourism, which is developing the most dynamically in the sector of the tourism (Murphy, 1985; Edginton and Chen, 2009; Bánhidi, 2012; Leber, 2012). Sport touristic researches started first abroad in the 80's (Turco et al., 2002). The model of phenomenon of sport tourism was described by Turco who said that the natural habitats and sport activities are interacting. On the base of this model several researchers dealt with these interactions of natural habitats and sport tourism (Dobay and Bánhidi, 2009). The concept of 'natural habitats' was used like an essential condition of sport activities examined by this approach. The increasing popularity of outdoor sport activities will probably increase their impact on natural habitats too. Due to this we think that examinations of effects of active sport tourism will become increasingly important.

Temperate grasslands cover large areas of the Earth's vegetation (Coupland, 1992), and they are typical in the regions where the impact of global climate change is predicted to be high (Campbell et al., 2000; Deák et al., 2014; Valkó et al., 2014). The temperate grassland surface has large and increasing areas with arid climate. Even in the

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middle of Europe, Hungary has wide variety of temperate grasslands from nutrient-rich loess grasslands to temperate semidesert sand grasslands (Tóth and Hüse, 2014; Albert et al., 2014) in areas where the relatively low and unevenly distributed yearly precipitation results in temperate semidesert conditions (Fekete et al., 1988; Zólyomi and Fekete, 1994). The open and closed grasslands, cliff vegetation are important elements of the original vegetation of Hungary. Various communities developed on the different bedrocks in the Carpathian Basin after the last glacial period. Due to the former climate changes differences were realized in floristic composition, since these mountain grasslands acted as refugia for many species by providing special microclimate. The grassland stands of strictly protected areas of Duna-Ipoly Natural Park are really valuable and remarkable communities in the Carpathian Basin, but these stands are exposed to a high pressure of tourism because of their vicinity to Budapest. There is a wide range of sport activities done illegally in these sites: off trail hiking, mountain biking, motocross, riding ATVs, horse riding and paragliding.

We explain the changes of grassland stands in the study sites using fixed plots. We have set the objective to look for the answer to the question which effects cause sport activities in the surveyed areas.

Material and methods

The examined four sites are under the competence of the Duna-Ipoly National Park Directorate and were the following: 1st site was on the southwest slope of Sas-hegy (GPS coordinates: sample plots N47 28,551 E019 1,122 control plots: N47 28,549 E019 1,120), the 2nd site was on the Szállás-hegy close to Csiki-hegyek (GPS coordinates: sample plots N47 28,202 E018 55,569 control plots: N47 28,201 E018 55,572), the 3rd site was on the Kutya-hegy (GPS coordinates: sample plots N47 35,317 E018 50,337 control plots: N47 35,448 E018 50,623) and the 4th site was on the Pilis (GPS coordinates: sample plots N47 41,127 E018 52,263 control: N47 41,129 E018 52,267). The species richness of the open and closed grassland called our attention to the stands of *Cleistogeni-Festucetum sulcatae* (Zólyomi, 1958) association. The size of all sample plots was 2 × 2 m in all sites. The date of surveying was in May and July in 2013. The relative ecological parameters of the species composition detected of all sample plots were following ones: social behaviour types and life form categories. For statistical evaluation the ecological characters of species were collected from Hungarian Database 1.2 (Horváth et al., 1995). The values of ecological indicator numbers of species (Borhidi, 1995) were given in percentage pro rata.

The species composition of the sample plots was subjected to cluster analysis, using SYN-TAX 5.0 program with percentage differences-index (Podani, 1993, 1994) trying to explain the coenological meaning of the formed groups. A hierarchic (cluster) analysis was performed. To explain more the difference between the species composition of plots we used a constrained ordination method to make ordination diagrams. For the redundancy analysis (RDA) we used CANOCO 5 software (Šmilauer and Lepš, 2014).

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Results and discussion

The Sas-hegy site

In the centre of the capital city, there is an intact dolomite hill with several valuable, endemic and rare plants of the Carpathian Basin. This site is a popular tourist attraction due to the view. Tourists often leave the trail and even camp there at night. To avoid these activities, the management of the Sas-hegy Nature Reserve mounted surveillance cameras and hired a night guard. Below the top of Sas-hegy, on the southwest slope our fixed transects were located cca. 10 m from each other. The sample and control plots had 30° degrees of slope. In the dendrogram at D=56 dissimilarity value we have 5 groups (*Fig. 1*). In the 1st group (1, 2, 8, 3, 13) the following species are present: *Artemisia alba, Asperula cynanchica, Bothriochloa ischaemum, Euphorbia cyparissias, Festuca valesiaca, Minuartia setacea, Potentilla arenaria, Sanguisorba minor, Stipa joannis* and *Thymus pannonicus.* The 2nd group (12, 14, 15, 17, 16, 18, 20) is corporated from control plots with the presence of the following species: *Asperula cynanchica, Galium glaucum, Euphorbia cyparissias, Linaria angustissima*, *Sanguisorba minor, Silene conica* and *Stipa joannis.* The 20th plot is in connection with the others only with presence of *Chrysopogon gryllus.* The 3rd group with a single plot (11) is characterised by the highest cover value of leaf litter. The plots of 4th group are in the vicinity of other parts of the sample plots (4, 5, 6, 7, 9, 10). In the 4th group the higher cover value of *Euphorbia seguieriana* and the presence of *Scorzonera hispanica* are characteristic. The last group (5th) has only one plot with the presence of *Lithospermum arvense, Stipa capillata* and *Allium scorodoprasum.*

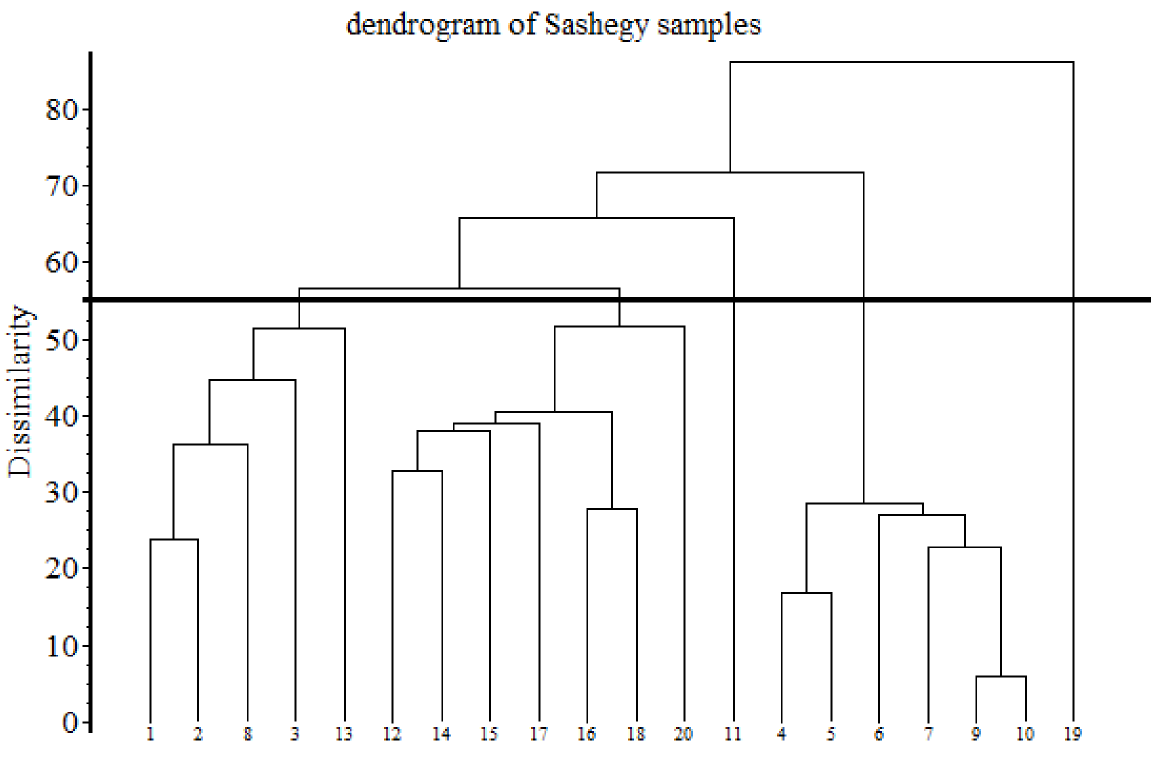

Figure 1. *Dendrogram of the sample (1-10) and control (11-20) plots of Sas-hegy*

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The distribution of life form categories shows more hemikryptophytes and chamaephytes in the sample plots than in the control plots.

The highest difference is in the category of therophytes, the ratio of therophytes in the control plots is significant (*Fig. 2*).

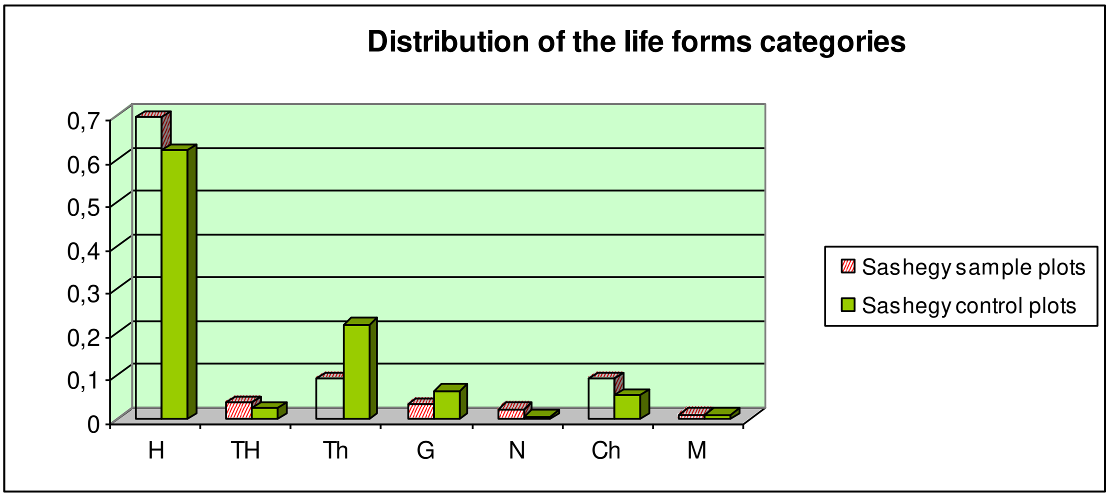

Figure 2. *Distribution of the life form categories of the sample and control plots of Sas-hegy*

The distribution of social behaviour types shows that generalists and stress tolerators are present in the greatest proportion in the sample plots. The ratio of natural pioneer species and specialists is lower in the sample plots than in the control plots (*Fig. 3*).

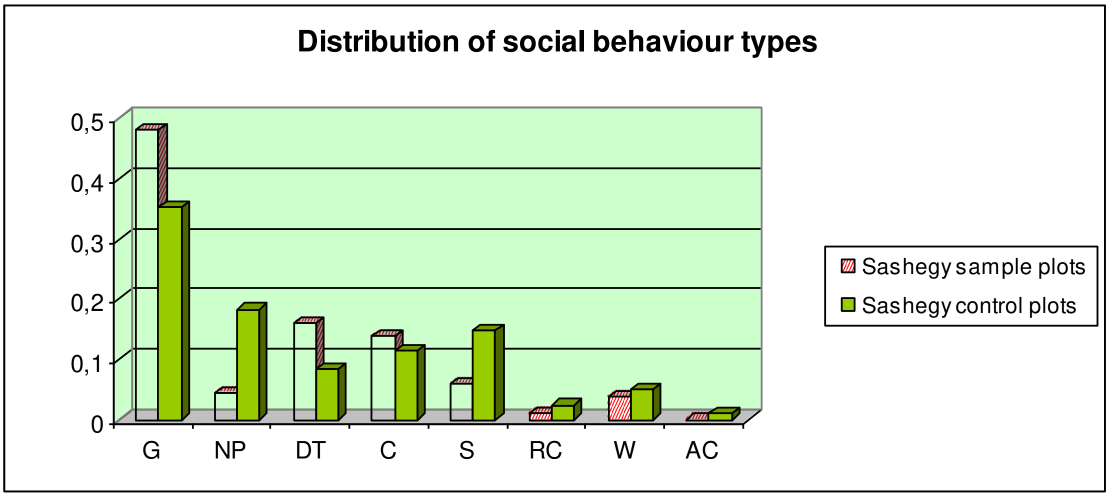

Figure 3. *Distribution of the social behaviour types of the sample and control plots of Sas-hegy*

The ordination diagram (*Fig. 4*) shows that the disturbed (D) and the control (C) plots are not forming two uniform groups according to the species composition. C1 plot is more similar to the disturbed ones; D9 and D10 are closer to the control plots than to the disturbed ones.

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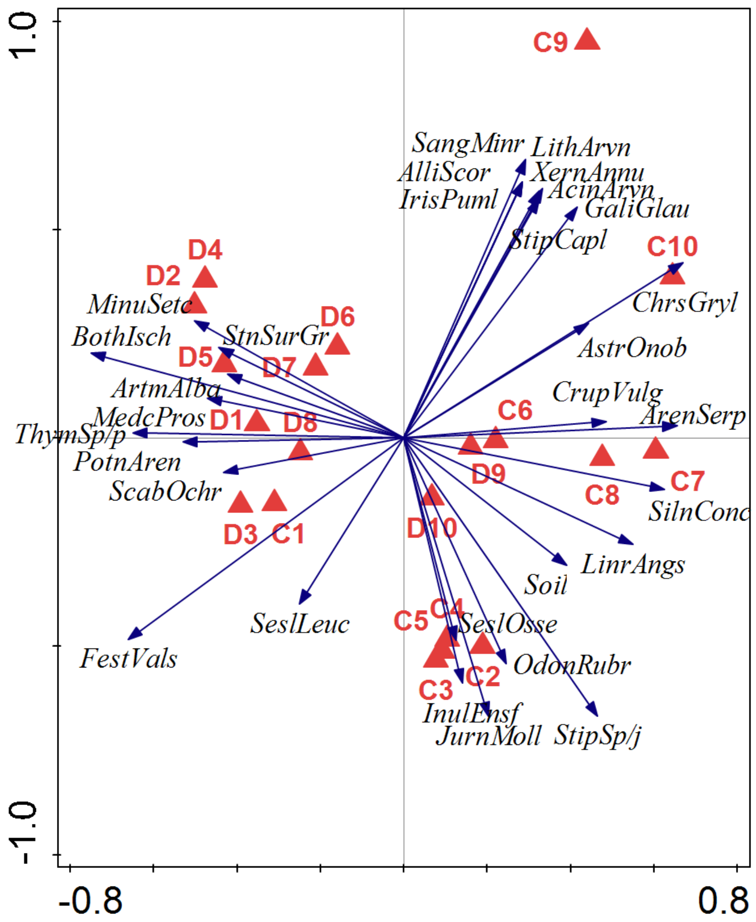

Figure 4. *RDA ordination diagram of plots of Sas-hegy (D-distrubed, C-control)*

The Szállás-hegy site

Szállás-hegy is a part of the ridge of the Csiki-hegyek, which is the southern part of Buda Mountains. The study area belongs to the Buda Protected Landscape Area. Despite strict protection, this area is traversed by more tourist paths. The biggest problems are caused by mountain bikers and motocross, because the thin soil layer of the dolomite surface is being snatched off. After damaging the plant cover, soil erosion starts quickly. The regeneration is nearly impossible on the thin soil and moving dolomite gravel. Despite the obligation of permission to visit this strictly protected area, we frequently met illegal bikers, off trail hikers, motocross and ATV riders close to our sample plots. There is no retentiveness of warning signs and traverses. The control plots were placed cca. 4 m from disturbed transect. In this area the grass layer was not disturbed but coherent.

In the trod area chosen a motocross-mountain biker path was clearly recognizable. 10 samples of 2×2 m plots were taken in this disturbed area (trod area) in a line transect form and 10 samples of 2×2 m plots of other transect was the control area without disturbance. (July of 2013, arranged in horizontally and parallel position).

The dendrogram of the two sample plots (disturbed and control areas) at D=35 dissimilarity value form 4 groups and one sample is strictly separate from the others

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(*Fig. 5*). The similar species composition and the mass of *Festuca rupicola* and *Inula ensifolia* bind the 1st and 2nd samples forming the first group.

The second group is composed from two parts. The first part consists control samples and the 8th and 9th sample plots. These plots are similar to control plots partly because of species composition, by the presence of *Anthyllis vulneraria, Centaurea sadleriana, Euphorbia cyparissias, Linaria angustissima, Muscari neglecta, Onosma arenarium, Plantago lanceolata, Teucrium chamaedrys* and *Thalictrum pseudominus* and the lack of *Euphorbia seguieriana, Fumana procumbens, Minuartia setacea, Potentilla arenaria* and *Scorzonera hispanica*, high rate of leaf litter and low rate of uncovered soil surface. The 8-9th and 12-14th plots are different from the other groups (15-20) with low cover of *Carex humilis* and *Teucrium chamaedrys* and high cover of bare soil surface. The 7th and 11th plots are similar to each other in the high cover values of *Stipa pennata* and form a separate group. The last group consists 3rd, 4th, 5th and 10th disturbed sample plots because of the presence of *Ajuga laxmannii*, *Alyssum montanum*, *Asperula cynanchica* species and the high cover of bare soil surface. In the degraded plots the cover of bare soil surface is higher than in the others. So the cover values are much smaller in degraded plots than the control ones. The cover values of *Carex humilis* are higher in the control plots than in degraded plots. These results are likely caused by the dry summer of 2013 and partly 2012. In Szállás-hegy site, the dry weather was likely a stronger factor than the erosive and/or disturbance processes in 2013.

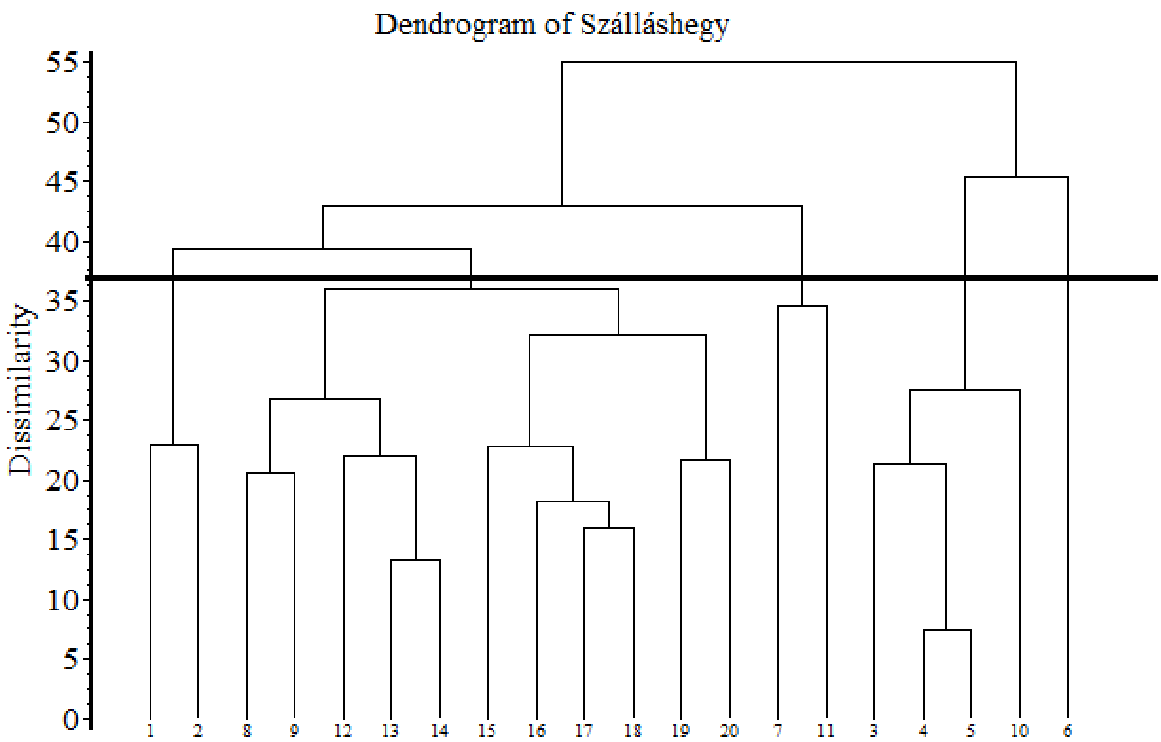

***Figure 5.** Dendrogram of the sample (1-10) and control (11-20) plots of Szállás-hegy*

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The distribution of life form categories doesn't show any significant difference between the sample plots and the control plots (*Fig. 6*).

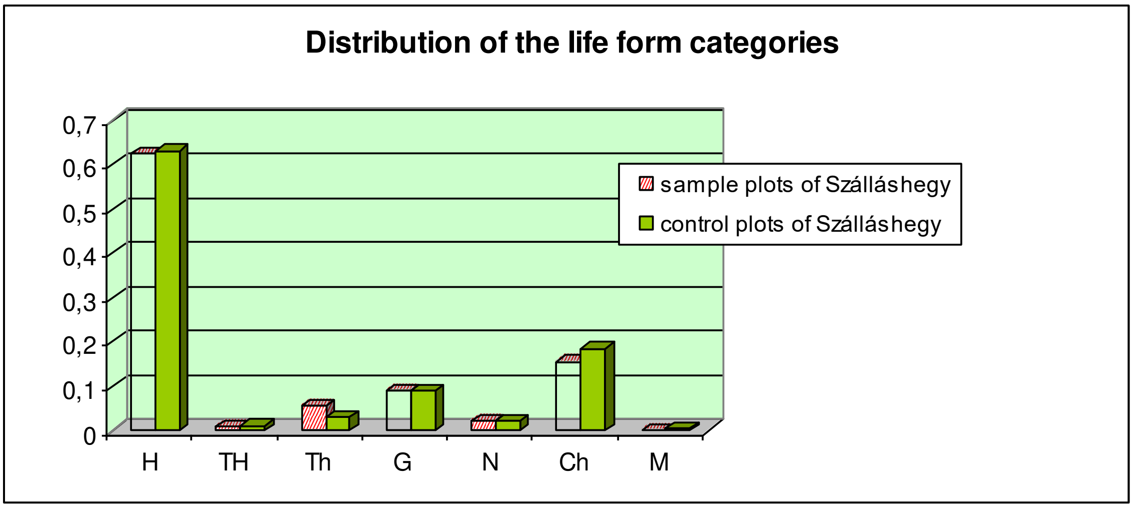

Figure 6. *Distribution of the life form categories of the sample and control plots of Szállás-hegy*

The distribution of social behaviour types shows that the ratio of generalists is significant higher in the sample plots than in the control plots *(Fig. 7).*

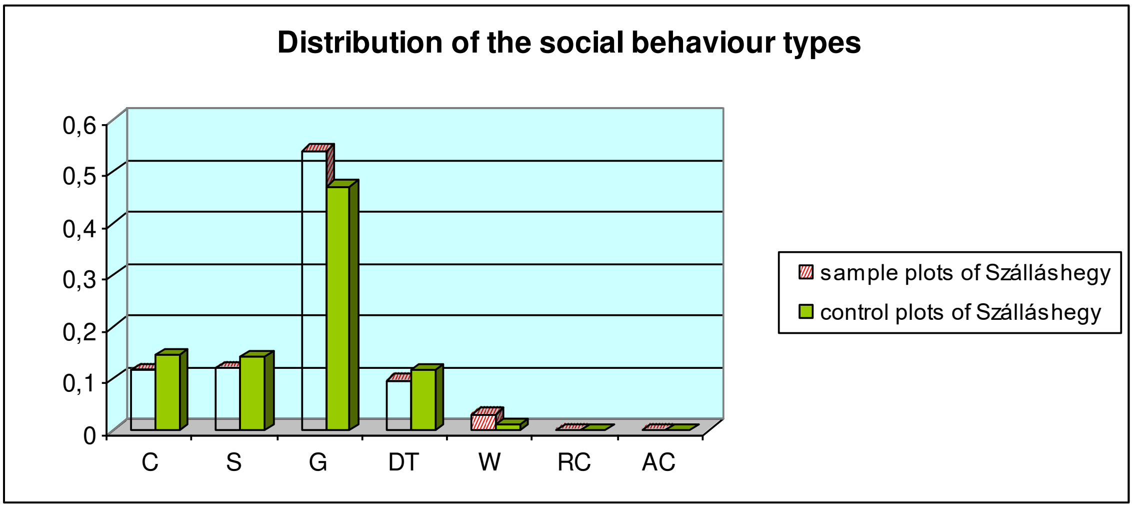

Figure 7. *Distribution of the social behaviour types of the sample and control plots of Szállás-hegy*

On the ordination diagram (*Fig. 8*) the most dividing variables are the proportion of leaf litter, uncovered soil surface, *Carex humilis* and *Festuca rupicola*. Beside of C3 and C4 plots, the disturbed and control plots are divided from each other.

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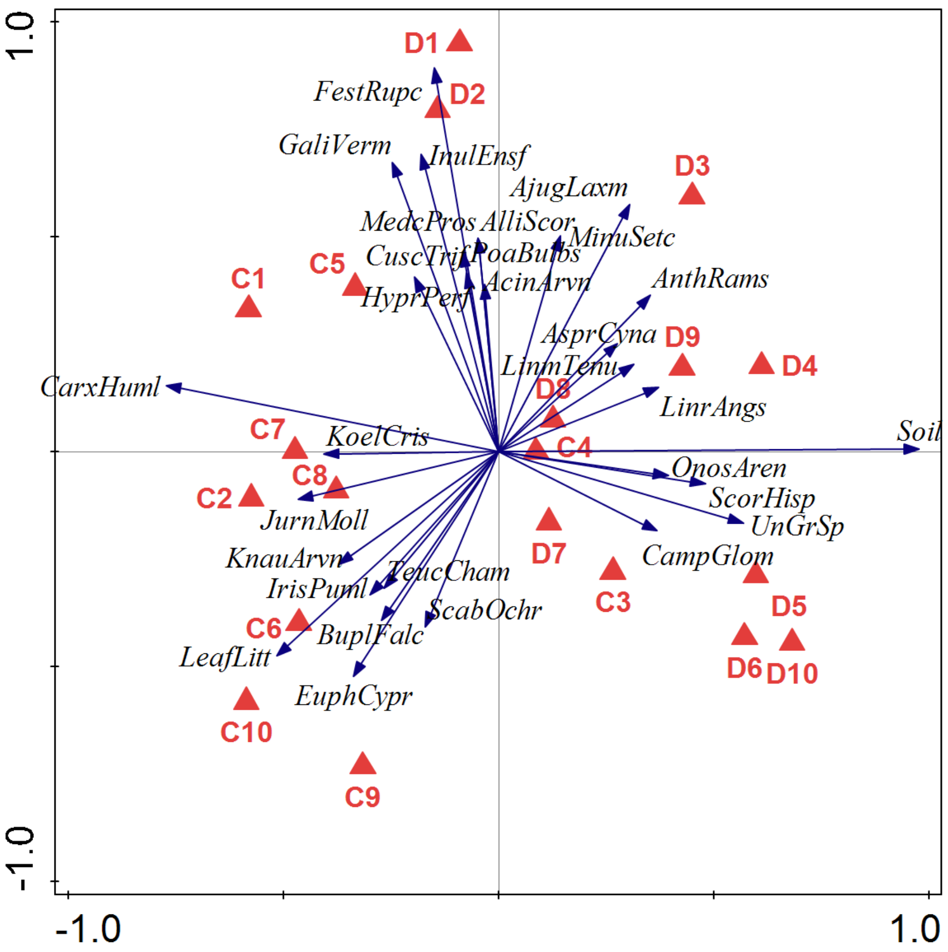

Figure 8. *RDA ordination diagram of the plots of Szállás-hegy (D-disturbed, C-control)*

An unexpected additional data according to Szállás-hegy site is that we found a new locality of the rare endemic species *Vincetoxicum pannonicum*. This rare species was first described by Borhidi and Priszter (1966) in the Csiki-mountains. According to the literature this species was found in the following places of Csiki-mountains: Ló-hegy, Futó-hegy, Szekrényes-hegy, Sorrento, Farkas-hegy, Odvas-hegy and in Fekete-hegyek. There is one known locality beside of the Buda Mountains in the Villányi Mountains (Harsány-hegy) (Priszter and Borhidi, 1967; Somlyay and Pifkó, 2002). According to the valid red list of IUCN, there are 4 known localities of *Vincetoxicum pannonicum* in the Buda Mountains in the present (Király, 2013). Therefore, our data means a new locality of the species. To find out more about the distribution of the species we searched the nearby hills and found two more new localities which did not have references yet. The three new localities are the following ones: Szállás-hegy, Út-hegy and Kő-hegy. The estimated number of individuals was different between the localities. On the Szállás-hegy there were approximately 150-200, on the eastern side of Út-hegy there were 75-100 and on the Kő-hegy there were 50-100 individuals. In several places we found plants which appeared to be different from *Vincetoxicum hirundinaria* and *Vincetoxicum pannonicum*. After we submitted our manuscript in 2013, later an article was published on the internet by Sramkó Gábor mentioning these localities too (http1).

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Kutya-hegy site

Close to Nagykovácsi (Buda Protected Landscape Area) the fixed samples were set on Kutya-hegy evidently near the tourist path. This region is a frequented resort used by hikers, horse riders and bikers. We observed several tourist groups leaving the paths and walking on the protected area, collecting flowers, including protected species, e.g. leopard's bane (*Doronicum hungaricum*). The control plots were set near to the tourist track in an undisturbed part of the area. On the dendrogram (*Fig. 9*) at value of D=35 we have 4 groups but the second group consists only one plot (2). The third group consists two smaller groups which are mixed from sample plots (8, 9, 10) and control plots (18, 20, 19, 11, 12). The first and fourth group contains only trod or control plots. In the plots of the first group the similarity is caused by presence of *Veronica verna,* high cover value of *Potentilla arenaria* and the low cover value of *Carex humilis.* The 2nd plot extremely differ from the 1st group because of the high cover value of *Fragaria vesca, Thymus glabrescens,* low cover value of *Sanguisorba minor* and lack of *Galium verum* and *Helianthemum ovatum.* In the third group there are mixed plots from sample plots (8, 9, 10) and some control plots (18, 19, 20). The following species are the same in these plots: *Euphorbia cyparissias*, *Galium verum* and *Iris pumila,* but they differ in presence or absence of *Achillea pannonica*, *Allium flavum*, *Asperula cynanchica*, *Botriochloa ischaemum*, *Cytisus nigricans*, *Filipendula vulgaris*, *Fragaria vesca*, *Helianthemum ovatum*, *Koeleria cristata*, *Medicago minima*, *Salvia pratensis*, *Sanguisorba minor* and *Thlaspi perfoliatum.* The 11, 12 plots are separated from others with high cover values of *Arabis hirsuta*, *Thlaspi perfoliatum* and *Viola tricolor* and lack of *Galium verum*. The 13, 14, 15 plots are cut from other groups because of higher values of *Botriochloa ischaemum.* The last group is composed from plots 16 and 17. Its position is caused by the higher rate of *Euphorbia cyparissias* and *Filipendula vulgaris.*

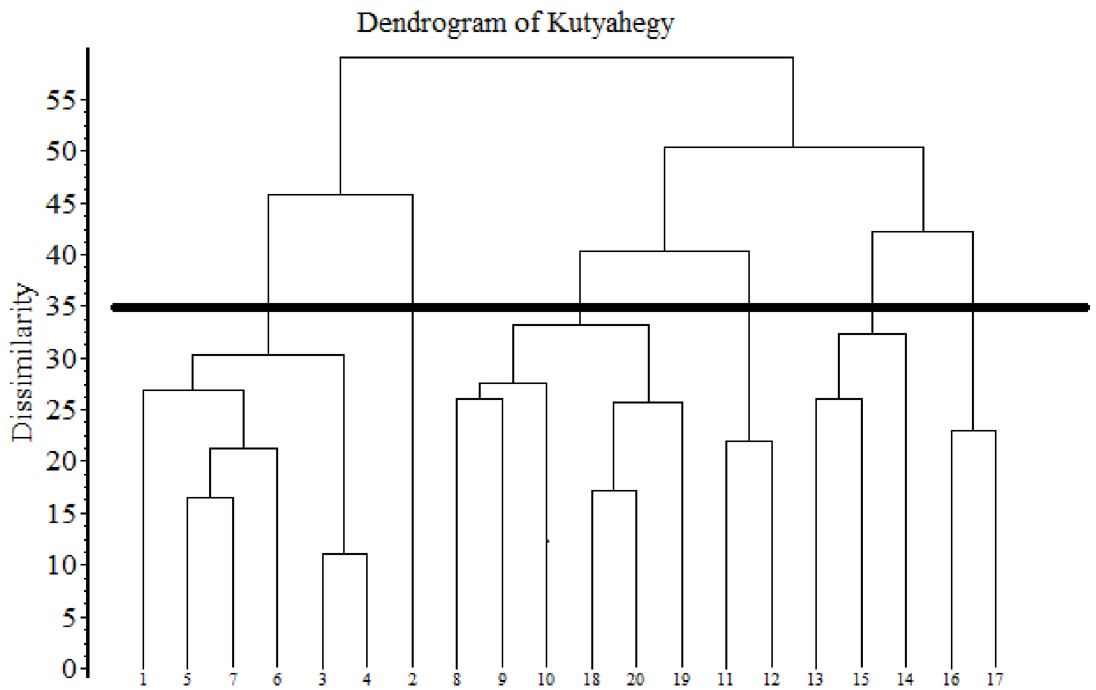

Figure 9. *Dendrogram of the sample (1-10) and control (11-20) plots of Kutya-hegy*

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The distribution of life form categories does not show any significant difference between the sample plots and the control plots *(Fig. 10).*

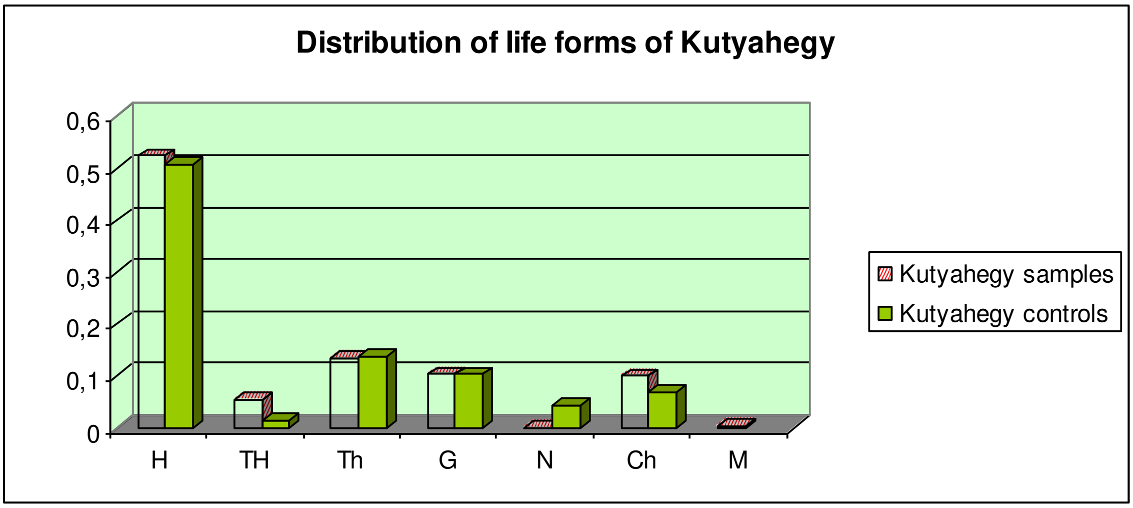

Figure 10. *Distribution of the life forms of sample and control plots of Kutya-hegy*

With regards to the distribution of plant species based on their social behaviour types, more generalists and fewer specialists can we observe in the sample plots than in the control plots *(Fig. 11).*

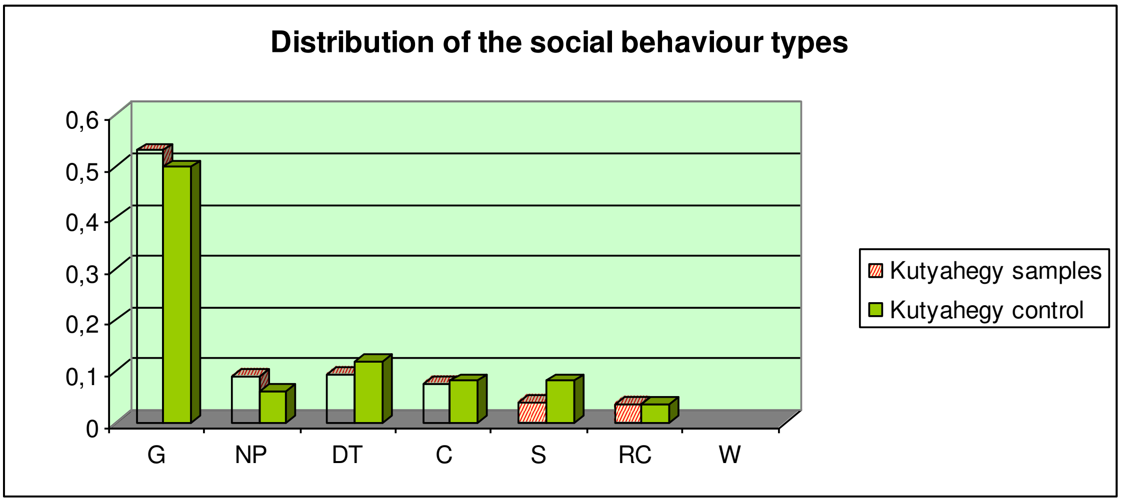

Figure 11. *Distribution of the social behaviour types of sample and control plots of Kutya-hegy*

On the ordination diagram (*Fig. 12*) there are 4 separate groups according to the species composition, but the control and the disturbed plots are divided from each other. According to the presence of *Carex humilis*, *Fragaria vesca*, *Veronica austriaca*, *Thymus glabrescens* and a few more species, some control plots are closer to some disturbed plots than to the other control plots.

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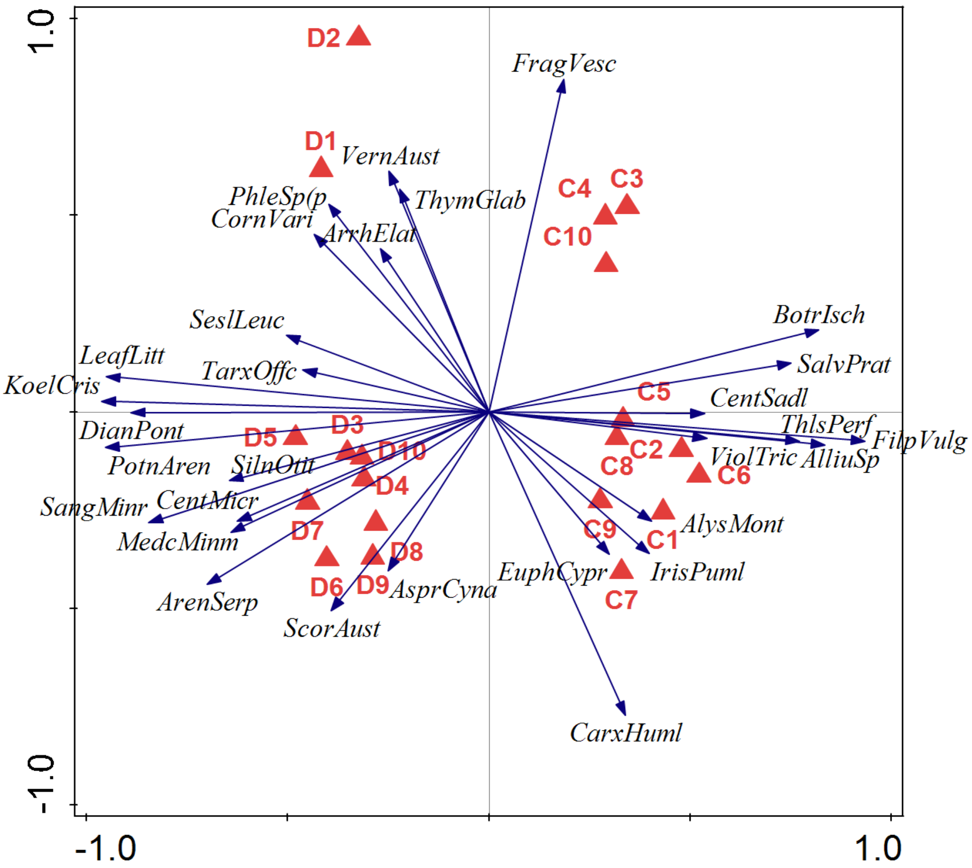

Figure 12. *RDA ordination diagram of the plots of Kutya-hegy (D-disturbed, C-control)*

The Pilis site

Pilis is the tallest peak (756 m) of the Pilis Mountains, so this is a frequented and beloved place for picnic and paragliding although it is part of the Duna-Ipoly National Park. Its plant communities are valuable and some of them are unique in the Carpathian Basin. There are some important localities of many rare plants of Hungary, e. g. *Dianthus pontederae, Orchis sambucina, Sesleria sadleriana* and the biggest population of *Ferula sadleriana,* which is a praeglacial relic endemism. The surveyed area of the peak in the last decades was a military post of Russian Army, so partly it was closed for the tourists. After the 90's the area was opened for tourists and paragliders, so this very valuable area of Pilis was impaired by treading acutely. The staff of the National Park Directorate settled a fence to defend this area from the grazing of mouflons. Some years later the grass layer of the peak of the Pilis changed to a „grass ocean" and the stand of *Ferula sadleriana* unfortunately decreased. Later the fence around the *Ferula* stand disappeared and probably now a new balance evolved. In these years a smaller part of the protected area is fenced because of paragliding. In the last years the paragliders destroyed the fence several times, removed the last barrier which could stop their illegal sport activities. Without the fence, this site is frequently visited despite the strict prohibition. The paragliders cut out shrubs and woods to "clear" their take off place, other visitors use to cut and collect wood to set up campfire in the middle of the highly protected area. The paragliding activity is actually the most serious risk to the local

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Ferula sadleriana population, by treading and pulling their equipments on grasslands the paragliders break the fragile stems of these plants. Our sample plots were taken at the tourist path also used by paragliders to jump off the peak. The control plots were placed 1.5 m in parallel from the sample plots. This year there were so many individuals of *Ferula sadleriana*, its stand may be firmed.

The dendrogram (*Fig. 13*) shows that the trod plots (1-10) stand apart from control (11-20) plots. The species composition of plots 1, 2, 4 and 10 has high rate of *Festuca rupicola* and a low rate of the soil surface that differ from the other trod quadrates. In the control plots the plot 11 strictly differs from the others because of the presence of *Orlaya grandiflora* and *Plantago argentea* and the higher rate of *Achillea pannonica* and *Thymus pannonicus.* In the plots 19 and 20 the higher cover value of *Geranium sanguineum* and a lower cover value of *Festuca rupicola* differs from the other control plots. The next species founder present only in the sample plots: *Artemisia absinthium, Berteroa incana*, *Bromus erectus*, *Bromus mollis*, *Capsella bursa-pastoris*, *Poa compressa*, *Poa pratensis, Poa trivialis, Polygonum aviculare, Silene vulgaris, Sisymbrium altissimum, Tanacetum corymbosum, Verbascum lychnitis, Veronica triphyllos* and *Viola arvensis. Campanula glomerata, Asperula cynanchica, Hypericum perforatum, Festuca valesiaca, Lolium perenne, Lotus borbasii, Medicago prostrata, Potentilla recta, Sanguisorba minor, Trifolium campestre, Scabiosa ochroleuca, Scorzonera hispanica, Silene conica, Tanacetum corymbosum* and *Verbascum lychnitis* only occur in control plots. In the sample plots the rate of uncovered soil surface is meaningful and the stony surface and the leaf litter cover are higher than in control plots. The specimens of *Ferula sadleriana* were found both in sample and control plots, but in the control plots the plants were higher and fertile. In the sample plots the *Ferula* plants were mainly short (5 cm) and dried.

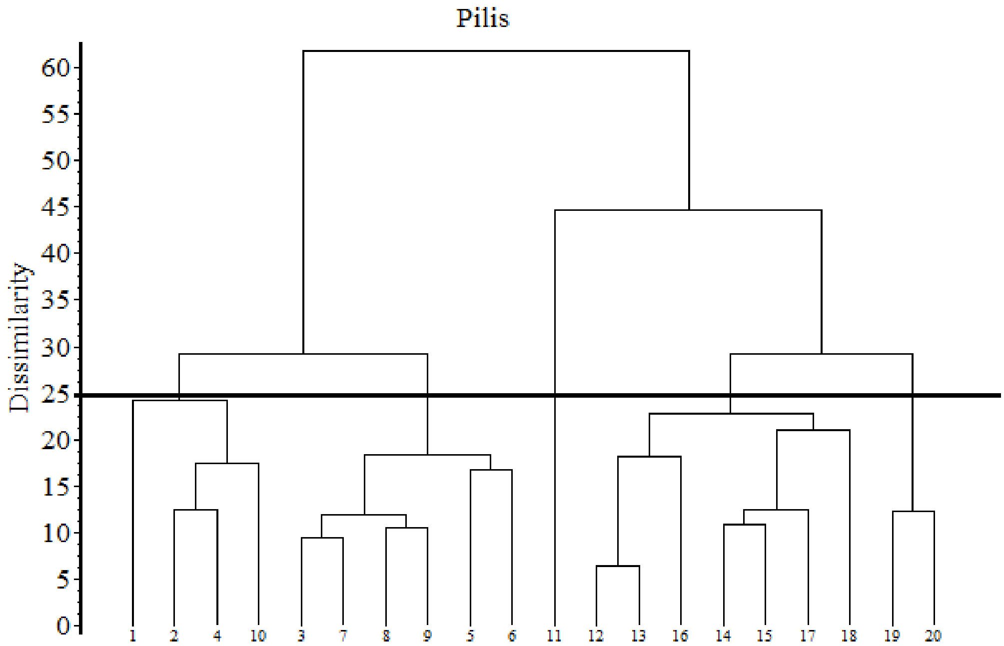

Figure 13. *Dendrogram of sample (1-10) and control (11-20) plots of Pilis*

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The distribution of life form categories shows significant difference in the category of hemikryptophytes and therophytes between the sample plots and the control plots. The ratio of hemikryptophytes in the sample plots is lower than in the control plots, besides, the ratio of therophytes is higher there than in the control plots *(Fig. 14).*

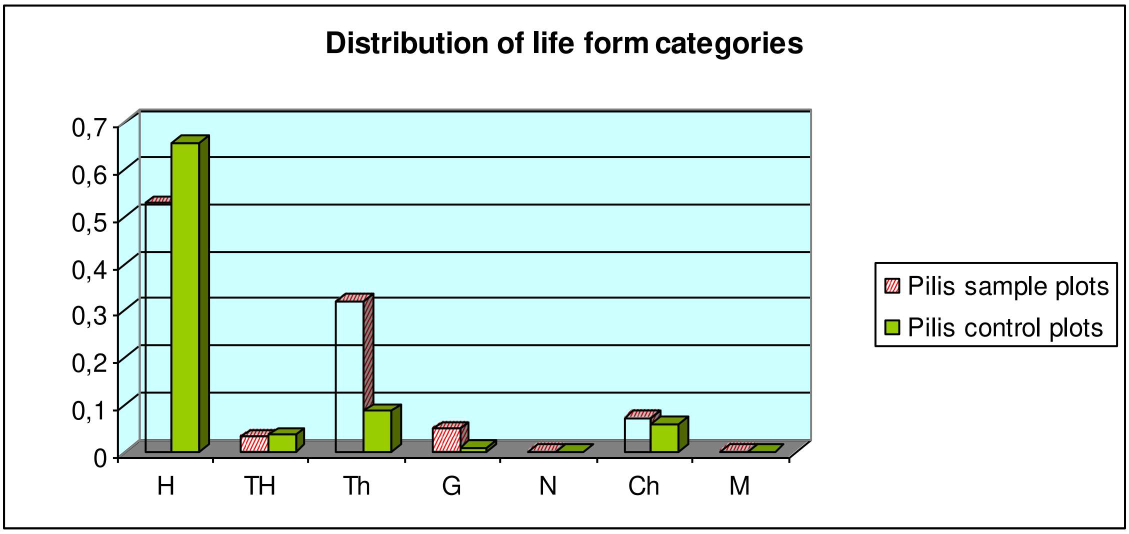

Figure 14. *Distribution of the life form categories of the sample and control plots of Pilis*

The distribution of social behaviour types shows that the ratio of stress tolerators, ruderal competitors and weeds is significant higher in the sample plots than in the control plots. There are fewer generalists, specialists and competitors in the sample plots than in the control plots *(Fig. 15).*

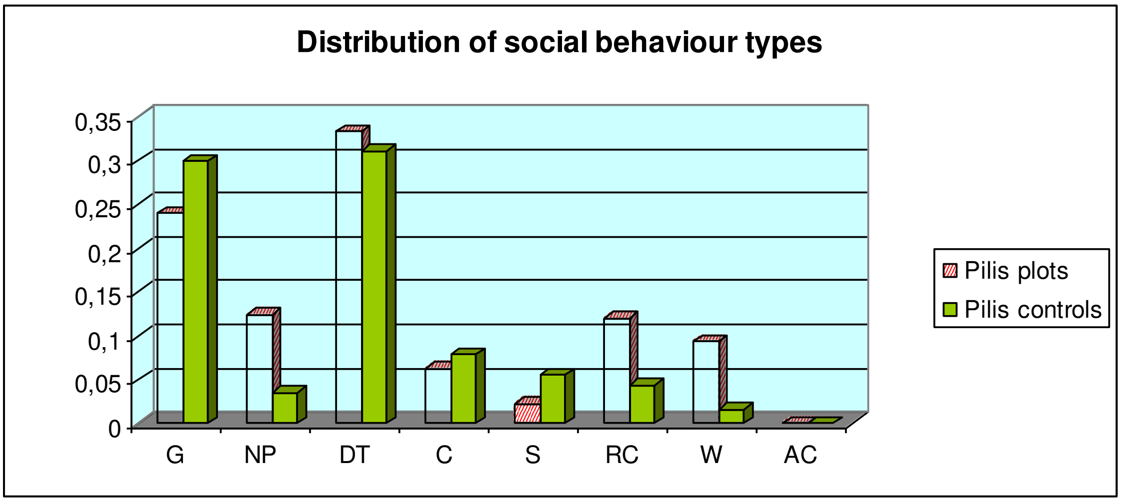

Figure 15. *Distribution of the social behaviour types of the sample and control plots of Pilis*

On the ordination diagram (*Fig. 16*) the disturbed and the control plots are divided from each other clearly, forming two uniform groups. Only the C1 plot differs with its species composition from the other control plots. The two groups of plots differ from

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each other mainly in the proportion of *Phleum phleoides*, *Elymus repens*, uncovered soil surface and *Veronica triphyllos*.

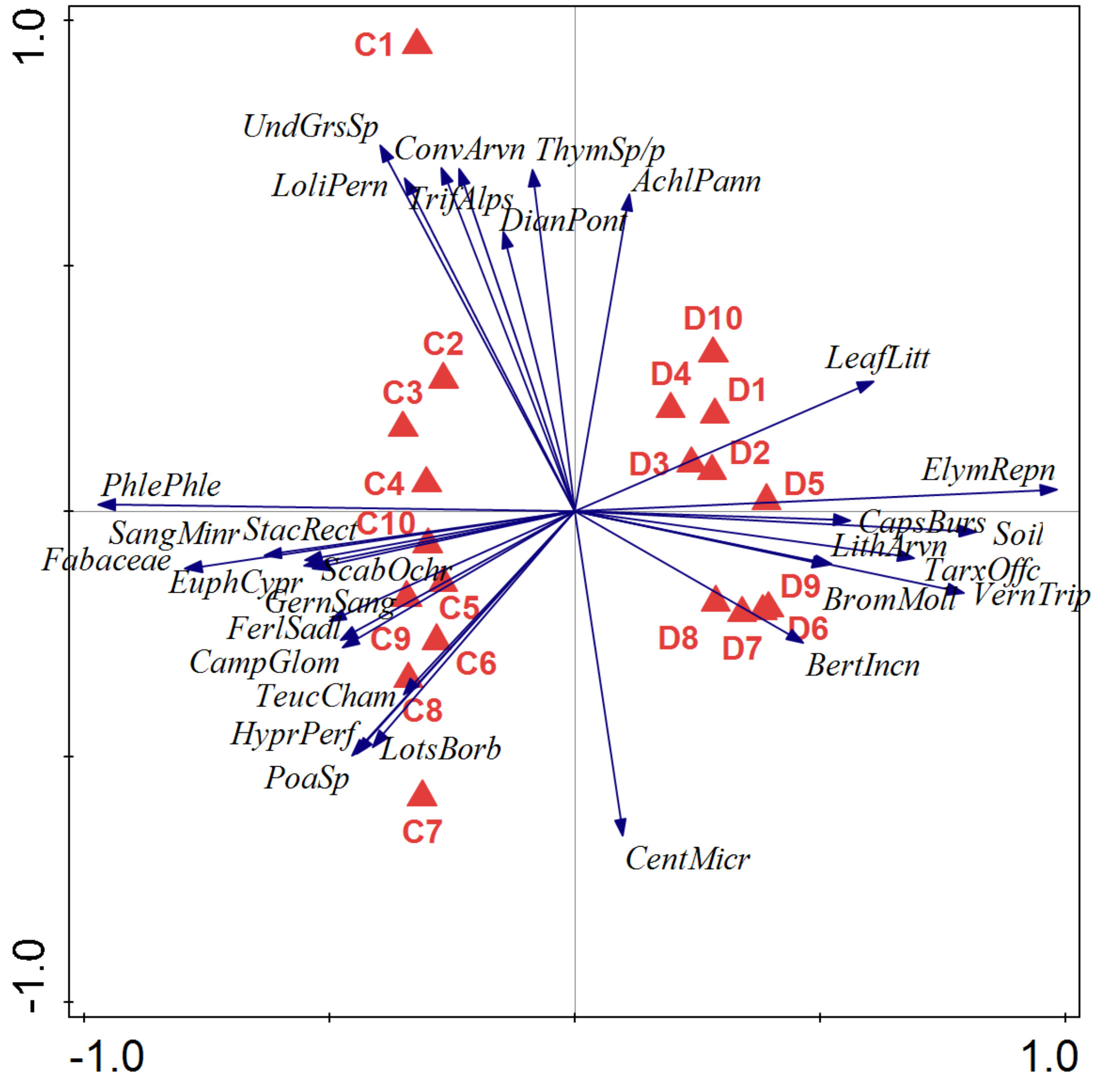

***Figure 16.** RDA ordination diagram of the plots of Pilis (D-disturbed, C-control)*

Conclusions

The distribution of social behaviour categories (*Fig. 3, 7, 11, 15*) normally shows higher ratio in the categories of generalists, ruderal competitors and weeds in the sample plots while the ratio in the categories of natural competitors and specialists decreases there. Thus, the number of species having higher naturalness value declines due to human influences. It was also found in urban habitats that species of natural grasslands were more typical in peri-urban habitats compared to the city centres characterised by higher human impacts (Deák et al., 2016). The life forms categories (*Fig. 2, 6, 10, 14*) normally shows higher ratio in the categories of therophytes and chamaephytes in the sample plots than in the control plots. Results of our sites shows complete distribution of trod and control plots of Pilis. In the sample plots the rate of uncovered soil surface

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was meaningful and the stony surface and the leaf litter cover were higher there than in the control plots. In the other sites this kind of distributions is not so clear in the sampling year because of extremely dry weather. These results are probably caused by the extreme dry weather during the summer of 2013 and partly in 2012. In sites of Szállás-hegy, Sas-hegy and Kutya-hegy the dryness was likely a stronger factor that year than the erosive and/or disturbance processes of treading. It was also found in other grassland types, that rainfall fluctuations can cause marked changes in the vegetation composition of dry grasslands (Lukács et al., 2015).

However, we can find similarities between the sample plots at different areas, too. *Potentilla argentea* besides *Festuca rupicola* seems to be frequent in the disturbed plots and the cover values of *Carex humilis* may be lower in these plots.

All of this leads to the conclusion that sport activities by human being can profoundly change the composition of the associations and the nature conservation work is very important in the controlling of weeds and in the preservation of protected plants. We should carry out more effective nature protection in these areas, especially in the very valuable site of Pilis.

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EXAMINING THE RELATIONSHIP BETWEEN ECONOMIC GROWTH, ENERGY CONSUMPTION AND CO_2 EMISSION USING INVERSE FUNCTION REGRESSION

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Abstract. Recently, the relationship between carbon dioxide emission (CO_2), aggregate energy consumption (EC) and economic growth (GDP) has been widely studied by many researchers using different approaches but the results were conflicting. Such controversy may due to the efficiency of the applied statistical approaches and using different dataset. The main objective of this experimental study is to examine the relationship between CO_2, EC, and GDP using different data transformation forms (natural logarithm versus inverse form) in reducing the heteroscedasticity in panel data. The panel data consist of 29 countries from two different economic levels of countries, 17 developed versus 12 developing countries. The data spanning from 1960 to 2008. A panel data approach is applied and estimations based on three models. First of all, the estimations are conducted by constructing three different models; First model is estimated by using the original data without any transformation, while the second and third model use the natural logarithm (Log) and inverse form to transform the data. Those two transformation forms are applied to reduce the heteroscedasticity problem. The main findings show a strong relationship between the three variables. The model with inverse function transformation is superior to the other two models using original data and Log transformation, as it has the highest R^2 which illustrates that more than 84% of CO_2 emission can be explained by GDP and EC. Since EC and GDP are influential on the CO_2 emissions, higher EC and lower GDP may lead to environmental problems such as air and water pollution. Therefore, prevention action should be taken to minimize the environmental degradation.
Keywords: *heteroscedasticity, panel data approach, economic growth, inverse form transformation*

Introduction

The relationship between carbon dioxide emissions (CO_2), aggregate energy consumption (EC) and economic growth (GDP) has been noticed growing attention in the recent energy economics literature ever afterward the crude oil prices had increased to double or ever more during the two energy crisis in the 1970s. Many researchers have examined that relationship by using different approaches but the results were conflicting. Such controversy may due to the efficiency of applied statistical approaches or using different dataset.

In the last few decades, rapid development has been observed plainly in many countries, and that is due to technology progress and industrialization etc. Besides, the energy resources such as oil, gasses and petrol are consumed in large scales as they are the main components need in the production of many goods especially in transportation,

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manufacturing and technology industry. A consequence, there are serious impacts towards the environmental degradation and in reducing the non-renewable energy resources. Thus it is very important to get a clear trend of that relationship to policymaker in monitoring the energy consumption/ efficiency and designing such a policy to minimize the trade-off effects of rapidly economic growth.

In light of the aforementioned literature, some of those studies used a bivariate framework or they included common variables in a single country or in a short panel of countries without considering the internal effects, and that has done by applying common methods. Therefore, this study is designed to overcome the shortcoming in the previous studies. To do that; first, we survey some of the related studies. Then, we detect the relationship between EC, GDP and CO_2 into two different groups of countries; developed and developing countries by using panel data approach with considering the data transformation by natural logarithm and inverse function to reduce the heteroscedasticity problem in the dataset.

To best of our knowledge this experimental study differs from earlier literature in several points; firstly, it is the first study uses original data without any transformation in analysis against (natural logarithm and inverse function transformation) in reducing the heteroscedasticity problem in the panel data which could provide more robust output. Secondly, it includes larger panel data in the analysis than that in previous studies, as it covers two groups of countries; (17) developed countries and (12) developing countries for the long time period from 1960 to 2008 in the multivariate framework, as the bivariate framework may suffer from omitted variable bias. Finally, it detects the different effects of (developed versus developing countries) into CO_2 emissions.

The remaining parts are organized as following; Section 2 overviews the strands of economic-energy literature. Section 3 provides data description. Section 4 explains the approach of panel data, and it provides the discussion of the empirical results. Section 5 recommends some suggestions to policymakers.

Literature Review

Seemly, there are three literature research strands which are interesting in the relationship between economic growth (GDP), energy consumption (EC) and environmental degradation, but some of them added other factors in the model such as; energy prices, capital, employment, foreign direct investment, industrial value added, agricultural value added and so on. The first strand is focusing on the relationship between GDP and environmental degradation which could be tested by environmental Kuznets curve (EKC) hypothesis. While the second strand is concentrating on causality relationship between EC and GDP. Finally, the third strand is exploring the relationship between GDP, EC and emissions. *Table 1* outlined some of the related literature.

The first strand of research is focusing on testing the EKC hypothesis. EKC is derived from original Kuznets Curve (KC) which is proposed by Simon Kuznets in 1955. EKC illustrates that in early stages of GDP the environmental quality is improving until a certain level (peak/turning point), then that case is reversed beyond the turning point, as it declines when GDP increase. This strand of literature is started by Grossman and Krueger (1991) who have applied EKC in path-breaking study of the potential influence of North American Free Trade Agreement (NAFTA) in the USA. The model includes SO_2, dark matter, and suspended particulate matter (SPM). The

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main findings support the existence of EKC for both SO_2 and dark matter, while there is a negative relationship between GDP and SPM. The turning points for SO_2 and dark matter are about 4000-5000 USD$, while the concentration of SMP appeared to decline even at low-income levels (negative relationship). Then this strand of literature followed by others. Majority studies support the EKC hypothesis (He and Richard, 2010), (Millimet et al., 2003), (Selden and Song, 1994), (Orubu and Omotor, 2011), (Alsayed and Sek, 2013), (Stern and Common, 2001), (Coondoo and Dinda, 2002), but some papers found there is no EKC existence (Liu, 2005), (Ghosh, 2010), (Fodha and Zaghdoud, 2010).

***Table 1**. The survey of some related studies to the relationship between GDP, EC and other variables*

Author	Methodology	Year	Scope	Additional variables	Findings and Results
Yu and Hwang (1984)	Sims and Granger causality	1947-1979 A	USA	EMP	GNP — EC EC → EMP
Masih and Masih (1997)	JJ, VDC and IRF	1961-1990 A	Korea Taiwan	Consumer prices	GDP ↔ EC GDP ↔ EC
Ghali and El-Sakka (2004)	JJ, VDC and VEC	1961-1997 A	Canada	Capital and EMP	EC ↔ GDP
Jobert and Karanfil (2007)	JJ	1960-2003 A	Turkey	IVA	EC — GNP EC — IVA
Odhiambo (2010)	Co-integration, ARDL and ECM.	1972-2006 A	South Africa Kenya Congo	energy prices	EC → GDP EC → GDP GDP → EC
Kaplan et al. (2011)	VECM	1971-2006 A	Turkey	Energy price, capital and labour.	EC ↔ GDP
Zaidi et, al. (2015)	Panel data	1960-2011 A	Austria, Sweden, Norway, France and Finland	GHG	GDP → EC GDP→GHG
Zaidi et, al. (2015)	Panel data and Granger causality	1950-2010 A	Austria, Sweden, Norway, France and Finland	SO_2	SO_2 ↔EC
Zaidi et, al. (2015)	Panel data	1960-2011 A	Europe countries	PM_{10}	GDP↔PM_{10}
Yang and Zhao (2014)	Granger causality	1979-2008 A	India	CO_2	EC → GDP EC → CO_2

Notes: The unidirectional causality, bidirectional causality and no causality between EC and GDP are represented by the symbols →, ↔ and — respectively. For the Abbreviations of methods; JJ: Johansen-Juselius causality test, VECM: vector error correction model. ARDL: autoregressive distributed lag bounds test. VDC: forecast error variance decomposition. ECM: error correction model. While the abbreviation of main variables and scope; GNP or GDP represent the economic growth. EC: energy consumption. IVA: Industrial value added. CO_2: carbon dioxide emissions. PM_{10}: particulate matter micrograms. GHG: greenhouse gases. SO_2: Sulphur dioxide. EMP: Employment.

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Moreover, the second strand of literature is concentrating on causality relationship between EC and GDP. The findings are restricted within four hypotheses; Feedback hypothesis which illustrates bidirectional causality between EC and GDP, that means there is a significant effect of EC into GDP and vice versa. Growth hypothesis which describes unidirectional causality running from EC to GDP, it suggests that EC may have an important role into GDP. Conservation hypothesis which supports the existence of unidirectional causality running from GDP to EC, as GDP may have influence into EC. Neutrality hypothesis which emphasizes that there is no significant effect between EC and GDP (Zaidi et al., 2015). An early study of this strand is conducted by Kraft and Kraft (1978) in the USA. The findings support the existence of unidirectional causality running from GDP to EC. Then it followed by others. Majority studies support the feedback hypothesis (Belke et al., 2011), (Eggoh et al., 2011), (Mahadevan and Asafu, 2007), but some support the growth hypothesis (Hossain and Saeki, 2011), while others support the Conservation hypothesis (Ozturk et al., 2010).

Furthermore, the third strand compiles the two above strands. This strand covers the related studies to the relationship between GDP, EC, and CO_2; (Arouri et al., 2012), (Omri, 2013), (Wang et al., 2011) and others. Most of those studies have report significant relationship between GDP, EC, and CO_2.

On the other hand, there are some studies focus on developed or developing countries. The studies based on single or panel of developed countries include (He and Richard, 2010), (Liu, 2005), (Millimet et al., 2003), (Selden and Song, 1994), (Belke et al., 2011), but some studies concentrates on developing countries; (Ghosh, 2010), (Fodha and Zaghdoud, 2010), (Orubu and Omotor, 2011), (Eggoh et al., 2011), whilst there are several studies have compared between developed and developing countries group; (Alsayed and Sek, 2013), (Stern and Common, 2001), (Coondoo and Dinda, 2002), (Mahadevan and Asafu, 2007), (Ozturk et al., 2010). By comparing the results, majority of studies support the existence of EKC hypothesis and bidirectional causality relationship in both of developed and developing group (Alsayed and Sek, 2013), (Stern and Common, 2001), (Ozturk et al., 2010), (Arouri et. al., 2012), (Omri, 2013). However, some results do not support a significant relationship between CO_2 and GDP in developing countries, but it existed in developed countries (Coondoo and Dinda, 2002). Moreover, there is bidirectional causality between GDP and EC in developed countries but it is not existed in developing countries (Mahadevan and Asafu-Adjaye, 2007).

Material and Methods

Scope of the study

This study contains several variables; the dependent variable is Carbon dioxide emission (CO_2) which measured by metric tons per capita. However, the independent variables are; Gross domestic product (GDP) per capita which measured by USD$, and Aggregate energy consumption (EC) which measured by kiloton of oil equivalent per capita. The study focuses on 29 countries which are divided into two groups; developed countries and developing countries, the classification is made based on World Bank definition. Developed countries group includes 17 countries; Austria, Belgium, Canada, Denmark, Finland, France, Greece, Ireland, Italy, Japan, Luxembourg, Netherlands, New Zealand, Norway, Portugal, Spain and Sweden. Developing countries group consists of 12 countries; Bulgaria, Eritrea, Ethiopia, Hungary, Iran, Jordan, Korea,

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Oman, Poland, Romania, South Africa, and Turkey. The panel data is obtained from World Bank website for annual data over the period 1960 to 2008.

Methodology

The heteroscedasticity problem in cross-section data exists when the variance of the unobserved error is not constant over a specific amount of time. The heteroscedasticity does not affect the estimated coefficients but it biases the variance of those coefficients. The Modified Wald test is applied to check the presence of heteroscedasticity as this test is more accurate even in the case of normality assumption is violated.

Panel data approach

The construction of Panel data is a combination of longitudinal data observed over a period of time. Panel data approach is applied to detect the relationship between the variables; GDP, EC and CO_2 by constructing three different models; First model is estimated by using the original data without any transformation, while second and third model transform the data by natural logarithm and inverse form, respectively. The best model fits the data is decided based on Coefficient of determination (R^2) and Root mean square error (RMSE).

One advantage of panel data analysis is to consider the spatial (individual) and temporal (time period) dimensions of the data, which allows to control the variation of time series and cross sections simultaneously, and it gives more robust regression. It could overcome the heteroscedasticity problems. Also, it allows covering more observations by pooling the time series data and cross sections which leads to the higher power of the test. Another advantage of panel data is controlling the individual heterogeneity which gives more informative data, less collinearity among the variables, more variability, more degree of freedom, more efficiency of estimate and broaden the scope of inference (Baltagi, 2005).

Panel data analysis follows several steps; First step is to estimate fixed effects models (FE) and Random effects model (RE), then compare them by Hausman test. After that, if the result is significant in favour to FE, we continue the analysis to compare FE with pooled model by the redundant test. If the later test is significant we opt for FE. If not, then the test suggests opting the pooled model. Finally, using White's robust estimation of the standard errors for FE could be performed to get a robust model for standard error against the heterogeneity.

The static panel model (pooled model) takes the form:

$$y_{it} = \alpha + x_{it}^{'}\beta + u_{it} \qquad \text{(Eq. 1)}$$

Where x_{it} is the independent variables with coefficients β , y_{it} is the dependent variable, i represent the county on cross section, t stands for time series dimension, α is both a time and an individual-invariant constant term, β is slope and u_{it} is the disturbance term.

Fixed effects model (FE) has the following formula:

$$y_{it} = \alpha_i + x_{it}^{'}\beta + u_{it} \qquad \text{(Eq. 2)}$$

$$u_{it} = \mu_i + \text{v}_{it}$$

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Where α_i is countries effects, taking into account the heterogeneity impact from unobserved variables and it may vary across countries, μ_i represents the unobserved countries effects for N cross sections, and v_{it} represents random disturbances.

On the other hand, the random effects model (RE) model is constructed as:

$$y_{it} = \alpha_i + x_{it}'\beta + u_{it} \tag{Eq. 3}$$
$$u_{it} = (\mu_i + \lambda_t) + v_{it}$$

Where λ_t represents unobserved time-series effects for T time periods.

The estimated models have the following formula based on the type of panel data effect; cross section and period effects after the transformation by natural logarithm and inverse form. The purpose of transforming the data is to induce the stationarity in data, and to reduce heteroscedasticity problem, so the estimated coefficients can be interpreted as elasticity estimates /percent of change. However, in case if the sample has zero or negative values, then there is no way to use the natural logarithmic transformation, which can be solved by applying the inverse form transformation to overcome the heteroscedasticity problem.

Cross section effect models:

$$\begin{aligned} CO_{2\,it} &= \alpha_i + \beta_1\, EC_{it} + \beta_2 GDP_{it} + \varepsilon_{it} \\ Ln\, CO_{2\,it} &= \alpha_i + \beta_1\, Ln\, EC_{it} + \beta_2 Ln(GDP_{it}) + \varepsilon_{it} \\ (1/CO_{2it}) &= \alpha_i + \beta_1\, (1/EC_{it}) + \beta_2\, (1/GDP_{it}) + \varepsilon_{it} \end{aligned} \tag{Eq. 4}$$

Period effect models:

$$\begin{aligned} CO_{2\,it} &= \gamma_t + \beta_1\, EC_{it} + \beta_2 GDP_{it} + \varepsilon_{it} \\ Ln\, CO_{2\,it} &= \gamma_t + \beta_1\, Ln\, EC_{it} + \beta_2 Ln(GDP_{it}) + \varepsilon_{it} \\ (1/CO_{2\,it}) &= \gamma_t + \beta_1\, (1/EC_{it}) + \beta_2\, (1/GDP_{it}) + \varepsilon_{it} \end{aligned} \tag{Eq. 5}$$

The coefficients (β_1 and β_2) represent the elasticity estimates of CO_2 emissions with respect to EC and GDP respectively.

Diagnostic tests

There are two diagnostic tests; Hausman and Redundant test to compare between the estimated models (FE, RE and Pooled model). Hausman test is used to compare between FE and RE which is based on Wald χ^2 statistics with degrees of freedom (k-1). If the results show a correlation between FE and RE, then it means that there is significant evidence in favor to choose FE model. Hausman statistics has the following formula:

$$h = \mathbf{q}'\left[\text{var}\,(\beta_{FE}) - \text{var}\,(\beta_{RE})\right]^{-1}\mathbf{q} \tag{Eq. 6}$$

Where, $\boldsymbol{q} = \beta_{FE} - \beta_{RE}$, FE and RE models are consistent under the assumption of the null hypothesis, and RE is more appropriate. However, in the alternative hypothesis FE is more appropriate than RE.

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In additional of that, Redundant test is used to compare between FE and pooled model. Pooled model assumed to be baseline for comparison. Rejection of the hypothesis illustrates that FE model fits the data better than pooled model (Baltagi, 2005). The formula of Redundant test is:

$$F_{(n-1,nT-n-K)} = \frac{(R_{FE}^2 - R_{Pooled}^2)/(n-1)}{(1-R_{FE}^2)/(nT-n-K)} \quad \text{(Eq. 7)}$$

Where n is number of countries. K is number of independent variables in model.

Discussion and Results

Descriptive statistics

Some of the descriptive statistics of the original data values for each variables GDP, EC, and CO_2 of the developed and developing countries are summarized in *Table 2*. We can note that the standard deviation for each of GDP and EC has high values, which due to the differences in people's incomes, while the variations in EC is due to the availability of the energy resources in the country. On the other hand, the results of Modified Wald test using the original data indicates the existence of heteroscedasticity in the panel data, as the test's value is (74.07) at 1% significant level. However, after transforming the data by using log and inverse form, the results of Modified Wald test support that the data have constant variance (homogenous).

Table 2. *Descriptive statistics*

Variables	Variance type	Mean	Median	Std. Dev	Minimum	Maximum
Developed countries (17)						
	overall			6021.5		
CO_2	between	9330.7	7982.1	1029.6	911.1	40457.8
	within			5934.5		
	overall			15224.6		
GDP	between	15,241.5	10,736.72	13207.1	360.5	118218.8
	within			7791.2		
	overall			1637.5		
EC	between	3,444.7	3,251.1	1460.5	411.4	12124.7
	within			767.5		
Developing countries (12)						
	overall			3429.7		
CO_2	between	5387.1	5584.7	1145.3	14.7	17349.3
	within			3241.4		
	overall			3520.8		
GDP	between	3,069.1	1,962.73	2258.6	79.3	22968.5
	within			2688.0		
	overall			2623.4		
EC	between	2,546.2	1,967.1	1227.9	119.7	13023.9
	within			2323.7		

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The results of the diagnostic tests

After estimation of both Random effects model (RE) and fixed effects model (FE), the comparison between them is performed by employing Hasuman test. The results of Hausman test suggest that FE model is more appropriate than RE model in most cases. The results are summarized in *Table 3.*

Table 3. *Results of Hausman test*

Model of data	Developed countries group		Developing countries group	
	Cross-section effects	Period effects	Cross-section effects	Period effects
1st model CO_2	17.8**	4.05	43.35***	26.74***
2nd model Ln CO_2	3.29	1.47	265.16***	140.65***
3rd model 1/ CO_2	3.80	11.04**	852.24***	168.81***

***, ** and * indicate the significant level of χ^2 test at 1%, 5% and 10% respectively

However, in case if the results show that FE model is appropriate more than RE, then the next step is to apply Redundant test to compare between FE model and Pooled model. Whilst some cases which have supported the using of RE model, will not be included in redundant test (dash line in table). The results of redundant test suggest that the FE models are more appropriate than the pooled model at 1% level in all remaining cases except in one model estimated by period effect using inverse form transformation. The results of redundant test are summarized in *Table 4*.

Table 4. *Redundant test*

Model of data	Developed countries group		Developing countries group	
	Cross-section effects	Period effects	Cross-section effects	Period effects
1st model CO_2	171.2***	----------	23.97***	4.48**
2nd model Ln CO_2	----------	----------	103.11***	5.23***
3rd model 1/CO_2	----------	0.43	93.93***	4.24***

***, ** and * indicate the significant level of F-test at 1%, 5% and 10% respectively.

Estimation models

This section illustrates the best model which fits the data with high accuracy among the three estimated models; 1st model against 2nd model and 3rd model. The results of estimated models is summarized in *Table 5*.

Generally, the results show that all of the coefficients are significant in all models. Moreover, the estimated models in cross section effects are accurate more than the estimated model in period effects according to the values of R^2 and RMSE. On the other hand, by comparing the cross section models among the three patterns, the results show that the models with data transformation (2nd and 3rd model) outperform than the model

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without data transformation (1st model) according to the scales, whereas the constant and RMSE are too large in the 1st model. However, in 2nd and 3rd model, we obtain more significant results according to R^2, RMSE and significance coefficients. Therefore, the discussion is based on the results of 3rd model which transformed the data by inverse form, as the models have higher R^2 in both developed and developing countries.

In the 1st model of cross section effects, CO_2 can be explained about 77% and 67% by the explanatory variables (GDP and EC) for developed and developing countries group, respectively. Whilst 2nd model supports that CO_2 can be explained almost 78% and 86% by GDP and EC for developed and developing countries. The 3rd model illustrates that CO_2 can be explained more than 84% by GDP and EC for developed and developing countries respectively.

In additional of that, it is clear in the 3rd model the coefficient of inverse GDP are (0.37) and (-0.65) in developed and developing countries respectively, which show that developing countries has larger effect than developed countries into CO_2, but it has a negative sign which illustrates that GDP has a negative effects towards the CO_2, the coefficient implies that in each one unit increase in (1/GDP) in developing countries leads to 0.65 unit decline in CO_2 compare to the case in developed countries. This implies that economic growth has negative environmental consequences, and the higher GDP ratio, the lower level of CO_2. This is particularly in developing countries due to their high levels of dependence on natural energy resources. As when developing countries start to develop their economic level by raising standards of living and improving quality of life, it results the depletion of energy resources and environmental degradation. After that, they start to mitigate the adverse environmental impacts through generating renewable energy, and induce waste management techniques etc.

In contrast, the coefficient of the inverse EC is (2.83) in developing countries which is about two times higher than that in developed countries (1.48). It has a positive sign which illustrates that the EC has a positive relationship with CO_2, with each 1 unit of kiloton oil per capita increase in the EC leads to the increase of 1.48 and 2.83 unit increase in CO_2 emission for developed and developing countries respectively.

In summary, based on the findings in *Table 5*, the elasticity of EC causes to higher pollutions and elasticity of GDP leads to improvement in pollution problem. So we can conclude that higher (elasticity) growth does not lead to higher pollution but higher (elasticity) energy consumption may cause to higher pollution in developed and developing countries. The increasing volume of CO_2 emissions has a significant effect on the environment. It could achieve the lower level of CO_2 emissions by reducing the consumption of fossil fuels, but that may result in a trouble to economic growth where the development relies on the cost of utilizing the fossil fuels.

Table 5. *Estimated Models of Panel Data Regression*

The Estimated Models	**Developed countries group**		**Developing countries group**	
	Cross-section effects	**Period effects**	**Cross-section effects**	**Period effects**
	Best Model Fitted The Panel Data			
1st Model	Robust	RE	Robust	Robust
α	4504.8*	3920.2*	2898.2*	660.12**
β_1 GDP	-0.21***	-0.11***	-0.18***	-0.21**
β_2 EC	2.31**	2.07***	1.73***	3.10***
	Statistics Tests			

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R^2	0.77	0.62	0.67	0.39
RMSE	2037.3	5744.5	1945.4	2780.2
2nd model	RE	RE	Robust	Robust
α	3.25***	1.06**	2.81***	-6.79*
β_1 ln(GDP)	-0.41***	-0.40**	-0.34*	-0.44**
β_3 ln(EC)	1.06***	1.44***	1.09***	2.52**
Statistics Tests				
R^2	0.78	0.69	0.86	0.67
RMSE	0.21	0.31	0.49	0.81
3rd model	RE	Pooled	Robust	Robust
α	0.0013**	0.0019**	0.0015***	-0.0023***
β_1 1/(GDP)	-0.37***	-0.83***	-0.65***	-1.02***
β_2 1/(EC)	1.48***	2.12***	2.83***	2.37***
Statistics Tests				
R^2	0.84	0.81	0.86	0.89
RMSE	0.0025	0.0033	0.0029	0.0041

***, ** and * indicate the Coefficient significant level from zero at 1%, 5%, and 10%. β_1 and β_2 are coefficients for EC and GDP. 1st model is estimated by using the original data without any transformation, 2nd and 3rd models use the natural logarithm and inverse form to transform the data, respectively.

Conclusions and policy implications

A number of challenges existed in detecting the relationship between energy and economic growth as energy is essential role for economic growth and development of a country. Therefore, the interest to investigate the relationship between energy consumption, economic growth and environment quality has been raised notably, especially with regards to the negative impact of energy consumption towards environmental degradation and climate change. These findings should become the framework that concerns competent authorities to take those issues into account.

The main objective in this experimental study is to examine the relationship between CO_2, EC and GDP using different data transformation forms (natural logarithm versus inverse form) in reducing the heteroskedasticity in panel data. The panel data consist of 29 countries from two different economic levels of countries, 17 developed versus 12 developing countries. The data spanning from 1960 to 2008. A panel data approach is applied and estimations based on three models. To achieve this objective, the panel data approach was applied by estimating three different models. The first model used the original data without any transformation, while the second and the third model used the data with transformation by natural logarithm and inverse form respectively. On the other hand, the results of Modified Wald test using the original data indicates the existence of heteroscedasticity. However, after transforming the data by using log and inverse form, the results support that the data have constant variance (homogenous). The main findings support that, the estimated models in cross section effects are accurate more than the estimated model in period effects. On the other hand, by comparing the cross section models among the three patterns, the results show that the models with data transformation outperform than the model without data transformation according to R^2,

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RMSE and significance coefficients. Therefore, the discussion is based on the results of the model which transformed the data by inverse form, as it has the highest R^2 which illustrates that more than 84% CO_2 emission can be explained by GDP and EC.

In conclusion GDP and EC might play an important role into environmental degradation particularly CO_2 emissions, thus it is recommended to policy makers to consider them as an effected factors, especially the developed countries, as the results support that the latter effects the environmental degradation (CO_2) more than that in developing countries group, that appear clearly as the developed counties assuming more energy resources than the developing countries, which most likely the main recourse to emits the CO_2 emissions.

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ANALYTICAL AND DETECTION SOURCES OF POLLUTION BASED ENVIRONMETRIC TECHNIQUES IN MALACCA RIVER, MALAYSIA

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Abstract. Environmetric techniques such as hierarchical cluster analysis (HCA), discriminant analysis (DA) and principal component analysis (PCA) methods are applied to investigate spatial variation and potential pollutant sources of surface water quality data of the Malacca River in Malaysia. HCA categorized three different cluster regions, namely Cluster 1 or LPS, Cluster 2 or MPS, and Cluster 3 or HPS. DA resulted in nine discriminant variables, namely turbidity, TSS, pH, BOD, COD, *E. coli*, As, Zn, and Fe. PCA indicated six components in HPS and MPS with total variance of 84.9% and 84.4%, while LPS result five components had a total variance of 77.1%. Generally, major sources of pollution are agricultural, residential and wastewater treatment plants, domestic and commercial waste, industry, as well as animal husbandry. The present study provides useful information for local authorities to identify sources of pollution of the examined area and effectively in proper management for land use area. Additionally, the study also helps in understanding river water quality within the basin and provides a database for future reference in developing water policies.
Keywords: *water quality, HCA, DA, PCA, spatial variation*

Introduction

Water resources have been depleting in recent year. According to worldwide statistics for water pollution developing countries produce 70% of industrial wastes that are dumped untreated into water and that an average of 99 million pounds (45 million kilograms) of fertilizer and chemicals are used each year (National Geographic Portal, 2016). This situation is no exceptional in Malacca River. Currently, the river has been reported to be contaminated and cause death to various fish species (Hua, 2015a, 2015b; Metro Online, 2015; Daneshmand et al., 2011; Nasbah, 2010). The state government has taken actions in terms of law enforcement (Hua, 2015a), policies for water resources (Hua, 2015b), exposure through religious and moral education (Ang, 2014), and public awareness about the importance of the environment (Hua and Marsuki, 2014), especially riverine water resources. However, the implementation of such projects to preserve river water quality by the state government still has not changed levels of water pollution to a lower level. The problem still persists even up to a higher level and has become more dangerous. Hence, the major pollutants from the main sources of pollution should be investigated and determined, especially in terms of spatial variation in the Malacca River.

Hierarchical cluster analysis (HCA), discriminant analysis (DA), and principal components analysis (PCA) are categories in environmetric methods that have been successfully applied in hydrochemistry especially in surface water, groundwater quality assessment, and environmental research (Mustapha et al., 2013; Najar and Khan, 2012; Samsudin et al., 2011). These methods have the ability to define all possible influences, including hidden information in an environmental water quality data set and offering greater possibilities in decision making process (Aris et al., 2013). Generally, HCA

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technique able to divides a large number of objects into a smaller number of homogenous groups on the basis of their correlation structure (Voyslavov et al., 2012), DA has the advantage of discriminating variables between two or more naturally occurring group (Singh et al., 2011), and PCA is used to extract important information from raw data, compress large size data by storing only important information, simplifying the description of data set, and analyzing the observations and variables together (Abdi and Williams, 2010). Therefore, this research study has been carried out to analyze the current condition of river water with quality based descriptive statistics, and to identify the main source of pollution using HCA, DA and PCA techniques in terms of spatial variation in the Malacca River.

Methodology

Description of Study Area

The Malacca River has a total catchment area of approximately 670 km^2. The river lies within latitudes 2°23'16.08"N to 2°24'52.27"N and longitudes 102°10'36.45"E to 102°29'17.68"E in Malaysia. Malacca River have 80 km in length and only 7 sub-basins are selected in the study, namely the Kampung Kelamak sub-basin, Kampung Sungai Petai sub-basin, Kampung Panchor sub-basin, Kampung Harmoni Belimbing Dalam sub-basin, Kampung Tualang sub-basin, Kampung Cheng sub-basin, Kampung Batu Berendam sub-basin (*Fig. 1*). There is a reservoir located between Alor Gajah and Malacca Central districts along the river, namely Durian Tunggal Reservoir, which has a catchment approximately 20 km^2 and acts as a source of water supply to Malacca residents.

The climate in the study area is characterized as uniformly average annual temperatures, high rainfall, and high humidity. These conditions impact on the hydrology and geomorphology of study area. The study area experiences two seasons, namely a dry season from January to March and a wet season from April to November. Normally, the weather consists of a South-West monsoon blowing across the Straits of Malacca, and the area easily experiences flooding. The selected study area can be categorized as impacted and non-impacted, which lie between Kampung Harmoni Belimbing Dalam sub basin to Kampung Batu Berendam sub basin with an area of 68 km^2 and Kampung Kelemak sub basin to Kampung Panchor sub basin with an area of 12 km^2, respectively.

Water quality data in this study were obtained from Department of Environment (DOE), Ministry of Natural Resource and Environment of Malaysia, and are concentrated on 9 stations along the main Malacca River (*Table 1*). The availability data were recorded from January to December of 2014 for all 9 sampling sites representing 7 sub-basins as previously described across the Alor Gajah and Malacca Central Districts. Generally, the parameters of river water quality consist of physic-chemical parameters (i.e. pH, temperature; electrical conductivity (EC); salinity, turbidity, total suspended solids (TSS); dissolved solids (DS); dissolved oxygen (DO); biochemical oxygen demand (BOD); chemical oxygen demand (COD); ammoniacal-nitrogen(NH_3N); trace elements (i.e. mercury (Hg), cadmium (Cd), chromium (Cr), arsenic (Ar), zinc (Zn), lead (Pb), and iron (Fe); and biological parameters (i.e. *Escherichia* coliform and total coliform). All samples are analyzed based APHA (2005) method.

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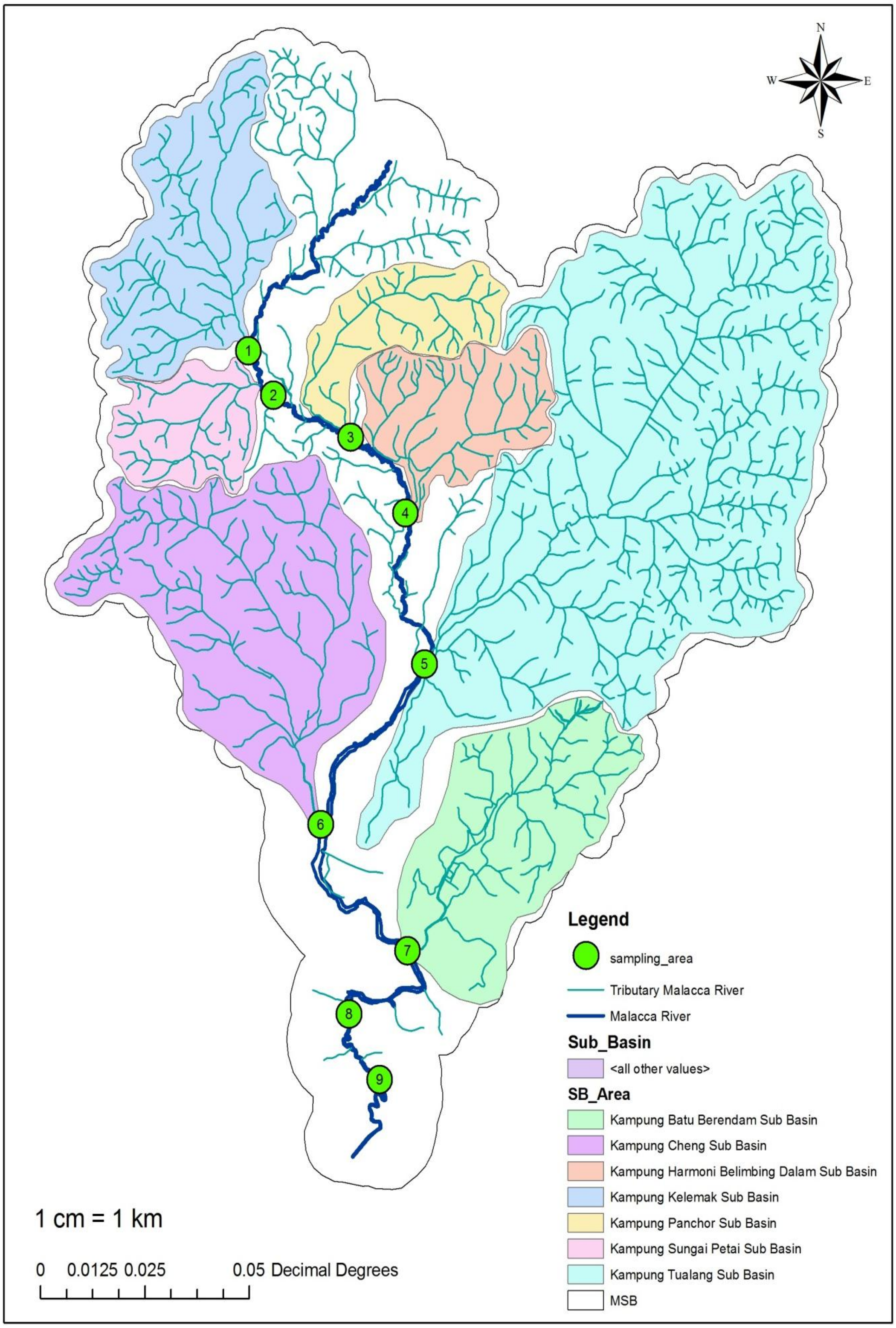

Figure 1. *7 sub-basin with 9 sampling stations along Malacca River. The Data and Monitoring Site*

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Table 1. *The latitude and longitude of sampling stations.*

Station	Latitude	Longitude
S1	02°21'57.41"N	102°13'7.10"E
S2	02°21'30.16"N	102°13'18.20"E
S3	02°20'49.52"N	102°14'36.44"E
S4	02°19'41.70"N	102°15'27.30"E
S5	02°17'48.86"N	102°15'39.51"E
S6	02°15'46.55"N	102°14'10.72"E
S7	02°14'5.02"N	102°15'24.67"E
S8	02°13'14.33"N	102°14'35.01"E
S9	02°12'23.42"N	102°15'0.80"E

Source: Global Positioning System

Water Quality Analysis and Data Analysis

The river water quality data was analyzed using Statistical Package for Social Science version 19 (SPSS 19) for descriptive analysis and environmetric techniques based HCA, DA, and PCA. In HCA, Wards method through variance analysis was used to evaluate distance between clusters with minimal sum of squares (SS) for any two clusters formed at each step (Mustapha et al., 2013; Najar and Khan, 2012; Samsudin et al., 2011); follow by squared Euclidean distance used to measuring similarity between two samples and distance that can be represented by different between analytical values from the samples (Mustapha et al., 2013; Najar and Khan, 2012; Samsudin et al., 2011); and the results are provided through a dendrogram grouping the high similarity with small distances between cluster (Gazzaz et al., 2012). The present study employed HCA to investigate grouping of sampling sites (spatial). Meanwhile, DA determines variables through discriminate between two or more groups or cluster (Gazzaz et al., 2012; Samsudin et al., 2011), as expressed in the equation below:

$$f\left(G_i\right) = k_i + \sum_{j=1}^{n} w_{ij} P_{ij} \qquad \text{(Eq.1)}$$

where i is the number of groups (G), k_i is the constant inherent to each groups, n is the number of parameters used to classify a set of data into a given group, and w_{ij} is the weight coefficient assigned by DF analysis (DFA) to a given parameter (P_{ij}). The present study applied DA to determine that the means of the variables differ within groups and to predict the pattern. HCA results are applied into DA using standard stepwise, forward stepwise, and backward stepwise modes to develop the DFs in evaluating spatial variations of river water quality. Generally, dependent variables are the sampling stations (spatial), while independent variables are all other parameters involved. Next PCA, with the ability to provide information on most significant parameters due to spatial and temporal variations, defines the whole data set by excluding less significant parameters with minimum loss of original information (Singh et al., 2011), which is explained by the equation below:

$$z_{ij} = a_{i1}x_{1j} + a_{i1}x_{2j} + \dots + a_{im}x_{mj} \qquad \text{(Eq.2)}$$

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where z is the component score, a is the component loading, x is the measured value of the variable, I is the component number, j is the sample number, and m is the total number of variables. Normally PCA will undergo procedure like (1) for original data to be reduced to dominant components or factors (source of variation) that influence the observed data variation, and (2) whole data set will be extracted through eigenvalues and eigenvectors from the square matrix produced by multiplying data matrix (Aris et al., 2013). The main condition is that eigenvalues should more than 1 to be considered as significant (Juahir et al., 2011) to perform new group of variable namely Varimax Factor (VFs). Generally, VFs coefficients that have a coefficient of more than 0.75 are considered as 'strong', while 0.75 to 0.05 are moderate and 0.50 to 0.30 are weak (Juahir et al., 2011). The present study applied PCA to the normalized data set (20 variables) separately based on the different spatial regions obtained from the HCA technique.

Results and Discussions

Descriptive analysis through mean and standard deviation for physico-chemical parameters, biological parameters and trace metal for year 2014 can be obtained from *Table 2*. Majority pH, temperature, and trace metal are in clean condition, except for iron in station 6 and station 9 that resulted in a class 3 ranking. Continuously, physical parameter showing salinity (in station 1 to station 3 and station 7), turbidity (in station 3, station 6 and station 8), electrical conductivity (in station 1 and station 6), dissolved solid (in station 1 and station 7), and total suspended solid (in station 6) resulted in class 5; while class 4 with total suspended solid and turbidity resulted in station 4, station 5, and station 9. Only electrical conductivity (in station 2 and station 3), turbidity (in station 1 and station 2), and dissolved solid (in station 2) are class 3; and other stations still remain clean (*Table 3*). Chemical parameter shows only biochemical oxygen demand and ammoniacal nitrogen are in class 5 and class 4, which is from station 1 to station 3 and station 6 to station 8. Meanwhile, mean analysis indicates biochemical oxygen demand (in station 4, station 5, and station 9), chemical oxygen demand (in station 1 to station 3 and station 7 to station 8), dissolved oxygen (in station 1 to station 3 and station 7), and ammoniacal nitrogen (in station 4) are in class 3, while the other stations remain class 2 and class 1. For biological parameters, majority total coliform is in class 5; and *E. coli* resulted in class 5 (in station 1 to station 2 and station 8), class 4 (station 3, station 6, station 7 and station 9) and class 3 (in station 4 and station 5).

Table 2. *Mean and standard deviation values of water quality data along Malacca River for years 2014 (n=20)*

Category		S1	S2	S3	S4	S5	S6	S7	S8	S9
pH	M SD	6.84 0.37	6.67 0.36	6.61 0.57	7.01 0.57	6.84 0.28	6.95 0.16	7.05 0.27	7.00 0.46	6.82 0.28
Temp	M SD	27.73 0.81	27.98 1.11	28.32 1.33	28.79 1.62	27.93 1.52	27.20 1.01	28.33 1.17	27.41 1.04	27.41 1.15
Sal	M SD	26.22 13.68	3.74 1.47	2.63 2.21	0.08 0.07	0.05 0.01	0.04 0.01	8.02 7.88	0.32 0.18	0.05 0.01
EC	M SD	30108.25 13370.57	2559.50 2211.23	3235.75 2376.98	150.75 113.77	104.83 23.82	96.42 27.01	13207.00 12635.49	966.08 293.43	99.25 22.64
TSS	M SD	36.00 10.25	33.58 12.53	56.83 14.97	163.58 180.69	171.50 233.95	304.33 363.56	38.08 36.84	48.58 15.22	165.75 235.83

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DS	M	19035.17	2870.25	1352.25	79.10	57.08	47.00	7587.42	514.25	53.53
	SD	9377.89	1874.00	741.68	57.44	12.47	10.86	7575.01	180.77	12.79
Tur	M	93.56	96.42	684.39	205.35	284.27	419.54	29.25	308.34	265.61
	SD	42.67	32.63	333.11	266.86	473.64	552.40	37.17	83.25	489.32
BOD	M	7.08	12.33	13.42	5.33	5.50	7.00	6.67	10.67	5.58
	SD	2.11	3.89	6.54	2.64	3.21	4.07	0.89	4.14	3.63
COD	M	35.00	45.08	33.67	19.00	19.75	20.58	25.83	49.67	19.25
	SD	6.93	22.91	8.28	10.63	11.14	10.35	5.37	15.44	10.45
DO	M	3.59	3.98	4.07	6.85	6.48	6.47	3.13	7.06	6.50
	SD	1.24	0.62	1.13	1.50	0.39	0.91	1.00	2.86	0.48
NH_3N	M	2.13	4.31	5.26	0.52	0.33	0.36	2.97	2.71	0.39
	SD	1.63	1.98	0.63	0.33	0.25	0.25	1.56	0.67	0.26
As	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hg	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cd	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cr	M	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pb	M	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Zn	M	0.07	0.05	0.04	0.06	0.05	0.05	0.05	0.04	0.06
	SD	0.05	0.02	0.00	0.05	0.02	0.03	0.02	0.01	0.02
Fe	M	0.33	0.17	0.51	0.78	0.88	1.18	0.15	0.30	1.35
	SD	0.48	0.13	0.33	0.72	0.63	0.94	0.19	0.27	0.95
Coliform	M	78483.33	124985.00	127756.67	85416.67	131695.83	147358.33	193783.33	195308.33	132550.00
	SD	78964.14	70669.60	89245.44	124363.13	150479.25	76150.54	206523.28	180044.57	71782.33
E. coli	M	168675.00	57133.33	38316.67	5034.50	7001.33	25816.67	49697.50	63091.67	13010.17
	SD	140418.16	57478.92	33913.66	7766.57	4670.18	38827.10	83867.26	35300.31	11429.03

(M means Mean; SD means Standard Deviation; Tur means Turbidity; DS means Dissolved Solid; Con means Electrical Conductivity; Sal means Salinity; Temp means Temperature; DO means Dissolved Oxygen; BOD means Biochemical Oxygen Demand; COD means Chemical Oxygen Demand; TSS means Total Suspended Solids; pH means Acidic or Basic water; NH_3N means Ammoniacal Nitrogen; *E. coli* means *Escherichia* Coliform; Coli means Coliform; As means Arsenic; Hg means Mercury; Cd means Cadmium; Cr means Chromium; Pb means Lead; Zn means Zinc; Fe means Iron; SD means Standard Deviation; S1 to S9 means Station 1 to Station 9)

Table 3. *National Water Quality Standards for Malaysia (adapted from Malaysian DOE, 2012).*

Category	**Class**					
	I	**IIA**	**IIB**	**III**	**IV**	**V**
pH (-)	6.5-8.5	6 – 9	6 – 9	5 – 9	5 – 9	-
Temp (°C)	-	Normal+2°C	-	Normal+2°C	-	-
Sal (%)	0.5	1	-	-	2	-
EC (µS/cm)	1000	1000	-	-	6000	-

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TSS (mg/L)	25	50	50	150	300	300
DS (mg/L)	500	1000	-	-	4000	-
Turbidity (NTU)	5	50	50	-	-	-
BOD (mg/L)	1	3	3	6	12	>12
COD (mg/L)	10	25	25	50	100	>100
DO (mg/L)	7	5 – 7	5 – 7	3 – 5	< 3	< 1
NH_3N (mg/L)	0.1	0.3	0.3	0.9	2.7	> 2.7
As (mg/L)	-	0.05	0.05	0.4 (0.05)	0.1	-
Hg (mg/L)	-	0.001	0.001	0.004(0.0001)	0.002	-
Cd (mg/L)	-	0.01	0.01	0.01 (0.001)	0.01	-
Cr (mg/L)	-	0.05	0.05	1.4 (0.05)	0.1	-
Pb (mg/L)	-	0.05	0.05	0.02 (0.01)	5	-
Zn (mg/L)	-	1	1	3.4	0.8	-
Fe (mg/L)	-	1	1	1	1(leaf)5(others)	-
Coliform (count/100mL)	100	5000	5000	5000(20000)	5000(20000)	> 50000
E. coli (count/100mL)	10	5000	5000	50000	50000	> 50000

(Tur means Turbidity; DS means Dissolved Solid; Con means Electrical Conductivity; Sal means Salinity; Temp means Temperature; DO means Dissolved Oxygen; BOD means Biochemical Oxygen Demand; COD means Chemical Oxygen Demand; TSS means Total Suspended Solids; pH means Acidic or Basic water; NH_3N means Ammoniacal Nitrogen; *E. coli* means *Escherichia* Coliform; Coli means Coliform; As means Arsenic; Hg means Mercury; Cd means Cadmium; Cr means Chromium; Pb means Lead; Zn means Zinc; Fe means Iron

Analysis of HCA is shown in *Figure 2* for nine sampling stations along Malacca River, indicating that 3 clusters have been identified from the techniques. Cluster 1 consists of S1 (Kampung Kelemak sub-basin), S2 (Kampung Sungai Petai sub-basin), and S3 (Kampung Panchor sub-basin); cluster 2 consists of S4 (Kampung Harmoni Belimbing sub-basin), S5 (Kampung Tualang sub-basin), and S6 (Kampung Cheng sub-basin); and cluster 3 consists of S7 (Kampung Batu Berendam sub-basin), S8, and S9. Generally, cluster 1 is considered as low-pollution sources (LPS) because a majority of land area is used for agriculture, animal husbandry, and residential activities; while cluster 2 is considered as middle-pollution sources (MPS) due to the land used area is residential and industrial activities; and cluster 3 are high-pollution sources (HPS) due to the residential, sewage treatment plant, commercial, and industrial activities.

DA techniques are used to evaluate the possibility changes in land used based on the 3 cluster that resulted from HCA output. The results indicate that clusters in standard mode for 20 variables are 91%, forward stepwise mode for 5 variables are 79%, and backward stepwise mode for 9 variables are 87%. Therefore, backward stepwise mode is considered for further analysis, which have turbidity, total suspended solid, pH, biochemical oxygen demand, chemical oxygen demand, *E. coli*, arsenic, zinc and iron. A box and whisker plot of water quality parameter for 2014 are shown in *Figure 3*.

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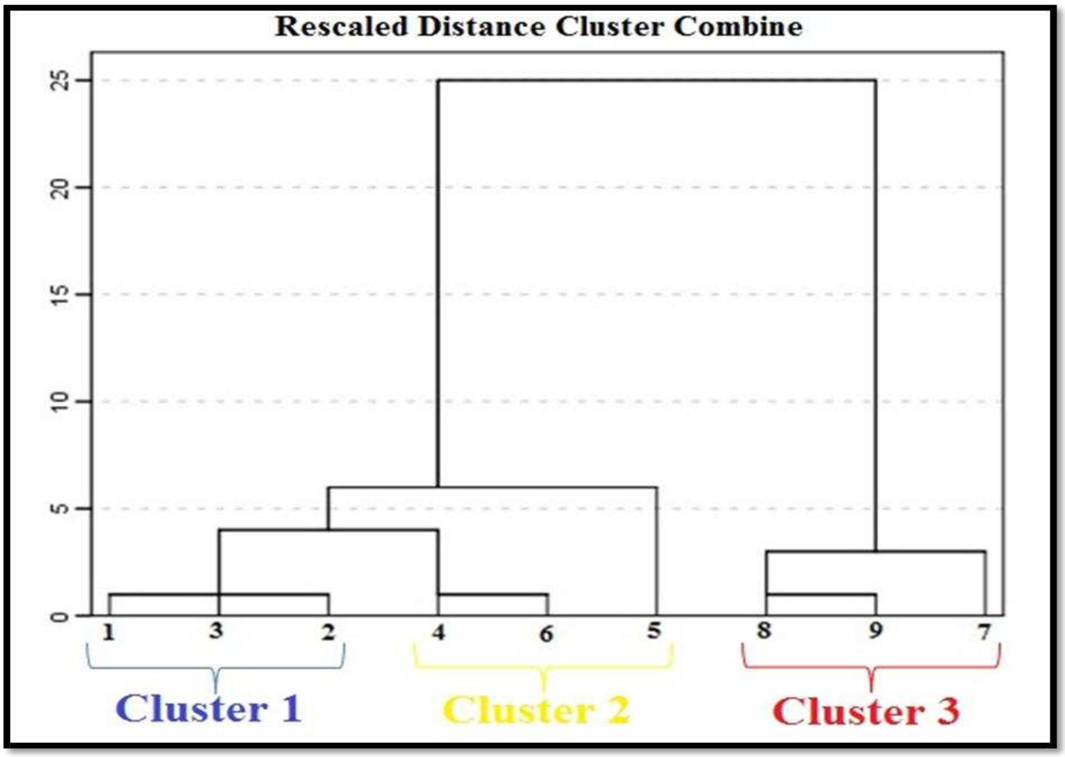

Figure 2. *Hierarchical cluster analysis (HCA) through Ward Linkage method to generate dendogram*

PCA was applied to the data set to compare the compositional patterns between the examined water parameters and to identify the factors that influence each of the identified regions (e.g. HPS, MPS and LPS). Six PCs were obtained for HPS and MPS regions, while only five are resulted from LPS region, which have eigenvalues more than 1 with the total variance of 84.9%, 84.4%, and 77.1%, respectively. Corresponding principal components, variable loadings, and variance are explained based on *Table 4*.

HPS

The principal component 1 loadings with 20.8% of total variance include strong positive loadings for salinity, EC and DS; weak positive loadings include pH and NH_3N; and weak negative loadings include DO. The elements of salinity, NH_3N, DO, and DS, are connected with extensive pesticide usage for agricultural activities in oil palm and rubber plantations, and animal husbandry (e.g. chicken, cow, goat and pig farm) carried out within the Malacca River basin. Meanwhile, EC components are possibly connected with the erosion of riverbank due to dredging in the river. Continuously, principal component 2 loadings with total variance of 19.5% have strong positive loadings for TSS, turbidity, and Fe; moderate negative loading for temperature; and weak negative loadings for pH and NH_3N. Turbidity and TSS are related with soil erosion caused by interruption from human activities and hydrologic modifications (e.g. dredging, water diversions, and channelization) (Deneshmend et al., 2011), urban development areas involving land clearing (Najar and Khan, 2012), and the erosion of road edges due to surface runoff (Juahir et al., 2011). The forest or agriculture land converted into urban areas may negatively impact the ecosystem (Ghumman, 2011) of the Malacca River basin in form of mud floods, landslides and river floods. The Fe

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element is possibly connected with industrial activities such as electroplating, and the NH_3N is likely related to domestic waste and agricultural runoff.

Next, principal component 3 loadings with total variance of 12% indicate strong positive loadings of pH and DO; moderate positive loading for COD; and weak positive loading for NH_3N. On the other hand, principal component 4 loadings with total variance of 11.5% resulted in strong positive loading for BOD; moderate positive loading for COD; weak positive loading for NH_3N; and weak negative loading for Fe. The factors explained by considering the chemical components of various anthropogenic activities that constitute point source pollution from industrial effluents, domestic waste water, commercial activities and wastewater treatment plants, including agricultural runoff area that located at Kampung Batu Berendam sub-basin and in the urban area. Basically, Fe representing one of the metal groups that originating from industrial effluents. Principal component 5 loadings explained total variance of 10.7% with strong positive loading for As; weak positive loadings of NH_3N and *E. coli*; and strong negative loading for Zn. The NH_3N is suspected to be from agricultural runoff using inorganic fertilizer (Aris et al., 2013), which is able to explain the decomposition of nitrogen containing organic compounds through degradation process of organic matter (Najar and Khan, 2012), and the conditions are strongly supported by the occurrence of As used in agriculture fields to produce pesticide waste. *E. coli* are strongly related to municipal wastes and animal husbandry. Lastly, principal component 6 loading has total variance of 10.4% with strong positive loadings for *E. coli* and coliform, which strongly explains that the factors are related to municipal sewage and wastewater treatment plants (Samsudin et al., 2011) along the Malacca River, especially in urban regions.

MPS

Principal component 1 loadings explain total variance of 19.7% with strong positive loadings of salinity, EC, and DS; moderate positive loading for coliform; and weak positive loading for pH. As describe in HPS, salinity, EC and DS are subjected from agricultural runoff and animal husbandry activities. The factor to cause coliform are related to municipal wastes, oxidation ponds, and animal husbandry, where large amount of oxygen used up by the bacteria decreases the DO availability to cause anaerobic fermentation process to produce organic acids (Juahir et al., 2011). Therefore, hydrolysis process leading to acidic material to cause water pH values to decrease. Continues, principal component 2 loadings resulted total variance of 16.1% with strong positive loadings of BOD and COD; and moderate positive loadings of TSS and turbidity. TSS and turbidity elements are subjected to construction activities and urban development that carry out in Kampung Tualang sub basin and Kampung Cheng sub basin, where most activities are happen near to the stream areas and increase the sediment deposited in the river. The condition become worst when overland inputs, stream-bank erosion, and bedload sediments during storm flow (Mustapha et al., 2013) are entering the river. BOD and COD are related to anthropogenic pollution sources and are possibly come from point source pollution such as sewage treatment plants and industrial effluents. Principal component 3 loadings with total variance of 15% have strong positive loading for NH_3N; moderate positive loading for temperature; weak positive loading for pH; moderate negative loadings of TSS and turbidity; and weak negative loading for Fe. As describe previously, NH_3N are related to domestic waste and agricultural runoff that highly usage of fertilizer and pesticides, which possibly to increase nitrogen levels and cause decreasing to water pH values.

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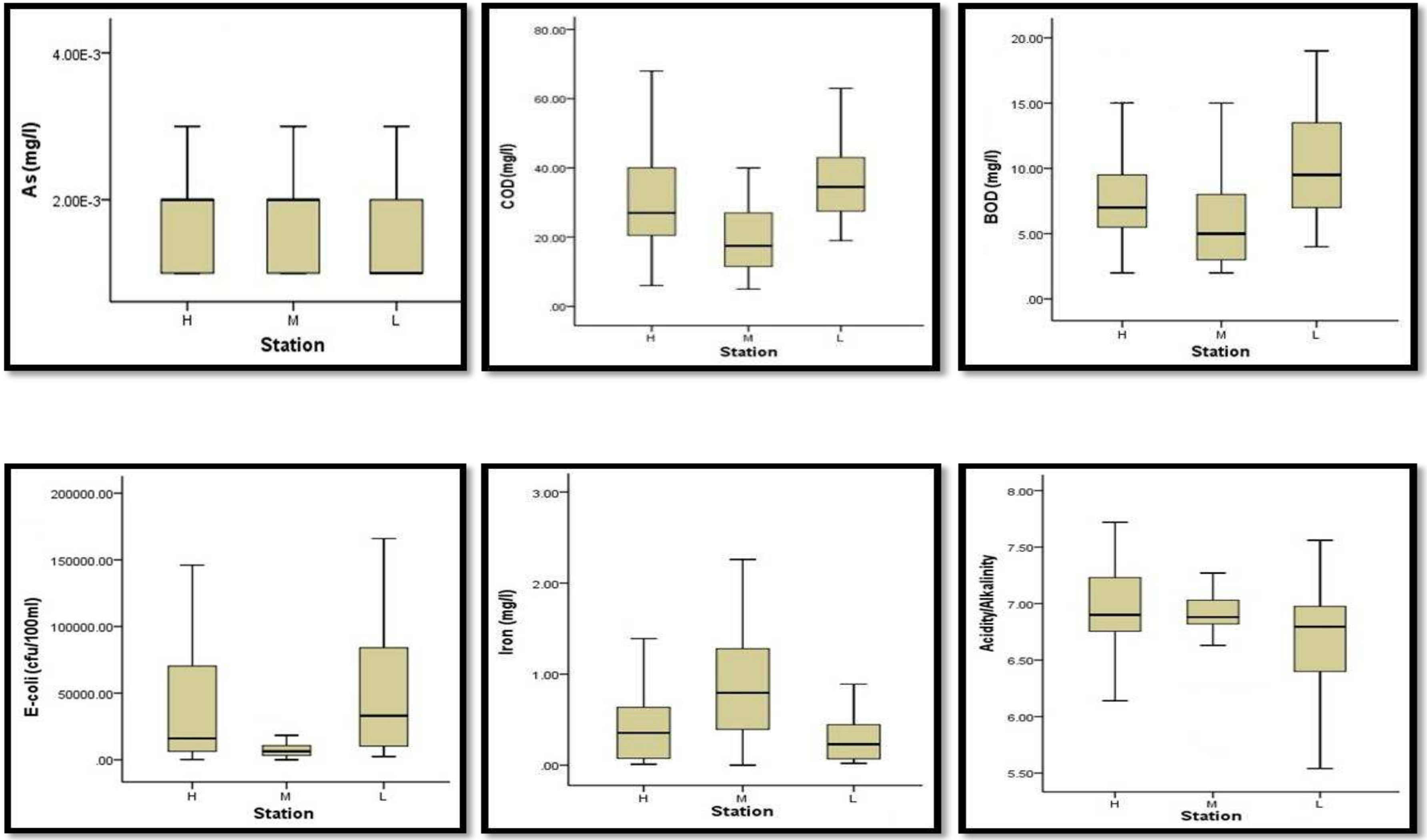

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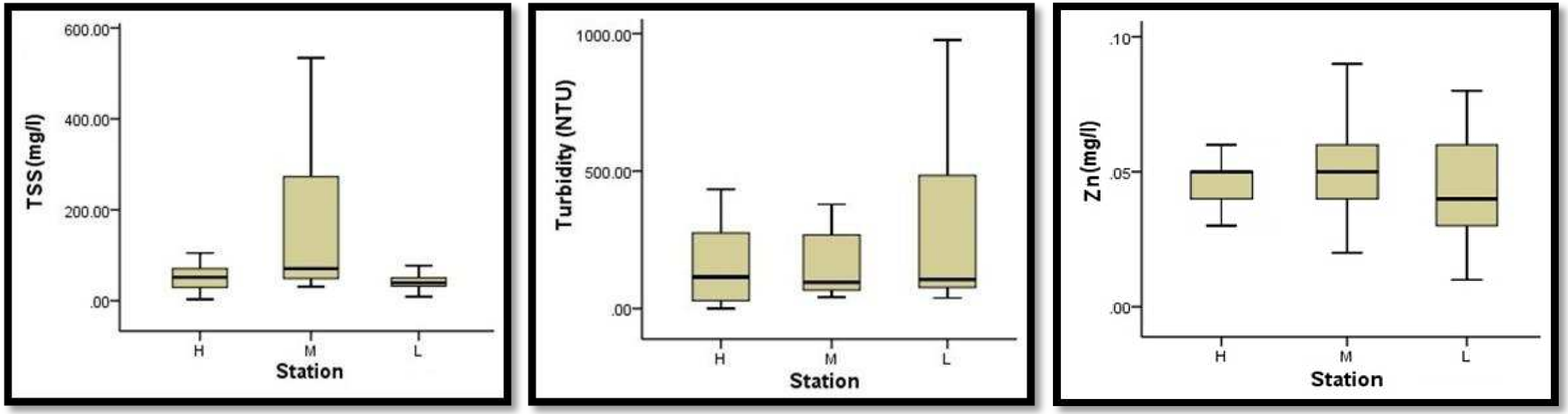

Figure 3. *Box and whisker plots of some parameters separated from DA associated with water quality data of Malacca River. H means high, M means middle, L means low pollution.*

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***Table 4**. Varimax rotation PCs for water quality data based on two clusters within Malacca River basin.*

Variables	**LPS**					**MPS**						**HPS**					
	1	2	3	4	5	1	2	3	4	5	6	1	2	3	4	5	6
pH	.088	**.415**	**.530**	-.044	**-.416**	**.362**	.261	**.306**	.088	**.302**	**.730**	**.372**	**-.391**	**.771**	-.063	-.011	-.142
Temp	-.058	-.142	**.650**	.266	.021	.022	-.042	**.635**	-.221	**.488**	.077	.045	**-.694**	-.226	-.162	-.221	.006
Sal	**.941**	-.009	-.207	-.018	.124	**.955**	-.149	.078	-.098	-.031	.064	**.961**	-.088	.006	-.006	.086	.052
EC	**.964**	-.080	-.089	-.067	.055	**.922**	-.214	.166	-.095	-.034	.121	**.971**	-.105	-.027	-.022	.039	.121
TSS	-.219	.132	**.843**	.130	.226	-.098	**.595**	**-.653**	.230	-.009	-.164	-.040	**.912**	-.122	-.002	-.193	-.050
DS	**.931**	.009	-.026	-.031	-.083	**.926**	-.203	.205	-.084	-.012	.043	**.938**	-.128	-.055	-.037	.010	.177
Tur	**-.381**	**-.481**	.253	**.420**	**.373**	-.099	**.565**	**-.602**	**.371**	-.006	-.237	-.190	**.874**	-.071	.283	.034	.065
DO	-.136	**.654**	**.429**	**.346**	-.122	-.113	.071	.295	-.097	**.757**	.069	**-.319**	.272	**.828**	-.099	-.073	-.041
BOD	**-.619**	**.683**	.072	.029	-.033	-.168	**.928**	-.188	.101	.008	-.075	-.050	.167	-.221	**.894**	-.002	.083
COD	-.233	**.629**	**-.473**	.295	-.090	-.189	**.905**	.081	.099	.135	.023	-.087	.009	**.624**	**.727**	.087	.083
NH_3N	**-.855**	-.089	-.083	.018	-.137	.264	-.031	**.860**	.013	-.053	.109	**.391**	**-.342**	**.300**	**.398**	**.482**	.124
As	-.059	-.008	-.027	.225	**-.818**	-.198	.136	-.017	**.896**	-.159	.025	-.138	.049	-.155	.116	**.767**	.229
Cr	.146	**.835**	.017	.010	.055	-.049	.114	-.271	**.866**	.050	-.152	.000	.000	.000	.000	.000	.000
Zn	.142	-.048	.085	.240	**.751**	-.099	.180	-.170	.124	.087	**-.841**	-.178	.009	-.067	.077	**-.805**	-.022
Pb	**-.602**	-.161	.133	**.591**	**.409**	-.233	-.193	**-.338**	**-.473**	-.130	**.525**	-.157	**.792**	.001	**-.453**	-.079	-.066
E. coli	.105	-.200	**-.312**	**-.768**	.011	-.015	-.140	.279	.122	**-.835**	.078	.201	-.095	-.107	.162	**.347**	**.797**
Coli	**.300**	**.498**	.011	**.574**	-.095	**.576**	.277	-.142	.066	**-.503**	.040	.135	.039	.001	.014	.017	**.932**
IE	4.580	2.760	2.073	1.856	1.846	3.354	2.736	2.545	2.113	1.929	1.681	3.324	3.123	1.925	1.848	1.704	1.667
%V	26.942	16.234	12.196	10.919	10.856	19.727	16.093	14.969	12.427	11.346	9.885	20.774	19.518	12.029	11.549	10.652	10.418
C%	26.942	43.176	55.373	66.291	77.148	19.727	35.821	50.790	63.217	74.563	84.448	20.774	40.292	52.321	63.869	74.521	84.939

(Tur means Turbidity; DS means Dissolved Solid; Con means Electrical Conductivity; Sal means Salinity; Temp means Temperature; DO means Dissolved Oxygen; BOD means Biochemical Oxygen Demand; COD means Chemical Oxygen Demand; TSS means Total Suspended Solids; pH means Acidic or Basic water; NH_3N means Ammoniacal Nitrogen; *E. coli* means *Escherichia* Coliform; Coli means Coliform; As means Arsenic; Cr means Chromium; Pb means Lead; Zn means Zinc; IE means Initial Eigenvalue; %V means % of Variance; C% means Cumulative %)

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Principal component 4 loadings with total variance of 12.4% to result in strong positive loadings of As and Cr; weak positive loading for turbidity; and weak negative loading for Fe. Generally, Cr exists in rock and soil, which have connections with soil erosion that cause turbidity; while As is typically from pesticide used in agriculture activities. Principal component 5 loadings have total variance of 11.3% with strong positive loading for DO; weak positive loadings of pH and temperature; strong negative loading for *E. coli*; and weak negative loading for coliform. Meanwhile, principal component 6 loadings explain total variance of 9.9% with string positive loading for pH; moderate positive loading for Fe; and strong negative loading for Zn. The factors involved in DO element are related with high levels of dissolved organic matter consuming large amounts of oxygen (Juahir et al., 2011), including *E. coli* and coliform that are suspected to be from the sewage treatment plant and pesticide usage in agricultural activities within Kampung Tualang sub basin. This condition will cause the river water quality to become acidified through pH reading. On the other hand, existing Fe element in water quality are suspected from industrial effluents.

LPS

Principal component 1 loadings indicate total variance of 26.9% with strong positive loadings of salinity, EC, and DS; weak positive loading for coliform; strong negative loading for NH_3N; moderate negative loadings of BOD and Fe; and weak negative loading for turbidity. As explained before, salinity, turbidity, EC, and DS are from agricultural runoff and animal husbandry activities; BOD and NH_3N are discharge from wastewater treatment and domestic waste water; and Fe are form industrial effluents. Next, principal component 2 loadings show total variance of 16.2% with strong positive loading for Cr; moderate positive loadings of DO, BOD, and COD; weak positive loadings of pH and coliform; and weak negative loading for turbidity. Principal component 3 loadings resulted total variance of 12.2% with strong positive loading for TSS; moderate positive loadings of pH and temperature; weak positive loading for DO; and weak negative loadings of COD and *E. coli*. Several areas in Kampung Kelemak sub basin and Kampung Sungai Petai sub basin are converting from agriculture field and forest into building and residential area, which highlighted the existing of turbidity and TSS elements in water quality (except Cr that naturally exist in soil). The condition caused chemical components of anthropogenic activities from domestic and commercial wastes, which indirectly increase the coliform and *E. coli* elements through wastewater treatment plants. Continuously, principal component 4 loadings with total variance of 10.9% have moderate positive loadings of Fe and coliform; weak positive loadings of turbidity and DO; and strong negative loading for *E. coli*. Lastly, principal component 5 loadings explain total variance of 10.9% with strong positive loading for Zn; weak positive loadings of Fe and turbidity; strong negative loading for As; and weak negative loading for pH. Zn element are connected with large number of houses and buildings constructed near to river that uses metallic roofs coated with zinc, when in contact with acid rainwater and smog, these could readily mobilize zinc into the atmosphere and waterways (Juahir et al., 2011). Meanwhile, Fe element is subject to industrial effluent, the As element is related to pesticide use in agriculture activities, *E. coli* and coliform are connected with sewage treatment plants, and turbidity come from hydrologic modifications such as dredging, water diversions, and channelization.

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Conclusion

HCA, DA, and PCA are applied to investigate spatial variation and potential pollutant sources of surface river water quality data for the Malacca River. HCA successfully categorized nine monitoring stations into three different cluster regions, namely Cluster 1 or LPS (comprised of S1, S2, and S3), Cluster 2 or MPS (comprised of S4, S5, and S6), and Cluster 3 or HPS (comprised of S7, S8, and S9). HPS is within Malacca Central basin, while MPS is between Alor Gajah basin and Malacca Central basin, and LPS is within the Alor Gajah basin. DA resulted in discriminating nine monitoring stations with nine discriminants assigned to 87% cases correctly using backward stepwise modes. The nine variables are turbidity, total suspended solids, pH, biochemical oxygen demand, chemical oxygen demand, *E. coli*, arsenic, zinc and iron. PCA indicated six components with 84.9% of total variance were extracted in HPS, while six components with 84.4% of total variance were extracted in MPS, and five components with 77.1% of total variance were extracted in LPS. Overall, the major sources of pollution come from agricultural, residential and wastewater treatment plants, domestic and commercial waste, industry, as well as animal husbandry. The present study provides useful information for local authorities in identifying sources of pollution of the examined area and effectively in proper management for land use area. Additionally, the study also helps in understanding river water quality within the basin and provides a database for future reference in developing water policies.

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DIRECT XPS ANALYSIS OF BIOLOGICAL MATERIALS FOR ENVIRONMENTAL PURPOSES

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Abstract. Fish tissues were analyzed directly with an X-ray photoelectron spectroscopy (XPS) system in order to find pollutant elements that may be bioaccumulated in the fish's body in a river that is in contact with the mining industry. XPS method could be used as a first technique to find a wide gamma of elements before using conventional methods. Fish tissue was homogenized in order to make it able to be analyzed by a surface analysis, Whole fishes were analyzed by two homogenizing methods: incineration at 350ºC, and by dehydration and crushing in a ball mill. Both methods tested show different results in the same samples. It has been observed that in the incinerated samples the bonding energy is more intense, this could be a result of the elements' oxidation due to the combustion. Other difference is that in the incinerated samples there are some elements that are not shown in the dehydrated samples. Because of this, we suggest that the incineration should be the adequate technique to continue analyzing this kind of materials.
Keywords: *X-ray photoelectron spectroscopy (XPS); environmental monitoring; pollution detection techniques; fish tissues; environmental chemistry*

Introduction

The junction of specialized cells is defined as a tissue (Walpole et al., 2011). Tissues are capable of encapsulate elements or substances that may be hazardous to an entire organism; this process is called bioaccumulation (Oost et al., 2003). Some techniques are used to identify the elements accumulated in a tissue and these are capable to determine even the concentrations (Han and Weber, 1988). The most popular technique is the called atomic absorption spectroscopy (AAS), this has been certified for legal purposes when detecting contamination in different matrices (water, soil, air, among others) (NOM-001-ECOL-1996). Although it is a universal and precise technique, it has some flaws, especially when it's about organic materials (tissues), maybe the most remarkable flaw is the amount of material and its sample destructiveness (Mugica et al., 2003). These problems come with some factors to be considered: the sample must be vast, it can only be measured once, and it becomes a bigger issue when it's about a protected specie tissue's sample (Carvalho et al., 2005). In this paper, we proposed as an alternative methodology the X ray photoelectron spectroscopy (XPS) that could work

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with a minimum amount of material that will not be destructed in the analysis. XPS is a surface analysis method used commonly in biology to measure the chemical composition of bacteria colonies (Rouxhet and Genet, 1991). This is because the colonies' formations are very thin and the organisms are composed just by one kind of tissue (Bundle, 1992). With bigger organisms, XPS has not been used due to its limitation as a surface technique (about only eight microns in deep); this is because elements stored in a tissue, might be in any part of it, not just on the surface. In the present work, fish tissues have been analyzed by two different methodologies to achieve a homogeneous material that could be representative of the whole animal (McArthur, 2006). We hope that these procedures help to obtain ecotoxicology information with less organisms' samples and samples that could be stored and compared in future broader and complex studies.

Methodology

The study was conducted in a river that has influence by miner activity. The river "Moctezuma", is located in the Nacozari, Cumpas and Moctezuma municipalities in Sonora, Mexico. Twelve sampling stations were worked along the river from the upper river, in theory less contaminated area, to San Pedro's dam, where all contaminants may be retained as a lower basin's reservoir (Taylor, 2008).

Fish were collected by using plastic minow traps, traps were settled facing upstream by 24 hours in order to catch fishes with diurnal and nocturnal habits, the deepth was about 40cm and vegetation was always present in order to have primary consumers and predators (Esacalera-Vazquez and Zambrano, 2010). Once captured, fish were classified for further analysis, and prepared in order to obtain a homogeneous material that could be sample representative, and measured by a surface analysis method where the materials depth was no relevant (Maurice-Bourgoin et al., 2000). We must mention that this paper is about the elements presents in the fishes tissues rather than the fish ecology, but we can mention the species we found and where XPS analysis were performed (*Table 1*) and due to the amount of indivudual we decide to perform XPS analysis with *Poeciliopsis occidentalis* (Baird and Girard, 1853).

Table 1. *Fish species collected in this study*

Specie	Number of specimens
Poeciliopsis occidentalis	355
Catostomus bernardini	11
Rhinichthys osculus	1
Campostoma ornatum	166
Gila intermedia	28
Agosia chrysogaster	33

To achieve XPS analysis, we prepared the fish by two different procedures. The first one consisted in burning the whole fish to ashes (350º C) and homogenized with a mortar until we got a smooth dust. Second, fish were dried in a crystal dryer' with silica gel and then homogenized with a spheres' mill. We must emphasize that this

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might be the first approach to analize organic matter from a complex animal by XPS technique due to the X-ray impossibility to travel out a material deeper than 10nm (McArthur et al., 2014).

After this, the resulting dusts were analyzed in the XPS, obtaining the subsequent spectrums. It must be noticed that in the whole preparation techniques, we did not use any solvent; we used an XPS Perkin-Elmer Phi-5100 with a non-monochromatic magnesium anode and an emission of Kα of 254 KeV. This preparation and other technical details are a accord to other methodologies that have used to find pollutants in other surface analysis such particulated material from air samples (Atzei et al., 2014 and Guascito et al., 2015).

Results

As a first result we collect six fish species and choosed one to perform the XPS analysis by dehidrating and incinerating. We can observe that dehydrated samples remain wet, so we had to use a vacuum chamber, and this delayed he XPS analysis. Once the samples were completely dry, the XPS showed different spectra (*Figs. 1a, 1b, 2a, and 2b*) for dehydrated and incinerated samples. Results show that there is a difference in the element composition in samples from the same place. We also detected that incinerated samples have a more intense spectra. There were elements that both techniques revealed, such as oxygen, carbon, phosphorus, sodium and calcium that are characteristic of organic materials. From all elements measured, dehydrated samples analysis did not show three elements that were present: chlorine, zinc and potassium, while in the incinerated samples there are no inconsistencies (*Table 2*).

Table 2. *XPS elemental analysis results*

Elements registered	Energy level (eV)	Incinerated samples	Dehydrated samples
Al	72.95	X	X
Cl	202	X	
Fe	706.8	X	X
Na	1070.8	X	X
P	136	X	X
S	163.6	X	X
Si	99.82	X	X
Zn	1021.8	X	
O	543.1	X	X
N	409.9	X	X
Ca	346.2	X	X
C	284.2	X	X
K	294.6	X	

On the other hand, the spectra intensities are different for incinerated and dehydrated samples, being more intense for the incinerated. This could be related with the oxygen levels result of the oxidation of the elements by heating (Hussein, 2007). We assume

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that this oxidation could maximize the elements's pikes that are missing in the dehydrated samples, and that is the reason they appear only in the incinerated analysis.

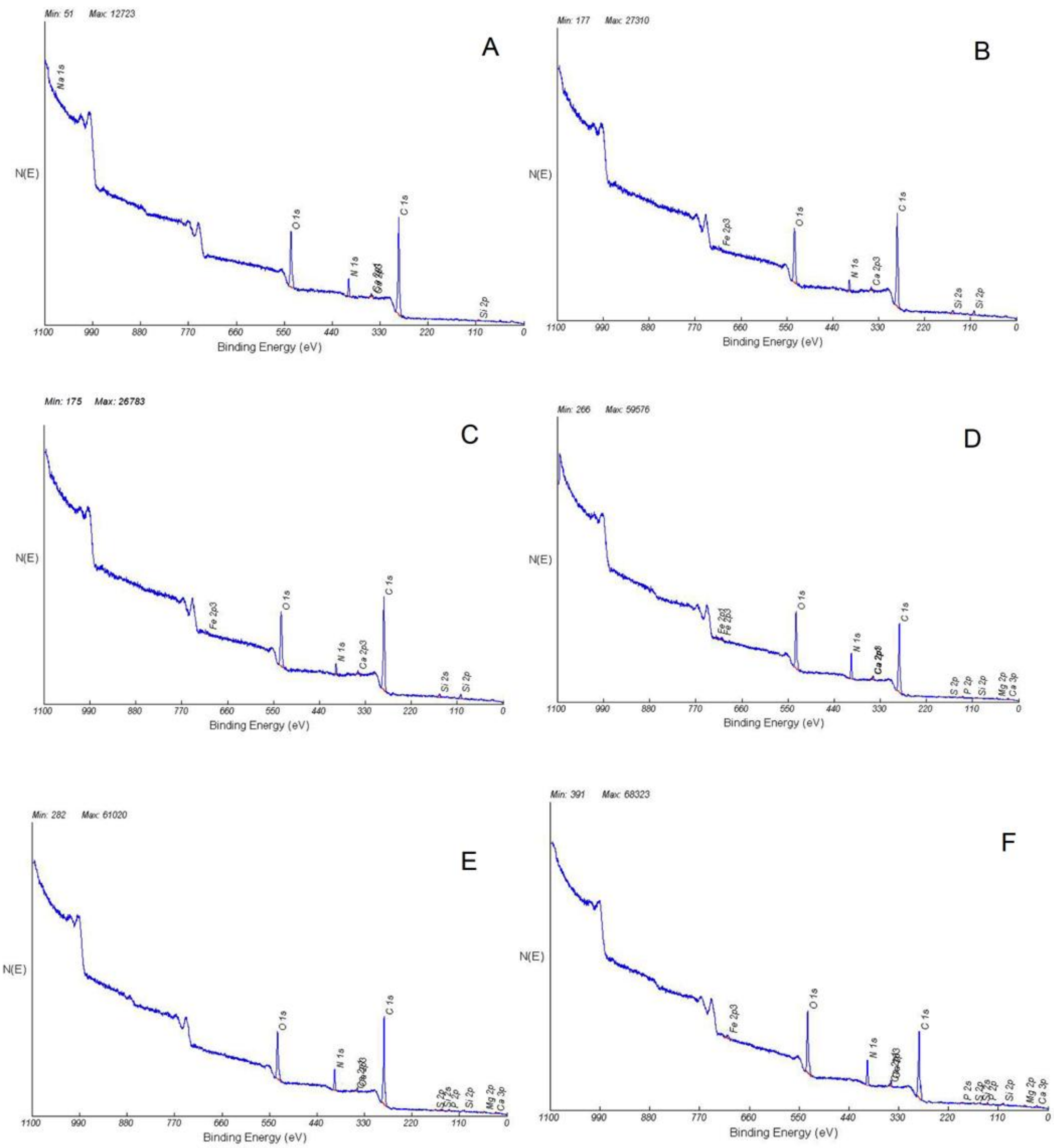

Figure 1a. *XPS spectra for incinerated samples*
(A is the most upper sample and F the lowest sample)

There are elements that are indispensable for life, these elements are hydrogen (that cannot be measured in XPS), carbon, nitrogen and oxygen (Vander Zanden and Rasmussen, 1999). There are also about 20 other that are necessary for biological processes, these are called macro and micro nutrients: calcium, phosphorus, cobalt, iron, manganese, chromium, magnesium, chloride, vanadium, iodine, sulfur, nickel, cupper, molybdenum, sodium, tin, fluorine, silicon, potassium, selenium, and zinc (Underwood, 1971). Some of these nutrients, when they are in excess, are metabolized and excreted, but some of them can be bioaccumulated and may cause illnesses. Such elements are

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cupper, cadmium, fluoride, selenium, molybdenum, vanadium and other considered severe contaminants, like lead, mercury and arsenic which may occasion dead. In the incinerated samples chloride, zinc and potassium have been detected (Tacon et al., 1984).

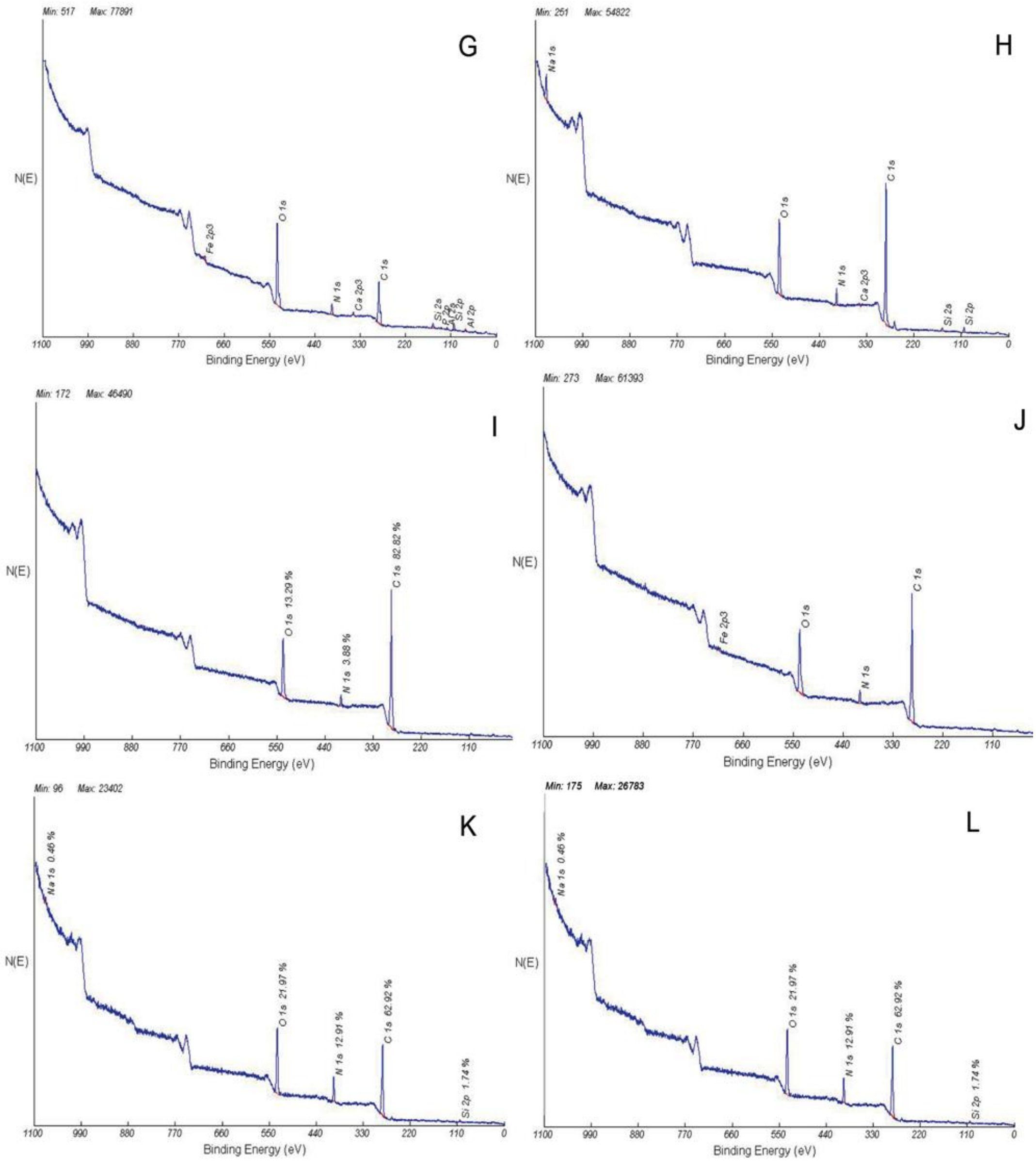

Figure 1b. *XPS spectra for incinerated samples*
(G is the most upper sample and L the lowest sample)

Analysis of incinerated samples shown presence of chloride, zinc and potassium, even when these elements do not represent contamination *per se*, is questionable that they couldn't be detected in the dehydrated samples because there must be present in organic material from living beings (Reinhold, 1975).

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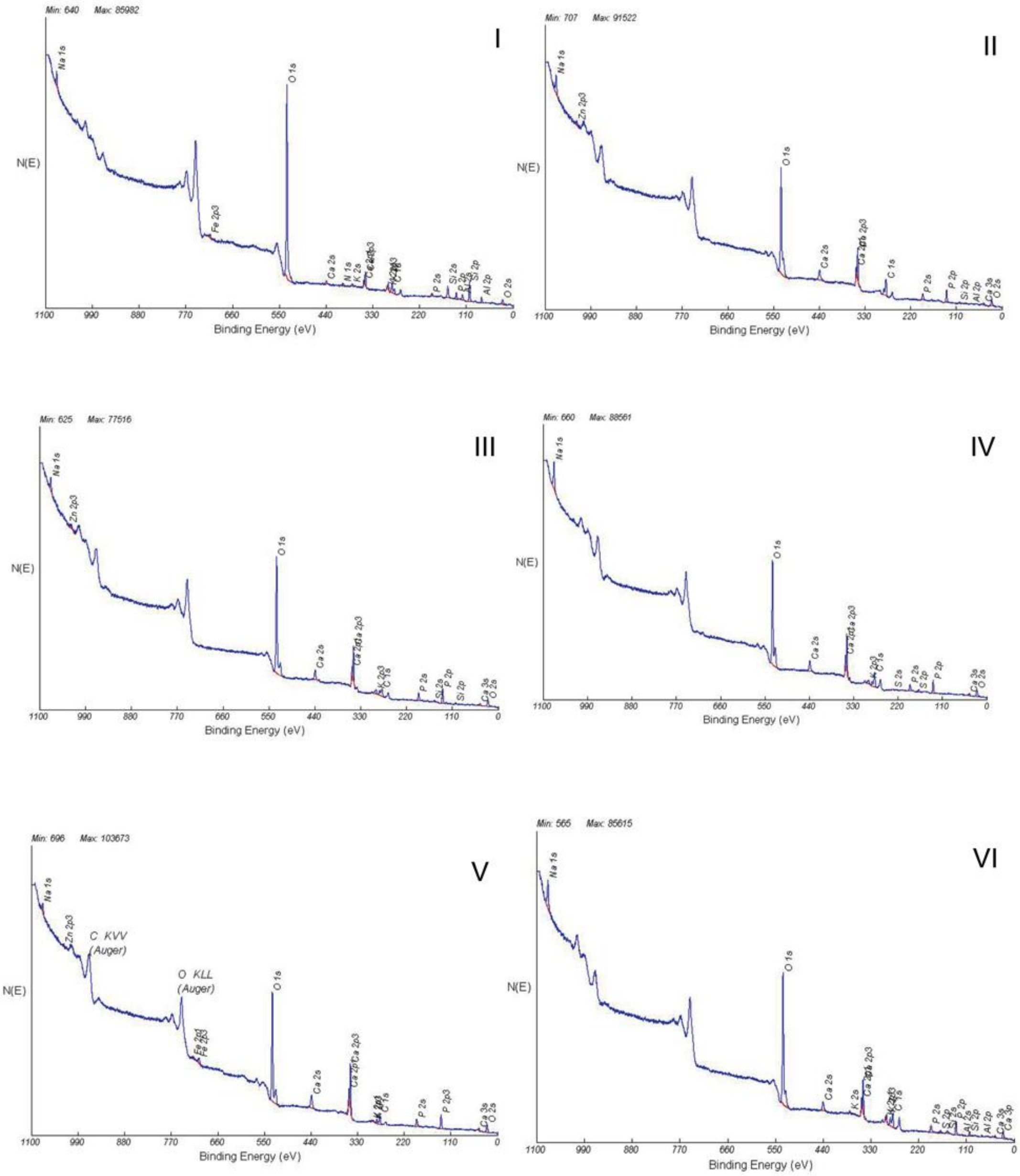

Figure 2a. *XPS spectra for dehydrated samples*
(I is the most upper sample and VI the lowest sample)

We have already mentioned that, even there is no clear evidence of pollution just considering the XPS results; it is missing the concentration value of each element characterized because this could evidence some environmental troubles in the region (Phiri et al., 2005). For example, the cyanide used in the gold extraction process is formed with sodium (Na), so a great amount of Na could indicate pollution by sodium cyanide (Chouinard and Veiga, 2008). To confirm this it is needed water quality analysis searching for each element (that could indicate environmental troubles) found in the organic materials (Charles et al., 2013).

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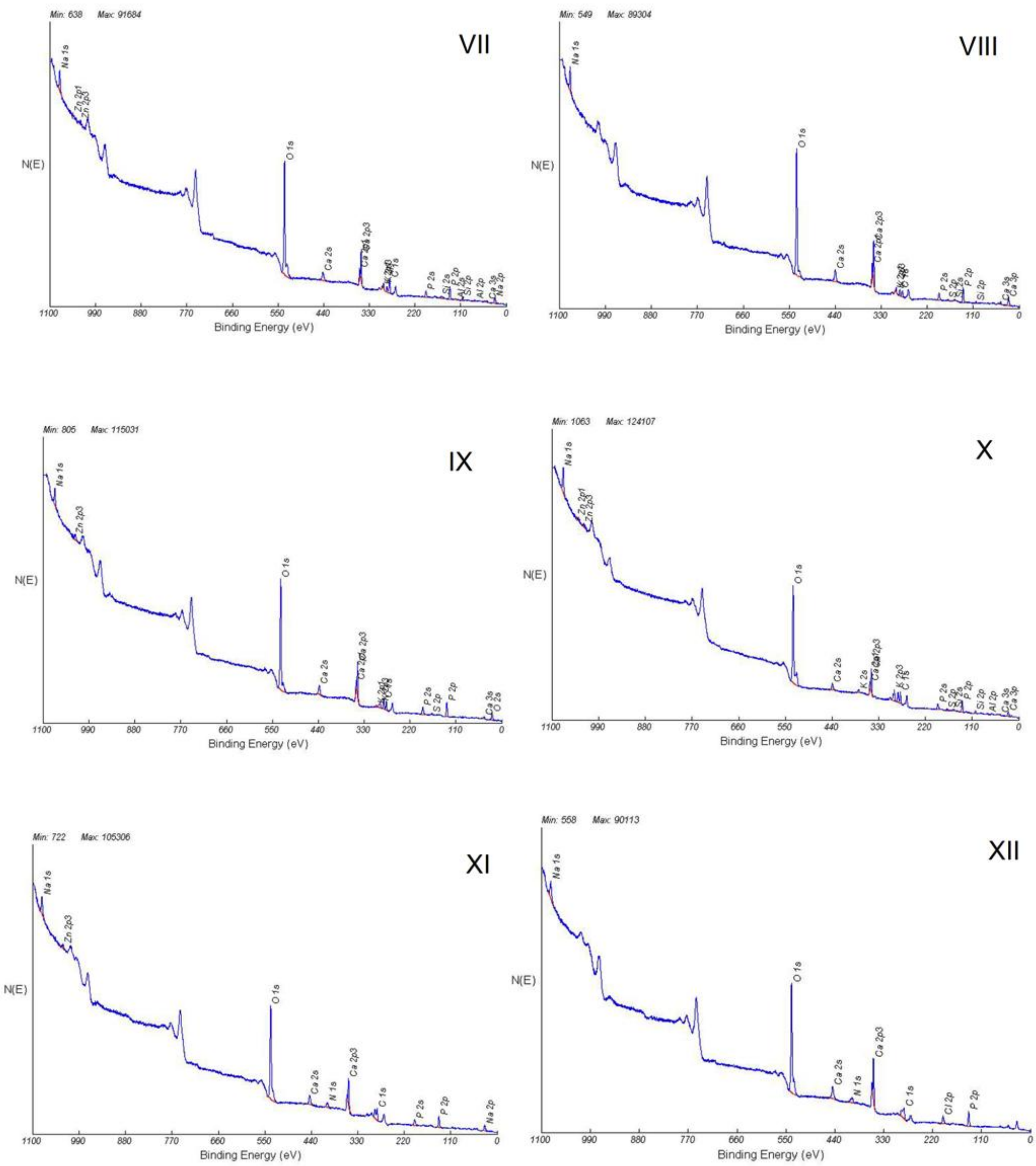

Figure 2b. *XPS spectra for dehydrated samples (VII is the most upper sample and XII the lowest sample).*

In conclusion, both procedures showed different results, which could not confirm water pollution at this time, but we can conclude that sample incineration must be the right technique to analyze organic materials by XPS because the high intensity of its spectra that may maximize the signal of the elements with low content. This is important to detect bioaccumulated elements that represent less the 1%, which is the ideal concentration to appear in the XPS analysis without previous treatment.

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FLORISTIC, DIVERSITY AND SPATIAL DISTRIBUTION OF TREE SPECIES IN A DRY FOREST IN SOUTHERN BRAZIL

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Abstract. This study was conducted in a fragment of deciduous seasonal forest (DSF), located between the municipalities of Piratuba and Ipira, Santa Catarina. The objective was to evaluate the floristic composition and the successional stage through the ecological groups, the Shannon diversity index (H') and the dispersal syndromes of species, also using the H' and the McGinnies index (IGA) to determine the pattern of spatial distribution of species. 14 transects were installed, each with 1,000 m^2, considering all trees with Diameter at Breast Hight (DBH) ≤ 4.0 cm. In total, 2,125 individuals were sampled, belonging to 113 species and 34 families. Myrtaceae and Fabaceae were the families with the highest species richness, with 14.2% and 11.5%, respectively. Euphorbiaceae and Lauraceae added approximately 25% of the individuals. The most abundant species were *Actiniostemon concolor* (Spreng.) Müll. Arg (6.9%) and *Luehea divaricata* Mart. (6.7%). The ecological group of the pioneers totaled 40% of the individuals and 36.3% of the species. The zoochoric syndrome accounted for just over 60% of individuals and species. The H' was 3.92 nats. ind^{-1} and the Pielou evenness (J) was 0.82. The IGA revealed that only over 40% of the species and 60% of the individuals showed a clumped dispersion pattern. The community is on successional transition phase, from the initial to the intermediate stage. In this scenario, management measures adopted for the microscale could be implemented in order to preserve this important repository for diversity. The application of McGinnies index can be of great use in conservation and forest management, as its interpretation may contribute to the development of restoration methods of degraded areas, enrichment of forest remnants, germplasm conservation and other activities.
Keywords: *Atlantic Forest; biodiversity; forest ecology; McGinnies index; phytosociology*

Introduction

The Atlantic Forest originally extended from Rio Grande do Norte to Rio Grande do Sul, also covering inland portions of Brazil, Argentina and Paraguay, totaling approximately 12% of the Brazilian territory (Ribeiro et al., 2009; Fundação SOS Mata Atlântica and INPE - Instituto Nacional de Pesquisas Espaciais, 2011).

However, the industrial and agricultural development, associated with urban expansions, has generated a reduction of more than 80% of its original forest cover (Ribeiro et al., 2009). Today, the forest remnants are scattered in different sizes and exposed to many different disorders (Colombo and Joly, 2010). Therefore, the Atlantic Forest was framed among 25 global hotspots, that is, areas with high

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biodiversity, high rate of endemism and at the same time, under strong anthropogenic impacts (Santos et al., 2012).

In the state of Santa Catarina, the Atlantic Forest originally covered about 80% of its surface area, but today the native forest cover is about 30% of it (Vibrans et al., 2008). Particularly, the original seasonal dry forests areas have suffered intense deforestation process, in order that, currently, the sum of the fragments coverage of the same size or smaller than 50 ha represent only 14% of this original forest typology (Gasper et al., 2013).

According to Freitas and Magalhães (2012), the floristic analysis provides important information about the classification and taxonomic distribution of a plant community and also about ecological attributes of species, such as: diversity, dispersal syndromes, and ecological groups, among others.

The species are organized in different patterns of distribution, as follows: clumped, uniform or random, affected by the influence of abiotic, biotic or even random factors (Matteucci and Colma, 1982; Townsend et al., 2010; Freitas and Magalhães, 2014). The distribution pattern of tree species is a useful tool for the management strategy and / or conservation of a forest population or community (Freitas and Magalhães, 2014).

The aim of this study was to evaluate the floristic composition and the successional stage through the ecological groups and dispersal syndromes of the species present in a fragment of DSF in western Santa Catarina, and also to use the Shannon diversity (H'), the Pielou evenness (J) and the McGinnies (IGA) indices to determine the species distribution pattern.

Materials and Methods

Study Area

The study was conducted in the Peixe river basin, between the cities of Ipira and Piratuba, both located in western Santa Catarina, between the coordinates 27°25'34" South and 51°47'18" West.

The predominant climate in the region is Mesothermal Humid Subtropical, Cfa (Köppen), with no distinct dry season. The monthly rainfall exceeds 60 mm, with average temperatures of the warmest month above 22^{o}C and the coldest month below 18^{o}C and above 3^{o}C (Seiffert and Perdomo, 1998).

The characteristic vegetation formation of this Upper Uruguay river region is the DSF (IBGE – Instituto Brasileiro de Geografia e Estatística, 1992), which extends throughout the valley of the Uruguay river, including the portion of the tributaries that is up to 500 - 600 m high.

Field Procedure

This study adopted the systematic sampling in tracks (IBGE 1992), using transects of 10 x 100 m, in a sampling of 1,000 m^2, per transect. 14 transects were allocated throughout the area, totaling 14,000 m^2 (1.4 ha).

In the tree layer, the height and the Circumference at Breast Height (CBH), or 1.30 m above the ground, were measured for all trees with CBH ≥ 12.57cm (or DBH ≥ 4.0 cm), including the standing dead plants.

The taxonomic identification was carried out with the aid of specialized botanical literature and comparisons with the Herbarium Collection of the Federal University of

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Santa Catarina (UFSC). The validation of the names of species and the exclusion of synonyms were obtained through the website of Flora Brazil (2015). The adopted classification system for families was the APG III (2009), except for Fabaceae, which is divided in three subfamilies: Faboideae, Mimosoideae and Caesalpinoideae (Cronquist, 1981).

Analysis Methods

The species found in the examined fragment were also classified according to their successional stages. According to Budowski (1965), the pioneer species grow in clearings or open spaces, and they are clearly dependent on high light conditions. The early secondary species prefer environments such as small gaps or areas of old clearings, next to pioneer species, while the late secondary species have shown to be shade tolerant in the juvenile stage, forming understory seedlings banks, with great mortality of individuals in the early years, presenting small to medium sized seeds with low viability. The climax species grow slowly, they are light intolerant when adults, with a high number of individuals in natural regeneration, with seed of large and short viability.

The determination of the dispersal syndromes of each species was carried out. Following the categories proposed by Van Der Pijl (1957), species can be classified as: anemochoric (dispersed by the wind), zoochoric (dispersed by animals), autochoric (self-dispersion) and those without classification.

This study also applied the H' and J indices (Brower and Zar, 1984), and the IGA index, which indicates that the distribution pattern is random when it is equal to one, uniform when it is below one, with tendency to cluster when it is above one and equal to or above two, and clumped when it is above two (McGinnies, 1934). The indices were calculated using Mata Nativa 3 (Cientec, 2012).

Results

This study recorded the presence of 2,125 individuals (2,754 stems), belonging to 34 botanical families, 83 genera and 113 species, disregarding the undetermined and dead individuals (*Tab. 1*).

The families presenting the highest species richness were Myrtaceae (16 or 14.2%), Fabaceae - Faboideae (13 or 11.5%), Euphorbiaceae (10 or 8.8%), Lauraceae (8 or 7.1%), Fabaceae - Mimosoideae and Rutaceae (6 or 5.3% each).

Of the sampled individuals, 1,711 (80.5%) are concentrated in only 10 botanical families, which are: Euphorbiaceae (297 or 14% of individuals), Lauraceae (223 or 10.5%), Meliaceae (204 or 9.6%), Sapindaceae (168 or 7.9%) Fabaceae - Faboideae (167 or 7.9%), Salicaceae (162 or 7.6%), Fabaceae - Mimosoideae (155 or 7.3%), Malvaceae (142 or 6.7%), Myrtaceae (138 or 6.5%) and Apocynaceae (55 or 2.6%). The other 550 individuals were distributed among the other 25 families.

In this study, the species with the greatest abundance were: *A. concolor* (146 individuals or 6.9%), *Luehea divaricata* Mart. (142 individuals or 6.7%), *Casearia sylvestris* Sw. (131), *Cupania vernalis* Cambess. (106), *Parapiptadenia rigida* (Benth.) Brenan (93), *Guarea macrophylla* Vahl (77), *Cabralea canjerana* (Vell.) Mart. (69), *Nectandra megapotamica* (Spreng.) Mez (69), *Sebastiana commersoniana* (Baill.) L.B. Sm. & Downs (62), *Ocotea puberula* (Rich.) Nees (56) and *Tabernaemontana catharinensis* A. DC. (49), representing about 50% of all individuals.

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Table 1. *Species sampled in a fragment of Deciduous Seasonal Forest in western Santa Catarina, Brazil, in alphabetical order.*

Scientific Name	**BA**	**N_i**	**GA_I**	**Rating IGA**	**DS**	**EG**
Actiniostemon concolor (Spreng.) Müll. Arg. - EUPHORBIACEAE	0.5532	146	15.05	Clumped	AUT	LS
Annona sp – ANNONACEAE	0.4813	38	5.63	Clumped	ZOO	ES
Apuleia leiocarpa (Vogel) J. F. Macbr. - FABACEAE – CAES.	19.952	36	2.64	Clumped	ANE	CL
Ateleia glazioveana Baill. - FABACEAE – FAB	0.0315	2	2.5	Clumped	ANE	PI
Balfourodendron riedelianum (Engl.) Engl. – RUTACEAE	0.0375	3	5.78	Clumped	ANE	LS
Brugmansia suaveolens (Bonpl. ex Willd.) Bercht. & C. Presl - SOLANACEAE	0.0472	9	3.71	Clumped	AUT	CL
Campomanesia guazumifolia (Camb.) O. Berg - MYRTACEAE	0.0332	7	5.82	Clumped	ZOO	LS
Campomanesia sp. – MYRTACEAE	0.0248	3	2.43	Clumped	ZOO	LS
Campomanesia xanthocarpa O. Berg – MYRTACEAE	0.7213	45	2.07	Clumped	ZOO	LS
Casearia decandra Jacq. – SALICACEAE	0.0612	19	2.89	Clumped	ZOO	ES
Casearia obliqua Spreng. – SALICACEAE	0.2529	12	3.79	Clumped	ZOO	LS
Casearia sylvestris Sw. – SALICACEAE	12.897	131	4.03	Clumped	ZOO	PI
Cedrela fissilis Vell. – MELIACEAE	0.2267	7	3.55	Clumped	ANE	ES
Celtis brasiliensis (Gardner) Planch. – CANNABACEAE	0.0152	2	3.55	Clumped	AUT	PI
Citronella paniculata (Mart.) R.A. Howard - CARDIOPTERIDACEAE	0.0241	1	2.06	Clumped	ZOO	LS
Cordia ecalyculata Vell. – BORAGINACEAE	0.0092	1	2.59	Clumped	ZOO	LS
Dalbergia frutescens (Vell.) Britton - FABACEAE – FAB	0.1248	14	7.35	Clumped	ANE	PI
Endlicheria paniculata (Spreng.) J.F. Macbr. - LAURACEAE	0.4692	9	2.97	Clumped	ZOO	LS
Erythrina falcata Benth. - FABACEAE – FAB	0.0964	2	2.67	Clumped	ANE	ES
Eugenia uniflora L. – MYRTACEAE	0.0163	4	3.86	Clumped	ZOO	ES
Euphorbiaceae 1. – EUPHORBIACEAE	0.0022	1	4.67	Clumped	AUT	NC
Hovenia dulcis Thunb. – RHAMNACEAE	0.8689	48	6.49	Clumped	AUT	CL
Luehea divaricata Mart. – MALVACEAE	51.292	142	2.36	Clumped	ANE	ES
Machaerium paraguariense Hassl. - FABACEAE – FAB	0.2378	32	2.19	Clumped	ANE	LS
Machaerium sp. - FABACEAE – FAB	0.0792	4	5.21	Clumped	ANE	PI
Machaerium stipitatum (DC.) Vogel - FABACEAE – FAB	0.0353	8	2.7	Clumped	ANE	ES
Dead	**0.1872**	**10**	**4.12**	**Clumped**	**-**	
Morus nigra L. – MORACEAE	0.5523	43	3.86	Clumped	ZOO	ES
Myrciaria floribunda (H. West ex Willd.)O.Berg - MYRTACEAE	0.0041	1	4.43	Clumped	ZOO	LS
Nectandra megapotamica (Spreng.) Mez – LAURACEAE	10.305	69	8.8	Clumped	ZOO	PI
Ocotea diospyrifolia (Meisn.) Mez – LAURACEAE	0.0685	8	4.72	Clumped	ZOO	ES

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Scientific Name	BA	N_i	GA_I	Rating IGA	DS	EG
Ocotea odorifera Rohwer – LAURACEAE	0.9478	33	3.2	Clumped	ZOO	LS
Ocotea sp. – LAURACEAE	0.3394	9	3.4	Clumped	ZOO	NC
Parapiptadenia rigida (Benth.) Brenan - FABACEAE – MIM.	57.39	93	3.88	Clumped	AUT	PI
Picrasma crenata Engl. in Engl. & Prantl - SIMAROUBACEAE	0.0445	3	5.3	Clumped	ZOO	LS
Prunus myrtifolia (L.) Urb. – ROSACEAE	0.2245	19	2.67	Clumped	ZOO	PI
Schinus terebinthifolia Raddi - ANACARDICEAE	0.0158	5	2.32	Clumped	ZOO	PI
Sebastiania brasiliensis Spreng. – EUPHORBIACEAE	0.1795	39	11.55	Clumped	AUT	PI
Sebastiania commersoniana (Baill.) L.B. Sm. & Downs - EUPHORBIACEAE	0.6654	62	10.02	Clumped	ZOO	ES
Strychnos brasiliensis (Spreng.) Mart. – LOGANIACEAE	0.0771	6	2.78	Clumped	ZOO	LS
Syagrus romanzoffiana (Cham.) Glassman – ARECACEAE	0.3002	7	2.07	Clumped	ZOO	ES
Tabernaemontana catharinensis A. DC. – APOCYNACEAE	0.6876	49	2.27	Clumped	ZOO	LS
Terminalia australis Cambess. – COMBRETACEAE	0.05	5	4.82	Clumped	ANE	PI
Trichilia claussenii C. DC. – MELIACEAE	0.7544	47	4.84	Clumped	ZOO	CL
Urera baccifera (L.) Gaudich. ex Wedd. – URTICACEAE	0.0196	7	3.24	Clumped	ZOO	PI
Vassobia breviflora (Sendtn.) Hunz. – SOLANACEAE	0.023	6	2.78	Clumped	ZOO	PI
Zanthoxylum petiolare A. St.-Hil. & Tul. – RUTACEAE	0.0173	3	2.89	Clumped	ZOO	ES
Zanthoxylum rhoifolium Lam. – RUTACEAE	0.0068	3	2.89	Clumped	ZOO	PI
Albizia edwallii (Hoehne) Barneby & J.W. Grimes - FABACEAE – MIM	0.1007	10	1.28	Tend. Cluster	ANE	PI
Albizia niopoides (Spruce ex Benth.) Burkart - FABACEAE – MIM	0.0494	4	1.18	Tend. Cluster	ANE	PI
Alchornea triplinervia (Spreng.) Müll. Arg. - EUPHORBIACEAE	0.305	3	1.39	Tend. Cluster	ZOO	ES
Boehmeria caudata Sw. – URTICACEAE	0.0234	5	1.39	Tend. Cluster	ANE	PI
Cabralea canjerana (Vell.) Mart. – MELIACEAE	19.576	69	1.48	Tend. Cluster	ZOO	PI
Calyptranthes tricona D. Legrand – MYRTACEAE	0.1839	19	1.91	Tend. Cluster	ZOO	LS
Celtis iguanaea (Jacq.) Sarg. – CANNABACEAE	0.0876	4	1.49	Tend. Cluster	ZOO	PI
Cestrum intermedium Sendtn. – SOLANACEAE	0.0176	1	1.93	Tend. Cluster	ZOO	ES
Chrysophyllum gonocarpum (Mart. & Eichler ex Miq.) Engl. - SAPOTACEAE	0.2351	24	1.18	Tend. Cluster	ZOO	CL
Cinnamomum verum J. Presl – LAURACEAE	0.0286	2	1.66	Tend. Cluster	AUT	PI
Citrus sp – RUTACEAE	0.0875	16	1.93	Tend. Cluster	ZOO	NC
Coussarea contracta (Walp.) Müll. Arg. – RUBIACEAE	0.0018	1	1.78	Tend. Cluster	ZOO	LS
Fabaceae 1 - FABACEAE – FAB	0.0522	3	1.85	Tend. Cluster	ZOO	NC
Holocalyx balansae Micheli - FABACEAE – CAES	0.0548	1	1.45	Tend. Cluster	ZOO	PI

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(*Cont…*) ***Table 1.*** *Species sampled in a fragment of Deciduous Seasonal Forest in western Santa Catarina, Brazil, in alphabetical order.*

Scientific Name	BA	N_i	GA_I	Rating IGA	DS	EG
Jacaranda micrantha Cham. – BIGNONIACEAE	0.0067	1	1.99	Tend. Cluster	ANE	ES
Maclura tinctoria (L.) D. Don ex Steud. – MORACEAE	0.056	2	1.18	Tend. Cluster	ZOO	ES
Manihot grahamii Hook. – EUPHORBIACEAE	0.0038	1	1.7	Tend. Cluster	AUT	PI
Myrcia oblongata DC. – MYRTACEAE	0.002	1	1.62	Tend. Cluster	ZOO	ES
Myrsine umbellata Mart. – PRIMULACEAE	0.0065	1	1.71	Tend. Cluster	ZOO	PI
Myrtaceae 3 – MYRTACEAE	0.1063	4	1.93	Tend. Cluster	ZOO	NC
Nectandra lanceolata Nees – LAURACEAE	0.9816	37	1.18	Tend. Cluster	ZOO	LS
Ocotea puberula (Rich.) Nees – LAURACEAE	21.442	56	1.7	Tend. Cluster	ZOO	ES
Ruprechtia laxiflora Meisn. – POLYGONACEAE	0.542	11	1.13	Tend. Cluster	ANE	LS
Sapium glandulosum (L.) Morong – EUPHORBIACEAE	0.9342	33	1.53	Tend. Cluster	ZOO	PI
Solanum mauritianum Scop. – SOLANACEAE	0.0514	2	1.93	Tend. Cluster	ZOO	PI
Trichilia elegans A. Juss. – MELIACEAE	0.0081	4	1.18	Tend. Cluster	ZOO	PI
Vitex megapotamica (Spreng.) Moldenke – LAMIACEAE	0.0186	4	1.85	Tend. Cluster	ZOO	CL
Aegiphila brachiata Velloso – LAMIACEAE	0.004	1	0.96	Uniform	ZOO	ES
Allophylus edulis (A. St.-Hil., A. Juss. & Cambess.) Hieron. ex Niederl. SAPINDACEAE	0.1691	21	0.97	Uniform	ZOO	PI
Allophylus guaraniticus Radlk.- SAPINDACEAE	0.0033	1	0.96	Uniform	ZOO	ES
Aloysia virgata (Ruiz & Pav.) Pers. – VERBENACEAE	0.0038	1	0.96	Uniform	ANE	PI
Aspidosperma australe Müll. Arg. – APOCYNACEAE	0.0165	6	0	Uniform	ANE	CL
Bauhinia forficata Link - FABACEAE – FAB	0.0583	8	0.93	Uniform	AUT	PI
Chrysophyllum marginatum (Hook. & Arn.) Radlk. - SAPOTACEAE	0.2617	20	0.96	Uniform	ZOO	PI
Cordia americana (L.) Gottschling & J.S. Mill. - BORAGINACEAE	0.8459	11	0.96	Uniform	ANE	CL
Coutarea hexandra (Jacq.) K. Schum. – RUBIACEAE	0.002	1	0.96	Uniform	ZOO	LS
Cupania vernalis Cambess. – SAPINDACEAE	11.032	106	0.96	Uniform	ZOO	PI
Dahlstedtia pinnata (Benth.) Malme - FABACEAE – FAB	0.0024	1	0.96	Uniform	AUT	ES
Duranta vestita Cham. – VERBENACEAE	0.0537	9	0.96	Uniform	ZOO	CL
Esenbeckia grandiflora Mart. – RUTACEAE	0.0012	1	0.93	Uniform	AUT	LS
Eugenia burkartiana (D. Legrand) D. Legrand - MYRTACEAE	0.032	5	0	Uniform	ZOO	LS
Eugenia rostrifolia D. Legrand – MYRTACEAE	0.0012	1	0.96	Uniform	ZOO	LS
Eugenia sp – MYRTACEAE	0.0179	4	0	Uniform	ZOO	LS
Eugenia subterminalis DC. – MYRTACEAE	0.2524	22	0.96	Uniform	ZOO	LS
Ficus citrifolia Mill. – MORACEAE	0.217	9	0.96	Uniform	AUT	ES

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Scientific Name	**BA**	**N_i**	**GA_I**	**Rating IGA**	**DS**	**EG**
Guarea macrophylla Vahl – MELIACEAE	0.2771	77	0.89	Uniform	ZOO	PI
Inga marginata Willd. - FABACEAE – MIM	0.4978	43	0	Uniform	ZOO	PI
Inga sp. - FABACEAE – MIMOSOIDEAE	0.0013	1	0	Uniform	ZOO	ES
Julocroton sp. – EUPHORBIACEAE	0.0015	1	0.96	Uniform	AUT	NC
Lonchocarpus nitidus (Vogel) Benth. - FABACEAE – FAB	0.2733	28	0.96	Uniform	ANE	ES
Lonchocarpus sp. - FABACEAE – FAB	0.1979	26	0.96	Uniform	ANE	NC
Matayba elaeagnoides Radlk. – SAPINDACEAE	0.3925	40	0.93	Uniform	ZOO	ES
Mimosa bimucronata (DC.) Kuntze - FABACEAE – MIM	0.0194	4	0.96	Uniform	AUT	PI
Myrocarpus frondosus Allemão - FABACEAE – FAB	0.428	30	0.96	Uniform	ANE	ES
Myrsine sp. – PRIMULACEAE	0.0012	1	0.96	Uniform	ZOO	NC
Myrtaceae 1 – MYRTACEAE	0.0113	2	0.96	Uniform	ZOO	NC
Myrtaceae 2 – MYRTACEAE	0.0035	1	0.96	Uniform	ZOO	NC
Myrtaceae 4 – MYRTACEAE	0.074	19	0.96	Uniform	ZOO	NC
Poecilanthe parviflora Benth. - FABACEAE – FAB	0.1629	9	0	Uniform	AUT	LS
Pouteria salicifolia (Spreng.) Radlk. SAPOTACEAE	0.0494	1	0	Uniform	ZOO	PI
Ricinus communis L. – EUPHORBIACEAE	0.0656	10	0.96	Uniform	AUT	PI
Sebastiania klotzschiana (Müll. Arg.) Müll. Arg. - EUPHORBIACEAE	0.005	1	0.96	Uniform	AUT	PI
Sessea regnellii Taub. – SOLANACEAE	0.0337	3	0.89	Uniform	AUT	PI
Trema micrantha (L.) Blume – CANNABACEAE	0.0427	2	0.93	Uniform	ZOO	PI
Vasconcellea quercifolia A. St.-Hil. – CARICACEAE	0.0199	1	0.96	Uniform	ZOO	PI
Zanthoxylum sp. RUTACEAE	0.0754	2	0.93	Uniform	ZOO	PI
INDETERMINATE	**0.5861**	**23**	**-**	**-**	**-**	**-**
TOTAL	**391.096**	**2.125**	-	-	-	**-**

BA: Basal Area (m^2 x ha^{-1}); N_i: number of individuals; GA_I: Mc Ginnies index; Rating IGA; SD: dispersal syndrome; EG: ecological group

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With regard to the ecological groups, 40% of the individuals and 36.3% of the species were categorized as early individuals and late successional species, amounted to 22.1% and 23%, respectively (*Fig. 1*). The zoochoric syndrome accounted for a little over 60% of individuals and species (*Fig. 2*).

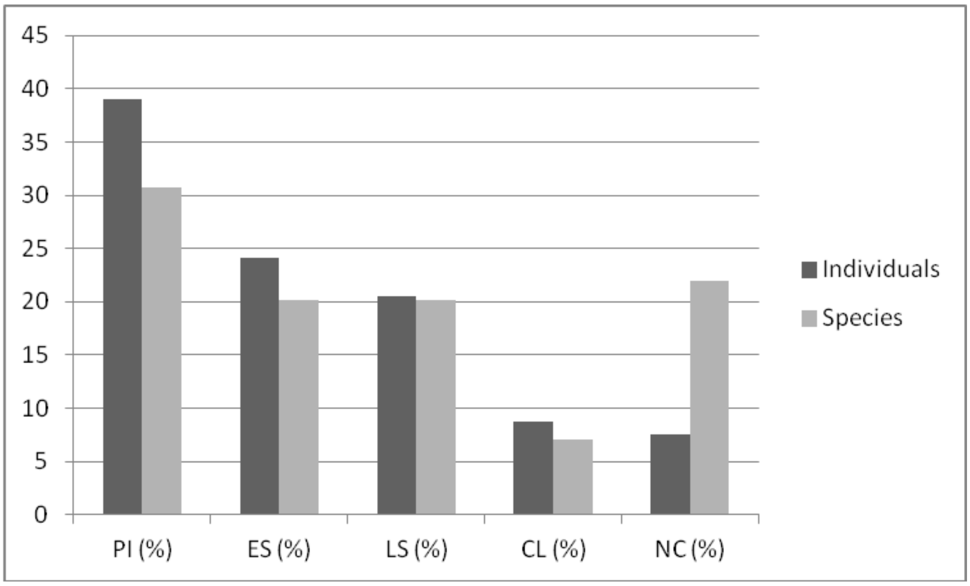

Figure 1. *Frequency distribution of the number of individuals and species, according to ecological groups, in a deciduous seasonal forest fragment in western Santa Catarina, Brazil. CL - Climax; LS - Late Secondary; ES - Early Secondary; PI -Pioneer; NC - Not Classified.*

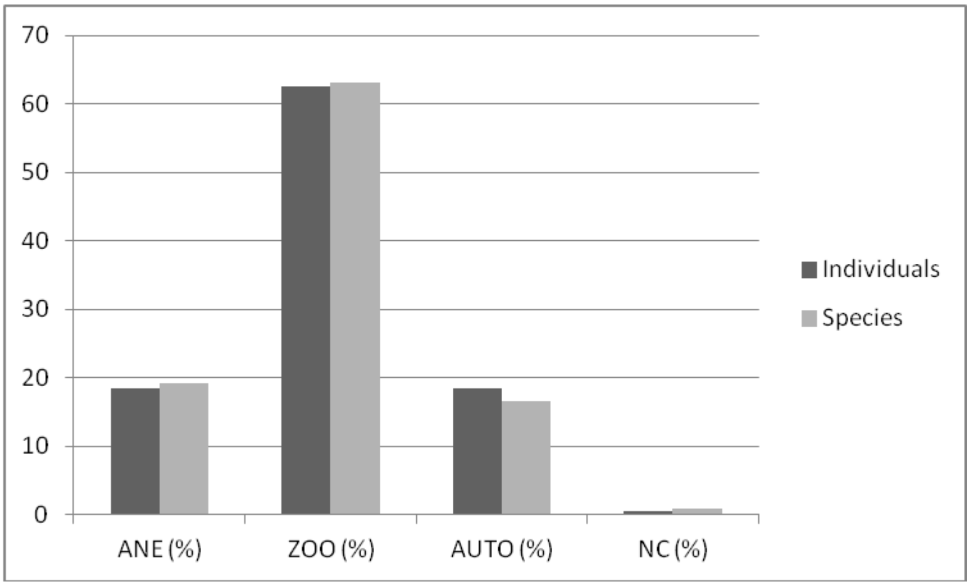

Figure 2. *Frequency distribution of the number of individuals and species, according to the dispersal syndrome, in a deciduous seasonal forest fragment in western Santa Catarina, Brazil. ANE - Anemochoric; AUTO - Autochoric; ZOO - Zoochoric; NC - Not Classified.*

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The H' and the J were, respectively, 3.92 nats. ind^{-1} and 0.82.

This community showed the predominance of a cluster spatial distribution pattern for a little over 40% of the species and for about 60% of the individuals (*Fig. 3*).

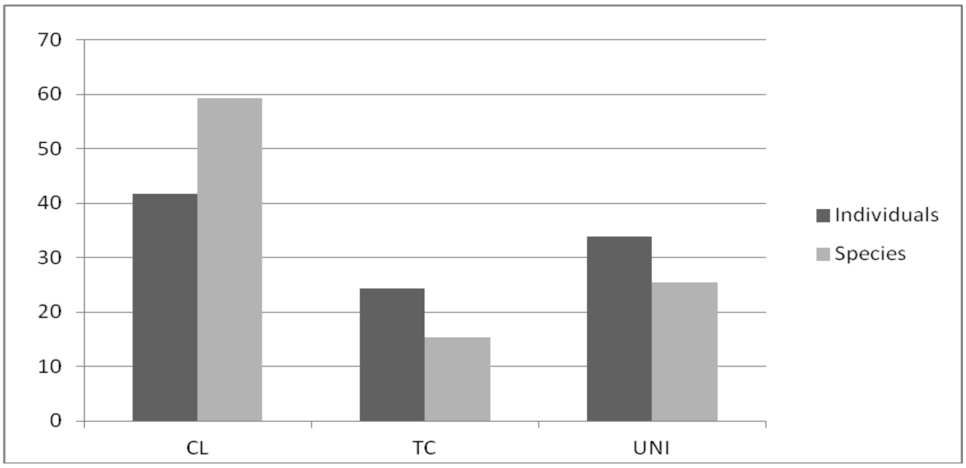

Figure 3. *Frequency distribution of the number of individuals and species, according to McGinnies spatial distribution pattern, in a deciduous seasonal forest fragment in western Santa Catarina, Brazil. CL - Clumped; TC - Tendency to Cluster; UNI -Uniform.*

This study recorded the presence of 11.6% of the species and 5.6% of the individuals considered exotic, such as: *Cinnamomum verum* J. Presl, *Citrus* sp, *Hovenia dulcis* Thunb. and *Morus nigra* L. (Flora Brasil, 2015). The species *Brugmansia suaveolens* (Bonpl. ex Willd.) Bercht. & C. Presl (1.05%) is considered a sub-spontaneous plant, also exotic, but that has adapted to the environment without causing adverse reactions in the community (Flora Brazil, 2015).

Discussion

The floristic comparisons between DSF remnants have shown that these areas are extremely diverse, with low similarity values, even between areas of high spatial proximity, and replacing the longitudinal distribution of deciduous formations (Prado and Gibbs, 1993; Oliveira-Filho and Fontes, 2000; Pennington et al., 2000; Oliveira-Filho et al., 2006). Bolzon and Marchiori (2002) affirm that the deciduous and semideciduous forests are closely associated with the climate history of South America, started in the tertiary period, during the Pliocene.

According to Jasper et al. (2013)**,** Santa Catarina's DSF structure has high species richness when compared to other seasonal forests, which may be associated with higher levels of rainfall in the regions where they occur, and a remarkable presence of widely distributed families and genera in neotropical dry forests.

The results corroborate that Myrtaceae and Fabaceae have a representative presence in many areas of DSF (Oliveira-Filho and Fontes, 2000). The Myrtaceae family has its participation justified by its broad longitudinal distribution spectrum (Negrelle, 2013). However, Gasper et al. (2013) claim that this family is best represented in the rain forests than in the seasonal forests, tending to decline in richness in the Cerrado and Amazon biomes.

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According to Ribeiro and Lima (2009) the Fabaceae (Leguminosae) family is more diversified in seasonal environments, which can be explained from the Tertiary, when the dry forests dominated the major regions of the world, from where the association of legumes with nitrogen-fixing bacteria created efficient evolutionary mechanisms, providing greater plasticity for the occupation in poor environments in nutrients and regeneration.

Five of the 10 species with the highest number of individuals recorded in this study were also cited by the Floristic and Forest Inventory of Santa Catarina (IFFSC) for the DSF, as follows: *O. puberula*, *N. megapotamica*, *L. divaricata*, *C. vernalis* and *C. sylvestris* (Vibrans et al., 2013). The species *C. canjerana* and *P. rigida*, common in DSF, are also present with relative abundance in the Dense Ombrophilous Forest (DOF) in Santa Catarina (Gasper et al., 2012; Gasper et al., 2013). For these authors, *A. concolor* is one of the main species in regenerating layers of DSF, but it rarely appears in DOF.

Some species found in this study are considered as having high timber value, as follows: *Apuleia leiocarpa* (Vogel) J. F. Macbr., *C. canjerana, Nectandra lanceolata* Nees, *P. rigida* and others (Fontana and Sevegnani, 2012).

According to Meyer et al. (2012), among the exotic species recorded in this study, only *H. dulcis* demands further attention. According to these authors, this species occurs with relative abundance in Santa Catarina DSF, deserving greater monitoring in the recruitment process and occupation in forest fragments in the initial and intermediate stages, their typical colonization in environments, and to adopt measures for its management, due to its high potential for competition.

Approximately 25% of the abundance of the examined fragment was represented by pioneer species, as *N. megapotamica*, *C. canjerana*, *G. macrophylla*, *P. rigida*, *C. vernalis* and *C. sylvestris*. Nevertheless, the species *L. divaricata* (early secondary) and *A. concolor* (late secondary) amounted to almost 15% of the sampled tree individuals (*Tab. 1*).

The analysis of dispersal syndromes (*Tab. 1*) indicated that most species and individuals (a little over 60%) use animals as dispersing agents, pattern already evidenced in several studies in the Atlantic Forest, as described by Almeida-Neto et al. (2008). As reported by Gentry (1982), the zoochory is the most important dispersal mode of woody species in the tropical region. In this sense, the community in question is very important for maintaining both its plant populations and the associated fauna.

Considering the participation of ecological groups and dispersal syndromes, it can be seen in the *Table 1* that there is a significant participation of species and pioneer individuals in the study area. There is also a relatively large proportion of more demanding species concerning the quality of their habitats (late secondary and climax) and, at the same time, the associated fauna plays an important role in spreading seedlings. According to Carvalho (2010), forests in the initial stages have lower species richness with biotic dispersion in relation to the preserved forests, and little participation of zoochoric species. Given this hypothesis, it can be suggested that the community in question adds favorable conditions to evolve in successional stages, considering the biotic mechanisms involved in the maintenance of ecosystems.

According to the IFFSC, the examined fragment is highly relevant in terms of diversity and evenness, as their maximum values recorded for samples in Piratuba ($H' = 3.13$ nats. ind^{-1} and $J = 0.63$) and Ipira ($H' = 2.63$ nats. ind^{-1} and $J = 0.78$) were lower than those found in this study ($H' = 3.92$ nats. ind^{-1} and $J = 0.82$) (Vibrans et al., 2013).

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For this community, the level measured by the value of evenness (J = 0.82) confirms the high value of H'. As stated by Werneck et al. (2000), high evenness values indicate more homogeneous abundance distribution among species and may be related to the high uniformity in the proportions of individuals in relation to the species within the community.

Oliveira-Filho and Fontes (2000) stated that Santa Catarina DSF establishes or provides a transition between the typical species from ombrophilous and seasonal environments, a fact that may be one of the arguments to explain the relative richness and diversity of species of Santa Catarina DSF.

Hay et al. (2000) consider three main scales: macro (biogeographical), meso (community) and micro (individuals within a community). The micro scale was considered in this study, restricted to the understanding of the distribution of species in a forest fragment of a small area (about 120 ha).

With respect to both individuals and species, a more uniform pattern was observed in the examined fragment, according to McGinnies index. Rondon Neto et al. (2000) report that the aggregation of tree species in tropical ecosystems may be associated to the kind of seeds dispersion, seeds sources distance, to variations in environmental conditions, particularly as regards their quality and intensity, in addition to the chemical and physical soil characteristics. For Silva et al. (2008), Cain et al. (2011), clusters indicate a mechanism of attraction, demonstrating that the chance of survival of an individual is increased by the presence of others of the same species or the availability of a common resource.

According to Fundação SOS Mata Atlântica and INPE (2015), deforestation rates in Ipira reduced 6% in the period from 2013 to 2014, while in Piratuba numbers remained the same during the same period, suggesting that anthropogenic changes in the region have been at least controlled.

From these results, it can be assumed that the fragment in question is in successional transition phase, from the initial to the intermediate stage, considering the species richness and evenness, and biotic mechanisms as facilitators: the predominance of zoochory, the participation of more demanding species concerning the habitat quality (late secondary and climax) and the clumped distribution of species.

Thus, management measures adopted for a microscale, for example, the choice of species to enrich fragments or the identification of forest species matrices for seed collection may be taken towards the handling and storage repository for this important diversity. Such actions can have a positive impact in the short term, on alpha diversity, i.e., on the number of species in a fragment, and they may, in the medium term, contribute to beta diversity (diversity of habitats). Whittaker (1972) postulated that maintaining the alpha and beta diversity is fundamental to the success of the gamma diversity (regional diversity).

This study corroborates the assumption that the Santa Catarina DSF still presents forest remnants with high richness and diversity of species, such as the case of the analyzed fragment (113 species and H '= 3.92 nats. $ind.^{-1}$). The studied fragment is in transition, from the initial to the intermediate stage, and it can be favored by biotic regulatory mechanisms, especially the zoochory and the clustered pattern distribution.

The application of McGinnies index can be of great use in the conservation and forest management, as their interpretation may contribute to the development of restoration methods of degraded areas, enrichment of forest remnants, germplasm conservation and other activities.

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AIR-DRYING EFFECT ON SOIL REACTION AND PHOSPHORUS EXTRACTABILITY FROM UPLAND-LOWLAND TROPICAL SOILS AS RELATED TO THEIR COLLOIDAL STABILITY

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Abstract. Although air-drying is a universally accepted practice for preserving soils before analyses, it can irreversibly affect certain soil processes and, hence, results of soil analyses. Reports about such effects on extractable/available phosphorus (P_{av}) are conflicting and are scanty for low-P_{av} tropical soils. Moreover, little is known about its relative effects for well and poorly drained soils or about any underlying role of soil colloidal stability. In this study, soil samples from well-drained uplands and adjoining poorly drained lowlands in four locations in southeastern Nigeria were analysed field-moist and air-dried for pH and P_{av}. The soils are mostly medium-textured. A trend existed of drying-induced decreases in soil pH for both uplands (4.8-5.8) and lowlands (4.6-5.6); whereas this effect was consistently non-significant in the former (unless tested for irrespective of location, 5.35 vs 5.53), it was location-specific in the latter. Air-drying tended to increase and decrease P_{av} in upland soils (0.62-12.75 mg kg^{-1}) and lowland soils (0.93-20.52 mg kg^{-1}), respectively, with a reverse effect in one lowland with values exceeding 15 mg kg^{-1}. However, these effects were non-significant except in one upland soil with evidence of appreciable organic matter. Correlations between soil pH and P_{av} were positive and non-significant, but strongest for field-moist lowland soils. Soil colloidal stability indices of water-dispersible clay and clay flocculation index proved useful in understanding the results for soil pH and P_{av}, respectively, with the effect of the former being more distinct than that of the latter. Air-drying is still recommended only for upland soils while we explore fully the interrelationships among drying-induced differences and colloidal stability for soils of contrasting drainage status.
Keywords: *available phosphorus, clay flocculation index, field-moist soil samples, microaggregate stability, poorly drained soils*

Introduction

Air-drying soil samples before analyses is a common practice in most laboratories. This is because air-drying allows soil samples to be stored for analyses even after a long time, since dried soil samples are perceived to undergo minimal changes caused by microbial and chemical reactions compared to field-moist samples (Erich and Hoskins, 2011). It equally increases the ease of handling of soil samples during analyses. However, air-drying soils before analysis often increases the concentration of solutes in soil solution which may later be precipitated or lead to an increased sorption on soil surfaces (Erich and Hoskins, 2011), and this may influence the results of soil analyses for easily sorbed soil chemical constituents. When solutes precipitate in air-dried soils, their complete solubility after rewetting such soils is unlikely. It has been shown repeatedly that drying increases surface acidity, and that this phenomenon can affect the solubility and availability of many nutrients including phosphorus (Bartlett and James, 1980; Haynes and Swift, 1985; Erich and Hoskins, 2011).

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Probably because of the agronomic and environmental importance of phosphorus, its extractability from soil as affected by air-drying has been studied extensively, although with conflicting reports. Studies reporting increases in available phosphorus with air-drying (Gilliam and Richter, 1988; Srivastava, 1997; Yongsong et al., 1998; Schaerer et al., 2005; Erich and Hoskins, 2011; Achat et al., 2012; Xiao et al., 2012) seem to outnumber those that found no such effect (Ekpete, 1976; Peltovuori and Soinne, 2005; Soinne et al., 2010; Xu et al., 2011), which in turn seem to outnumber those reporting decreases in extractable phosphorus with air-drying (Haynes and Swift, 1985). With the exception of the pilot studies by Ekpete (1976), Srivastava (1997) and Yongsong et al. (1998) which were carried out on tropical soils and that by Gilliam and Richter (1988) in a sub-tropical environment, the rest of the studies just cited were carried out on soils from temperate locations.

Considering the comparatively low concentration of available phosphorus in tropical soils particularly those of West Africa (Enwezor and Moore, 1966; Owusu-Bennoah et al., 1995), the issue of differing values of available phosphorus between air-dried and field-moist soils should be of greater concern to researchers in the tropics. On the other hand, the relationships between soil pH and phosphorus availability have been extensively studied on tropical soils (Mokwunye, 1975; Eze and Loganathan, 1990; Sato and Comerford, 2005; among others), and some of such studies show that the low availability of phosphorus in tropical soils is often exacerbated by the acidic nature of the soils (e.g., Sato and Comerford, 2005). Yet, as with phosphorus availability, there is paucity of data about the influence of air-drying soil samples before analysis on soil reaction in tropical soils.

Notably, research has implicated appreciable concentration of organic matter in soils as being responsible for the differences in soil available phosphorus induced by air-drying (Schaerer et al., 2003; Xiao et al., 2012). The majority of tropical soils show low values of organic matter concentration, often not exceeding 10 g kg^{-1} of soil. In tropical watersheds, however, organic matter and plant nutrients are usually lower in upland soils compared to adjoining lowland soils due to geological fertilization (Obalum et al., 2012a), as well as to poor drainage status of the latter. The quest for clear-cut information on the effect of drying on phosphorus availability in tropical soils should therefore not be limited to well-drained upland soils but also extended to adjoining poorly drained lowland soils. This approach is rare in the literature (Gilliam and Richter, 1988), but can have additional benefits. In lowland *sawah* rice systems of West Africa where phosphorus availability is suggested to promote rice yields (Obalum et al., 2014), for instance, such information also for lowland soils can help to develop strategies for managing phosphorus in *sawah* soils.

Furthermore, the present study considered a possible interdependence among soil pH, available phosphorus and structural stability at the colloidal level. Soil pH influences phosphorus availability (Sato and Comerford, 2005) as well as colloidal stability of the soil (Igwe and Udegbunam, 2008). Among the soil processes known to be important in colloidal stability is alternate wetting and drying (de Oliveira et al., 2005). Since the practice of drying initially wet soil samples and rewetting them before analyses simulates a wetting-drying cycle, at two extremes though, the response of soil pH and/or phosphorus to air-drying may be a reflection of change in soil colloidal stability induced by such drying. This hypothesis is yet to receive the attention of any research, going by our review of the literature. Because of their topographical and hydrological differences that promote geological fertilization, uplands and lowlands

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often differ not only in organic matter and drainage status but also in texture. Many other related soil properties, including colloidal stability, differ between them too. Again, this makes the concurrent study of uplands and lowlands in this study imperative.

In this study, four upland soils and adjoining lowland soils with clearly contrasting drainage status and occurring in four locations of southeastern Nigeria were sampled and analysed for pH and available phosphorus with and without air-drying. Some easily measurable indices of soil colloidal stability were equally derived. The objectives of the study were to assess: (i) the effect of air-drying on soil test results for pH and available phosphorus in well and poorly drained soils, (ii) the nature of the relationships between pH and phosphorus availability in air-dried and field-moist soils, and (ii) the role of soil colloidal stability in any effect of air-drying on these two soil properties.

Materials and Methods

Study sites and soil sampling

The study was conducted using soils from four locations in southeastern Nigeria, including Agbobu, Akaeze, Nando and Omasi (*Figure 1*). Some climatic and ecological features of these locations are briefly described in *Table 1*.

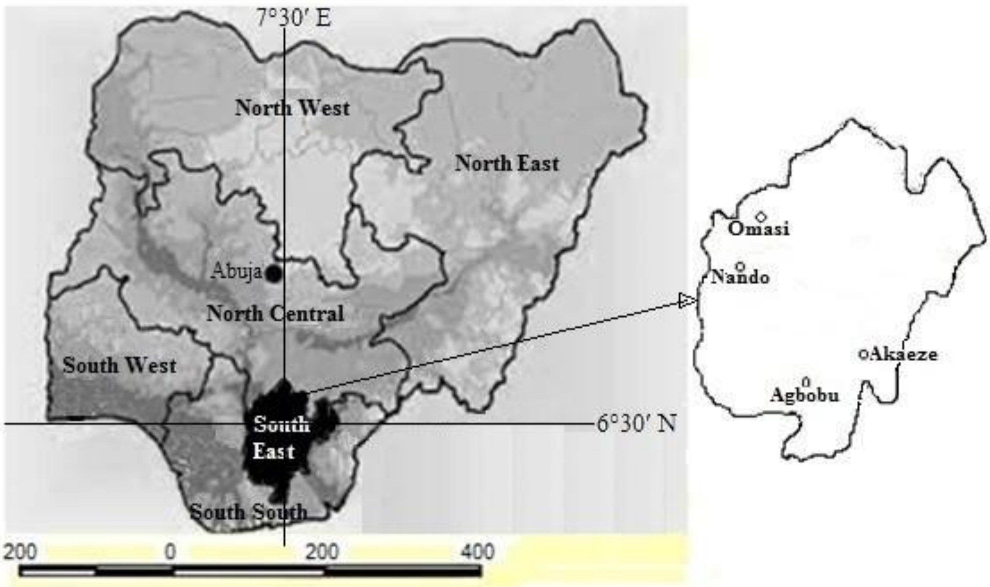

Figure 1. *Map of Nigeria showing southeastern Nigeria and the study locations*

Soils were sampled from an upland-lowland setting in all the four locations during the rainy season of tropical Africa, when the difference in drainage status of upland soils and adjoining lowland soils is usually most evident. At each location, three samples were collected from random spots along a short transect of about 20 m in each of the two topographic positions, giving 12 samples of upland soils and 12 samples of lowland soils. In all four locations, transects in the uplands were somewhat parallel to those in the lowlands, and were approximately 50 m apart. Soil samples were collected from 0-20 cm depth using an auger, packaged in black polythene bags and conveyed to the laboratory.

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***Table 1.** Brief description of study sites*

Location	Climate (mean annual)		Vegetation zone
	Rainfall[†] (mm)	Temperature (°C)	
Agbobu	2250 (~ 4 months)	27-28	Humid rainforest
Akaeze	1350 (~ 5 months)	29	Derived savannah
Nando	2300 (~ 4 months)	28	Humid rainforest
Omasi	2000 (~ 4 months)	29	Forest-Savannah Transition

[†]Dry season length is enclosed in parenthesis.

The soil samples were each partitioned into two equal parts by mass. One part was weighed and its field-moist mass, M_{fm}, recorded before being air-dried for 48 h under laboratory conditions after which its air-dried mass, M_{ad}, was also recorded. The other part was not air-dried but preserved in its field-moist condition. Both air-dried and field-moist soil samples were passed through a 2-mm sieve before laboratory analyses.

Soil laboratory analyses

Particle size distribution was determined on air-dried samples by mechanical analysis using the Hydrometer method described by Gee and Bauder (1986), and this was done twice per sample; one with and the other without 0.1N NaOH as dispersing agent, to enable the derivation of some soil colloidal stability indices. Soil pH was determined in suspensions of soil in both deionized water and KCl in a soil-liquid ratio of 1.0:2.5 (Mclean, 1982). Available phosphorus was extracted with Bray II, as this reagent is very effective for extracting phosphorus from acid soils of southeastern Nigeria (Enwezor, 1977). The determination was done using the method described by Olsen and Sommers (1982). For soil pH and available phosphorus, the amounts of air-dried soil samples used in the analyses were as recommended by the standard methods cited (10 g and 5 g, respectively), while the amounts of field-moist samples used were the equivalent masses, obtained thus:

$$EM_{fm} = \frac{RM_{ad} \times M_{fm}}{M_{ad}} \quad \text{(Eq. 1)}$$

where EM_{fm} is the equivalent mass of field-moist soil sample used for analysis; RM_{ad} is the recommended mass of air-dried sample for use in analysis; and M_{fm} and M_{ad} are the recorded masses of the sample in its field-moist and air-dried conditions, respectively before analyses.

Soil colloidal stability indices

The following easily measurable colloidal stability indices of the soils were derived after mechanical analysis for particle size distribution (Igwe and Obalum, 2013):

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(i) Water-dispersible silt (WDSi) = Silt content of the soil (%) obtained after mechanical analysis without dispersing agent. The lower the value of WDSi, the greater the colloidal stability of the soil.
(ii) Water-dispersible clay (WDC) = Clay content of the soil (%) obtained after mechanical analysis without dispersing agent. The lower the value of WDC, the greater the colloidal stability of the soil.
(iii) Clay flocculation index (CFI) = (Total clay − WDC)/Total clay. The higher the value of CFI, the greater the colloidal stability of the soil.

Statistical analysis

Preliminary comparison of the uplands and the lowlands using t-test revealed that they differed in soil pH (5.44 and 4.94, respectively; t_{cal} = 2.82*) but not in available phosphorus (5.91 and 8.32 mg kg^{-1}, respectively; t_{cal} = 0.80^{ns}). These differences observed, though in one out of the two soil parameters considered, informed the decision to analyse the data separately for the two topographic positions. One-way analysis of variance (ANOVA) was used to test for differences in pH and available phosphorus between air-dried and field-moist soils from each of the four locations. Treatment means were deemed different at $p \leq 0.05$. Such statistically significant differences between means were detected using Tukey's family error rate. Furthermore, we regarded locations as replications and subjected treatment means for soil pH and available phosphorus from all locations to t-test in order to compare the grand means for air-dried and field-moist soils. Again this was done separately for upland and lowland soils. Correlation and regression analyses were used to examine the relationships between soil pH and phosphorus extractability from the air-dried and field-moist soils. Regression analysis was also used to examine the nature of the relationships between treatment effect and selected indices of colloidal stability of the soils, on one hand and between mean values for air-dried and field-moist soils, on the other. All analyses were carried out using the two software, Minitab 17 and Microsoft excel sheet.

Results and Discussion

Both upland and lowland soils from the study locations are generally of medium texture except for Nando where the soil is fine-textured, especially at the upland region (*Table 2*). Instead of coarser soil texture due to natural flow of sediments, the uplands show similar soil texture as the lowlands in two of the locations (Akaeze and Omasi), and even finer texture than the lowlands in the other two, Agbobu and Nando, both of where the sampled sites are on a gently rolling terrain. Owusu-Bennoah et al. (2000) reported similar scenario on a gentle topography in Ghana and attributed it to mixing of the lowland part of the topography with alluvial sediments. In our opinion, the present results suggest that clay eluviation/illuviation is pronounced in the concerned soils, such that upland-lowland surface erosion exposes the illuvial, finer-textured layer in the uplands while leaving the adjoining lowlands coarser.

The upland soils showed generally lower values of water-dispersible clay (WDC) and higher values of clay flocculation index (CFI) compared to their lowland counterparts, implying greater colloidal stability of the uplands than the lowlands. The well-drained condition of the uplands as against the seasonally flooded condition of the lowlands would explain this observation (Obalum et al., 2014). The data in *Table 2* also show that soils from Nando had higher contents of water-dispersible silt (WDSi) than

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total silt and were more stable than soils from the other locations. That the silt content without dispersion is higher than that after 'complete' dispersion for these clayey soils is not an aberration. This observation rather suggests that, in the soil-water suspension without a dispersing agent, some flocculated clay particles could not be dispersed and so posed as silt particles, and this resulted in the high CFI and hence high colloidal stability of the soils. The next to Nando in terms of fineness of soil texture is Akaeze but it showed the lowest CFI and hence the lowest stability, an indication that clay flocculation and colloidal stability are not attributes of fine soil texture, but of other soil factors (Mbagwu and Schwertmann, 2006; Igwe and Obalum, 2013).

Table 3 shows that, for both uplands and lowlands of the four locations, soil pH tended to be lower in air-dried samples compared to the field-moist samples, and that the only cases of significant differences were in the lowland soils – Akaeze for pH in both water and KCl, Omasi for pH in water and Nando for pH in KCl. The decrease in pH of some of the soils due to air-drying and the associated increase in surface acidity has similarly been reported elsewhere (Erich and Hoskins, 2011). The chemistry of drying mineral surfaces and its acidifying effect on soil surface can be found in Dowding et al. (2005). For the upland soils, pH of air-dried samples only showed tendency for lower values which were not significantly different from those of field-moist samples, suggesting that the acidifying effect of drying on soil surface may not be pronounced in well-drained upland soils. Conversely, the significant treatment effect on soil pH in water for the lowland soils only at Akaeze and Omasi may be linked to the high tendency for clay dispersion in these two locations (particularly the former) compared to Agbobu and Nando, as evident in the values for WDC and CFI.

When the entire soils of the study were considered together irrespective of location, the differences in soil pH between the air-dried and the field-moist samples were significant for the upland soils but not for the lowland soils. The results suggest such that differences in parent material and hence soil texture are needed for the acidifying effect of drying to manifest in well-drained soils, but that such underlying influence of texture on the acidifying effect of drying can be masked in poorly drained soils. Overall, these results imply that the effect of air-drying on pH of well-drained soils may be unimportant at local scales but may be important at the regional scale, and vice versa for poorly drained soils.

Notably, values for the pH gradient (pH-H_2O minus pH-KCl) were generally higher for the field-moist samples than for the air-dried samples of the uplands soils; the reverse was true for the lowland soils except for Omasi. The upland soils thus conformed to the normal pattern of pH range in soils whereby such values increase with increasing pH in water (Thomas, 1996). This was not the case for the lowland soils in which air-drying had greater acidifying effect, implying that their air-dried samples had higher concentration of H^+ than the field-moist samples. Because H_2O displaces less H^+ from soil colloids than KCl, the soil solution in KCl probably had more displaced H^+ which lowered further the value for pH-KCl in the air-dried samples of the lowland soils, hence the observation.

For both air-dried and field-moist samples, upland soils consistently showed higher pH values compared to their lowland counterparts; the only exception was the field-moist samples of soils from Akaeze. This higher acidity level of the lowland soils is surprising, considering the relatively reduced soil condition of the lowlands, which was expected to lower soil acidity. Obalum et al. (2012b) reported similar acidity problem in some lowland *sawah* soils from northcentral Nigeria, in spite of the reduced condition

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of those soils. However, runoff and sediments from uplands normally accumulates in lowlands in typical upland-lowland setting (Obalum et al., 2012a), and often contain dissolved organic matter. Although the reduced condition of lowlands helps to preserve their soil organic matter, partial oxidation following intermittent periods of aerobiosis is also possible; and such a situation can increase soil acidity. Furthermore, since sandy soils are more prone to acidification (Marx et al., 1999), the generally finer texture of the upland soils compared to their lowland counterparts (which is particularly true for Agbobu and Nando soils; see *Table 2*) may have also contributed to the observed lower acidity of the upland soils than the lowland soils.

In the upland soils, values for soil available phosphorus were generally higher in air-dried samples compared to field-moist samples, but the differences were significant only for Akaeze (*Table 4*). Several authors have reported increased phosphorus extractability from soil with drying (Yongsong et al., 1998; Erich and Hoskins, 2011; Achat et al., 2012; Xiao et al., 2012). The major factors contributing to this phenomenon include mineralization of soil organic matter and lysis of microbial cells (Srivastava, 1997; Yongsong et al., 1998; Xu et al., 2011; Xiao et al., 2012). This is particularly true for soils high in organic matter (Xu et al., 2011; Achat et al., 2012). Soil organic matter was not determined in the present study, but the soils are known to be typically low in organic matter (Obalum et al., 2013), hence the generally insignificant drying-induced differences in extractable phosphorus. Peltovuori and Soinne (2005) argue that the destruction of organomineral complexes by air-drying simultaneously releases phosphorus and exposes new surfaces on which phosphorus could sorb.

The significant differences for Akaeze soil could be explained by its high clay content (see *Table 2*) which suggests a greater potential for formation of clay-organic complexes that can protect organic matter against rapid mineralization and loss. The soil also had the highest content of WDC, which tends to increase with an increase in organic matter in upland soils (Igwe and Udegbunam, 2008). The higher extractable phosphorus in the air-dried than the field-moist samples observed only for Akaeze soil was therefore attributed to the possible relatively high concentration of organic matter which was less stable to the oxidizing condition of air-drying (Gilliam and Richter, 1988). In such high-organic matter soils, the disruption of stable aggregates after drying and sieving also facilitates the release of inorganic phosphorus tied up in Fe-Al-phosphate complexes (Peltovuori and Soinne, 2005; Erich and Hoskins, 2011; Achat et al., 2012). Kaiser et al. (2015) document the influence of drying on aggregates, quantity and quality of dissolved organic matter, and soil microbiota.

Conversely, available phosphorus of the lowland soils tended to be higher in field-moist samples compared to air-dried samples, except for Omasi. Ekpete (1976) made similar observation in some waterlogged Nigerian soils, and so concluded that air-drying be still done when analysing such soils for phosphorus availability. It appears, therefore, that significant or non-significant decrease rather any increase in available phosphorus with air-drying should be expected in poorly drained tropical soils. The reason for the deviation of Omasi lowland soil from this trend is not clear, although it may be related to the 'high' value (> 15 mg kg^{-1}) of available phosphorus in the soil compared to the other locations. This reasoning is supported by the range of available phosphorus for Omasi lowland soil being similar to the range in other tropical soils (16.4-28.3 mg kg^{-1}, also Olsen-P) where air-drying was also reported to increase phosphorus extractability (Yongsong et al., 1998).

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Table 2. *Textural properties and colloidal stability indices of the experimental soils*

Location	Topographic position	Sand	Silt	Clay	Textural class	WDSi (%)	WDC (%)	CFI
			(%)					
Agbobu	Upland	51.8	31.4	16.8	Loam	29.4	4.8	0.71
	Lowland	73.8	17.4	8.8	Sandy loam	11.4	4.8	0.45
Akaeze	Upland	39.8	29.4	30.8	Clay loam	27.4	16.8	0.45
	Lowland	43.8	23.4	32.8	Clay loam	17.4	24.8	0.24
Nando	Upland	41.8	17.4	40.8	Clay	29.4	6.8	0.83
	Lowland	45.8	11.4	42.8	Sandy clay	29.4	10.8	0.75
Omasi	Upland	59.8	25.4	14.8	Sandy loam	21.4	6.8	0.54
	Lowland	59.8	21.4	18.8	Sandy loam	21.4	8.8	0.53

WDSi-water-dispersible silt; WDC-water-dispersible clay; CFI-clay flocculation index

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Table 3. *Air-drying effect on pH of some upland and lowland soils in southeastern Nigeria*

		Upland			Lowland		
Location	Treatment	pH-H_2O (I)	pH-KCl (II)	ΔpH (I − II)	pH-H_2O (I)	pH-KCl (II)	ΔpH (I − II)
Agbobu	Air-dried	$5.3^a \pm 0.12$	$4.7^a \pm 0.15$	0.6	$4.9^a \pm 0.10$	$3.8^a \pm 0.03$	1.1
	Field-moist	$5.6^a \pm 0.12$	$4.8^a \pm 0.17$	0.8	$4.9^a \pm 0.12$	$3.9^a \pm 0.05$	1.0
Akaeze	Air-dried	$4.8^a \pm 0.09$	$3.8^a \pm 0.09$	1.0	$4.6^b \pm 0.06$	$3.6^b \pm 0.06$	1.0
	Field-moist	$4.9^a \pm 0.12$	$3.8^a \pm 0.09$	1.1	$5.6^a \pm 0.03$	$4.7^a \pm 0.05$	0.9
Nando	Air-dried	$5.6^a \pm 0.03$	$4.7^a \pm 0.03$	0.9	$4.7^a \pm 0.03$	$3.9^b \pm 0.06$	0.8
	Field-moist	$5.8^a \pm 0.16$	$4.9^a \pm 0.07$	0.9	$4.9^a \pm 0.09$	$4.2^a \pm 0.09$	0.7
Omasi	Air-dried	$5.7^a \pm 0.09$	$4.9^a \pm 0.17$	0.8	$4.8^b \pm 0.06$	$3.8^a \pm 0.03$	1.0
	Field-moist	$5.8^a \pm 0.15$	$4.9^a \pm 0.17$	0.9	$5.1^a \pm 0.06$	$3.9^a \pm 0.03$	1.2
All locations[†]	Air-dried	$5.35^b \pm 0.20$	$4.52^a \pm 0.25$	0.83	$4.75^a \pm 0.06$	$3.78^a \pm 0.06$	0.97
	Field-moist	$5.53^a \pm 0.21$	$4.60^a \pm 0.27$	0.93	$5.13^a \pm 0.17$	$4.18^a \pm 0.19$	0.95
		($t_{cal} = 3.66$; $p = 0.04$)	($t_{cal} = 1.57$; $p = 0.22$)		($t_{cal} = 1.72$; $p = 0.18$)	($t_{cal} = 1.68$; $p = 0.19$)	

Values are means ± standard error of the mean. For each location, means for air-dried and field-moist samples that do not share a letter are significantly ($p \leq 0.05$) different. [†]Comparison based on the mean values from the four locations by t-test.

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Overall, the results of the t-test analysis indicated no significant differences in the values for available phosphorus between air-dried and field-moist samples for both the upland soils and the lowland soils (*Table 4*). These results imply that air-drying lowers the extractability of phosphorus from the soils (irrespective of drainage status), but such decreases are often not appreciable and are not a problem at the regional scale. Our data support Kaiser et al. (2015) who concluded in their review that air-drying effect is location-specific. Notably, phosphorus status was generally lower in upland soils compared to lowland soils across the locations. This would be explained by the fact that, in wet soils, redox reaction releases phosphorus while the dissolution of iron (III) oxyhydroxides further releases immobilized phosphorus (Obalum et al., 2012b; Rabeharisoa et al., 2012).

Table 4. *Air-drying effect on available phosphorus (mg kg^{-1}) of some upland and lowland soils in southeastern Nigeria*

Location	Treatment	Upland	Lowland
Agbobu	Air-dried	$4.04^a \pm 1.24$	$4.35^a \pm 1.12$
	Field-moist	$4.04^a \pm 1.73$	$4.35^a \pm 1.55$
Akaeze	Air-dried	$7.46^a \pm 0.54$	$7.77^a \pm 0.62$
	Field-moist	$4.97^b \pm 0.31$	$10.88^a \pm 1.73$
Nando	Air-dried	$0.93^a \pm 0.54$	$0.93^a \pm 0.54$
	Field-moist	$0.62^a \pm 0.62$	$1.24^a \pm 0.31$
Omasi	Air-dried	$12.75^a \pm 0.82$	$20.52^a \pm 3.53$
	Field-moist	$12.43^a \pm 1.35$	$16.48^a \pm 5.00$
All locations†	Air-dried	$6.30^a \pm 2.53$	$8.39^a \pm 4.28$
	Field-moist	$5.51^a \pm 2.49$	$8.24^a \pm 3.40$
		($t_{cal} = -1.35$; $p = 0.27$)	($t_{cal} = -1.05$; $p = 0.92$)

Values are means ± standard error of the mean. For each location, means for air-dried and field-moist samples that do not share a letter are significantly ($p \leq 0.05$) different.
†Comparison based on the mean values from the four locations by t-test.

The correlation analyses between soil pH and extractable phosphorus done separately for the air-dried and the field-moist soils ($n = 12$ in each case) indicated non-significant positive coefficients, *r*; 0.057 and 0.139, respectively for the upland soils and 0.079 and 0.206, respectively for the lowland soils. These poor relationships between pH and phosphorus availability in these soils are attributed to the low range of soil pH, a situation which gives no room for an appreciable reduction in phosphorus sorption by Fe-Al-phosphate complexes. Loganathan et al. (1987) reported similar observation in some soils from southern Nigeria. It also seems that soil pH has pronounced influence on phosphorus availability in weathered tropical soils only under conditions of high phosphorus levels attained by addition of phosphorus solution to soil (Eze and Loganathan, 1990), or when soils are treated with liming materials to enhance normal distribution of their pH values (Sato and Comerford, 2005).

Though non-significant, the above correlation coefficients suggest a tendency for weaker positive pH-phosphorus relationships in air-dried than field-moist samples, on one hand and in well drained upland soils than in poorly drained lowland soils of the tropics, on the other. The results provide a clue for defining priorities in any pH-based management of low

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phosphorus availability in upland and lowland soils of tropical watersheds, particularly lowland *sawah* systems of rice production in West Africa where phosphorus availability has been suggested to increase rice yields (Obalum et al., 2014).

Since the relationship between soil pH and available phosphorus was weak, the regression was not shown; instead, emphasis was shifted to the relationships between pH in air-dried and field-moist soils and between available phosphorus in air-dried and field-moist soils which were much stronger. The best regression between pH values in air-dried soils and those in field-moist soils was of the polynomial form of the second order; the corresponding regression for available phosphorus was of the power form (*Figure 2*). It can be deduced from these relationships that air-drying of soil samples before analyses has a less distinct effect on soil pH than on extractability of phosphorus from the soil.

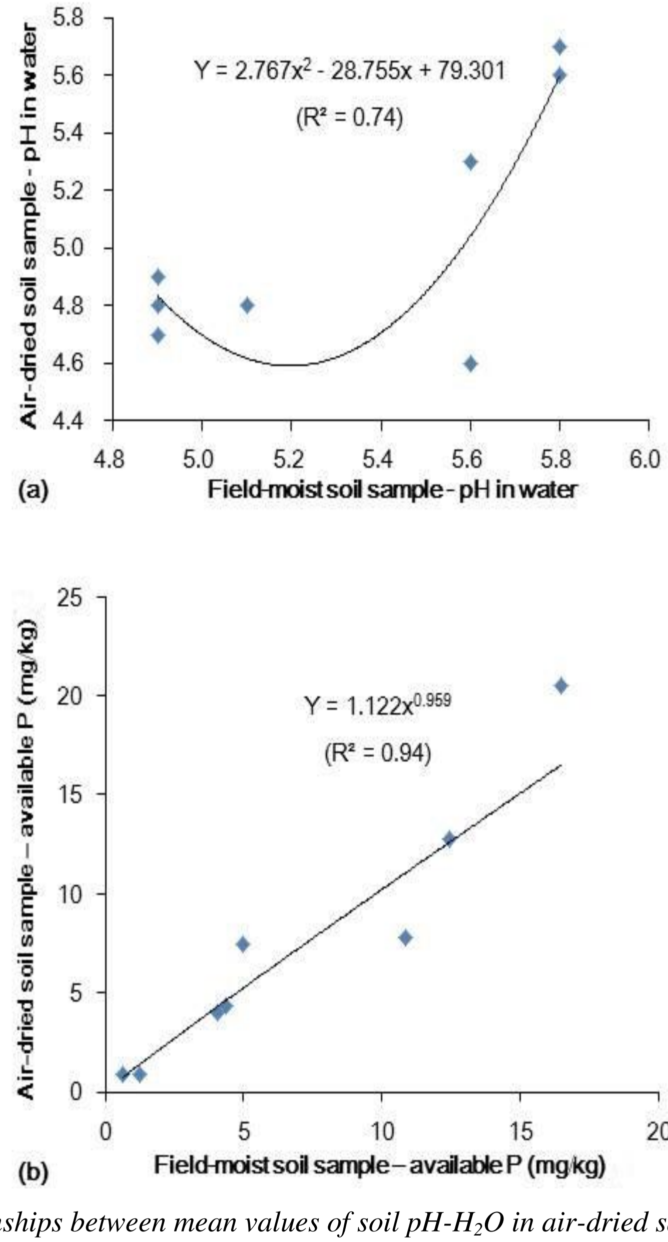

Figure 2. *Relationships between mean values of soil pH-H_2O in air-dried samples and in field-moist samples of the (a) upland soils and (b) lowland soils*

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The effect of air-drying on soil pH showed a very strong dependence on WDC contents of the soils, irrespective of drainage status, as evident in the regression of squared differences (between means for air-dried and field-moist samples) in pH on WDC contents of the soils (*Figure 3*). Thus, drying-induced differences in soil pH can be predicted very well from WDC content of the soil alone. The regression was of the polynomial form, implying that such differences are related to soil content of WDC in curvilinear manner. In other words, the drying-induced difference in soil pH increases polynomially with an increase in WDC content of the soil.

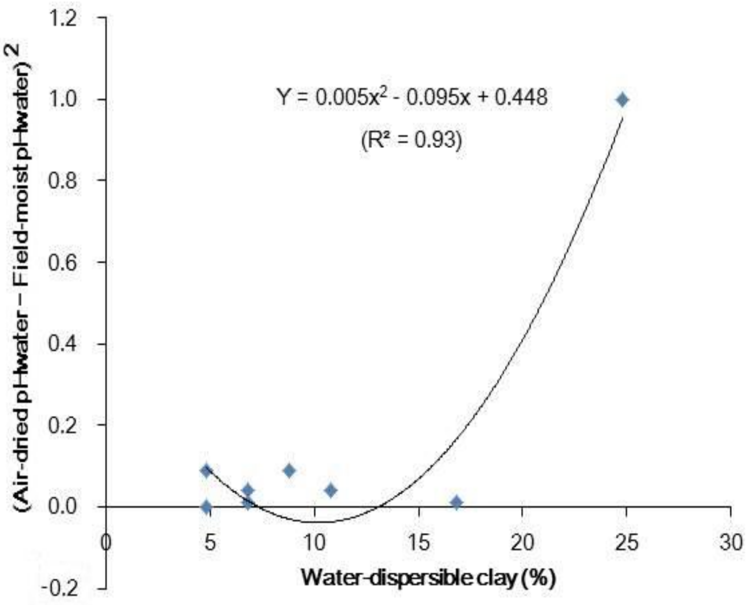

Figure 3. *Quadratic linear regression of the squared differences in mean soil pH-H_2O of air-dried and field-moist samples on water-dispersible clay (WDC) contents of the soils*

One way to appreciate further the role of soil content of WDC in the effect of air-drying on soil pH is to examine the linear (rather than polynomial) relationship between pH values in air-dried soils and those in field-moist soils with and without WDC as a regressor:

$$pH_{\text{air-dried}} = 1.084\ (\text{ns}) + 0.745pH_{\text{field-moist}}\ (*) \quad (R^2 = 0.53;\ s.e. = 0.32) \tag{Eq. 2}$$

$$pH_{\text{air-dried}} = 1.633\ (\text{ns}) + 0.711pH_{\text{field-moist}}\ (*) - 0.035\text{WDC}\ (*) \quad (R^2 = 0.86;\ = s.e. = 0.19) \tag{Eq. 3}$$

The role of soil content of WDC was such that it not only contributed significantly (*) to the regression when included as a regressor, but also resulted in an increased R^2 value and a decreased standard error (*s.e.*) of the estimate. This implies that field-moist soil pH alone could explain about 53% of the variations in pH of the air-dried soils, but together with WDC could explain up to 86% of such variations. Considering the rather small number of observations (n = 8) in these regressions, however, this relationship is not meant for use in predicting the effect of air-drying on soil pH, but to highlight the

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underlying influence of soil colloidal stability in drying-induced differences in soil pH. Notably, the WDC had a much stronger correlation with pH values of air-dried soils than with those of field-moist soils (data not shown), an indication that the observed role of WDC in explaining the drying-induced differences in pH was due mainly to its effect on pH of the air-dried soil.

In the case of available phosphorus (P), the linear regression of values in the air-dried soils on their counterparts in the field-moist soils in itself showed a good fit:

$$P_{\text{air-dried}} = -0.232\ (\text{ns}) + 1.102P_{\text{field-moist}}\ (*) \quad (R^2 = 0.91;\ s.e. = 2.17) \tag{Eq. 4}$$

Similar to this relationship, Gilliam and Richter (1988), who also worked with well- and poorly drained soils in South Carolina, reported that the intercept, slope and R^2 values for the regression of phosphorus extractable from air-dried and ground samples on that from field-moist samples of the well-drained soil were 0.35, 1.25 and 0.90, respectively. They, however, did not obtain any meaningful regression for the poorly drained soil of their study.

There was a corresponding further improvement in the above linear regression due to inclusion of the gradient in pH of the field-moist soil samples (pH-H_2O minus pH-KCl, referred to as $\Delta pH_{\text{field-moist}}$) and the clay flocculation index (CFI) in it; however, neither of these two parameters alone contributed significantly to the regression until both of them were included as regressors:

$$P_{\text{air-dried}} = -8.911\ (\text{ns}) + 0.933P_{\text{field-moist}}\ (*) + 10.492\Delta pH_{\text{field-moist}}\ (\text{ns}) \quad (R^2 = 0.95;\ = s.e. = 1.74) \tag{Eq. 5}$$

$$P_{\text{air-dried}} = -4.408\ (\text{ns}) + 1.217P_{\text{field-moist}}\ (*) + 6.014\text{CFI}\ (\text{ns}) \quad (R^2 = 0.93;\ = s.e. = 2.10) \tag{Eq. 6}$$

$$P_{\text{air-dried}} = -15.\ 684\ (*) + 1.056P_{\text{field-moist}}\ (*) + 12.147\Delta pH_{\text{field-moist}}\ (*) + 7.783\text{CFI}\ (*) \quad (R^2 = 0.98;\ = s.e. = 1.10) \tag{Eq. 7}$$

The increase in R^2 value and the corresponding decrease in *s.e.* of the estimate was also greater with both $\Delta pH_{\text{field-moist}}$ and CFI as regressors than with either of them alone. These results first suggest that air-drying effect on phosphorus extractability has to do with its influence on soil pH, and that such influence is mainly on the $\Delta pH_{\text{field-moist}}$, a parameter which represents the extent by which H^+ is solubilized less in H_2O than in KCl from colloids of the field-moist soil. Second, drying-induced differences in extractable phosphorus may not directly be influenced by soil colloidal stability, but whether the drying-mediated influence of soil pH would be evident or not depends on the soil colloidal stability. Again, these relationships are not meant for use in predicting the effect of air-drying on soil extractable phosphorus, but to highlight the underlying interplay between soil pH and soil colloidal stability. The correlation of CFI with available phosphorus was only slightly weaker for air-dried than for field-moist soils (data not shown). Considering that CFI was derived from data for air-dried soil samples, this similarity of the correlations appears to support the results attained for extractable phosphorus of its rather insensitivity to air-drying of soil samples before analysis.

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From the data of this study, Akaeze soil appears unique in some ways and it is apparent that this soil drives many of the patterns shown. As the only location in the derived savannah, Akaeze records longer dry season than the rest (see *Table 1*), a situation implying greater 'drying effect' and hence higher soil organic matter whose role in air-drying effect has already been highlighted. Again, soil organic matter relates positively with WDC, an index of clay dispersion (Igwe and Udegbunam, 2008), and Akaeze soil showed high WDC contents while showing low CFI compared to others. This not only accounts for the prominence of Akaeze in our study but also links this location to the observation here that WDC and CFI largely explained the overall results for soil pH and extractable phosphorus, respectively in response to air-drying.

Conclusions

The study reported here has the unique feature of assessing air-drying effect on soil pH and available phosphorus for both well-drained upland soils and adjoining poorly drained lowland counterparts in different locations, and relating such effects to colloidal stability of the soils. The practice of air-drying soil samples before analysis may or may not affect soil pH, depending on location and soil drainage status at the time of sampling. Although air-drying may not always have effect, air-dried samples of well-drained, less acidic soils show increased acidity upon rewetting; whereas those of poorly drained, more acidic soils behave in the opposite. The effect of air-drying on phosphorus extractability is location-specific too and often not pronounced, but with an overall tendency of enhancing this phenomenon in well-drained soils and reducing it in poorly drained soils with relatively 'high' phosphorus status. It appears, however, that this trend is reversed in poorly drained soils having field-moist available phosphorus beyond a critical value of 15 mg kg^{-1}. Overall, air-drying for pH and available phosphorus test is deemed safe and is thus still recommended for only well-drained upland soils with properties approximating those included in this study. Caution is needed only for soils with potentially high level (> 15 mg kg^{-1}) of available phosphorus. In such cases, the import of our data also lies in the possible impact of drying under field conditions when soil sampling programs overlap the interface between the distinct rainy and dry seasons of the tropics, even if the soils are analysed field-moist.

Soil pH and available phosphorus may not exhibit any meaningful relationships in these soils under natural conditions of rather narrow range of soil pH. However, the relationships are positive and tend to be stronger for field-moist soils, on one hand and for poorly drained soils, on the other. Soil colloidal stability has an underlying influence on effects of air-drying, with WDC content and CFI influencing such effects on soil pH and available phosphorus, respectively. The underlying influence of WDC can be more distinct compared to that of CFI, as the latter relies on a pH factor to manifest. These results are expected to provoke more studies aimed at exploring fully the roles of soil colloidal stability in drying-induced differences in soil pH and available phosphorus as well as the mechanisms involved.

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LOCAL AND SCIENTIFIC ECOLOGICAL KNOWLEDGE POTENTIAL AS A SOURCE OF INFORMATION IN A PERIURBAN RIVER, MEXICO CITY, MEXICO

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Abstract. Rivers in large cities face management problems that could be addressed through the integration of scientific and local ecological knowledge. The objective of this article is to emphasize the importance of different sources of knowledge in management alternatives for periurban watersheds. Specifically, this is done through (a) use of oral sources and archives to document the historic relationship between local residents and their natural resources, and (b) technical evaluation of the quantity and quality of water in the sub-basin through the time. We analyzed, through the 19 unstructured interviews, local residents' knowledge related to water quantity and quality. Additionally, we analyzed historical river flow data and water quality reports. The results show that the relationship between the local inhabitants and the river has been governed by public policies of both exploitation and conservation. For the last century, the river was used as the main source of electric power for local industry, the collapse of which, led to the decline of the importance of the river in the perception of the locals. Analysis of historical river flow data reveals a slight decrease in flow despite a trend of somewhat lower precipitation. This discrepancy may be due to hydraulic infrastructure construction and uncontrolled extraction. We conclude that the integration of two kinds of knowledge present a great opportunity to test a monitoring system that incorporates the environmental features that were more accurately described by the local residents: water quantity and forest land use.
Keywords: *environmental policies, Mexico City, illegal settlements, participative monitoring program, periurban river, water quality*

Introduction

Managing hydrologic resources has always been a priority in the establishment of human societies, requiring practices that are able to integrate the biophysical and socioeconomic components resulting from these interactions. Rivers, in particular, represent a challenge since based on the continuous interaction with human societies, as an important component of urban sustainability, particularly in preservation zones. As a feature of the environment, rivers are not bound to political divisions, nevertheless are highly sensitive to human activities, which in turn could alter the ecosystem outside certain political boundaries (Maass and Equihua, 2015). It is this interconnection between ecosystems and human beings at different spatial and temporal scales that shape socio-ecosystems (Maass, 2012). This concept emphasizes the importance of a coordinated management of water, land and related natural resources, maximizing social welfare without comprising sustainability and making local stakeholders a key part of the process (Bürgi et al., 2013; Molle and Mamanpoush, 2012; Swatuk and Motsholapheko, 2008).

The basis for developing an adaptive aquatic resource management strategy is the use of Local Ecological Knowledge (LEK) in scientific studies (Taylor and Loe, 2012). LEK is defined as *'the knowledge and perceptions from a particular group of people about local ecosystems and their interactions with the environmental, and how it is transmitted through the human generations using the oral communication'* (Taylor and Loe, 2012; Gómez-Baggethun et al., 2012; Robertson and McGee, 2003; Olsson and Folke, 2001). This understanding is relevant because it facilitates the participation of local communities in land-use planning and decision making, since it empowers them and increases the likelihood that the management actions will be responsive to local concerns (Taylor and Loe, 2012; Alberti, 2010; Robertson and McGee, 2003). Additionally, it allows recognizing the capacity of resilience of the socio-ecosystem that, in turn, depends on the individual and collective experiences of local stakeholders (Fernández-Llamazares et al., 2015). Resilience, understood as the adaptation of the socio-ecological system to disturbances, which in practice can be any event that breaks the structure of the ecosystem and modifies the availability of resources, land use and/or the physical environment (Gómez -Baggethun et al., 2012).

Local ecological knowledge has been traditionally recorded in the form of questionnaires, by examining written historical documents, photography, maps and oral history, which become important in places where oral communication is the only way to transmit knowledge (Robertson and McGee, 2003). Oral history can be defined as '*a structured conversation between two people, an interviewer pursuing a carefully defined line of inquiry, and a narrator with information that the interviewer seeks to acquire'* (Fogerty, 2001). This tool offers the possibility of having a complementary management perspective, since written sources often reveal only official views (Fernández-Llamazares et al., 2015; Bürgi et al., 2013). Some studies show the contribution of LEK in improving the sources of information on ecosystems, that provide management alternatives accepted by local people, as a way to validate historical documentation, validation of scientific and local knowledge in a socio-ecological context and the potential for implementation participative monitoring systems (Bürgi et al., 2013; Terer et al., 2012; Gómez -Baggethun et al., 2012; Alberti, 2010; Giordano, et al., 2010; Raymond et al., 2010; Robertson and McGee, 2003). In this sense, local participation could be conceived also as the "result" of a process aiming to empower people and communities through a learning process leading to increased

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capabilities to self-manage their ecosystems (Fernández-Llamazares et al., 2015; Lawrence, 2006; Hayward et al., 2004).

In large cities, resource management through participative processes that involves LEK and public policies applied under a governance scheme in order to progress towards sustainability becomes a complex task, such is the case in Mexico City (Aguilar, 2008). The city is a good example of socio-ecosystem interaction because it still has periurban ecosystems that support it from the perspective of ecosystem services (ES) provision. In particular, the provision of water, in quantity and quality, is one of the most valued ES in a city with increasing urban growth and water management problems due to intensive aquifer exploitation and mismanagement of periurban rivers (Starkl et al., 2013; Legorreta, 2009).

Despite these environmental management problems, the city has 87,000 ha of natural areas designated as ‘‘Federal Conservation Zones’’ (CZ), which are mainly mountainous, forested regions that provide fundamental ESs to the city inhabitants (Caro-Borrero et al., 2015a). These areas are located in a rural-urban fringe, being characterized by several different changes and processes (Aguilar and Santos, 2011): a dispersal pattern of urban occupation for housing and infrastructure; an establishment of illegal settlements with precarious dwellings and lack of public services; extraction of building materials and surface water and groundwater; as well as alteration of river courses. The best preserved zone of the Basin of Mexico is located in the southwest region and is represented in part by the Magdalena–Eslava River sub-basin (PUEC-UNAM-GDF, 2008). This sub-basin provides one percent of the surface water supply for human consumption in Mexico City; however, the conservation of water resources is compromised due to land-use change by illegal urbanization, agriculture, and the lack of suitable public policies in different periurban and socio-ecological contexts (Wigle, 2010; Aguilar, 2008). Given the importance of the last surface rivers and the provision of water to human needs, it should be a priority to develop baseline studies that take into account the watershed’s ecological present state, scientific opinion and historical socio-ecological knowledge. The objective of this article is to emphasize the importance of different sources of knowledge in management alternatives for periurban sub-basins and their conservation through (a) use of oral sources and archives to document the historic relationship between local residents and their natural resources, and (b) technical evaluation of the quantity and quality of water available in the sub-basin, both in the present and in the past. These hydrological parameters were selected because they are widely recognized and valued by people and data are available to compare the evolution in time and space.

Mexico City’s historic context and its relation with surface water and forests

The Basin of Mexico, where Mexico City is located, has experienced deep social and environmental transformations since pre-Columbian times. The Mexican culture (or Aztec) settled in what was once a lacustrine system (Legorreta, 2009). In the southern area a highly productive agro-ecosystem was built, what is known as *chinampas*, consisting of artificial islands man made as extensions of soil laid upon branches and roots, secured by stakes anchored to the lake’s bottom using *Salix bonplandiana* trees, thus allowing water to reach by capillarity the plants root system. The Mexica culture also created engineering structures such as the installation of aqueducts that doubled as a source of drinking water and served also as a flood control infrastructure, given the numerous rivers that fed the area (González-Reynoso et al., 2010). During the Spanish

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intervention many dykes and dams were built to contain the constant floods, although none proved to be very effective (Ezcurra, 1990). It was not until the 17th century that a more permanent solution was implemented, transforming the basin in an irreversible way into the exorheic basin instead of an endorheic system, with the construction of a deep and extensive drainage system (González-Reynoso et al., 2010).

The new exorheic nature of the lake system allowed the expansion of human settlements and city`s growth, which has continued until the present day. Nevertheless, during the first decades of the 20th century, there were still many green areas and areas of farmland fed by the numerous streams and rivers present in the Basin of Mexico. Many of these rivers were also used by the textile and paper industry from the early 1800s to the mid 20th century, when both industries started to decline due to river flow reduction and social activism with strikes at the industries. The decline of these industries coincided with a rapid urban growth process and by 1950, the city had spread beyond its political limits and invaded the adjoining state, with an exponential increase in population in the last 50 years. The demographic data shows in 2005 its whole metropolitan zone registered 19.2 million inhabitants (Aguilar, 2008). The increasing demand of water in quantity and quality for the population has led to disproportionate groundwater extraction and the importation of water from adjacent basins (Aguilar and Santos, 2011). This accelerated growth has exacerbated water management problems and promoted intubation of water courses and deforestation (Wigle, 2010), particularly in the CZ of the Magdalena-Eslava River sub-basin (*Figure 1*).

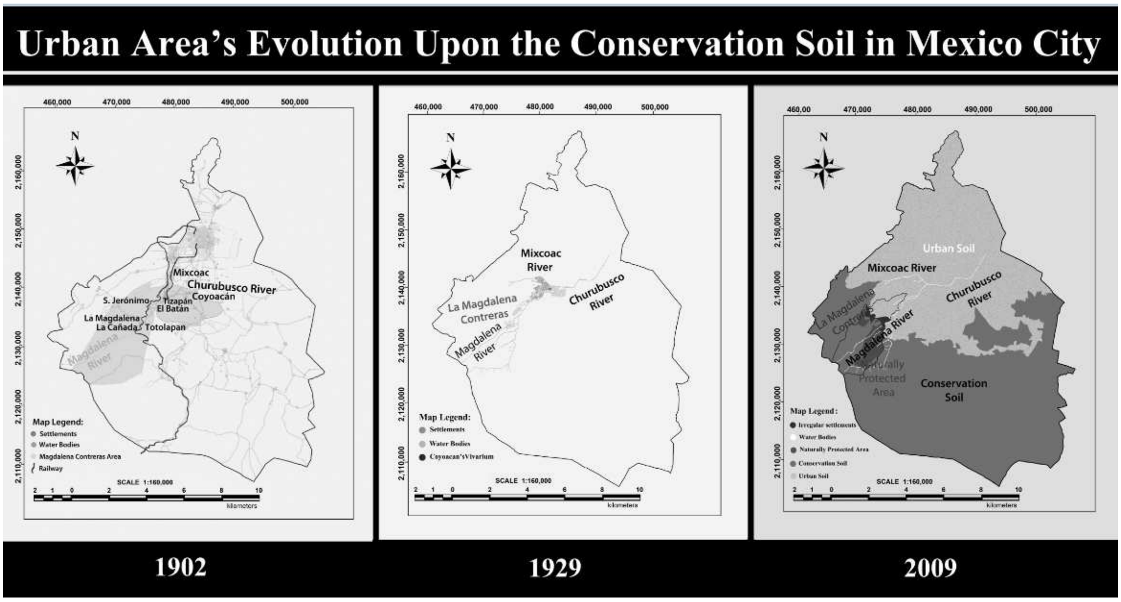

***Figure 1**. Major spatial demographic changes in Mexico City related to Conservation Zone and Magdalena-Eslava River sub-basin from 1902-2009.*

Mexico City at present

The Mexico City has an average altitude of 2,240 m and an area of 1,495 km^2 (Legorreta, 2009). Administratively, it is divided into an Urban Zone (UZ) with an area of 614.58 km^2(41%) and a Conservation Zone (CZ) with 872.95 km^2 (59%). This latter zone comprises most rural areas to the south of the city and includes large portions of

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the forest mountain slopes (Aguilar, 2008). Of the 2.2 million people who live in the CZ, 700,000 make direct use of it for their livelihood (i.e. agriculture, livestock breeding, extraction of non-timber forest products, etc.); they representing 8% of the total population in the entity that encompasses the central area of Mexico City (SEDATU, 2012). The land use policy consists of two regulations: (a) The General Program for Urban Development in Mexico City (2003), and (b) the Program for Ecological Planning (2003). However, an ambiguous interpretation and ignorance of social complexity, favors illegal occupations (Aguilar, 2008). For example, in the period 1970–1997, the CZ lost 239 ha of forest cover and 173 ha of agricultural land on an annual basis, while illegal settlements increased by 289 ha per year (Wigle, 2010).

The urban land uses within CZ mainly corresponds to 36 traditional towns that have existed in the Southwestern area of the city since ancient times (Aguilar, 2008). The Magdalena Atlitic community represented one of these traditional towns associated to the Magdalena-Eslava River sub-basin by since 1535. Requests to be recognized as a community were made to the government in 1945, but they were not granted until 1975. Two thousand three hundred and ninety-three hectares were granted to the sub-basin common landowners through a Communal Rights Confirmation Presidential Act, which partially overlap with the natural protected area highlighted above. The Magdalena community is organized autonomously and represented legally by a president, secretary and treasurer. Only 250 of the actual 1779 registered landowners attend regularly the community general assembly, where collective issues are negotiated, discussed and decided. The Magdalena-Eslava River sub-basin is mostly a woodland area with a current cover of 67% well preserved forests (Ávila-Akerberg, 2009). The logging ban imposed by the area’s inclusion in the CZ prohibits timber extraction (other than for ecological restoration purposes), but it does permit crop cultivation in some selected areas (SEDATU, 2012). However, the vast majority of residents work in the city and agricultural activities within the sub-basin have been progressively abandoned (Jujnovsky et al., 2012). In the Magdalena-Eslava River sub-basin, the provision of ES has been oriented mainly towards urban water supply and to some small extent to local ecotourism. However, uncontrolled tourism, unauthorized settlements and inefficient public policies have altered hydrological characteristics linked to the morphology of the sub-basin (Caro-Borrero et al., 2015a; Mazari-Hiriart et al., 2014). More recently the local government authorities have made some efforts to monitor and preserve the Magdalena-Eslava river sub-basin (PUEC-UNAM-GDF, 2009). Although, these efforts have been based on a combination of academic research and local government policy; paying very little attention to LEK and this has resulted in projects that have failed to achieve the desired objective of sub-basin conservations and river sanitation.

Methods

The scientific knowledge focused on the provision of water in quantity and quality, due to its historical importance since the emergence of Mexico City and its current relevance in the provision of water for a city with scarcity and a high population demand. The selection of basic criteria used to evaluate local ecological knowledge and ecological data were driven by an availability of official and academic information. Taking into account this limitation, we selected the water quantity and quality data as an ecological indicator of hydrological changes within the study area at a local scale (*Table 1*).

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***Table 1**. Basic criteria used to evaluate local hydrological and ecological knowledge evolution in the Magdalena-Eslava River sub-basin.*

	Indicator	Data source and method
Local ecological knowledge	Links to the river, industrial development and community life	Nineteen interviews using oral history tool (Fogerty, 2001). Selection of participants with snowball technique (Neuman, 2000). Interviews analyses with MAXQDA.11 software.
Historical and scientific context	Water quantity	One hydrometric station, National Water Commission (acronym in Spanish-CONAGUA), 1976-2014. (Caro-Borrero et al., 2015a; Mazari-Hiriart et al., 2014)
		Precipitation rate trends Precipitation, 1998-2010. Tropical Rainfall Measuring Mission (TRMM)
	Water quality	One site, 1945. Historical Archive Water (AHA, 1967).
		Ten sites, 2008-2012. Physical, chemical and bacteriological data (Caro-Borrero et al., 2015a; Mazari-Hiriart et al., 2014).
	Urban limits	Maps 1902, 1903, 1929 and 1936. Historical Water Archive (AHA, 1967).

Local ecological knowledge

This study was defined as an unstructured conversation between an interviewer and local people who through personal experience, memories or association may possess relevant historical information regarding a particular fact, environmental characteristic or period of time (Fogerty, 2001). We conducted a series of unstructured interviews with local residents, defining a resident as someone who was born in the study area or

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had lived there for more than 30 years. The number of interviews carried out does not correspond to a numerically representative sample of the total population that inhabits the area, it is representative of the perception of key actors with an in-depth discourse on the memories of their life associated with the existence of the river. The interviewees contributed with pictures and some historic documents from the past 40 to 80 years, which were incorporated to the analysis. Between 2012 and 2014, nineteen interviews were conducted, lasting up to 2 hours each, based on an interview schedule that sought to focus responses on their knowledge of local socio-ecological context. The first interviewees selected were those who had participated in previous studies and could be contacted personally or by phone, and subsequently the snowball method was used to contact further interviewees (Neuman, 2000). The interviews were recorded and transcribed, then analyzed qualitatively with the MAXQDA.11 software program. The analysis centered on the following points: past and present relationship with the water resources (quantity and quality) and with the forest, rural life (understood as the daily activities that includes work, domestic activities, crops and local celebrations) and the influence of industrial development on the latter. These results were explored qualitatively, in order to assessment coincidences and integrated different sources of information: local knowledge, historical records and scientific monitoring data of water amount and its quality.

Scientific knowledge: quantity and quality of water

Historical analysis of the water quality was based on data obtained from 10 sampling stations from 2008 to 2012 period for the Magdalena River and from eight sampling stations from 2010 to 2012 for the Eslava River. We used the annual average per sampling site per year of monitoring. The sites were selected because they are part of a long-term academic monitoring system. (for sampling points geographical location, see Caro-Borrero et al., 2015a; Mazari-Hiriart et al., 2014; PUEC-UNAM-GDF, 2009; PUEC-UNAM-GDF, 2008). Physicochemical and bacteriological parameters (fecal coliform and fecal enterococci), were considered in order to assess water quality during the dry and rainy seasons. The analysis was compared with the maximum permissible limits established by the official water quality Mexican regulations for human use and consumption (NOM-127-SSA1-1994, fecal coliforms 0 CFU/100 mL) (DOF, 2000) and input of water to water bodies (NOM-001-ECOL-1996, total nitrogen 15 mg/L, total phosphorous 5 mg/L) (DOF, 1997), because we expected that local perceptions about the river were mainly associated with human consumption. Temporal differences in physicochemical parameters were assessed through a Kruskal-Wallis test, with normality confirmed by a Kolmogorov-Smirnov test. Differences between years were compared using a Mann-Whitney two-tailed test with $p \leq 0.005$ significance. All statistical tests were performed using SPSS V.13 statistical package (Softonic, 2004).

Validation of flow rates and rainfall for the Magdalena-Eslava river sub-basin

Water quantity was measure thought the historic flow rate data were available through official records provided by a local monitoring station of the National Water Commission (CONAGUA, Santa Teresa 26440 station). These data were used as a reference in the analysis, based on daily flow magnitude and variability for 38 years, recognizing two distinct flow periods that correspond to a dry and rainy season. The validation of official data used the precipitation rates that were obtained from the USA-

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Japan Tropical Rainfall Measuring Mission (TRMM) satellite observing system. The TRMM data product used in this analysis were the 3B42 V7 3-hour precipitation rates (mm/hr) with a spatial resolution of 0.25 by 0.25 degrees. These data can be downloaded from http://disc.sci.gsfc.nasa.gov/precipitation/documentation/ TRMM_README/TRMM_3B42readme.shtml. TRMM average three hours' precipitation rate data were chosen from the latitude/longitude grid point most representative of the Magdalena-Eslava River sub-basin (19.375° LN, 99.375° LW). Average three hours' precipitation rate data were analyzed from 1998 to 2010, which coincides with the discharge data from the Santa Teresa hydrometric station. Given the strong seasonal cycle, with precipitation most heavily concentrated from June to September, inclusive, monthly means were calculated for the 13 years' record period. These monthly mean precipitation rates were then subtracted from each of the data points (eight per day) for the corresponding months. The trend in rainfall intensity of this period of record was assessed employing the Mann-Kendall test with the NCAR Command Language (NCL). Likewise, the same procedure for removing the annual cycle in flow rate was also carried out for the daily flow rate data. This analysis was performed to validate the official data flow records with independent satellite data.

Results

Local ecological knowledge

Nineteen residents of the lower part of the sub-basin were interviewed: 12 women and seven men, aged between 57 and 92 years old. Of this group, two persons previously worked in the old textile and paper factories and six reported having relatives or friends who were also employed in the factories. The perceptions and knowledge expressed in the interviews mostly considered the Magdalena River as the fundamental element and their relation with: (a) rural life developed (includes daily activities in a rural area); questions on forest cover and reforestation activities were included under this heading, and (b) industrial, domestic and recreational activities were developed, including how these were connected to the quantity and quality of available water (*Table 2*). The results shows, until the 1960s (closing of the last mills in the area) the main factor affecting the forest was its exploitation by the paper mill, although, according to interviewees this industry did not lead to a forest area reduction:'*There was never excessive logging. The main activity was dealing with tree diseases. The worst attack we had was in 1961 and the paper mill bought all the infected wood*' (Interviews with María Isabel Olvera and Lucia Rosas, Mexico City, La Magdalena Contreras). Later, the paper mill closed down and imposed a ban on unsuccessful logging '*There were times when trucks came down all night loaded with wood. In the 1990s a logging company extracted wood saying that they had acquired logging rights for 100 years*' (Interviews with Gregorio Valdez and Isabel Dolores Guzman, Mexico City, La Magdalena Contreras).

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***Table 2**. Evaluation of the perceptions of interviewees from the Magdalena-Eslava river sub-basin using the MAXQDA program coding system.*

Analysis code	Perception	Number of people who mentioned it
Forest		
Perception of the forest's current status	Existence of reforestation efforts	8
	Illegal logging contributes to forest losses	6
	The current forest cover is less now than when the factories were operational (before 1968)	11
Forestry legislation	Governmental control of logging	5
	Protected area status helps prevent illegal logging	4
Water quantity		
Present quantity	Flow rate is lower now than when the factories were operational	17
	The river dries up in the dry season	11
Reasons for decrease in flow rate	Caused by dam building	1
	Government diverts water to other basins	8
	It used to rain more	6
	Unauthorized settlements in the basin	7
Water quality		
Current water quality	Good in the upstream area of the sub-basin	8
	Poor in the downstream area because of:	
	Domestic sewage	10
	Garbage dumping	7
	Dead animals	3
	Human and animal feces	3
	Current water quality is worse than before the factories closed	16
Water quality in and before the 1960s	Excellent quality (clean and clear water) before the factories were established	18

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	Uses of good quality water:	
	Drinking	9
	Bathing	8
	Laundry	11
	Crop irrigation	1
	The river was contaminated below the factory area because of:	
	Garbage dumping	3
	Textile dyes and chemicals	2
River uses		
Present	Tourism (trout fishing and trekking)	12
	Religious festivities (related to catholic celebrations mostly)	9
Perception of risk associated to the river	Landslides involving unauthorized settlements	6
	Floods in the lower part of the basin	5
Responsibility for forest and river degradation	Unauthorized settlements allowed by:	
	Land owners	5
	Local government	7

Local and scientific knowledge: quantity and quality of water

The general perception of the local residents on water amount is that it was more plentiful before the factories ceased to operate, between 1963 and 1968, than it is today (*Table 2*). There are fewer streams feeding into the river and the flow has been reduced. As the residents put it: '*We miss the murmur (sound) of the river*' (Interviews with Maria Isabel Olvera and Lucia Ramos, Mexico City, La Magdalena Contreras); '*the river used to carry a lot more water; there was a lot more water before the water treatment plant was built, which has taken half of the river flow from here up to the first dynamo*' (Interview with Gregorio Valdez, Mexico City, La Magdalena Contreras) [Dynamo is an electrical generators that delivered power using the water flow of the river for both textile and paper industry. At the present time only remained the abandoned dynamos constructions that are out-of-use]. Local ecological knowledge shows that most of the interviewees perceive a tendency for the amount of water in the river to decrease, mostly because the river now ceases to flow in the downstream area during the dry season. On the other hand, the quantifiable data evaluation shows that the superficial flow annual average has remained constant in the last 38 years, with variations related to seasonal changes in water flow management. Leaving as a consequence an excessive runoff during rainy season and a drastically decreasing during the dry season. These observations are similar to those expressed by the residents, pointing to the local population's potential role in monitoring.

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Water quality issues have been a worry in the river for decades, although the interviewees showed less concern about contamination that the factories may have caused and, in some cases, they even considered it beneficial for the local people. For example, the residues of dyes used by the factories that were returned to the river were used for dyeing clothes and hair. Water quality reports from four factories that were discharging wastewaters into the Magdalena River in the 1960s show that the water was slightly acidic (pH 6.4), containing abundant organic matter, was murky and colored (gray, blue, purple or yellow) with a sulfuric acid odour, and was deemed not fit for human or animal consumption and in some cases not even for agricultural irrigation (AHA, 1967). Current perceptions of quality are related to contamination coming mostly from human settlements (authorized or unauthorized) lacking connections to the sewerage system. The interviewees mentioned that they do not use water from the river, although they did acknowledge that water quality was better upstream: '*For the last 20 years I have been forbidding my son to swim in the river because is not the same as before; he had a severe eye infection. With the dynamos the water was intercepted; when the water was released the river was cleaned and because of that the colored water produced by the factory discharges was flushed away and there were no infections*' (Interview with María Isabel Olvera, Mexico City, La Magdalena Contreras).

According to the human use and consumption, there is an increase in the number of enterobacteria found at the upstream. In the middle section of the sub-basin, there is evidence that the number of enterobacteria decreases and thus water quality is improved, which is a sign of the river's self-purification ability, considered a regulation ecosystem services. In the downstream section, water quality is again compromised because of sewage discharge, shown by the rise in enterobacteria numbers in which the self-purification is exceeded (*Figure 2*).

The Kruskal-Wallis test showed a significant variation in the physicochemical and bacteriological parameters in at least one year of sampling in each river (p=0.0-0.005). The Mann-Whitney test detected significant differences in total nitrogen (p=0.0-0.022) and total phosphorous (p=0.0-0.009) in the Magdalena River between 2008 and 2009 and in enterobacteria (p=0.0-0.001) between 2011 and 2012. In general, concentrations in the UZ and CZ were similar and displayed similar tendencies over time with a rise in total nitrogen observed in 2012 (*Figure 2*). There were significant differences in dissolved oxygen concentration (p=0.008) and fecal coliforms (p=0.032) in the Eslava River between 2010 and 2012 (*Figure 2*).

Official data validation: analysis of rainfall versus discharge

According to the results from the Tropical Rainfall Measuring Mission (TRMM) satellite data, precipitation intensity has decreased by -0.00148 mm/hr/year. This decreasing trend in precipitation was shown to be significant using the Mann-Kendall test at the 95th percentile. By contrast, the flow rate in the Magdalena River increased by 0.007 m^3/sec/year, this being significant to the 99th percentile. That is, rainfall rates appear to decrease over the basin and elevated terrain, yet there is an increase in the Magdalena River flow (*Figure 3*).

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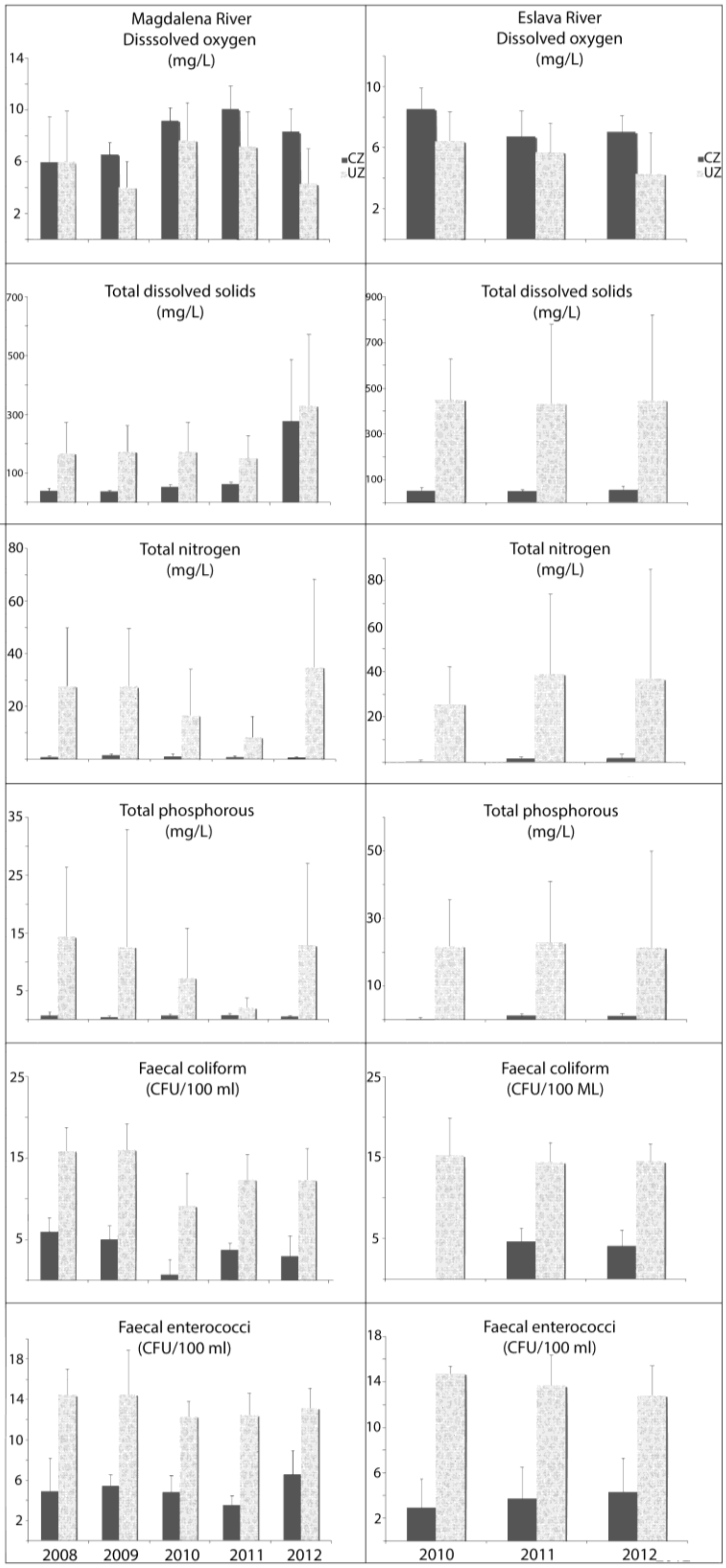

Figure 2*. Representative physicochemical parameters with the highest annual average variations. Left side: Magdalena River (n=30 corresponds to five sampling point in the CZ and five in the UZ, mean ±SE, five-year sampling). Right side: Eslava River (n=12 corresponds to five sampling point in the CZ and three in the UZ, mean ± SE, three-year sampling). CZ: Conservation Zone. UZ: Urban Zone.*

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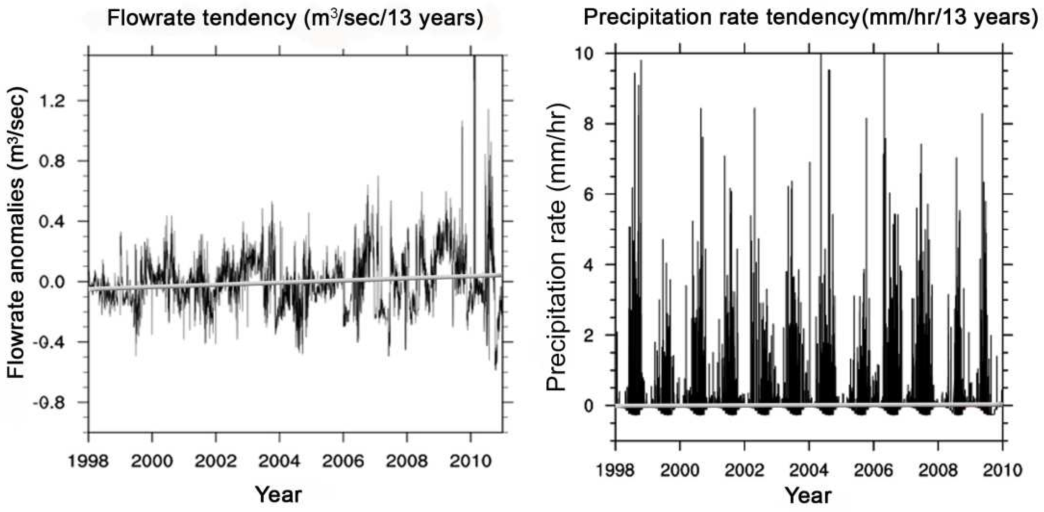

***Figure 3**. Comparison of the flow rate tendencies obtained from official data collected by local government and precipitation records obtained from the satellite system between 1998 and 2010.*

Discussion

In Mexico City, more than 15% of the CZ has been affected by urban growth on what was forested and agricultural land, and it is predicted that by 2020, the 32% of CZ will have been occupied by urban growth (Schteingart and Salazar, 2005). Currently, unauthorized settlements are the main cause of forest loss, land use change and increased demand for domestic water supplies and drainage (Aguilar, 2008). Although, Mexico City has some public policies for conservation like the Environmental Agenda which aimed '*protecting conservation land as a key space for the environmental equilibrium of the city, through protecting its ecosystems as well as preserving its natural flora and fauna in order to ensure the existence of environmental services*' (GDF, 2007). One of the key strategies was the '[…] *implementation of normative and regulatory tools, in terms of land use in conservation zone, for the control and ordering of irregular settlements* […]'. In the same way, the Green Plan objective states: '…*rescue conservation zone as a key space for the ecological equilibrium of the city…*' through several strategies that included '[…] *zero growth of irregular settlements* […]' in the CZ (GDF, 2009). When searching for management and conservation alternatives in the Magdalena-Eslava River sub-basin, LEK suggests a weak relationship between the residents and the natural resources in their area, in particular with water, although, water provision and drainage coverage in the CZ is highly deficient (Aguilar, 2008). The poor results can be due to the utilitarian view of the river, expressed by local people through their relationship with the river when local factories were operating. Here, it is relevant to point out the "tragedy of the commons" (Ostrom, 1991), because the sub-basin is important for the entire city (inhabitants´ health and socio-economic development) and only some of the landowners have the full weight of the conservation on their shoulders.

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Trends towards change within the sub-basin: water quantity

As LEK showed, the use of the river as an energy source for the factories was not a cause of significant reductions in water quantity. It did, however, have an impact on the river bank structure and stream bed. Local residents are aware and interested in giving qualitative tracking of these impacts and, as such, they could be persuaded to engage in a potential community monitoring, but another scenario is possible too. The local inhabitants can be aware 'passively' and finally not directly engaged actively in monitoring activities. In this sense, the awareness is not the only factor that plays a role in order to start the community monitoring program; an environmental education program that integrates local and scientific knowledge should be a requirement (Fernández-Llamazares et al., 2015). However, the effects of flow rate reductions by hydroelectric infrastructure such as dynamos and water extraction channels, could not be assessed either via LEK or by historical records. It could potentially cause habitat fragmentation and negatively affect biodiversity, as has been recorded elsewhere due to gabion dams, that have been constructed in the upper sub-basin (Caro-Borrero et al., 2015a; Wohl, 2006). Additionally, to the construction of gabion dams promoted by the local government in the 1990s as a flood control measure and the construction in 2011 of a marginal sewer in the river bed which was intended to transport sewage from unauthorized settlements upstream (PUEC-UNAM-GDF, 2009). Seasonal low surface runoff is a consequence of the proximity with the UZ, and of unauthorized and authorized settlements occupying woodlands and agricultural lands, *e.g.* because of the paved effect affecting groundwater recharge, which by the type of rock and soil, naturally is less a three percentage in the sub-basin (Jujnovsky et al., 2012). The observed surface runoff values in this case are 19 mm in areas currently undergoing urban growth and 325 mm in well-preserved areas (Caro-Borrero et al., 2015a; Jujnovsky et al., 2012).

According to local residents, changes in flow rates were attributed to water extraction operations by the government in addressing water demand in other areas of the city. The latter causes a feeling of uncertainty on the actual local river water distribution. Due to this situation, the locals solve their water supply by informal means: for example, small spring water extraction, carrying water from distant sources, public taps, or buying it from trucks (Aguilar, 2008). Analysis of official records concerning historical flow rates shows that in the period from1990 to 2010, natural flow became regulated, with a recorded flow rate average of 0.70 m^3/s but 0.67 m^3/s between 2002 and 2003, when the hydraulic works on the river bed became more evident (Mazari-Hiriart et al., 2014). Average monthly flow rate values show that the natural rate remained unchanged until 1989 with the expected rise during the rainy season and lower values in the dry season. However, observations from 1990 onwards show a constant flow rate across the different seasons, a clear indication of flow regulation activities (Mazari-Hiriart et al., 2014). This trend is also confirmed by the annual average flow rate data and the local residents' perceptions. Local residents' perceptions of the effects of hydraulic infrastructure and city water extraction on flow rates and water baseline are quite close to what was observed in reality and as such they can be valued as potential monitoring agents, as long as they decide to be active agents and not just passive observers (Giordano et al., 2010).

The comparison of flow rate and precipitation reveals that precipitation trends have little effect on flow rate reductions, and, as such, there is no evidence of climate-induced change. On the contrary, the analysis shows a slight increase in flow-rate trends

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and a decrease in precipitation amount. The latter may be a consequence of a higher runoff caused by the presence of non-porous materials, a sign of urban expansion in the CZ (Wohl, 2006). This means that sub-superficial and deep-water infiltration may be compromised, affecting the perennial character of the Magdalena-Eslava River system. As such, hydraulic infrastructure and urban expansion have a great impact on the structure and ecosystem health, taking water quality and quantity parameters to the limit, hence presenting a challenge when designing environmentally and socially sustainability public policies in the short and long term and compressing the ecosystem services the river provides to the city (Brauman et al., 2007).

Water quality

Local residents interviewed did not perceive historical water contamination caused by the factories as a problem; some seemed to be oblivious to it and others even took advantage of it: '*Sometimes water was dyed in different colors, mostly red and blue, so when the water was blue, we washed our clothes in it to color them and when it was red I dipped my hair in to dye It'* (Interview with Lucía Rosas, Mexico City, La Magdalena Contreras). Nonetheless, these dyes and chemicals, a byproduct of textile companies, were an evident sign of contamination (AHA, 1967). The industrial activity that took place in the Magdalena River led to deforestation and water chemical contamination that eventually caused complaints, not only about environmental problems but also because the river water could not be used for other activities such as agriculture and livestock (Barbosa-Cruz, 2005).

The present analysis of monitoring water quality data shows that the construction of perimeter drainage infrastructure in the Magdalena River greatly reduced the river's self-purification ability. During 2011 and 2012, an increase in organic matter and enterobacteria in the UZ was recorded (*Figure* 2), despite the perimetral drainage that was supposed to reduce water contamination from human waste originating in human settlements lacking adequate sewerage. In the same period, the Eslava River was also altered by perimetral and urban area drainage construction, with a similar outcome to the Magdalena River. As such, recovery measures undertaken in both rivers were unsuccessful and even unlawful since local regulations forbid permanently altering the natural flow, and riverbed of streams and land use change in the CZ (DOF, 2000). The failure of the perimetral drainage system explains the inefficiency of outdated river control practices, which do not incorporate an integral environmental approach, but it might also be a product of technical problems in designing the perimeter drainage system mostly due to hydro-geomorphological characteristics of the sub-basin (PUEC-UNAM-GDF, 2009). The elevated nutrients and microbiological contaminants, altered channel hydro-geomorphology and stability, and reduced biotic richness and increased dominance of tolerant species requires sub-basin scale solutions. A primary requirement of reversing the periurban stream is the management of wastewater effluent. The Mexico City authorities have also been unsuccessful in trying to implement integrated sustainability policies (such as Payments for Ecosystem Services Program, Caro-Borrero et al., 2015b), probably because they have neglected LEK, as budget deficits and law enforcement (*e.g.* the urban expansion on CZ) get in the way of political goals at federal, regional and local level of goverment implementation (Aguilar and Santos; 2011; Wigle, 2010). A clear example of this situation was the intervention on the riverbed with the perimetral drainage by the regional government through the Mexico City Water System local authority (SACMEX, for the acronym in Spanish), the

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hydraulics intervention changes the hydrological pattern of the river and that has serious consequences for the functioning of the aquatic ecosystem and biological communities (Caro-Borrero et al., 2015a; Mazari-Hiriart et al., 2014).

Precipitation and flow rate trends: evolution of the Magdalena-Eslava socio-ecosystem

Local residents' testimonials emphasize that the river has lost relevance in everyday life because there are no major practical uses to it, like when the factories operated and they got jobs. This perception can, to some extent, be linked to the relationship between water, forests and local economic development, due the rural inhabitants, in occasions, they seem to be more "sensitivity", it is surely more likely to be because their livelihoods depend more directly on environmental quality (Silvano et al., 2005). The mills and other industries relying on the river for their functioning were until 1960´s the main economy drivers in the area and for that reason the river was perceived as useful (Acosta-Colín, 2001).

As the result of continued urban expansion over the past 50 years (*Figure 1*), the sub-basin has been almost entirely absorbed into the city. This is a common problem in the city, where elevated areas are becoming part of the built environment despite the local regulations, including the ecological planning decree that forbids human settlement in the CZ (Starkl et al., 2013; Wigle, 2010; Aguilar, 2008). During the last 30 years of industrial operation in the area, two ordinances were issued to protect the forested areas, but these have only hindered management efforts, because of the contradictions in the size of the protected area resulting in the reduction of forests and even the intervention of the riverbed (Jujnovsky et al., 2012). However, the General Program of Ecological Planning of Mexico City, issued in 2000, established a Protected Forest Zone in an area of 215 ha from the middle section of the basin up to the UZ (Jujnovsky et al., 2012).

We suggest the use of socio-ecosystem framework, as an essential element to empower local residents and landowners in order for them to get identified once again with their environment (Maass and Equihua, 2015). For local inhabitants the identity is deeply rooted to a single location and their interest is to preserve their place histories, traditions and identities (Starkl et al., 2013; Aguilar, 2008). For example, the local residents express an interest in rescuing the river and recognizing one again its usefulness as a symbol of progress, and also to use it as a direct source of water: '*When the factories ceased operations the river died, outsiders began* to arrive to the community *and the river was no longer the main development driver in the area. People switched jobs and community unity was lost. People even stopped using public laundry facilities supplied by river water and stopped doing their laundry at the edge of the river'* (Interview with Isabel Dolores Guzman, Mexico City, La Magdalena Contreras). However, in the Magdalena-Eslava River system there are two particularly conflictive social groups. The first of them is formed by the original residents with legal ownership rights and the second is formed by illegal settlers. The original residents who lived from the land showed LEK, in general acquired through experience and oral tradition. Unauthorized settlers arrived from other rural regions and since they have no legal rights most of the times they are overlooked when implementing environmental management policies. Urban sprawl in many cases been favored by the landowners themselves, the illegal sale of land is theoretically for conservation or agriculture for self-consumption. In this way, it has been the massive sale of forest land to people highly marginalized (Aguilar, 2008; Varley, 1985). With respect to the Mexico City's

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land-use policy, some authors shows that it has been internally inconsistent, failing to take unauthorized settlements into account in the sustainable policies development for providing solutions to ecological degradation problems (Aguilar and Santos, 2011).

These unauthorized settlements are not anti-social elements, they simply need somewhere to live, but they are a convenient scapegoat for everything that goes wrong. This adds to the social injustice that led to their marginalization in the first place (Aguilar and Santos, 2011). Unauthorized residents might represent the largest potential for change when designing sustainable strategies, because they live within the CZ and may be the only ones capable of generating positive action and control for the forest and river conservation, as it has happened in other areas of the CZ with these land-use management problems (Wigle, 2010). However, these strategies requires that the land-use policies be consistence with urban and conservation zone designation (Aguilar and Santos, 2011; Aguilar, 2008).

Main threats to the CZ are associated with the dynamic of land use changes that has major repercussions for the environmental natural conditions of the CZ (Wigle, 2010; Aguilar, 2008). These processes represent a big challenge to integrate the LEK and scientific knowledge, because different social actors have had different ways of acquiring knowledge and not in all the occasions respond to an ancestral tradition and the same motivations to conserve the natural resources (Raymond et al., 2010). Then, iterative processes are required to consolidate and integrate knowledge, but these processes require continuous coordinated work between the local and scientific community (Giordano et al., 2010), within a confidence relationship that takes time to acquire. The testimonials gathered in this fashion, are enable to use in order to

capitalize on the experience of local residents and thus become useful when planning sustainable urban development's (Wall, 2014; Calvo-Iglesias et al., 2006). In this sense, the possible solution could be focus on mitigation actions, for example, thought the participative monitoring programs (Giordano et al., 2010), as a first step, rather than eliminating the impact of unauthorized settlements on conservation zone (Wigle, 2010), because this would be an unrealistic solution.

Conclusions

Although, a longer period of time, data and more deep analyses of community perceptions is needed for a more complete view, this work shows that local ecological knowledge is a useful tool to be incorporated into conservation and rescue of river basins management strategies, mainly because of the fundamental role local residents play in relation to the success of public policies implementation. *Figure 4* shows the conceptual scheme summaries the mainly topics to consider in order to improve the design of public policies based on our results and literature.

In the Magdalena-Eslava River sub-basin, local residents' perception of water quality differs from what historical records show. While residents did not recognize contamination, such as the textile dyes that were even considered as an advantage, official records show that there were serious contamination issues in the area. Which can result from the implementation of public policies that encouraged the exploitation of the river by factories and at present limit the contact and use by the local people as a conservation policy.

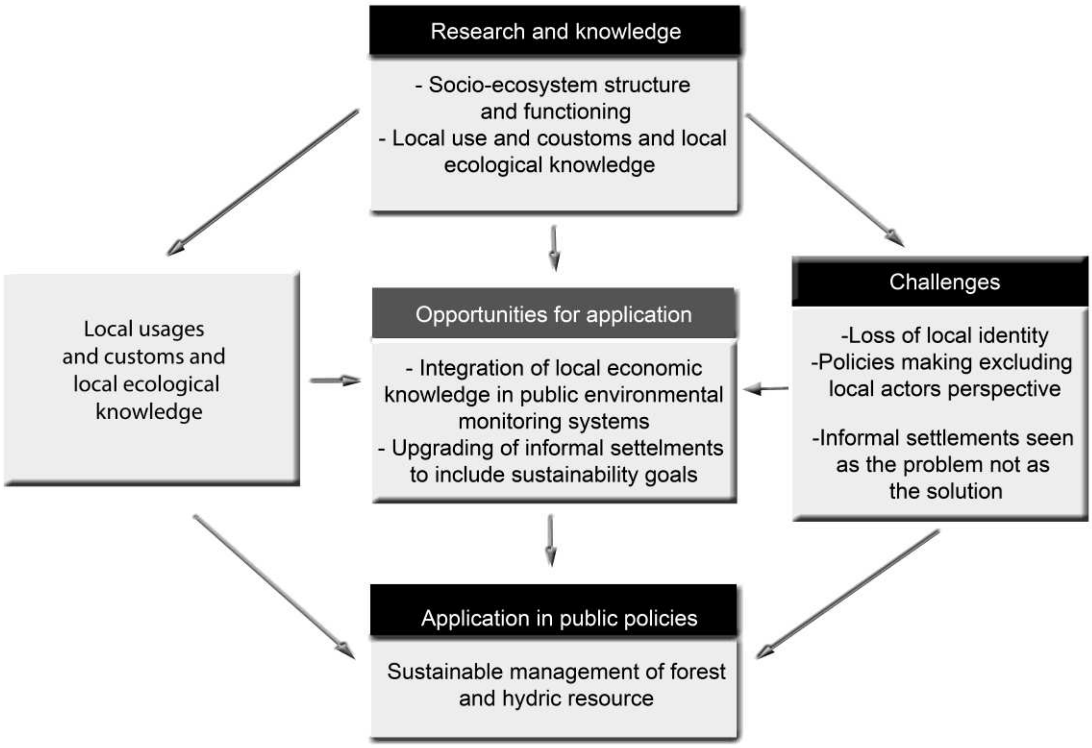

***Figure 4**. Conceptual framework that includes the socio-ecosystem relationship with local ecological knowledge (Modified from Terer et al., 2012).*

The analysis of official data revealed a slight trend towards increasing flow rates, whereas precipitation records show a trend towards somewhat lower rainfall rates. This might be explained by the proliferation of hydraulic infrastructure, especially unregulated water extraction and the regulation in surface runoff.

In order to achieve social, economic and environmental progress in the city, it is essential to consider the future of unauthorized settlements; it will otherwise be impossible to offer a comprehensive solution. Land use change, the conflicts between ownership rights and environmental needs result in a problem that is not only environmental, but also social and due to the complexity, with economic implications. More inter and transdisciplinary studies are required in the near future in the area. This case study is an example of the lack of clear public policies that which result in missing the opportunity to conserve and sustainably use related to a vital resource such as water.

This article has discussed two types of knowledge that may be useful in understanding the Magdalena-Eslava River sub-basin: local ecological knowledge and scientific knowledge. The integrations of these two presents a great opportunity to test a monitoring system that incorporates the environmental features that were more accurately described by the local residents: water quantity and forest land use. This could in practice translate into more job opportunities for authorized and unauthorized residents alike, as well as improving the chances of successful recovery strategies for the ecosystem services qualitatively and quantitatively provided by the river when including biological and physicochemical parameters identified in previous studies.

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Local ecological knowledge is a good starting point as a strategy to understand the perceptions, visions and values, such as patrimony and heritage, implicit in the socio-ecological evolution, and taking into account the scientific knowledge in order to have a big picture about the structure and function, including quality and quantity of the aquatic ecosystem and translate it into ecosystem services potential, particularly in a periurban context of a megacity. Then, both types of knowledge are necessary because they validate each other, when the conservation policies application requires both the scientific knowledge as a background and the LEK to understand the socio-ecological context in which conservation measures intend to be implemented.

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EFFECT OF FERTILIZATION AND SEED SIZE ON NODULATION, YIELD AND YIELD COMPONENTS OF CHICKPEA (*CICER ARIETINUM* L.)

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Abstract. This study investigated the effects of seed size and fertilizer usage with seed at planting on agronomic parameters of chickpea as a winter crop. The experiment was conducted according to split-split plots of randomized blocks with four replications. As factorial two chickpea varieties (Arda and Diyar 95), two fertilizer applications (with and without fertilizer) and three seed sizes (large, medium and small) are discussed in this study. The results showed that fertilizer applications along with seed at planting and large seed increased significantly the number of seed per plant, number of pods, biological yield, grain yield and 100-grain weight. However, fertilizer applications with large seeds had a negative effect on the output of the seeds and reduced grain yield per unit. In general, plants germinating from large seed had higher number of pods and number of seeds plant^{-1}, 100 seed weight, biological and seed yield plant^{-1}, while plants germinated from medium seed had higher emergence percentage, first pod hight, nodule fresh weight plant^{-1} and grain yield ha^{-1}. These effects tend to decrease in chickpea varieties having medium-grain weight as Arda. It was concluded that, application of fertilizer along with seed during the sowing time results in decreased emergence percentage of the seeds and lower the yield in chickpea.
Keywords: *field experiment, grain yield, leguminous plants, large seed, nitrogen, phosphorus*

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world. It is a highly nutritious grain legume crop and is one of the cheapest sources of protein. Therefore, it can help people to improve the nutritional quality of their diets (Siddique and Sykes, 199). Chickpea is practiced as an important rotational crop in many parts of the world. When placed in rotation with other crops, it can enhance soil fertility, reduce rate of weeds, diseases and pests. Between the genetically factors, seed size has a special role in crop production. There have been very important studies on seed size in various plant species. The effect of seed size on germination, ground cover and performance of plant has been confirmed. Seed size is one of the most important characteristics of seeds that can affect the duration of seed development. Generally big seeds with higher amount of initial food reserves germinate early with exhibiting uniformity and grow vigorously in field and shows early advantages of plant vigour with respect to plant performance and yielding ability as compared to small and medium seeds in several crops (Adebisi et al., 2011: Jerlin and Vadivelu., 2004). On the contrary in some crops even medium, small and bulk seeds were also found to have equal advantageous results as that of

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big seeds with respect to field performance. The small and medium seeds require less moisture for germination, germinate early, establish early, grow vigorously and yield equally as that of big seeds (Dar et al., 2002: Peksen et al., 2004).

Farokhi and Galeshi (2005) and Grant and James (2000) reported that there is a negative correlation between soybean tolerance to climatic factors and its seed size, because large seeds require more water resources for their vital activities and consequently they can be damaged by reduction of osmotic potential. Adebisi et al. (2013) with a study on soybean seed size differences reported that determination of the effects of seed size on yield and yield components of soybean and other important legumes has highest importance and seed size in soybean is influenced by genetic and environmental factors. As the seed size per seed is still a controversial issue and there is a need to investigatethe influence of seed size on plant growth, seed yield and quality in Kabuli and Desi chickpea varieties as they show much variations in seed size.

Nitrogen and phosphorus are among essential elements for the plant growth and development and plays an important role in the sufficient grain production. The evolution of science, especially in the last century, has openly showed the importance of phosphorus for all animal and plant life on the earth (Ryan et al., 2012). Particularly during the initial stages of plant growth, sufficient amount of phosphorus is required for development of the reproductive parts and it has a positive effect on root growth, early maturity, and reduced disease incidence. Methods of applying fertilizers can greatly affect their agronomic effectiveness. To avoid economic loss and soil depletion, it is necessary to determine the proper method of fertilizer application in chickpea. Shahzad et al. (2003) reported that fertilizer placement below the seed results in significantly high yield followed by side drilling on both sides of the seed rows, while minimum seed yield was recorded with the broadcast method. Chickpea is usually managed with low fertilizer input, and has shown variable growth pattern and yield response to fertilizer application. There is little research on combined effects of fertilizer application and seed size on yield and yield components of some legume crops as chickpea. Therefore, the present study aimed to assess the effects of seed size and fertilizer application along with seed at planting on yield and yield components of chickpea.

Material and Methods

This study was conducted for 2 consecutive years (during the winter of 2011-12 and 2012-13) at an experimental site in the GAP International Agricultural Research and Training Centre, Diyarbakır, Turkey (37°56' N, 40°15' E and altitude 612 m above mean sea level). Climatically, the area placed in the semi arid temperate zone with cold winter and hot dry-summer. The average annual precipitation is around 484 mm and most of the precipitation falls in the winter and spring months. The soil of trial area was clayey-loamy, with pH about 8.02 and EC about 2.6 ds m^{-1}. The trial was laid out in randomized complete block design with split-split plot arrangement keeping varieties in main plots, fertilizer aplications (unfertilized and fertilized) in sub plots and seed size (small, medium and large) in sub-sub plots with four replications. Seeds of each variety were divided into three seed size classes (small ≥7 mm, medium ≥ 8 and large≥ 9 mm) using by laboratory test sieves. Plots consisted of four rows, 6 m in length, with 45 cm row spacing and 10 cm between plants. Seeds of each variety were sown in the first week of December in both years and plant density for each variety was 45 seed m^{-2}. For fertilized plots 150 kg per hectare DAP (Di amonium phosphate, which contains 18 %

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N and 46 % P_2O_5) were applied in planting time by sowing machine. Weed control was supplemented over the growth period with hand weeding. Yield components such as emergence percentage, plant height, first pod hight, number of pods plant^{-1}, number of seeds plant^{-1}, biological yield plant^{-1} and seed yield plant^{-1} was recorded on 10 randomly selected plants. Grain yield per hectare and 100 seed weight was defined by harvesting the middle two rows of each plot. Nodule fresh weight plant^{-1} was conducted at the 50% flowering time. For all treatments, six plants were selected from two side rows, and tenderly uprooted. The roots were washed with water to take out the adhering soil. The nodules were cut off from the roots, and nodules fresh weight per plant were recorded. To see the effect of factors (cultivars, fertilizer application and seed size) on the examined parameters. The obtained results were subjected to the analysis of variance (ANOVA), using the MSTAT-C software. Treatment mean differences were calculated by the least significant difference (LSD) test at 0.05 probability level.

Results and Discussion

Effect of cultivars on nodulation, yield and yield components of chickpea

The results of ANOVA for nodulation, yield and yield components are briefly described in Table 1. The results of the variance analysis showed that the effect of cultivars on PH and FPH, NSP, BY, GY and 100 SW was significant in 1% probability level and there was no significant effect for EP, NPP, NFWP and SY.

The highest values were obtained from Diyar 95 cultivar for plant height (56.7 cm), first pod height (35.8 cm), BY (42.5 g) and 100 seed weight(37.8 g), while the highest values obtained from Arda cultivar for number of seeds plant^{-1} (43.4 seeds) and grain yield (3677 kg) (*Table 1*). El-Habbasha et al. (2012) also found the similar results.

Effect of fertilizer application along with the seed at planting on nodulation, yield and yield components of chickpea

Data presented in *Table 1* showed that the effect of fertilizer application along with the seed at planting on EP, NPP and NSP, NFWP, BY, SY and GY were significant in 1% probability level. But 100 SW, PH and FPH differences were not significant for fertilizer applications. Bicer (2014) reported that the effect of phosphorus application on PH and 100 SW was not significant, but the effect of phosphorus fertilizer application on NPP and NSP was significant. The highest NPP (40.4 pods), NSP (46.1 seeds), BY (44.1 g) and SY (17.7 g) were recorded at the fertilized plots, while the highest EP (94.6 %), NFWP (2.27 g) and GY (3891 kg) were obtained from unfertilized plots (*Table 1*). Similar findings have also been reported by Sahin and Gecit (2006). Pochiman (1991) reported that phosphoruse plays a key role in various physiological processes concerning root production, nodulation, seed formation and also improves the seed quality.

Effect of seed size on nodulation, yield and yield components of chickpea

Results of this two year study revealed that seed sizes significantly ($P < 0.01$) affected all measured parameters, except PH (*Table 1*). The highest values were obtained from medium seed sizes for EP (90.8 %) , FPH (34.1 cm), NFWP (2.23 g) and GY (3772 kg), while the highest values obtained from large seed sizes for NPP (40.8 pods), NSP (44.4 seeds), BY (42.7 g) and SY (17.7 g) and 100 SW (38.4 g). In general the lowest values were recorded at the small seed sizes, except EP, PH, FPH and GY.

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Table 1. *Effect of fertilizer application along with the seed at planting and seed size on nodulation, yield components in chickpea* (*Cicer arietinum* L.)

Parameters	EP	PH	FPH	NPP	NSP	NFWPP	BY	SY	GY	100 SW
Variety	Ns	**	**	Ns	**	Ns	**	Ns	**	**
Arda	89.9	52.4 b	30.5 b	37.2	43.4 a	1.75	35.0 b	15.30	3677 a	34.9 b
Diyar-95	87.6	56.7 a	35.8 a	37.6	40.3 b	1.59	42.5 a	16.10	3546 b	37.8 a
Fertilizer Application (F)	**	ns	Ns	**	**	**	**	**	**	ns
Fertilized	83.1 b	54.5	32.7	40.4 a	46.1 a	1.07 b	44.1 a	17.7 a	3332 b	36.30
Unfertilized	94.6 a	54.7	33.6	34.4 b	37.6 b	2.27 a	33.5 b	13.7 b	3891 a	36.40
Seed size (S)	**	ns	**	**	**	**	**	**	**	**
Large seeds (>9mm)	88.0 b	54.3	32.3 b	40.8 a	44.4 a	1.74 b	42.7 a	17.7 a	3336 b	38.4 a
Medium seeds (>8mm)	90.8 a	54.6	34.1 a	35.8 b	42.6 a	2.23 a	37.4 b	14.2 b	3772 a	36.2 b
Small seeds (>7m)	88.6 b	54.9	33.2 ab	35.5 b	38.6 b	1.03 c	36.2 b	15.3 c	3727 a	34.6 c
V x F	Ns	ns	Ns	**	**	*	**	**	Ns	ns
V x S	**	**	*	**	**	*	**	**	**	**
F x S	**	*	**	**	**	*	*	*	Ns	ns
V x F x S	**	*	*	**	Ns	Ns	**	ns	Ns	**
CV (%)	2.14	2.97	3.66	4.93	4.37	13.30	4.65	6.38	7.17	1.01

**: significant at level 0.05, **: significant at level 0.01, ns : non-significant, EP: Emergence percentage, PH: Plant height, FPH: First podheight (cm), NPP: Number of pod plant^{-1} , NSP: Number of seed plant^{-1} , NFWPP: Nodule fresh weight (g plant^{-1}), BY: Biological yield, SYP: Seed yield plant^{-1} , GY: Grain yield (ha^{-1}), SW: 100 seed weight (g)*

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Bhingarde and Dumbre (1993) reported that large sized seed gave higher NPP and smaller size seed gave lower NSP in mungbean. Kamal et al. (2001) reported that yield is positively associated with seed size in groundnut. The present findings are in agreement with the findings of Bhingarde and Dumbre (1993).

Effect of interactions on nodulation, yield and yield components of chickpea

Varieties with direct fertilizer applications during the time of sowing interact significantly ($P < 0.01$) for NPP and NSP, BY and SY, while for NFWP, there was significant differences in 1% probability level for this interaction (*Table 1*). The highest value of number pods (43.8 pods), NSP(48.4 seeds), BY (52.0 g) and SY (19.4 g) was obtained from Diyar 95 with fertilized application, while the highest NFWP (2.44 g) was obtained from Diyar 95 × unfertilized treatment (*Figures 1 and 2*) .

The interaction effect of cultivar and seed sizes on EP, plant hight, NPP and NSP plant, BY and SY, grain yield hectare^{-1} and 100 SW were significant in 1% probability level and FPH and NFWP significant in 5% probability level (*Table 1*). Hojjat (2011) reported that the germination parameters were significantly related by seed weight and large seeds germinated earlier and indicated better germination than small seeds of lentil genotypes. Similar results were obtained from Roozrokh et al. (2005) on chickpea.

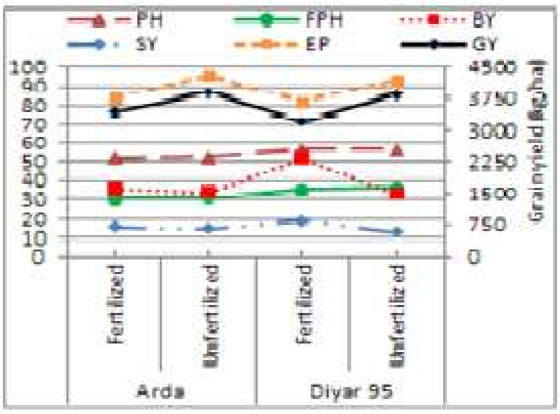

Figure 1. *Interaction effect of variety and fertilizer application on plant heiht, first pod hegiht, biological yield plant-1, seed yield plant-1 and grain yield hectare-1*

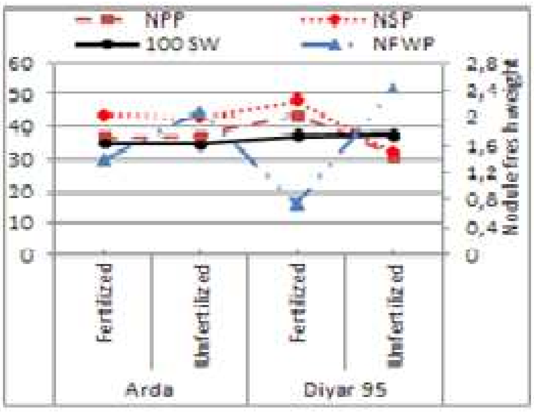

Figure 2. *İnteraction effect of variety and fertilizer application on number of pod plant-1, number of seed plant-1, nodule fresh weight plant-1 and 100 seed weigh*

The interaction effects of cultivar and seed sizes showed the highest 100 SW (40.9 g), NPP (45.4 pods) and NSP (45.3 seeds) , BY (48.2 g) and SY (18.6 g) in the Diyar 95 × large seed sizes; the highest GY (4000.0 kg), plant (57.7 cm) and FPH(37.7 cm) in the Diyar 95 × medium seed sizes; the highest EP (93.4%) and NFWP (2.53 g) in the Arda × medium seed sizes treatment (*Figure 3, 4 and 5*). Eser et al. (1991) reported that the genetic nature of the varieties and the high vigor of the large seeds as compared with the small seeds can affer to justify such differences.

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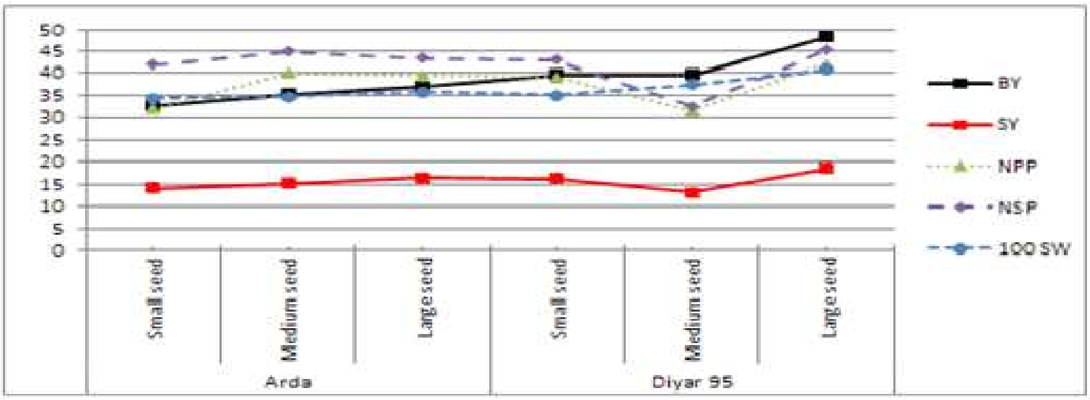
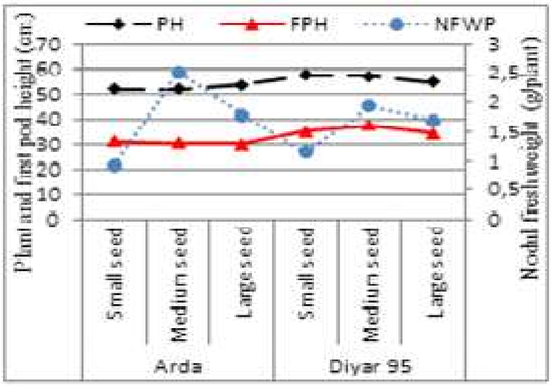


Figure 3. *Interaction effect of variety and seed size on number of pods plant-1, number of seeds plant1, biological and seed yield plant-1and 100 seed weight*



Figue 4. *Interaction effect of variety and seed size on plant height, first pod height and nodule fresh weight plant^{-1}*

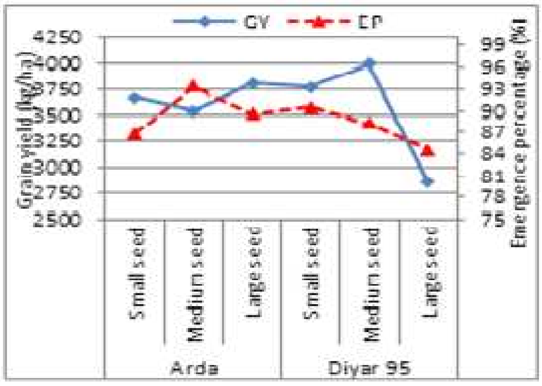


Figure 5. *Interaction effect of variety and seed on plant heiht, first pod hegiht and nodule fresh size emergence percenge and grain yield hectare^{-1}*

Those plots where fertilizer was applied during the sowing time and lage size seeds were sown achieved the maximum value of number pods plant^{-1} (44.9 pods), NSP(48.6 g), BY (48.6 g), seed yield (19.6 g) and 100 SW (38.5 g), while the highest plant (55.5 cm) and FPH (35.0 cm) was recorded from fertilized × medium seed size treatment (*Figs. 6 and 7*).

The highest EP (95.7 %) and grain yield (4010.2 kg ha^{-1}) were recorded from unfertilized × small seed size treatment. Generally FPH and grain yield in unfertilized treatment was higher than fertilized treatment (*Figure 8*). This is related to direct fertilizer application below the sown seed. This application leads to increase the risk of burning the roots of the sown seeds, especially during dry conditions. Mahli et al. (2001) reported that fertilizer application below the sown seed leads to increase the risk of burning the roots of the sown seeds. Thus the percentage of seed emergence was influenced by the used fertilizer placement and consequently, the yield per unit area decreased.

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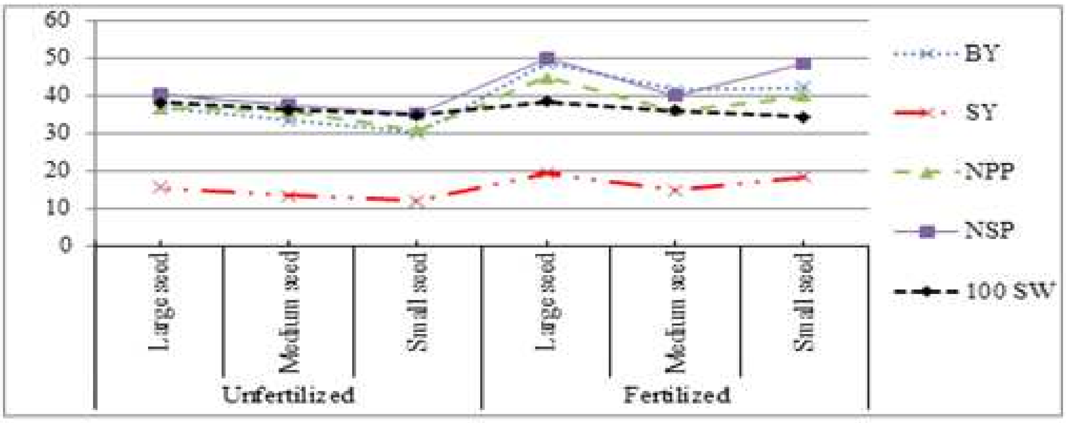

Figure 6. *Interaction effect of fertilizer application and seed size on number of pods plant^{-1}, number of seeds plant^{-1}, biological and seed yield plant-1and 100 seed weight*

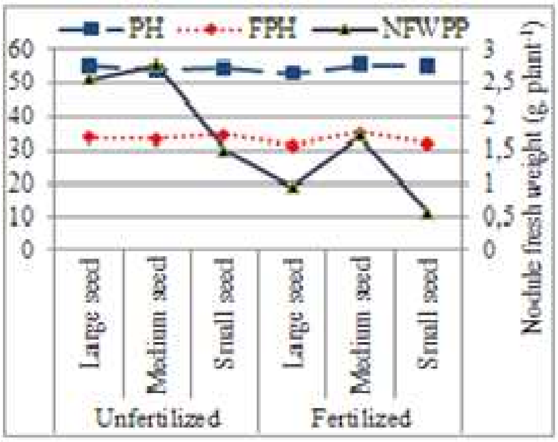

Figure 7. *Interaction effect of fertilizer application and seed size on plant heiht, first pod hegiht and nodule fresh weight plant^{-1}*

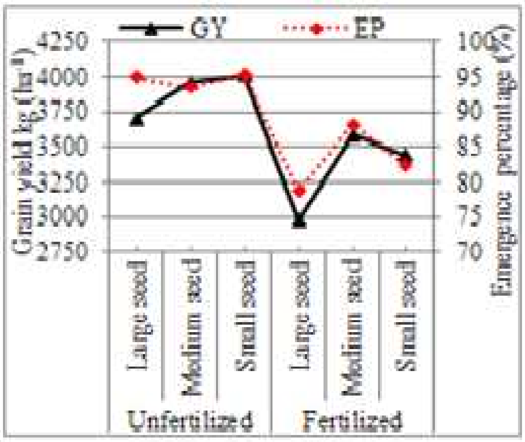

Figure 8. *Interaction effect of fertilizer application and seed size on emergence percentage and grain yield hectare^{-1}*

Conclusions

Results obtained from this study indicated that the effect of fertilizer application along with seed during the sowing time was significant for other examined characteristics, except plant height, FPH and 100-grain weight. This effect was negative on emergence percentage, grain yield and nodule fresh weight. Seed size affected all studied features of chickpea, except plant height. In general, plants originating from large seed had higher number of pods and number of seeds plant^{-1}, 100 seeds weight, biological and seed yield plant^{-1}, while plants originating from medium seed had higher emergence percentage, first pod hight, nodule fresh weight plant^{-1} and grain yield ha^{-1}. However, these effects tend to decrease in chickpea varieties having medium-grain weight (37 g 100 seeds^{-1}) as Arda. The increase in grain yield was associated with emergence percentage. Furthermore, variety × seed size and fertilizer application × seed size interaction had significant effects on yield and its components. Consequently application of fertilizer along with seed at planting can cause to decrease emergence percentage of the seeds and this can cause to yield loss. Therefore, do not let fertilizer contact with seed.

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CHANGES IN RODENT COMMUNITIES AS CONSEQUENCE OF URBANIZATION AND INAPPROPRIATE WASTE MANAGEMENT

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Abstract. One of the unpleasant byproducts of urban living is municipal solid waste. Unfortunately, many urban areas cannot effectively manage their waste, which causes creation of illegal waste sites. We investigated communities of small mammals from 14 illegal waste sites in Mediterranean Slovenia and Croatia. Five species of the family Muridae were recorded: black rat (*Rattus rattus*), domestic mouse (*Mus musculus*), wood mouse (*Apodemus sylvaticus*), striped field mouse (*Apodemus agrarius*), and yellow-necked mouse (*Apodemus flavicollis*). Black rats and house mice, the two commensal species, were exclusively found or prevalent in larger waste sites, while the other three *Apodemus* species were most frequent in natural or seminatural habitats (> 90% specimens). At large waste sites and in the human settlements, commensal species evidently outnumber the three native *Apodemus* species (<25% of specimens). Our results show that improper waste management drives the native rodents to local extinction and replacing them with invasive commensal species, thereby reducing biodiversity and ecosystem health. We conclude that higher rates of parasitism and infestation with different pathogens in waste sites, especially those in the close proximity of human settlements can raise the possibility of transferring the pathogens and parasites either to the people or their predators.
Keywords: *urban ecology, biodiversity treats, Mediterranean coast, anthropogenic influence, solid waste management*

Introduction

Among many anthropogenic pressures worldwide, urbanization is one of the most rapidly increasing threats on natural habitats, causing both biodiversity loss and biological homogenization (McKinney, 2002; Savard et al., 2000). By the year 2025, about 61% of the world's population is predicted to be living in urban areas, especially in developing countries (UNDESA, Population Division. World Urbanisation Prospects: The 2009 Revision, 2010). The expansion of urban areas into natural habitats causes significant changes in species compositions, species interactions, and also impacts ecological and evolutionary processes (Aronson et al., 2014; McKinney, 2008). Urban ecosystems lower the quality of ecological services, with limited capacity to prevent pest outbreaks (Morello et al., 2000).

The Mediterranean coast is a densely populated region with an intricate political history involving many different ethnic groups. Slovenia and Croatia are two, among 21 countries, that have coastlines on the Mediterranean Sea. In both countries high population density and economic activities near the coasts cause significant pressures on coastal areas and their landscapes in particular. As the coastal population grows and

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urbanises its environment, natural coastal habitats and landscapes get further fragmented. The land use changes with the corresponding change in the landscapes leading to decreasing integrity of coastal landscapes and ecosystems (UNEP/MAP: State of the Mediterranean Marine and Coastal Environment, 2012).

In addition to higher human population density, changes that occur along urban–rural gradients (McDonnell and Pickett, 1990), include the loss of biota and natural habitat, increased densities of roads, buildings and other surfaces, and microclimatic shifts (e.g. heat island effects) (Bradley and Altizer, 2007). Although the conversion of natural or semi-natural to urban ecosystems can be a slow process, it is usually irreversible (Morello et al., 2000; Power, 2010).

The rapid development of urban ecosystems generates waste, and landfills are the most frequent waste disposal method worldwide. The growth in waste has led to serious management problems in various cities and countries (Rodríguez et al., 2007). Despite the policies of reduction, reuse, and diversion away from the use of landfills, more than half of the European Union (EU) member states still direct more than 75 % of their solid waste to landfills (Mazzanti and Zoboli, 2008). The structure of urban areas and their outskirts consist of a variety of components, ranging from completely built environments to natural or semi-natural areas (McDonnell and Pickett 1990), the latter often used for the uncontrolled waste disposal. These sites affect biodiversity, ecosystem and human health, by facilitating the spread of invasive species, disease vectors, pathogens, and pollution (Vrijheid, 2000).

Solid waste management is one of the major public health and environmental concern in the EU. The public sector is unable to deliver services effectively, regulation of the private sector is limited, and illegal dumping of domestic and industrial waste is still a common practice (Reddy and Chirakkara, 2013). Despite the strong activity of European Union towards improving waste management policies, there is still a considerable amount of illegal waste dumping, and more than 7,000 dumping sites identified as ‘the tip of the iceberg’ (Giusti, 2009). Illegal waste sites are a fast growing problem in many European countries (Council Directive 1999/31/EC on the landfill of waste, 1999). Despite the existing infrastructure for dealing with waste, studies revealed an extensive network of illegal waste sites (Mazzanti and Zoboli, 2008).

These sites provide a ready source of nutrition and shelter for human-introduced species that support the spread of pathogen vectors and non-native/invasive species (Rusterholz et al., 2012), while discarded pollutants can disperse across the landscape (Diletti et al., 2008; Mattiello et al., 2013). As a consequence, illegal waste sites pose serious economic and social challenges through an increased burden upon the public health system (Ashworth et al., 2014; Elliott et al., 2001; Minichilli et al., 2005; Porta et al., 2009), and may lead to declines in ecosystem functions (Ettler et al., 2008; Kotovicova et al., 2011).

Many publications refer to the influence of specific chemicals or chemical mixtures in illegal landfills on human health (Baibergenova et al., 2003; Kramer, 1987; Sorsa et al., 1992; Sullivan, 1993) but there are almost no data about their influence on biotic homogenisation and the spread of pathogens throughout the rodents living area. It has been recently highlighted that urbanization is a major cause of biotic homogenization (McKinney, 2006; Smart et al., 2006) and that it usually leads to higher prevalence of alien animal and plant species (McDonnell et al., 1997; Melles et al., 2003). For example, garden waste deposited in semi-natural and natural habitats, such as forests, could be a source of dispersion for non-native plant species (Rusterholz et al., 2012).

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Some of these species have a high regeneration potential and may successfully colonize forests (Klimesová and Klimeš, 2007).

To predict the impact of urbanisation processes upon biodiversity and human well-being, it is crucial to understand the shifts in species composition and diversity (Desrochers et al., 2011). For instance, changes in landscape structure, such as an increase in urban patches with increased number and size of waste sites, alter the ability of some commensal organisms to disperse. In such a case, communities composed of species with specific habitat requirements have higher local extinction and turnover rates, whereas widespread and broadly tolerant species benefit from landscape disturbance and demonstrate higher stability (Dall and Cuthill, 1997; Krauss et al., 2004).

The Mediterranean coast, including both Slovenia and Croatia, is a densely populated region in EU where most of the population growth takes place in a dispersed small and medium-sized towns and cities (Cori, 1999; "UNEP/MAP/BP/RAC. A sustainable future for the Mediterranean," 2005) usually without adequate waste management (Ballantyne and Pickering, 2015).

An extensive network of urban landscape and natural habitat dissects the Istrian peninsula shared between Slovenia and Croatia (Kokalj and Oštir, 2005). Medium-sized towns are located on the coast and oriented towards tourism that considerably increase human concentration and waste production. An additional source for waste sites is created by small towns, villages, and small farms that are mainly are dispersed in the central part of peninsula (Gržinić, 2010). Study done on two small rodents species showed that road clearance without vegetation is an inhibiting factor for crossing the road (Macpherson et al. 2011). So, even the small roads, with relative little traffic, do act as a partial barrier for small mammals, reducing their movements between habitats on either side of the road.

There is little information on the effect of uncontrolled waste sites dispersal on rodent communities (Cavia et al., 2009). Rodents are attracted by solid and decomposing organic waste discarded at illegal waste sites. Data on rodent distribution in urban and semi-natural ecosystems is important, as these animals can cause damage to stored food, buildings, and infrastructure (Battersby, 2004; Drummond, 2001). Moreover, rodents are known to be involved in the transmission of diseases to humans and domestic animals (Battersby and Greenwood, 2004). They are a notorious reservoir for a number of pathogens and can act as either principal infected hosts or hosts for arthropod vectors (Desjeux, 2001). Rodent-borne zoonosis, transmitted from rodent to human hosts, can cause significant human morbidity and mortality globally, with thousands of cases diagnosed in Europe annually (Semenza and Menne, 2009).

Until now, no study has specifically investigated the distribution of rodents within the urban and semi natural-environment in relation to uncontrolled waste dumping. The aim of this study was to assess the potential effects of illegal waste dumping on the establishment of rural-like *versus* truly commensal rodent communities in semi-natural habitats. In particular, we looked at the spatial species turnover rates as a function of urbanisation and presence of illegal waste sites. Finally, we studied the effects of landscape structure on the composition of rodent communities. The higher parasitism and infection rates (Bužan et al., 2012) of animals in illegal waste sites could be significantly associated with the change in the rodents communities. Rodents at these sites are either more exposed or more susceptible to parasites or infections, which could have a possible negative impact upon their predators and people. Higher parasitism and infection rates can

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therefore be used as a measurable impact of changed rodent community that further impacts on their predators and people living nearby the waste sites.

We conducted our study in the coastal areas of Slovenia and Croatia, as they represent areas where urbanisation increases pressure on natural resources during limited time periods, and leads to higher rates of sewage and solid waste production.

Materials and Methods

Study area

Fieldwork was conducted in Istria, a peninsula in the Northern Adriatic, shared by Italy, Slovenia, Croatia, and in the Brijuni Archipelago located at the western shore of Istria with an area size of 3,160 km^2. According to the geological and geomorphic structure, the peninsula can be divided in three different areas (Sombke and Schlegel, 2007). The hilly northern and north-eastern part of the peninsula is characterised by scarce vegetation and bare karst surfaces. The south-west region is characterised by lower flysch mountainous tracts consisting mainly of impermeable marl, clay, and sandstone. Finally, the last part is the limestone terrace along the coastline covered with red earth (Krebs, 1907).

One third of the Istrian peninsula is covered by forest. Pinewoods, maquis, holm oak (*Quercus ilex),* and strawberry tree (*Arbutus unedo*) prevail along the coast and on the islands. The present grasslands are among the species-richest habitats of Europe and maintain high small-scale densities of plant species (Kaligarič et al., 2006). They are of semi-natural origin, as they have emerged through centuries or millennia of low-intensity land use (Bohn et al., 2004; Ellenberg and Leuschner, 2010). During the 20th century, the original grasslands were replaced by agro-systems, which are nowadays continuously being replaced by urban habitats. Today, the area represents a matrix of mixed urban, agricultural, and fragmented natural or semi-natural vegetation patches.
The sampling sites differed significantly according to the anthropogenic impact, and three groups of sites were immediately identified (*Figure 1, Table 1*): group A: natural habitats with low anthropogenic impact; group B: habitats with medium anthropogenic impact; and group C: habitats with high anthropogenic impact with large waste sites and/or human settlements.

Field data collection

Sites for data collection were located in illegal waste sites in groups A–C. In total, 17 areas (*Figure 1*) were sampled in the warmer part of the year (from April to November) between October 2011 and November 2012. Sherman traps of two sizes (dimensions of small Sherman traps: 50.08 x 6.35 x 22.86 cm; and dimensions of large Sherman traps: 7.62 x 8.89 x 22.86 cm) in equal shares were used. Sardines with breadcrumbs or peanut butter were used as bait. Traps were set in late afternoon/evening and rodents were sampled overnight. After each sampling night, traps were checked; trapped animals were euthanized in CO_2 chamber, and immediately transported to the laboratory. In one location traps were set 1–18 times (mean ± SD = 4.94 ± 4.41) with 20–80 traps (mean ± SD = 52 ± 20.8). The number of traps was defined by the size of the sampling area. Sampling areas differed considerably in habitat characteristics and level of anthropogenic impact (see *Figure 1, Table 1*). Three of the sampling locations on the

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Brijuni islands (*Figure 1*) were excluded from further analysis, as only the introduced black rat (*Rattus rattus)* was present on these islands.

Table 1. *The summary data on sampling sites in Istria. Each of the sampling site was assigned to one of five habitat types (*[1–5]*), data are presented in proportions to total investigated area). A1–A4 = natural habitats with low anthropogenic impact, B1–B5 = habitats with medium anthropogenic impact, can include small waste sites, C1–C5 = habitats with high anthropogenic impact with large waste sites and/or human settlements.*

Sampling site	Short description	Sampling period	Area (ha)	Housing estates (%) [1]	Villages (%) [2]	Open areas with human settlements (%) [3]	Open areas (%) [4]	Water (%) [5]
A1	reeds near the wood	Jul12	13,81					69.99
A2	meadow near the stream	Aug12	0,12					0.61
A3	wood, road, small amount of scattered waste	Sep12	0,05				0.25	
A4	wood	Oct12	0,04				0.20	
B1	wastesite's edge (near the wood)	Apr-Jul12	0,46			2.33		
B2	field and wood edge	Sep12	0,05				0.25	
B3	meadow, small wastesite, shrubs	Jul12	0,71			3.60		
B4	wood, road	Aug12	0,16				0.81	
B5	wood, road, meadow, small wastesite	Sep12	0,74				3.75	
C1	farm	Sep12, Nov12	0,74		3.75			
C2	wood, pasture	Sep12	0,14				0.71	
C3	large wastesite, shrubs	Oct-Nov11, Mar-Jul12	1,72		8.72			
C4	large wastesite, wood	Aug12	0,04				0.20	
C5	large wastesite, wood, backyard, shrubs	Oct11, Apr-Jul12	0,95	4.82				
		SUM	19.73	4.82	12.47	5.93	6.17	70.60

[1] detached, semidetached and terraced houses, with numerous fruit plants and trees in backyards; [2] farms, waste ground, roads; [3] setlements, fields, rehabilitated waste grounds; [4] grasslands, shrubs, agricultural fields, meadows, forest, waste grounds and floodplains; [5] rivers, canals, moat, river docks and reeds

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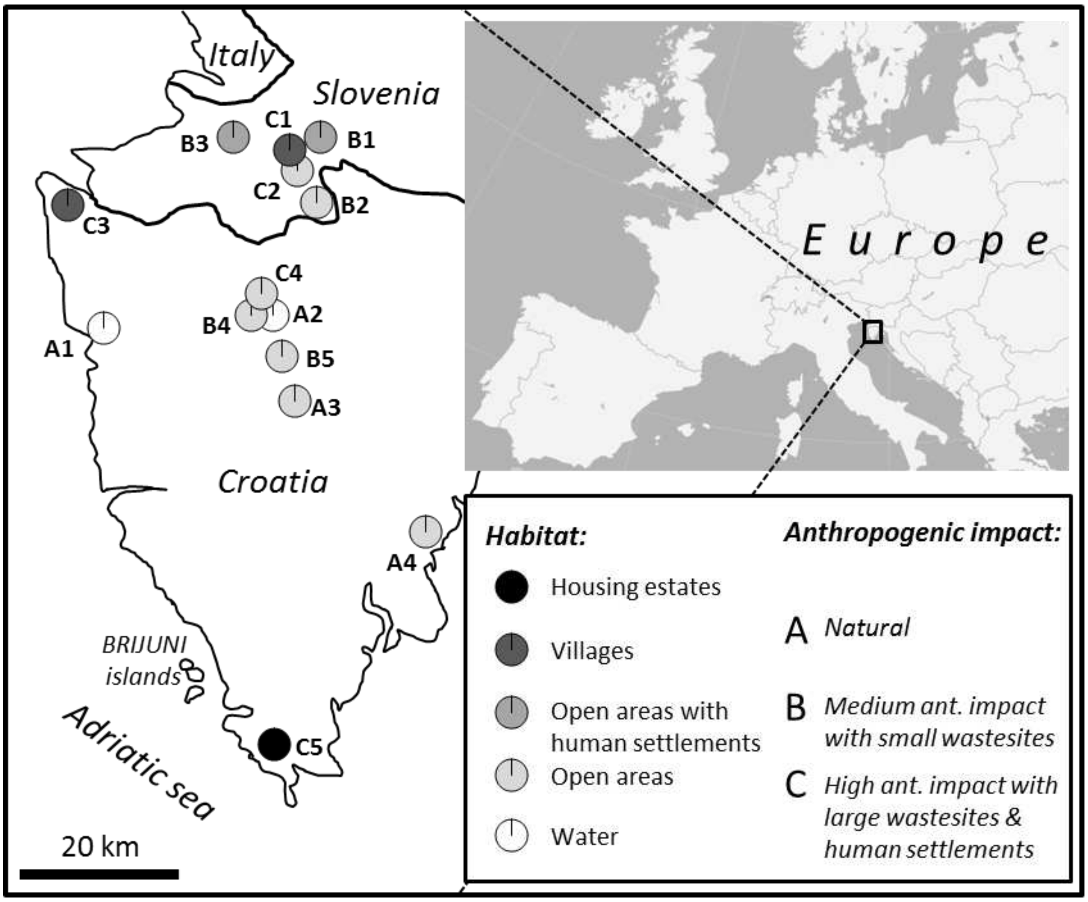

Figure 1. *Sampling sites in Istria.*

Laboratory work

Morphometry of key structures, like body length (from the tip of the nose to the anal opening), tail length (from the anal opening to the end of the tail), the length of the rear foot (from the beginning to the end of the finger phalanges without claws,) and the size of the ears (without the final hair) (Kryštufek and Janžekovič, 1999) were measured for species determination purposes. Most juveniles were identified to a species-level based on sequence similarity of mitochondrial cytochrome b gene (cyt b) (Bradley and Baker, 2001).

DNA extraction, PCR amplification, sequencing and sequence analysis

Tissues from rodent brain and muscle were isolated. DNA extraction was performed using PureLink® Genomic DNA Mini Kit (Invitrogen™, Life Technologies Corporation, Carlsbad, CA, USA). The cytochrome b gene (cyt b) was amplified with primers, L14727-SP and H15915-SP (Jaarola and Searle, 2002). The alignment yielded the sequence data for partial cyt b gene sequences of the length between 300 and 500 base pairs (total length in mammals is 1140 bp). A polymerase chain reaction (PCR) was performed in a total volume of 15 µl containing 3µl of DNA extraction, 0.3 µm 10 pmol forward and reverse primers and 7 µl of KAPA HiFi HotStart ReadyMix 2X (Kapa Biosystems, Inc., United States) containing KAPA

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dNTPs, reaction buffer, Mg^{2+} at a 1X final conc. of 2.5 mM and KAPA2G fast DNA Polymerase 5 units/ µl and 5.4 µl of water.

The PCR cycling conditions included an initial denaturation step at 94°C for 3 min, followed by 30 cycles of denaturation (15 s at 94°C), primer annealing (30 s at 48°C), and extension (1 min at 72°C). The final extension at 72°C ran for 10 min. The sequencing was performed on an ABI PRISM 3130 Genetic Analyzer using BigDye Terminators (Applied Biosystems, Foster City, CA, USA). The CodonCode Aligner 1.63 (Ewing et al., 1998) was used to align the forward and reverse sequences. The resulting consensus sequences for each individual were aligned using ClustalW 4.0, implemented in the Molecular Evolutionary Genetics Analysis (MEGA) package 5 (Tamura et al., 2011). Species identification was confirmed with Basic Local Alignment Search Tool (BLAST) implemented in National Center for Biotechnology Information (NCBI database). A threshold of 98% similarity for the same species was defined according to the results of BLASTn search for the sequences.

Data analysis

For each of the three groups of sites defined by the anthropogenic impact (A, B, C), species composition, abundance and proportions (calculated from abundances) were estimated. Shannon Wiener index ($H' = -\Sigma(n_i/n)*\ln(n_i/n)$; where n_i = number of individuals of a species in a sample i and n = number of all individuals in a sample) (Spellerberg and Fedor, 2003) was calculated for each site and the three groups (A, B, C). Average values, standard deviation and range of H' for sites within each of the groups were also calculated. For evaluation of possible interdependence (at $p < 0.05$) between H' and number of sampled individuals per site, Pearson correlation coefficient (r) was calculated between the two variables. Dominance ($D = \Sigma (ni/n)^2$) and evenness ($e^{H/S}$, S = no. of sampling sites) of the species per three groups were calculated.

Beta diversity (b_w) and species turnover rates between the three groups of sites were calculated following Whittaker (Koleff et al., 2003): $b_w = (S/\alpha_{AVG})-1$, where S is total number of species and α_{AVG} is average number of species.

Species communities were inspected through Unweighted Pair Groups Method (UPGMA), and the clusters of the sites were interpreted according to the level of anthropogenic impact. Distance matrix was computed using Raup Crick index for binary (presence/absence) data (Raup and Crick, 1979) as the sampling effort was uneven. The analysis was conducted in PAST ver. 2.17c, Excel 2010 and SPSS ver. 20.0.

Results

In total, three genera and five species of Muridae black rat, domestic mouse, striped field mouse, wood mouse and yellow-necked mouse (*Rattus rattus*, *Mus musculus, Apodemus agrarius*, *A. sylvaticus* and *A. flavicollis*) were sampled from 14 sampling sites (*Figure 2*). Most common species was *Apodemus agrarius*, recorded at 11 sites. At three sampling sites only one species was recorded (*M. musculus* in an illegal landfill at C4 and at the farm at the site C1, and *A. agrarius* in a wood at the site A4). The largest number of species (all five) were recorded in landfill at C3 and four species were recorded at landfill C5 (*R. rattus*, *M. musculus*, *A. agrarius* and *A. sylvaticus*), as well as near the landfills at sites B1 and B3 (*M. musculus* and all three *Apodemus* species).

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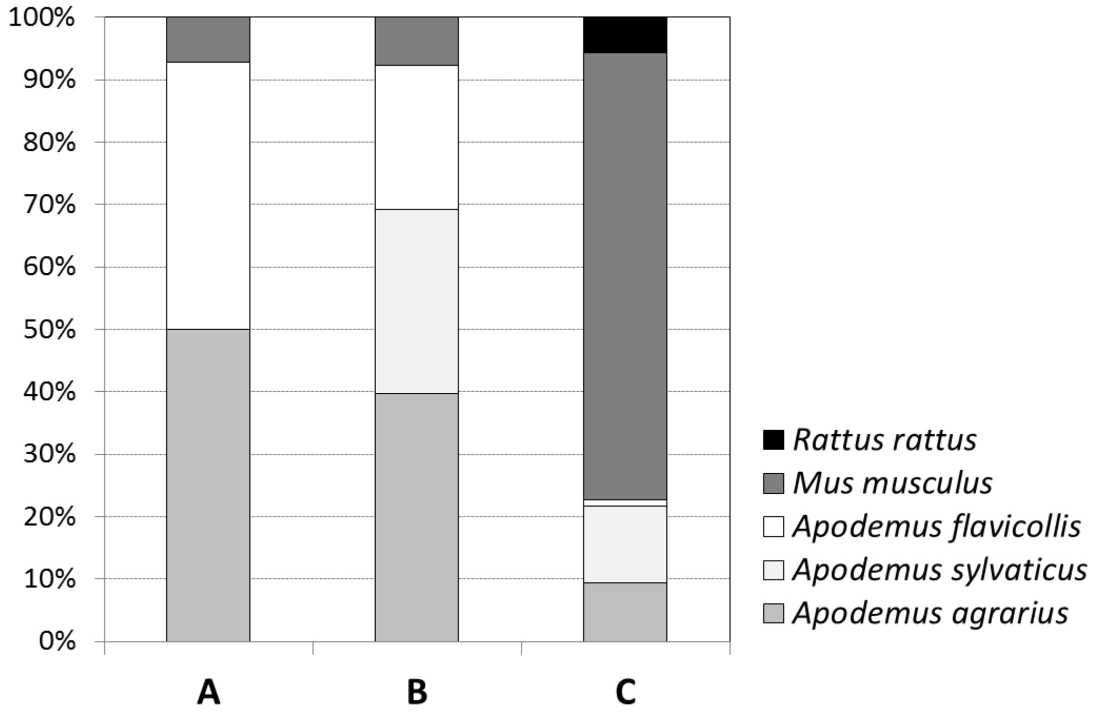

Figure 2. *Species composition and relative abundance of five Muridae species at 14 sampling sites in Istria, divided into three groups of habitats according to the human impact (A = natural habitats with low anthropogenic impact, B = habitats with medium anthropogenic impact, can include small waste sites, C = habitats with high anthropogenic impact with large waste sites and/or human settlements). Numbers of animals = 198: Rattus rattus: A, B = 0, C = 6; Mus musculus: A = 1, B = 6, C = 76; Apodemus flavicollis: A = 6, B = 18, C = 1; Apodemus sylvaticus: A = 0; B = 23, C = 13; Apodemus agrarius: A = 7, B = 31, C = 10.*

All five species were found in a group of sites with the highest anthropogenic impact (*Figure 2*), while in groups with medium and lowest impacts, four and three species were recorded, respectively. On average, 2 species (SD = 1.7) were recorded from the sites with highest impact, whereas 3.2 (SD = 0.8) and 2 species (SD = 0.8) were recorded from sites with medium and low anthropogenic impact, respectively. Shannon-Wiener species diversity index was highest at sites with medium impact ($H'_B = 1.26$ [$mean_{SITES} \pm SD$: 0.93 ± 0.26; range: 0.50–1.28]; $H'_A = 0.89$ [0.56 ± 0.40; 0–0.95]; $H'_C = 0.92$ [0.34 ± 0.58; 0–1.18]), where dominance (D) of the most common species was lowest ($D_B = 0.30$; $D_A = 0.44$; $D_C = 54$) and evenness ($e^{H/S}$) highest ($e^{H/S}{}_B = 0.88$; $e^{H/S}{}_A = 0.82$; $e^{H/S}{}_C = 0.50$). No significant Pearson correlation ($r = 0.412$, $p > 0.1$) was calculated between H' and numbers of caught individuals.

Global Whittaker beta diversity for all 14 sites is 0.25. The highest species turnover rate is recorded between the group of sites with smallest and largest anthropogenic impact (A-C: 0.25). The turnover rates are approximately half between sites with the medium and highest (B-C: 0.11) and medium and lowest anthropogenic impact (A-B: 0.14).

Cluster analysis of 14 sampling sites revealed two distinct clusters with less than 40 % of similarity (*Figure 3*). The first cluster consists of samples from the four largest waste sites and a farm (C1–C5) and the second cluster consists of all other samples. This can be further divided into two sub-clusters. The first sub-cluster consists of

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samples from fields, landfill edges, two small landfills (one in a wooded area and another in a wooded area near the road) (B1–B5). The second sub-cluster consists of samples from two wooded areas, the reeds near the wooded areas, and a meadow with a small stream; A1–A4). The latter sub-clusters join at the ~ 65 % of similarity rate.

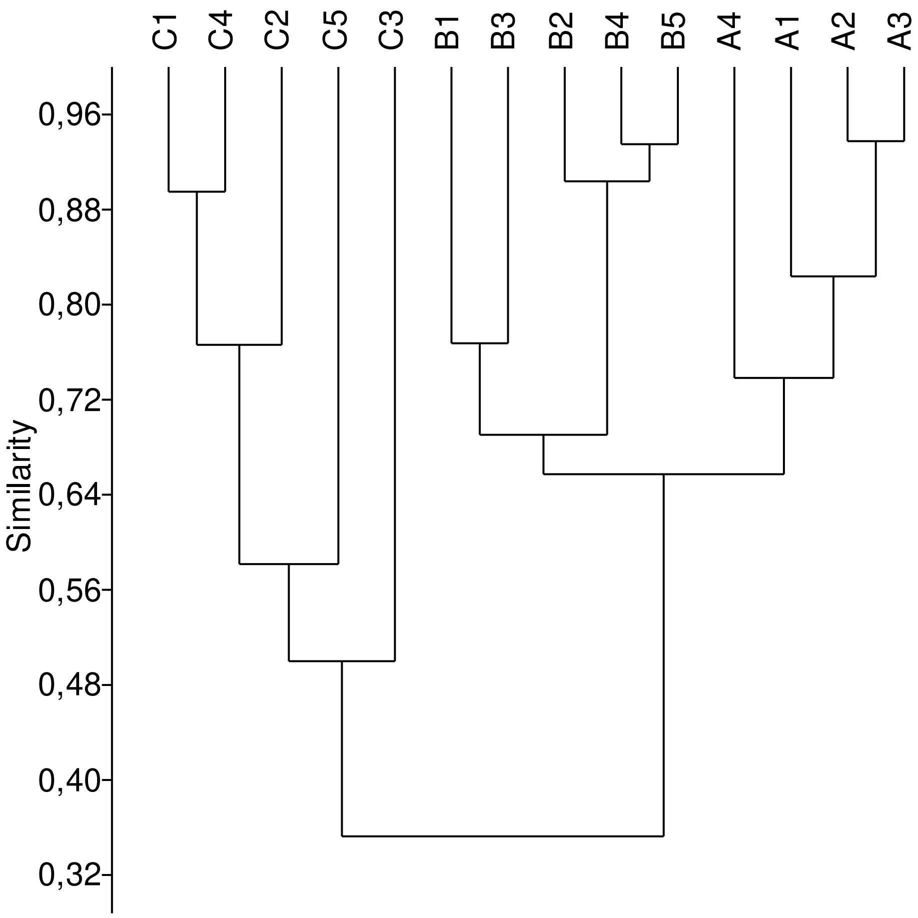

Figure 3. *Cluster analysis of species composition of fourteen Muridae samples locations from Istria (UPGMA, Raup-Crick index, binary data on presence and absence was used, coph. corr. = 0.81). For explanation of sample abbreviations see Table 1.*

Discussion

In the present study, the importance of understanding the impacts of urbanization and inadequate waste management on native wildlife and human health is researched. Most of the previous work dealing with urban ecology investigated the effects of urbanisation on habitat quality and other environmental conditions (Grimm et al., 2008; Sims et al., 2007) in cities, and how species' ecological and life history traits influence their ability to colonise and survive in urban areas (Croci et al., 2008; Kark et al., 2007; Lim and Sodhi, 2004; Thompson and McCarthy, 2008). The impact of solid waste disposal upon communities of small mammals at the outskirts of the cities and towns has not yet been shown. Cavia et al. (2009) reported that accumulated organic waste and litter provide food and harbourage for rodents. Although we agree that human impact through waste

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disposal almost necessarily changes local species community (Cavia et al., 2009), the consequences may vary between habitats and regions.

While human presence is usually connected with the abundance of food sources for small mammals (Feng and Himsworth, 2014), and thus species number may increase, the structure of community drastically changes. Some rodents however are treated as real commensals as they usually become highly abundant in close proximity of humans (Aplin et al., 2003). Black rat (*R. rattus*) and house mouse (*Mus musculus*) are two cases of commensal species that can take the advantage of potentially rich food resources provided by such habitats. Moreover, black rats are so much connected to humans that they are rarely found in the wild (Aplin et al., 2003); with its ability to adapt to a wide array of resources and environments, this species may be the only species within the habitat (Feng and Himsworth, 2014). Although introduced, *Rattus rattus* is the only Muridae representative on Brijuni archipelago offshore the southern Istria (see *Figure 1*) (Bužan et al., 2012)(Bužan et al., 2012). Also in our study, black rats were found only in the largest waste sites (≥1 ha: C3, C5) with food availability (organic waste) and shelter (infrastructure waste) (Aplin et al., 2003; Wilson and Reeder, 2005).

House mouse, however, is more ubiquitous since they are able to persist entirely in both commensal and non-commensal habitats (Pocock et al., 2004). This is in line with the results of the present study as the house mouse was present in a vast majority of sites, and with 83 trapped animals (42 % of total) proved to be the most abundant Muridae species within the area. The species also proved to be commensal as it was most abundant in areas with higher anthropogenic impact. As such, it may also be the most probable candidate for pathogen transmissions. Also two species, *Apodemus agrarius* and *A. flavicollis*, were found in groups of sites from highest to lowest anthropogenic impact. *A. agrarius* prefers moist river-valleys and areas with wet and dense vegetation (Zub et al., 2012) and proved to be the dominant species in natural and semi-natural groups of habitats. The forest and dense shrub stands, where there is sporadic occurrence of fallen woody material, however, are preferred by *A. flavicollis* (Flowerdew, 1985). Although the species was recorded in all three habitat types, its occurrence dropped evidently towards habitats with large waste sites or human settlements where it was trapped only once. Third *Apodemus* species (*A. sylvaticus*) was absent from natural habitats with low anthropogenic impact, and almost two times more frequent in habitats with medium anthropogenic impact than in habitats with settlements or large waste sites. Despite the absence from the natural habitats, this species in total was more frequent than *A. flavicollis*. It is a very adaptable species and inhabits a variety of semi-natural habitats including all types of woodland, moorland, steppe, arid Mediterranean scrublands, and sand dunes (Montgomery, 1989; Vukićević-Radić et al., 2006).

Interestingly, most species were recorded in sites with medium anthropogenic impact, where in our case habitat was changed due to inappropriate waste disposal (average ± standard deviation: 3.2 species ± 0.8). The mixture of different environments at these sites and potential food resources within the landscape units around these sites allowed the species present in the region to occupy the urban ecosystem (Alard and Poudevigne, 2002). These places can therefore serve as a border habitat for commensal and native species. Moreover, waste sites in this study were recognised as possible refuge for commensal and native rodent species, which enables integration and increases possibilities of spreading of rodent-related diseases. Hence agriculture and

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urbanisation with the connected waste disposal activities will put humans at risk of contracting a series of rodent-related diseases (Udonsi, 1989). Moreover, improper waste management is driving the native species to local extinctions and replacing them with invasive commensal rodents, thereby reducing biodiversity and ecosystem health (Joyce et al., 2000).

As a quantifiable impact upon changes in rodent communities at our study area, we can stress that while antibodies for Lymphocytic Choriomeningitis Virus (LCMV) was present in 24.1 % of animals from the entire area (N = 83 individuals, see Buzan et al. 2012; Buzan, unpublished), seroprevalence was always much lower at the reference sites (Croatia: 6.7 %; Slovenia: 9.1 %) than at the waste sites (Croatia: 39.0 %; Slovenia: 33.3 %). Moreover, also the rate of parasitism (N = 103 individuals) was more pronounced at waste sites (56 %) and less at the reference sites (44 %). Among 26 documented ecto- and endoparasites, eight could be potentially harmful also for people (Bužan et al., 2012). Increased rates of both at the waste sites can influence not only to the people but also to some predator species (e.g. foxes, common buzzards, cats; see Hanski et al. 1991 for the complete list). Generalist predators can either adjust their feeding choice to the available rodent species or switch to other available prey. Although in some cases generalist predators can even profit from the increased density of small mammals at the waste sites, they are also more exposed to the risk of transferring some parasites and diseases. On the other hand specialist small rodent predators (e.g. small mustelids; see Hanski et al. 1991) may be crucially influenced by the community change despite higher densities of small mammals as they are even more exposed to the possibility of parasite and disease transfers. Overall, our study results indicate that multiple features of waste sites influence infection risk and parasitism of host and suggest that synergism between traits can have important effects on predators and people.

In the majority of studies the number of rodent-borne diseases relates to the rodent population densities (Escutenaire et al., 2000; Olsson et al., 2002). Higher densities of rodents increase the likelihood of contact between rodents and man, which leads to a higher likelihood of disease transmission (Davis et al., 2004; Niklasson et al., 1995). Transmission can occur by direct contact, by bites, through the pathogen contamination of water or food sources (Slifko et al., 2000), or via arthropod vectors (Lindgren and Gustafson, 2001). Introducing commensal rodents to waste sites enables them to colonize the regions that are otherwise inhabited by native rodents, and gives them additional abilities for transmission of infectious agents from commensal rodents to native ones. Such contact favours the transmission of parasites by direct contact or via oral–faecal routes (Slifko et al., 2000). The monitoring of Lymphocytic choriomeningitis virus, flavivirus, *Leishmania* sp. and taxoplasmosis showed increased seroprevalence infections in rodents sampled at illegal waste sites (Bužan et al., 2012; Ivović et al., 2015).

Understanding rodent ecology and gene flow, including movement of commensal rodents with respect to human expansion in urban landscapes, is critical for understanding the dynamics of rodent-borne pathogens and is valuable for mitigating human disease outbreaks (Mills et al., 1999).

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Conclusions

The present study shows that illegal waste sites can present a potential place for spreading commensal rodents to the natural environment. These consequences of urbanization represent threats to human health due to spreading rodent-related diseases but will also drive the native species to local extinction and replacing them with non-native species. The ecological and economic damage caused by illegal waste sites is well known to the scientific community, but there is still a lack of awareness of these problems in landowners and the population in general. Inevitabelly we showed that not only the waste sites impact upon small rodents' community change, but also increase the risk of native species being overcompeted by the invasive small rodents that further influence on the entire ecosystem by exposing the native species (including their predators) and people to more likely get infected by some zoonoses or parasites.

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A SURVEY AND STUDY OF TOWERKILLS AND WIND TURBINE KILLS

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Abstract. The world is full of anthropogenic obstacles and ecological traps for wildlife but we rarely consider the possible ecological impacts on individual animals or regional ecosystems into account as designs are implemented. This research studies the impacts of electric transmission towers and wind turbines on animal casualties and behaviors. Within a one-year period, ecological surveys were conducted in areas adjacent to the Taichung Power Plant in Taiwan to analyze the interactions between the two facilities and avian collisions. Complete mechanisms of towerkill and wind-turbine kill are yet to be found, but the bird carcasses were all found next to waters especially the freshwater bodies. This high correlation may generate the conclusion that manmade structures close to water bodies are the most dangerous to birds. This conclusion may be very valuable for engineers in sitting the locations of the electric transmission towers and wind turbines..
Keywords: *electric transmission tower, ecological conservation, wind farm, avian collision, Taiwan*

Introduction

Humankind has completely dominated nature with advanced technologies. Development and construction have been carried out based on human needs and desires, leading to unprecedented anthropogenic destruction of existing ecosystems. According to estimations by biologist Edward O. Wilson (1989), approximately 10,000–15,000 species are facing extinction every year. Every day, 34 species disappear because of delays in conservation. There will be continuous and rapid deterioration of biodiversity, ecological integrity, and ecological health in the future globally. Ecological conservation is an imperative undertaking for the world. Along with social progress, ecological conservation will gradually become the popular consensus. Human rights and animal rights will gain traction at the same time, and humanity cannot exclude itself from the global ecosystem. Each species has its niche in the ecosystem, which is particularly true for birds. They are not only important members of ecosystems but also an indispensable element of social aesthetics and an indicator of environmental health. However, there are occasionally reports of killed birds, not only from direct human hunting, but also from anthropogenic facilities that have indirectly led to innumerable deaths. Many towers can attract birds or cause birds in flight to have blind collisions, which may be an ecological trap for many birds (especially high-flying migratory birds). What class of birds is most affected by towers and power lines? What is the impact on the number of species groups? These questions currently have not been systematically addressed.

According to research, human activities (mainly hunting) and anthropogenic facilities cause 200 million bird deaths annually, among which 61% is from hunting, 29% from traffic accidents involving animals, 3% from bird collisions with buildings, and 7% for other reasons (Banks, 1979). In general, the development of engineering design and construction is based on human subjective norms. Structures such as bridges,

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buildings, parks, green spaces, all sorts of towers, and so on may act as biological parks or animal traps at different levels. High-rise structures are an illustrative example. There had been observations of collisions between birds and towers since the 1940s in North America (towerkill.com). When there is dense fog or low atmospheric clouds at night, migratory birds are often attracted to the flashing lights of the tower and fly around it. Most bird deaths from towers occur when the birds cannot sense the presence of the power line. In the future, there will be increasing numbers of cell phone towers and wind turbines constructed, which may further affect the migration of birds at night. Electric transmission towers, telecom towers, bridges, and their attached cables are all obvious bird traps. In particular, power lines often stop birds mid-flight, lethally entangling them. Such cases are too numerous to name (Bevanger and Brøseth, 2004). In the United States of America in the 1970s, there were 1.3 million bird deaths each year caused by 1,010 TV towers (Banks, 1979). In addition, in the 25 years after 1960, there were more than 42,000 dead birds found around a single tower in Tallahassee, Florida (Crawfowrd and Engstrom, 2000). Hence, towers in certain locations can cause an alarmingly large number of bird casualties. However, after 28 years of investigation, the same researchers found that reducing the height of the tower can effectively reduce bird casualties (Crawford and Engstrom, 2001). Towers with a height of less than 61 meters pose no discernible danger to birds (Kerlinger, 2000).

There are two modes of mortality from bird collision with towers or power lines (towerkill.com). The first is from direct impacts due to the inherently poor eyesight of birds, which is termed “blind collision.” Certain fast-flying birds, such as waterfowl or shorebirds, may experience death by blind collision because they cannot see the towers or cables when there is daytime fog. Around a TV tower in Topeka, Kansas, in the USA, there were 2,808 deaths of birds from 9 species over a 4-night period (Ball et al., 1995). The other mode of death is collision death due to the attraction of the lights on the tower. Collision deaths would be more likely if a tower does not have a flashing light at night. The color of the flashing light emanating from the tower also has a significant effect on the flight path of birds. A red flashing light is more likely to create a nonlinear flight path for birds than a dazzling white light, or no light at all (Gauthreaux and Belse, 2006). Therefore, birds may be more sensitive to a red flashing light. However, another study (Evans et al., 2007) found that a blue, green, or white light was more effective in attracting birds than a red light when there is low cloud cover, which contradicts the previous study. Nonetheless, two other studies (Longcore and Gauthreaux, 2008; Gehring et al., 2009) suggested that avoiding using a red light on towers could help deter bird collisions, which seemed to imply that a red light might cause birds to fly closer to towers. However, in areas with mist, smoke, or high moisture, there will be a diffusion phenomenon due to the effect of light passing through the water particles, which may form a light source to guide the birds to fly and gather. As a result, some birds may die from mistakenly hitting the cables in circling flight. Early research found that migrating birds often circle around towers because they are attracted to the flashing lights on the towers (Graber, 1968). If the flashing lights on the tower were turned off, the circling birds near the tower would disperse (Cochran and Graber, 1958). Hence, a flashing light indeed attracts birds.

Weather is also another important factor for bird collision. The diffusion of the tower light by water particles in clouds and fog may cause the migrating birds to linger (Avery et al., 1976). Low clouds may also cause birds to circle around the tower (Larkin and Frase, 1988). The above phenomena are all due to the comprehensive effects of water

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particles and light. Moreover, the insulating wires of the tower are often places for birds to build their nests, and they may become conductive on damp or rainy days, thereby killing the birds. Towers on ridges, or those that are especially tall, are flight obstacles and are thus easy collision targets for birds, leading to their deaths (Longcore and Gauthreaux, 2008).

There have been no documented cases of bird collisions with wind turbines in Taiwan (Shi et al., 2008). However, the numbers of Charadriiform waterfowl may be reduced by the wind turbines, which may cause their main flight path to move far away from the wind turbines (Shi and Cheng, 2008). The presence of wind turbines changes landscape characteristics and reduces the number of local birds. In the same way, these changes probably lead to collisions with migrating birds at night, even though there are no actual descriptions of such events in Taiwan. It is necessary to conduct detailed investigation in order to clarify whether towers and wind turbines directly kill birds. This study examined bird deaths from transmission towers and wind turbines near the Taichung Power Plant in Taiwan. The results should clarify the effects of such anthropogenic structures on bird species and their migratory patterns, sum up the main factors, and determine what steps to take to fix the problem.

Scope and method of the study

Several studies have been performed to investigate bird collisions with transmission towers and wind turbines in Taichung, Taiwan. No collision events were found even in systematic investigations (Shi and Cheng, 2008; Shi et al., 2008), which may be attributable to low survey frequency or insufficient duration. Other reasons may be that bird remains may be removed or may be scavenged and eaten by other animals. Especially in the case of bird collisions with towers and wind turbines near water bodies, the carcasses may fall into the water where they cannot be collected. Therefore, the real impact can be studied only through intensive, long-term investigation.

Taichung Power Plant and the Chang Bin coastline at Longjing Township in Taichung, Taiwan were selected as investigation site. This area is an estuary of Choshui River and an important part of the ecological protection zone, which is located along the migration route of birds. The coast of Changhua (Chang Bin coastline) is located in the center of the East Asia-Australia migration path and is a wetland of international importance. Millions of birds move back and forth between the Northern and Southern hemispheres every year. However, there are densely distributed power transmission towers and wind turbines in this area. The tower density may be the highest in Taiwan, so it is a very suitable place to investigate bird collisions with high-rise anthropogenic structures. Hence, the Taichung Power Plant and the Chang Bin coastal areas were chosen as the survey areas.

This study focused on the transmission towers of the Taichung Power Plant (*Fig. 1*) and the wind turbines in the coastal areas of Changhua coast. The plan was to conduct field studies on a weekly basis for 1 year to better understand the real causes of bird collisions and number casualty distributions. There are 6 groups of transmission lines spreading out from the Taichung Power Plant (*Fig. 2*). There are also hundred of wind turbines located in nearby coastal areas in Chang Bin coastline (*Fig. 3*). The tower bases can be divided into 3 levels of accessibility: (1) those that can be directly accessed (A type); (2) those that can be observed only through a telescope (B type); and (3) those that are neither accessible nor observable (C type). The A-type towers were marked and

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surveyed on a weekly basis. The researchers directly accessed the base of these towers and searched for bird carcasses within a 20 m radius of the base. All dead bird carcasses were picked up and their location noted. After the preliminary identification and recording, the carcasses were brought back to laboratory for further identification. Since most of the wind turbines were accessible, the same process was also used for surveying bird carcasses around them.

***Figure 1**. Taichung Power Plant*

***Figure 2.** Tower cables to external connections*

After a preliminary walking survey on July 20, 2009, the research team decided to investigate west 4 groups of towers and nearest wind turbines near the power plant. This investigation consisted of 3 stages, as described below.

Stage 1: During the period from August 1, 2009 to September 15, 2009, a preliminary investigation was conducted daily by researchers on 30 transmission towers and 30 wind turbines. The results were only for reference purposes since the survey range was different from those used in Stage 2 and Stage 3.

Stage 2: The survey was restarted on December 26, 2009 and lasted until May 16, 2010. The survey range was increased to 31 towers and 35 wind turbines that were

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investigated and analyzed on a weekly basis (always on weekends). There were 21 surveys in 142 days.

Stage 3: This stage lasted from February 18, 2012 to February 17, 2013 with the same survey range as in Stage 2. The survey was also done on a weekly basis, for a total of 52 times in 366 days.

Figure 3. *Wind power generation facilities in coastal areas in Chang Bin coastline*

Investigation results

Taichung Power Plant is located between the Gaomei wetlands and Tatu estuary wetlands, both with abundant bird populations. The wind turbines at Chang Bin are located south of the Tatu estuary and extend for tens of kilometers southward along the Chang Bin coastline, and they provide a portion of the electric power generation in the center area. In this study, 31 towers near the Taichung Power Plant and 35 wind turbines surrounding Chang Bin Industrial Zone were chosen for the investigation of bird collisions. The study was divided into 3 stages, with Stage 3, the main study, being systematic and complete.

Three-stage investigation

Stage 1: There were 31 surveys from August 1, 2009 to September 15, 2009. A total of 5 bird carcasses were collected in this period, and their locations and species are shown in *Table 1*. All carcasses were found under the transmission towers where are located in land but outside the survey areas of stages 2 and 3. Stangely, no bird carcasses were spotted under wind turbines. Because this is the first survey undertaking, the carcasses were cummulated for long time and are mostly quite decayed, such that the number of carcasses is relatively higher comparing with stage 2 and 3. This can be considered to be clean the carcasses for the next surveys. The above results should only be used as a reference, owing to the presence of scattered surveys.

Stage 2: The survey range between December 26, 2009 and May 16, 2010 is as shown in *Figs. 36* and *37*. The collected bird carcasses around the transmission towers are listed in *Table 2*, while the bird carcasses near wind turbines are shown in *Table 3*.

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Table 1. *Details of the bird carcasses around the transmission towers in Stage 1*

Date	Location	Species
2009-08-02	Outside the investigation area of Stage 3	Sparrow (*Passer montanus*)
2009-08-12	Outside the investigation area of Stage 3	Golden turtle dove (*Streptopelia orientalis*)
2009-08-14	Outside the investigation area of Stage 3	Sparrow
2009-08-27	Outside the investigation area of Stage 3	Sparrow
2009-09-04	Outside the investigation area of Stage 3	Sparrow

Table 2. *Details of the bird carcasses around the transmission towers in Stage 2*

Date	Location	Species	Figure Number
2009-12-27	No. 26	Common moorhen (*Gallinula chloropus*) or White-Bellied Crake (*Amaurornis phoenicurus*)	4, 5
2010-01-10	No. 31	Little egret (*Egretta garzetta*)	6, 7
2010-05-16	No. 7	Heron (*Ardea cinerea*)	8, 9

Table 3. *Details of the bird carcasses around the wind turbines in Stage 2*

Date	Location	Species	Figure Number
2009-12-26	No. 34	Painted snipe (*Rostratula benghalensis*)	10, 11
2009-12-26	No. 34	duck or crow	12, 13
2009-12-27	No. 21	Little swift (*Apus affinis*)	14, 15
2010-03-13	No. 34	Little egret	16, 17
2010-04-11	No. 34	Heron (*Ardea cinerea*)	18, 19
2010-04-25	No. 33	Yellow-head fantail warbler (*Cisticola exilis*)	20, 21

Stage 3: The survey ran between February 18, 2012 and February 17, 2013 in the same locations as in Stage 2. The bird carcasses collected around the transmission towers and the wind turbines are listed in *Tables 4* and *5*, respectively.

Table 4. *Details of the bird carcasses around the transmission towers in Stage 3*

Date	Location	Species	Figure number
2012-02-18	No. 10	Dove (*Columba*)	22, 23
2012-05-20	No. 16	Dove	24, 25
2012-10-07	Between No. 10 and No. 12	Grey wagtail (*Motacillidae*)	26, 27
2012-10-07	Between No. 10 and No. 12	Pearl neck cushat (*Streptopelia chinensis*)	28, 29

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Figure 4. *No. 26 tower (right side)*

Figure 5. *The common moorhen (or white-bellied crake) at the No. 26 tower*

Figure 6. *No. 31 tower (the second from the left)*

Figure 7. *The little egret at the No. 31 tower*

Figure 8. *No. 7 tower (the second from the left)*

Figure 9. *The heron at the No. 7 tower*

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Figure 10. *No. 34 wind turbine*

Figure 11. *The painted snipe at the No. 34 wind turbine*

Figure 12. *No. 34 wind turbine*

Figure 13. *Duck or crow at the No. 34 wind turbine*

Figure 14. *No. 21 wind turbine*

Figure 15. *The little swift at the No. 21 wind turbine*

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Figure 16. *No. 34 wind turbine*

Figure 17. *The little egret at the No. 34 wind turbine*

Figure 18. *No. 34 wind turbine*

Figure 19. *The heron family carcass at the No. 34 wind turbine*

Figure 20. *No. 33 wind turbine*

Figure 21. *The yellow-head fantail warbler at the No. 33 wind turbine*

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Figure 22. *No. 10 tower*

Figure 23. *The dove near the No. 10 tower*

Figure 24. *No. 16 tower*

Figure 25. *The dove at the No. 16 tower*

Figure 26. *No. 10 and No. 12 towers*

Figure 27. *The grey wagtail between the No. 10 tower*

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Figure 28. *No. 10 and No. 12 towers*

Figure 29. *The pearl neck cushat beside the No. 12 tower*

Table 5. *Details of the bird carcasses around the wind turbines in Stage 3*

Date	Location	Species	Figure Number
2012-03-24	No. 21 turbine	Lark (*Alauda gulgula*)	30, 31
2012-03-24	No. 38 turbine	Egret (*Ardea alba*)	32, 33
2012-05-20	No. 38 turbine	Little swift	34, 35

Figure 30. *The first turbine is No. 21*

Figure 31. *The lark carcass at the No. 21 turbine*

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Figure 32. *No. 38 turbine*

Figure 33. *The bird carcass (possibly an egret) at the No. 38 turbine*

Figure 34. *No. 38 turbine*

Figure 35. *The little swift at the No. 38 turbine*

Consolidated results

A total of 9 bird carcasses were collected in Stage 2, and 7 were collected in Stage 3. Their distributions are shown in *Figs. 36* and *37*. Despite the small number, there was an obvious impact on the landscape and concentration of the bird collisions. For the transmission towers, all the collected bird carcasses were close to the freshwater river, while the bird collisions with the wind turbines were concentrated below the No. 34 turbine (4) and the neighboring No. 33 turbine (1). It is necessary to address the phenomenon of the concentrated bird collisions with an in-depth discussion.

Bird-collision analysis

Since no bird collision event was directly witnessed by the researchers, it is hard to know the exact situations or the modes of the collision with towers and wind turbines. However, it was estimated that the collected bird carcasses were less than the real number due to the weekly survey frequency. Between the surveys, bird carcasses might

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have been blown away by the wind, removed, taken away and eaten by wild dogs or other animals, or fallen into the water. The mechanisms of bird strikes with electric transmission towers and wind turbines are still inferable based on surveys and relevant literature, as described below.

Figure 36. *The distribution map of bird carcasses around the transmission towers where the symbols ● and ▲ stand for the survey results in Stage 2 and Stage 3, respectively. The numbers inside the symbols are the counts of the bird carcasses.*

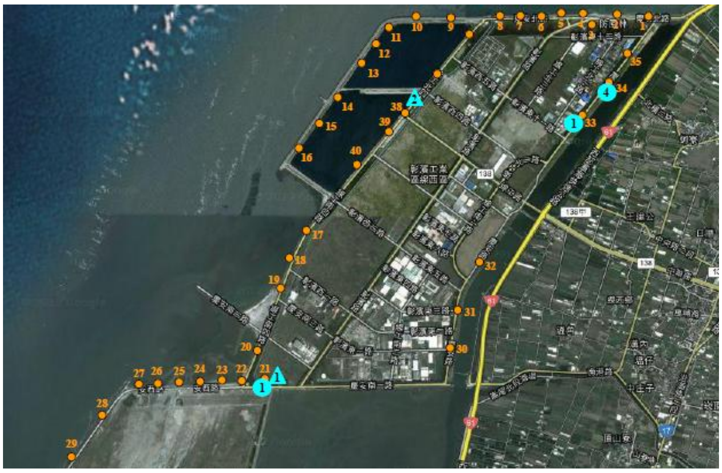

Figure 37. *The distribution map of bird carcasses around the wind turbines where the symbols ● and ▲ stand for the survey results in Stage 2 and Stage 3, respectively. The numbers inside the symbols are the counts of the bird carcasses.*

Transmission towers

A total of 9 bird carcasses were collected in the 21 surveys in Stage 2, with 3 around the transmission towers and 6 around the wind turbines. There were varied bird species, and it was difficult to summarize any species characteristics. All of the bird collisions around the transmission towers happened close to the rivers between the Tatu estuary and the river. Interestingly, no bird carcasses were found in the inland areas. Although it cannot be directly concluded that the river (or water bodies) is the only reason for the bird collisions with the towers, the landscape should be an important consideration in

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bird collisions. The birds may collide with the towers because they are attracted by the water body or they prefer water activities.

In Stage 3, 4 bird carcasses were found around the transmission towers and another 3 around the wind turbines in the 52 surveys. They also varied in species. The locations of bird collisions were at the No. 10 tower, between the No. 10 and No. 12 towers, and at the No. 16 tower that they are all along the river. During the survey the researchers noticed that birds congregated in the woods below the No. 16 tower, and the tower was close to the river. Such two ecological elements, woods and water, may lead to bird collisions with the towers or their cables. There was 1 bird carcass found between the No. 10 and No. 12 towers. It was observed in the field that there were 11 cables splitting out from the No. 10 tower to the No. 11 and No. 12 towers. The power lines were distributed in a web formation, such that the birds might be easily confused and collide with the cables. Water bodies must be an important factor in bird collisions with towers, while the collision behaviors still need continuous observations and an overall analysis of the landscape in order to understand the ecological implications.

Furthermore at night, most birds in flight are unable to sense the presence of transmission towers and power lines. In areas with high density of towers and power lines, bird strikes are unavoidable. Some small, agile birds (such as the dove, grey wagtail, sparrow, and golden turtle dove) are less likely to hit transmission towers during the daytime, they are less likely to avoid such strikes at night. For medium-sized and large birds, which are less agile, or birds that have poor eyesight (such as the common moorhen, little egret, and heron), collisions presumably happen during both day and night.

Wind turbines

The wind turbines in the survey range were mainly surrounding the Chang Bin Industrial Zone and the coastal area. What was surprising was the concentrated phenomena of the bird collisions with wind turbines. There were as many as 4 different species of birds that collided with the No. 34 turbine, and another at the neighboring No. 33 turbine. These two wind turbines were also close to the river, which is similar to the phenomenon of the bird collisions with transmission towers. In the field survey, no differences were observed in the rotation speed, positioning, and environment between the No. 33 and No. 34 wind turbines and others on the same route. Thus, it was speculated that the birds would have died from collisions with the blades of wind turbines when they gathered or foraged above the freshwater river, since all of the turbines with dead birds were close to a water body. In order to discuss whether the rotation speed of the turbine was higher for the turbine with dead birds than the others, researchers recorded videos of the No. 37–38 and 33–34 turbines and compared their rotation speeds. Only an extremely small difference was found in the rotation speeds between the No. 37-38 turbines, and No. 33-34 turbines. Hence, it was deduced that the rotation speeds of all the turbines were roughly the same, so this could not explain the differences in numbers of collisions. These results indicate the need for further in-depth study of the pertinent causes of bird collisions with wind turbines in the future.

Because of structural differences, wind turbines have a different mechanism of bird strikes than transmission towers. Bird injuries and deaths are primarily caused by collisions with rotating blades, which are unpredictable from the perspective of birds because the blades do not operate with a linear motion and the rotational motion confuses birds. Bird strikes are likely to occur during both day and night when the birds

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misjudge the rotational speed of the blades. Bird carcasses of various sizes were found in this investigation. This indicated that agile birds attempted to fly across the rotating blades. Medium-sized and large birds were hit by blades because they could not easily alter their flight paths.

Bird species

If only bird collision statistics for Stages 2 and 3 were included for discussion (excluding Stage 1), the order of main bird species based on the number of bird carcasses would be egret (3), little swift (2), dove (2), heron (2), and only 1 each of other species. It has to be emphasized that there might still be other bird carcasses that were not collected. Highly abundant egret and heron are usually seen in sporadic groups near sandy beaches, riverbanks, and paddy fields. They are not flexible while flying, and thus, are very likely to collide with man-made structures around water bodies such as transmission towers or wind turbines. Little swift and dove are local common birds with great abundance, and thus, they are at an increased risk of being involved in bird collisions. If the numbers and species of bird carcasses collected in Stage 1 are included for observation, the majority was sparrows. Transmission towers included in Stage 1 were all located inland. Coincidentally, sparrows in Taiwan are terrestrial bird species and extremely abundant; thus, their collisions with transmission towers are inevitable. Transmission towers included in Stage 1 were located in a significantly different landscape compared to that in Stages 2 and 3, in which the transmission towers were located in diverse landscapes and the wind turbines were mostly located along coastlines. Different landscapes have different ecosystems and bird compositions, and thus, birds involved in collisions can be classified as terrestrial (Stage 1) and aquatic (Stages 2-3).

Lights

On the other hand, it still cannot be concluded based on the results of this study whether lights on transmission towers and wind turbines will attract approaching birds and cause collisions. Since there were plenty of street lamps in areas where the transmission towers were investigated for all the stages, with mutual interferences among different lights, the lights from the transmission towers are likely to have been made imperceptible by the high number of street lights, and will not be as attractive to birds as single flashes in dark nights of transmission towers and wind turbines. However, wind turbines are located in coastal areas where the density of street lamps in neighboring areas are relatively low, and thus, it is possible the lights from wind turbines will attract birds. The above interpretations are only speculations based on observations. The true impact of flashing lights is yet to be profoundly and systematically investigated and studied.

Conclusions and suggestions

Construction projects precipitated by the need for economic growth and technological progress should not only affect the interests of humankind, but also consider their impacts on the conservation of native species and ecological communities. Electric transmission towers and their cables are essential facilities, and wind turbines are paramount to environmental sustainability. If safety, convenience, and

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conservation are all taken into account in engineering construction, we will be able to build more balance between construction development and ecological conservation. Hence, the goal of this project is to integrate conservation into engineering construction.

A total of 21 bird carcasses were collected during this research. Twelve were found around transmission towers and nine around wind turbines. Based on the locations of transmission towers and wind turbines where the carcasses were found, our speculations are summarized below.

(1) All bird carcasses were resident land birds; no seabirds or migrants were found. Nevertheless, the research location was the estuary of the Dadu River in a waterfront area, which is a hot spot for birds during migration. It was unusual that no seabird or migrant carcasses were found. The small numbers of seabirds in Taiwan’s coastal areas could be a possible explanation for this occurrence

(2) All transmission towers and wind turbines where the bird carcasses were found were near freshwater bodies, which were the preferred water bodies for most land birds. The probability of bird collision events was hence increased.

(3) We expected bird collision events for migrants in this area. However, a relatively small number of migrants were observed relative to that of resident land birds. Their stopover is only short-term. Furthermore, bird carcasses might have been removed by scavengers, such as wild dogs, or blown away by the strong winds. Therefore, the chance of finding them was significantly smaller than that of finding bird carcasses of land birds.

(4) Seabirds spend most of their time at sea. When they approach land, they typically fly low over the water or stay on the shore and are unlikely to hit transmission towers. Their flying heights are also likely to minimize the chance of strikes with wind turbines. Consequently, no sea bird carcasses were found. According to this study, onshore wind turbines should not cause injury or death to sea birds.

On the other hand, bird carcasses were concentrated around certain wind turbines. As many as 4 bird carcasses were found at the No. 34 turbine, 2 at the No. 21 turbine, another 2 at the No. 38 turbine, and 1 at the No. 33 turbine. None were found at the other turbines. The areas near the No. 33 turbine and the No. 34 turbine should be regarded as hot spots for bird collisions. However, it may seem arbitrary to affirm that the above wind turbines were hot spots for collisions since the survey was conducted weekly. It also cannot be determined whether there were any bird collisions between two surveys. Meanwhile, it was impossible to determine whether bird carcasses would be carried outside of the observation range by strong winds after collision with a turbine blade. However, the concentration phenomenon cannot be ignored. It can only be said that there is nothing statistically significant about the rotation speed and the positioning of the wind turbines. The more significant factor was that the No. 33, 34, and 21 wind turbines were all close to freshwater bodies, and their high probabilities of bird collisions were similar to those of the towers. Therefore, for the towers and wind turbines, being close to a water body is an important factor in collisions. Despite the variety of species, the aggregation of bird carcasses was very high. This result is important for engineering design. Field observations must be sustained for a long time to explore the reasons for such a high concentration, which is costly in terms of labor. However, with the planned survey in this study, some of the turbines were already chosen as our focus. Similarly, continuous observations could be carried out around transmission towers or wind turbines with concentrated bird collisions, and there would

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be real-time witnessed bird collisions at the turbines. This would be beneficial to bird conservation.

There were 31 towers in this survey. Stage 2 and Stage 3 lasted 14 months in total, during which a total of 6 bird carcasses were found. There are 21,844 towers in Taiwan. It is expected that the probability of bird collisions would be higher in mountainous areas than in the studied area, whereas that in urban areas would be lower. It is hypothesized that the bird collision frequency of the studied area is typical for Taiwan. If the situation of bird collisions with towers is similar to that of this area, and taking further consideration of the about 70-meter height of each tower in Taiwan and the total number of transmission towers according to Taiwan Power Inc. (www.taipower.com.tw/news/new198.htm), the annual bird deaths from collisions with transmission towers in Taiwan would be roughly estimated to be at least:

$$\frac{7}{31}\times\frac{12}{14}\times 21884 \cong 4236$$

Among these deaths, even the collision situations of the rare, conserved, and important species are unknown; they may have significant effects on the populations of these species.

There were a total of 9 bird carcasses found around the 35 wind turbines, while only about 200 wind turbines existed in Taiwan. Thus, the average annual bird deaths from collisions with wind turbine blades must be at least:

$$\frac{9}{35}\times\frac{12}{14}\times 200 \cong 44$$

However, if a turbine is located on the migration route of birds, the number of bird collisions must be higher. Besides, the number of bird collisions counted in this survey was lower than the actual number, which could be 2–3 times higher. In particular, bird carcasses may have been carried far away by wind after collisions with wind turbines and could not be collected by the researchers. Therefore, bird casualties from wind turbines may need more attention and could be much higher than what was estimated in this study.

After studying the relationships between the mechanisms of bird strikes with transmission towers and wind turbines; bird species; and geographic locations of artificial facilities, the following feasible conservation measures are proposed.

(1) Complete ecological surveys should be conducted before building transmission towers or onshore wind turbine generator systems. In addition to allowing for the ecological characteristics of resident birds, possible flight paths of migrants should also be considered. If migration paths can be avoided, it should reduce the incidence of migrant bird collisions.

(2) Because it is possible for various types of land birds to collide with transmission towers and wind turbines, if these facilities are built in the habitats of rare or endangered species, special designs should be implemented to avoid bird strike events to further decrease injuries and mortality of rare species. It is known from this study that towers should be far away from water bodies in order to avoid bird collisions, which is

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particularly important for areas of ecological abundance. This reduces the chance of birds getting close to the towers. Avoiding sitting wind turbines along migration routes or in areas where birds congregate could reduce the chances of bird collisions with the turbines.

(3) In the future, Taiwan will devote major efforts to the development of offshore wind turbines. Even sea bird may dodge the wind turbine (Desholm and Kahlert, 2005), such facilities are still likely to lead to collisions affecting seabirds and migrants (Marques et al., 2014). The difficulty in conducting surveys, however, will be the biggest obstacle to research. This issue requires further in-depth study. Furthermore, ecological issues should be of utmost consideration when designing and building offshore wind turbines.

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THE EFFECT OF ALTITUDINAL ZONE ON SOIL PROPERTIES, SPECIES COMPOSITION AND FORAGE PRODUCTION IN A SUBALPINE GRASSLAND IN NORTHWEST GREECE

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Abstract. The impact of altitudinal gradient on soil properties, species composition and forage production was assessed in the subalpine grassland of “Kostilata” in northwestern Greece. The area is important for endemic species and for its traditional transhumant livestock system. Soil properties, species composition and forage production were determined annually and monthly during three consecutive years (April 2013 – October 2015) from thirty experimental plots located in three altitudinal zones (i.e., lower, middle, and upper). Our results suggested that the altitudinal zone strongly affected soil physical and chemical properties, species composition and forage production. Indeed, altitude a.s.l. was positively correlated with soil sand content and negatively correlated with forage production. It is found that stocking rate exceeded the grazing capacity, which posed a hazard to grassland sustainability; a belief amplified by the high sand content in soil and a terrain with steep slopes, which would increase the risk of further soil erosion at all altitudinal zones.
Keywords: *altitude, grazing capacity, stocking rate, grassland vegetation, sustainable management*

Introduction

In Greece, subalpine grasslands comprise up to 21% of the total grassland area. They are characterized by rich flora and high proportion of endemic species (Georghiou and Delipetrou, 2010). In contrast to their small surface, in subalpine grasslands occurs approximately 2000 taxa, about 146 of them are Greek endemics (Papanikolaou et al., 2005). Furthermore, Greece harbors eight grassland habitat types, three of them being priority habitats (Dafis et al., 2001).

This rich heritage plays a key role in viability of livestock production in mountain areas and in sustainability of rural mountain areas resources where livestock products

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characterized from their quality, authenticity and originality (Chatzitheodoridis et al., 2007; McMorran et al., 2015).

Subalpine grasslands are utilized primarily under transhumant livestock system accompanied by the vertical movement of flocks from lowland to highland in order to take advance of the availability of forage production during the summer months (Hadjigeorgiou, 2011).

Indeed, according to the Report of on cohesion policy in mountainous regions of the European Union (European Parliament, 2016), mountain areas produce a large proportion of sheep and goat products (34% of milk and 25% of meat), a significant share for bovine products (9.5% of milk and 12% of meat) and less for other animal products such as pork meat.

Ruminant animals cover by grazing their annual feed requirements in a percent ranging from 25% to 75% (Zervas, 1998). This figure is up to 50% of their total production cost (Zioganas et al., 2001). In addition, dairy products can be characterized by special ingredients when livestock feeding with forage from species rich grasslands (Noziere et al., 2006).

Altitude a.s.l. has been recognized as a main factor that influences abiotic environment by altering climatic variables and topography (Holechek et al., 2010). As the main area of subalpine grasslands characterized by steep slopes, it seems that topography, based on altitude a.s.l. and slope degree, affects local climate by altering climatic variables such as precipitation and air temperature, as well as the soil properties and the botanical composition (Hadjigeorgiou et al., 2005; Roukos et al., 2011a).

Previous studies have shown that the forage production of Greek grasslands ranges between wide limits and it's influenced by management, soil properties, successional stages and climatic conditions (Papanastasis, 1981; Papanastasis and Koukoulakis, 1988; Mountousis et al., 2008; Roukos et al., 2010; Mountousis et al., 2011; Roukos et al., 2011a; 2011b; Mpokos et al., 2014; Karatassiou, 2016).

Little is known about the species composition and forage production of the subalpine grasslands in Greece. Even less comparative data are available concerning the impact of altitude zone across a subalpine mountain side on soil properties, species botanical components and forage production, which cannot fulfil forage demands of ruminants during summer and consequently limits their productivity.

The aim of this study was to determine the effect of altitudinal zone on soil properties, species composition and forage production, along a subalpine mountain side divided into three altitudinal zones based on the time they grazed by livestock.

Materials and methods

Study area

The research was conducted during the period 2013 – 2015 in 'Kostilata' subalpine grassland (longitude 21.159210°, latitude 39.422238°) located in northwestern Greece, on Mount Tzoumerka. Grassland extended at an altitude ranging from 1100 m to 2393 m a.s.l. and grazed by eight transhumant livestock flocks, from May to October, with 3,600 sheep.

The wider area is important for species associated with alpine and subalpine grasslands and due to its characteristic vegetation communities above the timber line in which many Greek endemics as well as rare and threatened plant taxa exist. Although

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the Kostilata subalpine grassland (*Fig. 1*) is known for its traditional transhumant livestock system, it seems that is intensively grazed suffering from high stocking rate values.

Figure 1. *General view of Kostilata subalpine grassland.*

Forage samples

During April 2013, 60 sites with eastern –southeastern aspects, which represented typical grassland conditions, were selected for monthly sampling. Sites were classified into three altitudinal zones based on altitude a.s.l.: lower (1100 – 1400 m), middle (1401 – 1800 m) and upper (1801 - 2393 m) according to seasonal use of the sheep flocks. In each zone, twenty experimental plots of 4 m X 5 m each, were fenced to avoid grazing during the experimental period. The forage production was measured at monthly basis by harvesting the above ground biomass of the vegetation from five randomly 0.5 m x 0.5 m quadrats in each protected plots. Those plant species present in the quadrats with a cover value above 15% were considered as dominant plant species (*Table 1*). Samples were immediately placed into individual paper bags, transported to the laboratory and manually separated into three plant groups: grasses, legumes and other forbs. Then, to determine the dry weight in each plant group, the samples were placed in an oven for 48 hours at 60 ºC (Deinum and Maasen, 1994).

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Table 1. *Plant species with a frequency of occurrence in herbage higher than 15% at blooming stage in each altitudinal zone.*

Botanical Group	*Species*	*Life Form*	Altitudinal Zone		
			Lower	Middle	Upper
Grasses	*Agrostis stolonifera,* L.	Perennial	●	●	●
Grasses	*Alopecurus gerardii* Vill	Perennial		●	●
Grasses	*Alopecurus pratensis* L.	Perennial	●	●	●
Grasses	*Anthoxanthum odoratum* L.	Perennial	●	●	●
Grasses	*Arrhenatherum elatius* (L) Beauv	Perennial	●	●	
Grasses	*Brachypodium pinnatum* (L.) P. Beauv	Perennial	●		
Grasses	*Briza media* L.	Perennial	●	●	
Grasses	*Bromus fibrosus* Hack	Perennial		●	●
Grasses	*Bromus hordeaceus* L.	Annual/Biennial	●	●	●
Grasses	*Bromus sterilis* L.	Annual	●	●	●
Grasses	*Bromus tectorum* L.	Annual		●	●
Grasses	*Calamagrostis varia* Host	Perennial			●
Grasses	*Cynosurus cristatus* L.	Perennial	●	●	
Grasses	*Dactylis glomerata* L. subsp. *glomerata*	Perennial	●	●	●
Grasses	*Dasypyrum villosum* L. P. Candagry	Annual	●	●	
Grasses	*Deschampsia flexuosa* L. Trin	Perennial		●	●
Grasses	*Festuca alpina* Suter subsp. *briquetii* (St-Yves ex Litard.) Markgr.-Dannenb.	Perennial		●	●
Grasses	*Festuca arundinacea* Schreb	Perennial	●	●	
Grasses	*Festuca heterofhylla* Lam	Perennial	●	●	
Grasses	*Festuca ovina* L.	Perennial	●	●	●
Grasses	*Festuca rubra* L.	Perennial		●	●
Grasses	*Festuca sancta* Meld	Perennial	●	●	
Grasses	*Festuca varia* Haenke	Perennial	●	●	
Grasses	*Lolium multiflorum* L.	Annual	●	●	
Grasses	*Lolium perenne* L.	Perennial	●	●	
Grasses	*Lolium rigidum* Gaudin subsp. *rigidum.*	Annual	●		
Grasses	*Phleum alpinum* L.	Perennial		●	●
Grasses	*Phleum montanum* C. Koch	Perennial		●	●
Grasses	*Poa alpine* L.	Perennial		●	●
Grasses	*Poa bulbosa* L.	Perennial		●	
Grasses	*Poa nemoralis* L.	Perennial		●	●
Grasses	*Poa pratensis* L.	Perennial		●	●
Grasses	*Sesleria argentea* Savi	Perennial		●	
Grasses	*Stipa pennata* L. subsp. *pulcherrima* (C. Koch) Freitag	Perennial	●	●	
Grasses	*Koeleria lobata* (Bieb.) Roemer & Schultes.	Perennial	●	●	
Legumes	*Anthyllis vulneraria* L. subsp. *Pindicola* Cullen	Perennial	●	●	
Legumes	*Lathyrus aphaca* L.	Annual	●	●	●
Legumes	*Lotus aegaeus* B.			●	●
Legumes	*Lotus corniculatus* L.	Perennial	●	●	
Legumes	*Lotus tenuis* Waldst. & Kit. ex Willd.	Perennial	●	●	●

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Botanical Group	Species	Life Form	Altitudinal Zone		
			Lower	Middle	Upper
Legumes	*Trifolium arvense* L.	Annual	●	●	
Legumes	*Trifolium repens* L.	Perennial	●	●	●
Legumes	*Vicia pubescens* (DC.) Link	Annual		●	●
Legumes	*Medicago arabica* L.		●	●	●
Forbs	*Chenopodium bonus-henricus* L. syn. *Blitum bonus-henricus* (L.) Rchb.	Perennial		●	●
Forbs	*Scandix macrorhyncha* C. A. Meyer	Annual	●	●	
Forbs	*Arum maculatum* (L.)	Perennial			●
Forbs	*Achillea millefolium* L. subsp. *millefolium*	Perennial	●	●	●
Forbs	*Bellis perennis* L.	Perennial	●	●	
Forbs	*Carduus tmoleus* Boiss.	Perennial		●	●
Forbs	*Crhysanthemum segetum* L.			●	
Forbs	*Anchusa azurea* Mill.	Perennial		●	●
Forbs	*Echium italicum* (L.)	Biennial	●	●	
Forbs	*Myosotis alpestris* F. W. Schmidt subsp. *suaveolens* (Waldst. & Kit. ex Willd.) Strid	Biennial / Perennial	●	●	
Forbs	*Sinapis arvensis* L.	Annual	●	●	
Forbs	*Capsella bursa-pastoris* L.		●	●	●
Forbs	*Capsella grandiflora* (Fauche & Chaub.) Boiss.	Annual	●	●	
Forbs	*Campanula albanica* Witasĕk	Perennial		●	●
Forbs	*Cerastium spp.*		●	●	
Forbs	*Arabis alpina* L.	Biennial, Perennial		●	●
Forbs	*Euphorbia herniariifolia* Wild.	Perennial		●	●
Forbs	*Euphorbia myrsinites* L.	Perennial		●	●
Forbs	*Erodium cicutarium* (L.) L'Hér. ex Aiton	Annual	●	●	
Forbs	*Pteridium aquillinum* (L.) Kuhn subsp. *aquillinum*	Annual	●	●	
Forbs	*Crocus veluchensis* Herbert	Perennial		●	●
Forbs	*Luzula campestris (indica)* (L.) DC.	Perennial		●	●
Forbs	*Marrubium velutinum* Sibth & Sm.	Perennial		●	●
Forbs	*Menta longifolia* (L.) Hudson	Perennial	●	●	●
Forbs	*Thymus striatus* Vahl	Perennial	●	●	
Forbs	*Fritillaria thessala* (Boiss.) Kamari subsp. *ionica* (Halácsy) Kamari	Perennial		●	
Forbs	*Muscari comosum* (L.) Mill.	Perennial	●	●	●
Forbs	*Ornithogalum oligophyllum* E.D.Clarke	Perennial	●	●	●
Forbs	*Ornithogalum sibthorpii* Greuter	Perennial	●	●	●
Forbs	*Scilla nivalis Boiss (Scilla bifolia* L.*)*	Perennial	●	●	●
Forbs	*Dactylorhiza saccifera* (Brongn.) Soo	Perennial		●	
Forbs	*Papaver rhoeas* L. *(Papaver; anemone)*	Annual		●	
Forbs	*Plantago atrata* ssp. *Graeca* (Halacsy) Holub.	Perennial	●	●	●
Forbs	*Plantago holosteum* Scop.	Perennial	●	●	●

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Botanical Group	*Species*	*Life Form*	Altitudinal Zone		
			Lower	Middle	Upper
Forbs	*Plantago lanceolata* L.	Perennial	●	●	●
Forbs	*Polygala nicaeensis* K. Koch subsp. *mediterranea* Chodat	Perennial		●	
Forbs	*Rumex acetosella L.*	Perennial		●	●
Forbs	*Rumex alpinus* L.	Perennial		●	●
Forbs	Primula veris L.	Perennial		●	
Forbs	*Primula vulgaris* Huds. συν. *P. acaulis* (L.) Hill	Perennial		●	
Forbs	*Helleborus odorus* Waldst. & Kit. Subsp. *cyclophyllus* (A. Braun) Strid	Perennial		●	
Forbs	*Ranunculus brevifolius* Ten.	Perennial	●	●	●
Forbs	*Ranunculus psilostachys* Griseb.	Perennial	●	●	
Forbs	*Ranunculus repens* L.	Perennial	●	●	●
Forbs	*Ranunculus spruneranus Boiss.*	Perennial	●	●	
Forbs	*Scrophularia canina* L.	Perennial		●	●
Forbs	*Verbascum densiflorum* Bertol.	Biennial		●	●
Forbs	*Urtica dioica var. dioica* L.	Perennial	●	●	●
Forbs	*Valeriana officinalis*	Perennial	●	●	
Forbs	*Viola epirota* (Halacsy) Raus	Perennial	●	●	

Soil samples analysis

In each plot, soil samples were collected from the surface layer (0 - 30 cm) in June 2013 and chemical analyses were performed in order to evaluate certain physical and chemical properties of the soil. Prior to soil physical and chemical analyses, all samples were air-dried at room temperature and passed through a 2 mm soil sieve. Soil particle size distribution was determined with the hydrometer method (Gee and Or, 2002) and organic matter by the Walkley Black method (Nelson and Sommers, 1996). The pH measurements were assessed in a water suspension using a soil/solution ratio of 1/2 (Thomas, 1996) and available P (ppm) was evaluated with the Olsen method (Kuo, 1996).

Climatic data

For our study, three automated weather stations (Onset HOBO weather station) were installed (one in each altitudinal zone) to record local precipitation and temperature fluctuations among the zones throughout the three years of the study (*Fig. 2*).

Calculations

Grazing capacity was calculated according to Holechek et al. (2010) taking into account the following assumptions: (a) the Animal Unit (AU) was defined as one 50-kg ewe and lamb (Minson, 1990); (b) forage intake by a ewe was 1.0 kg DM per 50 kg of liveweight (NRC, 1985); (c) sheep grazed only in grasslands for a period of four to five months in the grassland area; (d) the proper use factors were 50 % for the grasslands; and (e) the average grazing livestock population of the Kostilata grassland, in AU, for the period of 2013-2015 was taken from data provided by local selforganized authority (Municipality of Central Tzoumerka), in which farmers pay for grassland utilization (rangeland right) to receive European Communities subsidies.

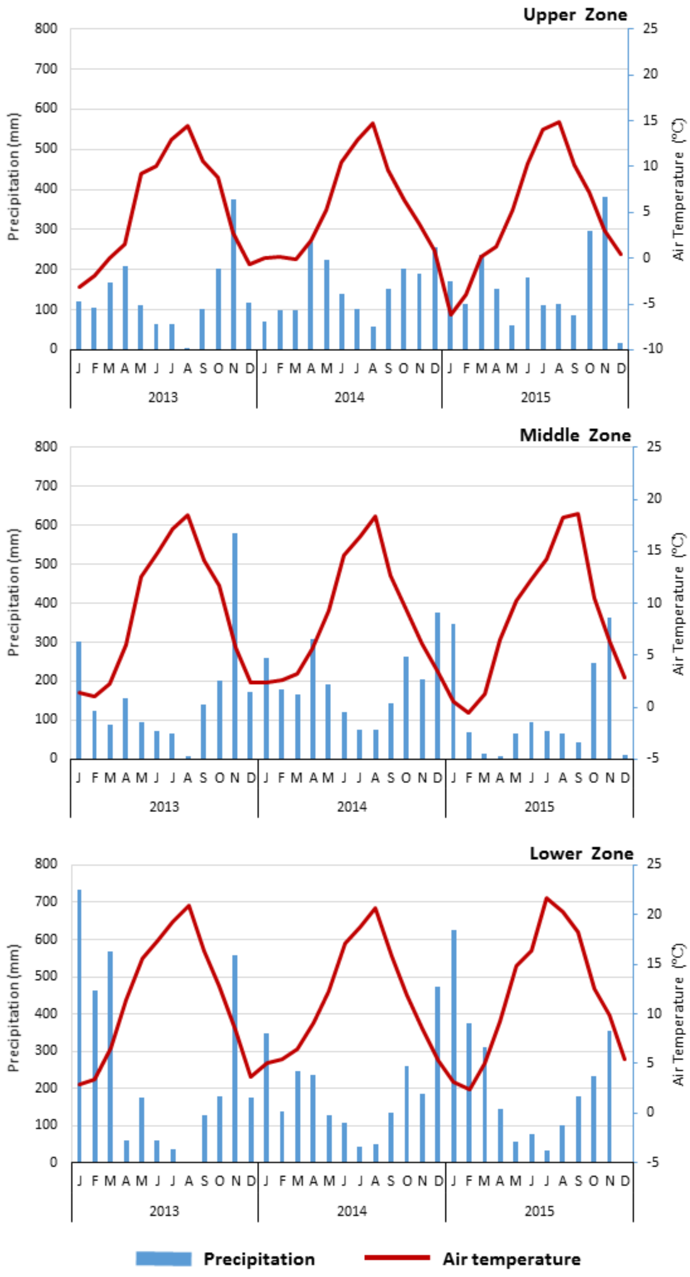

Figure 2. *Monthly precipitation and mean monthly air temperature during 2013 – 2015.*

Slope degree was extracted from the digital elevation model (DEM) of the study area (*Fig. 3*). The DEM obtained from Greek National Cadastre and Mapping Agency with a 5m × 5 m cell size was used in this study. Then, slope was divided into four levels, 0–10%, 11–30%, 31–60%, and >61% and estimated grazing capacity was calculated following the suggested reductions in grazing capacity for different percentages of slope (Holechek, 1988).

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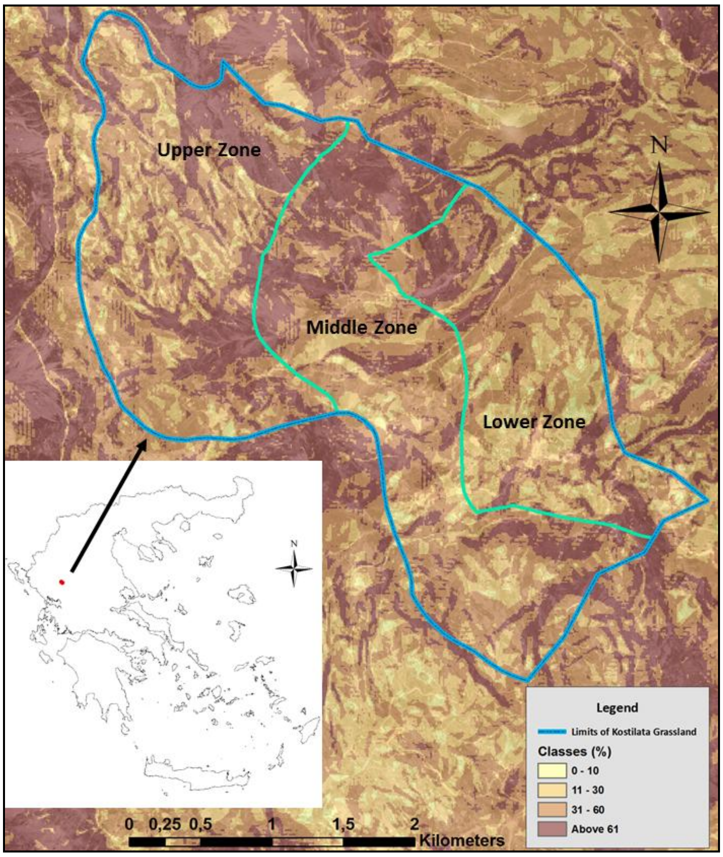

Figure 3. *Slope map of the studied grassland area divided into four slope levels.*

Statistical analysis

Statistical differences of climatic variables and soil properties were tested using Analysis of Variance (ANOVA). Least Square Differences (LSD) were used to determine significant differences among means when significant ANOVA results occurred ($p<0.05$).

Data of forage production and species composition were tested using a two-way analysis of variance with altitudinal zones ($n = 3$) as main plots and month of harvest ($n = 5$) as sub-plots (Snedecor and Cochran, 1980). The experimental plots and year of harvest were considered as random effects. The interaction altitudinal zone × month of harvest was significant ($p<0.05$); thus, analyses of variance were conducted among altitudinal zones and among altitudinal zones within month of harvest. Significant mean differences were detected using least square differences (Steel and Torrie, 1980). The Pearson's correlation was employed to examine relationships between climatic variables, soil properties, herbage production and altitude a.s.l.

Results and Discussion

Climatic conditions

Species composition and forage production are highly sensitive to climate variability and changes (Holechek et al., 2010). In the study area, the ombrothermic diagrams (*Fig. 4*) showed the absence of dry periods and consequently the plants species does not

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suffer from water stress periods especially in middle and upper zones. The mean monthly precipitation and air temperature were negatively correlated with altitude a.s.l. (r=-0.238; p<0.01 and r=-0.705; p<0.01, respectively). However, the temperature significantly (p<0.01) differed in the three altitudinal zones during the experimental period. The mean annual precipitation and temperature were significant higher (p<0.05) in the lower zone than in the middle and upper zones. In addition, significant difference in precipitation occurred between the lower and the other two altitudinal zones.

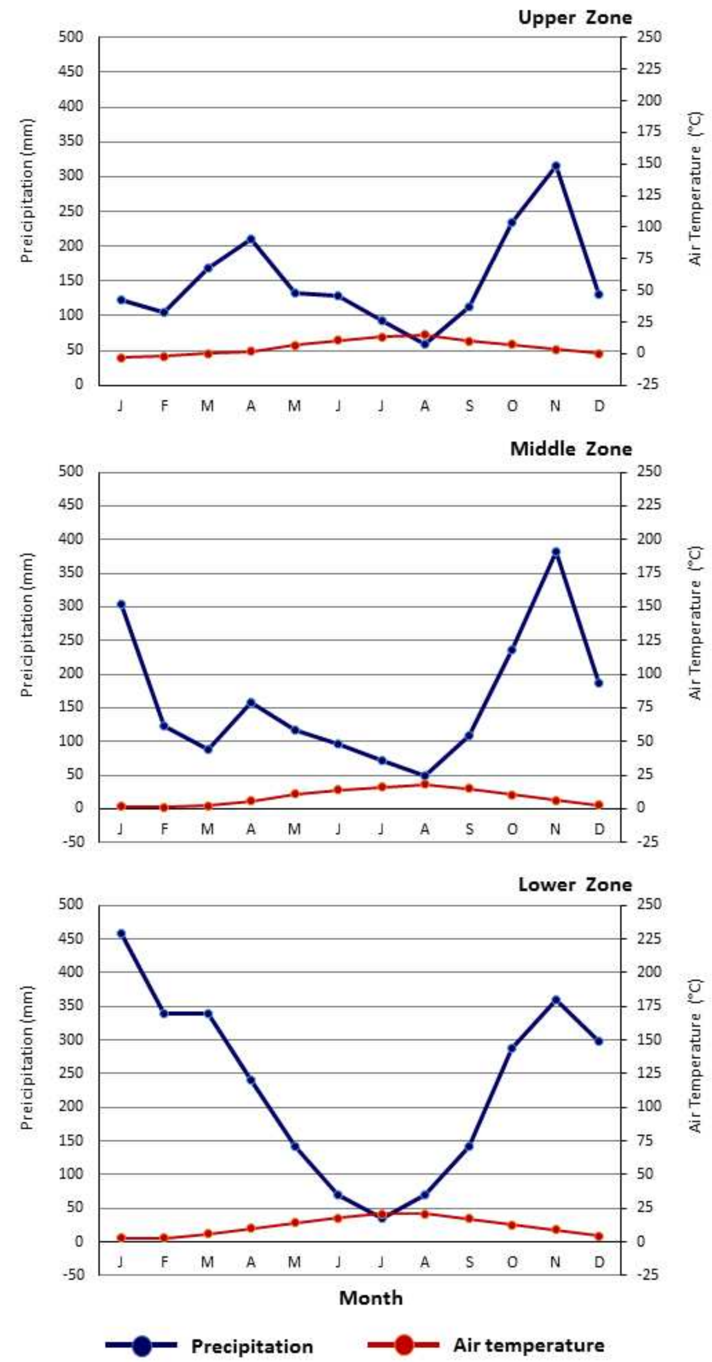

Figure 4. *Obrothermic diagrams for lower, middle and upper zone based on 3-years average values of precipitation and air temperature.*

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In the study area, it seems that the low air temperature is the main restrictive factor for plant growth and production because the higher amount of precipitation occurs during spring. On the other hand the cold temperatures of upper zone can influence plant productivity by delaying initiation of growth in spring (Sneva, 1982). This results is in agreement with the founding of Roukos et al. (2011a) and Mountousis et al. (2011) in northwestern Greece.

Grassland vegetation

In the 4 years of the experiment, the collected samples of forage production from Kostilata grassland consisted of 96 taxa which belong to 30 families. The most frequent family was the Poaceae with 35 species, followed by the Fabaceae with 9 species and Ranunculaceae and Liliaceae with 5 species (*Table 1*).

The subalpine grassland is dominated by perennial grasses, a finding that is in consistent with Papanastasis (1981) and Papanastasis et al. (2003). Grasses species have better adaptability to wide range of environmental conditions than legumes species which are influenced more by the low temperatures in winter and spring (Papanastasis et al., 2003; Holechek et al., 2010).

Changes in species composition

The contribution of each plant group in the overall forage botanical composition in relation to altitudinal zone and month of harvset is shown in *Figure 5*. The altitudinal zone and month of harvest significantly ($p<0.05$) affected the proportion of grasses, legumes and other forbs in forage composition. Over all altitudinal zones and sampling years, the major contribution to the forage mass was from grasses. Indeed, the participation of grasses in the species composition varied between 60.7% and 82.6% and it was significantly ($p<0.05$) higher in middle and upper zones than lower zone. On the other hand, the relative contribution of the legumes was higher ($p<0.05$) in the middle and upper than in lower zone. Furthermore, the proportion of other forbs was the highest ($p<0.05$) at upper zone.

The different contribution of the various plant groups is probably caused by the different stocking rate (Shakhane et al., 2013; Catorci et al., 2015; Lwiwski et al., 2015) and climatic and soil conditions (Vazquez-de-Aldana et al. 2000; Oztas et al.. 2003; Garamvölgyi and Hufnagel, 2013) between the three altitude zones (*Tables 4 & 6*).

Forage Production and Grazing Capacity

The altitudinal zone significantly affected ($p<0.001$) the forage production which fluctuated from 590 kg ha^{-1} at upper zone to 1621 kg ha^{-1} at middle zone (*Table 2*). Generally, forage production was negatively correlated ($p<0.01$) with altitude a.s.l.($r=-0.296$) and positively correlated with air temperature ($r=0.226$). Thus, on average, forage production was the highest at lower zone and the lowest at upper zone. This finding show that a district step gradient along to altitude a.s.l. occurs and is in accordance with the results of Pérez Corona (1998), Vazquez-de-Aldana et al. (2000), Mountousis et al. (2011) and Roukos et al. (2011a), who found significant variations among altitudinal zones in rangelands of Spain and Greece. However, other studies (Papanastasis 1982, Mountousis et al. 2008) found that forage production increases with the altitude a.s.l., a finding that in not confirmed with the results of this study. This difference may due to high precipitation at all zones and different soil properties in the study area.

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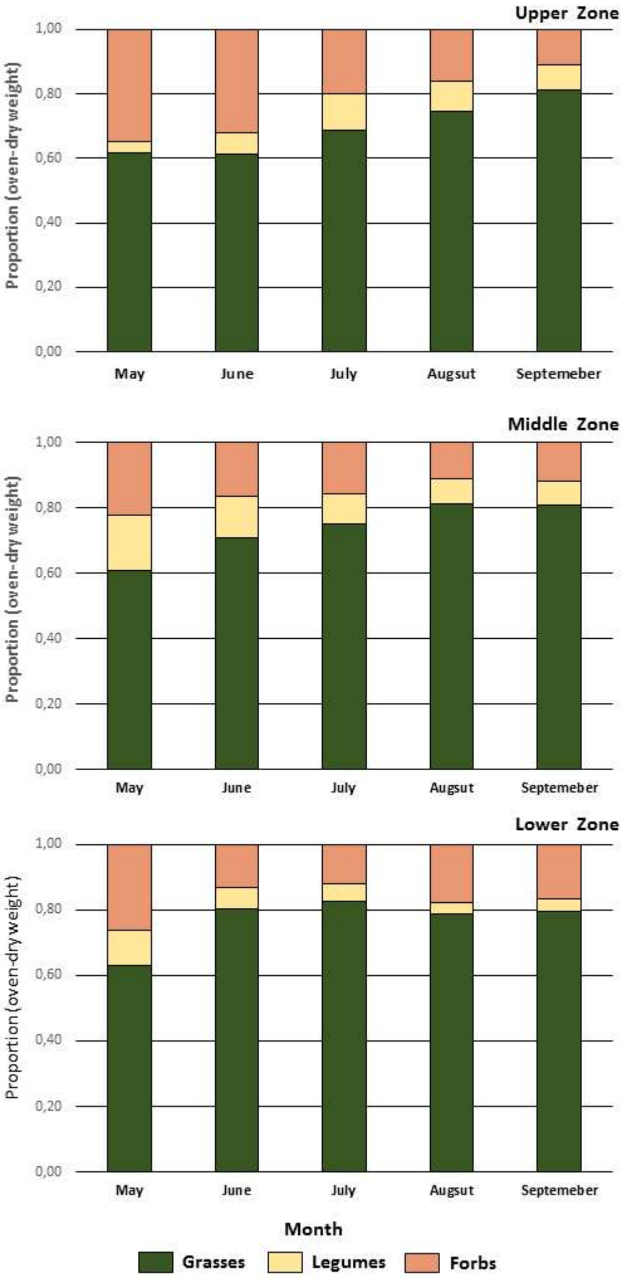

Figure 5. *Proportion (oven dry weight) of botanical groups (grasses, legumes and forbs) in the herbage mass in relation to altitudinal zone and month of harvest.*

For each altitudinal zone, the stocking rate and the grazing capacity values are shown in *Table 3*. The overall estimated grazing capacity of grassland (15400 AUMs), without slope adjustments, slightly exceeds the current stocking rate (14400 AUMs). The applied stocking rate, however, was fluctuated above the estimated grazing capacity at lower and upper zones. On the other hand, if slope adjustment will be taking into account, it is assumed that grazing capacity represents only the 40% of the current stocking rate. The results of grazing capacity estimation, suggests that the grassland is overgrazed at all altitudinal zones.

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Table 2. *Monthly and altitudinal zone means of herbage production (kg DM ha^{-1}).*

Zone	May	June	July	August	September	Mean	s.e.m.	Sig.
Lower	1.403	1.690	1.539	1.068	977	1.336	81	***
Middle	736	1.554	1.621	1.137	1.146	1.239	74	***
Upper		590	1.018	1.105	1.003	940	88	***
Mean	1070	1.278	1.393	1.103	1.042	1.172		
s.e.m.		96	93	78	62	49		
Sig.	***	***	***	NS	**	***		

Note: Different letters between zones denote significant differences (P < 0.05)
s.e.m. Standard error of the mean
Sig. Significant level, *: p<0.05, **: p<0.01, ***: p<0.001

Table 3. *Stocking rate and grazing capacity of the studied grassland in the three altitudinal zones.*

Altitudinal Zone	Area (ha)	Production (kg DM / ha)	Grazing period (months)	Animal Units (AU)	Stocking rate (AUM)	Grazing Capacity (AUM)	
						Without slope adjustment	With slope adjustment
Lower	2.502	1.690	6	1430	5720	4699	2024
Middle	3.729	1.621	5	1070	4280	6717	2311
Upper	3.523	1.105	4	1100	4400	3984	1311
Sum	9.754			3600	14400	15400	5646

Continued use of stocking rates above the grazing capacity generally leads to grassland degradation and decline in production per animal (Hunt et al., 2014). The overgrazing disappears the most palatable species and can result in significant changes in forage production, floristic composition, diversity, and the recycling of nutrients in the grasslands' ecosystem (Papanastasis et al 2002, Zhang and Dong, 2009, Catorci et al., 2015; Kairis et al. 2015). Furthermore, as slope degree increases with altitude a.s.l., an increased soil erosion hazard occurs and a further reduce in grazing capacity it is expected (Kairis et al. 2015).

Slope degree and soil properties

According to surface analysis, the slope degree of mountain grasslands vary according to altitudinal zone (*Table 4*). The lower zone is dominated by gentle slopes as the 37% of the total grassland area located in slope degree up to 30%. However, slope degree increases in middle and upper altitudinal zones. Thus, the vast majority of total grassland area is located in slope degree over 30%.

The soil textural fractions varied significantly (p<0.05) with altitudinal zone (*Table 5*). Clay and silt content were significantly (p<0.05) lower and higher, respectively, in lower zone than middle and upper zones.

The high mean annual precipitation, resulting from the orographic effect (Dotsika et al., 2010), has probably affected the soil sand content. The upper zone characterized by more steep slopes, high sand content and high precipitation, conditions that encourage

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soil erosion more than the other altitudinal zones. This has leaded to selectively transport clay and silt fractions from the higher altitudes down the slopes leaving behind sand fractions (Yimer et al., 2006). In high precipitation environments too much precipitation can result in nutrient leaching in sandy soils (Anderson et al., 1998) and soil stability is influenced by secondary clay minerals (Manyeverea et al., 2016). Additionally, as slope degree increases less water enters the soil and more runs off as overland flow. In this situation, when grazing pressure increases in mountain pastures, soils will be susceptible to degradation from processes such as runoff and erosion (Sheatch et al., 1998).

In mountainous areas, significant variations in soil texture in relation to topography have been reported both in Mediterranean basin (Badano et al., 2005; Acosta et al.. 2008; Oyonarte et al.. 2008; Roukos et al.. 2011b) and other countries (Oztas et al.. 2003; Yimer et al.. 2006; Guzman and Al-Kaisi, 2011). Our results concur with them.

Soil pH is considered to be an important factor that determines the floristic diversity and composition of grasslands (Critchley et al., 2002). The soil pH values showed significant variation (p<0.05) with respect to altitudinal zone (*Table 5*). The overall mean soil pH values ranges from 5.4 to 5.9 among all zones. Therefore, soils characterized are strongly acid in upper and middle zones and moderately acid in lower zone.

Table 4. *The distribution of slope classes in relation to altitudinal zone according to surface analysis.*

	Slope groups (%)			
Altitudinal Zone	**0 - 10**	**11 - 30**	**31 - 60**	**Abobe 60**
Lower	6%	31%	38%	25%
Middle	4%	20%	43%	34%
Upper	2%	16%	42%	40%
Sum	4%	21%	41%	34%

Table 5. *Soil properties in relation to altitudinal zone.*

Zone	Sand (%)	Silt (%)	Clay (%)	pH	Organic matter (%)	Available P (mg kg^{-1})
Lower	43.9a	38.3a	17.8a	5.9a	6.9a	4.3a
Middle	48.7b	35.1b	16.2ab	5.5b	6.7ab	5.6a
Upper	52.9c	33.9b	13.2b	5.4b	5.5b	6.2b
s.e.m.	1.45	1.17	1.59	0.17	0.48	0.71
Sig.	***	*	*	*	*	*

Note: Different letters between zones denote significant differences (P < 0.05)
s.e.m. Standard error of the mean
Sig. Significant level, *: p<0.05, **: p<0.01, ***: p<0.001

Acid soils are most often found in high precipitation areas as leaching of bases is more extensive (Ellis and Mellor, 1995). Consequently, low values of soil pH among altitudinal zones probably resulted from the fact that increasing altitude increases slope degree and thus, combined with the high precipitation, can cause increased leaching and

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reduction in soluble base cations leading to higher H+ activity and registered as decreased pH (Rezaei and Gilkes 2005).

Significant variation in soil pH among topographic aspects also found by Oztas et al. (2003), Yimer et al. (2006), Oyonarte et al. (2008) and Roukos et al. (2011b).

Soil organic matter content was influenced ($p<0.05$) by altitudinal zone. The mean soil organic matter varied between 5.5% at upper to 6.9% at lower zone (*Table 3*) and showed a significant ($p<0.05$) negative correlation with altitude a.s.l. (r =-0.394) and positive one with clay content (r=0.282).

It seems that the high precipitation and the more steep slopes of middle and upper zones have favored organic matter accumulation in lower zone because of runoff and erosion. Simiral results also demonstrated by Roukos et al. (2011b). Moreover, the high organic matter content have been directly related to higher surface cover rates in lower zone and because of higher amounts of available water content for plant growth (Oztas et al., 2003).

The concentrations of organic matter of all altitudinal zones were greater than those reported from Roukos et al. (2011b) on rangeland soils located near the study area. This result could be attributed to lower mean annual temperatures in all altitudinal zones and is consistent with the findings from Kirschbaum (1995, 2006) who reported that temperature influences organic matter accumulation, which increases with precipitation and decreases with temperature (Burke et al., 1989) as temperature is a key factor controlling the rate of decomposition of plant residues (Paré et al., 2006).

In grassland management, it is important to maintain proper levels of soil organic matter to sustain grassland soil productivity (Holechek et al., 2010). An additional decline in soil organic matter due to erosion at upper zone will significantly reduce the N supply and resulting in a deterioration of soil physical condition leading to yield reduction (Greer et al., 1996).

The result of this study is in agreement with results from other studies indicating that soil organic matter decreases from lowlands to uplands (Oztas et al.. 2003) and that tends to increase as the clay content increases (Prasad and Power, 1997; Roukos et al., 2011b).

The amounts of soil available phosphorus did not show significant variations among altitudinal zones (*Table 5*). Available P was significantly ($p<0.05$) higher at upper zone. However, the higher available P levels suggest that more P is present in forms available for plant uptake which may be probably be due to increased phosphorus fixation and lower rates of decomposition as suggested by Yimer et al. (2006).

Conclusions

In high rainfall subalpine mountainous environments, the altitudinal gradient strongly affects climatic conditions, soil properties, herbage production, and species composition of grasslands. In the studied grassland, the stocking rate exceeds the grazing capacity and thus a new management plan involving rotational grazing and the implementation of supplemental nutrition could be considered in order to sustain grassland production and avoid further degradation of grasslands ecosystem.

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SELECTION OF ALTERNATIVE LANDFILL SITE IN KANCHIPURAM, INDIA BY USING GIS AND MULTICRITERIA DECISION ANALYSIS

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Abstract. Identification of suitable landfill site for municipal solid waste disposal is a complex task. The proposal of alternative landfill site consists of multicriterial analysis of various parameters. In this study Geographical Information System (GIS) was utilized effectively for alternative landfill site identification. The analytical hierarchy process (AHP) technique was effectively used by assigning weights and rank for decision making. Criteria such as residential area, road network, Geology, Geomorphology and soil are considered for analysis. The developed system encompasses social, environmental, geological and accessibility conditions. By using this modelling approach, GIS once again proved an effective tool for evaluating multiple criteria in decision making. The research aims to introduce a model for selecting an appropriate landfill site in municipalities. The developed methodology is useful for identifying alternative landfill in the study area with an effective manner.
Keywords: *pollution, network analysis, buffering, model, solid waste management*

Introduction

Municipal solid waste management is one of the difficult tasks for the local authorities. Starting from planning of solid waste collection up to its disposal, it involves various stages. Because of rapid urbanization in the process of municipal solid waste management is being more tough process. Landfill site selection is one of the most complicated planning activities which should convince many related stakeholders (Eskandari et al., 2012). The urban sprawl emergence to identify the alternative landfill sites for fulfilling the present solid waste disposal requirement. The GIS and AHP method has been properly used in various landfill site identification projects (Sener et al., 2011). Because of the less availability of land for solid waste disposal proper planning is very essential. The process of identifying the alternative landfill site involves more time consuming and tedious process. Selecting the wrong site for landfill may result serious environmental hazards and public opposition (Babalola and Busu, 2011). Assessment on multiple criteria such as residential area, legislation, geology, geomorphology, etc., are required to choose the appropriate site. In developing countries, mostly engineered landfill methods are not adopted due to financial criteria. In this situation, adopting of landfill method is an alternative way for solid waste disposal. In many municipalities the existing landfill sites are already struggling to fit for the present situation. The inability of fulfilling the present

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demand of solid waste disposal, the severe unhygenic condition has been developed. Multicriteria spatial decision support systems help to take a decision in the conflicting problems (Demesouka et al., 2013). The analysis and decision making from the data for alternative landfill identification is a tedious process in conventional methodology. Both spatial and attribute data are considered for analysis in GIS software. High resolution satellite images, digitized maps and field survey information's are incorporated in GIS for decision making. GIS can be effectively used to identify the potential site by multi-criteria decision analysis. Multi criteria decision analysis and GIS were used to identify the landfill site by using suitable weightage (Alanbari et al., 2014). The government has taken many activities to protect the environment and ensure the hygiene in municipalities.

Kanchipuram municipality (*Fig. 1*) is one of the important place in south India, which is located in between 12º46'30''- 12º52'00'' North Latitude and 79º 39'00'' - 79º46'20'' East longitude. Present solid waste generation rate in the municipality is 120 to 130 metric tons per day. The existing solid waste landfill site is in operation from the year 2005 onwards. Nearby to the present landfill site more residential and commercial area are developed due to rapid urbanization. Now the municipality is in situation to identify the potential alternative landfill site to manage the present situation in solid waste disposal.

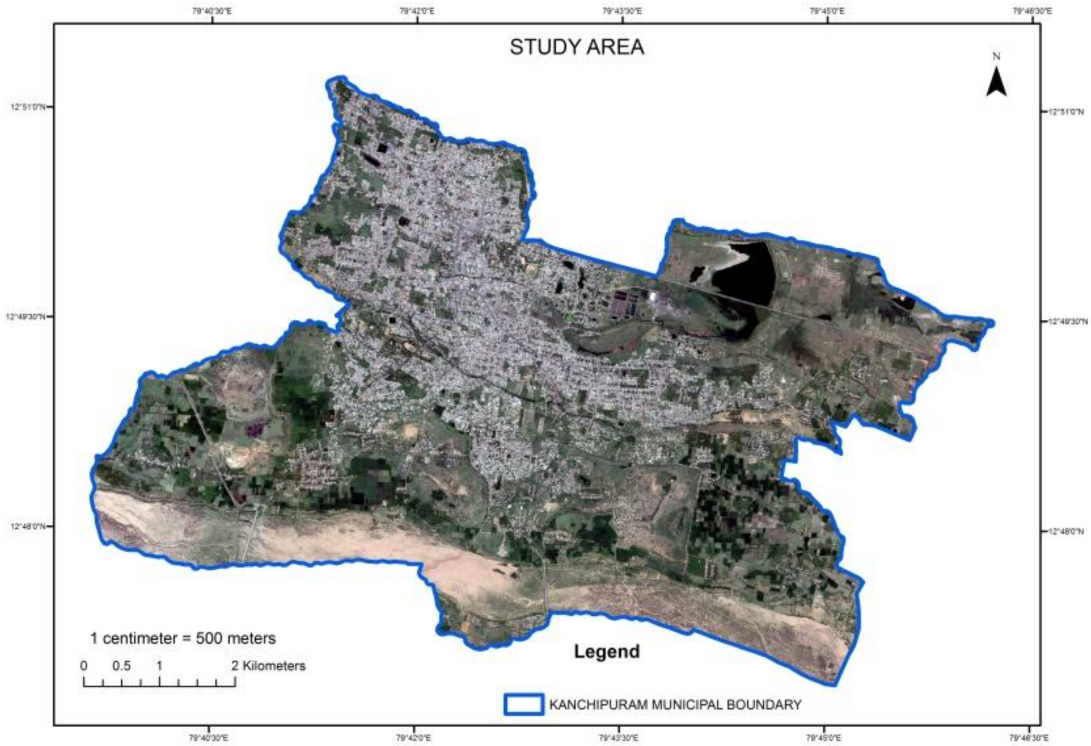

Figure 1. *Map of study area*

It has been identified both groundwater and soil gets polluted due to landfill activities in the surroundings of the present dump yard. Because of the high price in land value and environmental concern selection of alternative landfill in the study area is a complex process. In this case, the study was conducted to identify the potential alternative landfill site in the Kanchipuram municipality by using GIS technology.

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Materials and Methods

A field study was conducted primarily in existing landfill site to understand the reality in present dumping method. Both spatial and non-spatial data are essential to work in GIS platform, for that Kanchipuram boundary map was collected from the municipality to delineate the boundary of the municipality. An alternative landfill feasibility study has been conducted by using a GIS decision support tool (Kahvand et al., 2015). For preparing a base map of the study area, high resolution satellite imagery (quick bird) was used. From that prepared base map municipal boundary was extracted for further processing in GIS software. In India, the Central Public Health and Environmental Engineering Organisation (CPHEEO) provides the guidance for site selection of municipal solid waste. Thematic maps such as geology, geomorphology, residential, soil and road network was prepared for GIS analysis. According to the importance of each criterion, weights and rank assigned to AHP process.

Integration of GIS and AHP will produce the precious results when compared with the conventional method (Eskandari et al., 2013). The analysis results provide the spatial suitability for landfill in the study area. Based on that result further feasibility study was conducted to identify the best site for a landfill.

Results and Discussion

The identification of alternative landfill site is based on few criteria's such as restrictions and possibilities. These two criteria are evaluated properly in GIS to find the most suitable site for the landfill. CPHEEO provide restrictions such as 500m around the habitation areas are not allowed for landfill sites and no landfill should be constructed in a critical habitat area. It is mandatory to follow the guidelines framed by the government for landfill site identification. Appropriate technique is required to identify the potential municipal landfill sites (Gbanie et al., 2013).

The second factor is possibilities of selecting landfill based on the favourable condition such as soil type, geology, accessibility of road network to the site etc., available in the study area. The legislation, restrictions and possibilities are differing from one location to another, but the methodology is applicable for all the projects. The analytic hierarchy process in a GIS platform was used to identify the alternative landfill site (Aydi et al., 2013).

Residential Area Analysis

The proximity study in GIS software was applied to identify the suitable landfill site in the study area. Identification of the best place for burial of solid waste is based on analysis of many parameters which should not lead the environmental pollution (Gorsevski et al., 2012) Initially residential areas were digitized in the satellite image to understand the permanent settlement and the availability of land in the study area. By using the buffer analysis tool 500 m buffer was created around the residential area as CPHEEO guidelines. Buffer analysis on polygon was applied to generate the buffer of existing boundary of residential area. Optimal siting for waste treatment facilities was identified by using GIS techniques (Tavares et al., 2011).

This buffer results helps to identify the available land in the study area for landfill purpose. Four potential sites are selected in the study area based on the favourable condition. The spatial information is useful for proper planning and decision making in

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selecting the site. The four alternative sites with its location and residential area in the municipality are shown in *Fig. 2*.

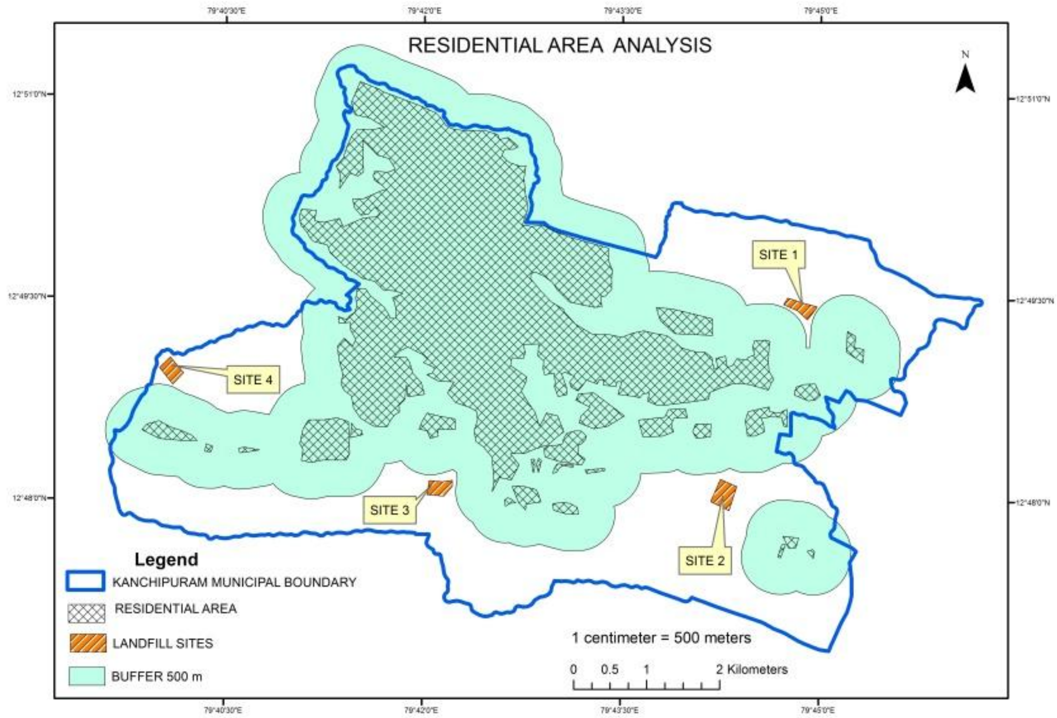

Figure 2. *Map of residential area analysis*

Road Network Analysis

The selected site must be suitable for transportation operation. Every day around 130 Mt solid waste has to be transported to the site, so that the road network facility to the alternative landfill site is an important condition.

As per CPHEEO guidelines for aesthetic reasons, no landfill site should be constructed within 200m on the right of way of any state or national highway. Buffer zone in between the roads and landfill sites was adopted to identify the suitable site (Turkish Landfill Directive, 2012). In the study area state highway is there, so that 200m buffer was created for state highways and identified the selected four potential sites are not falling within the buffer zone. All the selected four potential sites are full fill the road network accessibility (*Fig. 3*) in the municipality. Structural hierarchy was formed for the criteria like road, land uses, urban area and ecosystem to identify the suitable landfill site (Alavi et al., 2013).

Because of the proper road network the transportation of the solid waste made easy and time saving. In the prepared road network by using network analysis tools in ArcGIS software, it is possible to analyse origin and destination study for identifying the shortest path in the study area. This result will be useful for proper transportation planning for solid waste collection and transport to the dump yard.

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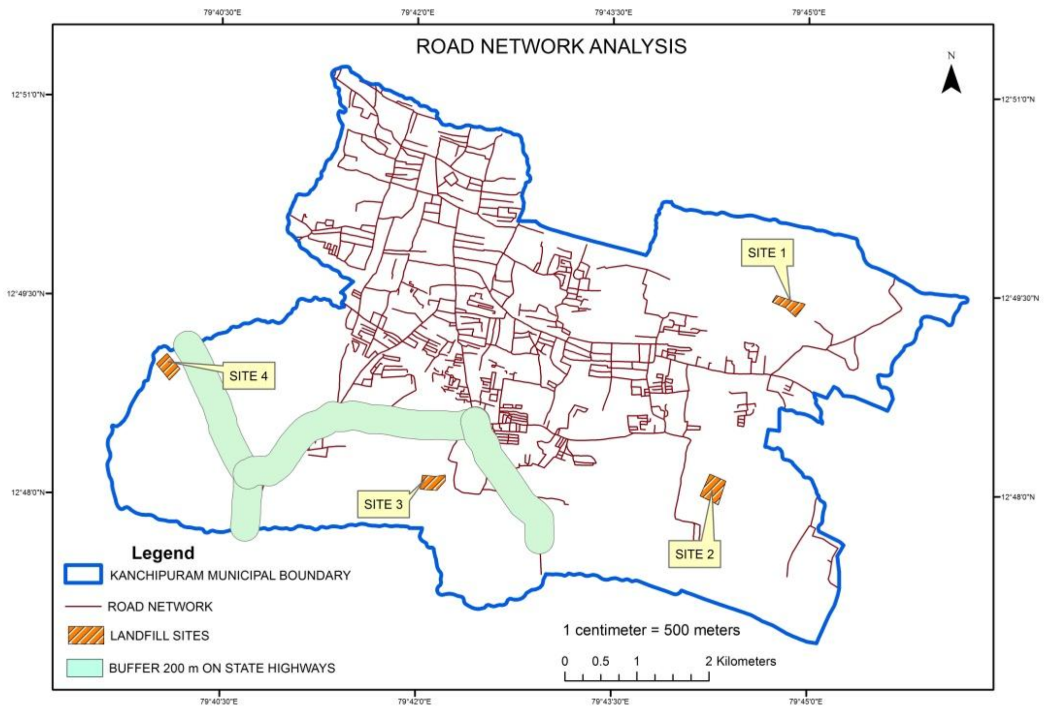

Figure 3. *Map of road network analysis*

Geological Analysis

The geological map is essential for identifying the geological settlement in the area. The presence of rock is a favourable condition for landfill site due to the impermeable of leachate. A bi-objective suitable model was developed for identifying the landfill location for municipal solid waste (Eiselt et al., 2014).

In the study area geological parameters such as gravel, sand, silt, sandstone with shale and coal partings are spatially distributed. Makan et al. (2012, 2013) was used PROMETHEE technique of suitable waste disposal site identification. Clay is considered as a most suitable ground for landfill due to its properties. In the absence of availability of suitable ground for landfill, it has been suggested to provide a compacted clay layer or suitable artificial materials to prevent the permeability of leachate. The suggested alternative four landfill locations are overlaid (*Fig. 4*) above the geology map by using an overlay analysis tool, it shows the presence of the geological feature below the landfill locations.

Geomorphological Analysis

The geomorphological map was used to recognize the water bodies, shallow flood plain, alluvial plain, moderately weathered/buried plain spatial distribution in the study area. Raster based GIS techniques was applied to identify the landfill (Yildirim, 2012). Site1landfill location fall on moderately weathered and buried plain area, site 2 landfill location falls on a shallow alluvial plain area, site3 landfill location falls on a shallow flood plain area and site4 landfills falls on a shallow alluvial plain area. This overlay analysis is useful for decision making to select the landfill site based on geomorphological condition. Structured query language (SQL) was utilised to retrieve the required

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information from the database. In this geomorphological analysis SQL was used to retrieve the geomorphological features from the entire database as shown in *Fig. 5*.

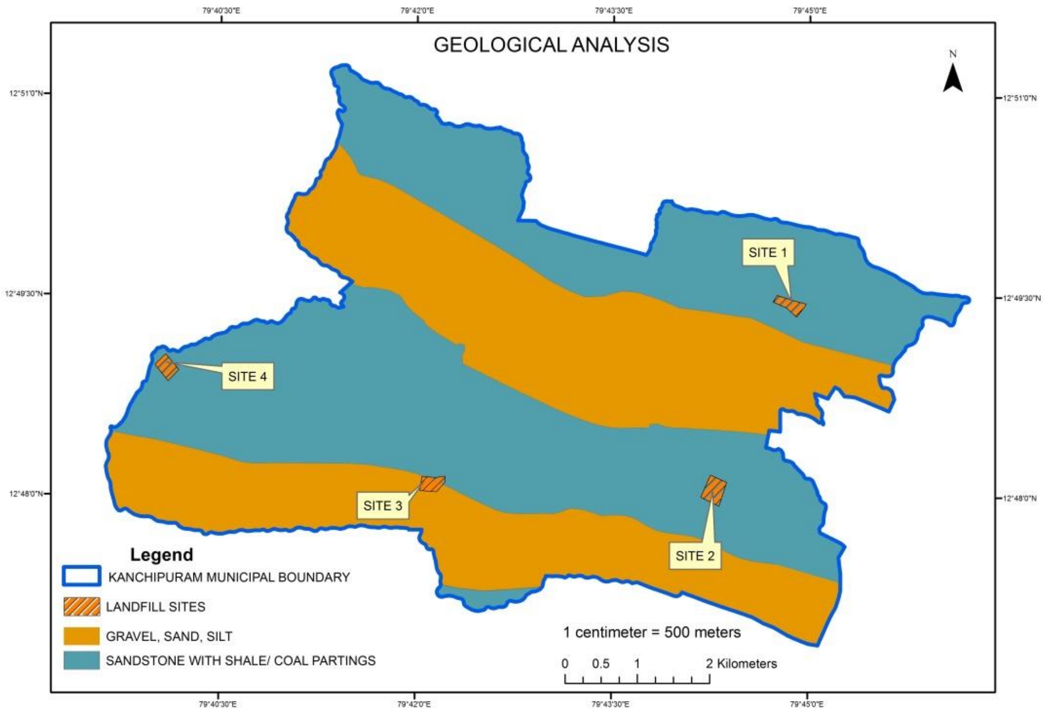

Figure 4. *Map of geological analysis*

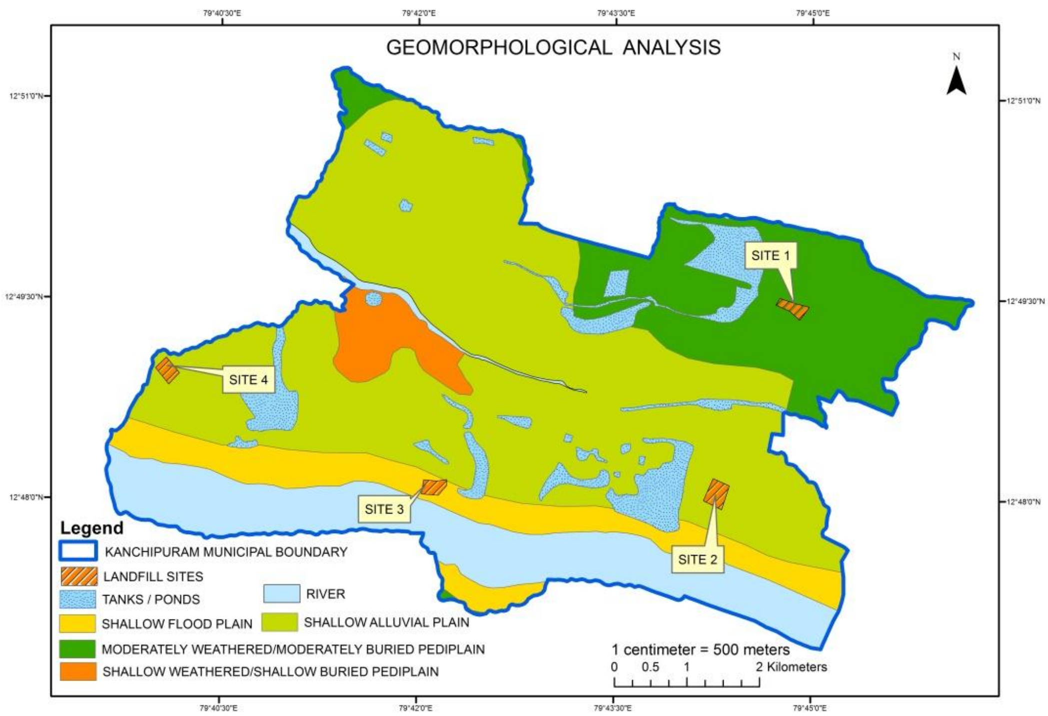

Figure 5. *Map of geomorphological analysis*

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Soil Analysis

The soil spatial distribution is identified in the study area by digitizing the soil map. The four potential alternative landfill sites are overlaid above the existing digitized soil map. In the study area five categories of soil (*Fig. 6*) are available and shown in the map. The landfill site1, site2 and site3 falls under sandy clay area. Clay is preferred as the most suitable ground for the landfill area (Istanbul Environmental Protection Company, 2014).

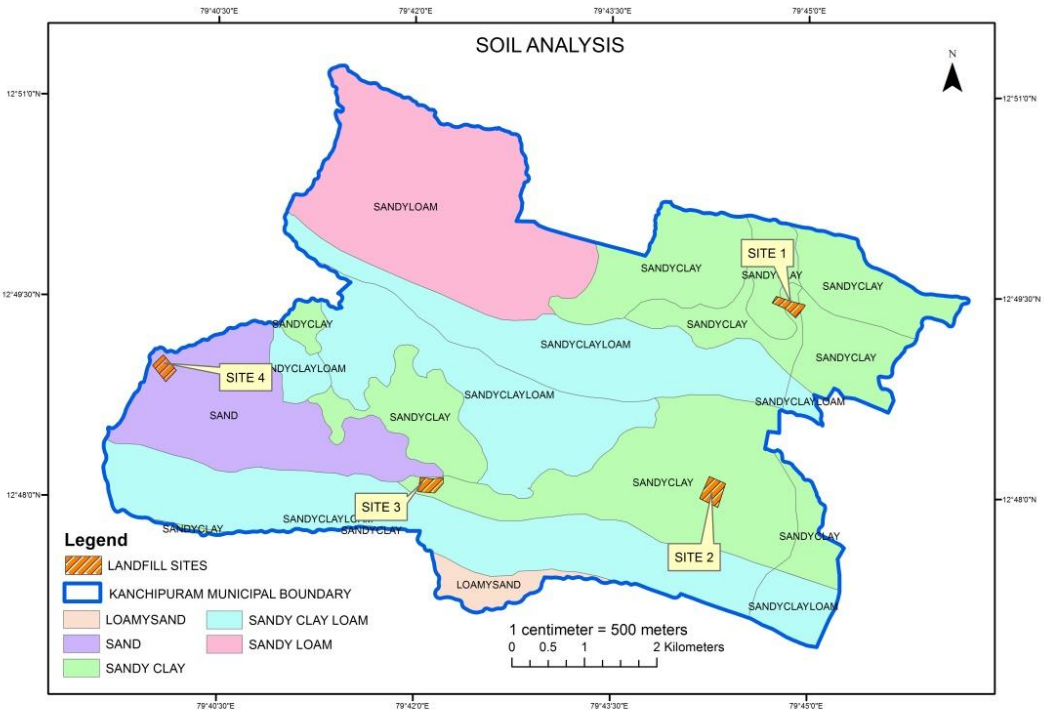

Figure 6. *Map of soil analysis*

The soil condition on site4 is sand and this is not suitable for landfill as per CPHEEO guidelines. This overlay analysis was performed to identify the soil condition in the selected landfill sites for decision making.

An AHP model for identifying a potential landfill site

The alternative potential landfill site was identified by using the AHP method in GIS platform. Each layer was weighted and ranked based on its importance. By using GIS multi-criteria decision making technique the optimum landfill site selection was identified (Demir et al., 2016). In AHP analysis residential area, road network, geology, geomorphology and soil are considered. Cost minimization is possible by doing proper model (Eiselt et al., 2015). By using a model builder in ArcGIS software a model was developed for the above mentioned five parameters (*Fig. 7*).

A raster layer was created for each parameter after reclassification process, weighted and rank was assigned to each parameter. The analysis results produce the appropriate landfill based on the mathematical overlap of the analysis parameters. Based on the

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analysis result four zones are classified in the study area as, low priority zone, medium priority zone, high priority zone and very high priority zone.

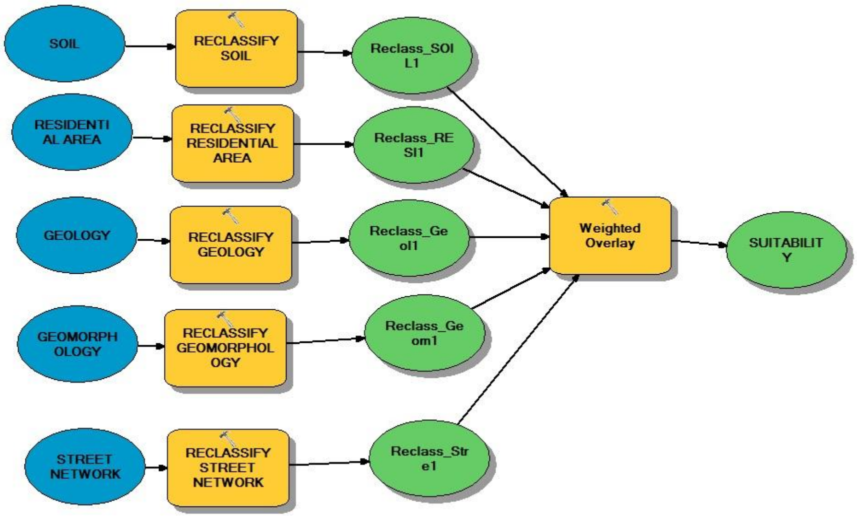

Figure 7. *GIS model for landfill site selection*

Overlaying various layers in GIS platform will produce a suitability map which shows the potential of each spatial location. The spatial location information is very much useful for decision making in landfill site selection. Justification of each alternative landfill site also made easier due to the AHP result. The prepared GIS model can be adopted in any municipalities for alternate landfill site identification. Based on the priority weighting and ranking may be adopted.

Conclusions

This study shows the potential of GIS analysis for identification of suitable alternative landfill sites. Integration of spatial and non-spatial data was effectively used to retrieve the appropriate results in landfill identification. Buffering results are clearly producing the spatial restriction of residential and road parameter based on CPHEEO. The AHP tool in GIS is used to identify five potential zones on the basis of priority. Out of the four potential zones, site1 was identified as a very high priority zone, site2 as low priority, site3 as high priority and site4 as medium priority. The AHP analysis resolves the decision making difficulties in an available large database. It has been identified, site1 is located in northeast of Kanchipuram municipality where all the conditions are favourable for the optimum alternative landfill site. This developed decision support system will be useful for decision makers in solid waste management system to identify the suitable alternative landfill site.

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GENETIC VARIATION OF *PALIURUS RAMOSISSIMUS* (LOUR.) POIRET (RHAMNACEAE): IMPLICATION FOR CONSERVATION STRATEGIES

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Abstract. *Paliurus ramosissimus* (Rhamnaceae) is a deciduous shrub found on the seashore of Jeju Island, located at the southern tip of the Korean peninsula, and has been designated a rare plant species by the Korea Forest Service. Random amplified polymorphic DNA was used to investigate the genetic variation within and among *P. ramosissimus* populations on Jeju Island. Populations of *P. ramosissimus* showed a relatively low genetic diversity. The percentage of polymorphic bands (PPB) ranged from 33.3% to 63.6% (average = 51.4%), Shannon's information index (I) ranged from 0.213 to 0.352 (average = 0.316), and Nei's gene diversity (H) ranged from 0.149 to 0.239 (average = 0.218). Analysis of molecular variance and Nei's gene differentiation coefficient showed low genetic differentiation among populations (Φst = 0.207 and Gst = 0.186). The level of gene flow was sufficient to counter population divergence due to genetic drift (Nm = 0.109). Population genetic information obtained from this study could provide a valuable baseline for conservation and management plans for this species; *P. ramosissimus* on Jeju Island have to be protected through *in situ* conservation. The populations Pyo-sun and Il-gwua with high genetic diversity have conservation priority. In particular, Pyo-sun population protection must be the highest priority, as this population was revealed to have a high genetic diversity despite its small size.
Keywords: *genetic diversity, genetic differentiation, plant conservation, RAPD, Jeju Island*

Introduction

Genetic variation provides the resources on which populations draw from to survive drastic environmental changes and for evolutionary adaptation (Li et al., 2013; Zhang et al., 2012; Frankham et al., 2002; Ellstrand and Elam, 1993; Milligan et al., 1994; Tansley and Brown, 2000). Species that have adapted to local environments or have been isolated for long periods are easily affected adversely by small environmental changes because they have low levels of genetic variation (Tansley and Brown, 2000; Hamrick and Godt, 1989; Frankham, 1996). Therefore, the level of genetic variation suggests that the geographical history and the circumstances that the taxon faced could provide the basic information to maintain genetic variation (Frankham et al., 2002) or to establish effective conservation strategies (Yu et al., 2011; Fritsch and Rieseverg, 1996) for target species.

Paliurus ramosissimus (Lour.) Poiret (Rhamnaceae) is a deciduous shrub found only on the Jeju Island in Korea (Lee, 2003). The geographical distribution of this species has been acknowledged in Taiwan, Japan, China, Vietnam, and other tropical and subtropical Asian locations (Nakanish, 1981, Chang and Kim, 2001). Jeju Island is known as the northern distributional limit of *P. ramosissimus* (Kim et al., 2002). The habitats of *P. ramosissimus* are situated above the high-water level on the shores and estuaries (Nakanish, 1981). The fruit of this species has a corky or woody mesocarp and

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is disseminated by seawater (Nakanish, 1981). Owing to its rarity, *P. ramosissimus* was listed as an endangered species in 2005 by the Ministry of Environment of Korea (Ministry of Environment, 2005). However, this species was lifted from the list in 2011, in accordance with newly discovered habitats and estimations of stable populations (Ministry of Environment, 2012). Nevertheless, this species is still listed on the rare plant list of the Korea Forest Service because of its small population size (Korea National Arboretum, 2009).

The habitat distribution of *P. ramosissimus* on Jeju Island, and population size and vegetation composition of *P. ramosissimus* have been studied in recent years (Chang and Kim, 2001; Kim et al., 2007; Kim, 2006). Nakanish et al., (2004), surveyed the natural habitat distribution and vegetation structure of *P. ramosissimus* on Jeju Island and compared the characteristics between habitats in Korea and Japan. However, molecular population genetic studies on *P. ramosissimus* have not been performed until now.

Molecular markers have been widely used to characterize the genetic structure of plant populations (Liu et al., 2012). The random amplified polymorphic DNA (RAPD) method has some advantages, such as the high polymorphism generation and the fact that it does not require previous knowledge of the genome. Therefore, it is now widely appreciated that understanding patterns of genetic variation is of critical importance to the conservation of threatened species (Trindade et al., 2009). In addition, molecular approaches are providing particularly valuable resources to fingerprint the consequences of historical events such as range expansion, fragmentation, and bottlenecks in population size (Haig, 1998), which may have significant implications to the development of conservation strategies (Newton et al., 1999).

RAPD markers were used to assess genetic diversity and genetic variation of the different *P. ramosissimus* populations in this study. The main objectives of this study were to (1) assess levels of genetic diversity of the natural populations; (2) reveal the partitioning of the genetic variations within and among populations; (3) provide elementary information for the conservation of *P. ramosissimus*.

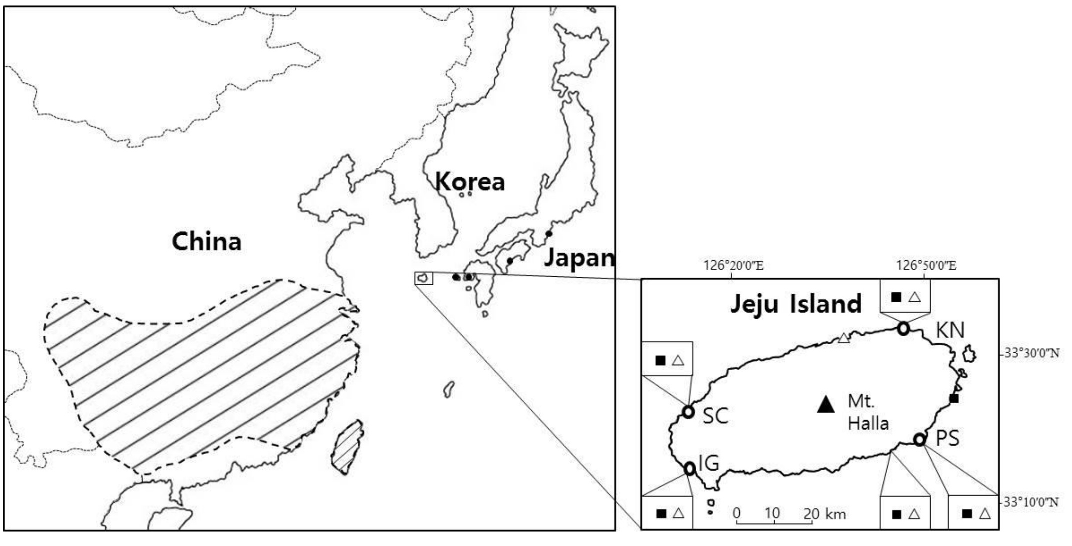

Figure 1. *Geographical distribution of P. ramosissimus. P. ramosissimus in southern parts of China, Taiwan (hatched area, from Chang et al. (2005), and southern parts of Japan (closed circle, Nakanishi 1981). The plant community studies on the P. ramosissimus on Jeju Island performed by Nakanishi (2004), closed squares, and Kim (2006), open triangles. Opened circles are sampled populations in this study. KN, Kim-nyung; PS, Pyo-sun; IG, Il-gwua; SC, Sin-chang.*

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Table 1. *The location of populations and sample size of surveyed of P. ramosissimus populations on Jeju Island, Korea (n=sample size; N=population size).*

No.	Population	Latitude	Longitude	n	N
1	Kim-nyung (KN)	33°33'49.7"	126°45'53.8"	18	30
2	Pyo-sun (PS)	33°18'54.9"	126°50'17.9"	16	30
3	Il-gwua (IG)	33°14'27.0"	126°13'37.2"	34	130
4	Sin-chang (SC)	33°20'34.1"	126°10'30.3"	17	40

Abbreviations of populations are in the parenthesis.

Materials and Methods

Plant sampling and DNA extraction

Eighty-five individuals from four populations of *P. ramosissimus* were collected from Jeju Island, which is the largest southern end island in Korea (*Fig. 1; Table 1*). For molecular analysis, two or three young and healthy leaflets were collected from each individual shrub of *P. ramosissimus*. The individual shrub samples were collected about 5 m away each other in a population, and the sampled leaves were put into an individual vinyl bag with solid moisture absorbent in the field. The leaf samples were preserved in the lab at -20^{o}C until DNA extraction.

The frozen leaf samples were ground for DNA extraction, using mortar and pestle, in liquid nitrogen. DNA was extracted using the DNeasy® Plant mini kit (Qiagen, Almeda, CA, USA). The extracted DNA concentration was adjusted to 25 ng/µl for RAPD PCR.

RAPD PCR

Fifty primers were screened using Operon RAPD® 10-mer kit (Quiagen Operon Technologies) and five primers were selected based on their reproducible and distinct banding patterns (Table 1). DNA amplification was performed in a 20-µl reaction, containing 1U Taq DNA polymerase (Takara bio Inc., Japan), 1.0 µl template DNA (25 ng genomic DNA), 2.0 µl 10X PCR buffer, 2.0 µl primer (20 pmol), 1.0 µl dNTPs mixture (2.5 mM each). Amplifications were conducted in T professional basic (Biometra, Goettingen, German). Amplification cycles were as follows: initial denaturation at 94°C for 2 min, followed by 39 cycles of 30 sec at 94°C (denaturation), 30 sec at 36°C (annealing), and 1 min at 72°C (extension), with a final extension at 72°C for 10 min. The PCR products were electrophoresed on 1.4% agarose gel in 0.5 for 10 min, and stained with ethidium bromide. The gels were viewed and photographed with Gel Doc 2000 (Bio Rad Laboratory Inc., California, USA) gel imaging system.

Data analysis

Each RAPD fragment was scored as “1” if present or “0” if absent, and was made into a binary data matrix. The data matrix was analyzed using POPGENE 1.32 (Yeh and Boyle, 1997) to assess genetic parameters: percentage of polymorphic band (PPB) for the total bands, observed number of alleles (*Ao*), Effective number of alleles (*Ae*), Nei's (1973) gene diversity (*H*), Shannon's information index (*I*), coefficient of gene differentiation (*G*st), and Nei’s unbiased genetic identity and genetic distance.

Analysis of molecular variance (AMOVA) was carried out to describe the genetic differentiation among populations of *P. ramosissimus* using AELEQUIN ver. 3.0 (Excoffier et al., 2005). The significance of Φst (*F*-statistic analogue, Ge et al., 2005),

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calculated by AMOVA, was tested by 1000 permutations. The level of gene flow, the proportion of new immigrant genes moving into a population, was determined using the formula: Nm = 1/4 (1- Gst)/Gst (Slatkin and Barton, 1989), where N is population effective size and m is migration rate.

To illustrate the genetic relationships between populations, we analyzed the matrix of RAPD bands with UPGMA (Unweighted pair-group method with arithmetic average) cluster analysis using POPGENE 1.32.

A principal coordinate analysis (PCoA) was performed by plotting Euclidian distance on 3-dimensional space, calculated based on a binary matrix of RAPD band pattern for all pair-wise individuals of experimental *P. ramosissimus*, by using GenAlex 6.5 (Peakall and Smouse, 2006).

Results

Genetic diversity

Fifty primers were screened, of which five primers showed reliable banding patterns. The five primers generated 31 clear and repeatable bands among 85 individuals. The average number of bands per primer was 6.6 (*Table 2*). At the species level, 26 of 33 bands showed polymorphic loci, and 78.8% polymorphism. The percentage of polymorphic bands (PPB) for a population ranged from 33.3% to 63.6%, and the mean percentage of polymorphic bands of all four populations was 53.8% (*Table 3*).

Table 2. *Primer sequences and amplified products of RAPD markers for the four P. ramosissimus on Jeju Island in Korea.*

Primer	Sequence (3'-5')	No. of loci	No. of polymorphic loci
OPA-10	GTGATCGCAG	7	6
OPA-17	GACCGCTTGT	6	4
OPAF-07	GGAAAGCGTC	6	6
OPP-14	CCAGCCGAAC	8	5
OPP-11	AACGCGTCGG	6	5
Mean		6.6	3.9

Table 3. *The genetic variations revealed through RAPD markers among populations of P. ramosissimus on Jeju Island in Korea. Population abbreviations are shown in Table 1 and Figure 1. (PPB=Percentage of polymorphic band; Ao=Observed number of alleles; Ae= Effective number of alleles; H=Nei's gene diversity; I=Shannon's information index).*

Populations	PPB	*Ae*	*Ao*	*H*	*I*
KN	54.6	1.3903	1.5455	0.217	0.314
SC	33.3	1.2722	1.3333	0.149	0.213
PS	63.6	1.4862	1.6364	0.266	0.383
IG	63.6	1.4093	1.6364	0.239	0.352
Average	53.8	1.3895	1.5379	0.218	0.316
Total	78.8	1.4482	1.7879	0.264	0.398

The IG and PS populations have high genetic diversity indices (PPB=63.6 and 63.6; Ae=1.4093 and 1.4862; Ao=1.6364 and 1.6364; H=0.239 and 0.266; I=0.352 and 0.383, respectively). On the other hand, SC and KN populations, which are on the northern shoreline of Jeju Island, exhibited lower genetic diversity than the southern shoreline of

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PS and IG. The genetic diversity indices at species level were higher than the average population diversity values, which showed the highest values of diversity among tested populations, 78.8 of PPB, 0.264 of *H*, and 0.398 of *I* (*Table 3*).

Genetic structure

The coefficient of genetic differentiation between populations (*G*st) was 0.186, as estimated by partitioning of the total gene diversity. The level of gene flow (*Nm*) was estimated to be 1.094 per individual per generation between populations. Similarly to Nei's genetic diversity statistics, analysis of molecular variance (AMOVA) made using RAPD data showed that 79.3% of the total genetic variability can account for the differences within populations of *P. ramosissimus*. The remaining 20.7% variations are due to variations among populations ($P<0.001$) (*Table 4*).

Table 4. *Analysis of molecular variance for populations of P. ramosissimus on Jeju Island in Korea.*

Source of variance	d.f.	Variance components	% of total variance	*P*-value
Among Populations	3	50.99	20.7	<0.001
Within Population	81	217.44	79.3	

A UPGMA dendrogram was made by using Nei's genetic distances among population (*Fig. 2*). Each population showed very near genetic relationships. The populations grouped below the 0.118 genetic distances between PS and SC (*Table 5*). This result means that the four populations show low genetic differentiation although KN, SC, IG grouped to one clade excluding PS. The populations of IG and PS, which are located at the southern coast, have relatively high genetic diversity. However, the two populations showed greater genetic distance, although at low levels, and IG showed the largest population size and short genetic distances between KN and SC in the northern shoreline. Therefore the IG population and PS population are important in the genetic relationships between *P. ramosissimus* populations on Jeju Island.

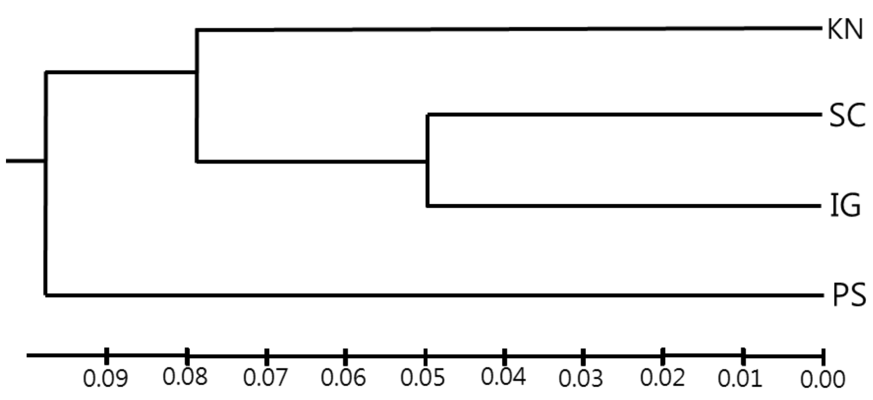

Figure 2. *UPGMA dendrogram of the four P. ramosissimus populations on Jeju Island. The scale bar represents Nei's genetic distance. Population abbreviations are shown in Table 1 and Figure 1.*

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Table 5. *Genetic identities (above diagonal) and Nei's genetic distance (below diagonal), using RAPD markers between four populations of P. ramosissimus on Jeju Island in Korea. Population abbreviations are shown in Table 1 and Figure 1.*

Population	KN	SC	PS	IG
KN	****	0.9117	0.9029	0.9420
SC	0.0925	****	0.8892	0.9521
PS	0.1021	0.1175	****	0.9394
IG	0.0598	0.0491	0.0625	****

RAPD banding pattern of each *P. ramosissimus* population on the PCoA analysis also did not show clear separation between populations with most of bands overlapped (*Fig. 3*). Axis one and axis two exhibit 17.51% and 17.05% of interpretabilities, respectively.

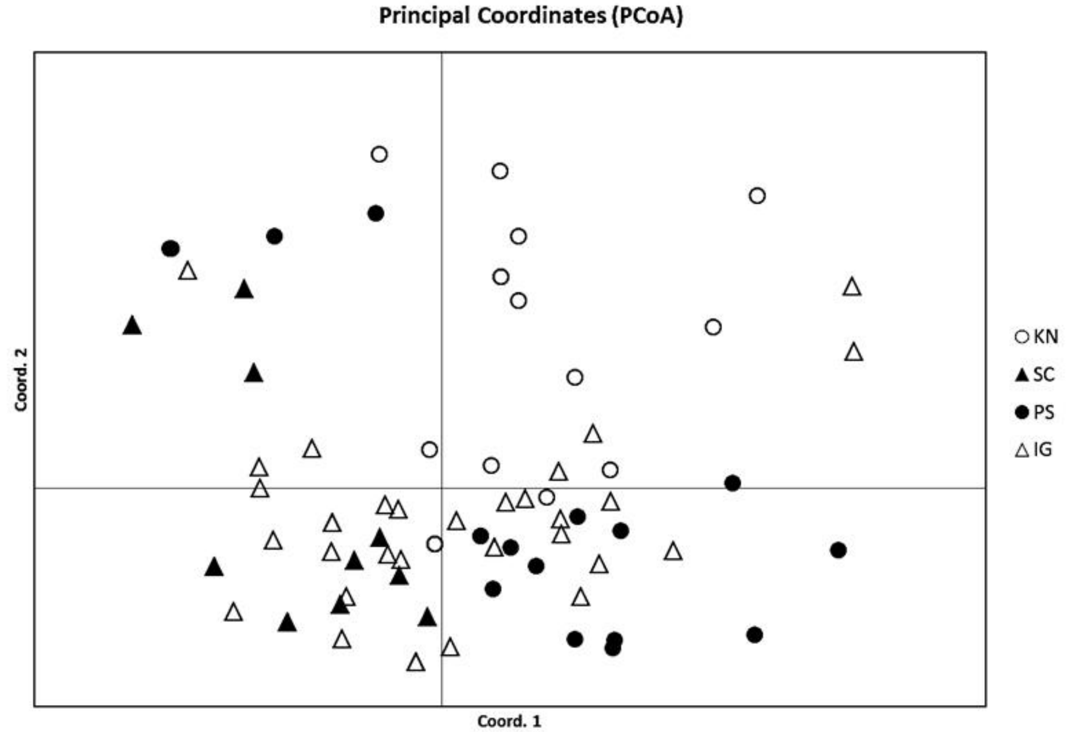

Figure 3. *Principal coordinates analysis (PCoA) plot for 85 individuals from four P. ramosissimus populations on Jeju Island in Korea based on RAPD markers. Population abbreviations are shown in Table 1 and Figure 1.*

Discussion

Genetic diversity

Genetic diversity depends on the regional distribution extent, the size of the population, breeding system, seed dispersal, and the life history traits of the species (Hamrick et al., 1992). In plant species, the breeding system is the most important major factor in explaining genetic variability at the population level (Ellstrand and Elam, 1993; Nybom, 2004). In general, self-fertilizing taxa tend to be the least diverse, whereas the outcrossing taxa tend to be the most diverse.

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Genetic diversity of *P. ramosissimus*-based RAPD markers on Jeju Island showed slightly low genetic variation at the population level. The Nei's diversity at population level of *P. ramosissimus* (*H*=0.217) was lower than that of the average long-lived perennial plants, *H*=0.25, and also lower than that of the average outcrossing species (*H*=0.27) but higher than that of selfing (*H*=0.12) and mixed (*H*=0.18) species (Nybom, 2004).

P. ramosissimus is the one species of Rhamnaceae assumed to be outcrossing. Bolmgren and Oxelman (2004), Meden and Bailio (2001), and Meden (1994) had indicated that most species in the family Rhamnaceae show the outcrossing breeding system. When we assume that *P. ramosissimus* is the one outcrossing species, the genetic diversity *H*=0.217 on Jeju Island is relatively low genetic variation.

In general, population size and within-population genetic diversity shows a positive relationship (Chang et al., 2005; Frankham et al., 2002), however, population PS showed high genetic diversity although it has a small population size. On the other hand, population IG showed high genetic diversity with the largest population size.

Comparing the genetic diversity of *P. ramosissimus* with other species in Rhamnaceae, the genetic diversity of *P. ramosissimus* was found to be higher at the population level and species level than that of endangered species such as *R. ludovici-salvatoris*, *R. glucophylla*, *R. persicifolia*, and *Ziziphus celata*, and endemic species such as *Alphitonia ponderosa* and *Colubrina oppositifolia* (*Table 6*). However, the diversity of PPB and *H* value of *P. ramosissimus* was found to be lower than that of the widespread species *R. alaternus* at the population level and species level, and on the other hand, showed significantly higher genetic diversity than that of the widespread species *Z. acidojujuba*. Endangered species *Berchemiella wilsonii* var. *pubipetiolata* showed contrary genetic diversities according to the AFLP marker, a DNA-based marker, which showed lower genetic diversity, compared to allozyme, which is protein-based and showed a higher genetic diversity. Our RAPD results for the genetic diversity of *P. ramosissimus* correspond with the DNA based AFLP marker, although many plant species of Rhamnaceae exhibited different diversity according to the used marker systems.

Table 6. *Comparison of genetic diversity in P. ramosissimus and literature data for other Rhamnaceae species (EN=Endangered species; ED=Endemic species; PPB=Percentage of polymorphic band; He=Expected heterozygosity; I=Shannon′s information index; Pop.=population level; Sp.=species level).*

Species	Marker	Status	PPB		*He*		*I*		*Φ*st	*G*st	*F*st	References
			Pop.	Sp.	Pop.	Sp.	Pop.	Sp.				
P. ramosissimus			53.8	78.8	0.218	0.264	0.316	0.398	0.207	0.186	-	This study
B. wilsonii var.*pubipetiolata*	AFLP	EN	26.9	36.9	0.163	0.202	-	-	0.396	-	-	Kang et al., 2007
B. wilsonii var.*pubipetiolata*	Allozyme	EN	71.3	85.0	0.348	0.378	-	-	-	-	0.130	Kang et al., 2005
R. persicifolia	ISSR	EN	30.7	67.1	0.111	0.207	0.165	0.314	-	0.458	-	Bacchetta et al., 2011
R. alaternus	RAPD		66.8	82.0	0.213	0.165	-	-	-	-	-	Ferriol et al., 2009
R. ludovici-salvatoris	RAPD	EN	34.6	69.4	0.045	0.096	-	-	-	-	-	Ferriol et al., 2009
R. glucophylla	ISSR	EN	22.6	48.7	0.080	0.116	0.119	0.184	-	0.290	-	Bedini et al., 2011
Z. acidojujuba	Microsatellite		-	-	0.674	0.659	-	1.386	0.091	0.271	-	Zhang et al., 2015
Z. celata	Allozyme	EN	-	25.0	-	0.079	-	-	-	-	-	Godt et al., 1997
Z. celata	Microsatellite	EN	-	-	0.390	-	-	-	-	-	-	Gitzendanner et al., 2012
A. ponderosa	RAPD	ED	29.0	47.1	0.136	0.183	-	-	0.457	-	-	Kwon & Morden 2002
C. oppositifolia	RAPD	ED	30.0	41.3	0.085	0.118	-	-	0.294	-	-	Kwon & Morden 2002

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Genetic differentiation and gene flow among populations

All the estimates of genetic differentiation coefficient (Φst and *G*st) between populations of *P. ramosissimus* on Jeju Island revealed a low level of genetic differentiation (20.7% and 18.6%). Low levels of genetic differentiation among populations have previously been found in several woody species (*Tetraena mongolica,* Ge et al., 2003; *Bretschneidera sinensis,* Hu et al., 2014; *Prunus mahaleb,* Jordano et al., 2000; *Araucaria araucana,* Bekessy et al., 2002). Also, Ge et al. (2003) suggested that genetic differentiation among populations using Φst, modified *F*-statics, and *G*st usually exhibit below 19% when RAPD markers have been analyzed for outcrossing plant species. These results suggest that the genetic variability of *P. ramosissimus* on Jeju Island was more attributed within population than among population, and showed similar results to referenced species in Rhamnaceae (*Table 6*).

Nybom (2004) surveyed the relationship of the genetic diversity index by RAPD marker within and between the populations according to the life history traits. This study found that the breeding system, life form, and seed dispersal mechanism has a great influence on the genetic diversity of the populations. According to Nybom (2004), our result of 0.207 Φst value of *P. ramosissimus* on Jeju Island is similar to that of the outcrossing plants (Φst value 0.27), while different from those of plants with selfing (Φst value 0.65) and mixed (Φst value 0.40) breeding systems. Nybom (2004) also noted that the average Φst values of long-lived perennial and water-dispersed plants are 0.25 and 0.27, and the average *G*st values for these are 0.19 and 0.22, respectively. *P. ramosissimus* on Jeju Island showed lower Φst and *G*st than long-lived perennial and water-dispersed plants surveyed by Nybom (2004) meaning that *P. ramosissimus* populations on Jeju Island have low genetic differentiation.

The gene flow (*Nm*) of 1.09 of *P. ramosissimus* on Jeju Island was slightly higher than one successful migrant per generation. Wright (1931) noted that the species of $Nm>1$ could be considered as genetic sameness, while those of $Nm<1$ keep the differentiation of the population due to the strong differential selection. Therefore, *P. ramosissimus* has considerable gene flow among populations that could effectively homogenize genetic traits of populations to some degree (Slatkin, 1987). *P. ramosissimus* on Jeju showed relatively low Φst and *G*st and high gene flow, maybe affected by outcross mating system and easily dispersed seeds.

Implications for conservation and the origin of P. ramosissimus on Jeju Island

A primary objective of nature conservation is the maintenance of genetic diversity. The results of this study could be useful in the decision making process of conservation and management strategies. *P. ramosissimus* on Jeju Island exhibit a relatively low level of genetic differentiation among populations. Therefore, protection of *P. ramosissimus* on Jeju Island has to firstly begin with protection through *in situ* conservation, and prevented fragmentation and ruptures. This is because decreases in effective population size lead to stochastic events, genetic drift, and inbreeding, resulting in a decrease of genetic diversity (Hartl and Clark, 1997; Rodrigues et al., 2013). The present habitats of *P. ramosissimus* need to be protected by conservation areas and there is need to ensure dispersion because of the expected destruction of habitat and decrease of population size. Additionally, the coastal area of Jeju Island has been exposed to the pressures of development in recent times (Kim, 2006). The populations PS and IG, which have high genetic diversity, have conservation priority. In particular, PS population protection

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must the highest priority, because this population revealed high genetic diversity despite its small population size.

P. ramosissimus is distributed geographically in the area of southern China and Thushima, Sikoku, southern Honshu of Japan, and Jeju Island in Korea (Fig, 1; Chang, 2005; Nakanishi, 1981). These distribution areas almost harmonize with Kuroshio Warm Current, which flows from the south coast of China to the south coast of Japan through Taiwan. The Tsushima Warm Current, which is the branch of Kuroshio Warm Current, flows past Jeju Island (Cho and Choe, 1988; Pang and Kim, 1993). The hypothesis that the *P. ramosissimus* on Jeju Island and Japan originated from southern China or Taiwan may be possible in consideration of sea water flow mentioned above and the characteristic of the seed of *P. ramosissimus*, which easily float in sea water. The fact that relatively high genetic diversity of southern shore than northern shore populations probably suggests the arrival history or route of migration of *P. ramosissimus* to Jeju Island have southern origin although there is no clear evidence of this. Further molecular studies are needed to clarify the dispersion and origin of *P. ramosissimus*. Furthermore, considering the expansion of the distribution of southern plant species due to climate change, there is a possibility that this species may become established in the southern shore of the Korean peninsula. This will require attention and monitoring.

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ADVANCE IN GLACIER MODELLING: GIS AND ORIENTED PROGRAMMING APPROACH APPLIED ON WHITTIER GLACIER, ALASKA

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Abstract. The global warming affects drastically the glaciers melting, a fact for which the glaciers from Alaska are continuously retreating. Based on Geographical Information System and oriented programming, we have defined a new method to model the glaciers. The Whittier Glacier from South Alaska was analysed in the last 30 years and it was modelled under climate conditions of the 21st century. The glacier modelling has come out from calculations of annual retreat rate, the hemisphere, the latitude, the annual temperature, the precipitation, the altitude data, and the aspect of flow direction. The prediction model of Whittier Glacier shows a value of 1.0544 km² of losing ice area up to year 2030, a value of 2.2184 km² of retreat area up to 2050, a value of 3.9704 km² up to 2080, and a value of 5.1524 km² up to 2100. We estimate a total loss ice volume related to this melting area of 0.3393 km³. The future scenario indicates a glacier volume reduction of 84.89% up to 2050. These findings are useful for glaciologists, but also the paper contributes to Alaskan glaciers knowledge.
Keywords: *climate change, satellite images, ice retreat, prediction model, melting*

Abbreviations
GIS – Geographical Information Systems
asl – above sea level
Rr – retreat coefficient

Introduction

Scientists use models for future predictions of different entities like climate, water resources or ecosystems. The environmental systems are continuously changing and consequently, human safety, in term of habitat and the real conditions of life, depends not only on atrophic systems but imply the global changes too. Climate change drastically affects groundwater and food resources and indirectly the coastal shore habitats in ice land masses' proximity.

In the Earth Science expertise area, many scientists have agreed with the models to see how to evolve the systems for future periods in hydrogeology, chemistry, volcanology, and seismology. IPCC (2007) claimed the global climate changes and up

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to 3 °C temperature change was predicted for the current century. The Alaskan glacier behavior observed shows that not all glaciers melt due to climate warming, some registered retreat due to landslides, eruptions, and surge dynamics (Molnia, 2006). The ocean branches penetrate into presents Prince William Sound fjords and the local climate became more humid during the summers. This thing accelerates the water flow rate in surface glacier crevices, a fact for which the glaciers located near the shore are more affected by regression. The snowfall during the cold seasons and precipitation quantity during the summers contribute to the glacier mass balance.

Here we present an Alaskan glacier model constructed for present century, which predicts the retreat rate of Whittier Glacier for different years up to 2100. We realized the glacier modelling using object-oriented programming and Geographical Information Systems (GIS). The program base was a preliminary study of Whittier Glacier through field research and drawing the outlines based on satellite images dated from 1973 to 2013. Taking into account the field's characteristics, local climate data, and a retreat rate along almost 40 years, a conceptual model formed by five classes in Eclipse software from Java platform was projected. Considering the past and recent physical characteristics of Whittier Glacier it was easy to calibrate the model closed to actual climatic conditions.

Many studies about climate (Oerlemans, 1994; Collins, 2008; Piao et al., 2010), water or landslides define firstly a conceptual model and further, the researches researchers start the modelling with the software. The regression of glaciers from the world is very complicated. The equilibrium between ice mass movements is difficult to generalize because each glacier was formed in a proper morphological condition, has proper slope aspect and its flow directions, and slip on itself-pattern. The surface rivers have a basic level in each cycle that could be a bigger river, a lake, sea or ocean, but the glacier pressure on the ground is not related to a base level. In Passage Canal were demonstrated that three glaciers situated close to each other had various retreat rates in the same period and under the same climatic conditions (Nistor and Petcu, 2015). In the ablation area of one glacier, many factors involved. For example, if the annual temperature is a positive and precipitation quantity is higher than heat energy, the glacier could easily retreat. On the other hand, if the annual precipitation such as snow is connected with low annual temperature, the glacier could be stable from area and volume point of view. Moreover, as the climate is warming some glaciers from Caucasus extends the tongue due to ice plasticity. The field characteristics and glacier thickness represent other factors that contribute to glacier behaviour. The geomorphological processes of side slopes and decompression forces of glacial wall's valley conduct at landslides and rock falls from lateral scree. Usually, the rock fall blocks have a dark colour in contrast with glacier brightness, a fact which implies higher heat energy of landslides and rock fall. Thus, in these sector of glaciers, the melting rate is greater.

The main objective of the present paper is to construct a model of the Whittier Glacier up to 2100. The glacier modelling is based on the past and future projections of climate data and it is based also on the GIS applications and satellite images. The GIS techniques and satellite images contribute to determining the annual retreat rate of Whittier Glacier and to draw the future glacier outlines.

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Materials and methods

Alaskan glaciers shifts and Whittier Glacier

The glaciers from Alaska were studied by the United States Geological Survey (USGS) and in the last 50 years, various retreat rates were found. Mayo et al. (1977), Kennedy et al. (2006) reconstructed the Portage Glacier terminus status starting in 1914. Portage Glacier is located near Whittier Glacier and the terminus limits predicted in 2020 was exceeded in 2009 (Nistor and Petcu, 2014). Nistor and Petcu (2015) completed a quantitative analysis of three glaciers from Passage Canal in the last decades. They found a continuous annual retreat rate which varies from 0.025 km^2yr^{-1} to 0.083 km^2yr^{-1}. This is a tragic result regarding the Whittier Creek flooding system discharge and the Whittier town harbour activity (Nistor, 2013).

Whittier Glacier is located in Passage Canal Fjord from Prince William Sound, on the northern Kenai Peninsula. The Passage Canal Fjord has Dfc climate, characterized by fully humid and cool summer, in which the evapotranspiration has lower values.

Whittier Glacier (*Figure 1*) is an outlet glacier flowing from the Harding Ice field, with a small slope and it has flow direction from south-southwest to north-northeast. Whittier Glacier extends in altitude between 470 m and 1013 m above sea level (asl), and it presents a large terminus limit, a fact which makes and have various sensitive sites. The Whittier Glacier extends from the south of Whittier town in the Kenai Mountains. From the Landsat satellite images were carried out the limits of Whittier Glacier starting in 1973 to 2014. The oldest data of Whittier Glacier bring forward that in 1910 the terminus of this glacier was only at 1 km distance to shore (Barnes, 1943). Barnes (1943) recalls in his works that the terminus was shifted between 1913 and 1939

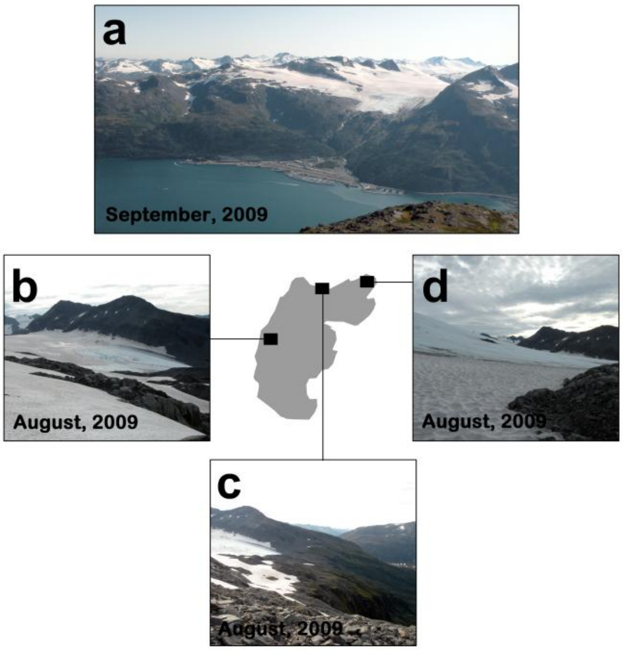

***Figure 1.** Views with Whittier Glacier. **a.** Oblique south-looking aerial photograph of Whittier Glacier (September, 2009). **b.** West-looking ground photograph of central sector of Whittier Glacier (August, 2009). **c.** Terminus of glacier near the glacial threshold (August, 2009). **d.** Terminus of glacier in the north-eastern extremity (August, 2009). Photo courtesy: Nistor.*

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from 180 m asl to 300 m asl. Interestingly, based on GIS and Landsat images the eastern and north-eastern part of the glacier was depicted more sensitive than other parts (Nistor and Petcu, 2015).

Climate data

The mean annual air temperature, mean annual precipitation, and annual snow depth from 1985 to 2010 was used together with monthly temperature data, precipitation, and snowfall from 1960 to 2010 to assess the climate conditions in which is the major retreat of Whittier Glacier. The climate data used in the calibration belongs to Whittier meteorological stations and are courtesy of Western Regional Climate Center (2014). In the calculations of future climate data parameters the CGCM2 in the GHG+A IS92a models has been adopted (The Canadian Centre for Climate Modelling and Analysis, 2015a; The Canadian Centre for Climate Modelling and Analysis, 2015b) created in transient runs and its relative change plots served into future absolute calculations that are based on meteorological data from Whittier station. It is safe to mention that the Western Regional Climate Center data of temperature and precipitation are expressed in Fahrenheit degrees respectively in Inches and in the present work, the temperature and precipitation data are converted to Celsius and millimetres respectively.

Landsat data

Whittier Glacier outlines were extracted through remote sensing in one band using GIS and satellite images. Using ArcGIS 10.2 software and optical remote sensing with Landsat satellite images (United States Geological Survey, 2015), the spatial extension of Whittier Glacier was drawn in various set times from 1973 to 2014.

During the last decades, many glaciers and ice lands studies have been made based on satellite images and remote sensing (Echelmeyer et al., 2004; Holobâcă, 2013). Molnia (2008), Kargel et al. (2005) present in their papers the contribution of satellite images in ice land measurements. Nistor and Petcu (2015) carried out the annual retreat rate of Whittier, Learnard, and Billings glaciers from 1985 until 2013, using the Landsat images.

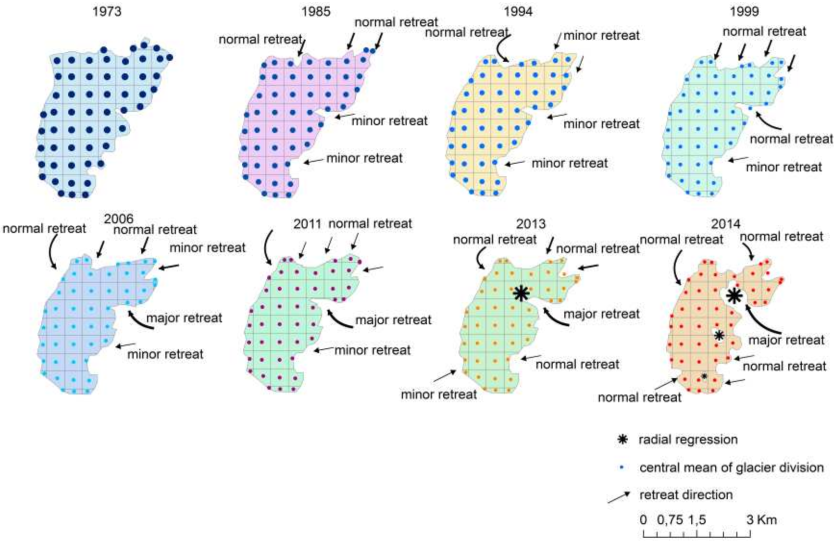

Figure 2. *Division in polygons of glacier outlines and founding the sensitive retreat zones using the 'Central mean' analysis.*

To complete this study with the spatial extension of Whittier Glacier in the future, the GIS techniques was linked to results carried out through Java model constructed and calibrated in Eclipse software. This procedure was essential for the glacier modelling because it gives the future perspective of Whittier Glacier and in case of flooding or the Pacific Ocean rise risks the planning precautions could be.

Based on GIS techniques, two innovative methods were combined to delimit the future outlines of Whittier Glacier. Firstly, the vector data of glacier outlines were divided into network quarts of 500m dimension through 'Intersect' function. Data Management Tools served to find the centres of these polygons using the 'Make XY Event Layer' function. This spectacular and innovative method was used to identify the terminus retreat directions (*Figure 2*) for past years and after was taken into consideration for future glacier projections. A 'Central Mean' function was applied and tested to identify the general retreat and sensitive areas of Billings Glacier, Learnard Glacier, and Whittier Glacier by Nistor and Petcu (2015).

Figure 3 depicts the simple steps used in this methodology. Through these methods, the retreat sites of the glacier were found, as soon as the mathematical calculations argue these locations. For the analysis of Whittier Glacier outlines in different set times were used ArcGIS 10.2 software applications and thus we calculated the retreat rates of Whittier Glacier. Digital elevation model, thickness grid, 'Central mean' positions of vector divisions, backed with field researches was considered in the spatial representation of the Whittier Glacier. The outlines of Whittier Glacier were extracted by manual vectorization considering as much as possible the satellite images from autumn or late summer period. Also, for the outlines precision, the local field research observations were done. These things helped to define more clearly the limits of Whittier Glacier.

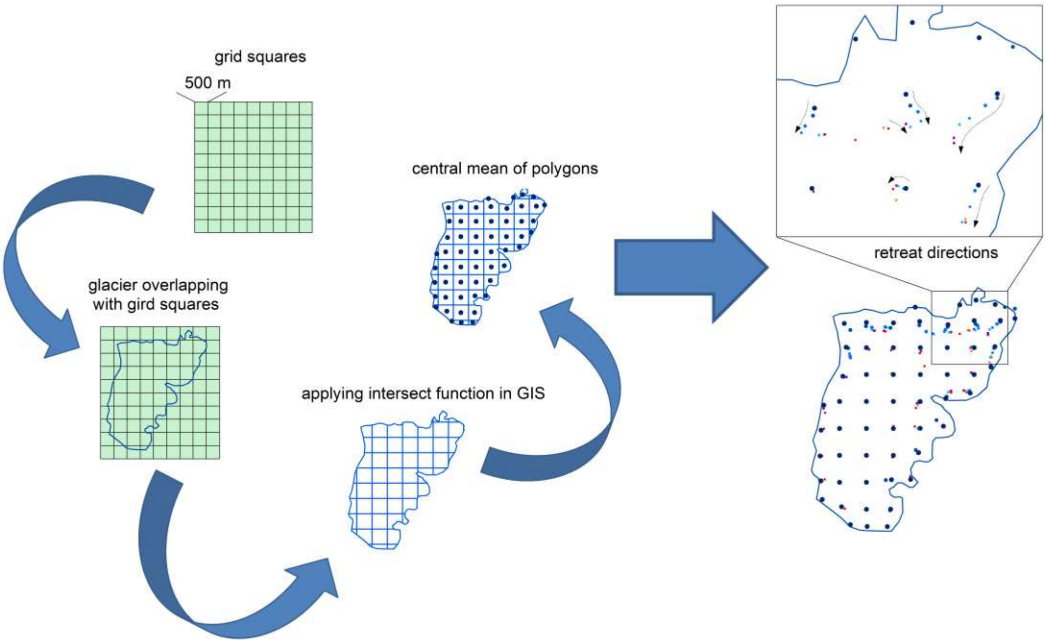

Figure 3. *General framework of the 'Central mean' methodology.*

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The conceptual model of Whittier Glacier

Glacier modelling is a complex matter under climate warming, local field's field conditions and processes. The climate data analysis and the calculated retreat rates served to build a conceptual model of Whittier Glacier, which further permits to project the retreat of Whittier Glacier and its extensive area for future periods, up to 2100.

For this reason, this study started with a model that predict the retreat rate of Whittier Glacier under climate change taken into account the temperature and precipitation climate data, the hemisphere glacier location, the glacier latitude, the glacier altitude, and the glacier slope aspect. These data influences are elementary to the ice mass melting. The field research and Whittier Glacier drawing boundaries in different set times helped to understand how evolved the ice melting during the last 40 years and in which conditions the major retreat is. The climate data are taken into account to observe the relationship between the climate parameters and Whittier Glacier retreat.

The Whittier Glacier thickness data grid obtained by Farinotti et al. (2009) and Huss and Farinotti (2012). They applied an algorithm based on volume-area scaling relation for the entire globe's glaciers.

Glacier modelling method using oriented programming objects

In an aim to understand correctly the Whittier Glacier retreat pattern, this study proposed an advanced glacier model using GIS and oriented programming, transposing the conceptual model of Whittier Glaciers into Java programming language aimed to project its annual retreat rate for the future period.

This program constructed in Eclipse software and contains five classes as follows: (i) "FieldSettings" class, (ii) "ClimateData" class, (iii) "Glacier" class, (iv) "Processes" class, and (v) "WindowManager" class. The glacier modelling based on combination and relationship between the global position of the glacier, field characteristics, and climate data. "FiedSettings" class includes Whittier Glacier field characteristics like aspect and altitude. In the "ClimateData" class the annual mean temperature and precipitation were inserted. In "Glacier" class the hemisphere data, latitude, area, and glacier length were declared. The "Processes" class refers to retreat rate and sensitive areas. The "WindowManager" class contains the main function which leads to the program running. The program returns the calculated retreat rate values of melting area under climate data, global position, and field characteristics which were added when the program was running. The model was calibrated for 1985-2010 set time. Also performed was the Pearson correlation between the measured retreat rates and the simulation retreat rates for the same period.

Table 1. *Measured and simulated retreat rate of Whittier Glacier during the 1985-2010 period.*

Period	Measured annual retreat ($km^2\ yr^{-1}$)	Simulated annual retreat rate ($km^2\ yr^{-1}$)
1985-1994	0.06	0.07
1995-1999	0.09	0.08
2000-2006	0.04	0.07
2007-2010	0.07	0.07

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The Pearson correlation coefficient reaches the value 0.85 and approves the observed agreement between the measurements and estimations. *Table 1* shows the measured and simulated retreat rates during 1985-2010.

This study's model returns the annual retreat rate corresponding to the parameters mentioned. The first step is finding the relationship between annual temperature and annual retreat rate for the years from 1985 to 2010. In this relationship the retreat coefficient was introduced (Rr), which is coming from the additional factor product influencing the Whittier Glacier retreat. Thus, the precipitations, the latitude, the aspect, and the altitude was abstracted in the Java programming language, considering at the same time the retreats of Learnard and Billings glaciers, which are located in the same fjord with Whittier Glacier. The Equation 1 was used in the future predictions of Whittier Glacier and this equation may be used for various glaciers from the different places of the world. In the program code, the Rr was constructed to increase proportionally with set values only if the rainfall, latitude, and altitude decrease and also if the slope aspect of the glacier is changing from 0 to 360 degree. The various coefficients for the Rr factors (Eq. 2) of rainfall, altitude, and altitude were attributed in an aim to homogenize the calculation and to have the class range of these parameters. The model considers also the inverse impact of global position related to the glaciers situated in the Northern hemisphere and to the glaciers situated in the Southern hemisphere.

$$\textit{Annual retreat rate} = 0.0076 * T + Rr \qquad \text{(Eq. 1)}$$

T temperature [°C]
Rr retreat coefficient

$$Rr = Rp \times Ra \times Reas \qquad \text{(Eq. 2)}$$

Rp Retreat precipitation coefficient
Ra Retreat altitude coefficient
Rea Hemisphere-latitude and aspects coefficient (see Eq. (3))

$$Rea = Re \times Ras \qquad \text{(Eq. 3)}$$

Re Hemisphere-latitude coefficient
Ras Aspect coefficient

Results

The GIS and Landsat satellite images were used to reconstruct the historical Whittier Glacier outlines (*Figure 4*). The GIS measurements indicate that the terminus retreat in north-eastern part has a value of 1446.22 m and in north-western part a value of 520.47 m during the 1973-2014 periods. This is possible because of the flat form and large extension of the Whittier Glacier, which influence the thickness reduction in the glacier north-eastern sector. The past Whittier Glacier behaviour analysed through satellite images and various retreat rates were carried out in different set times. In 1973, an area of 9.652 km^2 was extracted and until 1985 the glacier diminished with 1.092 km^2. The Whittier Glacier retreat rates registered in the past four decade values between 0.038 – 0.655 $km^2\ yr^{-1}$. The most striking retreat was in 2010', when the retreat rate reached the

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maximum value (0.655 km^2 yr^{-1}). Climatological data show no significant modifications in temperature trend that are almost constant from 1960 to 2010 (*Figure 5*) and a value around 5° C of annual temperature was calculated for this period. The changes in rainfall trend identified, as to expect from the glacier melting area. Due to the slight decrease of precipitation after the year 2000 and constant temperature above 0 °C, Whittier Glacier shows a continuous retreat from 1973.

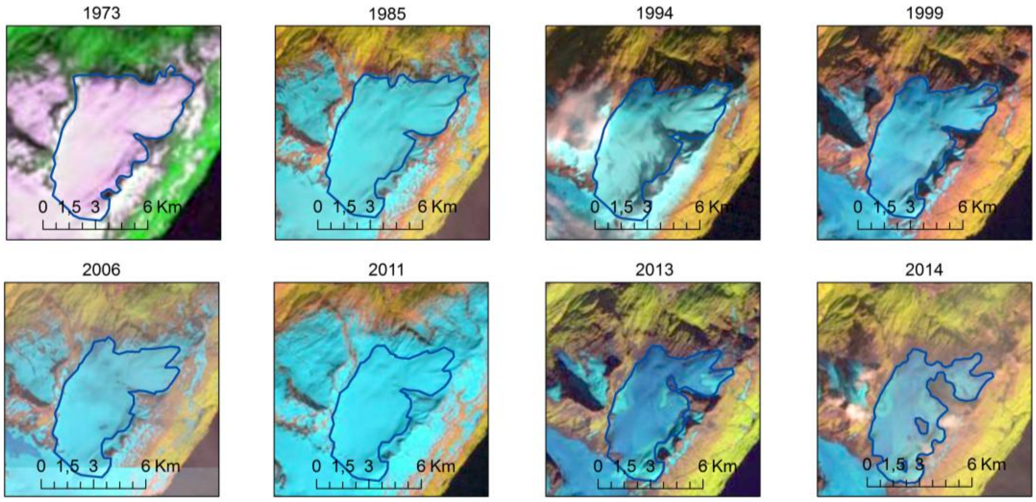

Figure 4. *Landsat mosaic images of Whittier Glacier and outlines in 1973, 1985, 1994, 1999, 2006, 2011, 2013 and 2014. Landsat images courtesy of the U.S. Geological Survey.*

A correlation between temperature, precipitation, and melting area was important to carry out in estimating the Whittier Glacier future retreat. From the climate data analysis, it was observed that the annual mean temperatures are not changed significantly. The most important changes were identified from the mean annual precipitation in 1986-1995 and 2001-2010 time intervals (*Figure 5a*). A reduction in precipitation from 5834.63 mm to 4910.24 mm were found in the analysis of both set times. These findings are important in the negative balance of Whittier Glacier because even if the temperatures are changed a little (*Figure* 5b), the glacier cannot be extending more in an area without precipitation and snowfall during the cold seasons. The snow depth ranges from 10 cm to 85 cm, with a slight decrease after 2000 years (*Figure 5c*). In contrast, if the Whittier Glacier extends because of the high melting rate, the ice volume diminished considerably. On the other hand, from the mean monthly data analysis along 1960-2010 is observed that the precipitation in summer ranged between 267.71 mm and 371.09 mm (*Figure 6a*), while the temperature in summer period exceeded 10 °C and in July, in August the temperature was higher than 12 °C (*Figure 6b*) and snowfall was 0 mm (*Figure 6c*). This fact drastically influences the glacier retreat because of the water flow action on glacier ice and no accumulation in ice mass.

Starting from the glacier annual retreat data rate and future model climate analysis offered by The Canadian Centre for Climate Modelling and Analysis (2015a) and The Canadian Centre for Climate Modelling and Analysis (2015b), it projected the

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precipitation and temperature data, further used in the glacier modelling. The temperature and precipitation projections for different years up to 2100 are reported in *Table 2*. Based on the future climate data projections the annual retreat rate and total retreat area of Whittier Glacier were calculated.

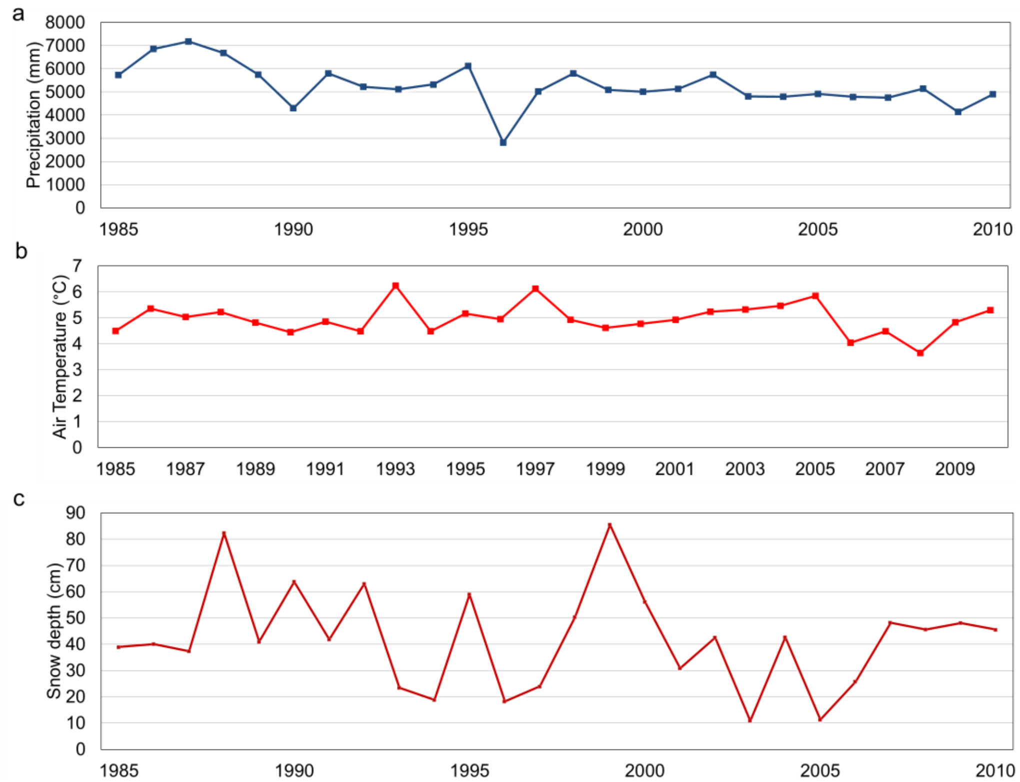

Figure 5. a. *Annual mean precipitation measured at Whittier station between 1985-2010.* ***b.*** *Annual mean air temperature measured at Whittier station between 1985-2010.* ***c.*** *Annual mean snow depth measured at Whittier station between 1985-2010. Source: Western Regional Climate Center.*

In the future, it was found that a value of the annual retreat rate of 0.065 km² yr-1 for 2014-2030, a value of 0.058 km² yr-1 for 2031-2080, and a value of 0.059 km² yr-1 for 2081-2100 and a total retreat area of 5.1524 km² is expected to melt by the end of this century.

Table 2. *Temperature and precipitation projections for 2030, 2040, 2050, 2080, and 2100.*

Year	Relative change (T °C)	Mean temperature projection (°C)	Relative change PP (mm/year)	Mean precipitation projections (mm/year)
2030	0,02 (+)	4,9317	73 (+)	5338.692
2040	0,03 (+)	4,98005	0	5265.692308
2050	0,03 (+)	4,98005	0	5265.692308
2080	0,05 (+)	5,07675	0	5265.692308
2100	0,06 (+)	5,1251	91,25 (-)	5174.442308

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A total melting area of 1.10544 km2 up to 2030 and 2.2184 km2 up to 2050 was estimated. This study's model predicts 3.9704 km2 for 2080 and 5.1524 km2 for 2100 of total loss area. A volume of 0.3393 km³ will melt between 2014 and 2100 under the present century climate condition. The main spatial melting area for the future was identified in the north, north-eastern, and the eastern part of the Whittier Glacier, while in the southern part the melting area is minor. The high spatial reduction is due to the flattened shape of the Whittier Glacier, with inconsistent thickness. The existing "inselberg" landforms in the glacier slide bed of the Whittier Glacier will contribute to the radial regression of ice mass in the eastern and southern area especially for 2030, 2050, and 2080 outlines projections. *Figure 7* highlights the predicted Whittier Glacier outlines for 2030, 2050, 2080, and 2100. For the year 2100 it will be expected that the main ice mass of Whittier Glacier will be in the south-western part compared with the actual position. In *Figure 8* are drawn the future outlines of Whittier Glacier on an aerial photograph.

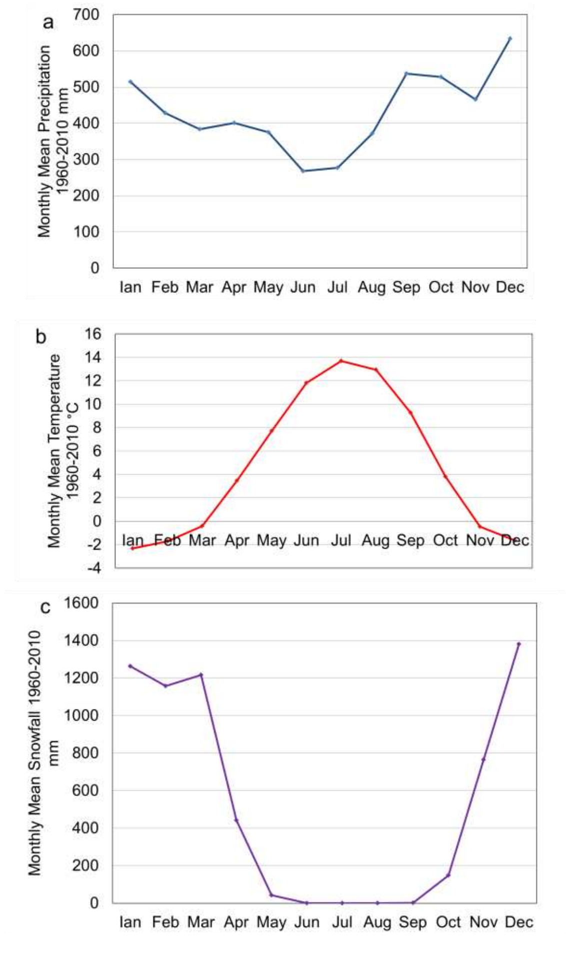

***Figure 6.** Climatological monthly data between 1960-2010 measured at Whittier station. **a.** Monthly mean precipitation measured at Whittier station between 1960-2010. **b.** Monthly mean air temperature measured at Whittier station between 1960-2010. **c.** Monthly mean snowfall measured at Whittier station between 1960-2010. Source: Western Regional Climate Center.*

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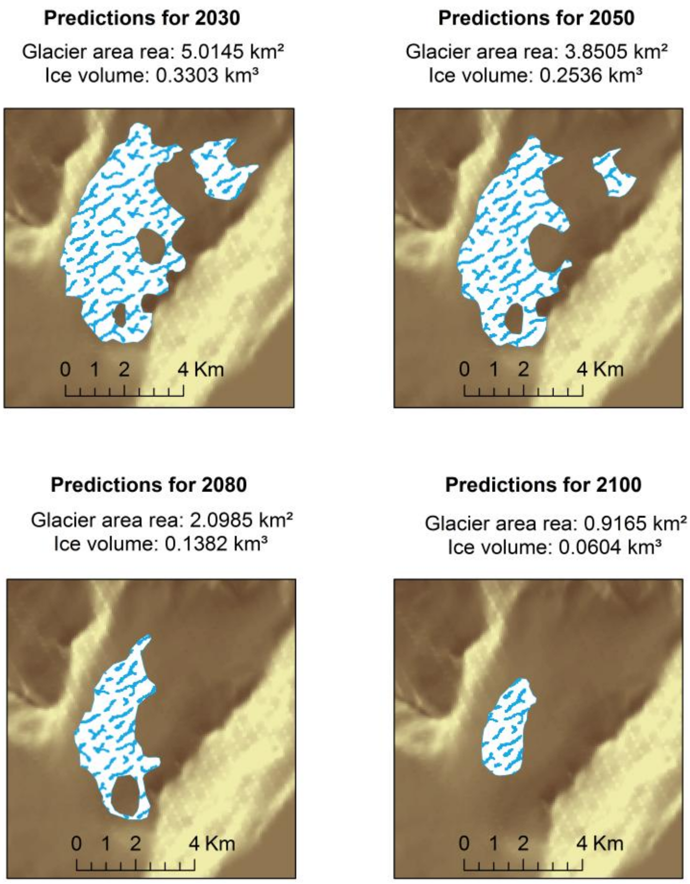

Figure 7. *Future projections of the Whittier Glacier outlines for 2030, 2050, 2080, and 2100.*

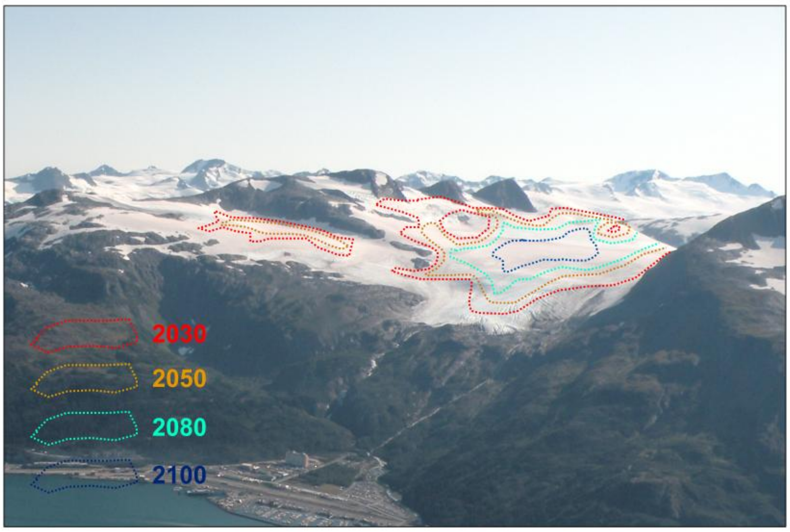

Figure 8. *3D view of Whittier Glacier outlines predictions for 2030, 2050, 2080, and 2100. Photo courtesy: Nistor.*

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Discussion and conclusions

Comparing the results with available climate data from the Whittier meteorological station during the last 40 years, the glacier behaviour under climatic conditions was understood. Based on these findings and field characteristics, the model that will serve future periods was calibrated. The recent global climate changes and continuous warming in the arctic regions results in the ice melting of large areas. Temperatures and precipitations are main climatic data that influences the glacier behaviour. During the last century, many glaciers of the entire world registered high lose areas related to climate warming (Haeberli, 1999; IPCC, 2001; Oerlemans, 2005). Many future scenarios show an increase of 1 to 3 °C in global temperature trend (IPCC, 2007). For the current century was predicted a rise in temperature and a decrease in precipitation quantity in North America. In addition to climate warming, population growth and its impulsive activity on landscape contribute to glaciers modifications in the 21st century.

Due to climate data temperature projections and precipitation based on models, this study's predictions for future Whittier Glacier outlines and retreat areas have some limitations. In this study, obtaining the Whittier Glacier 3D model is difficult, because of missing hole measurements, but also for more elasticity of ice during hot periods and complex flow glacier movements on the surface roughness of sliding bed. At the same time, the Whittier Glacier spatial retreat area shows the general tendency of future outlines, a fact for which this study's projections did not indicate the top glacier surface.

In contrast, this study's model strait is that the method integrates both glacier field characteristics and the Whittier station climate data. Moreover, this study's methodology for glacier modelling could be applied to other glaciers without loss in performance. The oriented object program was implemented in Java Eclipse to predict the glacier retreat rate related to the Whittier Glacier under geographical position, climate conditions, and field characteristics. The past outlines of Whittier Glacier changes were delineated using satellite images and GIS applications. A new method was proposed to estimate the sensitive melting area and major retreat areas through 'Central Mean' of squares networks applied on vector data outlines of the glacier.

The first and most important conclusion carried out in this study is the Whittier Glacier's drastic reduction in the 21st century. From this finding it would seem that a high discharge of the Whittier Creek and other streams threatens the Whittier town by possible flooding and erosion. Moreover, the marine biomes and the salmon prohibition of Passage Canal could be seriously affected by heating the water in this fjord. Considering the Whittier Glacier's characteristics and local meteorological data conditions, the present paper aims to contribute to glacier modelling based on GIS and Java programming. This model was calibrated to the Whittier Glacier and adapted for use in any of the world's glaciers.

Acknowledgements. We want to express sincere gratitude to Assoc. Prof. Bogdan Enescu for the review of the paper and his suggestions that improved the manuscript. Many thanks are coming also to Alex Berni for supporting in figures editing, but also to the COGNOSCENTI PROOFREADING Org. for the English corrections. We would also like to thank Western Regional Climate for climate data and we thank to U.S. Consulate from Bucharest for the visas support in 2009 and 2010, that were indispensable for the field research. We mention also that Ştefan Dezsi's contributions for this paper are the same like the first and corresponding author.

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APPENDIX

The climate data used in the paper for climatic analysis belongs to "Western Regional Climate Center". Monthly summary data lister (SOD-TD3200) of temperature during the 1985-2010 period at Whittier meteorological station from Alaska can be found using

URL: http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ak9829.

The future projections of climate were done based on climate models carried out by "The Canadian Centre for Climate Modelling and Analysis". The useful links of these data are:

URL: http://www.cccma.ec.gc.ca/diagnostics/cgcm2/animation_st_na.shtml.

URL: http://www.cccma.ec.gc.ca/diagnostics/cgcm2/animation_pcp_na.shtml.

The Landsat images are courtesy of United States Geological Survey. The satellite images can be found on the LandsatLook Images web site.

URL: http://landsatlook.usgs.gov/.

Photos with Whittier Glacier taken during the field survey. Pictures courtesy by Nistor, M. M.:

Whittier Glacier terminus in 2010: Northern side (left) and North-Eastern side (right).

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'Moutonnée rochers' and similar landforms presented at terminus of Whittier Glacier (2009 and 2010 years).


Epi-karst landforms of ice mass formed at terminus of Whittier Glacier (left). Slide bed of Whittier Glacier at terminus (right) (2010).


Oblique east-looking ground photograph of Whittier Glacier (left). North-looking ground photograph of Whittier Glacier and the corresponding author picture during field survey (right) (2010).

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GENETIC AND BIOCHEMICAL DIVERSITY OF *HYPERICUM PERFORATUM* L. GROWN IN THE CASPIAN CLIMATE OF IRAN

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Abstract. *Hypericum perforatum*, also known as St John's wort, is an important medicinal plant that produces many secondary metabolites that have anti-viral, anti-bacterial, and anti-depression properties. The most effective drug substance in this plant is hypericin that changed in the various genotypes. To investigate the genetic and biochemical diversity of *Hypericum perforatum*, samples were collected from different genotypes around the Caspian climate region. A total of 15 genotypes from the Hyrcanian Province in the Caspian climate region were evaluated using 15 randomized amplified primers design (RAPD primers). A total number of 90 bands with the average of bands amplified by each primer was 9-band. The number of polymorphic bands per primer ranged from 1 to 13 and the bands were 250 to 3000 bp in size. Based on the results, OPAD-10 primer with 13 bands and OPV10 with 7 bands were used as the maximum and minimum number of amplified fragment, respectively. Molecular marker genotypes showed a high degree of polymorphism. Based on the RAPD results, the genotypes were divided into 4 groups. Most similar genotypes with a coefficient of 75% were in subgroup A_3 (Noshahr and Kelardasht). Variation in hypericin was very significant in the genotypes grown under identical conditions. The highest amount of hypericin was measured in the Kelardasht genotype and the lowest amount in the Roodsar genotype.
Keywords: *biodiversity, population genetics, St John's wort, RAPD, plant, genotype*

Introduction

The side effects of synthetic drugs have led to the widespread use of medicinal plants and several drugs with plant origin have been recently produced and marketed. *Hypericum perforatum*, which is part of the family Hypericaceae, is one of the most important plants in the pharmaceutical industry. It is produced by developed countries and can be found as an ingredient in several anti-depressants (Azizi and Omidbaigi, 2002; Tonk et al., 2011). There are about 200 medicinal plants in the Hypericaceae family. The flowers are widely appearing to coincide on June 24th (St. John's birthday) (Bais et al., 2003). Tiny spots on the petals, which can be observed as dark lines in the secretory glands, contain terracotta-colored sap. This is the secondary metabolite that contains a substance called hypericin (Omidbeigy, 2001; Mitch, 1994). Hypericin is one of the most important active substances that nowadays, are an integral part of biologically active substances, are of great interest. They can reduce mutagenic influence, regulating the oxidation process of free radicals (Mairapetyan et al., 2016). In Greek history, Discourse, Polini, and Hippocrates were using hypericin (Weed, 2000). Until about a century ago, for the first time in Germany for industrial products and about three decades ago, it was used in many products marketed in the US and European countries (Peterson et al., 2001; Vardanyan et al., 2014). Since ancient times, this herb has been used to treat wounds, burns, abdominal pain, and bacterial diseases (Gleason et al., 1991; Mitch, 1994, Stanley et al., 1997). Recent evidence has shown its clinical and pharmacological effects in anti-depressant and -viral medications. Such properties are attributed to the particular combination of the same hypericin (Gleason et

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al., 1991). *H. perforatum* can be found at high altitudes; and in Iran, it is scattered throughout the northern latitude. Due to climatic and environmental factors and interactions with phisiomorphologic-specific plants in Northern Iran, *H. perforatum* has biochemically adapted to the area, which has resulted in the formation of many important phenolic compounds such as hypericin (Zargari, 1996). *H. perforatum* is exposed to a high degree variation of genotypic and phenotypic, particularly among varied populations (Walker et al., 2001). For example, some researchers have reported that the qualitative variation between the two subspecies of *Hypericum* (*H. perforatum* ssp. and *H. veronense* ssp.) with small oval-egg leaves there are not hypericin and hyperforin (Couceiro et al., 2006). However, factors such as geographic location for growing the plant, harvest time, and at the second level of importance, the second metabolites production conditions are important (Filippini et al., 2010). In this respect, several studies were conducted in relation to the differences of hypericin (Campbell et al., 1997; Southwell et al., 1991; Jensen et al., 1995). The results showed that the amount of hypericin produced can change due to varying environmental conditions (Erken et al., 2001). Different possibilities of hypericin in genotypes should be considered on influencing factors: environment, genotype, or their interactions (Buter et al., 1998).

Currently, morphological, biochemical, and DNA markers are used to identify genetic diversity in plants. Molecular markers are preferred over other methods because they are infinite and do not depend on the growth period of the plant and environmental conditions. Therefore, molecular markers are used extensively in studies on genetic diversity (Aas et al., 1994; Ghalachyan et al., 2014). Genetic diversity is necessary for plant breeding derived from natural evolution and is an important biological system component of sustainability (Rubatzky and Yamaguchi, 1997). Other advantages of genetic diversity include species conservation management, within-species genetic diversity knowledge, assessment danger of extinction, and evolutionary potential (Hedrick, 2001). RAPD markers are a widely used in the genetic evaluation because they use small amounts of DNA to identify variations between plants at the DNA level and do not require information of the genome of interest. RAPD applications in plant breeding consist genetic mapping, Marker Assisted Selection (MAS) and transfer of useful genes and germplasm evaluation (Boonparkob, 1996; Garcia et al., 2004). RAPD marker is based on DNA amplification by non-specific primers and uses the polymerase chain reaction. Advantages of this marker include simultaneous assessment of multiple loci in the genome samples, no probing, radioactive materials, the low cost, and also is the speed of application execution from a special position in the molecular evolution of genetic diversity (Williams et al., 1990). One disadvantage of the RAPD markers is the low reproducibility (Naghavi et al., 2005). In recent years, numerous studies using RAPD markers have been used to assess the genetic diversity of medicinal plant species such as *Achillea fragrantissima* (Morsy, 2007), *Satureja hortensis* L. (Hadian et al., 2008), *Carthamus tinctorius* L. (Maali Amiri et al., 2001), *Ocimum gratissimum* L. (Vieria et al., 2001), *Ferula gummosa* Boiss (Talebi Kohyakhy et al., 2008), and *H. perforatum* clones (Tonk et al., 2011).

Since the *Hypericum perforatum L.* as well as distributed in the region of Hyrcanian in Iran, but yet not been conducted to identify in plant varieties, until preparing superior cultivar to replicate cultures spread and utilization of medicinal. The aim of this research was to discover distant cultivars that can be used in an ongoing *H. perforatum*

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L. hybridization program and hypericin content in the Hyrcanian located in the Caspian climate north of Iran.

Materials and Methods

Plants and Growth Conditions

Plant materials of *Hypericum perforatum* L. were collected in September-October of 2014 from 3 provinces and 15 places (*Table 1, Fig.1*) in the north of Iran (Hyrcanian Province). Temperatures ranged from 20°C to 25°C. Leaf samples were placed on ice, during transport to the laboratory of Islamic Azad University Tonekabon Branch (IAUTB). Reference specimens were placed in the (IAUTB) herbarium.

Table 1. *The clone collected from different provinces in Hyrcanian*

Roudsar 110 m	Gonmad 1660 m	Hashtpar 1610 m	Astara 1430 m	Lahijan 1050 m	Ghaemshahr 240 m	Siahkal 1870 m	Noshahr 1230 m	Ramsar 2470 m	Golestan 1640 m	Sari 55 m	Rasht 230 m	Kelardasht 1860 m	Naharkhoran 1540 m	Amol 260 m
P_{57}	P_{53}	P_{49}	P_{45}	P_{41}	P_{37}	P_{33}	P_{29}	P_{25}	P_{21}	P_{17}	P_{13}	P_{9}	P_{5}	P_{1}

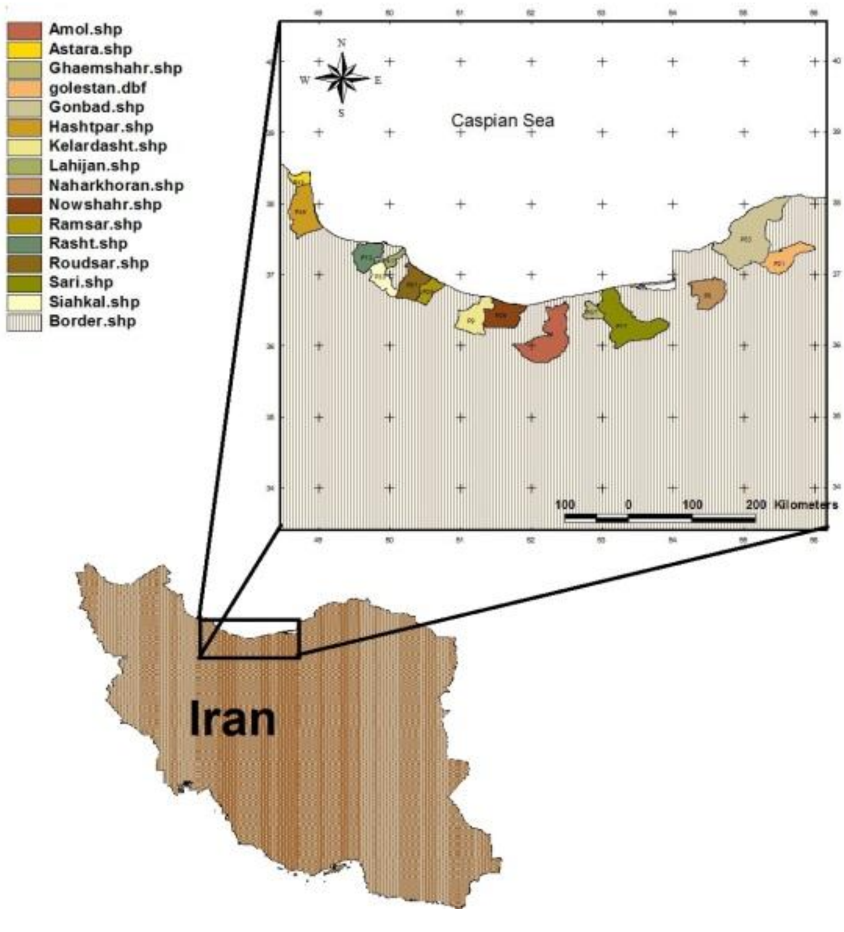

Figure 1. *Hypericum perforatum L. sampling sites under Caspian climate*

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DNA Extraction and RAPD Analysis

Fresh leaves from mature plants were frozen in liquid nitrogen and stored at -80°C. DNA was extracted using the method described by Khan et al. (2004). To decrease the effects of secondary metabolites in the process of extraction and to prevent DNA degradation as well as the subsequent inhibition of the polymerase chain reaction (PCR), we used a protocol described by Cheng et al., (2003). Co-precipitated RNA was separated, adding 0.5 units of RNAse per sample. The DNA extracted was dissolved in TE and for quality assessment, 12.5 ng/µl DNA in ddH_2O was verified spectrophotometrically. Twenty-five primers that were 10 bases in length (GENEray biotechnology Co.) were chosen (*Table 2*). The selection of primers was made from a primer pool that gave strong and consistent amplification. PCR was performed using a thermal cycler (Bio RAD, MyCycler) and a reaction volume of 15 µl that contained 4 µl plant genomic DNA, 7.5 µl ready-to-use master mix (Cinnagen Co., Tehran, Iran), 1 µl primer (concentration of 0.5 mM), and 2.5 µl ddH_2O. The conditions used were as follows: one cycle for 5 min at 95°C and 40 cycles of 1 min at 94°C, 2 min at 34°C, and 2 min at 72°C. Cycling was finished with a final extension for 10 min at 72°C. The PCR amplification products were separated via electrophoresis using 1.5% agarose gels. The DNA was stained with ethidium bromide and photographed under UV light in a gel documentation system (UVIdoc, UK).

Table 2. *RAPD analysis with 15 Primers*

No.	Primers	Primer (3′-5′) sequence RAPD	Amplified bands per primer	Polymorphic	Polymorphism %	PIC*	Tm (°C)*
1	OPAA10	TGGTCGGGTG	11	11	100	.39	31.52
2	OPAD10	AAGAGGCCAG	13	13	100	.41	24.24
3	OPM10	TCTGGCGCAC	8	7	88	.35	32.77
4	OPV10	GGACCTGCTG	7	5	71	.31	24.51
5	OPZ10	CCGACAAACC	8	8	100	.40	25.75
6	B12*	CCTTGACGCA	5	1	20	.12	27.94
7	E09*	CTTCACCCGA	5	2	40	.18	25.95
8	A04*	AATCGGGCTG	4	1	25	.14	30.91
9	A07*	GAAACGGGTG	6	2	33	.21	25.75
10	OP-B12	CCTTGACGCA	8	7	88	.36	27.94
11	OP-C11	AAAGCTGCGG	10	8	80	.30	32.28
12	OP-C19	GTTGCCAGCC	8	7	88	.34	30.93
13	OP-D20	ACCCGGTCAC	9	8	88	.33	28.38
14	OP-Q11	TCTCCGCAAC	8	7	88	.34	26.24
15	A12*	TCGGCGATAG	5	1	20	.11	28.17
	Mean		9	8.1	89.1	.35	28.43

*These primers were not entered in the analysis. *PIC: Polymorphism Information Content. *Tm: Temperature melting.

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Data Analysis

All the samples scored for the presence or absence of RAPD fragments with UVIsoft (version 12.6), and the data entered into a binary data matrix as discrete variables ("1" for the presence and "0" for the absence of a homologous band) with Excel (version 2003). Jaccard's coefficient of similarity calculated with Popgen software (ver. 1.44), and the species grouped by cluster analysis using based on Nei's unweighted pair-group of Arithmetic Means Averages (UPGMA) method and DICE Similarity coefficient with NTSYS Ver.2 Polymorphism Information Content for each primer combination was calculated from the formula PIC=1-$\sum_{i=1}^{n} Pi^{2}$ (*Pi*: allele frequency, n: the number of bands) (Anderson et al., 1993).

Hypericin Analysis

To evaluate the effect of genetic variations on production Hypericin, Cuttings are taken from of the genotypes mentioned provinces (5-8 cm length cuttings) were cultivated in grow bags (Depth of 50 cm and the area of square 0.5 m^2) filled with homogenized soil (*Table 3*). Genotype samples were cultivated in the geographic profile 39 S 0483774, UTM 4074954 (Tonekabon) and 25 meters from the sea level was selected. This experiment was conducted to completely randomized design with three replications, hypericin measurement samples were taken from flowering branches. Hypericin percentage was determined by the method of the European Pharmacopoeia (2008). The concentration of hypericin was calculated and compared with hypericin standard (Roth).

Table 3. *Soil Profile*

Soil texture: Loam													
Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Sampling depth cm	E.c (ds/m)	pH	N %	OM %	P (ppm)	K (ppm)	Sand %	Silt %	Clay %
27	6.28	1.04	.6	0-30	.21	7.18	6	33.3	42.17	121	56.5	32.3	11.2

Results

DNA Analysis

From the 15 RAPD primers used in this study, 10 primers were suitable and used in the analysis for all genotypes with a total of 90 different bands. Using a primer pool, 25 primers were identified as suitable for analysis and used to amplify a total of 90 different bands for all the genotypes. The mean number of bands for each primer was 9 bands. The number of polymorphic bands per primer ranged from 1 to 13 and the bands were 250 to 1000 bp in size (*Fig. 2*). The amplified bands are shown in Table 2. Based on the results, OPAD-10 primer with 13 bands and OPV-10 with 7 bands were the maximum and the minimum number of amplified bands, respectively. The lowest frequency among the polymorphic locus was 0.025 in the OPAD-10-2 and OPC-19-5 loci and OPAD-10-11 showed the highest frequency rate of 0.953. The mean of amplify frequency by RAPD primers are shown in *Fig. 3*. The value for each set of primers was the mean of amplification frequency of all loci related to the primer.

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Polymorphism information content (PIC) in the total population of 0.31 in primer OPV-10 to 0.41 for OPAD-10 was varied with the mean equal 0.35 for all primers (PIC > 0.1 indicates heterozygosity). Nei's gene diversity (H) is one of the important indicators in determining allele diversity. H values for each primer, which was obtained by averaging the H values, were calculated for each primer. The lowest H value (0.3011) was in the OPV-10 primer. The value of H estimated in each primer matches with the PIC, so that the primer OPAD-10 has the highest amount of PIC and equal to H (0.4001) (*Table 1*). Cluster analysis was performed using UPGMA and the Jaccard's similarity coefficient was calculated, with the highest correlation Cophenetic coefficient of 0.831. The dendrogram (*Fig. 4*) obtained from the RAPD primers were sectioned at 0.67 similarity coefficient and the genotypes were grouped into four main groups: A, B, C, and D (*Fig. 4*).

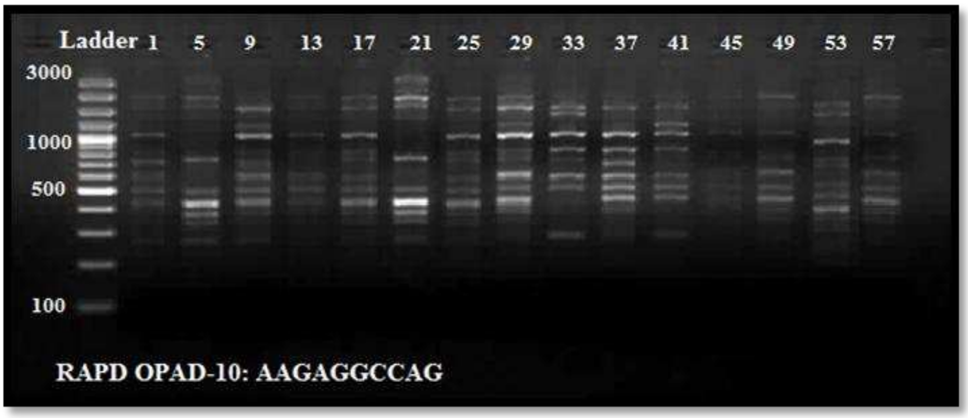

Figure 2. *Banding pattern of amplified genomic DNA for Hypericum perforatum with using OPAD-10 RAPD primers.*

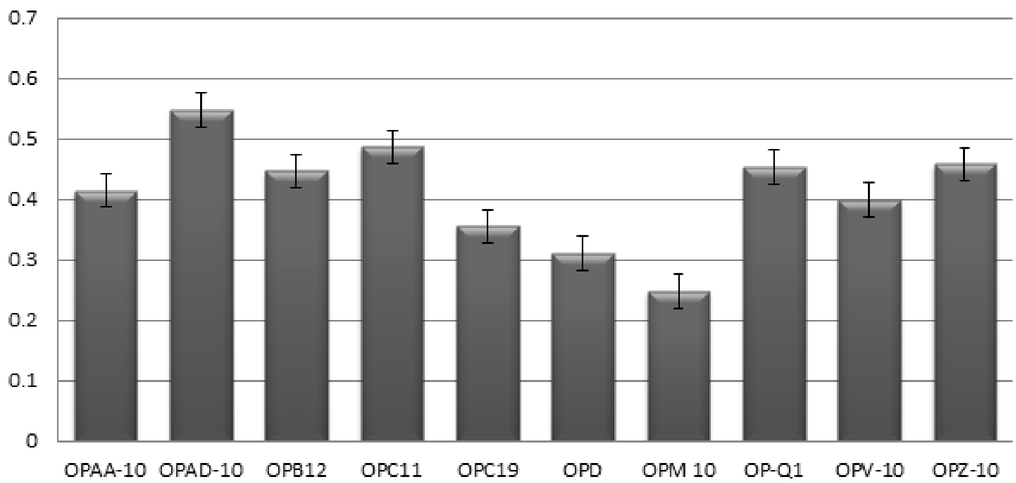

Figure 3. *Mean of Amplify Frequency by RAPD Primers*

The A group consisted of three subgroups, A_1, A_2, and A_3, and contained 40% of the genotypes. A_1 consisted of P_1, P_{13}, and P_{17} genotypes. It was separated from the other

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two subgroups by a similarity coefficient of 76%. In this subgroup, P_1 and P_{13} genotypes have a greater affinity towards each other. A_2 had a similarity coefficient of 72% with genotype P_{25}, which is separated from A_3. A_3 had a similarity coefficient of 75% with genotypes P_9 and P_{29}. The B group contained two subgroups, B_1 and B2. This constituted about 40% of genotypes with 69% similarity coefficient. 'The B_1 subgroup contained P_{33}, P_{37}, and P_{41} genotypes. P_{37} and P41 genotypes were the most similar to one another. The B_2 subgroup included P_{45}, P_{49}, and P_{57} genotypes. P_{49} and P_{57} genotypes were very similar to each other. The third group C contained 6.7% of the genotypes and had a similarity coefficient of 62%. P_{53} was placed in this cluster. The fourth group (D) contained 13.3% of the genotypes and had a similarity coefficient of 76%. P_5 and P_{21} genotypes were part of this group. According to the dendrogram, the genotypes were separated into groups and subgroups. As a result, it becomes clear, cluster analysis confirmed that the analysis of molecular similarity and genetic distance (calculated by the software is POPGENE).

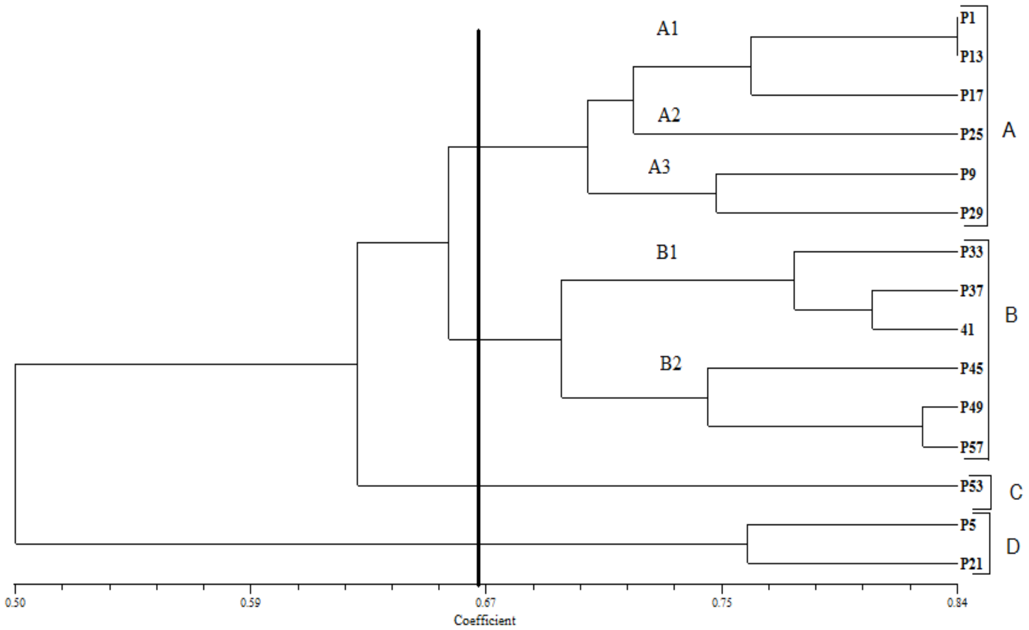

Figure 4. *Dendrogram obtained by cluster analysis based on the presence/absence matrix. The numbers on the left side correspond to different genotypes.*

Arrangements of the 15 cumulative population using genetic similarities based on the RAPD primers are shown in *Fig. 5*. This figure shows that the principle coordinates analysis and the drawn two-dimensional plots are similar to the cluster analysis results. The aggregation of *Hypericum* genotypes in one area of the two-dimensional plot indicates that these genotypes are genetically similar. In this study, the maximum similarity was observed between regions P_1 and P_{13}. Since these two populations of morphological traits are different, i.e., leaf length and width, the distance between the upper and lower leaf and black leaf glands are similar, but in terms of the growth area. In this regard, there may be several factors, including the movement of seed in these two regions or do not cover of the primer (due to the inadequacy of the number and function) for the entire genome. In other populations, the highest similarity was

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observed between populations P_{49}-P_{57}, P_{41}-P_{37} and P_5-P_{21}. According to the genetic similarity in different climates, we can conclude that molecular diversity was not associated with geographical distribution.

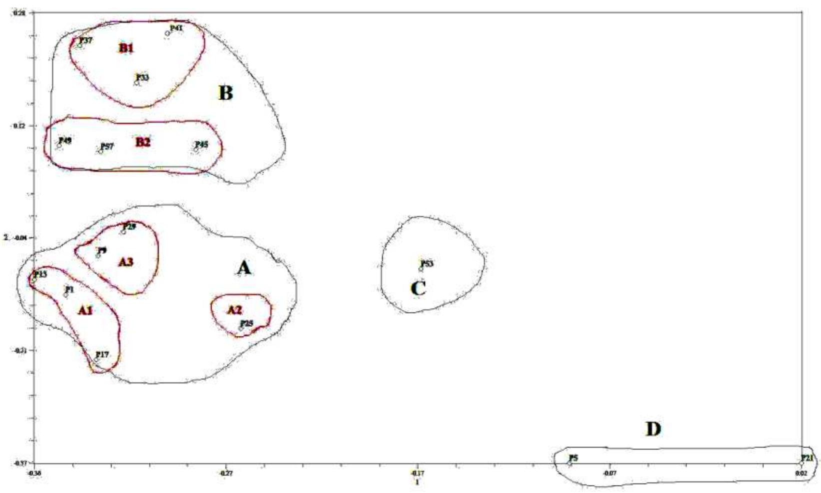

Figure 5. *The two-dimensional scatter plot of the principal coordinates analysis RAPD primers.*

Hypericin Measurement

Hypericin changes were very significant in genotypes grown under identical conditions. The highest amount of hypericin was measured in the Kalardasht genotype and the lowest amount in Roodsar and Noshahr genotypes (*Fig. 6*). Therefore, these changes in hypericin are related to the genotypes and not to the environmental conditions. Although the production of hypericin changes under ambient conditions, for determining superior genotypes, gives the best results from the comparison of the results genotypes cultivated under identical conditions with molecular analysis.

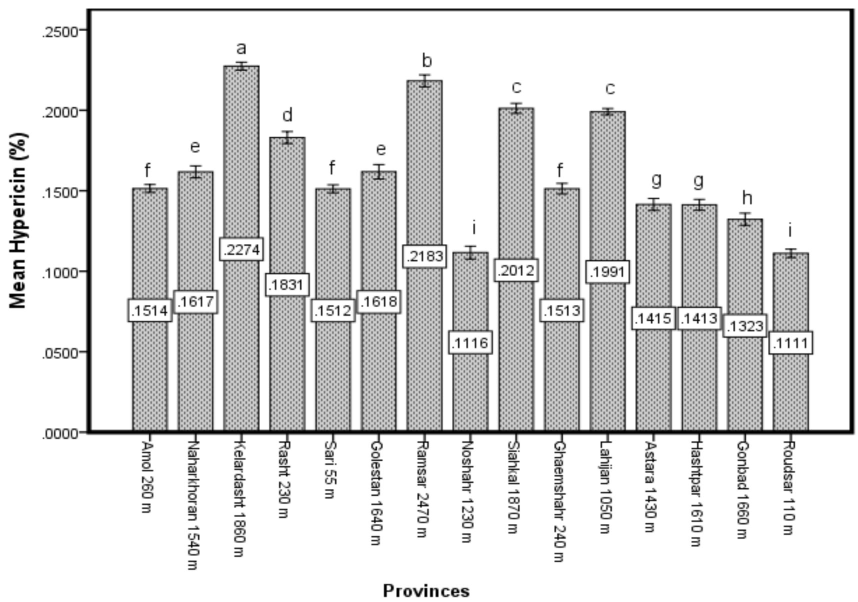

Figure 6. *Hypericin levels in different Provinces*

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Discussion

It is best to use primers that are distributed evenly throughout the genome in order to sample the entire genome, so if the markers are selected from different parts of the genome will be low between correlations. As a result, the principal coordinates analysis, a greater number of components required for variations justification in the data. Several factors affect the estimation of genetic relationships between individuals including the number of markers used such as the distribution of markers in the genome and nature's evolutionary mechanisms. Plant genetic diversity is impacted by anthropogenic climate change and its effects. Therefore, the search for gene banks containing new genotypes is required for the future of agriculture and the development of resistant plants. *Hypericum* has a high degree of phenotypic and genotypic changes. In this regard, several studies have compared the properties of *Hypericum* from California, Oregon, and Montana. Several regional differences were observed in chemical properties (i.e., hypericin content) and morphology (i.e., gland density, leaf area, stems length and ratio of leaf length to width) (Walker et al., 2000, 2001). It is worth mentioning that *Hypericum* is exposed to changes in the morphological and genetic variation and it's the response of the plant to deal with herbivores (Buckley et al., 2003). The results of the genetic screening in this study showed that there are genotypic differences between *H. perforatum* grown in different regions of Hyrcanian. This is in agreement with the results reported by Tonk et al. (2011). Our research revealed that genotypes and environmental factors can affect the efficiency of secondary metabolite production. These factors can significantly increase the concentration of hypericin in *H. perforatum* in various provinces in Iran and at different altitudes. Based on the results obtained, the best altitude for growing, genotype, location for growth, and ecotype was determined in this study.

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INVESTIGATION OF HEAVY METAL CONTAMINATION IN THE GROUNDWATER RESOURCES, OSHTORINAN, SOUTHWEST IRAN

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Abstract. For the first time, a comprehensive groundwater heavy metal and trace element study was conducted in the Oshtorinan plain situated in the north corner of Boroujerd basin located between the two important structural zones, Sanandaj-Sirjan zone in the east and Zagros in the west, aiming to investigate the impacts of natural weathering of minerals, industrial and agricultural activities on distributions of these elements. Groundwater samples were collected including ten wells, five Qanats and one spring in different seasons, and analyzed for heavy metal and trace element values using inductively coupled plasma mass spectrophotometer (ICP-MASS) and their levels compared with WHO specified maximum contaminant level. The levels of heavy metals under study in water used for drinking purpose are below the maximum allowable limits recommended by WHO except for Fe, Mg, Pb, Sr, and V.The levels of Pb, B and Fe in groundwater in the area understudy increase from east and west to center.It is found that elevated levels of the strontium concentration (Sr) in groundwater were considered to be mainly the result of natural processes such as the weathering of minerals such as feldspars in granites and Granodiorites. Wells diesel engines are a probable source of contamination in the area, because in the region about 88 wells of total 144 wells have been excavated are diesel engines. Alloy steel industries, especially in the aquifer from the north entrance that release their effluent on ground surface Could be a potential source of contaminants such as heavy and trace metals and threat the environment and groundwater.
Keywords: *groundwater quality, agricultural acivities, Sanandaj-Sirjan, rock-water interaction, trace element*

Introduction

Rapid industrialization process coupled with spiraling growth in global population has been instrumental in increased agricultural production. These have had the legacies of pollution of various kinds that has reportedly undermined the integrity of environment and eco-system. This has moreover, brought about an increasing pollution index in natural environments, such as water bodies, soil structures, air, etc. (Thibodeaux, 1996; Dawson and Macklin, 1998; Murena, 2004; Hassanzadeh et al., 2011; Wauchope, 1978). Groundwater aquifers and water bodies are among the most important sources which are under frequent threat of pollution and contamination (Momodu and Anyakora, 2010; Vodela et al., 1997). Heavy metals are said to play a major role in degrading these vital renewable resources that has attracted the attention of various research investigations. One of the major attributes of these contaminants is the severity of their toxicity even at low concentrations (Marcovecchio et al., 2007; Botté et al., 2007).

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Heavy metals are among the chemical elements with atomic weights ranging between 63.546 and 200.590 and a specific gravity greater than 4.0 i.e. at least 5 times that of water. Their existence in water can be in the form of colloidal, particulate and dissolved phases (Greenwood and Earnshaw, 2012). with their occurrence in water bodies being either of natural origin (e.g. eroded minerals within sediments, leaching of ore deposits and volcanism extruded products) or of anthropogenic origin (i.e. solid waste disposal, industrial or domestic effluents, harbor channel dredging) (Marcovecchio et al., 2007).

Increase in anthropogenic growth of the heavy metals, has called for more investigation of the effects of those pollutants in the environment (Marengo et al., 2006). Sources of heavy metals in water bodies consist of natural sources including eroded minerals within sediments, and leaching of ore deposits. Heavy metals in nature are not usually significantly hazardous to the environment and human health. Having said so, a limited amount of these heavy metals at low concentrations are necessary as catalysts for enzyme activities in human body (Co, Cu, Fe, Mn and others). Under the circumstances where the levels of these metals exceed the normal ranges, they can affect the physiological functioning of the body organs due to the toxicity they bond to bring about in the human body (Pirsaheb et al., 2013).

Oshtorinan plain within the north of Boroujerd basin with an area spanning approximately 389 km2 is bounded by the Eastern and Western different structural zones. The chemical quality of these ground water sources are affected by the characteristics of the media through which the water passes on its way to the ground water zone of saturation (Adeyemi et al., 2007). Thus, the heavy metals discharged through industrial effluents, traffic, municipal wastes, hazardous waste sites as well as unsystematic application of agri-inputs and accidental oil spillages from oil tankers. All of these have the potential in gradual rise in contamination of groundwate aquifers.

Groundwater is considered as the major source of water supply for domestic, agricultural and industrial sectors. It can be in various forms of which the wells, springs and Qanats are the principal examples. Groundwater resources of Oshtorinan are reportedly under serious threats by contamination from municipal sewage, industrial effluents and excessive application of agri-inputs. Despite the seriousness and gravity of the potential pollution hazards that these post-modern activities can bring about, no proportional investigation of the scale it deserves have been undertaken. This is certainly the case for the Oshtorinan water basin baring a summary report on the existence of major ions in the groundwater (Asarab, 2012). Based on such a background, there is an urgent need to undertake a systematic investigation of the nature of the pollutions and the manner in which they threaten the integrity of the environment and livelihood of those whose survival depends on these vital renewable sources.

The paper aims to achieve the following objectives. (i) to determine the heavy metals level in water samples using ICP_MS. (ii) to examine the spatial distribution of heavy metal level in the groundwater. (iii) To identify the potential pollutant sources. The methodology involves using a correlation between metals as a means of identifying the possible sources of metal pollutants in the groundwater. The study is undertaken with an objective of identifying the manners in which to enhance the integrity of these water bodies through systematic environmental monitoring, effective remediation, and viable planning to ensure sustainable groundwater abstraction in this strategically important region in Iran.

Materials and methods

Study area

Oshtorinan plain, is one of the small sub basin in the Lorestan province of Iran that situated in the north corner of Boroujerd basin (*Fig. 1*). The area is located between the two important structural zones, Sanandaj-Sirjan zone in the east and Zagros structure as the west border that can be affected differently by them. It is situated between 33° 56′–34° 03′ N and 48° 56′–49° 07′ E and covers 389 km2 with about 1600 m above the sea level. Average annual rainfall of the study area is around 464 mm that only fifteen percent of it is recharging to groundwater.

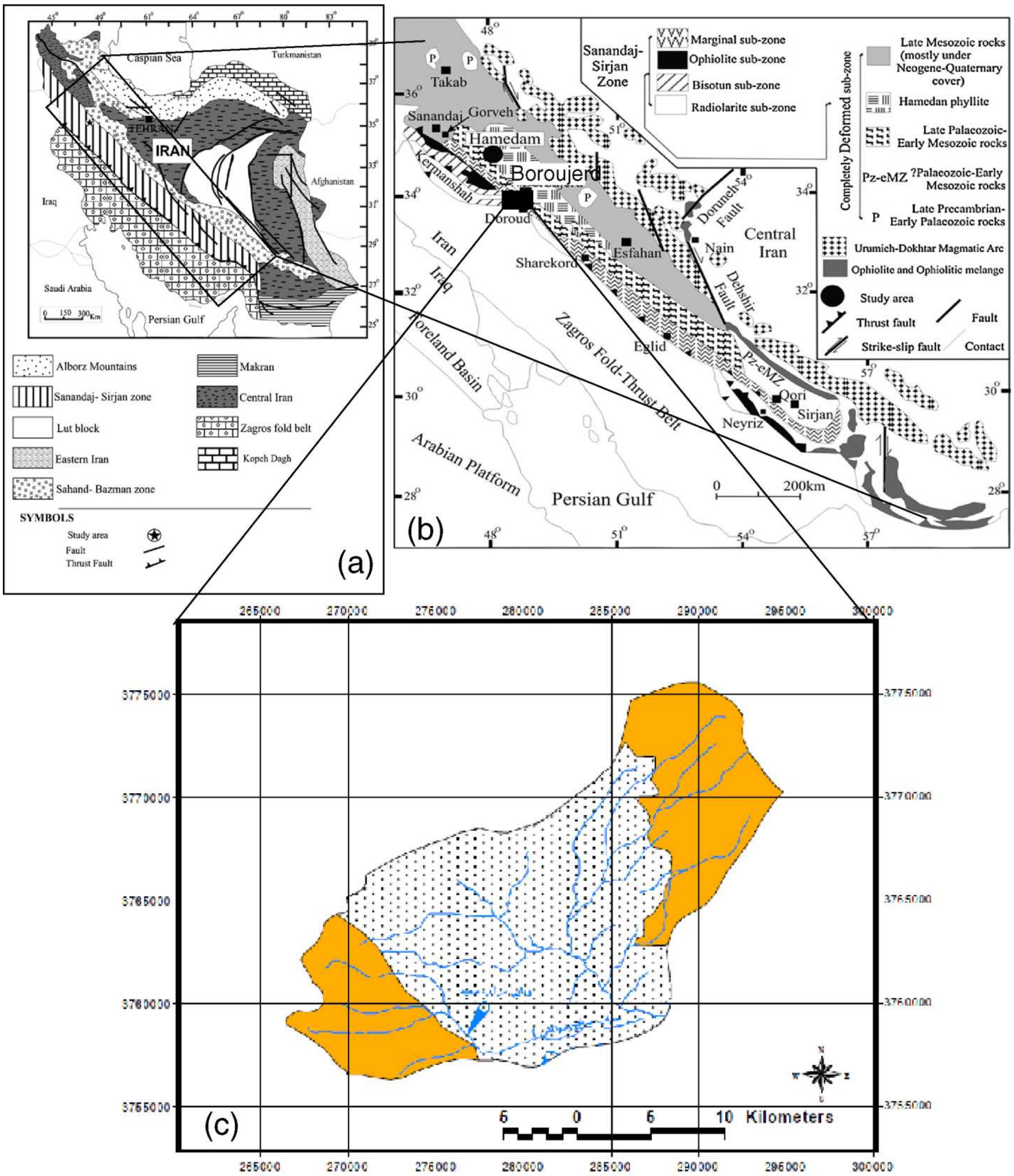

***Figure 1.a)** Generalized geological map of Iran (modified from (Khalaji et al., 2007) showing major lithostructral zones.* ***b)** Schematic geotectonic sketch of western Iran (modified from (Mohajjel et al., 2003) showing location of the Oshtorinan.* ***c)** Oshtorinan plain*

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Geology

The study area can be divided into two parts with significantly different modes of development. High unconformity of second and third era has been observed in the central Iran but none of them is seen in the Zagros. In the southwest heights, rocks are mainly composed of limestone, marl, sandstone and conglomerate with Miocene age. In the North East and East, Jurassic and Cretaceous igneous rocks consist of Granite and Granodiorite and also metamorphic rocks such as Schist, Gneiss and Hornfelse have existed. Limestone formations outcrop near Zagros with high developed cracks due to the many large springs have abundant yield, While the outcrop of igneous and metamorphic rocks of central Iran have the lower springs discharge. Sanandaj-Sirjan metamorphic area has hummocky landscape, but sometimes more intrusive rocks embedded into the rocks caused the rough morphology. Southwest and western Zagros Range Configuration is a folded mountain belt with highest heights.

Fig. 2 is a geological map of the rocks type distribution of Oshtorinan basin(Hajmollali et al., 1991). Eighteen lithological units were mapped that are, recent deposits, Hornfelse, Clay flats, Granite Granodiorite, limestone, limestone, marble, marl, sandstone, conglomerate, dotted schist, dolomite, philite, metamorphed sandstone, colored mélange, new and old terraces.

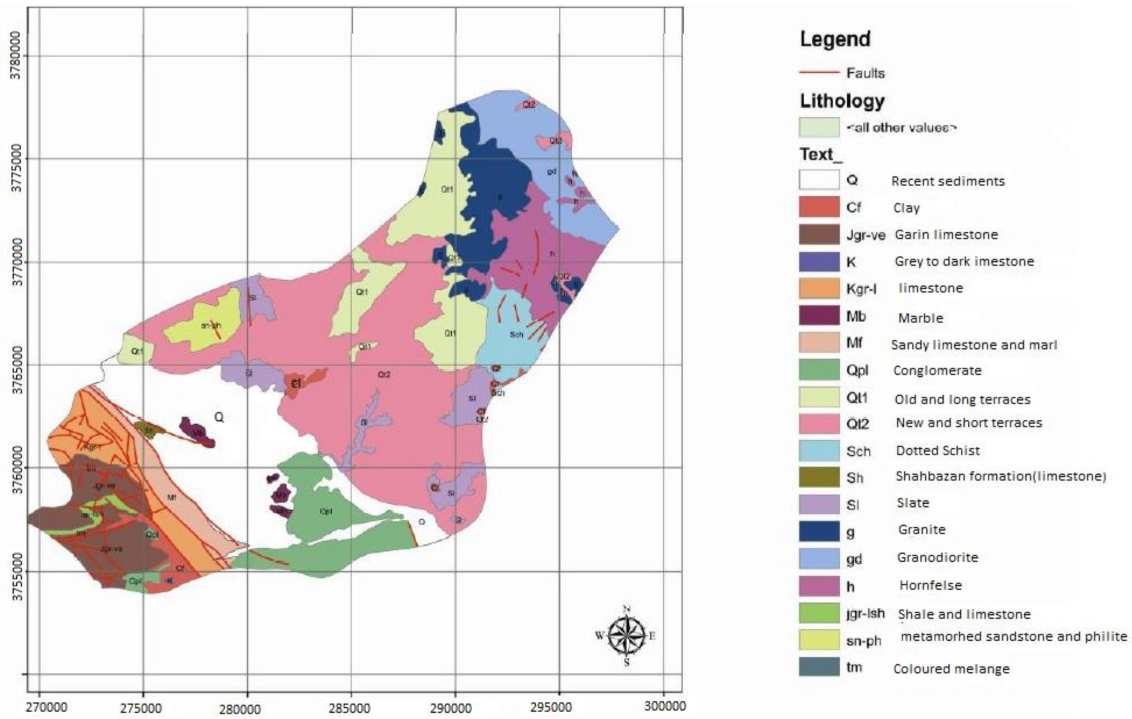

Figure 2. *Geological map of the Oshtorinan plain*

Sampling

In their study, a total of 32 samples were obtained from various resources (13 wells, two Qanats and one spring) from Oshtorinan plain to provide good spatial coverage in the studied area. During sampling all the precautions were taken according the standard

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guidelines, to avoid any possible contamination. Sample batches were regularly interspersed with standards and blanks, and all data were corrected for instrument drift. A three-point calibration curve was constructed for each element. The results of three replicate analyses indicated that the precision of cation measurements was generally better than 5%.

The latitude and longitude values for all locations are introduced in (*Table 1).* The groundwater samples were collected from the outlets of the wells after flushing water for 10–15 min in order to remove the stagnant water. Tight-capped high quality polyethylene bottles (HDPE) were used for sample storage. Before use, the bottles were washed by distilled deionized water. Samples were filtered through 0.45 mm cellulose filter paper. To prevent precipitation of metals and biological growth, few drops of concentrated nitric acid were added to samples to obtain pH around 2 (Eaton et al., 1995). Thereafter, samples were immediately transported to the laboratory in iceboxes and stored at 4° C up to analysis.

At field, the pH, Eh, temperature, electrical conductivity (EC), and dissolved oxygen (DO) of water samples were measured by using a portable multimeter (Hach- HQ40d, USA) by portable colorimeter (Hach-890) (*Table 1*). Acidified aliquots were analyzed for heavy metals and trace elements by inductively coupled plasma mass spectrometry (ICP-MS, HP_4500) except for boron (B) which was analyzed by inductively coupled plasma atomic emission spectrometry (ICPAES). Unacidified aliquot was analyzed for major anions by Ion Chromatography.

The GIS mapping technique was adopted to demonstrate the spatial distribution of major ions and heavy metals concentrations in the groundwater by using ArcGIS (version 9.3). Descriptive statistics and correlation coefficients among all the parameters were calculated using software SPSS 16 (*Table 2*).

Table 1. *Parameters measured at field*

No	Location	Source	X	Y	Z	pH	Eh	T^{oc}	EC	Do
Q1	Darehgarm	Qanat	288134	3765103	1844	8.11	-27.2	14.9	557	7.82
Q2	Deh Torkan	Qanat	289424	3766506	1925	8.14	-24.5	14.7	535	7.85
Q3	Davaei	Qanat	289488	3768166	1972	8.16	-32.4	13.4	480	7.84
Q4	Ghorchali	Qanat	290552	3769731	2074	8.19	-37.3	14.5	269	7.55
Q5	Ahmadi	Qanat	291382	3768334	1981	8.12	-29.2	15.1	381	7.45
W1	Bayatan	Well	289749	3774370	2164	7.66	-29	14.7	572	6.82
W2	Deh Yousef Ali 1	Well	283840	3772978	1914	7.88	-32.1	16.2	402	7.42
W3	Deh yousef	Well	285792	3773387	1969	7.81	-27.1	15.9	426	7.42
W4	Crashing	Well	283266	3772564	1922	7.84	-39.7	18.7	413	7.54
W5	Dehriz	Well	281887	3773040	1906	7.99	47.6	17.3	511	7.58
W6	Reza abad	Well	280051	3771338	1862	7.7	-27.7	18.3	629	7.51
W7	Tudehzan	Well	281865	3761840	1729	7.62	-29.3	19.7	1085	7.26
Q6	Qayed Taher	Qanat	276201	3764760	1807	7.34	-12.1	14.5	350	8.4
Q7	Charboreh	Well	279011	3763455	1749	7.3	-8.4	14.9	595	9.93
W8	Bondizeh	Well	278444	3759497	1768	7.82	-38.2	18.7	424	6.33
S1	Kartoil	Spring	275890	3760589	1838	8	-78	11.5	241	8.8

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***Table 2.** correlation coefficients of heavy metal and trace elemnts in groundwater*

	pb	**Mg**	**Fe**	**sr**	**V**	**B**	**EC**	**Eh**	**pH**
Pb	1								
Mg	0.203547	1							
Fe	0.023844	0.186625	1						
Sr	0.054451	0.890862	0.207162	1					
V	0.464314	-0.59098	-0.26617	-0.62216	1				
B	-0.08217	-0.28374	0.327181	-0.00609	0.123416	1			
EC	-0.08137	0.205978	0.341102	0.427316	-0.00459	0.608266	1		
Eh	0.278282	-0.40089	0.356247	-0.57399	0.342548	0.038067	0.34559	1	
pH	-0.04767	0.180383	0.423703	0.064507	-0.50253	0.041902	0.33329	0.49271	1

Results and discussion

Sources of heavy metals and trace elements in the Oshtorinrn area

Heavy metals and trace elements in subsurface environments come from natural (weathering of minerals) and anthropogenic (fertilizers, industrial effluent and leakage from service pipes) sources. In the area, industrial and agricultural activities are likely to be the major contamination sources because of the presence of these activities. The main features of the area are 1) rock sources 2) agricultural activities 3) extensive areas of pervious surfaces 4) lack of subsurface drainage systems 5) High traffic rate. Motor vehicles are likely to be the contributor to the pollutants on roads. Pollutants accumulated on road surfaces could be washed by storm as road runoff and finally collected and penetrate into the aquifer that these pollutants include heavy metals and organic chemicals (Ball et al., 1998; Preciado and Li, 2006; Leung and Jiao, 2006; Kiem, 2002;Yannopoulos et al., 2004). In this paper, focus is placed on heavy metals and trace elements. Many studies have investigated the concentrations of heavy metals in stormwater. In Iran, heavy metal contents in road runoff and plants have been studied, some studies measured heavy metals in plants bodies collected in the highways (Saeedi et al., 2009; Maleki and Zarasvand, 2008; Soltani et al., 2015). These data may help to assess the heavy metal compositions of road runoff in Oshtorinan that may be also bring them into the surrounding environments and pollute the groundwater.

6) Wells with diesel engines are probable sources of contamination in the area because in the region, about 88 wells of total 144 wells have been excavated are diesel engines. In addition, a lot of dug wells are also seen that can easily transfer pollution to underground sources and there are not performed the health monitoring, extraction and maintenance.

7) Alloy steel industries, especially in the north entrance that release their effluent on ground surface could be a potential source of contaminants such as heavy and trace metals and threat the environment and groundwater.

Some studies that reported the allowable limits of the levels of some heavy metals in drinking and irrigation water are available elsewhere (Gray, 2008; WHO, 2011). It has been found that the levels of heavy in most groundwater samples are suitable for irrigation and drinking use. However, the levels of Fe, Mg, V, Sr and Pb are to higher than the allowable limits. High levels of Fe, Mg and Pb metals could be attributed to the contribution from anthropogenic sources, Industrial and/or domestic activities may in

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the environment of the study area. It could be concluded that the levels of the most elements in the groundwater in Oshtorinan plain are suitable for all purposes. But low relative standard deviation (%RSD) of heavy metals in different sources (well, spring and qanats) is an indicator of inefficient mixing process of groundwater (*Table 2*).

Heavy metal and trace element distributions in groundwater in the Oshtorinan area

The main objective of this study is to evaluate the contaminations of groundwater in terms of heavy metal and trace element. Groundwater samples of natural springs and wells nearby hillsides are comparable to urbanized, industrial and cultivated area and then could be free from any anthropogenic effects. Thus they could be used to evaluate the presence of heavy metal and trace element contaminations in the groundwater.

Tables 3 present the statistical summary of heavy metal and trace element concentrations in groundwater samples collected in dry and wet seasons, respectively. According to analyses, groundwater samples collected in the Zagros slopes and Sanandaj-Sirjan zones had slightly higher DO content than that in the developed areas for both seasons. Most of the groundwater samples in the study area are weakly basic (pH ranged from 7 to 8.2) and oxic (DO above 5 mg/L) in nature. Since the developed areas are located downhill to the natural slopes, it seems that the distribution of heavy metals and trace elements may be modified by natural processes such as water–rock interactions, but most of the heavy metals and trace elements measured, their average concentrations in the middle parts of basin were higher than that in the natural slopes. This may be the results of natural processes and/or anthropogenic pollution.

According to measurements, some heavy metals, including Mg, V, Fe, Pb, Sr and B, in groundwater in the some areas were at significantly higher concentrations than that in the natural slopes. A few sites were even found to have Pb levels higher than the drinking water guideline value of 10 ppb recommended by WHO (2011). Some of these heavy metals could be derived naturally and suggested that Mg could be released by incongruent or disproportionate reactions from silicate or oxide minerals and emerge as potential residence-time indicators. Besides, some elements are redox-sensitive and local chemical conditions could affect their availability and mobility in groundwater (Leung and Jiao, 2006; Bourg and Loch, 1995; Hu et al., 2008; Cherry et al., 1984). Statistical analysis indicated that Fe is negatively correlated with DO in both seasons.

Groundwater temperature from granite and granodiorite heights from the east and also from limestone sedimentary rocks at the west to the center show the meaning increasing. In the (Sp) Kartil spring water temperature is 11 ° C while in the (W8) that is close to it shows 18.7° C that is questionable. The amount of dissolved oxygen in the surrounding areas from Zagros limestone show higher values than igneous and metamorphic areas can be attributed to more quickly flow in high fractured limestone. Certain groundwater samples from the middle part and cultivated areas (e.g. sites W4, W5 and W8) contained relatively high Pb and Fe concentrations, which may possibly be related to their low, DO contents. This suggests that the concentrations of some heavy metals could be affected by local redox conditions. The concentration of Fe is found to be positively correlated with pH (R42.3). For most of the other heavy metals, only weak or negative statistical correlations with pH were observed *(Fig 3a, 3b and 3e).*

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Table 3. *Concentration of trace and heavy elements in the Oshtorinan groundwater resoures(dry and wet seasons)*

		As	B	Ba	Bi	Fe	Li	Mg	Mo	Nb	Ni	Pb	Rb	Sc	Se	Sn	Sr	Ta	Te	Th	U	V	W
Dry season	Q1	4.8	69	31.8	9.2	0.255	16.4	30.5	6.1	4.9	12	4.5	19.3	14	2.4	5.7	0.86	4.5	8.7	11.4	<1	95.8	30
	Q2	3.5	62	32.7	3.7	0.243	21	36.3	6	2.8	13	4.1	12	14	0.7	3.3	0.89	3.3	3	2.9	2.3	85.8	23.2
	Q3	3.5	166	25.7	2.7	0.275	20.7	19.5	4.9	2.3	12	4.7	9.1	14	0.5	2.3	0.73	2.5	1.7	1.4	3.1	104.9	21.9
	Q4	3.4	150	9.9	2.3	0.165	8	6.9	2.2	1.9	11	5.1	7.7	16	0.7	3	0.27	3.2	1.1	0.9	1.1	126.5	20.6
	Q5	3.2	138	29.6	2.3	0.219	13.2	19.7	5.2	2	15	6.2	6.3	18	1.1	2	0.72	2.1	1	0.5	5.3	133.8	21.2
	W1	3.3	62	35.5	2	0.242	12.3	18.3	3.9	1.4	13	5.6	4.7	22	1.3	2.5	0.67	1.7	0.7	0.3	4.5	113.3	19
	W2	3.7	64	24.8	1.9	0.293	9.6	14.4	3.3	1.6	11	4.5	5.5	22	1.1	1.8	0.47	1.6	0.6	0.1	5.2	123.9	20
	W3	4.8	49	26.1	1.8	0.218	15.1	8.5	4.6	1.3	18	5.2	5.3	16	1.2	2.2	0.35	1.5	0.5	0.1	5.1	142.9	27
	W4	5.6	193	36.3	2.1	0.328	30.8	7.4	6.1	1.4	17	15.7	5.2	14	2.1	2.2	0.37	1.4	0.4	0.2	8.8	154.7	21.4
	W5	4.3	233	32.9	1.8	0.312	26.5	15.5	4.6	1.1	11	25.9	4.9	17	1.1	2.3	0.63	1.3	0.4	<0.1	3.6	150.7	19.6
	W6	3.9	381	47.6	1.7	0.284	20	9.6	4.6	1.1	11	4.3	4.6	17	1.1	2.4	0.45	1.6	0.3	<0.1	6.6	151	20.5
	W7	5.1	394	17.7	1.7	0.241	12.4	12.4	4.8	1.2	13	4.5	3.5	22	1.7	3.1	0.59	2.4	0.3	<0.1	1.4	171.4	19
	Q6	2.9	5	35.8	1.7	0.119	15.1	13	3.3	1	15	8.3	3.4	28	0.9	2.3	0.52	1.1	0.3	<0.1	<1	162.7	19.3
	Q7	3.1	41	62.7	1.7	0.069	10.8	23.4	1.9	0.9	20	8.2	3.7	31	1	2	0.7	1	0.3	<0.1	1.1	166.1	18.8
	W8	3	89	134.4	1.7	0.314	18.9	18.6	3.1	0.8	14	17.3	4.3	30	1	2.4	0.5	1.4	0.3	<0.1	<1	186.6	18.2
	S1	3.2	<1	3.2	1.6	0.265	3	7.4	1.2	0.9	15	4.9	2.9	34	1.8	2.2	0.12	1.2	0.2	<0.1	<1	195.5	18.1
Wet season	Q1	3.7	71	30.3	8.6	0.33	15.5	27.3	5.7	4.7	11	47.1	16.6	12	2.4	5.2	0.93	4.2	7.8	9.9	<1	86.5	25.2
	Q2	2.9	68	31.1	3.5	0.3	19	28.9	5.4	2.6	10	31.4	9.3	13	0.5	3.1	0.87	2.9	2.7	1.8	1.7	79.3	19.5
	Q3	2.3		21.7	1.9	0.244	14	28.5	3.8	2	15	29.5	6.1	14	0.3	1.6	0.73	1.4	1.1	0.8	2.4	96.8	18.7
	Q4	3.9	152	12.3	1.7	0.21	11	28.5	2.2	2.2	14	25.21	7.9	14	0.8	3	0.68	3.2	1.3	1.1	0.9	121.6	19.3
	Q5	3	144	27.7	2.1	0.2	17	28.5	5.1	2.1	15	27.8	5.9	17	0.8	1.7	0.72	1.9	0.8	0.4	4.6	135.9	21.3
	W1	3.3	53	30.1	2.2	0.351	13	17.7	4.2	1.3	13	27.3	4.7	18	1.1	2.2	0.67	1.7	0.8	0.4	4.1	108.5	18
	W2	3.5	61	25.6	2.8	0.351	17	14	3.8	1.9	14	32	5.8	18	0.9	2.1	0.47	1.1	0.9	0.3	5	121.1	19
	W3	5.1	49	22.6	1.6	0.3	15	6.8	4.6	1.2	17	28	4.7	14	0.8	2	0.35	1.2	0.6	0.2	4.9	138.6	25
	W4	3.3	222	27.3	1.6	0.22	16	6.647	5.5	0.9	14	25.9	3.8	12	1.4	1.6	0.32	0.9	0.3	0.1	6.3	138.6	18.8
	W5	4	257	33.9	1.7	0.362	27	14.32	4.2	1	10	38.6	4.6	15	1.1	2.1	0.58	1.4	0.5	<0.1	4.1	143.5	18.2
	W6	4.7	387	52.2	2.3	0.29	35	33.37	4.5	1.5	13	29.2	4.6	16	1.2	2.2	0.45	1.9	0.5	<0.1	6.8	142.7	21.7
	W7	6.1	387	21.6	2.3	0.35	35	33.37	5.1	1.6	14	25.2	3.7	18	1.4	3.3	0.8	2.2	0.4	<0.1	0.9	159.6	19.6
	Q6	2.9	4	34.2	1.5	0.98	8	12.21	2.9	1.4	11	31.3	3.1	21	0.9	1.8	0.38	0.8	0.2	<0.1	<1	149.6	21.2
	Q7	4.2	30	66.4	1.9	0.07	18	23.27	2.7	0.6	22	33.4	3.8	22	1.2	2.3	0.74	0.8	0.6	<0.1	1.2	168.5	20.3
	W8	1.9	103	121.9	1.4	0.21	13	18.29	2.3	0.6	9	45.2	3.8	19	0.7	1.9	0.48	1	0.2	<0.1	<1	177.3	15.7
	S1	2.2	1	2.1	1.4	0.246	1	8.821	0.8	0.6	11	41.9	2.4	26	1.6	2	0.09	0.9	0.1	<0.1	<1	182.3	16.8

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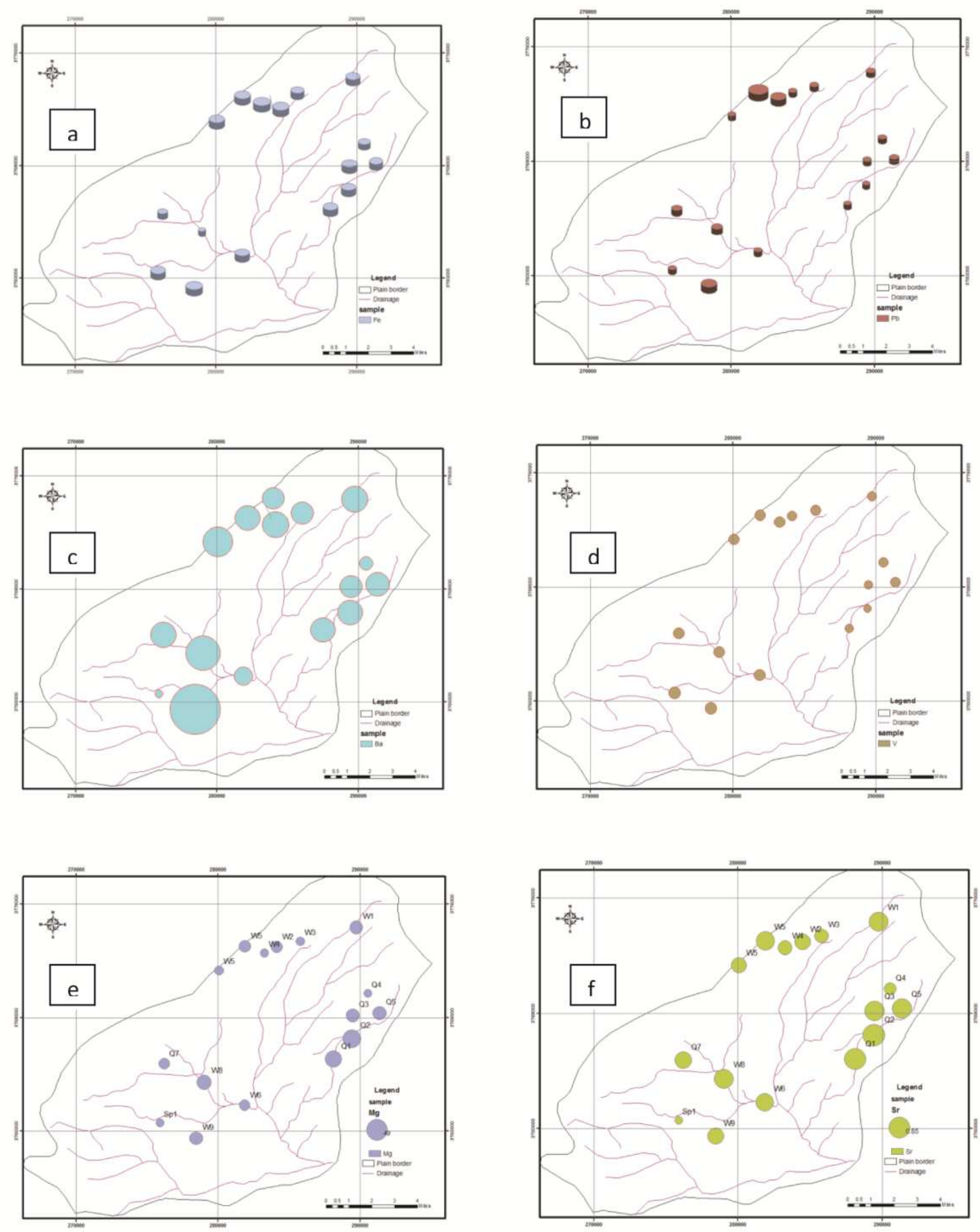

***Figure 3. a, b, c, d, e, f**. show heavy metal (Fe, Pb, Ba, V, Mg and Sr) distribution in the Oshtorinan groundwater*

However, natural processes alone may not be able to account for the observed concentrations. Besides, Fe and Pb could also be derived from steel corrosion. These heavy metals are used as additives on steel production. An alloy steel factory is located at the upstream side of groundwater direction and inlet of the Oshtorinan that release its wastewater into the aquifer without any quality control and pollution supervision. Erosion and weathering of and Sanandaj-Sirjan rocks in the area may result in

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increasing of dissolved Fe in the groundwater. *Fig. 3a* and *3b* show the spatial distribution of Fe and Pb using interpolation method in GIS environment. Lead contamination of the ground water may be the result of entry from industrial effluents, old plumbing, household sewages, agricultural run-off containing phosphate fertilizers and human and animal excreta (Ritter, 2002; Sirajudeen and Abdul Jameel, 2006; Falta, 2004; Savci, 2012). In addition to the symptoms found in acute lead exposure, symptoms of chronic lead exposure could be allergies, arthritis, hyperactivity, mood swings, nausea, and numbness, lack of concentration, seizures and weight loss. The sample W8 has maximum concentration in the southeastern of the plains and adjacent military barracks. According to measurments, three samples are above the maximum permissible limit.

Magnesium, except in samplesQ1, Q2 shows the allowed values that their concentration were 30.5 and 36.3 ppm respectively, which relate to Dareh Garm and Dareh Gorg road Qanats. Weathering of hornfels and schist of Sanandaj-Sirjan zone can cause magnesium washed down into the groundwater. Concentration of Ba ranges from 3 to 132ppb. The lowest concentrations of Ba are noticed in the southwest (Sp) whereas, the highest concentration is recorded at its neighbor (W8) (*Fig. 3c*). Ba values were below the MPL for drinking and irrigation water. The differences for some elements between the Kartil spring (Sp) and the nearby water sources could be a good knowledge of hydrogeological conditions and hydraulic connection with limestone formations and recharge from them. Sb was not detectable Except for sample Q1 with concentration 5.1 ppb, which is influenced by weathering of schist and hornfels.

The concentration of V varies between 79 to 196ppb that highest value appears in the southwest. The concentration of vanadium in drinking water depends significantly on geographical location and Shawn in *Fig 3d*. Groundwater samples in the samples near Sanandaj-Sirjan zone contained Sr concentrations ranging from 120 to 890 ppb with an average of 578 ppb, which is significantly higher than near the Zagros resulted from natural sources such as the weathering of plagioclase feldspar. Therefore, presence of Granite and Granodiorite rocks in east part can justify the high level of Sr.

Industrial and agricultural activities effect on groundwater quality

Boron is a good indicator of the presence of sewage (Barber et al., 1988; Pitt et al., 2000; Verstraeten et al., 2005; Leung and Jiao, 2006). In the middle of plain, the concentration of boron in groundwater ranged from 1 to 394 ppb with average of 134.07 ppb. In the Zagros and Sanandaj-Sirjan slopes, it ranged from 5 to 69 ppb with an average of 41 ppb. The average boron concentration in the center was about five times higher than that in the natural slopes, indicating that groundwater in the developed areas was likely to be contaminated by the leakage from sewage. Boron is also found to be low positively correlated with Fe, suggesting that boron and iron may be derived from the same source, possibly from sewage. Spatial distribution map of boron *(Fig 3e)* depicts that maximum concentration of boron at Central, and western part of Oshtorinan basin. Boron concentration indicates that the samples fall within the permissible limit set by WHO.

Seasonal differences of heavy metal concentrations in the study area

The samples collected both in wet and dry seasons are compared to examine the seasonal effects on groundwater heavy metal and trace element contents. A total of

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16sample sites in the basin are selected for comparison. According to *Table 3*, both the Zagros and Sanandaj-Sirjan zones, groundwater samples collected in the wet season had slightly lower pH and higher DO contents than dry season. The seasonal comparisons of trace element contents in the Oshtorinan plain are presented in *Table 4* for both the natural slopes and the developed areas, the average concentrations of most of the heavy metals and trace elements are higher in wet season than in dry season. It is suggested by (Vaze and Chiew, 2003) that the concentrations of heavy metals in road runoff are particularly high when short duration; intense summer storms follow a long dry period during which pollutants have accumulated on the road surface. However, as discussed, the impacts of leakage from stormwater drains on the heavy metals and trace elements in groundwater appear to be insignificant. Another explanation is that more heavy metals and trace elements could be leached out in wet season because of the generally higher water table during the season. In addition, more chemicals may be washed out directly from the vadose zone by infiltrated rainwater during the wet season. Higher concentrations were obtained in the wet season than in dry season, and in hand dug wells than in boreholes.

Table 4. *Seasonal variation of trace element concentration in Oshtorinan Groundwater Resources*

	Dry Season					***Wet Season***				
Element(ppb)	Min	Mean	Max	SD	CV	Min	Mean	Max	SD	CV
As	2.9	3.831	5.6	0.834	0.689	1.9	3.562	6.1	1.1068	0.8453125
Ba	3.2	36.668	134.4	29.466	16.836	2.1	35.062	121.9	27.331	16.9140625
Zn	7.5	12.53333	20.1	6.671082	5.044444	6.2	12	18.8	6.359	4.533333333
Sr	0.12	0.5525	0.89	0.211865	0.17125	0.09	0.578	0.93	0.228	0.18890625
Se	0.5	1.23125	2.4	0.519896	0.392969	0.3	1.0687	2.4	0.492	0.35625
Li	3	15.8625	30.8	7.013119	5.407813	1	16.968	35	8.866	6.1484375
V	85.8	141.6	195.5	31.80082	25.9	79.3	134.4	182.3	30.666	24.075
Rb	2.9	6.4	19.3	4.163012	2.8125	2.4	5.675	16.6	3.403	2.19375
Mo	1.2	4.1125	6.1	1.502831	1.235938	0.8	3.925	5.7	1.401	1.121875
Pb	30.6	43.725	62.9	9.708587	8.01875	25.2	32.438	47.1	7.037	5.501171875
Mg(ppm)	6.9	16.3375	36.3	8.430016	6.492188	5.6	15.175	26.6	5.368	4.1125
Ni	11	13.8125	20	2.713393	2.164063	9	13.315	22	3.198	2.3125
Sn	0.12	0.5525	0.89	0.211865	0.17125	1.6	2.381	5.2	0.911	0.634375
Ta	1	1.9875	4.5	0.970137	0.759375	0.8	1.718	4.2	0.981	0.7484375
Te	0.2	1.2375	8.7	2.116562	1.210938	0.1	1.175	7.8	1.872	1.034375
U	1.1	4.008333	8.8	2.37083	1.908333	0.9	3.575	6.8	2.090	1.795833333
Bi	1.6	2.49375	9.2	1.865643	1.014844	1.4	2.406	8.6	1.742	0.96015625
Nb	0.8	1.6625	4.9	1.026889	0.698438	0.6	1.6375	4.7	1.019	0.709375
Sc	14	20.5625	34	6.762334	5.632813	12	16.8125	26	3.885	3.0625
Th	0.1	1.977	11.4	3.645	2.298	0.1	1.666	9.9	3.133	1.859259259
W	18.1	21.112	30	3.245	2.253	15.7	19.893	25.2	2.568	1.9171875
Fe	0.069	0.27	0.4	0.0905	0.07	0.07	0.313	0.98	0.193	0.10546875
B	1	134.0625	387	122.6390198	95.8203125	5	139.733	394	118.581	90.48

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Conclusion

The current study reports for the first time the investigation of the levels of heavy metals and trace element concentrations in groundwater samples in Oshtorinan basin, Iran. From this investigation, the following results can be made. The levels of heavy metals under study in water used for drinking purpose are below the maximum allowable limits recommended by WHO except for Fe, Mg, Pb, Sr, and V. This shows that the vadose zone could effectively remove many of the heavy metals and thus protect the underlying groundwater from contaminations. The study revealed that the groundwater in Oshtorinan affected by heavy metals enrichment by anthropogenic sources or may also be derived from remobilization from natural weathering and changes in local redox conditions. Thus, in time, geogenic processes together with anthropogenic activity will lead to elevated heavy metal accumulation in the study area.

The levels of Pb, B and Fe in groundwater in the area understudy increase from east and west to center. To some extent, higher levels of Sr, V and Mg were recorded in the Eastern part in the area under study. It is found that elevated levels of the strontium concentration (Sr) in groundwater were considered to be mainly the result of natural processes such as the weathering of minerals such as feldspars in granites and Granodiorites. Moreover, igneous, metamorphic and carbonate rocks in the one hand and human activities like mining, the other hand, have had a significant effect on the concentrations of these elements in the groundwater, as seen in the analysis of W3.

Higher concentrations of elements in the W1 than other near samples originated from the weathering of Hornfelses and Schists that clearly visible in the field survey, as a result of surface weathering and erosion and dissolution of the elements in minerals.

The proposed studies would be crucial for further Geochemistry studies in the Oshtorinan area and helps to identify the significant parameters of getting better information about source of pollution in the areas with a similar geology bounded by different structures. The spatial and seasonal variations in most of the investigated variables suggest point source contamination in wells and dug wells.

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PLANT VIRUS ECOLOGY: A GLIMPSE OF RECENT ACCOMPLISHMENTS

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Abstract. Plant virus ecology mainly focuses upon populations and their interactions with host plants within environment. The subject includes interesting insights as many factors which affect the virus behavior, population, virus-vector interactions, biodiversity and host plants genotypes are involved in it. Moreover the achievements in the field of molecular ecology by application of recent molecular biology techniques are included which enhance the strength in understanding the economically important virus populations, growth and their world wide spread. Virus infection results direct and indirect effect on insect vectors by evolution of changes in their life cycles, health and interacting behaviors that support their spread. Similarly, the description of the recent information about how plant viruses disseminate towards the important agro-ecological zones in naturally managed vegetations and which factors play important role in ecological aspects are also included in this review. The modern era of science and technology requires a better understanding about movement of viruses in both directions which has become a highly important issue to levitate such kinds of aspects thus making plant virus ecology an exciting research discipline in future.
Keywords: *biodiversity, insect vector, ecosystems, ecological factors, molecular*

Introduction

Plant virus ecology is an indispensible discipline, focusing upon the viruses populations within a particular environment. This discipline further targets the complicated associations as well as interactions between the viruses and the hosts causing the economically important disease spread (Wilson, 2014). Ecology of viruses can be taken as the study of different factors which influence or affect the behavior of a virus under specific environmental conditions (Hull, 2002). It is also considered as a study of virus behavior in its ecosystem and the factors affecting its population (Wilson, 2014). During the earlier decades of last century, plant virus ecology looked disconnected with the basic discipline of plant ecology due to differentiation between plant ecology and plant virology. The least availability of viral symptoms on less economically important plants (Cooper and Jones, 2006; Jones, 2014b; Malmstrom et al., 2011; Prendeville et al., 2012; Roossinck, 2013) resulted in reduction of covering the important aspects in this research field. In recent literature, the plant virus ecology is

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described with spread of viruses in commonly cultivated crops and weeds (Thresh, 2003, 2006a,b,c; Jones, 2004, 2014b; Gallitelli, 2000; Thresh and Fargette, 2003; Morales and Jones, 2004; Coutts et al., 2008; Makkouk and Kumari, 2009; Traore et al., 2009; Adkins et al., 2011; Culbreath and Srinivasan, 2011; Olarte-Castillo et al., 2011; Hull, 2014). Plant virus ecology gives an insight of a complex of virus-host-environment correlation and interactions. This field includes the aspects of plant virus biodiversity, including sampling against the viruses from infected plants directly and from other ecosystems; how plant viruses invades the emerging and invasive species; interactions between plants communities involving mixed and wild plant populations, insects and insect vectors ; persistent viruses showing epigenetic effects; soil born plant viruses and their surrounding soil ecosystems; molecular basis of viral genomics ;ecological factors impacting upon plant viruses and modern technological innovations helping in research about the viruses. This review includes the important aspects on the plant virus ecology in modern era and concludes by understating the rapid advancement and innovation in the technology.

Plant Virus Biodiversity

History reveals that most of the research findings were done accidently while studying some other aspects. For example, *Tobacco mosaic virus* was found accidently while searching for the disease causing agent of a tobacco disease (Beijerinck, 1898). However, due to technological innovations, viruses infection to the non-crop hosts is being studied, leading to the contradictory results against the opinion of the most plant scientists illustrating our knowledge about the plant viruses is still very little (Roossinck, 2012a). Result oriented approach is being followed to study biodiversity, where samples of different environments are analyzed against particular virus sequence after enrichment for viruses but plant virologists adopted another way by individual plants sampling (Roossinck et al., 2010). In this way, each sequence can be tracked towards its specific host thus allowing deeper ecological and evolutionary analyses (Roossinck, 2012a). The data from recent sequencing of samples from marine samples supports that around 2,000 viral species may exist on our planet (Breitbart and Rohwer, 2005). Regarding viral diversity, a little data is available about mixed virus infections or temporal viral infections in native plants. So it is really important to attain more and more knowledge about viruses, their hosts and existing host and virus combinations causing disease to next level that can be very damaging in future (Jonathan et al., 2012). In this regard, the plant virus biodiversity and ecology project played an important role in conducting the vascular plants survey in the nature conservancy's tall grass prairie preserve of Oklahoma, the reservoir of over 700 plant species. Most of the plant species in Costa Recon region showed positive response towards double-stranded RNA viruses. These show that there is a huge difference in our understanding regarding ecology, evolution and viral diversity.

Emerging Plant Viruses

International Committee for Taxonomy of Viruses (ICTV) has enlisted approximately 900 species of plant viruses (King et al., 2012). Several metagenomic studies have identified viruses resembling to plant viruses in a wide range of sample (Human and other mammals feces; fresh, marine, reclaimed water; soils from paddy

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fields and plant-feeding insects). All of these viruses from previously mentioned samples are from a particular genera having exclusively stable capsid, e.g. *Tobamo viruses*. A prevalent example is *Pepper mild mottle virus* found in waste water (Rosario et al., 2009). Reviewing the emerging plant pathogens, studies show that 50 % of the newly emerging viruses belong to a category of DNA or RNA viruses (Anderson *et al.*, 2004). RNA viruses perform host shifting mechanism easily than others (Longdon et al., 2014). Viruses show evolution of changes before invading new plant species, guaranteeing their survival within their new hosts (Longdon et al., 2014; Roossinck et al., 2015). Varma and Malathi (2003) emphasized upon the fast emergence of geminiviruses and explained that they were the most important crop pathogens. Boulton (2003) referred that the disease outbreaks were due to evolutionary changes in the begomoviruses and their dissemination abilities. The emergence of geminivuses in tropics and subtropics was documented in a number of publications. Examples are infection of begomoviruses to: tomato and pepper in North Africa (Tahiri et al., 2006), south-east Asia (Tsai et al., 2011), South America (Martínez-Ayala et al., 2014); legume crops in south-east Asia (Tsai et al., 2011, 2013) and in South America (Rodríguez-Pardina et al., 2011; Martínez-Ayala et al., 2014) and cassava crops in sub-Saharan Africa (Ssweruwagi et al., 2004). In Taiwan, Cheng et al. (2014) observed the infection of this particular virus shown mottling symptoms in fruit and leaf as well as deformation in pepper and given this a provisional name Pepper chlorotic spot virus (*Fig. 1*). So, new viruses are spreading towards the new plant hosts under the umbrella of natural ecosystems thus threatening the plant biodiversity (Jones and Coutts, 2015).

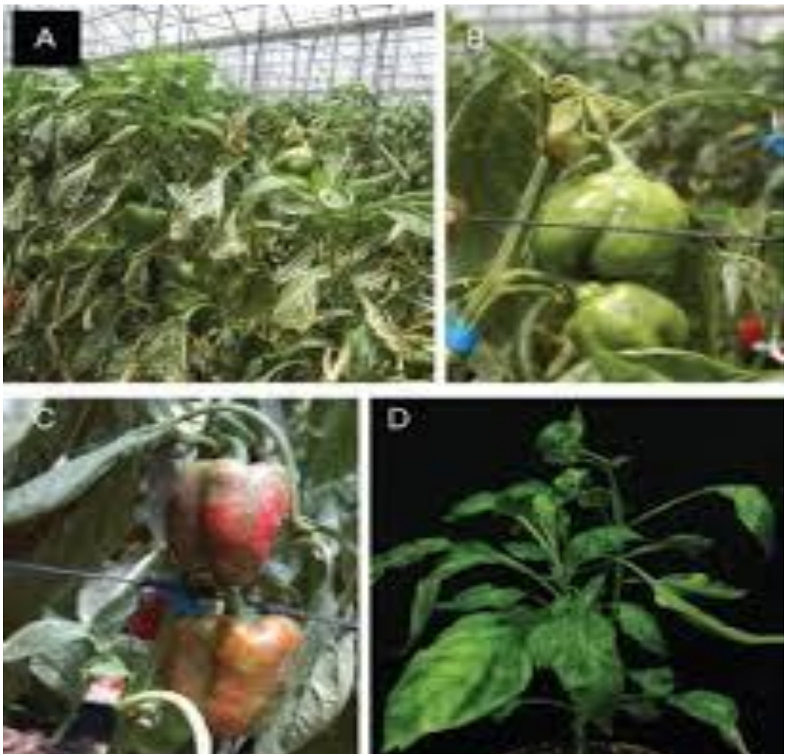

Figure 1. *(A) Pepper chlorotic spot virus (PCSV-TwPep3) symptoms on sweet pepper observed under field conditions; (B) Bud necrosis; (C) Fruit mottle; (D) In vivo leaf mottle and bud necrosis. Source: Cheng et al., 2014*

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Insect Vector Ecology

Recent studies illustrate that viruses can turn plants more attractive for the insects by impounding some changes in volatile compounds produced by the plants. This may lead to the theory that insects feed more at infected plants (Mauck et al., 2012). Further studies describes that plant infected by persistent viruses are more attractive than infected by non persistent ones (Mauck et al., 2012). The transmission of plant viruses can be carried out by insects in two principal methods; one is circulative pathway which includes circulation of viruses through the haemocoel of the insect and the other is non circulative which only involves the foregut or mouth parts of the insect (Fereres and Raccah, 2015).

Viruses can reflect changes in host plants behavior towards the insects. Examples include the transmission of persistent viruses by aphids in wheat (Barley yellow dwarf virus) and potato (Potato leaf roll virus) (Bosque-Pérez and Eigenbrode, 2011). Similarly, it was found that when the insect vector feeds on the infected plant and get virus acquisition, its feeding behavior changed directly afterwards (Stafford et al., 2011; Ingwell et al., 2012; Shestra et al., 2012; Moreno-Delafuente et al., 2013; Rajabascar et al., 2014; Carmo-Sousa et al., 2014). Regarding indirect modifications that include the alterations in the vector (white fly) behavior or its performance after landing and feeding on infected plants is documented (Jiu et al., 2007; Wang et al., 2012; Zhang et al., 2012). Sometimes, the changes inserted in the host plants by the viruses lead towards the changes in virus vector interactions, transmission towards new hosts e.g. (Cilia et al., 2012; Westwood et al., 2013; Zhou, 2013). For example, the increased settling of viral proteins inside the aphid reduces the *Cocumber mosaic virus* (CMV) transmission while discouraging settling would enhance CMV transmission to healthy plants (Westwood et al., 2013).

Viruses can lead towards the modifications of insect vector life cycles, fitness and behavior directly or indirectly. Direct changes occur when the virus remain inside the insect for its whole life span. For example, in tomato TYLCV (*Tomato yellow leaf curl virus*) remained in the body of white fly thus influencing vector settling, probing and feeding (Moreno-Delafuente et al., 2013). Further research revealed that the interaction between virus and vector was mutually beneficial for each other specifically for the biotype Q only (Pan et al., 2013). Similarly in thrips interaction, the feeding behavior of male *Frankliniella occidentalis* (Pergande, 1985) infected by TSWV (*Tomato spotted wilt virus*) was changed thus influencing the transmission of the virus (Stafford et al., 2011). Natural enemies of insect vectors also play an important role in altering virus spread patterns by using different strategic methods. According to Dáder et al. (2012) Aphid parasitoid, *Aphidius colemani* Viereck 1912 increased the spread of non-persistently transmitted CMV in cucumber, but reduced the spread of persistently transmitted Cucurbit aphid-borne yellows virus.

Molecular Ecology

As the genome of plant virus is simple, it makes it ideal subject for molecular ecology studies. Molecular ecology research shows that the genomic sequence of viruses acquire signatures which describe their histories that help in understanding the ecological patterns and evolutionary processes. These patterns can be understood if natural host range is known (Traore et al., 2009). The ecological patterns and virus–plant interaction systems were described previously as well in which improvements in

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virus population traceability, epidemic disease appearance patterns and testing the durability of virus or vector selective control measures were included. e.g. (Fargette et al., 2006; Gibbs et al., 2008; Desbiez et al., 2009; Traore et al., 2009; Lecoq et al., 2011; Olarte-Castillo et al., 2011; Acosta-Leal et al., 2011; Malmstrom et al., 2011; Thapa et al., 2012; Roossinck, 2012 b; Rodelo-Urego et al., 2013; Jones, 2014b) .Garcia –Arenal in his project of plant virus interactions and co-evaluations focused upon a two-host evaluation systems model for cucumbers mosaic virus showing plants Infection possibilities by virus genotypes Y, A and N competing in mixed infections (M). Genetic diversity shown how basic information from different field experiments lead to the use of molecular diagnostic tools to study the virus populations and their influences on different crops (Pagan et al., 2012). A bright future of plant virus ecology is ahead because the combination of molecular and traditional approaches in the discipline are being brought together to collect the details of virus genetic variations and epidemic breakouts which were impossible in the previous years (Jones, 2014a).

Factors Affecting Plant Virus Ecology

Viruses cause diseases in most plants due to the interactions of susceptible host, virulent pathogen and a suitable environment. Similarly biotic and abiotic stresses post a huge impact upon the crop health and yield (Ludmerszki et al., 2014; Noman et al., 2015). The interaction of all these factors and many more other factors shows a complex connection. These may include climate changes (temperature, wind speed, light duration, rainfall), insect vector movement pattern, feeding behavior, population of host plant their genotypes, weeds, human activity, mixed plant populations, wild plant communities, invasive plant species and impacts of worldwide trade. The disease triangle illustrates the importance of all the interactions required for disease development (Jones and Barbetti, 2012) (*Fig. 2*).

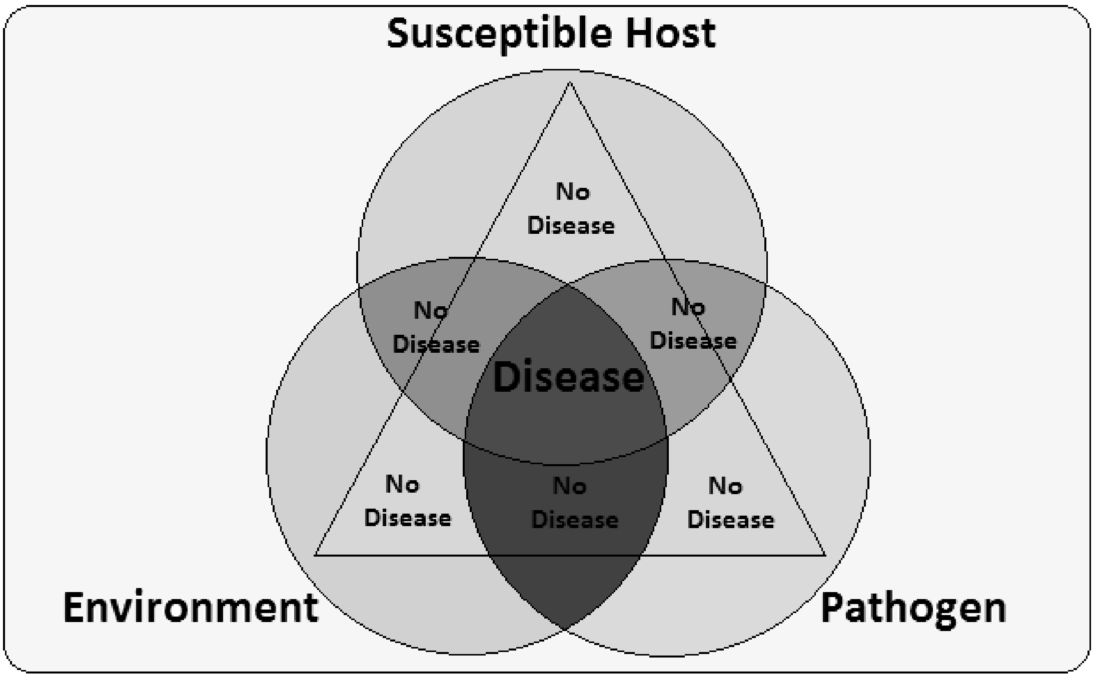

Figure 2. *Plant Disease Triangle*

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1. Climate Change

According to NASA Earth Observatory, in 21st century the global temperature will rise by 2-6°C which will lead to the devastating changes for ecology of plant viruses. For example, the population of whitefly starts building up in high temperature and high relative humidity levels and shows were declining at low temperature and high rainfalls. Temperature also effects virus proliferation by causing changes in gene silencing (Chellappan et al., 2005; Vanitharani et al., 2005). Furthermore, light and humidity also manipulates viral symptoms through induced gene silencing (Fu et al., 2006). Extreme weather events that include adverse rains, wind storms, heat wave patterns and drought spans are predictable due to better technology now a day. Such events divide the world in different ecological sensitive zones (*Fig. 3*). However, capability of plants as well as viruses in adaptation to extreme weather patterns is great, such as adoptability of plants in geothermal soils of Yellowstone National Park against fungal endophytes infected by viruses (Márquez et al., 2007). The extraordinary changes in ecosystem can significantly bring changes in the range of cultivated plants and their cultivated area, resulting in the introduction of new weeds, and increased activity of insect vectors thus promoting the disease spread (Harrington et al., 2001).

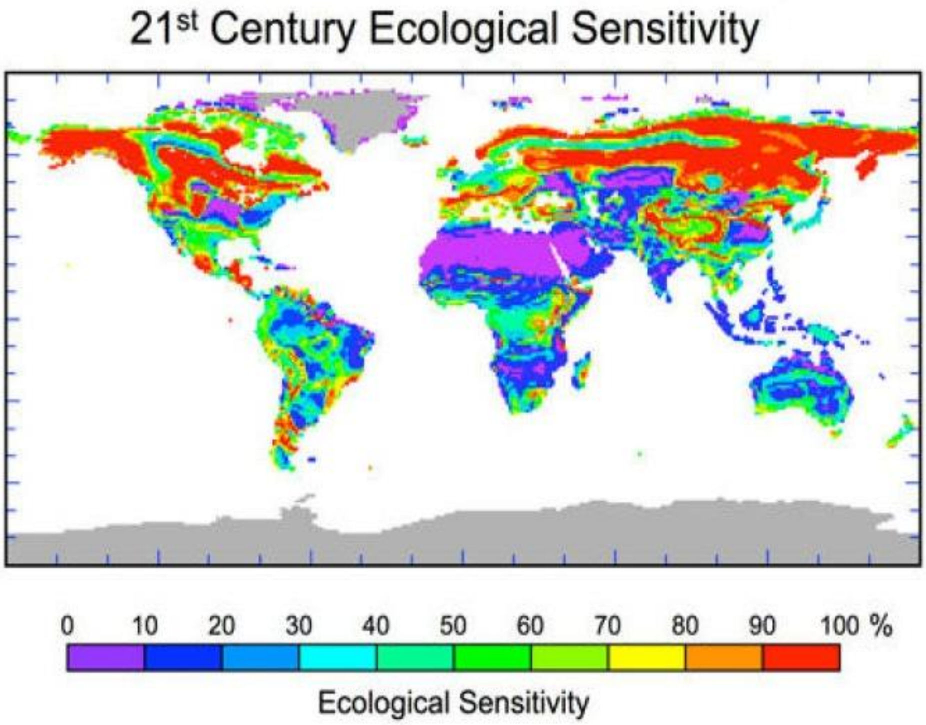

***Figure 3.** 21st Century Ecological Sensitivity - Changes in Plant Species (John, 2011)*

Tropical cyclones in the future are expected to become more extreme. Storms are projected to move pole wards in future, with consistent changes in winds, precipitation and temperature patterns (Metz et al., 2007; Parry et al., 2007). These insecure weather patterns test human ability to manage plant virus diseases effectively (Jones and Barbetti, 2012).

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2. Movement and Feeding Behavior of Insect Vector

The spread of any disease in time and space is dependent upon the positive correlation of insect vector movement to population size. The vectors movement involves some steps for appropriate landing upon the host and then starts its feeding (Fereres and Moreno, 2009). Moreover plant virus transmission is specific by the help of certain insects and their transmission modes, persistence and localizations can be varied accordingly by Insect vector which is explained in *Table 1*. Some studies reveal that plant viruses can modify insect behavior directly. For example, viruliferous aphids by BYDV were attracted to uninfected host plants, whereas non viruliferous aphids were attracted to infected plants (Ingwell et al., 2012). Carmo-Sousa (2014) explained that when the vector came in contact to the CMV infected plants, a change in the probing and settling behavior of Aphids was initiated and increased by the time. The plants infected by CV viruses excrete some volatile compounds that increased the attraction of aphids towards them (Eigenbrode et al., 2002). But the Aphids may feel the plants less attractive for them after they have fed upon them and got virus acquisition (Rajabaskar et al., 2014). Mauck et al. (2015) examined the CMV infected plants of *Cucurbita pepo* and found that the susceptibility of vector (Aphid) towards its parasitoid *(Aphidius colemani)* was increased.

Table 1. *Different Modes of Virus Transmission*

Viruses	**Localization**	**Vector**
Persistently Transmitted Viruses (Takes Weeks Time)		
Begomovirus	Salivary gland	Whitefly
Curtovirus, Mastreovirus	Unknown	Leafhopper
Enamovirus, Luteovirus, Nanovirus	Salivary gland	Aphid
Ilarovirus	Unknown	Thrips
Semi-Persistently Transmitted Viruses (Takes Few Days Time)		
Badnavirus	Unknown	Mealybug, leafhopper
Crinivirus	Foregut/Cibarium	Whitefly
Closterovirus	Foregut	Aphid, Mealybug
Comovirus, Sobemovirus, Tymovirus	Unknown	Beetle
Ipomovirus	Unknown	Whitefly
Machlomovirus	Unknown	Leafhopper
Sequivirus	Foregut	Aphid
Torradovirus	Stylet	Whitefly
Waikavirus	Foregut	Leafhopper
Non Persistently Transmitted Viruses (Takes Few Hours Time)		
Alfamovirus, Cucumovirus, Fabavirus, Potyvirus	Stylet	Aphid
Carlavirus	Stylet	Aphid or whitefly
Caulimovirus	Acrostyle	Aphid
Macluravirus	Unknown	Aphid

3. Host Plant Genotype and Populations

The plant viruses can infect hosts of varying taxonomic status (Woolhouse et al., 2001). For example, the evolution of Pelargonium flower break virus (PFBV) and adaptation to C*henopodium quinoa* (Rico et al., 2006). Genetic diversity of virus is

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imposed by its own evolving genomic make up for better sustainability (Schneider and Roossinck, 2001). Frequent availability of host plants leads to disease epidemics. For example, Cotton leaf curl virus (CLCuV) epidemics in Pakistan was resulted due to introduction of new cotton genotypes which proved extremely susceptible towards local virus strains, leading to wiping off cotton crop by CLCuV (Mansoor et al., 2006).

4. The Role of Weed Plants

Viruses can infiltrate the annual weeds by passing through seeds and undoubtedly play a vital role in maintaining the population of insect vectors as well in agro-ecosystems of their cultivated hosts (Norris and Kogan, 2005). The dissemination of several viruses is linked by the movement of insect vector and continuous availability of the host plants. Many weeds are reported as virus hosts of plant viruses by Kucharek and Purcifull (2001). Removal of volunteer plants and weeds from the borders of a particular crop field helps in management of viral diseases by reducing the vector infestation (Momol and Pernezny, 2006). Seasonal changes in the ecosystem are also important in vector dissemination. For example, *Bamicia tabaci* population exhibit lower levels upon different cultivated and weed plants in winter and spring months. Their migration towards cotton starts in summer leading to the buildup of population. Trebicki (2010) studied some factors that were important in epidemics of *Tobacco yellow dwarf virus* in Australia and found that the two weed species *Amaranthus retroflexus* and *Raphanus raphanistrum* were the virus harbors from which *Orosius orientalis* (vector) disseminated the virus towards other crops.

5. Role of Human Beings

Currently the world is experiencing rapid human activity day by day, resulting in significant impacts upon plants, vectors and viruses leading to un-stability to the virus-vector-plant ecosystems (Patel and Fauquet, 2011). The fast and furious human activities include adopting more diverse, expansive and intensive agronomic practices such as: mono or dual cropping systems; enhanced tillage operations; un-judicial chemical usage; raw irrigation methods and similar cropping patterns. All of these help in virus disseminations and epidemic disease break outs (Jones, 2009). Several examples of vast agricultural activities and practices done by human beings inadvertently caused the emergence and epidemics of new diseases (Alexander et al., 2014). Newly introduced vegetations in tropics and sub tropics by humans become more attractive to new virus encounters (Morales and Jones, 2004; Morales, 2006; Jones, 2009; Vincent et al., 2014). Begomoviruses are the best example of adaptations to the new host vegetations (Navas-Castillo et al., 2011). Increasing Human population demands more food which is enhancing the international trade of plant products thus favoring the travelling of viruses. Jones (1980) reported about *Pepino mosaic virus* movement via trade from Peru. The particular virus infected different *Solanaceae* species which was proved by the sap inoculation experiments, including potato, tomato etc. Till 2000, the issue remained suppressed but then it appeared in tomato crop in Netherlands and contaminated the seeds (Van der Vlugt et al., 2000). Later on it travelled to European states, northern states of America and China. It further moved to other continents through the activities of international seed companies who sold the infected seeds in the other continents (Mumford and Jones, 2005).

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6. Wild Plants and Invasive Plant Species

Preliminary research upon wild plant species describe that hundreds of viruses are needed to be studied and discovered yet (Roossinck et al., 2010, 2012a). In wild Plants, Majority of viruses show persistent life style (Roossinck, 2012b). Maskell et al. (1999) researched upon the virus infection to *Brassica oleracea* (Wild cabbage) and found that it was vulnerable to the infection of *Turnip mosaic virus* and a wide range of other viruses. Moreover recently in Nigeria identification of alternative hosts has been done against ACMV (*African cassava mosaic virus*) and EACMV (*East African cassava mosaic virus*) species (Alabi et al., 2008). In Uganda, studies have even confirmed about CMV in their wild species such as *Manihot glaziovii* (Sserubombwe et al., 2008). Moreno et al. (2004) described that in Spain wild plants were the virus harbor, disseminating it to cultivated Lettuce and Brassica. Similarly *Sweet potato mild mottle virus* about 300 years ago when it entered the Africa, spread from native *Convolvulaceae* species (Tugume et al., 2013).

Generally invasive species adopt phenomena called pathogen release by which they wash off the pathogens in their surrounding habitat (Mitchell et al., 2003). But in their invasions invasive species can be aided by plant viruses by different mechanisms. For example, they may carry virus's infections apparently that lead to the disease spread in their surroundings or they can show extraordinary tolerance to the viruses existing in that particular environment (Rua et al., 2011). Studies illustrated that population of aphids was increased due to invasive grass species (Malmstrom et al., 2005).

Concluding Remarks

In the recent years, a lot of publications which included the new molecular methods have enhanced the development of plant virus ecology. Molecular ecology provides the benefits of improving the traceability in virus populations, establishing the channel for diagnosis of the risk of viral diseases epidemics and magnifying the durable control measures against economically important diseases. The current innovation in the technology makes plant virus ecology a wide research disciple for researchers. For example, spatial and temporal virus spread patterns and which factors contribute in their spread can be understood with the help of remote sensing techniques (Jones, 2014a). Also the diverse and modern molecular tools are very suitable for virus detection, quantification and analysis helpful for data collection regarding the genetic variability of virus populations. The plant virus ecology has entered in modern era in which innovations and advances in technology provide much more effective predictions of epidemic virus breakout on continental or regional levels (Jones, 2014b).

An exciting future is there for the researchers by using the combination of molecular approaches and traditional measures for better understanding the technical points upon genetic variations of particular virus populations. In future, the sudden outbreak of newly emerged viruses in both cultivated and wild plants is worth to research upon because rapidly increasing plants and plant products international trading will results in establishment of different zones of virus populations across different sub continents of the world. Acquiring the ability of prediction about epidemic break outs, require extensive efforts in future. Rapidly increased rate of innovations in the technology is providing a great opportunity to address the plant virus disease threat towards the managed vegetations thus leading to acceleration worldwide accomplishments regarding plant virus ecology which insure that the discipline has an exciting future.

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SEA SURFACE TEMPERATURE CHANGE IN THE MEDITERRANEAN SEA UNDER CLIMATE CHANGE: A LINEAR MODEL FOR SIMULATION OF THE SEA SURFACE TEMPERATURE UP TO 2100

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Abstract. The warming in the inner seas could not be more important and time is certainly running out for it. This study highlights important results about the detectable and predictable warming of the sea surface temperature (SST) in the Mediterranean Sea. The featured analysis of the SST is based on the remote sensed and corrected SST data for the period 1986-2015, and predicted data by using a linear black box model for period 2015-2100. The 30-year (1986-2015) SST analysis shows an increase of about 0.4 ºC per decade. The most fluctuation in the monthly SST was generally detected at the beginning of the summer period. In the last 30 years of this century (2071-2100), the relative increase in average SST is predicted to be about 5.8 ºC in the Mediterranean Sea by the model. The analysis and prediction of the recent studies showed a perceptible warming in the sea surface during the last decades, and the warming will also continue to increase under the present environmental condition.
Keywords: *linear climate model, SST, MODIS, remote sensing, Mediterranean warming*

Introduction

Climate change generally indicates significant changes in the measures of climatic conditions for an extended period. The classical period for defining the climate is about 3 decades, which is determined by the World Meteorological Organization (WMO). In various studies, the climate change is mostly considered as the major changes in precipitation, temperature or wind conditions in a region for at least a 30-year period.

Scientists have been intensively studying the impacts of climate change on the terrestrial and aquatic ecosystems, as the biotic and abiotic basis for life are directly related to the change in the environmental conditions, i.e., climate. The climate change studies particularly feature global warming, which is mainly caused by anthropogenic activities since the beginning of the industrial era. The studies indicate a 0.3-4.8 ºC increase in global average surface temperature up to 2100 (Collins et al., 2013). Not only the terrestrial ecosystem, but also the aquatic ecosystem has been strongly being influenced by climate change since the beginning of the industrial era.

Sea Surface Temperature (SST) is an indicator of the water temperature close to the ocean's surface layer (i.e., from 10 µm to 20 m below the sea surface) in the oceanography. The layer plays an important role for the physical and chemical conditions of the oceans owing to the mixing of atmospheric CO_2 into the seawater (Bricaud et al., 2002). Furthermore, SST has a strong interaction with the carbon biogeochemical cycle between the atmosphere and marine ecosystems. For instance, the increase of the CO_2 in the atmosphere has not only an impact on the temperature but also affects the CO_2 absorption capacity and the aragonite saturation state of the oceans, which has enormous impacts on the marine ecosystems. That mainly leads to a decrease

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in pH, and is directly influenced by water temperature (Feely et al., 1988). Likewise, various studies have intensively presented the dependency of the primary production on the SST in the marine ecosystem in the last century (Greg et al., 2003; Arrigo et al., 2008; Demarcq, 2009).

Hence, the change in surface temperature of the seas under climate change has been analyzed and studied in the ecology and oceanography subjects in the recent decades. The Mediterranean Sea is a semi-enclosed sea that is especially sensitive to astronomically induced climatic variations, which are well documented in its sedimentary record. Emeis et al. (2000) documented in a study about the SST of the Mediterranean Sea that the SST was varying between 12.3 ºC and 24.4 ºC in the sub-basins over the last 16,000 years. Also, Hayes et al. (2005) analyzed the annual average of the SST in the last glacier period, and pointed out a variation in the SST from 9 ºC to 19 ºC in the Mediterranean sub-basins. Marullo et al. (2007) investigated the correlation of remote sensed SST (i.e., AVHRR Pathfinder version 5.0) and measured SST in the Mediterranean Sea for a 21-year time period. The results of the study pointed to a very high correlation (i.e., ca. 99%) between the datasets. On the other hand, Shaltout and Omstedt (2014) specifically analyzed the change and anomalies of the remote sensed SST in the Mediterranean Sea by using 0.25º gridded advanced high-resolution radiometer data from the recent past (1986 to 2015). The study indicated an approximately 0.24º C temperature increase in the Mediterranean Sea per decade. Moreover, they reported that the SST change in the six sub-basins varied from 13.8 ºC to 22 ºC.

The main purposes of this study are the investigation and illustrative presentation of the sea surface temperature change and anomalies in Mediterranean Sea under climate change by using a very high gridded dataset to develop a linear black box SST model, which simulates the SST up to 2100.

Material and Methods

Near-term SST data

To investigate the change in sea surface temperature, we used the generated/provided SST data by Copernicus Marine Services in ca. 4x4 km very high spatial and temporal (i.e. daily). This data is based on AVHRR Pathfinder Version 5.2 (PFV52) data set obtained from the US National Oceanographic Data Center and GHRSST (http://pathfinder.nodc.noaa.gov) over the period of January 1986 - December 2015. Also, the inter-annual variability of the SST was analyzed for the 30-year period. This was performed with the Climate Data Operators version 1.6.0 (CDO, 2015).

Visualization of the data

Spatial distribution of the data was done for the time average of the 30-year period. The inter-annual variation and seasonal distribution, i.e., DJF, MAM, JJA, SON (capitals presents each capital of the month's name) were also illustrated in this study.

Average SST were calculated for two 30-year periods (i.e., 2031-2060 and 2071-2100) by using the CDO software to simulate the differences between near past 30-year climate period and near future two 30-year climate periods. Anomalies in SST were also computed by using the following equation:

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$$SST_{Anom(i)} = SST_{mon(i)} - \frac{\sum_{k=1982}^{n=2015} SST_{mon(i,k)}}{n-k+1} \quad \text{(Eq.1)}$$

where i is the number of each grid cell, n and k are start and end of the data collected years, respectively. SST_{mon} is the monthly SST for each grid cell, and SST_{Anom} is the SST anomaly for each grid cell.

Future simulations

To simulate the SST up to 2100, we analyzed the change in each grid cell and defined a linear function for it over the 30-year period of 1986-2015. Afterwards, we run the linear regression model for simulation of the SST in each grid cell from 2016 to 2100. We used the following equations to calculate the linear regression parameter.

$$y = a + bx \quad \text{(Eq.2)}$$

$$a = \frac{\sum y - b \sum x}{N} \quad \text{(Eq.3)}$$

$$b = \frac{N \sum xy - (\sum x \cdot \sum y)}{N \sum x^2 - (\sum x)^2} \quad \text{(Eq.4)}$$

where N is number of the observations, x is a year index, y is the SST for given census years.

Results

Inter annual variability of the remote sensed annual average of SST was conducted using linear regression in the Mediterranean Sea over the period 1986 to 2015. Results of the analysis indicated that the annual average of the SST, since 1986, has been linearly increasing ca. 0.4 ºC per decade in the entire Mediterranean Sea (see *Figure 1*).

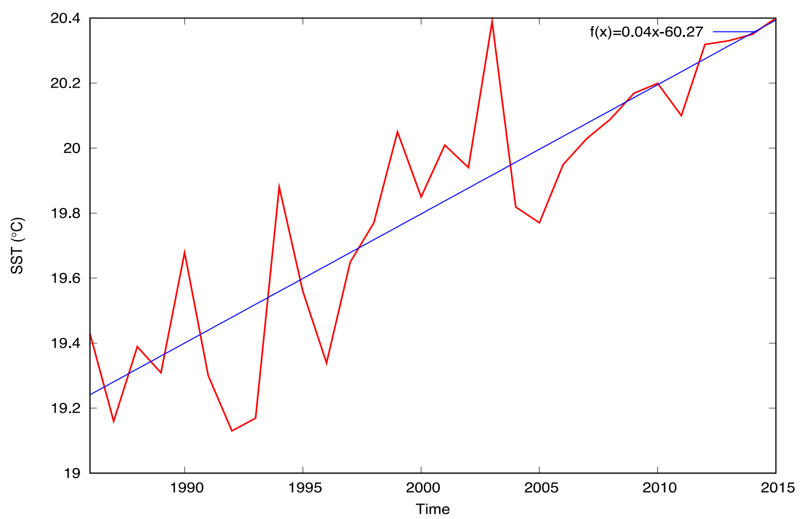

Figure 1. *Annual average sea surface temperature fluctuation in the Mediterranean Sea over the period 1986 to 2015.*

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Spatial distribution of the 30-year average SST was illustrated in a very high resolution, i.e., 4x4 km in the Sea (see *Figure 2*).

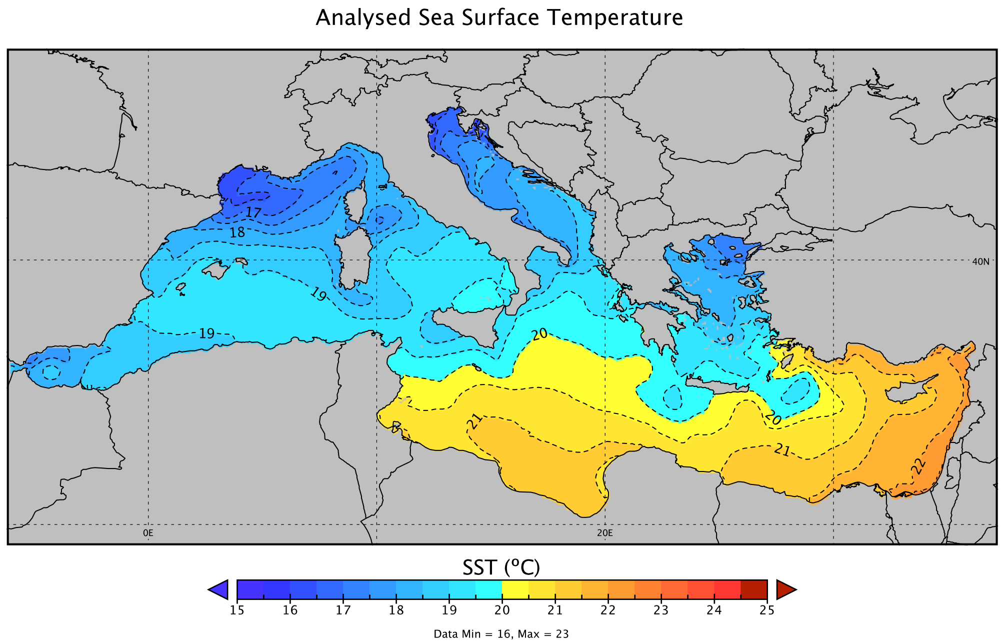

Figure 2. *Spatial distribution of very high resolution (4x4 km) 30-year average sea surface temperature in the Mediterranean Sea.*

The distribution of the SST data showed that south and southeast Mediterranean Sea was approximately 3-5 ºC warmer than other regions in the Sea during the studied period (see Figure 2). Annual cycle of the monthly SST was investigated over the 30-year period, and plotted on Figure 3. On average, lowest (14.1 ºC) and highest (ca. 24.3 ºC) SST were recorded in the Sea in March and August over the 30-year period, respectively (see *Figure 3*).

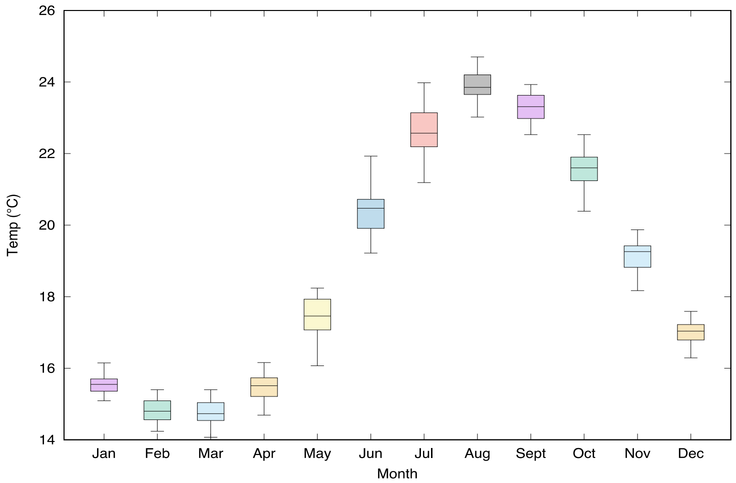

Figure 3. *Inter-annual variation of 30-year average sea surface temperature in the Mediterranean Sea.*

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In the Figure, it can also be seen that the largest amplitude of the SST was found in June and July. Other important points to mention are that the SST in the Mediterranean Sea mainly showed negative amplitude, in other words a cooling, in May, October and November. The SST anomalies were computed by using the Eq. 1. To see the annual anomaly trend for the 30-year study period (1986-2015), results of the annual anomaly analysis were plotted in *Figure 4*.

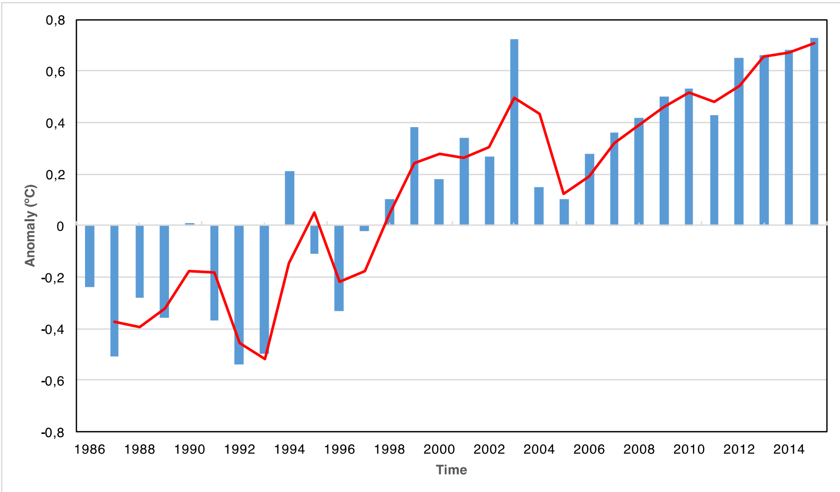

Figure 4. *Anomalies for 30 year and running mean (red solid line).*

It can be seen that the annual average SST generally recorded below the 30-year average SST between 1986 and 1997. The largest annual anomaly was found in 2003. The figure also illustrates that the annual anomaly increased almost continually from 2005 to 2015, except in 2011.

The annual and seasonal cycle of anomaly in monthly mean SST is presented in *Figure 5 and Figure 6* over the 30-year period.

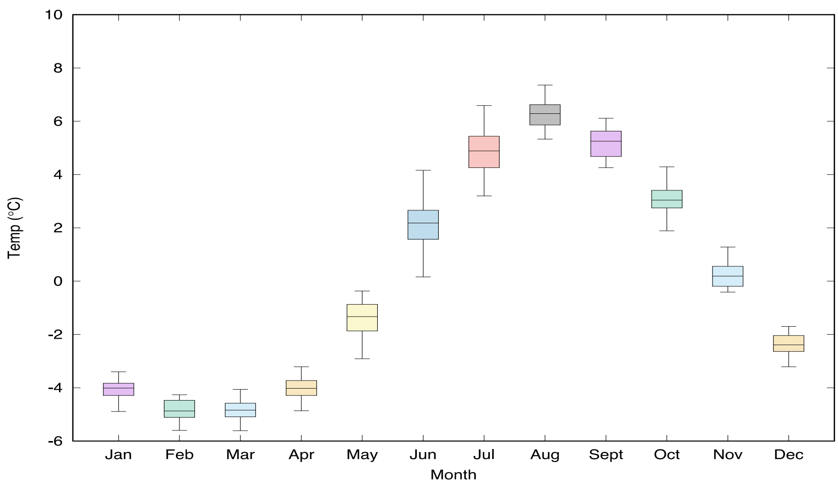

Figure 5. *Inter-annual variation of the 30-year sea surface temperature anomalies in the Mediterranean Sea.*

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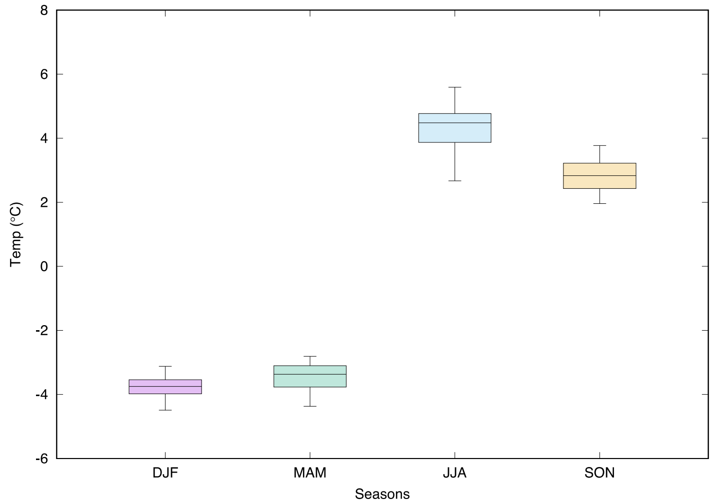

***Figure 6.** Seasonal distribution of the 30-year sea surface temperature anomalies in the Mediterranean Sea.*

The largest amplitude of anomaly in monthly mean SST can be seen in June, July, 0 ºC to 4.2 ºC, and 3.1 ºC to 6.9 ºC, respectively. On the other hand, the anomaly in seasonal mean SST indicates a relatively smaller range (from -3.2 ºC to -4.4 ºC ca. 1.2 ºC) in winter time, compared to other seasons (see *Figure 6*). Also, distribution of the seasonal mean SST was computed and plotted in this study (see *Figure 7*).

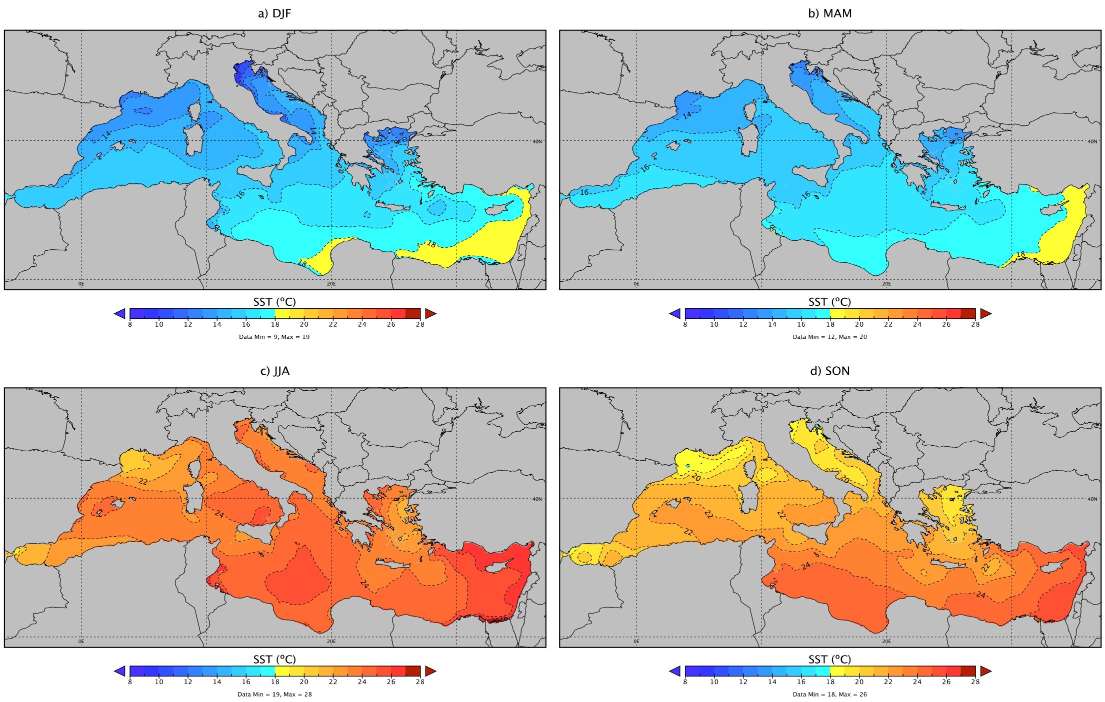

***Figure 7.** Distribution of the 30-year (1986-2015) seasonal average sea surface temperature in the Mediterranean Sea. a) DJF (December-January-February) season, b) MAM (March-April-May) season, c) JJA (June-July-August) season, d) SON (September-October-November) season.*

The Figure shows that the north and west Mediterranean Sea is generally 5 ºC to 10 ºC colder than the south and east of the Sea during the same seasons, and the maximum temperature (ca. 28 ºC) is observed during the summer period.

The annual cycle of the SST from 2016 to 2100 was computed by using the functions Eq. 2-4. In *Figure 8-a and 8-c*, spatial distribution of the annual average SSTs of the future simulation are shown in the Mediterranean Sea for 2031-2060 (i.e., 1st future period) and 2071-2100 (i.e., 2nd future period) periods, respectively.

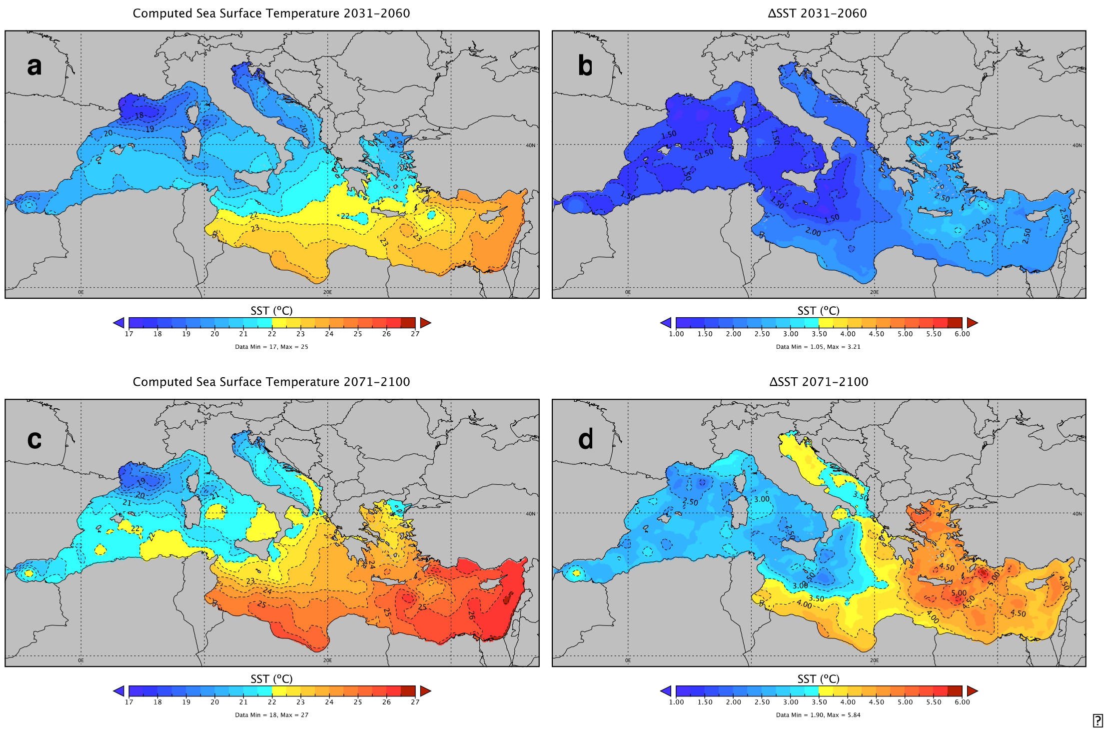

***Figure 8.** Distribution of the predicted 30-year, i.e. a) 2031-2060 and b) 2071-2100 average sea surface temperature in the Mediterranean Sea, and the relative differences (c and d) to the 30-year study time period (1986-2015), respectively.*

In the 1st future period, the 30-year mean SST generally has a range of 22 ºC to 25 ºC from Gulf of Gabes to Gulf of Iskenderun toward southeast, and from Aegean Sea toward the south (see *Figure 8-a*). Compared with near past period, warming of the Mediterranean Sea reaches up to +2.5 ºC in the 1st future period (see *Figure 8-b*). In the Sea, South and southeast regions also become warmer than west and north (including Aegean Sea) regions during this period. In the 2nd future period, the warming of the Mediterranean Sea continuously increases towards the west (see *Figure 8-c*). In this period, the mean SST reaches up to 27 ºC, and the relative difference to the near past period gets nearly up to 6 ºC (see *Figure 8-d*).

Discussion

Remote sensed surface temperature of the Mediterranean Sea is detailed analyzed in the last decades. Marullo et al. (2007) validated the remote sensed SST from AVHRR (Advanced Very High Resolution Radiometer) with more than 21 000 matchups (in-situ data) for 21

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years, and found out that the remote sensed SST has quite a good correlation (ca. $r^2 = 0.99$) with the in-situ dataset. This is a concrete proof for the quality of the remote sensed data, which is often used for determining the climate change in the near past.

The temperature gradients between the east and west Mediterranean basins are mainly caused owing to diffusing of the Atlantic water into the sea through the strait of Gibraltar (Millot, 1999). On the other hand, the north-south sea surface temperature gradients are among others caused owing to the effects of the North African hot winds (e.g., Sirocco winds), and flowing warm pacific water through Suez Canal into the sea (Russo and Artegiani, 1996; Ferrarese et al., 2009; Schicker et al., 2010; Galil et al., 2015).

Warming of the Mediterranean Sea is reported as 0.24 ºC per decade from 1986 to 2015 (Shaltout and Omsted, 2014), and 0.03 ºC per year (ca. 0.3 ºC per decade) in the study from Nykjaer (2009); however, a linear 0.4ºC warming per decade was detected in this study from 1986 to 2015 (see *Figure 1*). The difference between SST of the studies could be caused from the differences in spatial resolution and origin of the datasets. It is important to mention that this study has ca. 36 and 16 times higher spatial resolution than the two studies, respectively.

Within the analysis, annual SST mostly shows high negative anomalies before 1998, and high positive anomalies after 1998. That clearly points to a warming of the sea surface in the Mediterranean, and it is related–with a high probability–to the El Niño (warm)-South Oscillation (ENSO) periods over the Pacific Ocean (Brönniman, 2007; Brönnimann et al., 2007; Marti, 2007). Moreover, inter annual fluctuations of SST anomalies presents the highest variability in monthly SST during the summer periods of the last 30 years. Chronis et al. (2011) reported similar impacts of Summer North Atlantic Oscillation (SNAO) on surface temperature (i.e., sea and terrestrial surface temperature) in East Mediterranean region during summer periods from 1979 and 2008. It is still not clearly known which oscillation may have impacts on the increase in surface temperature over the entire Mediterranean Sea.

Recent studies about the rapid increase in sea surface temperature during last centuries shows that it will drastically continue to rise in the future (Meissner et al., 2012; Collins et al., 2013; Mizuta et al., 2014). Within the World Climate Research Program, a large number of comprehensive climate and Earth system models have been used in studying the interaction between climate and biosphere recent years (Collins et al., 2013). Analysis of the results from Coupled Model Intercomparison Project Phase 5 (CMIP5) shows an up to 4 ºC increase in SST under consideration of the Representative Concentration Pathway Scenario 8.8 (RCP8.5) on global average at 2100 (Collins et al., 2013). In this study, the predicted SST of the Mediterranean Sea by using the linear black box model suggests that there will be a 3.5 ºC increase at the middle of the century (see Figure 8-a) and 5.8 ºC increase at the end of the century (see *Figure 8-b*). This means that the SST of inner Seas like the Mediterranean will be more influenced by the climate change in the future. Shaltout and Omsted (2014) also predicted an increase in SST between 0.5 ºC (RCP2.6) and 2.6 ºC (RCP8.5) by analyzing the CMIP5 SST data with various RCP scenarios (i.e., RCP2.6, RCP4.5, RCP6.0 and RCP8.5) and 1.25ºx1.25º spatial resolution for the Mediterranean Sea. The linear black box model in this study predicts the sea surface warming–approximately–being two (RCP8.5) to ten times (RCP2.6) higher than the CMIP5 analysis estimates. By comparing SST results with different spatial resolutions, it can be seen that the change in predicted SST can vary enormously, and impacts of the sea surface warming can lose their meaning in the future period.

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Conclusion

Not only the oceans' surface temperature, but also inner seas' surface temperature is strongly influenced by climate change. Recent study shows that the increase of the SST is about 0.4 ºC per decade in the Mediterranean Sea. During the continual increase in the SST, the analyses show that the fluctuation in inter-annual SST and anomaly particularly appear in the sea during June, July and October. The warming in the Sea will definitely continue in the future, and will rise about 5.8 ºC (i.e., relative change to the average SST for the period 1986-2015) on average at 2100. A large part of the warming will be presumably irreversible in the Mediterranean Sea. Regarding the previous studies, a question about "which oscillation has stronger impacts on the surface temperature in Mediterranean region?" is still open, and has a high priority for investigation and clarification in the future.

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PREFERENCE OF TREE SPECIES FOR TROPICAL FOREST ENVIRONMENTS

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Abstract. In order to characterize the environment where plant species have better adaptation, one way is to associate species abundance and distribution with environmental parameters. To do this, we worked in a tropical forest fragment, with point-quarter sampling. The sampling points were parameterized by topographic, hydrographic and soil components, using slope, aspect, distance and water body altitude differences, and fertility and grain size parameters. With canonical correlation analysis and principal component we could detect preferences of some species for water supply, which in turn correlates with some grain size and fertility parameters. The environmental variation related to species abundance and distribution allowed the indication, especially associated with water characteristics, of species for ecological restoration.
Keywords: *species ecology, phytosociology, environmental analysis, multivariate analysis, semideciduous forests*

Introduction

In order to become effective within the ecological precepts, ecological restoration and recovery of degraded areas are issues that require scientific and technological investment. Exploitation of natural resources, agricultural and industrial production and the urban population, with their environmental liabilities, cause impacts that require a task force for reconstruction of natural environments.

There is research about ecological restoration experiencing methodologies with successional processes, phytosociology, and seed rain, among others (Durigan and Dias, 1991; Barbosa et al., 1992; Tabarelli et al., 1993; Palmer et al., 1997; Barbosa and Lieberg, 1998; Kageyama and Gandara, 2001; Rodrigues and Gandolfi, 2001; Rodrigues and Leitão Filho, 2001; Coutinho et al., 2002; Almeida, 2004). However, the use of a restricted floristic diversity base, without considering ecophysiological information, is usual.

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Other factors that hinder ecological restoration activities are growing trends of warming and drought periods (Getirana, 2016), and competition with invasive grasses, contributing to the increased cost and methodological inefficiency.

A requirement to indicate species, improving the survival rate and seedling development in the field, is to know the relationship of the plant species with the natural environment, such as water regime, soil, fertility and topography (Aquino, 2006).

Among the information that aids in survival and development of plant species is adaptation to drained or poorly drained environments, soil acidity tolerance and fertility requirements, phytosociology of the species through horizontal and vertical parameters, sun or shade tolerance, and adaptation to the succession stage. For this information, the phytosociological inventory and environmental characterization are the first data sources (Oliveira Filho et al., 1994).

The soil component may be parameterized by fertility, particle size and soil water dynamics, among others, while the relief can be parameterized by altitude, slope, aspect, plans and curvature profiles. The components to characterize the preferred species environment will be gathered considering soil, geomorphology, geology, hydrology and climate.

However, the preferred environments for species characterization may be more accurate (Carvalho et al., 2005), since variations in the parameters create distinct and abrupt conditions that can only be perceived with increased scale. Thus, the objective was to analyze tree species in a tropical forest fragment, relating them to topographic, hydrographic and soil parameters, for the selection of species according to their ecological characteristics, aiming at restoration actions in similar environments.

The test was conducted at “Parque da Cascata”, which belongs to the Environmental Protection Area of “Serra Santa Helena”, in Sete Lagoas, Minas Gerais, Brazil, with high vegetative and hydrological importance (Mahé, 2009).

Materials and Methods

The natural vegetation in this region is savanna with grasslands and enclaves of Atlantic tropical forest (IBGE, 2004). It has been highly degraded or replaced, mainly by pasture.

Soils are grouped into three types: residual soils of metapelitic rocks, colluvial soils and residual soils of limestone, with color ranging from yellow to dark red (Associação de Desenvolvimento Ambiental, 2007).

Climate classification, according to Köppen, is Cwa, i.e., savanna climate with dry winter and rainy summer. The average annual temperature is 21.1 °C; the lowest average temperature is approximately 11.5 °C, recorded in June and July. The highest temperatures are between 28.5 °C and 30 °C, and occur in January to March and October to December. August and September are the driest months with relative air humidity (RH%) between 57.6 and 58.8%. January, February, March and December have the the highest RH (76.2 , 74.3, 74.8 and 76.7%). The dry season extends from May to September. The total average annual rainfall is 1.384 mm. The average daily evapotranspiration (ETp) is lower in June, 2.7 mm, and higher in October, whose daily average is 4.7 mm (Gomide et al., 2006).

The Municipal “Parque da Cascata”, created by Law 593 on September 27, 1977, has an area of 205 ha, with 92 ha of tropical forest area (ADESA, 2007). The inventoried area is shown in figure (*Fig. 1*).

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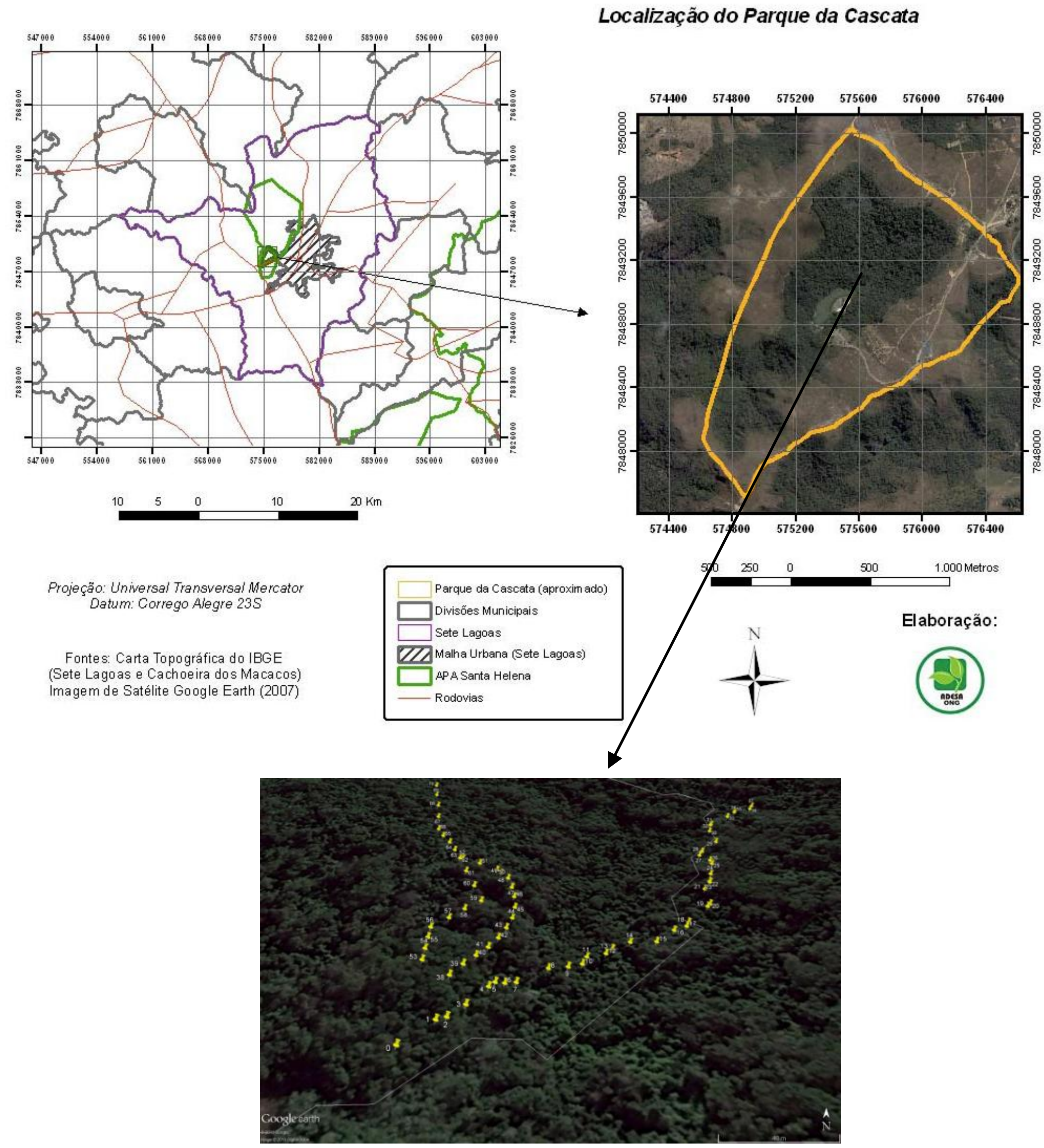

Figure 1. *Inventoried quadrant points and the stream in Municipal "Parque da Cascata" (Figure Credit: ADESA, 2007)*

A phytosociological inventory was performed through the Point-centred Quarter Method (Cottam and Curtis, 1956). Three transects were drawn (*Fig. 1*), the first near the watercourse, with 38 points, the second in an intermediate section, with 15 points, and the last transect is farthest from the stream bed, with 18 points.

In the first transect (points 0 to 37), flooded areas prevail near the stream that runs through a part of this transect. The second transect (points 38 to 52) has a forest with lianas, and the third transect (points 53 to 71) is the most remote area of the creek and with higher altitude terrain.

Points were marked at a distance of 10 m, and in each quadrant the nearest tree to the point was selected, measuring the diameter at breast height (DBH), total height using a digital hypsometer and the distance between the point and the tree. All trees with DBH ≥ 5 cm were considered by collecting botanical material for identification at the

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Herbarium PAMG of the Agricultural Research Company of Minas Gerais (Epamig) and support of the virtual herbarium Tropicos.org (2016), The Field Museum (2016) and The New York Botanical Garden (2007).

The fertile materials were herborized and included in the collection of the PAMG Herbarium /Epamig. The scientific names and phylogenetic relationships follow the APG III classification system (Souza and Lorenzi, 2012), and the confirmation of the scientific names and botanical synonymies were based on the Flora do Brasil 2020 (2016).

The phytosociological parameters of density, dominance, frequency and importance value (IV) were obtained for each species. Environment variables were analyzed with the most abundant species.

Soil samples were collected at points 1, 9, 19, 28, 39, 43, 47, 51, 56, 60, 65 and 68. We evaluated the following parameters: fertility (Lopes and Guilherme, 2004), particle density, particle size, macro and micro-porosity. With the aid of volumetric rings, thirty-six undisturbed samples were taken at three depths 0-10, 10-20, 20-30 cm and, separately, thirty-six deformed soil samples were collected.

The TanDEM-X derived DEM digital elevation model was used (Viana et al., 2015), with a spatial resolution of 12.5 x 12.5 m. Aspect and slope in degrees were calculated, and the distance of the water body and the difference in altitude between the point and the water body were generated with the help of a GIS (Geographic Information System). The information per point was extracted at the intersection of layers with these data and the quadrant points. The aspect was ranked in faces: North, Northeast, East, Southeast, South, Southwest, West and Northwest, by the framework of the original angles in classes defined by 22.5º angles. Thus angles that are in classes 0-22.5º and 337.5º-0 were classified as North face, or 0º. Angles 22.5º to 67.5º were classified as Northeast face, or 45º, and so on.

To explore the relationship among the physical medium parameters, we analyzed the correlation between the hydrography, topography, granulometry and soil fertility parameters considering the 12 points and the three soil depths sampling.

Finally, canonical correlation (CCA) multivariate analysis was applied to soil data, correlating with topographic and hydrographic attributes, and analysis of principal components (PCA) was applied to topographic, hydrographic and dendrometric attributes for more abundant species.

Results and Discussion

Structure of arboreal community

Two hundred thirty-five individuals belonging to 27 families, 47 genera and 65 species were inventoried. Nine species had only the genus defined and 8 species were not determined, remaining as morpho-species (*Tables 1 and 2*).

The largest families were Fabaceae with 6 species, Euphorbiaceae, Rubiaceae and Vochysiaceae with 5 species each, Lauraceae and Meliaceae, with 4 species each, and Annonaceae, Apocynaceae, Bignoniaceae and Cannabaceae with 2 species each. The other 14 families were of single-species. The most diverse families are among the 30 most diverse families of Minas Gerais, according to Oliveira Filho et al. (2008a). In general they are also quite representative families regarding number of species in the seasonal forests of Triângulo Mineiro (Araújo et al., 1997; Araújo and Haridasan, 1997), Zona da Mata (Silva et al., 2004), Southern Minas Gerais (Vilela

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et al., 2001; Machado et al., 2004) and in the Brazilian Cerrados (Costa and Araújo, 2001; Pereira-Silva et al., 2004).

Table 1. *Species identified with their popular names, conservation status (Cons.) and ecophysiological category (Ecol.). AB = Abundant; CO = Common; FR = Frequent; VR = Very rare; OC = Occasional; RA = Rare; ER = Extremely rare; C = Climax; P = Pioneer; IS = Initial Secondary; LS = Late Secondary (Oliveira Filho et al., 2008a; 2008b; 2008c; 2008d; 2008e)*

Family/Species	Common name(s)	Cons.	Ecol.
Anacardiaceae			
Astronium fraxinifolium Schott ex Spreng	Aroeira-vermelha, gonçalo-alves	AB	IS
Annonaceae			
Annona sylvatica A.St.-Hil.	Araticum-cagão-macho, araticum-do-mato, embira	FR	IS
Guatteria ferruginea A.St.-Hil.	Imbuí-amarela	ER	LS
Apocynaceae			
Aspidosperma subincanum Mart.	Guatambu-vermelho, pau-pereira-do-campo, perobinha	OC	LS
Bignoniaceae			
Jacaranda macrantha Cham.	Caroba, carobão	OC	IS
Cannabaceae			
Celtis pubescens Spreng.	Cipó-espinho, grão-de-galo	FR	P
Trema micrantha (L.) Blume	Crindiúva, pau-pólvora, periquiteira	OC	P
Caricaceae			
Jacaratia spinosa (Aubl.) A.DC.	Barrigudo, jacaratiá, mamãozinho, mamoeiro-de-espinho	FR	IS
Euphorbiaceae			
Alchornea glandulosa Poepp.	Amor-seco, maria-mole, tapiá	FR	P
Croton urucurana Baill.	Capixingui, urucurana, sangra-d'água	OC	P
Gymnanthes klotzschiana Müll.Arg.	Branquilho, branquinho, branquio	AB	IS
Fabaceae			
Copaifera langsdorffii Desf.	Copaíba, copaíba-vermelha, óleo-de-copaíba, pau-de-óleo	AB	P
Plathymenia reticulata Benth.	Vinhático, vinhático-branco	CO	P
Platycyamus regnelli Benth.	Angelim-rosa, folha-de-bolo, pereira, pereira-vermelha	RA	IS
Lauraceae			
Cryptocarya aschersoniana Mez	Canela-amarela, canela-fogo, canela-pimenta	FR	P
Nectandra megapotamica (Spreng.) Mez	Canela-cheirosa, canela-ferrugem, canela-loura, canela-preta	RA	IS
Ocotea corymbosa (Meisn.) Mez	Canela-corvo, canela-fedorenta	OC	P
Ocotea velutina (Nees) Rohwer	Canela-amarela, canelão-amarelo	RA	LS
Lecythidaceae			
Cariniana estrellensis (Raddi) Kuntze	Estopeira, jequitibá, pau-de-cachimbo	ER	C
Malvaceae			
Luehea grandiflora Mart.	Açoita-cavalo, açoita-cavalo-graúdo	AB	P
Melastomataceae			
Miconia chamissois Naudin	Maria-preta	VR	P
Meliaceae			
Cabralea canjerana (Vell.) Mart.	Canjerana, cedro-canjerana	VR	IS
Guarea guidonia (L.) Sleumer	Camboatã, canjerana-miúda, cedro-branco, jitó, marinheiro	RA	LS
Trichilia claussenii C.DC.	Catiguá-vermelho, quebra-machado	RA	IS
Trichilia pallida Sw.	Baga-de-morcego, catiguá	OC	P

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Monimiaceae			
Mollinedia widgrenii A.DC.	Corticeira, pau-de-espeto, pimenteira	OC	IS
Moraceae			
Sorocea bonplandii (Baill.) W.C. Burger et al.	Folha-de-serra, laranjeira-do-mato	OC	IS
Myrtaceae			
Eugenia florida DC.	Guamirim, pitanga	OC	P
Nyctaginaceae			
Guapira opposita Vell.	Flor-de-pérola, joão-mole, maria-mole	OC	IS
Ochnaceae			
Ouratea tenuifolia Engl.	Guaratinga, guatinga	ER	IS
Polygonaceae			
Coccoloba mollis Casar	Falso-novateiro, folha-de-bolo	OC	P
Primulaceae			
Myrsine umbellata Mart.	Capororoca, capororoca-branca	FR	P
Rubiaceae			
Amaioua guianensis Aubl.	Canela-de-veado, marmelada-brava, pimentão-bravo	FR	IS
Faramea hyacinthina Mart.	Limãozinho-bravo, marmelada-de-cachorro	OC	IS
Ixora brevifolia Benth.	Ixóra-arbórea	FR	IS
Psychotria carthagenensis Jacq.	Carne-de-vaca, erva-de-rato-branca, rainha	FR	IS
Rutaceae			
Galipea jasminiflora (A. St.-Hil.) Engl.	Jasmim-do-mato, mamoninha, quina-de-três-folhas	FR	IS
Metrodorea stipularis Mart.	Laranjeira-do-mato, limoeiro-do-mato	OC	IS
Salicaceae			
Casearia decandra Jacq.	Café-do-mato, chá-de-bugre, guaçatonga, pau-de-espeto, pau-vidro	OC	P
Solanaceae			
Solanum rugosum Dunal	Jurubeba-do-mato	ER	IS
Urticaceae			
Cecropia pachystachya Trécul	Árvore-da-preguiça, embaúba	CO	P
Vochysiaceae			
Qualea dichotoma (Mart.) Warm.	Pau-terra e pau-terra-da-areia	CO	IS
Qualea multiflora var. *pubescens* Mart.	Pau-de-tucano, pau-terra-do-campo	FR	P
Qualea parviflora Mart.	Pau-terra-de-flor-miúda, pau-terra-mirim	OC	P
Vochysia tucanorum Mart.	Amarelinho, pau-de-tucano, pau-doce	CO	P

Using the classification of successional categories, by Oliveira Filho et al. (2008a; 2008b; 2008c; 2008d; 2008e), of Minas Gerais forest inventory we found that of the 46 species identified (*Table 1*), 21 (45.6%) are initial secondary, 20 (43.5%) are pioneers, 4 (8.7%) are late secondary and one (2.2%) is climax.

As for the conservation status (*Table 1*), 4 (8.7%) species are abundant, 5 (10.9%) are common, 11 (23.9%) are frequent, 15 (32.6%) are occasional, 5 (10.9%) are rare, 2 (4.3%) are very rare and 4 (8.7%) are extremely rare (Oliveira Filho et al., 2008a; 2008b; 2008c; 2008d; 2008e). The Parque da Cascata houses species of extremely rare occurrence (*Guatteria ferruginea*, *Cariniana estrellensis*, *Ouratea tenuifolia* and *Solanum rugosum*), very rare occurrence (*Miconia chamissois* and *Cabralea canjerana*)

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and rare occurrence (*Platycyanus regnelli*, *Nectandra megapotamica*, *Ocotea velutina*, *Guarea guidonia* and *Trichilia claussenii*), and this indicates special attention to the development of conservation and management strategies.

Of the 46 species identified, 14 (30.4%) are endemic to Brazil: *Annona sylvatica* (Lobão, 2015), *Casearia decandra* (Marquete, 2016), *Celtis pubescens* (Santos, 2014), *Eugenia florida* (Sobral, 2014), *Galipea jasminiflora* (Pirani, 2011a), *Guatteria ferruginea* (Lobão, 2016), *Jacaranda macrantha* (Lohmann, 2015), *Metrodorea stipularis* (Pirani, 2011b), *Mollinedia widgrenii* (Peixoto, 2014), *Ocotea velutina* (Quinet, 2014), *Platycyamus regnelli* (Moura, 2016), *Psychotria carthagenensis* (Zappi, 2014), *Qualea dichotoma* (Souza, 2014) and *Trichilia claussenii* (Stefano, 2012), and occur mainly in phytogeographical areas of the Atlantic Forest and Cerrado.

Table 2 shows the horizontal phytosociological parameters for the inventoried species. Among the 46 species identified, 35 are suitable for reforestation, restoration and consolidation of degraded areas, because they present rapid growth, are pioneering or produce fruits eaten by animals (Lorenzi, 2008; 2009a; 2009b).

Table 2. *Abundance values (Ni), Relative Density (RD), Relative Frequency (RF), Relative Dominance (RDom) and Importance Value (IV) for the inventoried species*

Code	Scientific Name	Ni	RD (%)	RF (%)	RDom (%)	IV
21	*Ixora brevifolia* Benth.	45	19.15	8	20.52	47.67
39	*Gymnanthes klotzschiana* Müll.Arg.	26	11.06	9.6	4.06	24.73
17	*Galipea jasminiflora* (A.St.-Hil) Engl.	32	13.62	4.8	3.86	22.28
25	*Metrodorea stipularis* Mart.	18	7.66	4	8.48	20.14
1	*Alchornea glandulosa* Poepp.	14	5.96	6.4	4.45	16.81
12	*Copaifera langsdorffii* Desf.	5	2.13	3.2	11.31	16.64
32	*Plathymenia reticulata* Benth.	4	1.70	3.2	6.88	11.78
2	*Amaioua guianensis* Aubl.	8	3.40	2.4	1.38	7.18
14	*Cryptyocharya aschersoniana* Mez	3	1.28	2.4	2.40	6.07
7	*Cariniana estrellensis* (Raddi) Kuntze	3	1.28	0.8	3.73	5.80
19	*Guarea guidonia* (L.) Sleumer	4	1.70	3.2	0.53	5.43
28	*Nectandra megapotamica* (Spreng) Mez.	3	1.28	2.4	1.03	4.70
36	*Qualea multiflora* var. *pubescens* Mart.	2	0.85	1.6	2.20	4.65
30	*Ocotea velutina* (Nees) Rohwer	2	0.85	1.6	2.05	4.50
20	*Guatteria ferruginea* A.St.-Hil.	2	0.85	1.6	1.92	4.37
22	*Jacaranda macrantha* Cham.	3	1.28	2.4	0.43	4.11
15	*Eugenia florida* DC.	4	1.70	0.8	1.49	3.99
33	*Platycyamus regnelli* Benth.	1	0.43	0.8	2.47	3.69
23	*Jacaratia spinosa* (Aubl.) A.DC.	1	0.43	0.8	2.46	3.68
9	*Cecropia pachystachya* Trécul.	3	1.28	1.6	0.63	3.51
29	*Ocotea corymbosa* (Meisn.) Mez.	1	0.43	0.8	2.27	3.49
3	*Annona sylvatica* A.St.-Hil.	2	0.85	1.6	0.92	3.37
6	*Cabralea canjerana* (Vell.) Mart.	2	0.85	0.8	1.48	3.13
47	*Vochysia* sp.	1	0.43	0.8	1.77	3.00
41	*Solanum rugosum* Dunal	2	0.85	1.6	0.25	2.70

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43	*Trema micrantha* (L.) Blume	3	1.28	0.8	0.57	2.65
16	*Faramea hyacinthina* Mart.	2	0.85	1.6	0.12	2.58
47	*Aspidosperma* sp.	2	0.85	0.8	0.74	2.39
47	Undertermined 5	1	0.43	0.8	1.00	2.22
47	Undertermined 2	1	0.43	0.8	0.98	2.20
35	*Qualea dichotoma* (Mart.) Warm.	1	0.43	0.8	0.70	1.92
11	*Coccoloba mollis* Casar.	1	0.43	0.8	0.60	1.82
47	*Alchornea* sp.	1	0.43	0.8	0.57	1.80
27	*Mollinedia widgrenii* A.DC.	1	0.43	0.8	0.53	1.76
47	Undertermined 6	1	0.43	0.8	0.51	1.73
47	*Ouratea* sp.	1	0.43	0.8	0.45	1.68
47	Undertermined 7	1	0.43	0.8	0.41	1.63
47	*Swartzia* sp.	1	0.43	0.8	0.32	1.55
47	Undertermined 4	1	0.43	0.8	0.28	1.50
24	*Luehea grandiflora* Mart.	1	0.43	0.8	0.27	1.49
26	*Miconia* cf. *chamissois* Naudin	1	0.43	0.8	0.26	1.49
40	*Senna multijuga* (Rich.) H.S. Irwin & Barneby	1	0.43	0.8	0.25	1.47
47	*Croton* sp.	1	0.43	0.8	0.23	1.45
47	Undertermined 3	1	0.43	0.8	0.22	1.44
47	Undertermined 8	1	0.43	0.8	0.21	1.43
47	*Bauhinia* sp.	1	0.43	0.8	0.20	1.42
47	*Jacaranda* sp.	1	0.43	0.8	0.19	1.42
10	*Celtis pubescens* Spreng.	1	0.43	0.8	0.19	1.41
46	*Vochysia tucanorum* Mart.	1	0.43	0.8	0.15	1.38
44	*Trichilia claussenii* C.DC.	1	0.43	0.8	0.12	1.35
47	Undertermined 1	1	0.43	0.8	0.12	1.34
18	*Guapira opposita* Vell.	1	0.43	0.8	0.09	1.32
47	*Myrcia* sp.	1	0.43	0.8	0.08	1.31
5	*Astronium fraxinifolium* Schott ex Spreng.	1	0.43	0.8	0.08	1.30
47	*Chomelia* sp.	1	0.43	0.8	0.08	1.30
38	*Myrsine umbellata* (Mart.) Mez	1	0.43	0.8	0.07	1.30
37	*Qualea* cf. *parviflora* Mart.	1	0.43	0.8	0.08	1.30
4	*Aspisdosperma subincanum* Mart.	1	0.43	0.8	0.06	1.29
31	*Ouratea* cf. *tenuifolia* Engl.	1	0.43	0.8	0.06	1.29
34	*Psychotria* cf. *carthagenensis* Jacq.	1	0.43	0.8	0.06	1.29
42	*Sorocea bonplandii* (Baill.) W.C. Burger et al.	1	0.43	0.8	0.04	1.27
45	*Trichilia pallida* Swartz.	1	0.43	0.8	0.05	1.27
8	*Casearia decandra* Jacq.	1	0.43	0.8	0.03	1.26
13	*Croton urucurana* Baill.	1	0.43	0.8	0.04	1.26
47	*Daphnopsis* sp.	1	0.43	0.8	0.04	1.26

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Species with higher IV

The species with the highest importance values (IV) (*Table 2*) were *Ixora brevifolia* (47.67), *Gymnanthes klotzschiana* (= *Sebastiania commersoniana* (Baill) L.B. Sm. & Downs (Oliveira, 2014)) (24.73), *Galipea jasminiflora* (22.28), *Metrodorea stipularis* (20.14), *Alchornea glandulosa* (16.81) and *Copaifera langsdorffii* Desf. (16.64). The abundance of these species, totaling 160 individuals, represent 68% of the sampled individuals.

Relating these species with other surveys in the same typology, Machado et al. (2004) inventoried a fragment of seasonal forest near a pond in Lavras, Minas Gerais, Brazil, in an area of Atlantic Forest, with Köppen Cwa climate, on nitosoils and argisoils, and found only one individual of *S. commersoniana*, and many *G. jasminiflora*. Rocha et al. (2005) inventoried a swamp forest on cambisoils and argisoils in Coqueiral, Minas Gerais, Brazil, less than 50 km from Lavras, in the transition of the Cerrado to the Atlantic Forest area, and found that *G. jasminiflora*, *S. commersoniana* and *M. stipularis* had the highest IV, and occupied mainly the hillside areas.

Meira-Neto and Martins (2002), inventorying a fragment from natural regeneration, about 60 years old in the municipality of Viçosa, Zona da Mata, Minas Gerais, in an Atlantic Forest area, with Aw climate, did not obtain registration of any of the species with the highest abundance in this present survey.

In surveys of shrubby tree flora, even in similar environments, there are low floristic similarities, indicating that there are other large-scale conditionings interfering with the distribution of the species.

Regarding some features of the most expressive species, *I. brevifolia* is considered late secondary, and exclusive to semi-deciduous forest of altitude, occurring mainly in well-drained and medium fertility terrain (Prado Júnior et al., 2012).

S. commersoniana is classified as a pioneer species, deciduous with autochoric dispersion (Cappelatti and Schmitt, 2009) and occurs almost exclusively in humid areas, in riparian forests and flooded forests (Barddal et al., 2004; Kanieski, 2013). It is usually found in groups, reaching pure populations, developing in fields and on the border of clumps; it is rare within dense primary forest (Lorenzi, 2008). In the alluvial plain of the Paraiba River in São José dos Campos, São Paulo, Brazil, *S. commersoniana* presented the second highest IV, occurring in the dominant stratum in both the canopy and the understory (D'Orazio and Catharino, 2013). The *Sebastiania* genus includes species considered exclusively of marsh, such as: *S. brasiliensis* Spreng, *S. edwalliana* Pax & K. Hoffm., *S. klotzschiana* (Müll. Arg.) Müll. Arg. (Torres et al. 1992) and *S. serrata* (Baill. ex Müll. Arg.) Müll. Arg. (Ivanauskas et al., 1997).

Regarding soil conditions, *S. commersoniana* occurred mostly in neosoils (Botrel et al., 2002), indicating a preference for soils with higher sand content (Kolb et al., 1998). According to Callegaro (2012), the presence of this species may be related to the secondary stage of forest succession, being an indicator species of this stage in a mixed broadleaf forest stretch, classifying it as a pioneer species (Longhi-Santos, 2013).

G. jasminiflora is an early secondary species (Oliveira Filho et al., 2008d), endemic to Brazil, which occurs in phytogeographical areas of Cerrado and Atlantic Forest, in the vegetation of semideciduous forest (Pirani, 2011a). This

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species showed preference for dry soil, occurring at low densities in swamp forest (Teixeira and Assis, 2003).

Both *M. stipularis* and *G. jasminiflora* are early secondary species (Oliveira Filho et al., 2008d), occur mainly within the dense forest and are less frequent in open and secondary formations, with very low population density (Lorenzi, 2008). *M. stipularis* can be found in the phytogeographic areas of Cerrado and Atlantic Forest in the vegetation of semideciduous forest (Pirani, 2011b).

A. glandulosa is a pioneer species (Oliveira Filho et al., 2008d), which may appear in swamps, but occurs mainly in areas with temporary waterlogging, such as ciliary or riparian and gallery forests, and even drier forests, where waterlogged soil never occurs. It can be found in Cerrado (*lato sensu*) vegetation, riparian or gallery forest, rain forest and restinga (sandbank vegetation) (Paula-Souza, 2014).

C. langsdorffii is a pioneer species (Oliveira Filho et al., 2008e), characteristic of Cerrado transition to broadleaf semideciduous forest formations, occurring in secondary formations and in dense primary forests (Lorenzi, 2008). According to Oliveira Filho and Ratter (2001), *C. langsdorffii* is a habitat generalist, and, in general, is dominant in the face of most of the remaining forests in the Central South of the state of Minas Gerais.

Relationship of species and the environment

The correlations between the physical medium parameters, hydrography, topography ("Appendix *Table 1*"), granulometry and soil fertility parameters ("Appendix *Tables* 2 and 3") are in "Appendix *Tables 4, 5 and 6*" considering the 12 points and the three soil sampling depths. Via quantification of sand, silt and clay levels, the soil textural classes ranged from franco silty-clayey to silty-clayey. The relief is predominantly wavy, especially the aspect to the southwest.

Twenty-nine significant correlations greater than or equal to 0.70 in the 0-10 cm layer, 62 correlations in the 10 to 20 cm layer, and 46 at 20 in the 30 cm layer were found. The smaller amount of correlations in the topsoil may be due to the contribution of nutrient cycling by litter deposition, which is the layer least dependent on the source material.

In the canonical correlation between fertility and particle size parameters (*Fig. 2*), we found positive associations among fine sand, micro-pores, particle density and silt levels with Mg, Ca, SB, V, Zn, Fe, Mn, Cu, and P. In the opposite direction, there are positive associations among coarse sand, clay and macro-pores with H + Al, K, Al, and Aluminum Saturation. The pH, CEC and organic matter did not show associations with other parameters, both in magnitude and in direction.

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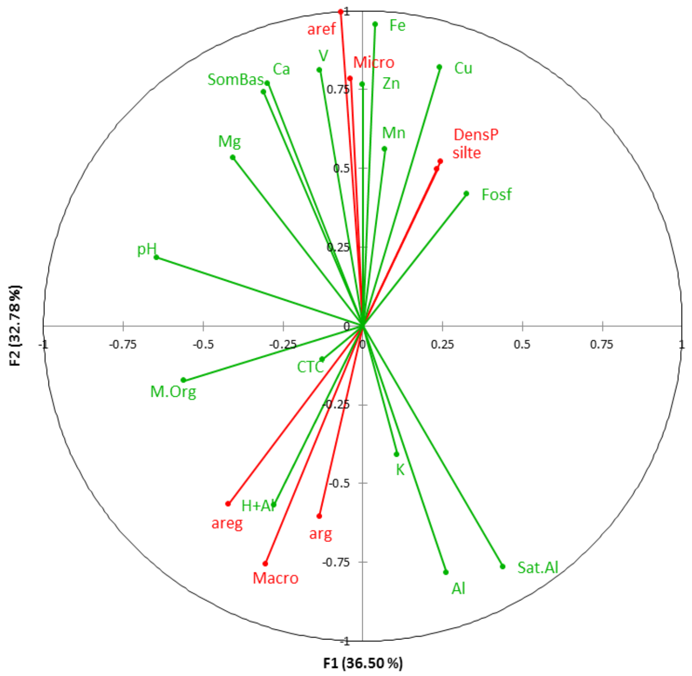

Figure 2. *Canonical correlation among particle size and fertility parameters for the soil depth up to 30 cm (average levels)*

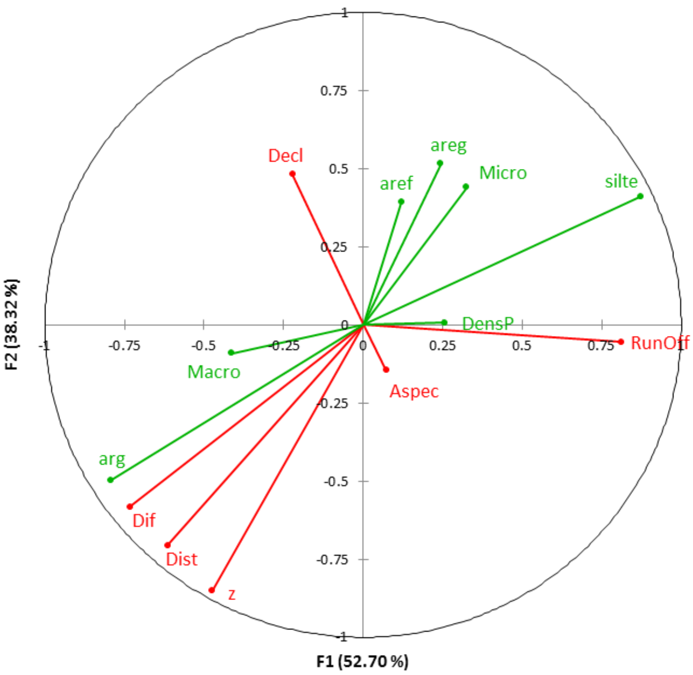

Figure 3. *Canonical correlation among parameters particle size, topography, altitude difference and distance from the water body, for the soil depth up to 30 cm (average levels)*

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In the canonical correlation among particle size, relief and hydrographic parameters in *Fig. 3*, it is observed that the difference in altitude and the distance from the water body are positively correlated, and validate the negative correlation with runoff. It was found that, with increasing distance from the stream, the clay content and the amount of macro-pores increase, hence silt, sand and micro-pore content reduce.

Thus, a negative correlation between micro-pores and clay occurred. We expected the opposite effect, increasing the amount of micro-pores and reducing macro-pores with stream clearance and increase in clay content, since the function of the macro-pores is to facilitate aeration and water infiltration into the soil, and the function of micro-pores is retaining water. The aspect and slope were not associated with the soil particle size.

In the relationship of species of higher IV to physical parameters by principal components (*Fig. 4*), related to altitude difference and distance from the water body, runoff, aspect, slope and dendrometric parameters (height and sectional area), we found two species. They showed greater preference for river proximity: *Alchornea glandulosa* (1) and *Gymnanthes klotzschiana* (39), with few individuals of species 39 away from the stream, showing tolerance to well-drained sites. In addition to these species, some *Ixora brevifolia* (21) and *Galipea jasminiflora* individuals (17) occurred in areas with increased runoff and nearby water, which shows tolerance of these species to poorly drained environments.

An uncontrolling source interfering in the distribution of species is seed dispersal, which may be adding a contribution to the aggregation of individuals, inherent in a larger-scale approach, it not being possible to isolate this effect. *Alchornea glandulosa* and *Ixora brevifolia* have zoochory mainly by avifauna (Pascotto, 2006; Prado Júnior et al., 2012), with less chance of this effect, but *Gymnanthes klotzschiana*, *Galipea jasminiflora* and *Metrodorea stipularis* have autochoric dispersion (Cosmo et al., 2010; Piedade, 1991), with a greater chance of aggregation in the plant community.

The size of the trees did not show a strong negative correlation with increasing steepness, and no significant negative correlation with the faces turned mainly to the north, but the direction of the vectors showed this trend. The tallest species and with more sectional area were *Ixora brevifolia* and *Metrodorea stipularis.*

A feature of the analyzed environment is its face, mainly on the southwest. The species with greater abundance also occurred on the southeast and south faces, but with greater overlap on the southwest face, indicating that they prefer less exposure to sunlight throughout the year.

The difference in altitude and distance from the water body indicated the species *Alchornea glandulosa* and *Sebastiania commersoniana* as having lower dispersion, being closer to the water body and at different altitudes. The species with greater occurrence and farther from the water body was *Metrodorea stipularis*.

Interestingly, *Alchornea glandulosa* had the lowest amount of runoff, indicating it does tolerat places with greater drainage and/or tendency to waterlog, contrary to information found in the literature. An assumption for this may be in the 12.5 x 12.5 m resolution of the digital elevation model, and the distance of trees to the quadrant point, selecting cells (pixels) with lower runoff adjacent to the cells containing the tree position in the ground.

In preference in relation to the slope, the species that occupied sites with greater steepness was *Achornea glandulosa*, and among the other species, there were no large variations as to slope preference.

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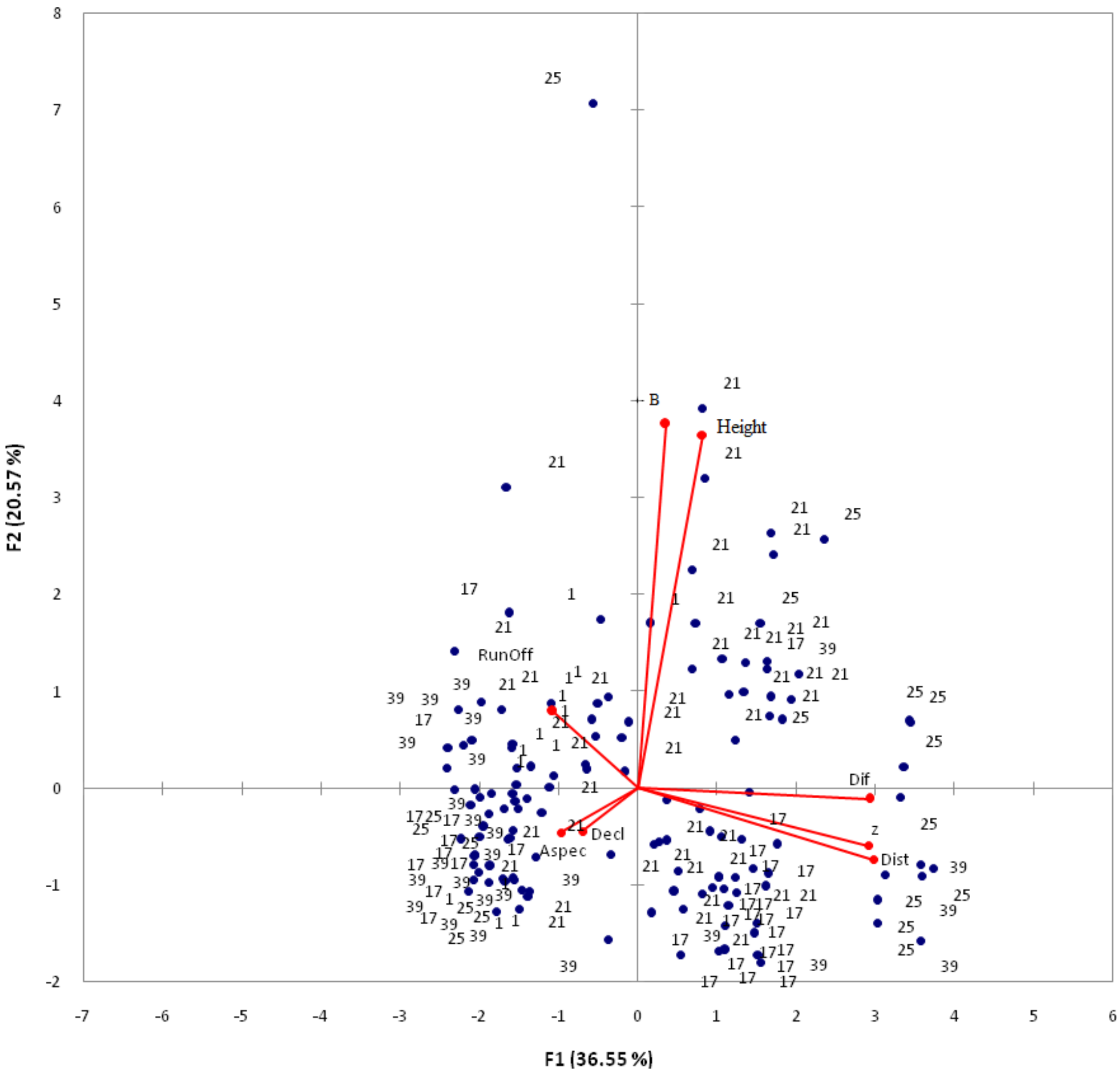

Figure 4. *Analysis of major components of physical parameters (distance and altitude difference of the water body, slope, aspect) and tree size (basal area and height) for the species with the highest importance value*

Continuing with we discuss relationships between particle size parameters associated to topography and water features and also identify relationships between fertility parameters and particle size parameters.

For *Alchornea glandulosa* and *Gymnanthes klotzschiana* species, which prefer the shortest distance from the stream and less difference in altitude, we can expect small differences in clay, silt and sand content, reducing the percentage of clay and increasing silt and sand. In these environments, particle densities and micro-porosity will be greater, following the trends in figure 3. Continuing the correlations, for fertility, Al contents are lower, and Ca, Mg, P, SB, V, Cu, Zn, Fe and Mn contents are higher, although all Fe and Mn levels in the samples are above the need of the plants.

In all samples, the micro and macro-porosity are high, but not enough to retain water by surface tension forces, and particle density indicates higher organic matter content.

Acidity is low, the Ca content is high, the Mg content is moderate and the P content is low. Zn and Cu have high values and base saturation is high.

After the analysis, it follows that for degraded environments with similar topographical, hydrological and soil conditions, colonization would start by including these major species, all of them initial secondary, except *Alchornea*, which is pioneer. That is, all have requirements to start the ecological succession, theoretically heliophytic species in their early development. *Achornea* and *Gymnanthes* could be planted closer to the stream, *Galipea* and *Ixora* could mainly occupy the middle range, and *Metrodorea* would be planted farther from the stream, based on the analyses presented.

Conclusion

Ecological restoration processes and recovery of degraded areas go through issues such as indication of native species, which require well-founded forest inventories, within the floristic, phytosociology, ecophysiology, and even faunal relationships.

The results in this study demonstrate a relationship between biotic and abiotic factors of the area, and confirm the influence of the stream that flows through the fragment on the vegetation, determining species occurrence and distribution. To validate the indication of species to compose ecological restoration proposed in this work, an assay with long-term monitoring is necessary.

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ANNEXES

Table 1. *Physical parameters: altitude (z), distance from the stream (Dist), difference of altitude between the point and the stream (Dif), slope in degrees (Decl), sun exposure in azimuth (Aspec), runoff (runoff), for the soil sampling points, coordinates UTM Zone 23/WGS 84*

POINT	x (m)	y (m)	z (m)	dist (m)	dif (m)	decl (m)	aspec	*runoff*
1	575656	7849054	939	17	1	17	180	9
9	575725	7849113	950	43	9	17	135	1
19	575810	7849151	942	12	-2	6	135	2
28	575835	7849231	948	0	0	8	180	3266
39	575673	7849101	952	39	15	19	180	1
43	575696	7849135	962	74	24	8	180	1
47	575703	7849177	959	102	18	3	270	1
51	575690	7849205	964	120	19	11	180	1
56	575657	7849129	957	71	20	7	0	1
60	575683	7849168	958	112	15	8	135	436
65	575654	7849234	974	160	23	9	135	1
68	575639	7849275	978	185	20	12	180	1

Table 2. *Mean grain size among the three depths (0-10, 10-20, 20-30 cm): fine sand (sand), coarse sand (csand), clay, silt, particle density (DensP), macro-porosity, micro-porosity and total porosity (poreden)*

POINT	sand	csand	clay	silt	DensP	macrop	microp	poreden
	--------------------%----------------------				g/cm^3	----------%----------		
1	3	4	38	55	2,24	19,2	40,3	59,5
9	8	1	33	57	2,33	9,9	46,5	56,4
19	4	2	36	58	2,25	12,4	41,5	53,9
28	4	3	30	63	2,29	14,2	37,7	51,9
39	3	2	45	50	2,21	19,2	34,3	53,5
43	3	3	44	51	2,30	16,4	38,3	54,7
47	3	3	46	48	2,25	16,4	37,3	53,7
51	3	2	49	45	2,23	20,0	33,6	53,6
56	3	3	45	49	2,13	15,5	35,5	51,0
60	3	3	46	48	2,29	15,3	37,8	53,1
65	3	2	48	48	2,26	16,5	34,9	51,4
68	2	2	49	47	2,26	16,6	37,8	54,4

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Table 3. *Mean fertility values among the three layers (0-10, 10-20, 20-30 cm): phosphorus (P), potassium (K), aluminum (Al), calcium (Ca), magnesium (Mg), Cation Exchange Capacity (CEC), Hydrogen + Aluminium (H + Al) Sum of Bases (SB), Saturation by Aluminum (m), Bases Saturation (v), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), pH and organic matter (om)*

POINT	P	K	Al	Ca	Mg	CEC	H+Al	SB	m	v	Cu	Fe	Mn	Zn	pH_{H2O}	om
	-----mg/dm^3-----		-------------------------cmolc/dm^3--------------------------						------%------		----------------mg/dm^3------------------					Dag/kg
1	2.78	58	1.95	2.20	0.44	9.98	12.3	2.78	31.45	18	0.84	89.70	20.72	0.84	6.7	4.01
9	3.81	50	0.30	5.89	0.77	10.71	3.9	6.79	5.60	62	3.42	870.50	74.64	2.45	5.7	3.86
19	7.48	63	3.17	1.29	0.25	10.22	8.5	1.70	65.27	17	3.21	386.00	21.47	1.69	5.0	2.74
28	2.92	51	3.29	1.27	0.41	8.36	6.5	1.81	65.44	21	1.63	124.37	59.06	0.65	4.7	3.05
39	2.02	60	5.67	0.85	0.22	10.67	9.4	1.23	81.89	12	1.32	95.79	27.60	0.73	4.6	3.73
43	1.74	97	3.30	1.85	0.49	8.73	6.1	2.59	62.20	28	1.23	80.90	51.52	0.63	5.4	3.50
47	1.74	104	3.92	3.46	0.63	12.03	7.7	4.35	48.62	36	1.19	88.39	61.29	1.23	5.2	4.23
51	2.07	73	5.48	2.70	0.51	13.96	10.6	3.40	64.87	22	1.27	177.53	46.17	1.50	4.8	5.23
56	2.06	80	3.74	1.55	0.50	9.81	7.6	2.26	65.91	23	1.05	82.44	32.00	0.21	5.6	3.80
60	2.50	97	5.06	1.12	0.25	14.91	13.3	1.62	76.65	10	1.15	108.86	49.37	0.55	5.4	4.19
65	2.26	97	4.77	1.79	0.29	10.98	8.7	2.33	67.22	22	0.70	126.63	25.14	0.59	5.5	3.62
68	2.68	85	4.03	2.54	0.52	12.21	8.9	3.29	55.13	27	1.40	154.96	38.09	1.20	5.4	3.86

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Table 4. *Pearson Correlation among the hydrographic parameter (distance from the stream, dist), topographic parameters (z, slope, aspect, runoff), soil parameters (sand, coarse sand (csand), clay, silt, pore density (poreden), macro and micro-pores, aluminum (Al), Ca, Mg, P, K, Cation Exchange Capacity (CEC), H + Al, sum of bases (SB), aluminum saturation (m), base saturation (V), micro-nutrients (Cu, Fe, Mn, Zn, pH), organic matter (om), in the first 10 cm of soil (n=12)*

Variables	dist	slope	aspec	runoff	sand	csand	clay	silt	poreden	macrop	microp	Al	Ca	Mg	P	K	CEC	H+Al	SB	m	V	Cu	Fe	Mn	Zn	pH	om
z	**0.95**	-0.21	0.04	-0.24	-0.34	-0.30	**0.74**	**-0.71**	0.46	0.10	-0.56	0.36	-0.02	-0.10	-0.55	0.41	-0.01	0.00	-0.02	0.10	0.04	-0.34	-0.30	0.05	-0.09	0.03	0.29
dist		-0.19	0.07	-0.39	-0.33	-0.20	**0.81**	**-0.81**	0.31	0.04	-0.45	0.41	0.03	-0.09	-0.44	0.52	0.19	0.17	0.03	0.10	-0.01	-0.35	-0.28	0.01	0.02	0.18	0.43
slope			0.03	-0.15	0.37	0.29	-0.23	0.11	-0.06	-0.37	**0.58**	-0.13	0.29	0.18	0.08	-0.52	0.33	0.12	0.27	-0.21	0.09	0.13	0.28	-0.06	0.45	0.22	0.27
aspec				0.10	-0.15	0.21	0.03	-0.01	0.11	0.15	0.11	0.02	0.19	0.09	-0.18	0.02	0.28	0.13	0.18	-0.10	0.02	-0.13	-0.13	0.43	0.34	-0.24	0.24
runoff					-0.10	0.04	-0.54	**0.66**	0.44	0.01	-0.06	-0.07	-0.25	-0.16	-0.06	-0.32	-0.08	0.12	-0.25	0.09	-0.23	0.01	-0.11	0.34	-0.26	-0.24	-0.45
sand						-0.42	**-0.60**	0.44	0.13	-0.48	0.55	**-0.70**	0.55	0.33	0.16	-0.50	0.00	-0.40	0.52	-0.55	0.52	**0.70**	**0.91**	0.35	0.56	0.29	-0.17
csand							0.03	-0.04	-0.48	0.30	0.31	0.00	0.18	0.41	-0.11	0.19	0.46	0.28	0.21	-0.20	0.04	-0.53	-0.55	-0.03	-0.05	0.27	0.49
clay								**-0.98**	-0.20	0.31	**-0.66**	**0.67**	-0.15	-0.15	-0.41	**0.67**	0.20	0.30	-0.13	0.32	-0.17	-0.57	**-0.58**	-0.20	-0.23	-0.14	**0.59**
silt									0.25	-0.25	0.54	-0.56	-0.03	0.01	0.44	**-0.64**	-0.29	-0.26	-0.04	-0.16	0.03	0.51	0.46	0.12	0.10	0.03	**-0.70**
poreden										-0.21	-0.02	-0.12	-0.08	-0.22	-0.13	-0.19	-0.25	-0.17	-0.11	-0.01	0.01	0.16	0.21	0.19	0.02	0.15	-0.40
macrop											-0.46	0.22	-0.15	0.08	-0.55	0.40	-0.30	-0.21	-0.11	0.07	0.11	**-0.80**	**-0.71**	-0.07	**-0.72**	-0.14	0.15
microp												**-0.63**	0.41	0.34	0.49	-0.42	0.20	-0.10	0.39	-0.46	0.25	0.46	0.57	0.02	**0.62**	**0.59**	-0.09
Al													**-0.71**	**-0.72**	-0.02	0.34	0.13	**0.68**	**-0.71**	**0.86**	**-0.80**	-0.49	**-0.60**	-0.42	-0.46	-0.46	0.18
Ca														**0.92**	-0.40	-0.10	0.38	-0.39	**1.00**	**-0.95**	**0.88**	0.13	0.36	**0.59**	**0.62**	0.46	0.50
Mg															-0.45	-0.01	0.25	-0.48	**0.94**	**-0.95**	**0.90**	-0.05	0.11	0.49	0.38	0.44	0.47
P																-0.27	-0.12	0.20	-0.41	0.37	-0.49	**0.61**	0.45	-0.51	0.24	0.04	-0.52
K																	0.27	0.31	-0.06	0.11	-0.05	-0.48	-0.54	0.05	-0.37	0.09	0.49
CEC																		**0.70**	0.38	-0.19	-0.04	-0.12	-0.06	0.42	0.42	0.18	**0.76**

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H+Al																		-0.40	0.55	**-0.73**	-0.20	-0.31	-0.04	-0.04	-0.18	0.35
SB																			**-0.95**	**0.89**	0.10	0.32	**0.59**	**0.59**	0.46	0.52
m																				**-0.94**	-0.15	-0.34	**-0.58**	-0.49	-0.52	-0.31
V																					0.08	0.29	0.49	0.32	0.42	0.27
Cu																						**0.90**	0.14	**0.62**	-0.03	-0.49
Fe																							0.20	**0.68**	0.21	-0.36
Mn																								0.34	-0.08	0.29
Zn																									0.26	0.18
pH																										0.15

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Table 5. Pearson Correlation among the hydrographic parameter (distance from the stream, dist), topographic parameters (z, slope, aspect, runoff), soil parameters (sand, coarse sand (csand), clay, silt, pore density (poreden), macro and micro-pores, aluminum (Al), Ca, Mg, P, K, Cation Exchange Capacity (CEC), H + Al, sum of bases (SB), aluminum saturation (m), base saturation (V), micro-nutrients (Cu, Fe, Mn, Zn, pH), organic matter (om), in the first 20 cm of soil

Variables	dist	slope	aspec	runoff	sand	csand	clay	silt	poreden	macrop	microp	Al	Ca	Mg	P	K	CEC	H+Al	SB	m	V	Cu	Fe	Mn	Zn	pH	om
z	**.95**	0.21	.04	0.24	0.29	0.33	**.75**	**0.76**	0.42	.34	0.23	.41	0.08	.05	0.43	**.61**	.03	.06	0.05	.17	0.07	0.45	0.26	0.07	0.26	0.08	.06
dist		0.19	.07	0.39	0.31	0.33	**.81**	**0.84**	0.30	.41	0.26	.43	0.04	.02	0.41	**.59**	.27	.25	0.02	.14	0.08	0.46	0.24	0.16	0.22	.02	.20
slope			.03	0.15	.38	0.28	0.17	.13	.10	.11	0.14	0.16	.23	.01	0.21	**0.59**	.16	.02	.19	0.19	.14	.04	.33	0.05	.20	0.02	.11
aspec				.10	0.11	.11	.03	0.01	.38	.24	0.13	.10	.11	0.02	0.15	.23	0.28	0.32	.10	0.03	.18	0.06	0.13	.16	.14	0.31	.15
runoff					.04	.52	0.57	**.60**	.06	0.32	0.02	0.04	0.19	0.09	0.01	0.37	0.44	0.26	0.19	.15	0.09	0.01	0.12	.30	0.15	0.36	0.35
sand						0.51	**0.60**	.49	.40	**0.74**	**.79**	**0.77**	**.85**	**.71**	.34	0.36	.05	0.49	**.83**	**0.86**	**.82**	**.75**	**.96**	**.73**	**.84**	.11	.24
csand							0.22	.26	.01	.09	0.37	.17	0.53	0.38	0.28	0.10	0.02	.32	0.52	.47	0.51	0.52	**0.65**	0.19	**0.65**	.22	0.34
clay								**0.99**	0.35	**.67**	0.55	**.69**	0.35	0.28	0.51	**.58**	.21	.40	0.33	.46	0.39	**0.58**	0.50	0.41	0.44	0.04	.31
silt									.29	**0.59**	.47	**0.62**	.24	.18	.55	**0.58**	0.27	0.38	.22	0.36	.30	.55	.41	.30	.37	0.03	0.40
poreden										0.29	.36	0.28	.26	.02	.26	0.16	0.09	0.22	.23	0.24	.25	.42	.41	.47	.43	0.03	.30
macrop											**0.84**	**.83**	**0.61**	**0.63**	0.37	.06	.16	.53	**0.61**	**.68**	**0.66**	**0.64**	**0.62**	**0.74**	0.46	0.33	.15
microp												**0.88**	**.77**	**.77**	**.60**	.04	0.14	**0.63**	**.78**	**0.85**	**.83**	**.82**	**.78**	**.70**	**.71**	.27	0.04
Al													**0.78**	**0.76**	0.46	.17	.06	.55	**0.78**	**.87**	**0.81**	**0.70**	**0.69**	**0.58**	0.57	0.42	.20
Ca														**.91**	.24	0.06	.19	0.47	**.00**	**0.98**	**.97**	**.64**	**.83**	**.66**	**.82**	.23	.33
Mg															.14	.05	.08	0.52	**.93**	**0.92**	**.92**	.51	**.65**	**.70**	**.62**	.27	.21
P																0.15	0.20	0.33	.23	0.36	.30	**.80**	.50	.03	.56	0.13	0.28
K																	0.11	0.09	0.02	.10	.01	0.23	0.32	.01	0.23	.26	0.07
CEC																		**.77**	.18	0.14	0.04	0.27	.10	0.28	.04	.54	.43
H+Al																			0.48	.51	**0.66**	**0.64**	0.43	**0.68**	0.48	.32	.18

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SB																				**0.98**	**.97**	**.62**	**.81**	**.67**	**.80**	.24	.32
m																					**0.97**	**0.70**	**0.84**	**0.65**	**0.81**	0.27	0.20
V																						**.70**	**.79**	**.73**	**.80**	.13	.21
Cu																							**.82**	.50	**.84**	0.16	.06
Fe																								**.60**	**.93**	.03	.30
Mn																									.55	.01	.29
Zn																										0.15	.40
pH																											0.14

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Table 6. *Pearson Correlation among the hydrographic parameter (distance from the stream, dist), topographic parameters (z, slope, aspect, runoff), soil parameters (sand, coarse sand (csand), clay, silt, pore density (poreden), macro and micro-pores, aluminum (Al), Ca, Mg, P, K, Cation Exchange Capacity (CEC), H + Al, sum of bases (SB), aluminum saturation (m), base saturation (V), micro-nutrients (Cu, Fe, Mn, Zn, pH), organic matter (om), in the first 30 cm of soil*

Variables	dist	slope	aspec	runoff	sand	csand	clay	silt	poreden	macrop	microp	Al	Ca	Mg	P	K	CEC	H+Al	SB	m	V	Cu	Fe	Mn	Zn	pH	om
z	**0.95**	-0.21	0.04	-0.24	-0.45	-0.43	**0.78**	**-0.76**	0.15	0.10	-0.21	0.30	0.16	0.34	-0.40	**0.81**	0.53	-0.09	0.39	0.05	-0.02	-0.44	-0.22	0.22	-0.15	-0.33	0.49
dist		-0.19	0.07	-0.39	-0.48	-0.44	**0.84**	**-0.83**	0.09	0.16	-0.20	0.31	0.26	0.40	-0.36	**0.83**	0.53	0.11	0.42	-0.02	0.02	-0.46	-0.20	0.14	-0.12	-0.15	**0.59**
slope			0.03	-0.15	0.38	-0.33	-0.11	0.08	0.20	0.51	-0.31	-0.02	0.27	0.05	-0.16	-0.55	-0.36	0.08	0.01	-0.32	0.37	0.15	0.38	-0.13	-0.02	0.33	0.14
aspec				0.10	-0.08	-0.26	0.08	-0.04	0.41	0.24	-0.11	0.21	0.25	0.47	-0.12	0.10	0.16	0.28	0.20	-0.10	0.08	-0.06	-0.13	0.19	0.24	-0.05	0.10
runoff					0.18	0.37	**-0.58**	**0.60**	0.01	-0.12	0.06	-0.14	-0.24	0.07	0.10	-0.28	-0.18	-0.30	-0.16	0.12	-0.08	0.04	-0.13	0.27	-0.20	-0.32	-0.19
sand						-0.43	**-0.63**	0.56	0.32	-0.37	**0.62**	**-0.77**	0.53	0.24	0.38	-0.57	-0.31	**-0.63**	0.46	**-0.75**	**0.77**	**0.76**	**0.87**	**0.62**	0.57	0.03	-0.22
csand							-0.32	0.36	-0.56	0.04	-0.30	0.20	**-0.69**	-0.41	0.14	-0.24	-0.36	0.29	**-0.73**	**0.63**	**-0.66**	-0.23	-0.56	**-0.64**	-0.46	0.20	-0.24
clay								**-0.99**	-0.21	0.46	-0.46	**0.66**	-0.09	0.08	**-0.60**	**0.78**	0.53	0.37	0.06	0.34	-0.32	**-0.67**	-0.45	-0.10	-0.30	-0.10	**0.65**
silt									0.23	-0.46	0.44	**-0.62**	0.05	-0.11	**0.60**	**-0.76**	-0.51	-0.32	-0.10	-0.29	0.26	**0.64**	0.39	0.05	0.28	0.09	**-0.68**
poreden										-0.36	0.37	-0.43	0.54	0.20	0.09	0.02	-0.09	-0.29	0.47	-0.55	0.55	0.32	0.44	0.37	0.37	0.02	-0.37
macrop											**-0.86**	**0.72**	-0.28	0.06	-0.56	-0.03	-0.05	**0.58**	-0.38	0.43	-0.38	-0.57	-0.45	-0.38	-0.47	0.20	**0.68**
microp												**-0.77**	0.43	0.12	0.52	-0.12	0.11	-0.48	0.48	-0.59	0.53	**0.70**	**0.64**	**0.61**	**0.62**	-0.12	-0.52
Al													**-0.61**	-0.31	**-0.60**	0.40	**0.64**	**0.79**	-0.57	**0.80**	**-0.78**	**-0.77**	**-0.82**	-0.56	**-0.62**	-0.44	0.34
Ca														**0.73**	0.41	-0.01	-0.04	-0.39	**1.00**	**-0.95**	**0.93**	0.55	**0.77**	0.53	**0.73**	0.43	0.16
Mg															0.12	0.15	0.07	-0.21	**0.78**	**-0.61**	**0.61**	0.17	0.32	0.58	0.40	0.26	0.50
P																-0.46	0.04	-0.24	0.38	-0.50	0.39	**0.84**	**0.58**	-0.02	**0.74**	-0.12	-0.43
K																	0.48	0.05	0.21	0.23	-0.21	**-0.61**	-0.44	0.17	-0.25	-0.22	0.39
CEC																		0.15	0.33	0.27	-0.37	-0.03	-0.12	0.18	0.26	**-0.73**	0.15

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H+Al																			-0.50	0.58	**-0.66**	-0.46	-0.57	**-0.66**	-0.35	0.35	0.18
SB																				**-0.93**	**0.91**	0.51	**0.70**	**0.64**	**0.73**	-0.24	0.18
m																					**-0.99**	**-0.72**	**-0.91**	**-0.61**	**-0.77**	-0.45	0.01
V																						**0.65**	**0.89**	**0.67**	**0.68**	0.44	0.04
Cu																							**0.87**	0.30	**0.86**	-0.12	-0.48
Fe																								0.48	**0.78**	0.04	-0.20
Mn																									0.39	-0.34	0.10
Zn																										-0.22	-0.22
pH																											0.08

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PLANKTON AND MACROINVERTEBRATE COMMUNITY COMPOSITION IN THE PELAGIC AND NON-VEGETATED LITTORAL DRAWDOWN ZONES OF A SHALLOW RESERVOIR, MANJIRENJI, ZIMBABWE

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Abstract. We examined the influence of physicochemical variables on zooplankton, phytoplankton and macroinvertebrate community composition in a shallow dam, Manjirenji in Zimbabwe. And further explored the relations between selected water parameters on chlorophyll *a* production as a proxy for phytoplankton biomass in the non-vegetated littoral drawdown zone in comparison to the pelagic zone. We observed high similarity overlaps between some water parameters, and a uniform dominance pattern of zooplankton comprising of rotifers > copepods > cladocera in littoral and pelagic sites, though the dominance order for phytoplankton varied significantly between lake zones. Pollution sensitive macroinvertebrate families dominated in the non-littoral vegetated drawdown zone. Physicochemical variables account for low plankton (50.37%) and macroinvertebrates (59.59%) ecogeography suggesting the influence of latent (unexplored) environmental and biotic factors on plankton and macroinvertebrate community composition in this lake. There was no significant predictor factor among Secchi depth, total nitrogen, and total phosphorus towards chlorophyll *a* production in the Dam. Non-vegetation in littoral drawdown zones of the shallow turbid lake reduce habitat complexity conferring functional and structural similarity with the pelagic zone. There is a need to comprehend transitional nutrient and water hydrodynamics and their effects on aquatic biodiversity in shallow lakes.
Keywords: *eulittoral zone; lake level fluctuation; alternative equilibria; ecosystem; fauna; assembly*

Introduction

Shallow tropical lakes which are located in arid areas have extensive periods of low water levels intertwined with increased levels in the rain season (Antenucci et al., 2000). Natural water level fluctuations have associated effects on the water quality and resident aquatic communities (Wantzen et al., 2008), besides constantly shifting the drawdown zone. Though the effects of natural water level fluctuations on water quality and aquatic organisms are magnified if the dams have other non- seasonal water withdrawing activities present (Abrahams, 2008). Water level fluctuations serve to promote high species diversity in littoral zones of shallow lakes and for doubling the productivity of these lakes thus projecting fish densities in water bodies (Antenucci et al., 2003; Kolding and van Zwieten, 2012).

In shallow turbid lakes, spatial isolation, stochastic nutrient stoichiometry and water level fluctuations due to the constant shifting of the ecotone up and down the shoreline affects aquatic biodiversity (Wang et al., 2011; Kolding and van Zwieten, 2012). For this exploratory study, we examined the effects of physicochemical variables on the

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community composition of phytoplankton, zooplankton and macroinvertebrates in different zones of a shallow lake, Manjirenji in Zimbabwe, which is prone to extreme water level fluctuations. This shallow lake is unique as it has a non-vegetated shoreline (Utete and Tsamba, 2016). Regardless, we assumed that there were two alternative stable states in this shallow lake, largely adopting the alternative stable equilibria states framework by Scheffer et al. (1993). The relatively deeper pelagic zone acts as the first equilibrium state which predominates at low nutrient concentrations, and is characterised by abundant macrophytes and clear water. This state is stabilised by high zooplankton grazing rates, low planktivorous and benthivorous fish abundances and high piscivorous fish abundances, and thus a low macroinvertebrate abundance (Scheffer et al., 1993). And we assumed the second state, which is characterised by abundant phytoplankton and turbid water at relatively high nutrient concentrations to be prevalent in the littoral zone. This turbid state is stabilised by light limitation due to algal blooms and has high macroinvertebrate densities. We also assumed that at intermediate (similar) nutrient levels, both alternative stable states can occur within a shallow lake. Scheffer et al. (1993) considers that within a given background of biotic conditions, abiotic interactions may strongly influence both function and community structure at different trophic levels, including the zooplankton. Thus in this study we hypothesised that: shallow lakes with non-vegetated shorelines, show no demarcation or dissimilarity for plankton and macroinvertebrate community composition, and physicochemical variables between the open deeper pelagic and shallow littoral sites. Conversely, we explored the suggestion that a continuum in shallow lakes and non-significant spatial isolation confers similarity in nutrient stoichiometry, thus, we envisage similarities in plankton and macroinvertebrate community composition (Quiros, 2003).

Although most research on plankton and macroinvertebrates has focused on river reservoirs (transition from river to lake/reservoir) (Grzybkowska et al., 1990), other reservoir studies have been limited to pre- and post-impoundment surveys or have been done on systems that have irregular drawdown patterns or low winter water levels that subject the benthos to freezing (Kolding and van Zwieten, 2012). Few, or sparse studies have been conducted in shallow lakes with non-vegetated shorelines to explore the effect of stochasticity of nutrient stoichiometry on plankton and macroinvertebrates assemblages (Baldwin et al., 2008). The objective of this study was to investigate the influence of physicochemical variables and abundance on phytoplankton, zooplankton and benthic macroinvertebrates community composition in the non-vegetated littoral drawdown zone relative to the pelagic zone of a turbid shallow dam, Manjirenji in Zimbabwe, with multiple designated uses

Materials and methods

We carried out this study in Manjirenji Dam located in Zimbabwe, constructed from 1964-67 with the primary aim of irrigating sugar cane estates in Mkwasine and Triangle in Zimbabwe (ZINWA, 2014). The lake's hydrological history and morphometry is shown in *Table 1*. The Dam, a relatively shallow reservoir located in the located in the arid south eastern part of Zimbabwe is subject to wide water level fluctuations reaching a drastic 12.6% or 4.66 metres from an average of 24. 2 metres in November 2012, 18.9% in September 2013 and increasing to just below 40% in January 2014 (ZINWA, 2014). Though the main purpose for its construction was to supply irrigation water for

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the vast sugar cane producing Mkwasine Estates, other multiple designated uses comprising of domestic water abstraction, lakeshore irrigation, downstream wheat irrigation and small scheme hydroelectric power generation have subsequently evolved (Svubure et al., 2010; ZINWA, 2014). We deliberately established ten non-vegetated littoral sites so as to cover as much as logistically possible the drawdown shoreline zone of the Manjirenji Dam, and chose seven pelagic sites as field controls (*Figure 1*). We designated each site less than 2m deep as a littoral site, with all other sites deeper denoted as pelagic sites and sampled each site once per month from August 2013 to April 2014.

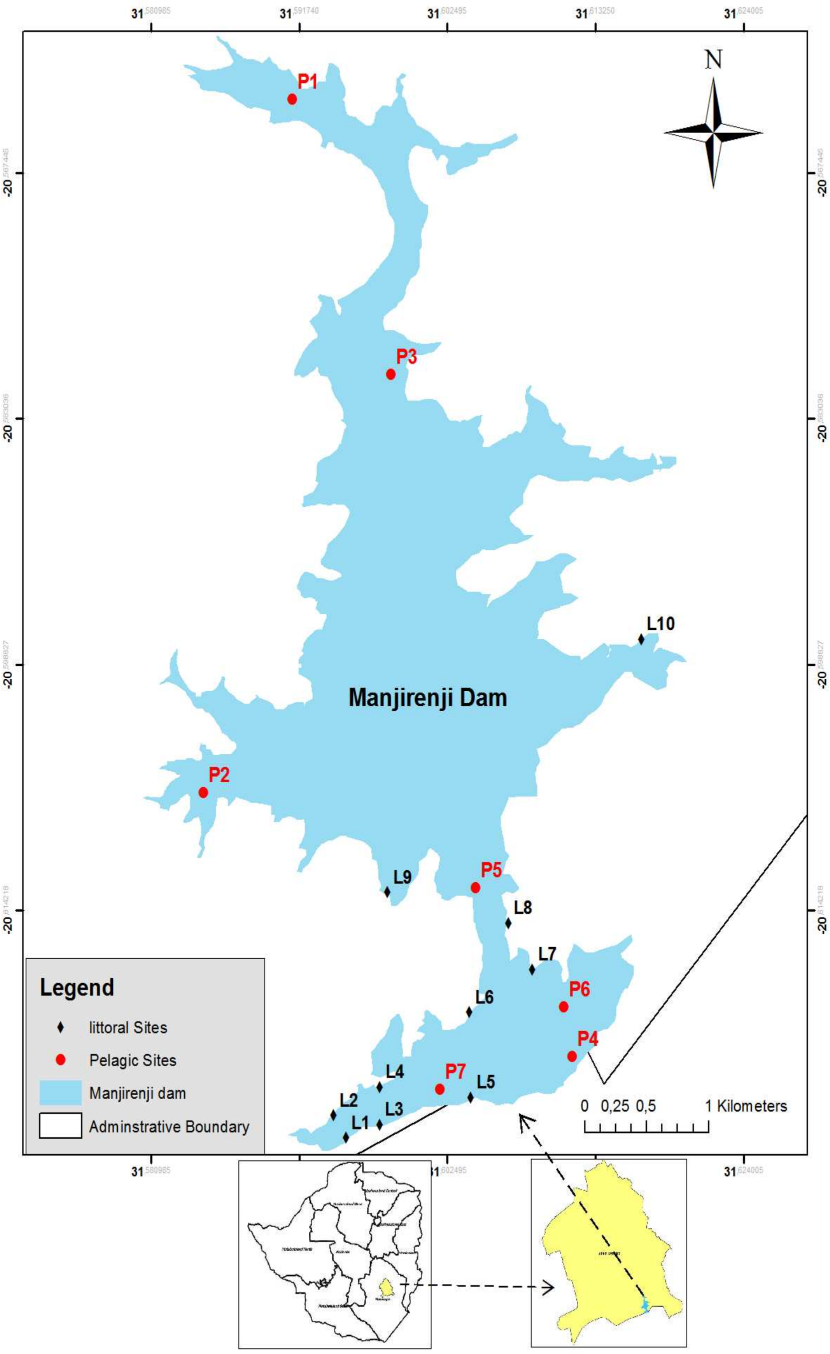

Figure 1. *Sampling sites in the Manjirenji Dam. P=Pelagic and L = Littoral.*

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Table 1. *Morphometry of the Manjirenji Dam source (ZINWA, 2014).*

Name	Manjirenji Dam
Location	Chiredzi
Construction began	1964-1967
Height	51 metres
Length	382 metres
Capacity	284.2million cubic metres
Catchment area	1536km^2
Surface Area	2020ha
Max water depth	47metres
Discharge capacity	2730m^3/s
Average depth	1.35m

Data collection

Assessment of physicochemical parameters

At each site we collected three replicate integrated water samples at a minimum depth of 0.5m using a 5L Ruttner sampler. All effort was made to take the three replicates at each site and to sample before mid-morning ie 1000hrs to ensure uniformity. In situ measurements of pH, conductivity, total dissolved solids, temperature, turbidity, dissolved oxygen and Secchi depth were done using a pH meter, Conductivity meter, Nephelometer, DO meter (HACH, LDO, Germany) and a Secchi disc. Nitrogen, nitrates, ammonia, sulphates total and reactive phosphorus were determined using standards methods from EPA, Hach and Standard Methods in the laboratory (APHA, 1995).

Assessment of phytoplankton and zooplankton

Phytoplankton were sampled using phytoplankton nets with a diameter of 40 cm and mesh sizes of 20µm, thus excluding picoplankton and nanoplankton. After collection, phytoplankton samples were preserved with Lugol's solution before transfer to the laboratory.

Zooplankton (cladocerans, copepods and large rotifers) were collected with a 12 L Schindler-Patalassampler (Vanni et al., 1997). Three replicate samples were taken at each site in the pelagic and littoral zone of the lake. The samples from a given site were combined and filtered through a 62 µm mesh net, thus excluding picoplankton and nanoplankton, and preserved in 5% formaldehyde (final concentration). After collection and preservation the phytoplankton and zooplankton samples were transferred to the laboratory for identification and enumeration. Zooplankton were identified and enumerated following the standard Edmondson-Winberg method (1971). Plankton

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species were identified following Needham and Needham (1962). The drop count method (Trivedi and Goel, 1984) was used for the quantitative estimation of the phytoplankton and zooplankton.

Macroinvertebrate sampling

We used Surber samplers with an area of 0.916 m^2 to collect macroinvertebrates from sand and cobble samples in the littoral sites. Woody debris was sampled using forceps to pick organisms off submerged logs for 5 minutes. Surber samplers were used for 5 minute durations at each site. At each site we took a minimum of 5 Surber samples in order to collect a wider variety of macroinvertebrates. Drift net samplers were used to collect macroinvertebrates in the deeper pelagic sites. The drift nets were anchored at each pelagic site for 5 minutes to capture macroinvertebrates. Macroinvertebrate samples were separated from the water, mud and detritus, identified to family level following studies by Gerber and Gabriel (2002), sorted and counted in the field.

Data analysis

For the purpose of this paper we did not analyse and discuss individual water quality parameters but the detailed water chemistry data are presented in the Appendix section. Spatial and temporal differences in physical chemical parameters were investigated using one way ANOVA at 5 % level of significance using Past 2.16 software (Hammer et al., 2001). Similarly, a Raup Crick cluster analysis to evaluate the similarities and dissimilarities of sites for environmental factors in both the pelagic and littoral zone was done in Past 2.16 (Hammer et al., 2001). We organized the paper along parameters that show great similarity and dissimilarities between sampling sites and exploited the effects of documented water parameters comprising of Secchi depth, total nitrogen (TN), total phosphorus (TP) on chlorophyll *a* levels. A multiple regression statistical approach was used to elucidate the relationships between Secchi depth TP, TN vs chlorophyll *a* as a proxy for phytoplankton biomass in the non-vegetated littoral drawdown zone in comparison to the pelagic zone of the turbid shallow dam, Manjirenji in Zimbabwe.

The density of zooplankton and phytoplankton was calculated using the formula by as stated below:

$$\text{Density} = \text{Number of Organisms (Org) / Litres (L)}$$

The densities calculated were then used to compute the diversity indices. Zooplankton and phytoplankton diversities were calculated using Past 2.16 (Hammer et al., 2001). Shannon Wiener index, evenness, and dominance indices were used for the study as a measure of plankton diversity. Species richness was measured as the total number of zooplankton and phytoplankton species in a particular zone and the detailed results are shown in the Appendix section. The relationship between phytoplankton and zooplankton diversity and environmental factors was investigated using the Canonical Correlation Analysis following the study of Sharma and Sharma (2012), with the CANOCCO 5 software by Ter Braak and Smilauer (2012).

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Results

Physicochemical parameters

The full set of water quality parameters is shown in the Appendix section. The pH values were slightly neutral with the highest value recorded at site L7 of 8.08±0.85 (*Appendix A*). Highest mean water temperature of 30.47±12.80 °C were recorded at L3, and DO values averaging 4.2 mgL^{-1} were recorded across sites in both the pelagic and littoral zones (*Appendix A and C*). Electrical conductivity values averaging above 79 $\mu S\cdot cm^{-1}$ were recorded across all littoral and pelagic sites (*Appendix A and C*). Turbidity was highest at L4 averaging 221.07±160.63 NTU, and high suspended solid (SS) values of 166±118.58 and 140.33±74.14 mgL^{-1} were recorded at sites L4 and L9 respectively (*Appendix C*). Relatively higher mean total phosphorus concentrations of 0.47±0.74 and 0.31±0.25mg L^{-1} were recorded at L6 and P6 respectively (*Appendix A and C*). Comparatively consistent mean ammonia and nitrate values of 0.03 mgL^{-1} were recorded across all littoral and pelagic sites (*Appendix B and D*). Mean water transparency was highest in pelagic sites 1, 3 and 4 (with respective average Secchi depth values of 2.11±1.02, 2.03±1.11 and 2.66±1.23m – see *Appendix C*). Whilst a high mean sulphate value of 153.93±155.68 $mg.L^{-1}$ was recorded at P1 (*Appendix D*). High mean chlorophyll *a* values of 3.45±1.56, 3.57±0.99, 3.32±1.01 $\mu g\ L^{-1}$ were recorded at L5, L9 and P3 respectively.

There were no significant differences (Anova, $p > 0.05$) of pH at different sites in the littoral zone. There were significant differences of pH in the pelagic zone, for example site P6 in the pelagic zone differed significantly from sites P2 and P5. Whilst, we detected no significant differences of DO concentrations in the pelagic sites sampled, DO concentration in some sites in the littoral zones such as L2, L4 and L9 differed significantly from sites L8 and L6. We detected no significant variations of TDS values at all the littoral and pelagic sites. However, there were significant differences in the levels of suspended solids (SS) recorded between sites in both the littoral and pelagic zones. For instance in the pelagic zone, the levels of suspended solids at site P6 differed from SS values at P2, P4 and P7. The mean Secchi depth was relatively consistent among littoral sites and did not differ significantly (Anova, $p>0.05$) in the littoral sites of the Manjirenji Dam. However, the mean Secchi depth differed significantly among pelagic sites in the Manjirenji Dam.

We recorded significant variations in total phosphate concentrations among sites in the littoral zone. There were no significant variations in nitrate concentrations among sampling sites in the pelagic zone, though we recorded significant variations in nitrate concentrations among littoral sites. Significant variations in TN concentration were recorded among sites in the pelagic zone, and between site L6 and sites L8 and L9 in the littoral zone of the Manjirenji Dam. Though we recorded no significant differences in EC values among sites in the pelagic zone, the EC values at some littoral sites differed significantly. For instance EC values at site L8 differed significantly from L2 and L6, whilst EC values at sites L1 and L4 differed significantly from site L9 and L10 respectively. We recorded no significant differences in ammonia concentrations across all pelagic and littoral sites. There were significant variations in turbidity among littoral sites, for instance turbidity values at site L7 differed significantly from those recorded at L1 and L3. Chlorophyll *a* concentrations did not differ significantly ($p>0.05$) among

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littoral sites. The Raup Crick cluster analysis computation of site similarity (*Figure 2*) shows no clear distinction of physical/ chemical parameters between pelagic and littoral sites in the Manjirenji Dam as evidenced in the extensive inter-site dichotomy above the 0.95 similarity level characterised by numerous branching off of sites.

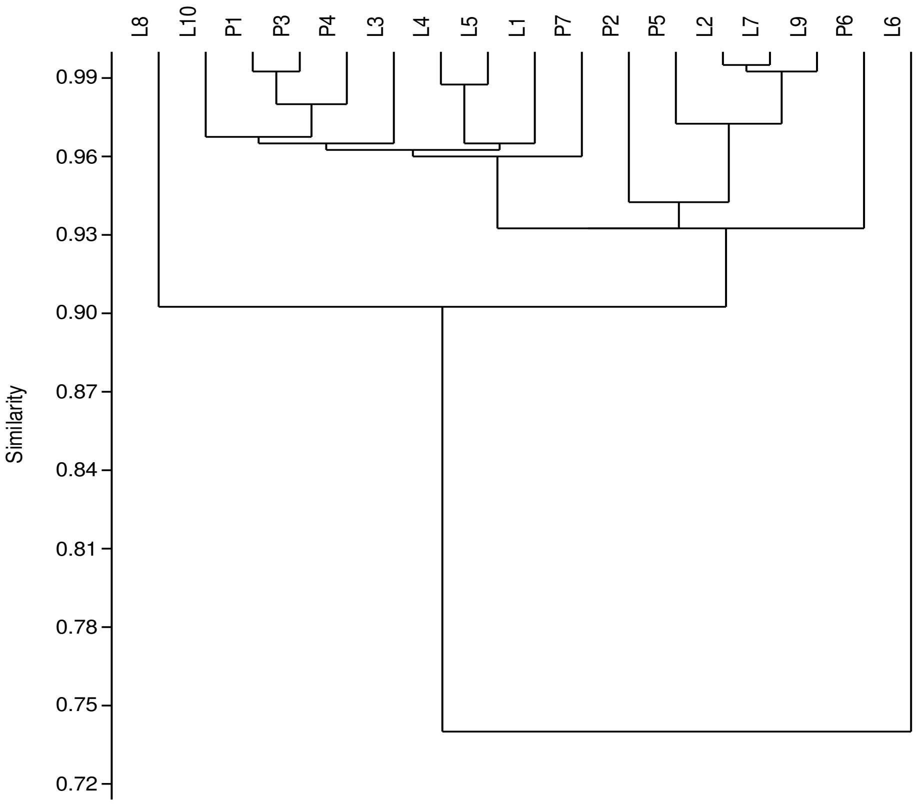

Figure 2. *Raup Crick cluster diagram showing site similarities / dissimilarities among pelagic (P) and littoral (L) sites sampled in the Manjirenji Dam.*

Phytoplankton community

Phytoplankton community in Manjirenji Dam comprised of diatoms, chlorophytes, cyanophytes, desmids and euglenophytes. Unknown filamentous algae were grouped in an unspecified group termed ‘other groups’ in this study. Diatoms consisted of 9 taxa with 10 species. The order of dominance for phytoplankton in the littoral zone comprised of diatoms (73.5%) > cyanophyta (10.1%) > chlorophyta (8.8%). Euglenophytes (3.8%), desmids (2%) and the other group (1.8%) were present in smaller quantities. The pelagic zone was also dominated by diatoms (57%) followed by euglenophyta (23.4%) and chlorophyta (17.2%). Cyanophytes (1.8%), desmids (0.1%) and the other group (0.5%) were present in lower quantities. Five taxa of diatoms were recorded in both the pelagic and littoral zone and they also recurred in all the sampling months. These were *Tetraedon regulare, Diatoma, Synedra, Fragillaria* and *Cymbella*. The scatter diagram for phytoplankton dominance reflects overall dominance for diatoms, cyanophyta and desmids in the littoral zone of the Manjirenji Dam (*Figure 3*).

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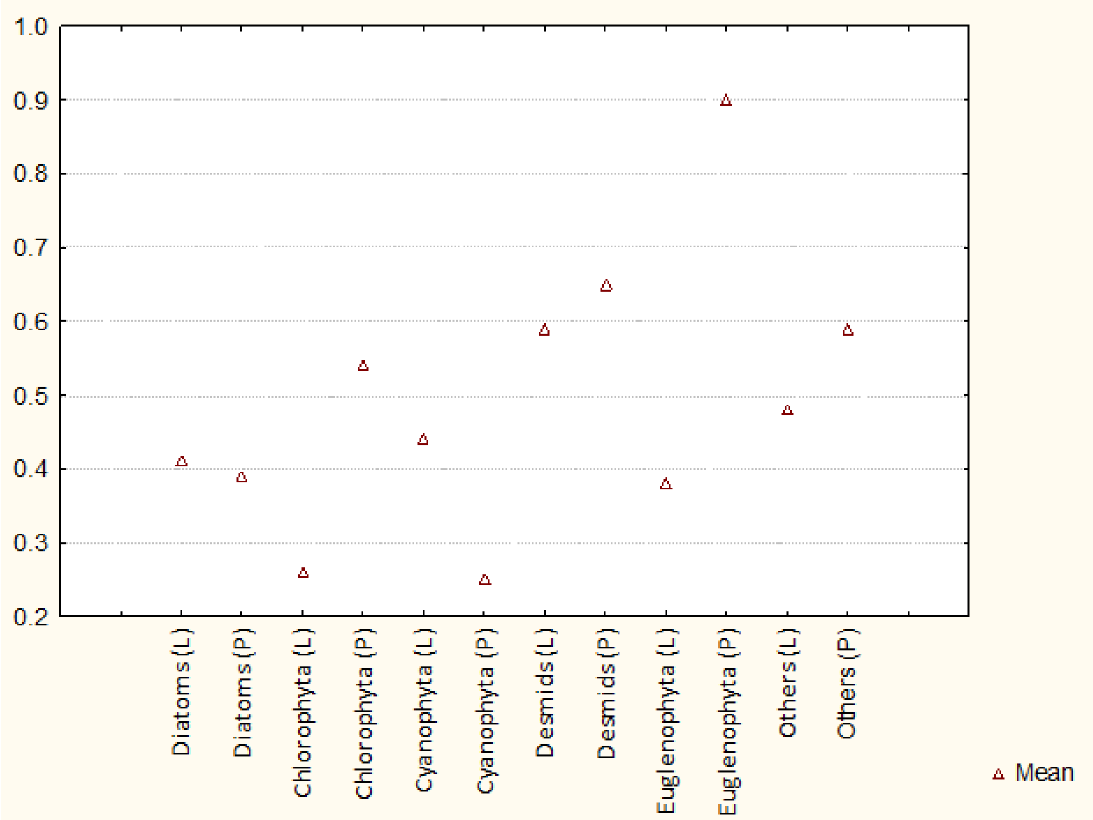

Figure 3. *Phytoplankton taxon dominance in pelagic (P) and littoral (L) sites sampled in the Manjirenji Dam.*

Diatoma were evenly distributed in both the littoral and pelagic zones and were characterised by low dominance. Although diatoma had high diversity index values, they were surpassed by chlorophyta which was the most diverse group in the littoral zone with a Shannon index of 1.58±0.3 (*Appendix E*). Cyanophytes were the most diverse group in the pelagic zone with a Shannon index of 1.58±0.18 (*Appendix E*). Cyanophytes had a low dominance and higher evenness in both the littoral and pelagic zones (*Appendix E*). They were more diverse in the pelagic zone than in the littoral zone. Chlorophytes were more diverse in the littoral zone than in the pelagic zone, and were more evenly distributed in the littoral zone than in the pelagic zone and had low dominance in the littoral zone than in the pelagic zone (*Appendix E*).

We used chlorophyll *a* as a proxy for phytoplankton biomass in the Manjirenji Dam following Carlson (1977). There were no significant correlations ($p > 0.05$; Spearman test) among the Secchi depth, TN, TP and chlorophyll *a* for littoral sites. However, chlorophyll *a* was significantly correlated to TN in the pelagic zone. High R values (.> 0.60) in the pelagic zone indicate a strong relationship among the Secchi depth, TN, TP and chlorophyll *a* in the pelagic zone (*Table 2*). Multiple regression indicates no significant predictor factor ($p < 0.05$) among Secchi depth, TN, TP towards chlorophyll *a* production in both littoral and pelagic zones in Manjirenji Dam as illustrated by derived equations 1 and 2.

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Regression equation 1 for chlorophyll a in the littoral zone:

Chlo a (µg/g) = 0.20 Secchi depth (m) + 0.16 TN (µg/g) + 0.48 TP (µg/g) -5.072
R2 = 0.32, P value =0.16

Regression equation 2 for chlorophyll a in the pelagic zone:

Chlo a (µg/g) = 0.16 Secchi depth (m) + 0.85 TN ((µg/g) + 0.309 TP (µg/g) -3.807
R2 = 0.60, P value = 0.29

Zooplankton community

We recorded 3 groups of zooplankton comprising of rotifers, cladocera and copepods in both the littoral and pelagic zones of the Manjirenji Dam. Rotifers consisted of 8 taxa with 14 species, whilst copepods consisted of 6 taxa. Rotifers consisted of *Filinia pejleri, Branchionus havaensis, Branchionus calyciflorus, Trichocerca similis, Keratella tropica, Polyartha vulgaris, Keratella quadrata, Tetracerca tropis, Trichocerca elongata, Lecane luna,* an unidentified *Trichocerca species and Euchlaris dilatata*. The *Trichocerca similis* made up the bulk of rotifers in both the littoral and pelagic zone. Copepods consisted of *Afrocyclops, Eudiaptomus, Megacyclops, Macrocyclops, Metaboeckella* and *nauplii*. Cladocerans were composed of 4 taxa namely *Daphnia species, Ceriodaphnia, Sididae* and *Chyrodidae.* The scatter diagram for zooplankton dominance show rotifer dominance in the littoral zone, whilst copepods and cladocera dominate in the pelagic sites (*Figure 4*).

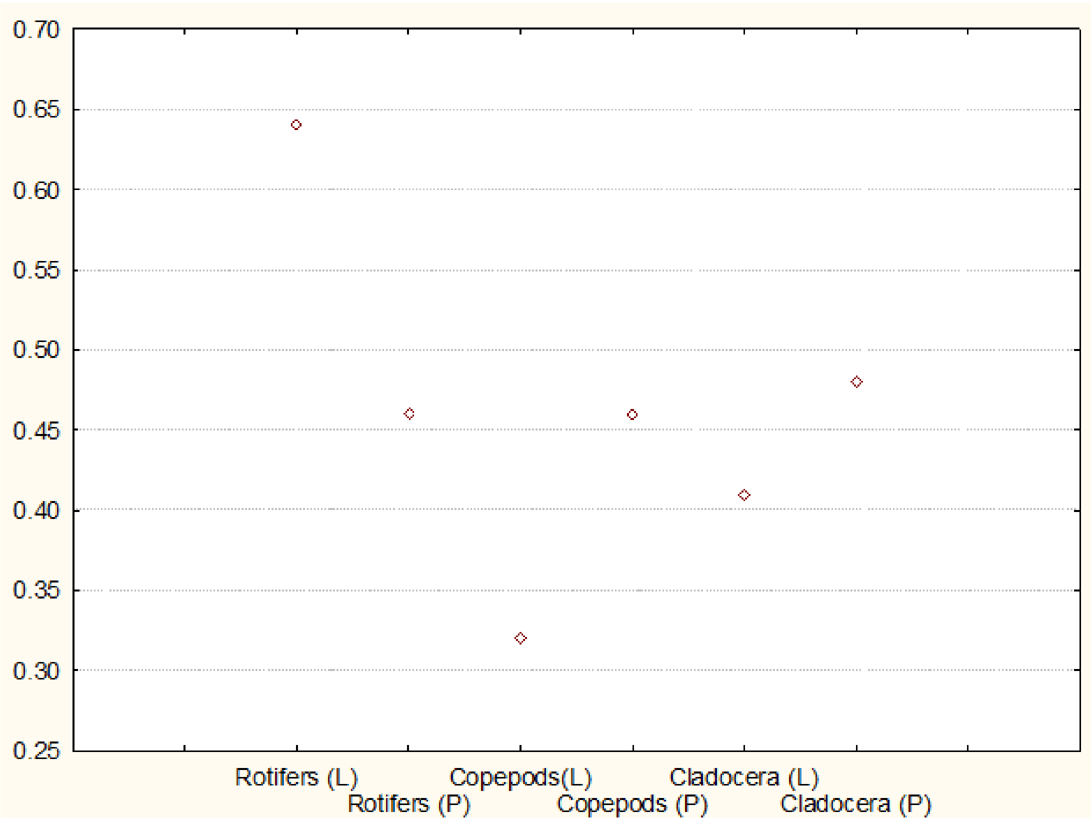

Figure 4. *Zooplankton taxon dominance in sites sampled in the Manjirenji Dam*

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*Table 2. Spearman rank correlation among chlorophyll a, Secchi depth, total nitrogen and total phosphorus in littoral (L) and pelagic (P) sites sampled in the Manjirenji Dam. Significant relations are denoted *.*

Pair of variables	Spearman R (L)	P level (L)	Spearman R (P)	P level (P)
Chlo *a* & Sec depth	0.19	0.60	0.75	0.052
Chlo *a* & TN	0.22	0.53	0.82	0.02*
Chlo *a* & TP	0.47	0.17	0.60	0.18

Species richness of zooplankton was 14±2 (mean ± standard deviation) and 11±2 for the littoral and pelagic zones respectively (*Appendix F*). The abundance of zooplankton followed a distinct pattern over the sampling period which consisted of Rotifer > Copepod > Cladocera for both littoral and pelagic sites. Although rotifers were dominant in the littoral and pelagic zones numerically, their diversity in the littoral zone was the lowest among the three groups. Rotifers were however, the most diverse group in the pelagic zone (*Appendix F*). Copepods and cladocerans were more diverse in the littoral zone than in the pelagic zone. The cladocerans however, were more dominant and also more evenly distributed in the pelagic zone than copepods and rotifers (*Appendix F*). Rotifers were characterised by higher dominance and lower evenness in the littoral zone and exhibited opposite traits in the pelagic zone. Whilst copepods had low dominance in the littoral zone but were evenly distributed in both the littoral and pelagic zone (*Appendix F*).

Canonical Correspondence Analysis (CCA) reflect that physicochemical variables accounted for up to 50.37% of the variation in phytoplankton and zooplankton abundance and distribution in the Manjirenji Dam (*Figure 5*). Temperature, turbidity and nitrate concentrations positively influenced abundance and distribution rotifers and chlorophytes particularly in the littoral sites (*Figure 5*). Electrical conductivity, total dissolved solids and dissolved oxygen levels positively influenced the abundance and distribution of diatoms and cladocera in the pelagic sites and at littoral sites near the dam wall. Copepods and euglenophytes were positively influenced by sulphur concentrations in the pelagic sites (*Figure 5*). However, there was no environmental variable positively associated with zooplankton and phytoplankton at some littoral and pelagic sites like L1. L2. L4, L7, L8, L10, P3 and P4 (*Figure 5*).

Macroinvertebrate community

A total of 242 macroinvertebrates belonging to 25 families were recorded in the 10 littoral shorelines sites of the Manjirenji Dam. We recorded a very low number (7) of macroinvertebrates in the pelagic sites to be useful for inclusion in this research. The most dominant taxa with dominance index > 0.50 were the *Notoneumoridae, Belostomatidae, Hydropsychidae, Hydrophilidae, Sphaeridae*, and *Pyralidae*. Physicochemical variables explained 59.59% of the variation in macroinvertebrates at the ten littoral sites sampled. Total dissolved solids, dissolved oxygen concentrations, electrical conductivity were positively associated with the occurrence and abundance of the *Dytiscidae, Elmidae* and *Helodidae*. Nitrate and total phosphorus concentrations positively influenced the distribution of the *Hydrophilidae, Physidae, Belostomatidae, Ancylidae, Pyralidae, Lubellidae*, *Cordullidae*, and *Sphaeridae*. Ammonia concentrations in the littoral zone positively influenced abundance of pollution tolerant macroinvertebrate families such as *Chironomidae, Corixidae, Aeshnidae*, and *Hydrophilidae* (*Figure 6*). Turbidity, total nitrate concentration, suspended solids, and

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the water pH levels influenced the diversity, abundance and distribution of the pollution sensitive macroinvertebrate taxa that include the *Gomphidae, Pisaulidae, Hydropsychidae* and *Tetragnathidae.*

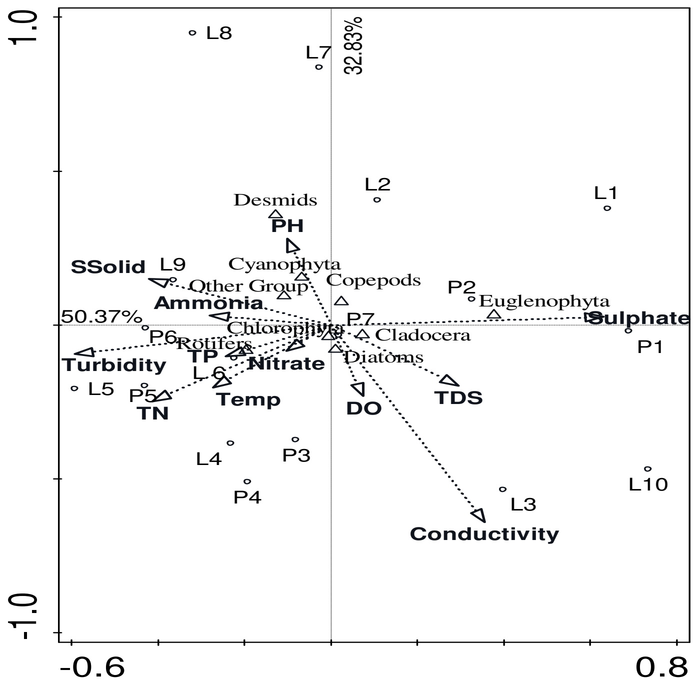

Figure 5. *CCA triplot showing the relationship between environmental variables and plankton in the pelagic and littoral zones of the Manjirenji Dam.*

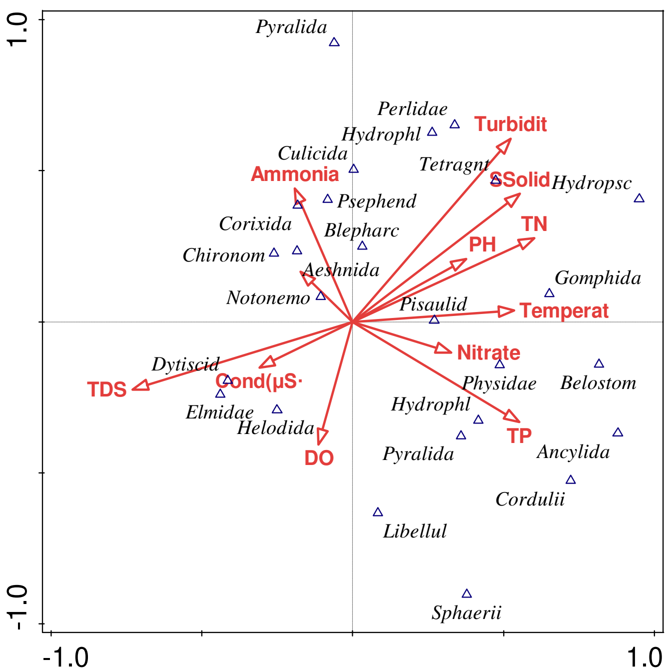

Figure 6. *CCA triplot showing associations between physical chemical variables and macroinvertebrates in littoral sites of the Manjirenji Dam.*

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Discussion

The main objectives of this paper were to determine the influence of physicochemical variables on phytoplankton, zooplankton and macroinvertebrate community composition in a shallow dam, Manjirenji in Zimbabwe. And to explore the relations between selected water parameters on chlorophyll *a* production as a proxy for phytoplankton biomass in the non-vegetated littoral drawdown zone in comparison to the pelagic zone. Our hypothesis was that: shallow lakes with non-vegetated shorelines, show no demarcation or dissimilarity for plankton and macroinvertebrate community composition, and physicochemical variables between the open deeper pelagic and shallow littoral sites.

Physicochemical parameters

Despite subtle differences in physicochemical parameters in both lake zones, the Raup Crick cluster analysis computation of site similarity shows no clear distinction for most physical/ chemical parameters between pelagic and littoral sites as evidenced in the extensive inter-site dichotomy above the 0.95 similarity level characterised by numerous branching off of sites. This reflects insignificant loss or gain of nutrients in the pelagic system, an observation attributable to intense sediment - water contact, which ensures a rapid reflux and resuspension of most sedimentated nutrients into the open pelagic system (Sheffer, 1998). Moreso, the non-significant spatial differences in water quality parameters indicate the high connectivity between different zones in shallow lentic systems facilitating longitudinal, vertical and lateral exchanges of water and materials (Scheffer and van Nes, 2007). Distinct dissimilarities among littoral sites for nutrients such as nitrates, phosphorus and sulphates reflect intrinsic site specific hydrochemical, hydraulic, and morphometric variations (Dierberg, 1992). As well non-vegetation of both the pelagic and littoral zone, cannot be discounted as a key latent factor driving lateral and vertical nutrient dispersion in this shallow lake, as submerged and emergent macrophytes tend to play crucial roles in either absorbing or releasing nutrients, and through indirect control of redox conditions in aquatic systems (Wetzel, 2001). Thus for a non-vegetated shallow lake, a host of interacting abiotic and biotic factors may impact nutrient stochasticity.

Phytoplankton community composition and chlorophyll a production

Significant spatial heterogeneity of phytoplankton community in the Manjirenji Dam, reflect an integral site specific uniqueness, spatial isolation, and its concomitant influence on consequent dominant biota (Nowlin et al., 2013; Berggren et al., 2014; Kolzau et al., 2014).

Since physicochemical variables explain 50.37% of plankton abundance and ecogeography in the dam, it suggests the limited influence of nutrient stochasticity on plankton community composition (George et al., 2012). Moreso it indicates a gap in this study which excluded vital site specific factors such as light intensity, tidal flushing, grazing pressure (Phlips et al., 2002), drawdown landscape disturbance, sedimentation rates and lake level fluctuations (Wang et al., 2011), which can influence plankton communities in shallow water habitats. We do not exclude the impact of water level fluctuations on algal biomass in shallow lakes, although we did not utilise information on them due to lack of supporting water extraction data and hydrological water flow regime for the dam during the study period. For instance, morphometrically, the

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discharge capacity for this shallow dam is 2730 m^3/s (ZINWA, 2014). This means that about 83 per cent of the whole water volume of the dam could be released per day as 2730 x 3600 s x 24 h = 234 x10**6 m^3 = 83% of 285 x 10**6 m^3. Therefore, the theoretical implications, are that water abstraction and extraction dynamics, affect the water level fluctuations in this shallow dam. How the water level fluctuations affect nutrient stoichiometry and aquatic biodiversity needs further exploration. Suggestions of bathymetrical measurements to determine thermal stratification and infer its influence on phytoplankton community composition, though, prudent, were hampered by the high operating cost, inefficiency, and inapplicability to shallow waters (Zhang et al., 2012).

Regional differences in the relative importance of light and nutrient availability strongly influence spatial and temporal patterns observed in the abundance and composition of phytoplankton in several shallow water bodies (Phlips et al., 1995a; Philps et al., 1997; Sheffer, 1998). Contrastingly, our study observed no significant spatial heterogeneous correlation between water transparency and chlorophyll *a* concentration. We envisage that the non-vegetation of the littoral drawdown zone and the lack of substantial macrophyte cover in the pelagic zone provide no inhibition to light penetration across the Manjirenji Dam. Despite previous studies on this dam showing a clear absence of shoreline macrophytes, with a muddy drawdown zone devoid of vegetation and an open deep pelagic zone (Utete and Tsamba, 2016), the extended drawdown zone and the shallow littoral sites, dissuaded us from using light climate in predicting photosynthesis and the distribution and development of phytoplankton. The recorded Secchi depth was very low (similar to the euphotic depth), which increase downward attenuation coefficient, surface reflectance or bottom reflectance (Utete and Tsamba, 2016), thus, limiting the influence of light dynamics on phytoplankton community.

The uniform non-algal turbidity values recorded among sites in both the pelagic and littoral zone, reflect uniformity of distribution of nutrients, and further suggests the crucial role of sediment resuspension on phytoplankton abundance and community composition in shallow tropical lakes (Bachmann et al., 2010). Sedimentation of cells is a major factor in the dynamics of pelagic phytoplankton (chiefly diatoms) because diatoms dominated in pelagic and littoral zone sites of Manjirenji Dam. In their study of Lake Apopka, a shallow lake, Schelske et al. (1995) and Bachmann et al., 2010, noted that the abundance of diatoms was positively correlated to wind because of resuspension of tripton and meroplankton in sediments. This may explain the high frequency of recurrence of diatoms in the shallow Manjirenji Dam, as the non-vegetated littoral zone provides no hindrance to wind mixing across the dam, which can further enhance pelagic diatom abundances through sediment resuspension and desorption. We observed algal blooms at some littoral sites which reflect localised pollution, nutrient upwelling, periodic flushing in drawdown zones and pulse release from sediments processes which are significant determinants of nutrient stoichiometry in the littoral zones in shallow lakes (Dierberg, 1992; Paerl and Huisman, 2008).

Our study show that chlorophyll *a* is strongly related to total nitrogen concentration in the Manjirenji Dam, though a strong correlation between total nitrogen and chlorophyll-*a* (bottom-up control) in the pelagic zone shows eutrophication avoidance through controlling N input to the dam (Phlips et al., 1997; Scott and McCarthy, 2010). Though, high Spearman test R values (.> 0.60) in the pelagic zone suggest a strong relationship among the Secchi depth, TN, TP and chlorophyll *a* in the pelagic zone, this is inadequate to explain relationships between lake nutrient concentrations and

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chlorophyll *a* concentration, as reflected by distinct predictive powers of the multiple regression equations we derived. In as much, several studies estimate primary production in lakes integrating chlorophyll *a* concentrations as a proxy for phytoplankton biomass (standing crop) and trophic state (Carlson, 1977). Needless to say there is extended debate over whether nitrogen (N) or phosphorus (P) is the nutrient that ultimately determines productivity in tropical shallow lakes (Schindler, 1977; Vollenweider and Kerekes, 1980; Reynolds, 1999; Lewis and Wurtsbaugh, 2008; Sterner, 2008; Tendaupenyu, 2012). Although the nature of limiting factors can vary spatially and temporally within individual lakes (Piontkovski et al., 1995), the dissimilarity in nitrogen and phosphorus concentrations we recorded among pelagic and littoral sites in tandem with a lack of a significant predictor variable among Secchi depth, TN, TP towards chlorophyll *a* production in Manjirenji Dam, reflect the complex interplay of internal mechanisms and influence of external forces on nutrient stoichiometry in lakes (Wetzel, 2001). Rather than a single nutrient determining lake productivity, the limiting nutrient in a lake may vary spatially, trophically and seasonally (Quiros, 2003). Complex multiple regressory and curvilinear relationships among chlorophyll *a*, total phosphorus, total nitrogen and water transparency have been shown for many lake data sets, though there exists a tangential rudimentary understanding of the factors that determine TP-TN and TP-Chl *a* sigmoid patterns (Kolzau et al., 2014). McCauley et al. (1989) recommends for an elucidation of the underlying biological mechanisms that produce the nonlinear response with TN and TP. Furthermore, Watson et al. (1992) suggested that non-linearity reflects an underlying systematic variation in the biomass of functional algal groups. To obtain an insight into how factors like lake depth, flushing, resuspension, grazing interact with nutrient dynamics in determining algal biomass it is prudent to go the empirical regression approach and in future studies analyse algal growth in a more mechanistic way for shallow lakes such as the Manjirenji.

Zooplankton community composition

Zooplankton dominance analysis show rotifer dominance in the littoral zone, whilst copepods and cladocera dominate in the pelagic sites. Individual taxon versus nutrient analysis was impossible due to the low number of samples we obtained at some sites. However, canonical correspondence analysis indicate that some zooplankton taxa resonate positively with some environmental variable in particular sections of the lake. As the different spatial zones in the shallow Manjirenji Dam share the same water source, geology and history, and appear to be interconnected (reducing chance effects of dispersal with colonisation), differences in zooplankton community structure can be attributed to local biotic and abiotic interactions. Moreso partial explanation of zooplankton abundance and ecogeography by spatial and environmental variable heterogeneity offers insights into the potential effects of factors (we excluded in this study) such as substrate type, sediment- recolonisation (Nhiwatiwa and Marshall, 2010), feeding apparatus adaptations, fish predation pressure (Soares et al., 2010) and the drawdown zone extent (Zohary and Ostrovsky, 2011) and retraction (Wetzel, 2001), in structuring plankton communities in the shallow littoral zones of the Manjirenji Dam. A more explicit consideration of the biologically active resource pools to heterotrophic and autotrophic elements of both phytoplankton and zooplanktonic systems is integral towards a flexible and mechanistic understanding of aquatic ecosystem response to a range of potential environmental changes (Beaver and Havens, 1996).

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Potential interactions between phytoplankton and zooplankton

Strong interactive relationships exist between phytoplankton and zooplankton as the main systematic groups of zooplankton including many taxa, feed on phytoplankton. Selective grazing by zooplankton is an important factor affecting the structure of phytoplankton communities (Scheffer and van Nes, 2007). Reciprocally, phytoplankton structure also influences the taxonomic composition and dominance of the zooplankton. For instance grazing by cladocerans creates a selective pressure on the phytoplankton community, causing elimination of organisms that do not exceed a precisely defined size (Jeppesen et al., 2002). Predatory copepods influence phytoplankton composition as they target lager sized phytoplankton, whereas nanoplanktonic algae increase in abundance.

For this study copepods and cladocera dominated in the pelagic sites, explaining the significant spatial variation in phytoplankton dominance, as rotifers which are filter feeders dominate the littoral sites. Hence large sized algal species like diatoms, cyanophyta and chlorophyta were dominant in the littoral zone. However, even smaller sized algal species were present in the littoral zone indicating that algal species which are resistant to grazing and predation are more likely to survive, but also can make filter feeding more difficult (Kozak and Gołdyn, 2004). Because of the constant feeding pressure of zooplankton on phytoplankton, the more resistant algae may become more and more abundant during the growing season. This, in combination with the pressure exerted by fish on large-sized zooplankton, results in the restructuring of the community of zooplankton towards the dominance of small-sized organisms resistant to disturbances and trophic interactions.

The domination of small species in the zooplankton community can be associated with fish predation pressure and by the negative influence of cyanophyta or cyanobacteria morphological complexity (Kozak and Gołdyn, 2004). Cyanobacteria was abundant throughout the study period and when their numbers exceed a threshold value, they exert a negative influence on the feeding, development and abundance of large cladocerans. Also, cyanobacterial filaments make their foraging difficult (they block the closing of the carapace), so these algae can influence the decline of the cladoceran community. Thus for our study we observed high diversities in rotifers and cyanobacteria in the littoral zone, where there is localised pollution. Though larger-sized cladocerans (mainly *Daphnia* spp.) were quite abundant in the littoral zone and according to Meijer, 2000, in some conditions, they can contribute to the low level of phytoplankton biomass despite a high trophic state of the water. This suggests competitive (biotic) displacement of rotifers from the pelagic zone, with resource partitioning between copepods and cladocera in the pelagic zone. An important consideration would be to determine the ratio of the large filter-feeders in zooplankton community and their distribution across a shallow lake. Moreso, future studies must explore the effect of predation pressure, competition between zooplankton and the importance of filter-feeders in both the littoral and pelagic zone of this shallow lake.

Macroinvertebrate community

Pollution sensitive macroinvertebrate families dominated the benthic macroinvertebrate community in littoral sites in the Manjirenji Dam as they mostly favour gravel beds or live under aquatic vegetation (Geber and Gabriel, 2002). They colonise solid submerged gravel substrate (Geber and Gabriel, 2002) which was

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abundant in the drawdown zone of the Manjirenji Dam. The negligible abundance of macroinvertebrates recorded in the pelagic zone, shows that most macroinvertebrates prefer macrophyte cover to avoid predation, solid substratum for anchorage and feeding, and well oxygenated water for respiratory functions (Chakona et al., 2009). Despite some physical factors accounting for the presence of pollution tolerant macroinvertebrate families that include the *Chironomidae, Corixidae, Aeshnidae*, and *Hydrophilidae*, the non-association of some benthic macroinvertebrates with some physicochemical variables in the littoral sites reflect that the presence / absence of a taxa can be a natural phenomenon (Chakona et al., 2009).

Low levels of variation explained by physicochemical variables (59.59%) suggest that other, unmeasured latent environmental and biotic factors affect macroinvertebrate community composition in the Manjirenji Dam. Factors such as substrate composition, porosity, and stability, lake bathymetry, drawdown habitat complexity, sediment recolonisation and trophic interactions are important determinants of the range and competitive abilities of benthic macroinvertebrates (Wetzel and Likens, 2000). It implores for further process based studies to better understand causal mechanisms underlying the observed low influence of physicochemical variables on macroinvertebrate abundance in both the littoral drawdown zone and pelagic sites of this shallow dam.

Conservation implications for shallow water lakes

High overlap and similarity for plankton, macroinvertebrates and physicochemical variables for shallow lakes indicate a modicum to suggest that non-vegetation or reduction of macrophyte beds in the littoral drawdown zone reduces habitat complexity (i.e., surface area, niche availability) allowing unhindered nutrient reflux, circulation and enhancing connectivity with the pelagic zone. Thus, a significant consideration for shallow lakes such as Manjirenji with marked drawdown zones, is the complexity in site selection owing to repeated water level fluctuations, which rapidly turn previously littoral sites into pelagic sites upon inundation, and vice versa upon withdrawal. This force rubric horizontal differences and similarities in nutrient stoichiometry, phytoplankton and zooplankton composition. We suggest that the shallowness of the lake, and the lack of emergent and submerged macrophytes induce hydrodynamic instability and inflexible nutrient stochasticity across the entire dam, thus conferring similarity upon physicochemical parameters in turn distorting the alternative stable equilibria among zooplankton, phytoplankton and macroinvertebrate communities. Hence the functional and habitat similarities between the pelagic and non-vegetated littoral zones. Understanding the transitional nutrient and water transparency dynamics is key to predicting the consequences of management strategies that alter nutrient stoichiometry and the translative effects on aquatic biota within shallow lake ecosystems.

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***Appendix A**. Water physical variables (mean ± SD) for littoral zones in the Manjirenji Dam.*

Site	pH	Temperature (^{0}C)	DO (mg.L^{-1})	Conductivity (µS·cm^{-1})	TDS (mgL^{-1})	SS(mgL^{-1})	Turbidity (NTU)	Secchi depth (m)
L1	7.29±0.79	26.53±10.92	4.62±10.18	79.30±28.05	57.33±4.04	84.00±26.06	114.13±51.74	1.99±0.37
L2	7.44±0.55	28.03±11.68	4.47±10.87	78.53±27.80	54.67±3.51	90.67±33.50	119.7±49.89	1.54±0.34
L3	7.50±1.03	30.17±12.65	4.86±11.73	80.87±28.44	53.00±4.58	77.67±43.92	110.2±50.75	1.99±0.48
L4	7.74±0.73	29.77±12.39	4.77±11.51	82.03±28.78	54.67±2.89	166.00±118.58	221.07±160.63	1.93±0.41
L5	7.70±0.69	30.47±12.80	4.05±12.02	80.30±28.46	54.33±3.21	84.33±52.00	162.87±112.21	1.66±0.37
L6	7.54±1.06	29.47±12.40	4.78±11.48	77.90±27.67	54.33±3.51	67.00±58.08	102.27±72.46	1.66±0.35
L7	8.08±0.85	28.37±11.65	4.37±10.94	77.47±27.70	53.33±4.93	84.00±48.59	112.53±64.9	1.71±0.42
L8	7.76±0.63	27.63±11.36	4.44±10.62	78.23±27.77	51.00±7.55	117.33±30.89	123.00±29.46	1.78±0.47
L9	7.79±0.87	28.10±11.58	5.40±10.70	79.13±27.62	52.33±10.69	140.33±74.14	181.00±93.66	1.78±0.47
L10	7.44±1.00	27.43±11.35	5.43±10.47	82.00±28.86	57.67±10.07	57.67±5.13	72.67±14.57	1.54±0.34

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Appendix B. *Water nutrients (mean ± SD) for littoral zones in the Manjirenji Dam.*

Site	TP (mg L^{-1})	TN (mg L^{-1})	Nitrate (mg.L^{-1})	Ammonia (mg.L^{-1})	Sulphate (mg.L^{-1})	Chlorophyll *a* (µg L^{-1})
L1	0.12±0.06	0.94±1.16	0.03±0.02	0.02±0.01	73.24±34.69	1.40±0.70
L2	0.11±0.10	1.54±1.32	0.03±0.03	0.02±0.01	56.88±48.25	1.87±0.11
L3	0.12±0.13	1.6±1.38	0.01±0.01	0.02±0	60.36±15.63	2.31±1.01
L4	0.28±0.34	1.53±1.32	0.03±0.02	0.02±0	53.13±14.47	1.23±0.34
L5	0.04±0.03	1.33±1.06	0.01±0.00	0.03±0.02	50.52±38.35	3.45±1.56
L6	0.47±0.74	0.8±1.22	0.04±0.02	0.02±0	53.62±26.9	0.97±0.11
L7	0.12±0.14	0.76±1.24	0.04±0.04	0.01±0	65.23±25.95	2.48±0.87
L8	0.06±0.03	0.77±0.99	0.02±0.02	0.02±0.01	53.19±18.49	2.21±0.33
L9	0.28±0.39	0.81±1.17	0.03±0.03	0.02±0.01	67.66±23.09	3.57±0.99
L10	0.04±0.01	0.57±0.94	0.01±0.00	0.02±0.01	61.66±23.22	3.01±0.77

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Appendix C. *Water physical variable (mean ± SD) for pelagic zones in the Manjirenji Dam.*

Site	pH	Temperature (^{0}C)	DO (mg.L^{-1})	Conductivity (µS·cm^{-1})	TDS (mgL^{-1})	SS (mgL^{-1})	Turbidity (NTU)	Secchi Depth (m)
P1	7.95±0.69	28.17±11.56	4.32±10.87	81.70±28.68	54.00±3.46	70.67±42.15	87.90±34.83	2.11±1.02
P2	7.83±0.84	28.60±11.82	4.40±11.07	79.87±28.1	57.33±15.70	76.33±66.26	117.37±73.02	1.97±0.93
P3	7.92±0.63	26.00±10.46	4.27±9.88	81.03±28.43	51.33±7.02	81.67±70.87	129.63±86.09	2.03±1.11
P4	7.43±1.04	28.30±11.71	5.08±10.81	78.63±27.87	54.67±3.79	76.33±62.88	107.23±65.19	2.66±1.23
P5	7.80±1.10	27.90±11.38	4.64±10.65	80.13±28.29	55.33±11.93	84.33±66.23	117.97±83.32	1.89±1.02
P6	7.82±0.94	27.80±11.34	4.18±10.71	80.10±28.27	57.00±7.00	78.00±64.16	113.83±74.83	1.71±0.89
P7	7.50±0.60	27.603±11.45	3.98±10.76	80.73±28.61	55.33±7.51	77.00±59.86	108.97±72.44	1.78±0.98

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Appendix D. *Water nutrients (mean ± SD) for pelagic zones in the Manjirenji Dam.*

Site	TP ($mg\ L^{-1}$)	TN ($mg\ L^{-1}$)	Nitrate ($mg.L^{-1}$)	Ammonia ($mg.L^{-1}$)	Sulphate ($mg.L^{-1}$)	Chlorophyll *a* ($\mu g\ L^{-1}$)
P1	0.11±0.03	0.70±1.06	0.03±0.03	0.02±0.01	153.93±155.68	2.10±0.30
P2	0.06±0.01	0.77±1.14	0.04±0.04	0.01±0.00	48.89±37.38	1.70±0.20
P3	0.15±0.07	0.76±1.02	0.04±0.05	0.01±0.00	63.36±39.22	3.32±1.01
P4	0.12±0.04	0.78±1.26	0.05±0.07	0.01±0.00	55.20±19.74	2.03±0.34
P5	0.06±0.04	1.63±1.12	0.04±0.06	0.02±0.01	56.63±46.75	1.27±0.56
P6	0.31±0.25	1.62±1.13	0.05±0.03	0.02±0.01	59.91±44.32	1.56±0.11
P7	0.04±0.02	1.61±1.21	0.03±0.01	0.02±0.01	55.02±32.78	1.21±0.87

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***Appendix E**. Phytoplankton diversities in littoral and pelagic zones.*

	Dominance		Shannon		Evenness	
Phytoplankton	L	P	L	P	L	P
Diatoms	0.41±0.07	0.39±0.07	1.19±0.15	1.17±0.12	0.4±0.08	0.52±0.05
Chlorophyta	0.26±0.08	0.54±0.29	1.58±0.3	0.87±0.49	0.64±0.12	0.45±0.22
Cyanophyta	0.44±0.22	0.25±0.05	1.26±0.46	1.58±0.18	0.51±0.3	0.78±0.12
Desmids	0.59±0.17	0.65±0.36	0.67±0.27	0.67±0.76	0.75±0.06	0.86±0.13
Euglenophyta	0.38±0.07	0.9±0.16	1.12±0.17	0.18±0.28	0.75±0.18	0.60±0.37
Other_groups	0.48±0.24	0.59±0.32	1.12±0.5	0.81±0.67	0.58±0.24	0.69±0.14

***Appendix F**. Zooplankton diversities in littoral and pelagic zones.*

Zooplankton	Dominance		Diversity		Evenness	
	L	P	L	P	L	P
Rotifers	0.64±0.34	0.46±0.12	0.82±0.71	1±0.15	0.36±0.23	0.56±0.23
Copepods	0.32±0.07	0.46±0.03	1.41±0.18	0.86±0.07	0.59±0.1	0.73±0.13
Cladocerans	0.41±0.22	0.48±0.17	1.18±0.49	0.84±0.38	0.65±0.05	0.82±0.2

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PREDICTION OF GREENHOUSE MICRO-CLIMATE USING ARTIFICIAL NEURAL NETWORK

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Abstract. The aim of this study is to develop an Artificial Neural Network (ANN) model for prediction of one day ahead mean air temperature and relative humidity of greenhouse located in the sub-humid sub-tropical regions of India. The adequacy of back propagation neural network to model the inside temperature and humidity of a production greenhouse as a function of micro-climatic parameters including temperature, relative humidity, wind speed, and solar radiation was addressed. Micro-climatic data of greenhouse and outside were collected on daily basis and used for analysis of best fit ANN model. After the network structure and parameters were determined reasonably, the network was trained. The activation functions were respectively the hyperbolic tangent in the hidden layer and the linear function in the output layer. The Root Mean Square Error (RMSE), Mean Absolute Error (MAE) and Correlation Coefficient were chosen as the statistical criteria for measuring of the network performance. A comparison was made between measured and predicted values of temperature and relative humidity, and the results showed that the BP neural network (for network (6-4-2) model given a best prediction for inside temperature and relative humidity. Statistical analysis of output shows that, the RMSE and Mean Absolute Error (MAE) between the measured and predicted temperature was 0.711 ^{o}C and 0.558 ^{o}C, and the relative humidity RMSE and MAE was 2.514% and 1.976% which can satisfy with the demand of greenhouse climate control.
Keywords*:* *ANN, temperature, relative humidity, wind speed, solar radiation*

Introduction

Prediction of temperature and relative humidity under the greenhouse is a complex process and a challenging task for researchers. The inside temperature and relative humidity of the greenhouse is one of the key parameters that directly influence the crop production. Prediction of ambient temperature can be useful in the thermal analysis of greenhouse enabling heating and cooling load calculations. The prediction and control of temperature and relative humidity is also important to maintain other micro climatic parameters at acceptable level, for reducing plant stress, checking growth of harmful organisms and to improve heating system. The greenhouse microclimate is a typical complicated nonlinear system, which provides plants with good environmental conditions for growing. ANN models have been applied in controlled environments like greenhouse and glasshouse to predict micro-climatic parameters such as temperature, relative humidity etc. (He and Ma, 2010; Seginer et al., 1994; Seginer, 1997; Linker and Seginer, 2004; Parsons, 2009). ANN does not require any prior knowledge of the system under consideration and are well suited to model dynamical systems on a real time basis. It is, therefore, possible to set up systems so that they would adapt to the events which are observed and for this, it is useful in real time analyses, e.g., weather forecasting, different fields of predictions, etc. (Devi et al., 2012). ANN provides a methodology for solving many types of non-linear problems that are difficult to solve by traditional techniques. ANN has capability to extract the relationship between the

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inputs and outputs of a process. Thus, these properties of ANN are well suited to the problem of weather forecasting under consideration (Ahmad et al., 2014). Its applications are numerous in various fields including engineering, management, health, biology and even social sciences (Diaz et al., 2001; Imran et al., 2002; Topalli and Erkmen, 2003; Islamoglu, 2003; Gwo-Ching et al., 2004; Yalcinoz and Eminoglu, 2005; Lauret et al., 2008; Jassar et al., 2009).

Frausto, and Pieters (2004) and Linker and Seginer (2004) trained a neural network using experimental data to model the internal temperature of greenhouse and they found that ANN model has great potential to predict the temperature under the greenhouse with very less degree of error. Behrang et al. (2010) developed Multi-layer perceptron (MLP) and radial basis function (RBF) neural networks for daily global solar radiation prediction. They considered various meteorological variables including Daily mean air temperature, relative humidity, sunshine hours, evaporation, and wind speed values between 2002 and 2006 for Dezful city in Iran. The results of comparison between ANN and other prediction methods showed ANN suitability in prediction due to less Mean Absolute Percentage Error (MAPE). Imran et al. (2002) used ANN for the prediction of hourly mean values of ambient temperature 24 h in advance. This neural network is trained off-line using back propagation and batch learning scheme. The trained neural network is successfully tested on temperatures for years other than the one used for training. It requires one temperature value as input to predict the temperature for the following day for the same hour.

The aim of this study is to develop an Artificial Neural Network (ANN) model to predict greenhouse mean temperature and relative humidity in one day advance using inputs as maximum and minimum temperature (T) and relative humidity (RH) of greenhouse, outside average wind speed (WS) and solar radiation (RS).

Materials and Methods

Experimental Greenhouse

The experimental sawtooth shape naturally ventilated greenhouse (N-S oriented) cladded with 200μ diffused (PAR transmissivity of 90% and 42% diffusivity) film was fabricated and installed at the Field Water Management Laboratory of Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, India (*Figure 1*).

Micro-climatic parameters such as temperature, relative humidity and solar radiation under greenhouse and outside were recorded and analyzed. Automatic weather station of M/S Campbell Scientific, Canada comprising a data-logger (model CR1000) and sensors were installed in the greenhouse to monitor air temperatures & relative humidity (model HMP 45 C), global radiation and Photosynthetically Active Radiation (SPLITE and PARLITE of Kipp and Zonen). The outside micro-climatic parameters such as temperature, solar radiation and wind speed were recorded manually using handheld instruments.

Data and Methodology

In this study, the daily micro climatic data of greenhouse and outside conditions for the years 2011- 2015 were used to train and test the model for prediction of one day ahead greenhouse mean temperature and relative humidity. The inputs for model were

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maximum and minimum temperature (T), and relative humidity (RH) of greenhouse, outside average wind speed (WS) and solar radiation (RS) however outputs were one day ahead greenhouse mean temperature and relative humidity. Neural networks generally provide improved performance with the normalized data. The use of original data as input to neural network may cause a convergence problem (Khan and Ondrusek, 2000). All the weather data sets were therefore transformed into values between -1 and 1 through dividing the difference of actual and minimum values by the difference of maximum and minimum values (Litta et al., 2013). At the end of algorithm, outputs were denormalized into the original data format for achieving the desired result. After pre-processing of data set in desired time lag format, three different data sets were extracted from the input and target data for training, validation and test phase. Training set consists of 60 percent of data to build the model and determine the parameters such as weights and activation function, validation data set includes 15 percent to measure the performance of network by holding constant parameters. Finally, 25 percent of data is used to increase the robustness of model in the test phase. Analysis of daily temperature and relative humidity data of greenhouse is presented in *Figure 2* and *3* respectively. It can be seen from *Figure 2*, the maximum and minimum temperature of greenhouse varies from 45.5 ^{o}C to 18 ^{o}C and 32 ^{o}C to 6 ^{o}C respectively whereas the mean temperature varies from 37 ^{o}C to 12 ^{o}C. Daily mean relative humidity of greenhouse varies from 84% to 31%. Outside daily solar radiation and wind speed data is presented in *Figure 4*

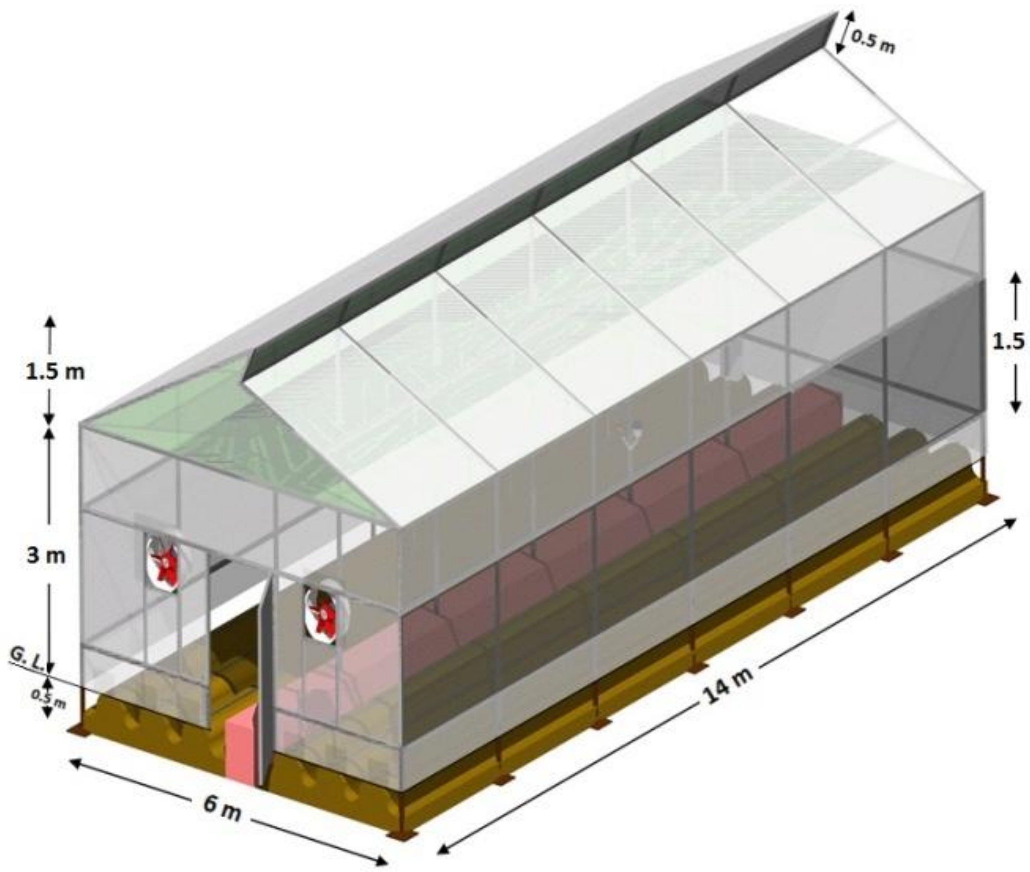

Figure 1. *Layout of experimental greenhouse*

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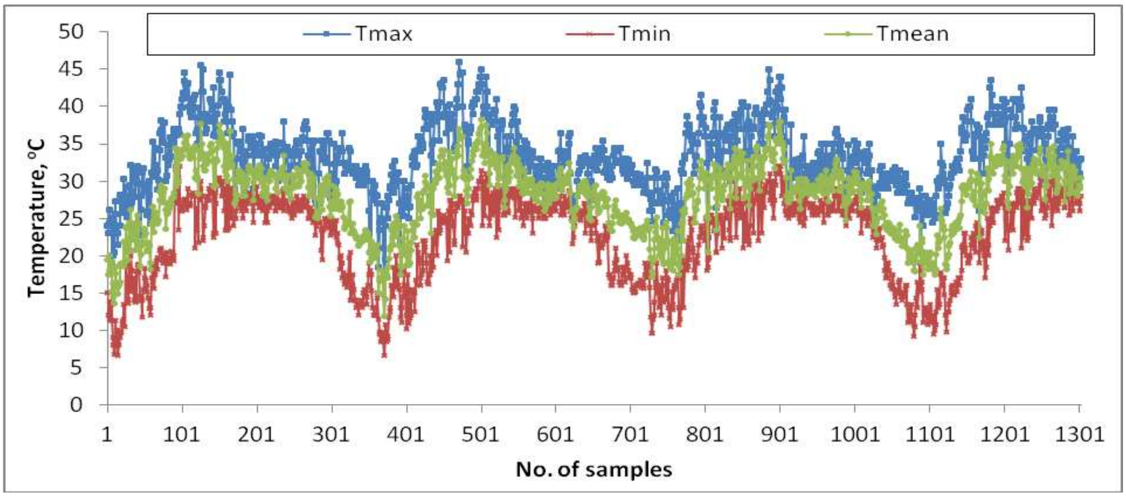

***Figure 2.** Maximum, minimum and mean temperature of greenhouse*

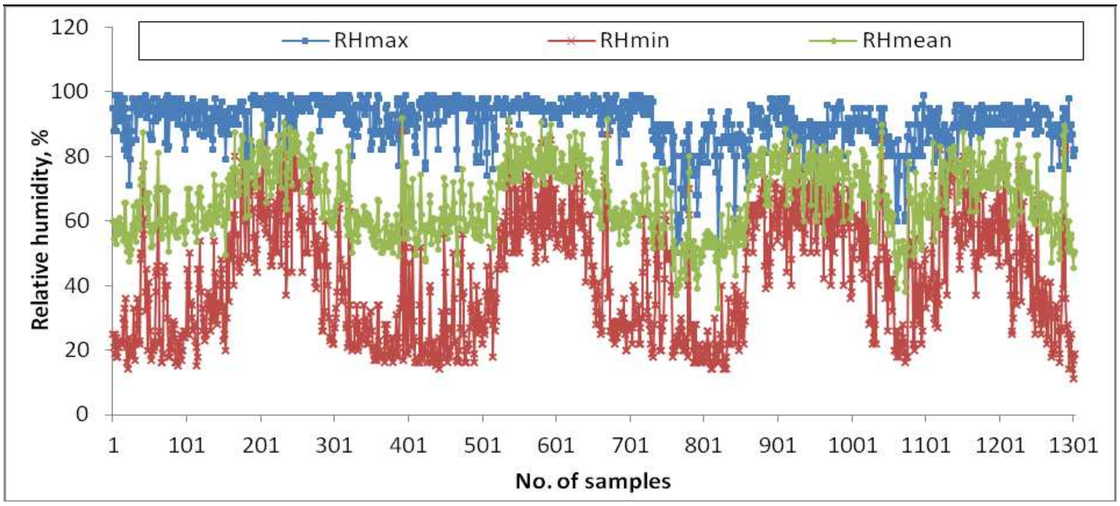

***Figure 3.** Maximum, minimum and mean relative humidity of greenhouse*

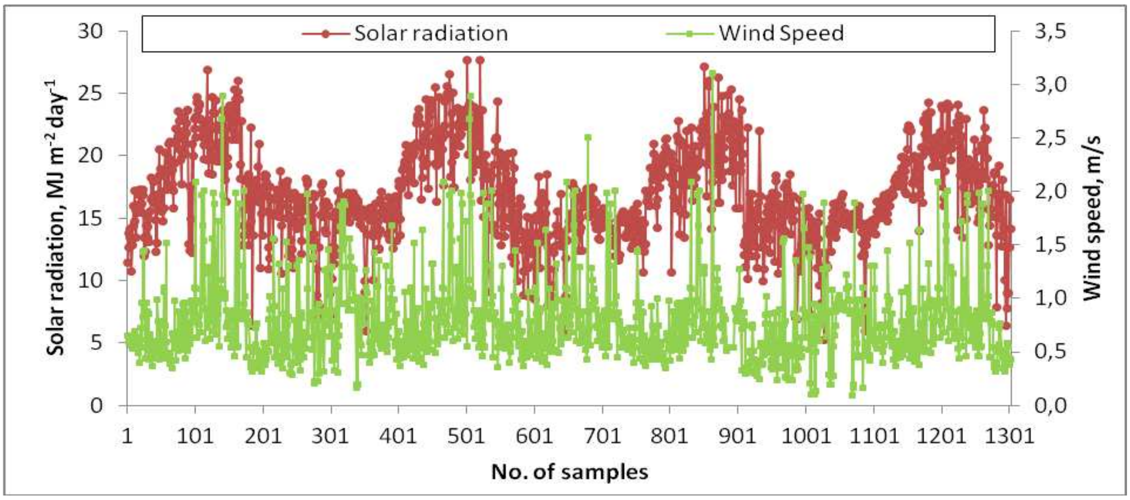

***Figure 4.** Outside daily solar radiation and wind speed experimental data*

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Determination of Neural Network Structure

Experimental data were used to determine the optimal ANN structure and simulations were carried out using Neurosolutions software developed by Neuro Dimensions Inc. of Florida. In order to determine the optimal network architecture, various network architectures were designed; the number of neurons in the hidden layer and transfer functions in hidden/output layer was changed. Based on the Kosmogorov approaching theory, the BP neural network with a single hidden layer including a sufficient number of neurons can approximate any function with the desired accuracy; the neural network with only one hidden layer was chosen (He et al., 2007; Said, 1992; Imran et al., 2002). According to the factors affecting the greenhouse micro-climate, the network input layer neuron was set as 6. The output layer neuron was 2. For the determination of number of neurons in the hidden layer, we chosen different number of neurons of hidden layer to try after inputting the same training and validating data. For each network RMSE, MAE and R^2 values of the outputs were calculated and compared. *Table 1* was the error comparison of different ANN structure. When the number of neurons in hidden layer was 4, the network output error was smallest, which showed the network structure was better. According to analysis, the neural network model was 3 layers BP neural network with the structure of 6-4-2. A typical architecture of ANN model showing inputs and output of this study is presented in *Figure 5*. The learning rate η is taken to be 0.9.

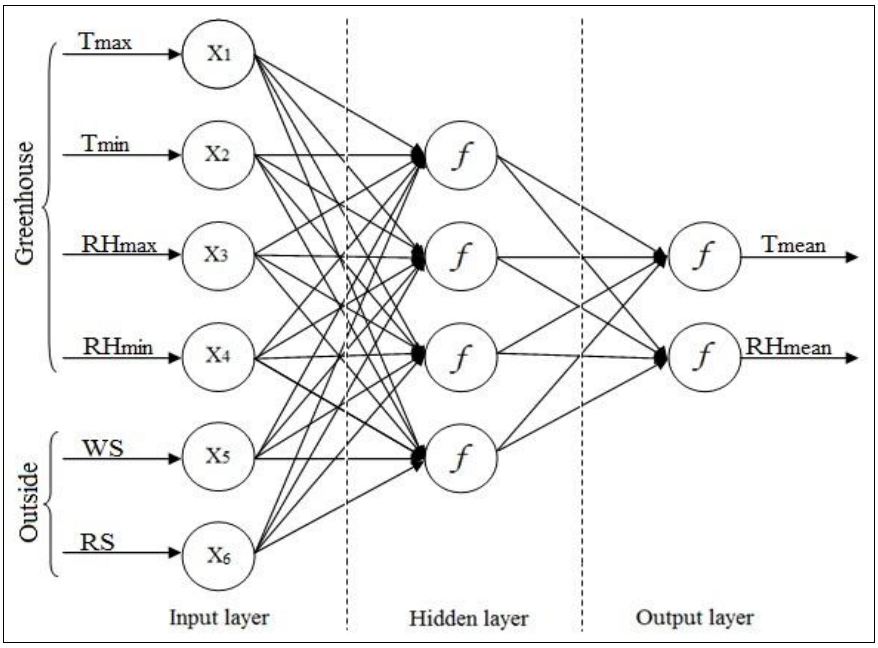

Figure 5. *The architecture of ANN model*

Training of the Network

Training of the network was performed using Levenberg–Marquardt feed-forward back propagation algorithms. It is the fastest and ensures the best convergence to a minimum of mean square error (MSE) for function approximation problems (Sahai et al., 2000, 2003). The flow chart of ANN prediction system is presented in *Figure 6*. Then, the ANN models were tested and the results were compared by means of correlation coefficient and root mean square error (RMSE) statistics. The best suited network was selected based on the minimized

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values of root mean square error (RMSE), and the maximum value of correlation coefficient (CC). Root Mean Square Error (RMSE), Mean Absolute Error (MAE), R^2 and correlation coefficient statistics were used to measure the performance of the models.

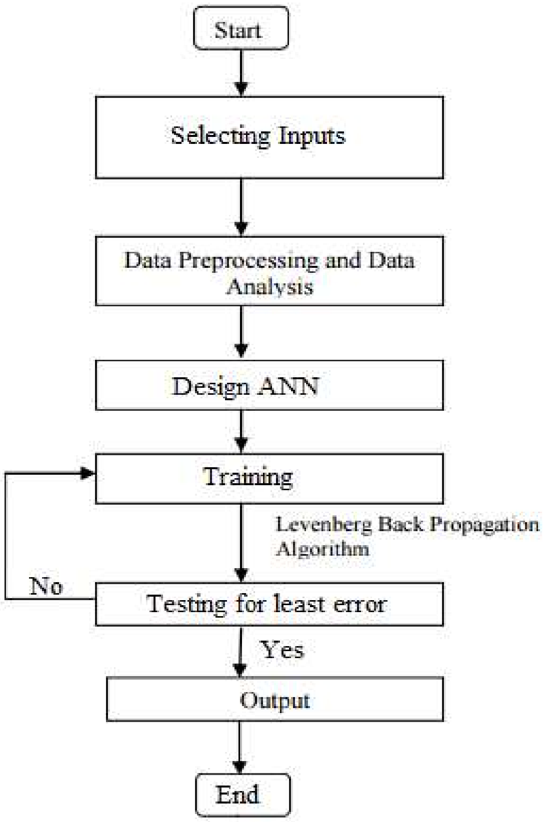

Figure 6*. Flowchart of prediction system*

Let x_i (i = 1, 2, ... n) are inputs and w_i (i = 1, 2, … n) are respective weights. The net input to the node can be expressed as *Equation 1*:

$$\mathrm{net} = \sum_{i=1}^{n} x_i w_i \qquad \text{(Eq.1)}$$

The net input is then passed through an activation function $f(.)$ and the output y of the node is computed as $y = f(net)$.

A three-layer structure (one input layer, one hidden layer, and one output layer) was selected with hyperbolic tangent (tanh) transfer function for hidden layer and linear transfer function for output layer. Therefore, an ANN with 6 inputs, *h* hidden neurons and two output units defines a nonlinear parameterized mapping from an input *x* to an output *y* given by the following relationship (*Equation 2)*:

$$y = y(x, w) = \sum_{j=0}^{h} \left[w_j \,.\, f\left(\sum_{i=0}^{6} w_{ji} x_i \right) \right] \qquad \text{(Eq.2)}$$

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There are two main steps to obtain the ANN optimal model: The learning phase and the generalization phase. During the learning phase, the ANN is trained using a training dataset of N inputs and output.

The vector *x* contains samples of each of the six input variables. The variable *t,* also called the target variable, is the corresponding measurement of the temperatures. This phase consist of adjusting *w* so as to minimize an error function *J*, which is usually the sum of square errors between the experimental output t_i and the ANN model output, $y= y(x_i;\, w)$ (*Equation 3)*:

$$J(w) = \frac{1}{2}\sum_{i=1}^{N}\{y_i - t_i\}^2 = \frac{1}{2}\sum_{i=1}^{N} e^2 \quad \text{(Eq.3)}$$

The second phase is the generalization phase. It consists of evaluating the ability of the ANN model to replicate the observed phenomenon, that is, to give correct outputs when it is confronted with examples that were not seen during the training phase.

The performance measure is usually given by the linear correlation coefficient (r), coefficient of determination (R^2), mean absolute error (MAE) and root mean square error (RMSE) (*Equation 4, 5, 6, and 7*):

$$r = \frac{n\sum \hat{y}y - (\sum \hat{y})(\sum y)}{\sqrt{n(\sum \hat{y}^2) - (\sum \hat{y})^2}\sqrt{n(\sum y^2) - (\sum y)^2}} \quad \text{(Eq.4)}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y}_i)^2} \quad \text{(Eq.5)}$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|\hat{y}_i - y_i| \quad \text{(Eq.6)}$$

$$RMSE = \sqrt{\sum_{i=1}^{n}\left[\frac{(\hat{y}_i - y_i{}^2)}{n}\right]} \quad \text{(Eq.7)}$$

Where, ŷ is predicted value, y is measure value, $\bar{y}$ is the mean of measured variables and n is the number of samples.

Results and Discussion

The ANN model was trained using the daily micro-climatic data of the years 2011–2014, and the trained model was then tested with the daily micro-climatic data of the year 2015. This process was begun with a network which had 3 neurons in its hidden layer, and repeated, increasing the number of neurons up to 10. The calculated Root Mean Squared Error (RMSE), Mean absolute Error (MAE) and fraction of variance (R^2)

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values of the errors of the ANN forecasted daily mean temperatures and relative humidity are listed in *Table 1*, for each network. The LM algorithm with 4 neurons in the hidden layer for network (6-4-2) has produced the best results, and it is used for generating the graphical outputs.

Mean square error (MSE) during training and cross validation period is presented in *Figure 7*. The back propagation Training-Error graph explains that the error is high when the iteration is less and vice versa. In the graph mentioned in *Figure 7*, it explains that when the iteration count is below 400 the training MSE is greater and when the count reaches 600 the error is minimum and constant till 1000 iteration. This results that for more accurate results, the iteration count should be high.

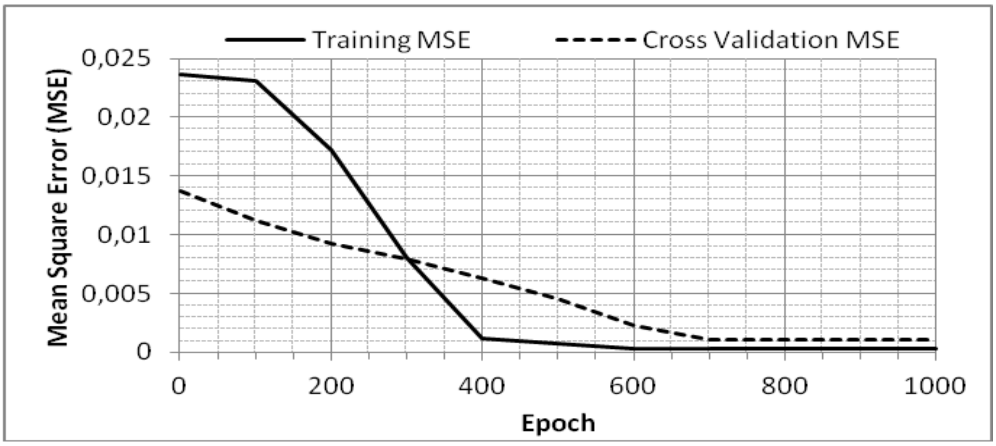

Figure 7. *Convergence of error (MSE) with Epoch during training and cross validation*

Figure 8 and *Figure 9* are the comparison curves between measured and predicted values of the inside temperature and relative humidity. *Figure 10* and *Figure 11* were the fit curves between measured and predicted inside temperature and relative humidity. From the results shown in *Figure 8* and *Figure 9* it is observed that the predicted values are in good agreement with measured values and the predicted error is very less. The predictions of greenhouse temperature and relative humidity results simulated using the developed ANN model were strongly correlated with the experimental data. Therefore the proposed MLP Back Propagation Neural Network model with the developed structure can perform good prediction with least error.

By utilizing the RMSE algorithm to analyze the values, the RMSE and Mean absolute Error (MAE) between the measured and predicted temperature was 0.711 ^{o}C and 0.558 ^{o}C respectively, and between the measured and predicted relative humidity it was 2.514% and 1.976%, which illuminated the neural network model had the ability to predict the inside mean temperature. The coefficient of determination (R^2) obtained from the regression line between the measured and predicted temperature and relative humidity was found to be 0.980 and 0.967 respectively. The results demonstrated the neural network model can predict the change of micro-climate of greenhouse accurately. It is clear from test result that the higher values of correlation coefficients (0.989 and 0.974 for temperature and relative humidity respectively) and lower values of root mean square error suggests the applicability of ANN model for prediction of greenhouse mean temperature and relative humidity.

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Table 1. *Variation of RMSE, MAE and R^2 values with number of hidden neurons in test phase*

Hidden layer neurons	ANN structure	RMSE		MAE		R^2	
		Temperature (^oC)	R. H. (%)	Temperature (^oC)	R. H. (%)	Temperature	R. H.
3	6-3-2	0.7189	2.528	0.573	1.983	0.968	0.948
4	**6-4-2**	**0.7111**	**2.514**	**0.558**	**1.976**	**0.980**	**0.967**
5	6-5-2	0.7145	2.537	0.564	1.992	0.973	0.956
6	6-6-2	0.7231	2.529	0.592	1.988	0.978	0.952
7	6-7-2	0.7211	2.561	0.576	2.008	0.971	0.951
8	6-8-2	0.7119	2.582	0.568	2.095	0.965	0.945
9	6-9-2	0.7213	2.584	0.597	2.097	0.973	0.943
10	6-10-2	0.7293	2.579	0.599	2.086	0.971	0.952

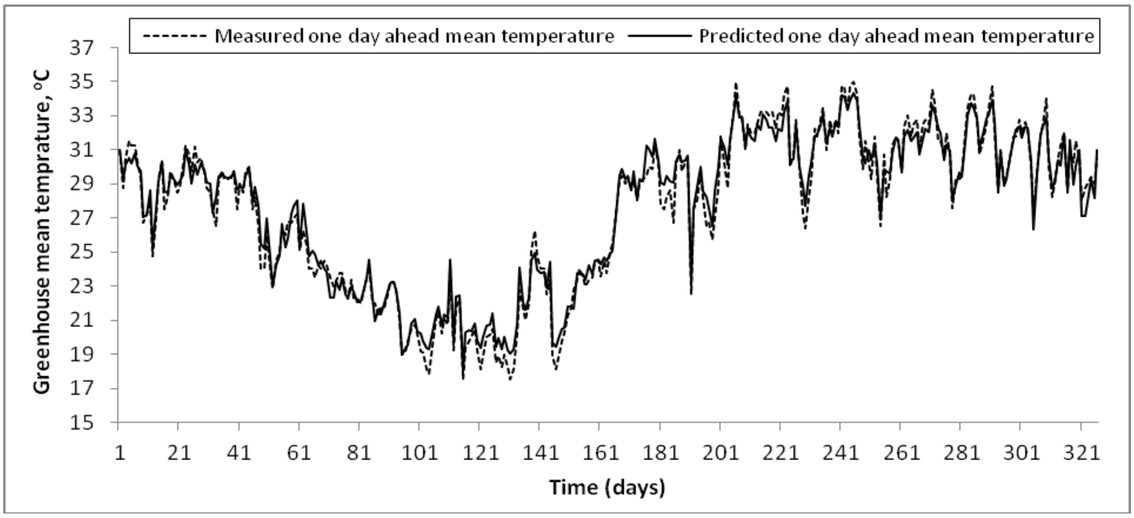

Figure 8. *Comparison between one day ahead measured and predicted mean temperature of greenhouse for ANN structure 6-4-2*

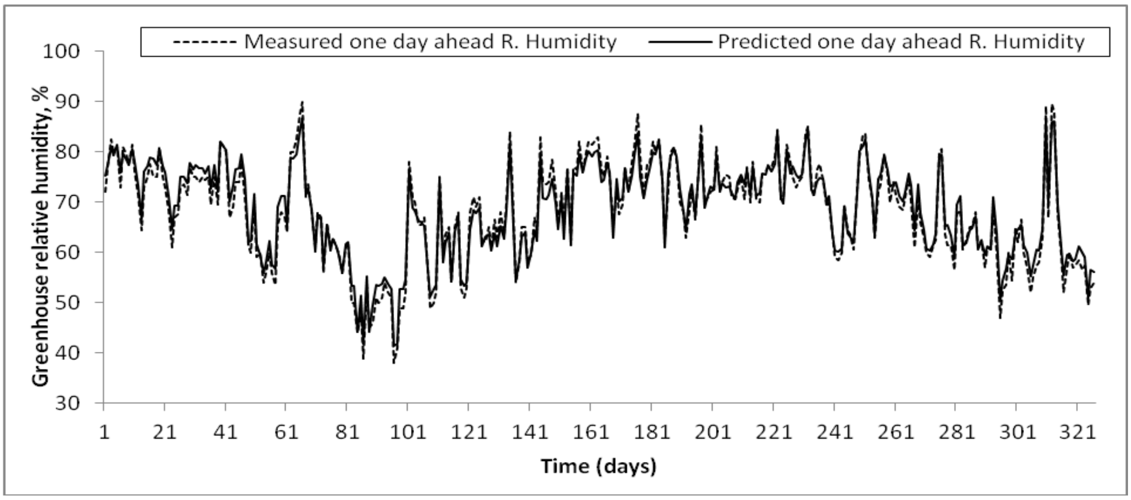

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Figure 9. *Comparison between one day ahead measured and predicted relative humidity of greenhouse for ANN structure 6-4-2*

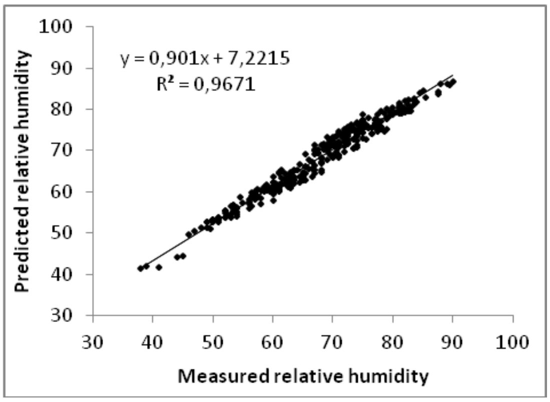

Figure 10. *The relationship between measured and predicted mean temperature for ANN structure 6-4-2*

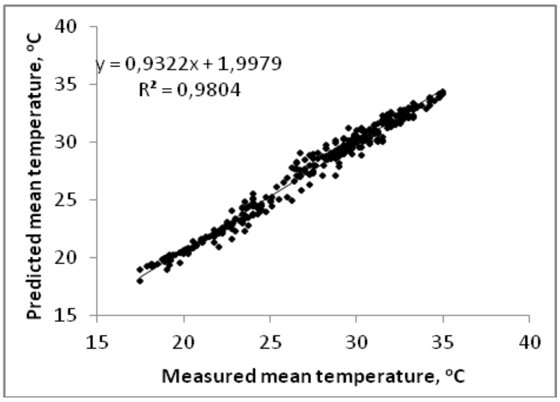

Figure 11. *The relationship between measured and predicted relative humidity for ANN structure 6-4-2*

Conclusions

The present study discusses the application and usefulness of artificial neural network based modeling approach in predicating the greenhouse mean temperature and relative humidity. This approach is able to determine the nonlinear relationship that exists between the micro-climatic data (temperature, humidity, wind speed and solar radiation) supplied to the system during the training phase and on that basis, make a prediction of what the temperature and humidity would be in future. A 3-layered neural network is designed and trained with the existing dataset and obtained a relationship between the existing non-linear parameters of micro-climate. Now the trained neural network can predict the future temperature with less error. After 1000 epochs, the Artificial Neural Network has been found to produce a forecast with small prediction error. The study, therefore, establishes that the NN structure with 4 hidden neurons in single layer is the best predictive model over the other structures.

RMSE and MAE statistical indicators showed small values that demonstrates the correct behavior of the developed forecasting models. The values of the correlation coefficient demonstrate the good agreement between predicted and measured values between temperature and humidity.

This study also concludes that a combination of minimum and maximum greenhouse air temperature and relative humidity and outside wind speed and solar radiation provides better performance in predicting the greenhouse temperature and relative humidity for next day. The results are quite encouraging and suggest the usefulness of artificial neural network based modeling technique in accurate prediction of the temperature and relative humidity in the greenhouse.

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ASSESSMENT OF THE EXTERNAL EXPOSURE DOSE TO HUMANS FROM ^{137}CS AND ^{90}SR IN THE COASTAL WATERS OF THE BALTIC SEA NEAR LITHUANIA

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Abstract. At present the Baltic Sea is considered to be the most contaminated with anthropogenic radionuclides in comparison to any other part of the World Ocean. Anthropogenic radionuclides (mainly ^{137}Cs and ^{90}Sr) found in the seawater are sources of the external exposure that contributes to the total radiation exposure to humans. The variations of activity concentrations of ^{137}Cs and ^{90}Sr in the water of the Baltic Sea near Lithuanian coast in 1985–2013 were analyzed. External exposure dose from these radionuclides to humans due to immersion in the seawater were calculated using the results of the measurements. An average external exposure dose from ^{137}Cs ranged between 6.28 $nSv \cdot h^{-1}$ to 1.5 $nSv \cdot h^{-1}$, from ^{90}Sr – between 1.73 $nSv \cdot h^{-1}$ to 0.53 $nSv \cdot h^{-1}$.
Keywords: *activity concentration,ionizing radiation, radionuclides, radioactivity, marine ecosystem*

Introduction

All living organisms, including humans, are being exposed to ionizing radiation at all times. Natural radiation comes from many sources including more than 60 naturally-occurring radioactive materials found in air, soil and water. Humans are also exposed to natural radiation from cosmic rays. According to the United Nations Scientific Committee on the Effects of Atomic Radiation report (UNSCEAR, 2010), the worldwide average radiation exposure from natural sources is approximately 2.4 mSv per year. However, the exposure to humans from ionizing radiation of natural sources is a continuing and inescapable feature of life on Earth.

Humans exposure to ionizing radiation also comes from anthropogenic sources. ^{137}Cs and ^{90}Sr are one of the most hazardous anthropogenic radionuclides due to their long physical and biological half-life (about 30 years) (IAEA, 2005). Anthropogenic radionuclides are penetrated in the marine environment mostly as a result of nuclear explosions, accidents at nuclear power plants and due to the operation of nuclear industry (HELCOM, 2009; Zalewska and Suplińska, 2013).

Review of Literature

For the first time ^{137}Cs and ^{90}Sr were penetrated into the Baltic Sea with the global fallout as a result of the tests of nuclear weapons in the atmosphere, particularly in the 1950s and 1960s (Livingston and Povinec, 2000; Ikäheimonen et al., 2009; Ilus, 2007). According to data published in Helsinki Commission proceedings (HELCOM, 2013), the total inputs of weapons test ^{137}Cs and ^{90}Sr into the Baltic Sea were 800 TBq and 500

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TBq, respectively. During a long period of time this sea was contaminated by the North Sea waters where radioactive waste was discharged from various nuclear reprocessing plants (HELCOM, 2009; Zalewska and Lipska, 2006; Saniewski, 2013). The increase of the global fallout proceeded up to 1963, when a treaty concerning nuclear and thermonuclear weapon test stopped in the atmosphere, hydrosphere and cosmos were signed. This moment was the beginning of the decrease in radioactive contamination of the marine environment. The process was observed up to 1986. The average activity concentrations of ^{137}Cs and ^{90}Sr in the seawater until 1986 were 12 $Bq \cdot m^{-3}$ and 24 $Bq \cdot m^{-3}$, respectively (Styro et al., 1990).

The radiological situation in the Baltic Sea changed after the Chernobyl Nuclear Power Plant (ChNPP) accident, which became the main source of ^{137}Cs after 1986 (Ikäheimonen et al., 2009; Juranová et al., 2015). After the accident, an average activity concentration of ^{137}Cs in the surface waters of the Baltic Sea grew by more than an order of magnitude relative to the radioactive background (12 $Bq \cdot m^{-3}$) formed after nuclear weapons tests in the atmosphere (Styro et al., 1990). According to data calculations (HELCOM, 2013), direct input of ^{137}Cs into the Baltic Sea from ChNPP accident was estimated to be 4700 TBq. The Chernobyl fallout was scattered very unevenly over the Baltic Sea area (Weiss, 2011). The Bothnian Sea and the Gulf of Finland were both regarded as the most contaminated regions of the Baltic (Zalewska and Lipska, 2006). In 1986, the respective average concentrations of ^{137}Cs in these regions were 480 $Bq \cdot m^{-3}$ and 500 $Bq \cdot m^{-3}$, whereas in the Baltic Proper the average activity concentration of this radionuclide was only 150 $Bq \cdot m^{-3}$ (Zalewska and Suplińska, 2013). Much less contaminated were the Bothnian Bay, the Belt Sea and the southern Baltic Proper where, in 1986, the average concentration was 84 $Bq \cdot m^{-3}$ (Zalewska and Suplińska, 2013). The activity concentration of ^{90}Sr following the accident of the ChNPP changed negligibly (Saniewski, 2013; Zaborska et al., 2014). The direct input of this radionuclide was estimated to be 80 TBq (Ikäheimonen et al., 2009). During a period of time, a leveling of the ^{137}Cs activity concentration in the surface waters took place; the end of this process may be ascribed to 1989, when its activity concentration appeared to be about 150 $Bq \cdot m^{-3}$ (Styra et al., 2008). This time was used as the beginning of self-purification process of the Baltic Sea from ^{137}Cs (Styra et al., 2008). During the period 1992–2010 concentrations of ^{137}Cs in the surface waters decreased in all parts of the Baltic Sea. The average activity concentration of ^{137}Cs in the Baltic Proper decreased to 40 $Bq \cdot m^{-3}$ in 2010. Concentrations in the Western Baltic and in the Gulf of Finland were lower at around 35 $Bq \cdot m^{-3}$ and 29 $Bq \cdot m^{-3}$, respectively. The highest concentrations were reported in the Archipelago and Aland Sea (equal to 44 $Bq \cdot m^{-3}$) (HELCOM, 2013). At present, concentrations of ^{137}Cs are relatively uniform in all regions of the Baltic and remain at approximately similar levels mainly because of the transport and mixing of water masses (Zalewska and Suplińska, 2013). ^{90}Sr concentration in the Baltic seawater varied in general from 5 $Bq \cdot m^{-3}$ to 15 $Bq \cdot m^{-3}$. The ^{90}Sr concentration decreases slowly with time and its behavior in seawater is different from ^{137}Cs (HELCOM, 2013).

The decrease of activity concentrations of these radionuclides in the coastal waters near Lithuania was observed too (Styro et al., 2012). However, radionuclides in the coastal waters are sources of external exposure that contribute to the total radiation exposure to humans.

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The aim of this work is to analyze the average activity concentrations of ^{137}Cs and ^{90}Sr in the waters of the Baltic Sea near Lithuania and to assess the external exposure dose to humans due to these radionuclides.

Materials and Methods

The samples of the sea surface water for determining activity concentrations of ^{137}Cs and ^{90}Sr were taken in the Baltic Sea at the distance of 10 m from the seashore of Lithuania at 0.5 meter depth. Water samples were taken near Juodkrante (*Fig. 1*). The minimum volume of the analyzed water sample was 40–50 liters. The temperature, specific electric conductivity, water current direction, wind speed and direction were registered during the time of sampling.

Figure 1. *Sample locations of the seawater*

During the whole research period, the determination of activity concentrations of ^{137}Cs and ^{90}Sr in the coastal surface waters of the Baltic Sea were carried out using a single technique – radiochemical analysis and the same type of measuring equipment (Styro et al., 2011; Styro et al., 2012). This research technique consists of some main stages: the concentration of ^{137}Cs and ^{90}Sr together with a stable carriers, radiochemical cleaning and activity measuring.

The ^{137}Cs and ^{90}Sr were concentrated from the same seawater samples. After the radiochemical cleaning the yield of cesium was determined gravimetrically in the form of $Cs_3Sb_2I_9$. It varied within a range of 60–80 %. The activity of ^{137}Cs samples was registered by gamma spectrometer (CANBERRA) with HPGe detector (resolution 2 keV, efficiency 15 %). The determination error for ^{137}Cs amounted to 10 %.

^{90}Sr was measured by ^{90}Y emission using a low level background beta radiometer. A stable strontium yield was determined by the atomic absorption spectrometer and Y – gravimetrically in Y_2O_3 form. The yield values varied within a range of 60–80 %. The determination error for ^{90}Sr activity concentration amounted to 15 %.

The external exposure dose to humans from ^{137}Cs and ^{90}Sr was calculated with the following equation (Eckerman and Ryman, 1993; IAEA, 2001):

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$$E_{im} = f\, DF_{im}\, C_V, \qquad \text{(Eq. 1)}$$

where E_{im} – external exposure dose to humans from radionuclide ($Sv \cdot h^{-1}$); f – exposure time to external radiation ($h \cdot y^{-1}$); DF_{im} – dose conversion coefficient for water immersion ($Sv \cdot m^3 \cdot Bq^{-1} \cdot y^{-1}$), C_V – activity concentration of the radionuclide in the seawater ($Bq \cdot m^{-3}$).

Results and Discussion

The average values of ^{137}Cs and ^{90}Sr activity concentrations in the coastal waters of the Baltic Sea near Lithuania (Juodkrante) in 1985–2013 are illustrated in *Fig. 2*. An average activity concentration of ^{137}Cs was 18 $Bq \cdot m^{-3}$ and ^{90}Sr – 28 $Bq \cdot m^{-3}$ before the ChNPP accident.

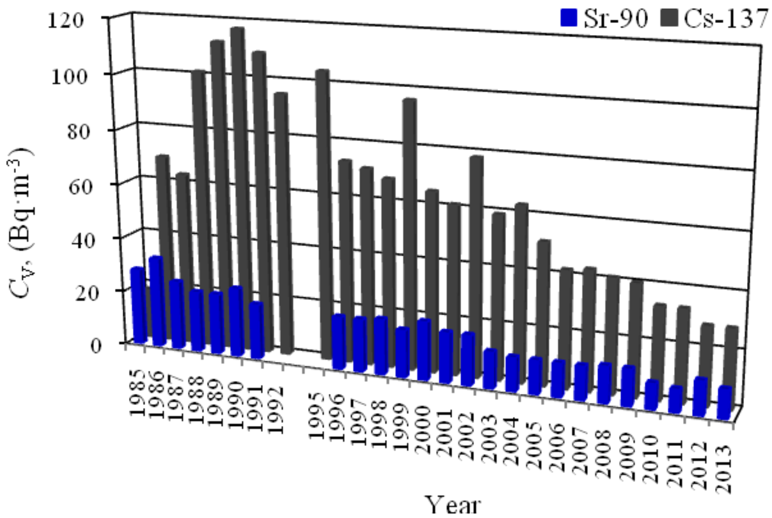

Figure 2. *Average values of ^{137}Cs and ^{90}Sr activity concentrations in the coastal waters of the Baltic Sea near Lithuania (Juodkrante) obtained in 1985–2013 (data from 1985–2003 after Nuclear Hydrophysics laboratory of Vilnius Gediminas Technical University)*

After this accident the average activity concentration of ^{137}Cs considerably increased. The highest average values of ^{137}Cs in the coastal waters varied in the range from 95 $Bq \cdot m^{-3}$ to 117 $Bq \cdot m^{-3}$ were found from 1988 to 1995. This increase was attributed to the transport of more polluted water from the northern Baltic as well as to considerable riverine discharges (Styra et al., 2008). Since 1995 the situation stabilized and the activity concentration of ^{137}Cs started to decrease as a consequence of many processes, mainly radioactive decay, bioaccumulation and sedimentation (Styro et al., 2012). However, an unexpected increases of the average values of ^{137}Cs were also observed in 1999 and 2002. During 2003–2013, an average activity concentration in the coastal waters varied in the range from 63 $Bq \cdot m^{-3}$ to 30 $Bq \cdot m^{-3}$. However, in 2013 this average value exceeded almost two times the value found in 1985.

After ChNPP accident activity concentration of ^{90}Sr slightly increased. In 1986, the average concentration was 33 $Bq \cdot m^{-3}$. However, in 1987 it reached the value found before the accident (*Fig. 2*). During 1987–2002, an average activity concentration in the coastal

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waters varied in the range from 25 Bq·m^{-3} to 18 Bq·m^{-3}. In 2003, an average value of ^{90}Sr decreased to 13 Bq·m^{-3}. Since then, the average activity concentration of ^{90}Sr in the seawater varied within a relatively narrow range from 13 Bq·m^{-3} to 10 Bq·m^{-3}. The average activity concentration of ^{90}Sr in 2013 exceeded almost three times the value found in 1985.

According to data published in (Zalewska and Suplińska, 2013; Druteikienė et al., 2011; Lujanienė et al., 2010) and variations of average activity concentrations of ^{137}Cs and ^{90}Sr in the coastal waters near Lithuania, the Baltic is still the most contaminated seas in the world.

Certainly, variations of activity concentration of radionuclides in seawater have an impact on external exposure to humans. The summer season in the coastal Baltic Sea near Lithuania is from June to September. According to (Kahru et al., 2015), the number of days with the seawater warmer than 17 °C have almost doubled (from 29 to 56 days) over the past 30 years. Approximately, humans are being exposed when swimming in the seawater from few to few dozen hours per year.

External exposure dose to humans calculated using the average activity concentrations of ^{137}Cs and ^{90}Sr in the seawater and equation (*1*) are presented in *Figs. 3* and *4*.

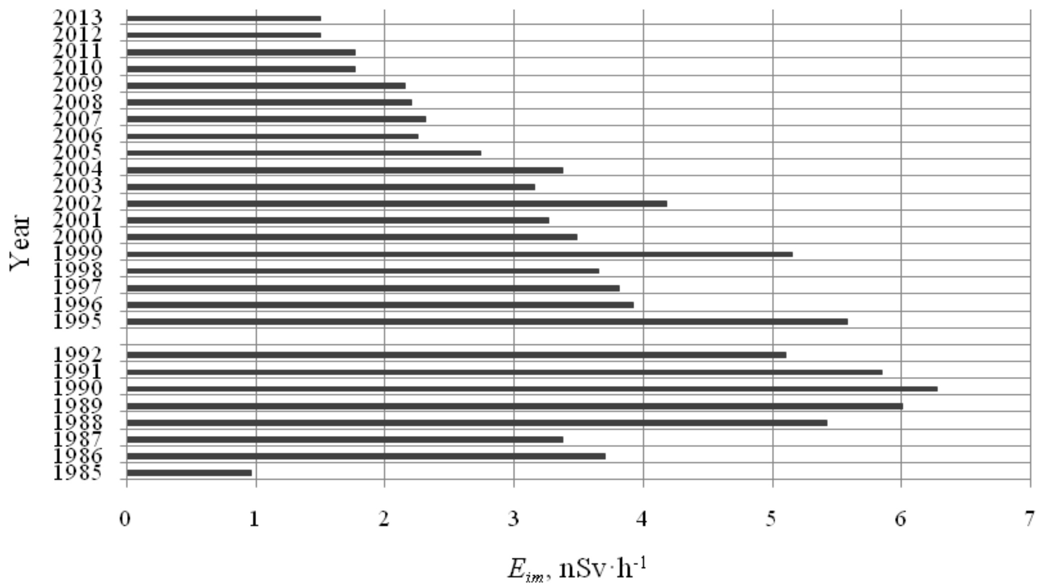

Figure 3. *External exposure dose to humans from ^{137}Cs in the coastal waters of the Baltic Sea (Juodkrante) in 1985–2013*

According to data obtained by the calculations, an average external exposure dose to humans from ^{137}Cs in the coastal waters in 1985–2013 ranged between 6.28 nSv·h^{-1} and 1.5 nSv·h^{-1} (*Fig. 3*), from ^{90}Sr – between 1.73 nSv·h^{-1} and 0.53 nSv·h^{-1} (*Fig. 4*). The highest exposure dose from ^{137}Cs was calculated in 1990, from ^{90}Sr – in 1986. After thirty years after ChNPP accident the exposure dose from ^{137}Cs decreased four times. The external exposure dose from ^{90}Sr due to immersion in the water decreased slower, almost three times.

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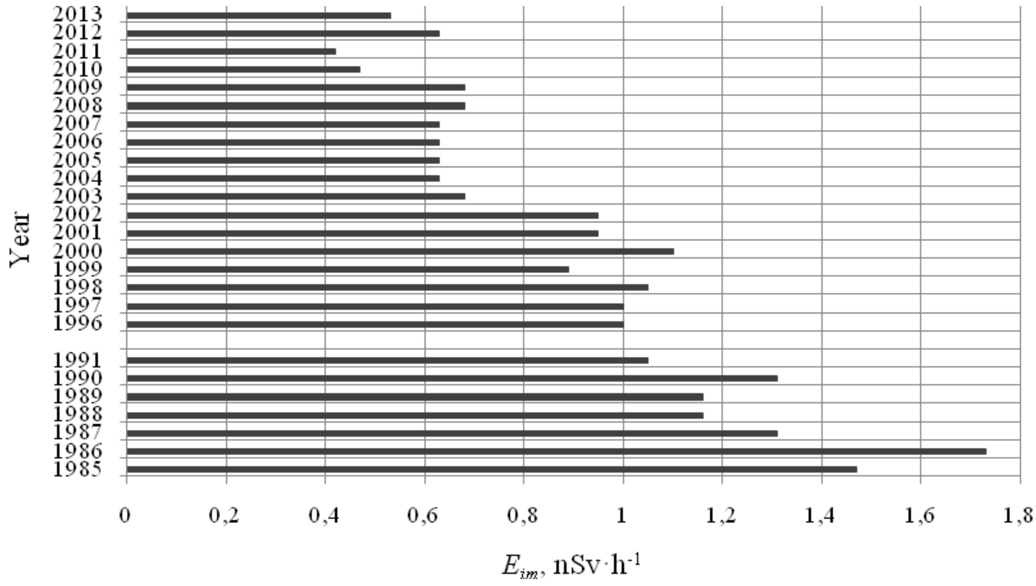

Figure 4. *External exposure dose to humans from ^{90}Sr in the coastal waters of the Baltic Sea (Juodkrante) in 1985–2013*

The external exposure from ^{137}Cs and ^{90}Sr in the seawater to humans when swimming is unavoidable. However, the average external exposure dose was very small in comparison to natural sources and did not make any risk for humans health.

Conclusions

Activity concentrations of ^{137}Cs and ^{90}Sr in the Baltic Sea waters decrease in time due to their natural radioactive decay and the decrease of the environmental radioactive pollution. According to average values, the activity concentration of ^{137}Cs in the coastal waters near Lithuania has been decreasing since 1990. The average activity concentration of ^{137}Cs decreased from 117 Bq·m^{-3} found in 1990 to 30 Bq·m^{-3} in 2013. However, the average activity concentration of ^{137}Cs in 2013 exceeded almost two times the value found in 1985. During the same period the average activity concentration of ^{90}Sr decreased almost three times, i.e. to 10 Bq·m^{-3}.

During the period from 1985 to 2013, an average external exposure dose to humans from ^{137}Cs in the coastal waters varied in the range from 6.28 nSv·h^{-1} to 1.5 nSv·h^{-1}, from ^{90}Sr – from 1.73 nSv·h^{-1} to 0.53 nSv·h^{-1}. The level of the external exposure was considerably lower than its limit (1 mSv·y^{-1}) and did not make any risk for humans health.

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MORPHOLOGICAL ASSESSMENT OF THE ROOTS, STEMS AND LEAVES OF *SOLANUM NIGRUM* L. CULTIVATED ON DIFFERENT SOIL TYPES

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Abstract. *Solanum nigrum* is a wild vegetable well-known for its nutraceutical properties. This plant's proper identification is essential not only to plant scientists but to a wide range of users. A pot experiment was therefore conducted to determine the influence of sandy clay loam, silty clay loam, clay loam and loam soils on the morphology of the plant's roots, stems and leaves. Roots, stems and leaves were viewed under the Scanning Electron Microscope while the leaves were further viewed under the Light Microscope. Silty clay loam recorded the highest stomata, guard, and subsidiary cell densities on the abaxial as well as on the adaxial surface. The LM revealed the hypo-amphistomatous and anisocytic type of stomata in *S. nigrum.* The SEM analysis revealed the presence of different types of glandular and non-glandular trichromes on both surfaces of the leaves regardless of the soil types. The soil types did not have a significant effect on the root and stem characteristics, however, the stomata, guard and subsidiary cells were significantly affected by the soil types. The presence of trichomes is a good indicator of the ability of *S. nigrum* to secrete bioactive compounds for which this plant is well known.
Keywords: *scanning electron microscopy, light microscopy, stomata, trichomes, adaxial and abaxial surfaces*

Introduction

Solanum nigrum belongs to the most complex and largest genus of the Solanaceae family. Furthermore, it comprises over 2000 identical species and this often causes confusion in their taxonomy (Edmond and Chweya, 1997; Bohs, 2001). Most of these species are of great economic and nutritional value and/or importance (Maiti et al., 2002). This plant is one of the African flora known for numerous health benefits (Lester, 2011). Its therapeutic benefits therefore necessitate its proper identification. The responsibility to minimize ambiguity caused by numerous species, overlapping features, ecological distribution, similar genomes and their various growing habitats fall on the researchers (Levin et al., 2005; Oyelana and Ugborogbo, 2008). However, much emphases must be laid on the micromorphology of parts of special interest, especially those that contribute to the plant's therapeutic, nutritional and economic importance. Leaves are some of the most important organs in plants, playing a functional role in addition to containing a number of bioactive compounds (Koduru et al., 2006; Aliero et al., 2006). Leaves contain chlorophyll and stomata which determine the plant's productivity and life. Stomata as leaf components are also associated with many physiological functions in plants. Stomata are responsible for plant biomass accumulation through the process of photosynthesis (Al Afas et al., 2006; Bussis et al., 2006). Like any other plant in the Solanaceae family, *S. nigrum* stores its bioactive contents in trichomes (Calo et al., 2006). Trichomes are also recognised for storing and

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secreting a higher level of secondary metabolites than quantities produced by other plant tissues (Jakoby et al., 2008). Among other functions, trichomes are also responsible for heat regulation in plants (Wagner et al., 2004). Environmental and hereditary factors are the two major factors that influence stomatal distribution in mature leaves (Fernadez and Muzica, 1973). Various environmental conditions alter density, size, location on the leaf surface and conductivity of stomata (Maherali et al., 2002). These environmental conditions in turn affect biomass production hence the important role of soil types in relation to plant growth. Plant organs such as the stomata, guard and subsidiary cells increase in size and density in response to favourable environmental conditions. This research therefore is interested in those functional parts of plants that determine the growth and yield of the plant. Therefore, stomata, guard cells, subsidiary cells and trichomes; the plant parts which are likely to differ morphologically within the same species will be investigated. Establishing facts on these morphological parts could shed more light into their taxonomy and identification to distinguish different species in the genus *Solanum*.

Reports indicate that there are differences in seed size and colour, spermodem cell arrangement in the macro-microscopy study of the fruits and powder of different *Solanum* species (Anuradha et al., 2012). There are reports on plant stomata response to various environmental conditions from molecular level to whole plant perspectives, ecosystems and global level (Nilson and Asmann, 2007; Woolward, 1987). Other reports include changes in stomatal density to elevated carbon-dioxide, salt stress and plant density (Zhao et al., 2006; Zhang et al., 2003). Many studies have also been carried out relating water deficit to an increase in stomatal density, size and adaptive nature of the specific plant to moisture stress (Spence et al., 1986; Yang and Wang, 2001; Zhang et al., 2006). However, no study has been conducted to examine the influence of soil types on root, stem and leaf morphology of *S. nigrum.* Earlier studies indicate that warmth stimulates and assists African indigenous vegetables to exhibit their potentials including *S. nigrum* (Flyman and Afolayan, 2006). The response of *S. nigrum* cultivated on a particular soil type calls for morphological assessment due to differences in water and nutrient retention capacities of different soil types. Distribution, density, size and type of stomata and the magnitude of the adaptation mechanism exhibited by stomata in response to different soil types are of major interest. The present study therefore aimed to morphologically identify *S. nigrum* stems, roots, trichomes and stomata cultivated on different soil types. The types of trichomes acquired by the plant are expected to be elucidated using energy dispersing X- ray microscopy and light microscopy. Information leading to the proper identification of *S. nigrum* from other species of the genus *Solanum* is expected to be gleaned from this study.

Materials and methods

Soil collection, treatments and study area

The soil was collected from a fallow land at a depth of 30 cm from the University of Fort Hare farm, Alice campus in South Africa located at $32^{0}46'47''$S and $26^{0}50'\ 5''$E and 524 m a.s.l. Soil treatments were relative combinations of sand, silt and clay in different proportions. The physico-chemical properties of soil types are shown in *Table 1.*

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Table 1. *Textural compositions of experimental soils*

Soil types	Sand particle %	Silt particles %	Clay particles%
Control ST_0	60	30	10
Sandy clay loam ST_1	66	13	21
Silty clay loam ST_2	10	60	30
Clay loam ST_3	36	30	34
Loam ST_4	40	40	20

Plant materials

Seeds were extracted from mature berries of plants by hand squeezing, washing in distilled water and air-drying for 3 days and planted in nursery trays in the green house. Seedlings of *S. nigrum* were transplanted at 4 leaf stage into experimental pots containing different textural soil compositions (*Table 1*). The trial was conducted between February and March, 2016 at the University of Fort Hare, Alice campus, Eastern Cape, South Africa, located at 32°46’47’’S and 2650’ 5’’E and 524m a.s.l. The plant had earlier been identified (BVE11/017) and deposited at the Giffen herbarium of the same University (Bvenura and Afolayan, 2013). For the micromorphological study of *S. nigrum* root and stem specimens cultivated on different soil types were harvested at the plant’s early flowering stage (4^{th} week). The first pair of mature, fresh and fully expanded leaves were collected and specimens measuring 10 X 10 mm^2 cut for SEM analysis.

SEM and Energy Dispersive X-ray Spectroscopy (SEM-EDX)

Following the procedure adopted from Dyubeni and Buwa (2012) the specimens (root, stem and leaves) of sizes 4, 6 and 10 mm respectively were immersed in 6% w/v glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.5) for 24 hours. The samples were then rinsed with distilled water and dehydrated in different graded ethanol series of 20-100% at 20 minutes per rinse. Specimens were then immersed in 100% ethanol solvent till the time of drying. Samples were prepared for drying by mounting on aluminium specimen stubs with a double-sided carbon tape and sputter coated with a layer of gold. Samples were critical point dried using liquid CO_2 in a Hitachi HCP- 2 critical point drier, and sputter coated with gold palladium in a Hummer V-sputter coater. Both the adaxial and abaxial surfaces were observed in a Hitachi (S-450) Scanning Electron Microscope (SEM), operating at 10-15 KV acceleration voltage. Images were captured digitally with an image slave computer programme for windows.

Light microscopy

For better leaf micromorphological assessment of stomata and the companion cells, freshly harvested leaves of plants from different soil types were prepared for observation under the light microscope. A strip of lower and upper epidermis from the middle portion of the leaves were carefully peeled off to show the transparent part (Marenco et al., 2009; Coopoosamy and Naidoo, 2011). Images were viewed with a Zeiss West Germany optical microscope digital camera (DCM 510) at 100X and 400X magnification. An image showing the number, arrangement of stomata with guard cells

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and subsidiary cells was captured with the digital camera, fitted in the light microscope. The images were captured digitally using Microsoft image programme for Windows.

Effect of soil types on density and size of stomata, guard cells, and subsidiary cells

Leaf stomatal density was determined and expressed as the number of stomata per unit area (Radoglou and Jarvis, 1990). This was achieved by counting the number of full or half shown stomata within a 50 µm unit area at 500X magnification on the lower and upper surfaces of the specimens analysed by the SEM. Density of stomata per mm^2 was calculated and recorded for each soil type. The density of guard cells and subsidiary cells was determined as each of two double the density of the stomata since they both occur in pairs, surrounding the stomata. The size of each of these parameters is determined by length, therefore, 3 stomata cells, were selected and the mean value represents the size.

Effect of soil types on distribution and types of stomata

Using the concept of Silva et al. (2008), assessment was made based on the number, type of arrangement and position of accessory cells surrounding the guard cells of the stomata. Critical observations were made on the lower and upper surfaces of leaf specimens from the SEM and light microscopy analysis for possible changes in relation to soil types.

Effect of soil types on roots and stems

S. nigrum roots and stems were observed for possible changes in their micromorphology under the SEM following the method of Otang et al. (2012).

Effect of soil types on distribution and types of trichome

Across the soil types, both lower and upper surfaces of leaf specimens of *S. nigrum* were observed for distribution and types of trichomes present in them under the SEM and light microscope following the method of Otang et al. (2012).

Statistical analysis

All data on density and size were subjected to statistical analysis using MINITAB Release 17. A one-way analysis of variance (ANOVA) was used to compare the mean values among the soil treatments. Means were segregated using Fisher's Least Significant Difference (LSD) paired wise comparison. The means were treated as significantly different at $p < 0.05$.

Results and discussions

Soil types used for the growth of *S. nigrum* in this study were composed of different sand, silt and clay proportions as shown in *Table 1*.

Effect of soil types on density and size of stomata, guard cells, and subsidiary cells

Based on the results of the SEM analysis, stomata density, guard cell and subsidiary cell density were influenced by soil types in both lower and upper surfaces of the leaves.

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As shown in *Table 2*, the density of stomata, guard and subsidiary cell on the adaxial surface decreased in the order: $ST_2>ST_3>ST_4>ST_1>ST_0$. In addition, on the abaxial surface these constituents decreased in the order: $ST_2>ST_3>ST_1>(ST_4=ST_0)$. The present results indicate that stomata density, guard cell and subsidiary cell densities declined as the sand ratio increased. Highest stomata, guard and subsidiary cell densities on the lower surface (287.8, 575.6 and 575.6) and on the upper surface (205.6, 411.2 and 411.2) were recorded in samples cultivated on silty clay loam soil. The control soil recorded the lowest stomata density, guard cell and subsidiary cell densities (164.5, 329 and 329) on the lower surface and sandy clay loam soils recorded the lowest stomata density, guard cell and subsidiary cell densities (61.7, 123.4 and 123.4) on the upper surfaces as shown in *Table 2*. Silty clay loam soil had the highest stomata density per mm^2 in both lower and upper leaf surfaces. Silty clay loam soil recorded significantly increased density of stomata, guard cell and subsidiary cells when compared with the control. However, the average shortest distance between stomata (21.7 mm) was recorded in silty clay loam on the lower leaf surface. The longest distance (46.7 mm) was recorded in control soils on the upper surface (*Table 2*). Higher densities of stomata, guard and subsidiary cells recorded in silty clay loam, clay loam and loam soils could be as a result of the ability of these soils to retain water and nutrients as compared to other soils with higher sand proportions which is characterised by larger pores that drain water and leach nutrients (Soil Conservation Service Engineering Division, 1964; Bengough et al., 2011). The ability of silty clay loam soil to retain water for plant uptake in comparison with other soil types could possibly explain the increased stomata, guard and subsidiary cell densities in the plants grown on this soil. This could also be responsible for increased biomass accumulation (Marenco et al., 2006). Regardless of the soil types, stomata were more distributed on the adaxial than on the abaxial surfaces. This is a general phenomenon with angiosperms (Metcalfe and Chalk, 1979; Volekonifa and Ticha, 2001). Moreover, it is a characteristic of hypo-amphistomatous leaves, of which *S. nigrum* is a member (Volenikova and Ticha, 2001). This high mean stomata density recorded in silty clay loam soil in addition to the high mean sizes of the stomata could possibly explain the high growth and yield potentials exhibited by *S. nigrum* cultivated on these soils. However, in general, there is an inverse relationship between stomatal density and stomatal size which is in contrast with the results of the current experiment (Marenco et al., 2006). The present results differed from those of Zhang et al. (2004) which affirmed an inverse relationship between stomatal size and density. Moreover, the effects of any abiotic factors on stomatal size have been reported to be dependent on plant species or varieties (Maherali et al., 2002).

Effect of soil types on distribution and types of stomata

The arrangement of the stomata on adaxial and abaxial leaf surfaces of *S. nigrum* are shown in *Fig 1*. Two large and one small subsidiary/ accessory cells with their common walls parallel to the guard cells surrounded the stomata as described by Metacalf and Chalk, (1979). The five soil types did not significantly affect ($p<0.05$) stomatal, guard as well as subsidiary cell characteristics throughout the trial. Also, the stomata arrangement was anisocytic as described by Silva et al. (2008).

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Table 2. *Effect of soil types on stomata, guard and subsidiary cell characteristics*

Leaf indices	Upper surface					Lower surface				
Soil types	ST_0	ST_1	ST_2	ST_3	ST_4	ST_0	ST_1	ST_2	ST_3	ST_4
Stomatal density mm^{-2}	61.7±0.0 ^{d}	82.3±1.0^c	205.6±0.2 ^{a}	102.8±0.1^b	61.7±0.0 ^{d}	164.5±1.0 ^{e}	185±1.00^d	287.8±1.0 ^{a}	267.3±0.2 ^{b}	246.7±0.6^c
Stomatal length mm	8.2±0.7^b	9.0±1.0 ^{b}	13.6±1.0 ^{a}	10.0±2.4^b	9.0± 1.9 ^{b}	7.3± 0.7 ^{c}	8.3±0.1^{ab}	9.3±0.7^a	9.0±1.0^a	9.0±1.7^a
Guard cell density mm^{-2}	123.4±1.0^e	164.6±0.6 ^{d}	411.2±1.0 ^{a}	205.6±1.0 ^{b}	123.4±0.9^c	329.0±1.0^a	370±0.6 ^{d}	575.6±1.0^a	534.6±1.0^b	493.4±0.9^d
Guard cell length mm	8.4±0.4^c	9.3±1.0^{bc}	14.0±0.7^a	11.0±1.4^b	13.0±1.2^a	8.0±1.0^c	13.5±1.0^a	11.3±0.3^a	10.7±0.2^b	12.0±1.0^a
Subsidiary cell densitymm^{-2}	123.4±0.7^c	164.6±0.1^{bc}	411.2±0.7^a	205.6±1.0^b	123.4±1.7 ^{a}	329.0±1.0^e	370±0.6^d	575.6±1.0^a	534.6±1.0^b	493.4±0.9^d
Subsidiary cell length mm	12.0± 1.0 ^{c}	13.5±0.1 ^{b}	16.0±0.2 ^{a}	12.6± 0.1 ^{b}	13.0± 1.0 ^{a}	9.7± 1.7 ^{b}	9.3± 1.3^b	12.3± 0.3^a	12.0± 0.0 ^{a}	13.3± 0.3^a
Distance between stomata mm^2	46.7± 0.8 ^{a}	36.3± 1.0 ^{b}	25.0± 0.2 ^{c}	26.0± 0.3 ^{c}	45.0± 2.0 ^{a}	23.5± 0.5 ^{c}	31± 1.4 ^{a}	21.7± 1.0 ^{d}	23.7± 0.1 ^{c}	26.0±1.0 ^{b}

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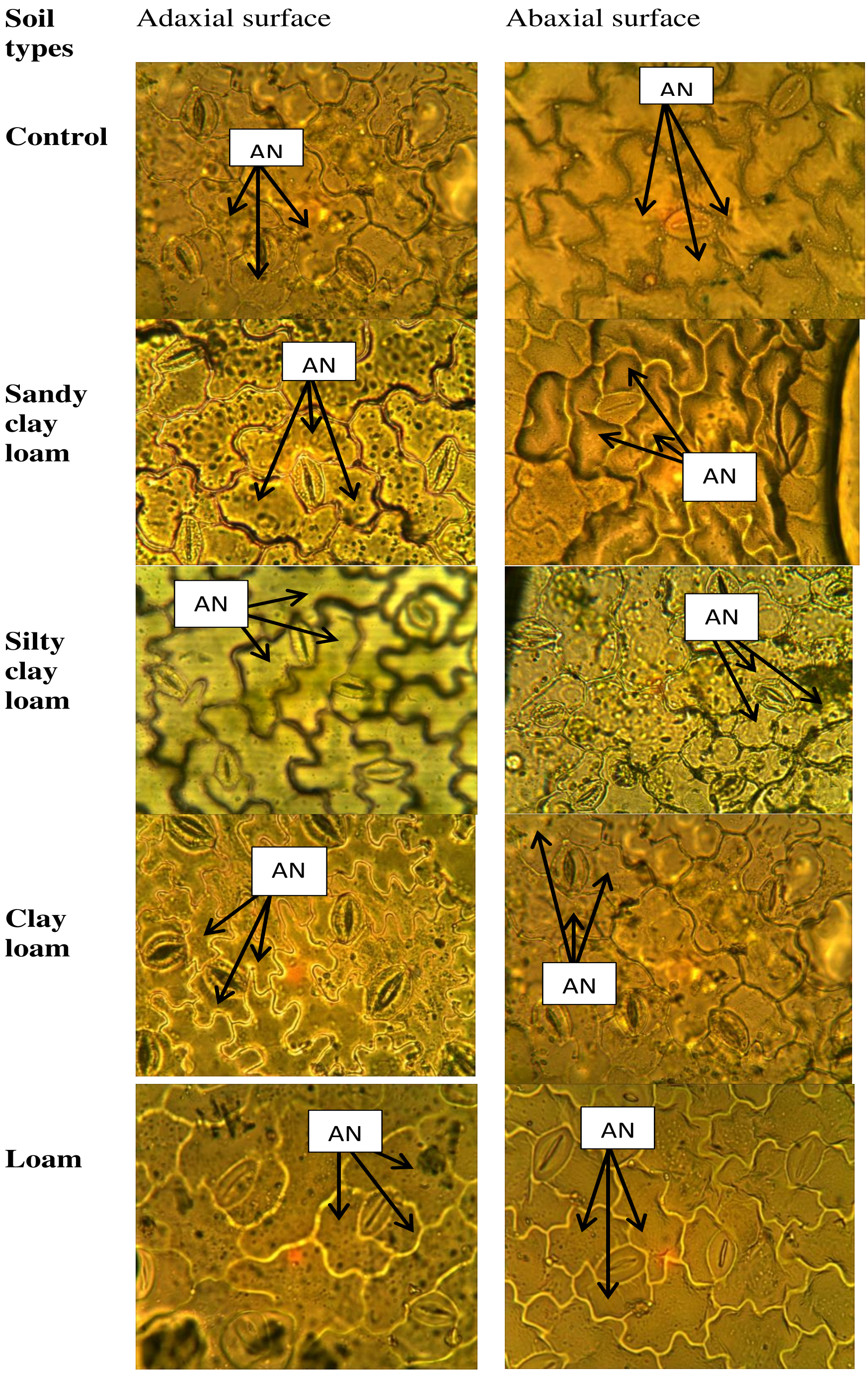

***Figure 1.** Stomata distribution on Solanum nigrum leaves showing Anisocytic (AN) stomata surrounded by 2 large and 1 small accessory cells*

Effect of soil types on roots and stems of S. nigrum

The effects of soil type on root and stem characteristics of *S. nigrum* are shown in *Fig. 2*. The results indicate that the soil types did not significantly affect the root and stem characteristics except for minor variations in the sizes of the xylem.

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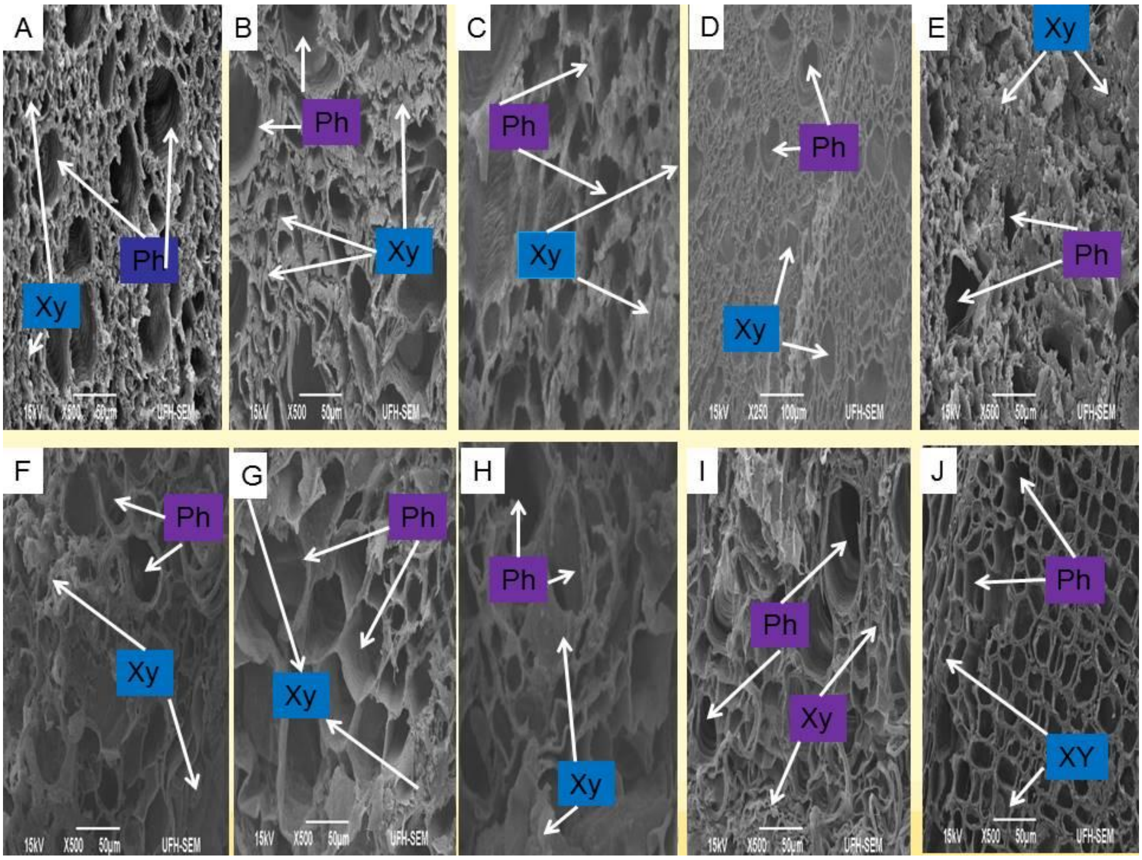

***Figure 2.** Effect of soil types on roots and stems of S. nigrum.*
Ph= Phloem, Xy= Xylem

The xylem vessels in the roots of silty clay loam, clay loam and loam soil cultivated plants appeared more compacted as compared to the control and sandy clay loam soils. The basic function of xylem is to transport water while phloem transports photosynthate and nutrients.

Effect of soil types on distribution and types of trichomes

Distribution of trichomes was not significantly affected by the soil types as shown in *Fig. 3a*. Trichomes were found on both adaxial and abaxial surfaces in the foliage of *S. nigrum* as it occurs in some other dicotyledonous leaves. These vary from glandular to non-glandular with arrow/sharp end, knob-like head and point end *Figure 3a*. The two types of trichomes (glandular and non-glandular) were simultaneously found on both adaxial and abaxial surfaces of leaves *Figure 3b*. It was also observed that these trichomes were multicellular but they exist in solitary form.

SEM analysis showed the occurrence of glandular trichomes on both leaf surfaces. The abundance of trichomes in a plant is generally linked to increased secretion of bioactive compounds, the therapeutic substances responsible for the plant's economic importance (Maiti et al., 2002). The abundance of trichomes in the present study is therefore an indicator of the high therapeutic disposition of *S. nigrum* and therefore a good source of secondary compounds (Afolayan and Meyer, 1995; Ascensao et al., 1999; Calo et al., 2006). The possession of glandular trichomes is a characteristic of the

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genus *Solanum* with the exception of *Nicotiana glauca* and *Solandra nitida* (Maiti et al., 2002). The solitary form of existence of all the present trichomes is deviant from the type of trichomes reported in *Solanum aculeastrum*, which were multicellular but some exist in stellate form (Koduru et al., 2006).

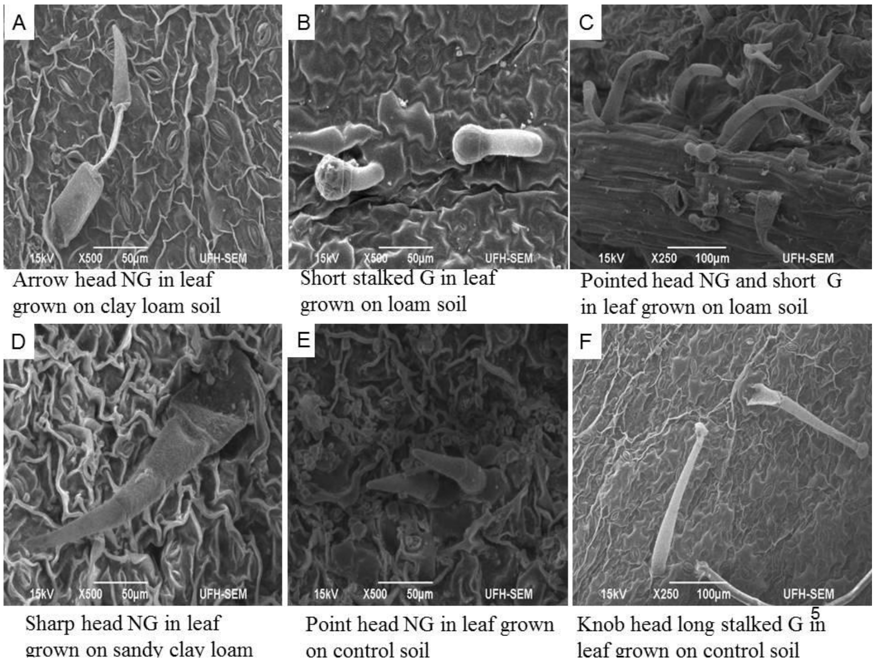

Figure 3.1. *Effect of soil types on distribution and types of trichome. NG= non glandular trichome; G= glandular trichome*

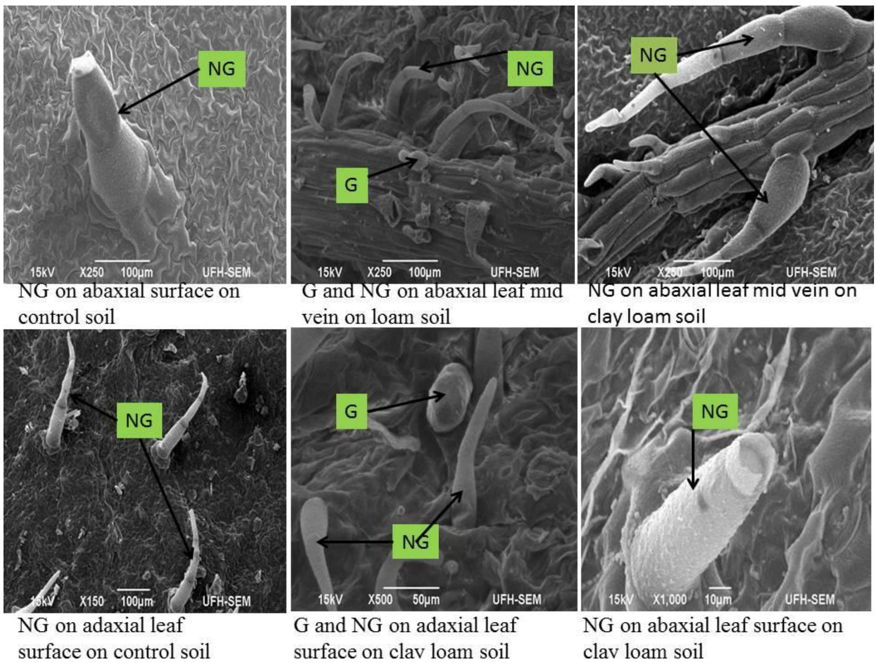

Figure 3.2. *Effect of soil types on distribution and types of trichome. NG= non glandular trichome; G= glandular trichome*

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Conclusion

Soil types significantly influenced density, size and distribution of stomata as well as guard and subsidiary cells. The results of the present study confirmed that *S. nigrum* leaves possess hypo-amphistomatous and anisocytic stomata. This study also observed the occurrence of glandular and non-glandular trichomes on the leaves, whose heads were either sharp, point or knob-like. The occurrence of glandular and non-glandular trichomes was on both abaxial and adaxial surfaces of the mid-vein of the leaves. The presence of glandular trichomes might be responsible for the therapeutic importance of *S. nigrum,* since glandular trichomes are known for secreting bioactive compounds. However, soil types did not have any influence on root and stem characteristics of *S. nigrum* cultivated on them.

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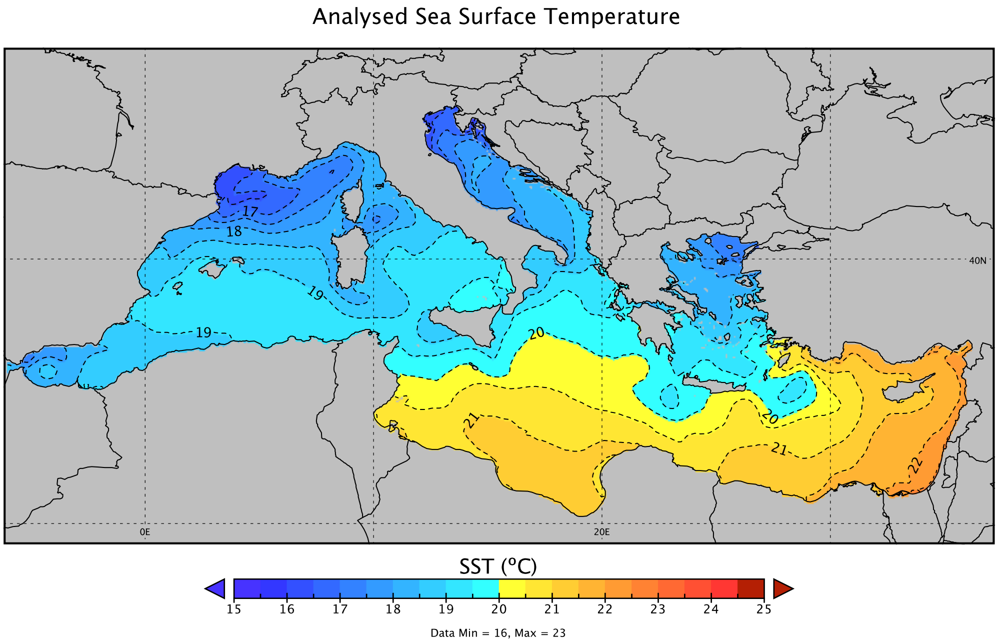

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