Editorial Communication

RETRACTED: Effects of climate change on invasion potential distribution of *Lantana Camara*

This article has been retracted by the Editorial Board of Applied Ecology and Environmental Research, because it has been simlutaneously submitted to another scientific journal, where it has already been published, and has not been withdrawn from our journal.

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Apologies are offered to readers of the journal that this was not detected during the submission process.

DYNAMICS OF ECOSYSTEM SERVICE VALUES IN RESPONSE TO LANDSCAPE PATTERN CHANGES FROM 1995 TO 2005 IN GUANGZHOU, SOUTHERN CHINA

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Abstract. This study analyzed the landscape pattern changes, the dynamics of the ecosystem service values (ESVs) and the spatial distribution of ESVs from 1995 to 2005 in Guangzhou, which is the capital of Guangdong Province and a regional central city in South China. Remote sensing data and geographic information system techniques, in conjunction with spatial metrics, were used to facilitate the analysis. The forest was the main landscape with a total coverage of 37.36% during the study period. Between 1995 and 2005, the forest and garden became less fragmented while the cultivated land, grassland, water body and built-up area became more fragmented. Such landscape pattern changes have resulted in a significant increase in the overall annual ESVs in Guangzhou of approximately 3.37%. Two important landscape types (forest and water body) were the main contributor to the total ESVs, the proportion of both of which was over 75%. During the period from 1995 to 2005, the ESVs tended to decline from the north and south to the central part in Guangzhou. The average ESVs was more than 15 000 Yuan/ha in the north for the mountainous forest area and 20 000 Yuan/ha in the south for the water body at the Pearl River mouth, but the average ESVs was lower than 5 000 Yuan/ha in the central part of the study area due to low vegetation coverage and widely scattered built-up area for rapid urbanization. So, it is important to protect the key landscape types, such as forest, water body and garden, to avoid fragmentation. Furthermore, this study offers important insights to those in fast-urbanizing regions for achieving more successful landscape to obtain the increasing of ESVs.

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Keywords: *Ecosystem service values (ESVs), spatial distribution, landscape pattern, spatial metrics, Guangzhou*

Introduction

Ecosystem services are the resources and processes supplied by natural ecosystems which benefit humankind directly or indirectly (Daily, 1997; MEA, 2005; Burkhard et al., 2010; de Groot, 2010; Sherrouse et al., 2011). Although the term “ecosystem services” was primarily introduced by Ehrlich and Ehrlich (1981), it didn’t attract worldwide attention until the publishing of Daily’s book *Nature’s Services: Societal Dependence on Natural Ecosystems* (Daily, 1997) and the paper *the value of the world’s ecosystem services and natural capital* by Costanza et al. (1997) in *Nature*. The monetary figures of global ecosystem services, which were presented by Costanza et al. (1997), resulted in a high impact on both science and policy making. Especially after the release of the Millennium Ecosystem Assessment (MEA, 2003), which focused on the services(i.e. supporting, provisioning, regulating, and cultural) that ecosystems provide to humans, the literature concerning ecosystem services has increased exponentially (Chazdon, 2008; Fisher et al., 2009) and the concept of ecosystem services has been found increasing attention in environmental science, policy making and practical applications (Daily and Matson, 2008; Fisher et al., 2009; ICSU, UNESCO and UNU, 2008).

To date, the ecosystem service values (ESVs) in various aspects have been studied with different methods. Landscapes, containing many important functions and providing numerous goods and services to society (de Groot, 2006, Gimona et al., 2007; Willemen et al., 2008), play a fundamental role in ESVs and the concept of landscape functions or services, used as synonym to ecosystem services, raised much attention in the field of landscape ecology and landscape planning (Hermann et al., 2011). Accurate assessments of the ecosystem service values is one of the critical endeavors in ecological economics and could help ecological planners (Bryan et al., 2010; Kozak et al., 2011; van der Horst, 2011) by given its capacity to combine ecological processes and economic outcomes (Wainger et al., 2010), and the key aspect of which is to evaluate how ecosystem services respond to specific landscape pattern designs (Jones et al., 2012). Landscape pattern, a major focus of landscape ecology, refers to the spatial structure characters of different landscape combinations, including the diversity and spatial distribution of landscape composition units (Wu, 2007). Monitoring landscape pattern changes provides an indirect approach for characterizing the ecological consequences of human activities (e.g. urbanization) because those changes would influence a variety of ecological processes and functions, such as land quality, biodiversity, vegetation carbon storage and greenhouse gases emission(Su et al., 2012). However, few attempts have been made to jointly analyze changes in landscape pattern and ESVs (Su et al., 2012). So, there is a need for integrating the concepts of landscape pattern and ecosystem services for landscape pattern may affect ESVs in different ways and at different geographical scales (Zhang et al., 2011).

Guangzhou, a large central city in South China and China's Southern Gateway to the world, have experienced significant economic growth and rapid urbanization since 1980s, which led to a dramatic change in landscape pattern and significantly affected the providing of ecosystem services. Research on urban ecosystem service values is highly demanded (Larondelle and Haase, 2013). In recent years, although many researches on ESVs in Guangzhou have been engaged (Ye et al., 2008; Wang et al., 2009; Ye et al., 2011), little attention has been paid to the ESVs' spatial distribution. The linkages between landscape pattern and ESVs in Guangzhou remain largely unknown. In this paper, we analyzed the landscape pattern changes, the ESVs and its spatial distribution in Guangzhou from 1995 to 2005, using remote sensing and geographic information system techniques in order to provide useful information for policy makers who concern about sustainable development. It aims to: (1) retrieve landscape pattern changes of Guangzhou; (2) evaluate variations of ESVs in response to landscape pattern changes; and (3) analyze the spatial distribution of ESVs during 1995 and 2005.

Materials and methods

Study area

Guangzhou, the capital of Guangdong Province and the biggest city and political, economic, and cultural center of South China, is located in the southeast of Guangdong Province and at the center of the Pearl River Delta. Crossed by the Tropic of Cancer, it is laid between longitude 112°57′ to 114°13′ east and latitude 22°26′ to 23°56′ north, bordering on the South China Sea and adjacent to Hong Kong and Macao (*Fig.1*). The city, covering approximately an area of 7437.14 km^2 and with a population of 1.27×10^7, administrates 10 districts, namely Yuexiu district, Liwan district, Haizhu district, Baiyun district, Tianhe district, Huangpu district, Huadu district, Luogang district, Panyu district and Nansha district, and 2 county-level cities including Zengcheng county and Conghua county(Gong et al., 2009). The topography is higher in the north and lower in the south. The north and northeast are mountainous area and south is an alluvial plain of the Pearl River Delta. Guangzhou has a subtropical monsoon humid climate, which is determined that there is either intense heat in summer or severe cold in winter. The average temperature is 22.8 ℃, the average relative humidity is about 68%, and the annual rainfall at the urban area is over 1,600 mm.

As a regional central city in South China and China's Southern Gateway to the world, Guangzhou has enjoyed economic, political, social and cultural prominence for more than 2000 years. However, the rapid population growth and urban expansion in Guangzhou are now exerting pressure on its natural environment and there are always conflicts among the demands for good natural environment, urbanization, and ecosystem conservation.

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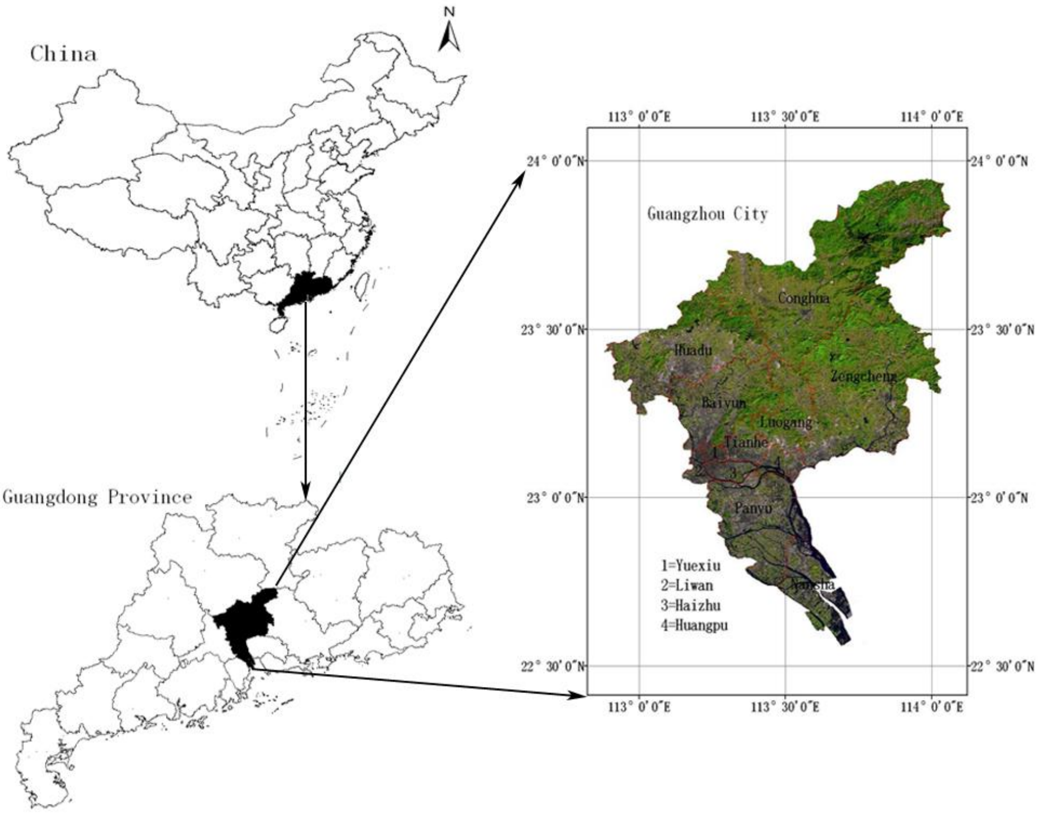


Figure 1. *Sketch map for the location of Guangzhou City, China*

Data acquisition and preprocessing

The basic data used to analyze landscape pattern and dynamics of ecosystem services in this paper were extracted from two cloud-free Landsat Thematic Mapper (TM) images taken on December 30, 1995 and October 22, 2005, respectively, and the World Wide Reference System (WRS) = 122/04385. The resolution of the images was 30 m. Although these satellite images were pre-geo-referenced, they could not be compared directly because the coordinate reference system and resolution used in each image was not consistent. To reduce potential position errors between the two data sets, we used a three-step image preparation procedure. Firstly, we identified the X and Y coordinates of pairs of points that represent prominent features on both the TM image in 1995 and the 1:250,000 topographic map of Guangzhou in 1985, compiled by Guangdong Province Municipal Institute of Surveying and Mapping. Secondly, we used the same topographic map as the geo-referenced standard together with the geo-referencing and Re-sampling modules of IDRISIs Release 2 software (Clark Labs, 2001; Li et al.,2011) to resample the 1995 TM data set into a Universal Transverse Mercator (UTM) coordinate system with original longitude 117ºE, original latitude 0ºN, WGS84 (World Geodetic System-1984) geodetic datum and WGS84 ellipsoid. Finally, using the same procedure as in the second step and the geo-rectified 2005 TM data as the master dataset, the 2005 TM image was resampled and rectified. Average Root Mean Square (RMS) error of less than 0.5 pixel in step 2 and 3 was achieved for all the images and the pixel size was kept as 30×30m grid.

Classification of landscape types

The landscape types used in this study were extracted from the two TM images in 1995 and 2005. In order to describe and represent the actual physical landscape of Guangzhou more profitably, the landscape was classified into six classes, namely cultivated land, garden, forest, grassland, built-up area and water body. The cultivated land class includes areas utilized for agricultural activities, particularly for the growing of grain crops, generally refers to paddy fields and dry land. The garden class includes areas utilized for the growing of cash crops such as fruit, vegetable, tea and flower, etc. The forest class includes thickly forested area, riparian forested area and shrub forested area, which are mostly protected areas and parks. The grassland class includes high, middle and low coverage plain grassland. The built-up class includes urban, residential, industrial, mining and institutional areas, traffic land (roads, the airport) and other concrete structures. The water body class includes rivers, channels, lakes and beaches.

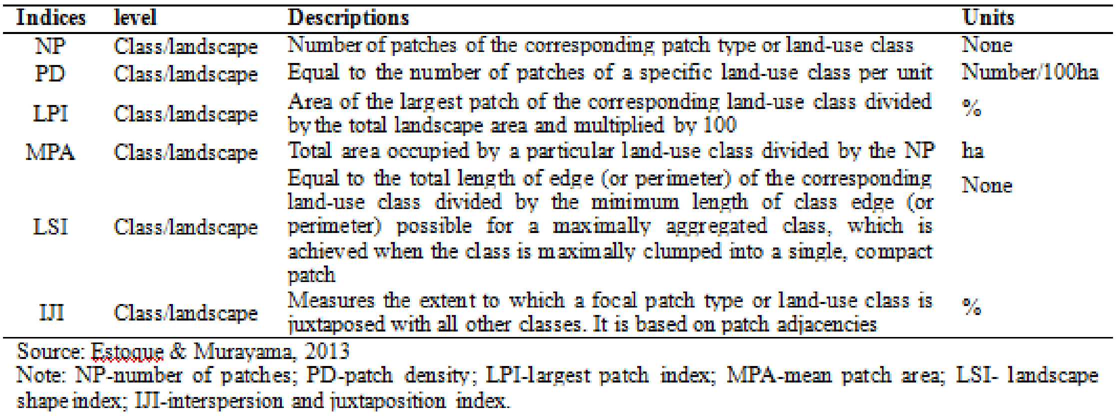
Landscape pattern analysis

There are a growing number of literatures that discuss the landscape metrics for ecological process and landscape fragmentation analysis (e.g., Ribeiro et al., 2009; Solon, 2009; Su et al., 2012; Thapa and Murayama, 2009; Zhang et al., 2011) in recent years. Spatial metrics at the landscape level provide more general information, whereas class level metrics provide more specific information about landscape development patterns, variations at the local level, spatial patterns and the distribution of land-use classes (Estoque and Murayama, 2013). So, in this study, based on landscape maps established by the above steps, we selected six indices for class/landscape level landscape metrics to monitor the changes in the landscape patterns of Guangzhou (*Table 1*). The eight-neighbor rule was used to derive the patch number and all the indices were calculated using Fragstats3.3.

Ecosystem service values assessment

Ecosystem services and their values have been studied since the 1970th, but the Earth's ecosystem service values are difficult to assess accurately because of the lack of a corresponding theory systems and methods (Huang et al., 2009). Costanza et al. (1997) classified the global biosphere into 17 types of service functions and then estimated their ecosystem service values, which made the principles and methods of assessing ecosystem service values clearer in a scientific sense. The result of Costanza et al. (1997) has also attracted the Chinese ecological researchers' attention over the years. However, it is unsuitable to directly adopt this method, which is intended for a global scale to a specific region for some ecosystem services may be less valued or even ignored (Du et al.,2008; Lin et al.,2013). Based on actual conditions in China and through the survey of 700 Chinese ecologists, Xie et al. (2008) extracted the ecosystem service values (ESVs) coefficients, which are the values per unit area of various ecosystem services in China to facilitate its practical application.

Table1. *Indices of class-level landscape metrics*



Indices	level	Descriptions	Units
NP	Class/landscape	Number of patches of the corresponding patch type or land-use class	None
PD	Class/landscape	Equal to the number of patches of a specific land-use class per unit	Number/100ha
LPI	Class/landscape	Area of the largest patch of the corresponding land-use class divided by the total landscape area and multiplied by 100	%
MPA	Class/landscape	Total area occupied by a particular land-use class divided by the NP	ha
LSI	Class/landscape	Equal to the total length of edge (or perimeter) of the corresponding land-use class divided by the minimum length of class edge (or perimeter) possible for a maximally aggregated class, which is achieved when the class is maximally clumped into a single, compact patch	None
IJI	Class/landscape	Measures the extent to which a focal patch type or land-use class is juxtaposed with all other classes. It is based on patch adjacencies	%

Source: Estoque & Murayama, 2013
Note: NP-number of patches; PD-patch density; LPI-largest patch index; MPA-mean patch area; LSI- landscape shape index; IJI-interspersion and juxtaposition index.

Considering about the real-world situation of Guangzhou, this paper referred to the ESVs coefficient of Xie et al. (2008) and revised some coefficients. For the cultivated land, which is mainly paddy fields and almost two crops planting in Guangzhou, the ESVs coefficient of cultivated land is 2 times greater than the ESVs coefficient of Xie et al. (2008). The ESVs coefficient of forest and grassland is 1.45 times greater than the ESVs coefficient of Xie et al. (2008) for the evergreen broad-leaved forest in Guangzhou and the ESVs coefficient of garden is the average of forest and grassland (Ye and Dong, 2010; Ye et al., 2011). So, the ESVs coefficient per unit area of each landscape class in Guangzhou is assigned (*Table 2*). The ESVs of different land use and the ESVs of Guangzhou for each of the two time periods (i.e., 1995 and 2005) were determined using Eq. (1), (2) and (3).

$$ESV_k = \sum_f A_k \times VC_{kf} \qquad \text{(Eq.1)}$$

$$ESV_f = \sum_k A_k \times VC_{kf} \qquad \text{(Eq.2)}$$

$$ESV = \sum_k \sum_f A_k \times VC_{kf} \qquad \text{(Eq.3)}$$

where ESV_k, ESV_f, and ESV refer to the ecosystem service value of landscape type k, the value of the function type f, and the total ecosystem service value, respectively. A_k denotes the area of landscape type ‘k’. VC_k is the value coefficient (Yuan·ha^{-1}·a^{-1}), which refers to the service value per unit area of function type ‘k’ on landscape type ‘f’ (Kreuter et al., 2001).

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***Table 2.** Ecosystem service value of unit area of different land use in Guangzhou ($Yuan\cdot ha^{-1}\cdot a^{-1}$)*

First class	Ecosystem services types	Forest	Grassland	Cultivated land	Water body	Garden	Built-up
Supply	Food production	214.89	280.01	898.20	238.02	247.45	Not available
	Raw material	1 940.56	234.44	350.30	157.19	1 087.50	Not available
Regulation	Gas regulation	2 813.16	976.79	646.70	229.04	1 894.98	Not available
	Climate regulation	2 650.37	1 015.87	871.26	925.15	1 833.12	Not available
	Water supply	2 663.39	989.81	691.62	8 429.61	1 826.60	Not available
	Waste treatment	1 120.05	859.57	1 248.50	6 669.14	989.81	Not available
Support	Soil formation and retention	2 617.80	1 458.67	1 320.36	184.13	2 038.24	Not available
	Biodiversity protection	2 936.89	1 217.74	916.16	1 540.41	2 077.31	Not available
Culture	Recreation and culture	1 354.49	566.54	152.70	1 994.00	960.52	Not available
	Total	18 311.60	7 599.45	7 095.78	20 366.69	12 955.53	Not available

Results

Changes in landscape area

From the composition of landscape in 1995, the forest, with a total coverage of 37.36%, was the most extensive distribution area. The next was the cultivated land, built-up area, water body, grassland and garden, which accounted for 18.37%, 16.73%, 11.48%, 9.01% and 7.05% of the total area, respectively. In 2005, forest was still the biggest, which accounted for 38.23%. Built-up area, cultivated land, water body garden and grassland accounted for 17.60%, 16.63%, 14.17%, 8.39% and 4.98% of the total area respectively.

From *Table 3*, we can also see that the area of grassland and cultivated land decreased during the last decade, while areas of forest, garden, water body, and built-up area increased. Water body and garden increased fastest with a rising rate of 23.40% and 19.05% respectively, the primary cause of which is that the grassland and cultivated lands had low economic profits and were artificially transformed into garden and water body. The built-up area increased by 5.18%, which reflected the needs of economic development, population growth and urbanization in Guangzhou, and the forest increased by 2.33%.

Changes of landscape pattern at the class level

The results showed that the forest and garden became less fragmented, as shown by the overall decrease in the number of patches (NP), patch density (PD) and landscape shape index (LSI) and the increase in mean patch of area (MPA), largest patch index (LPI). The increase in interspersion and juxtaposition index (IJI) suggests that the forest has become more contiguous while the decrease in IJI showed that the garden has become less contiguous.

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Table 3. *Total area of each landscape change in 1995 and 2005*

Landscape	1995		2005		2005-1995	
	Area (ha)	**Proportion (%)**	**Area (ha)**	**Proportion (%)**	**Changing area (ha)**	**Changing rate (%)**
Forest	277841.09	37.36	284323.54	38.23	6482.45	2.33
Grassland	67037.46	9.01	37041.09	4.98	-29996.40	-44.75
Cultivated land	136620.08	18.37	123715.27	16.63	-12904.80	-9.45
Garden	52401.38	7.05	62383.99	8.39	9982.61	19.05
Water body	85391.97	11.48	105377.93	14.17	19985.96	23.40
Built-up area	124421.9	16.73	130872.06	17.60	6450.16	5.18
Total	743713.88	100	743713.88	100	-	-

The grassland, water body and built-up area were all more fragment in 2005 than that in 1995, as shown by the overall increase in NP and PD, as well as the reduction in MPA, LPI, LSI and IJI. The reduction in MPA, LPI, LSI and IJI indicated a result of continuous losses of grassland, water body and built-up.

The cultivated land was more fragmented in 2005 than that in 1995, as shown by an overall decrease in NP, PD, MPA, LSI and IJI. The decrease of NP and PD revealed a continuous loss of fragmented cultivated patches. The decrease in LIS indicated the formation of more regular shapes at the edges of cropland, whereas the overall decrease in IJI showed that the cultivated land patches were not contiguous (*Table 4*).

Table 4. *Comparison of class-level landscape metrics in 1995 and 2005*

landscape	NP		PD(No./100ha)		MPA(ha)		LPI(%)		LSI		IJI(%)	
	1995	**2005**	**1995**	**2005**	**1995**	**2005**	**1995**	**2005**	**1995**	**2005**	**1995**	**2005**
Forest	540	466	0.07	0.06	514.52	610.14	15.05	23.07	44.54	18.75	87.10	88.78
Grassland	1825	1897	0.25	0.26	36.73	19.53	0.37	0.16	49.54	15.78	70.99	64.73
Cultivated land	1381	1351	0.19	0.18	98.93	91.57	0.49	3.58	81.62	28.48	87.41	81.91
Garden	760	548	0.10	0.07	68.95	113.84	0.48	1.26	54.37	21.66	87.57	85.60
Water body	1537	2470	0.21	0.33	55.56	42.66	7.89	5.65	76.91	26.85	82.66	78.67
Built-up	2078	2166	0.28	0.29	59.88	60.42	0.27	0.08	77.05	21.19	91.80	90.34

Note: NP-number of patches; PD-patch density; LPI-largest patch index; MPA-mean patch area; LSI- landscape shape index; IJI-interspersion and juxtaposition index.

Changes in ecosystem service values

The total annual ESVs of Guangzhou was 8984.63 million Yuan in 1995 and 9320.18 million Yuan in 2005 (*Table 5*), which showed an increasing trend in the total ESVs. Although the reduction of grassland and cultivated land area lead to reduce 319.53 million Yuan and the changing rate was -44.75% and -9.45%, the value of garden, water body and forest increased 655.08 million Yuan and the changing rate was 19.05%, 23.41% and 2.33%, respectively, which made up the loss value caused by grassland and cultivated land. The total ESVs of Guangzhou maintained sustaining growth of 335.55 million Yuan from 1995 to 2005 and the increasing rate was 3.73%.

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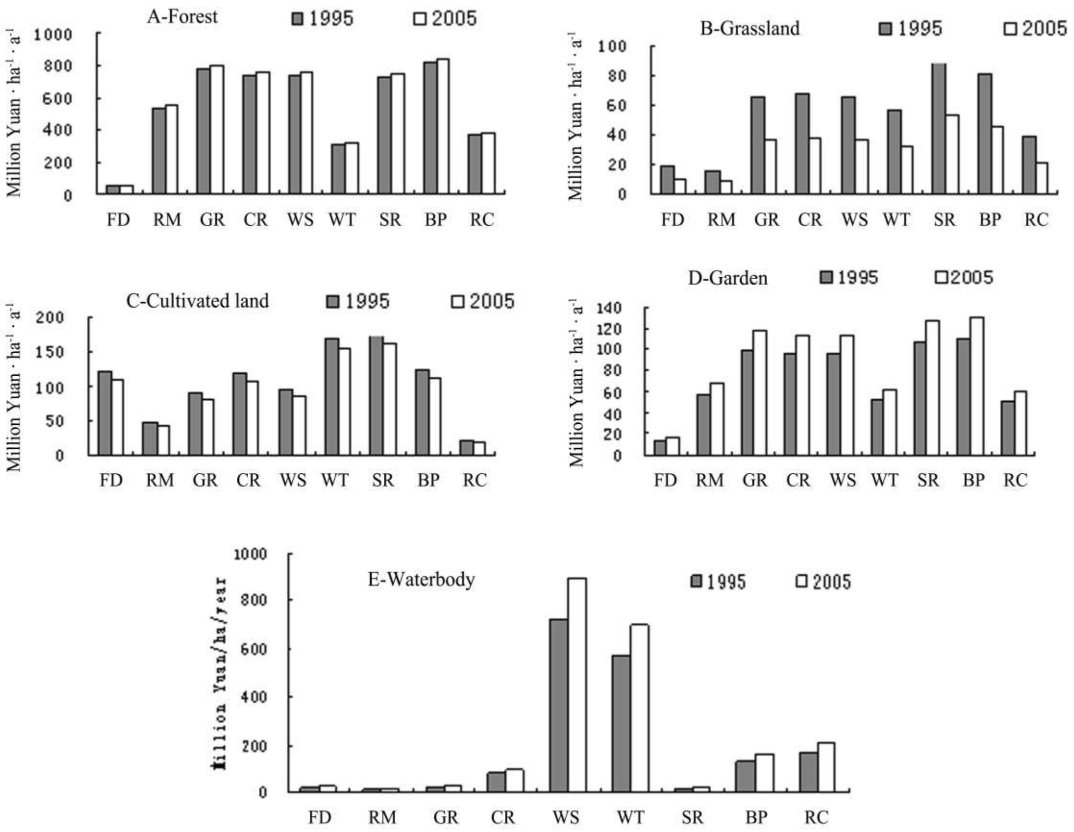
Forest and water body were the two main ESVs contributors in the study area. ESVs from both were over 75% of the total ESVs. The area of forest accounted for 37.36% in 1995 and 38.23% in 2005, instead, it generated 56.63% of the total ESVs in 1995 and 55.86% in 2005. Meanwhile, the area of water body accounted for 11.48% in 1995 and 14.17% in 2005, it generated 19.36% of the total ESVs in 1995 and 23.03% in 2005. Contribution rate from the cultivated land ranked in third, accounted for 10.79% in 1995 and 9.42% in 2005. Grassland contribution rate was smallest, accounted for 5.67% in 1995 and 3.02% in 2005.

Table 5. *Changes in ecosystem service values from 1995 to 2005 (10^6 Yuan/year)*

Landscape	**Ecosystem service values**				**Changes**	
	1995	**Percent (%)**	**2005**	**Percent (%)**	**2005-1995**	**Changing rate (%)**
Forest	5087.72	56.63	5206.42	55.86	118.70	2.33
Grassland	509.45	5.67	281.49	3.02	-227.96	-44.75
Cultivated land	969.43	10.79	877.86	9.42	-91.57	-9.45
Garden	678.89	7.56	808.22	8.67	129.33	19.05
Water body	1 739.15	19.36	2 146.20	23.03	407.05	23.41
Total	8 984.63	100	9320.18	100	335.56	3.73

The ESVs provided by single ecosystem function were calculated by the Eq. (1) and Eq. (3). The contribution of single ecosystem function by forest was mainly focused on the gas regulation, climate regulation, water supply, soil formation and retention, and biodiversity protection, each of which increased from 1995 to 2005 (*Fig. 2A*). The single service function of grassland and cultivated land was mainly the soil formation and retention and biodiversity protection. All the service functions provided by grassland and cultivated land decreased for their area reduced from 1995 to 2005 (*Fig. 2B and Fig. 2C*). The principal service functions of garden were raw material, gas regulation, climate regulation, soil formation and retention, and biodiversity protection, which all increased more from 1995 to 2005 (*Fig. 2D*). For the water body, the water supply and waste treatment were the most extensive distributors (*Fig. 2E*).

As seen from *Table 6*, the value of the individual ecosystem service functions in Guangzhou did not change the basic structure. The contribution order of the every ecosystem function value to the total value was followed by Water supply > Biodiversity protection > Waste treatment > Soil formation and retention > Climate regulation > Gas regulation > Raw material > Recreation and culture > Food production. The value of water supply was the largest category in Guangzhou and the principal reason is the location of Guangzhou at the center of the Pearl River Delta, where the water courses are densely and have many rivers and lakes. However, the value of food production was the smallest category because of the reduction of cultivated land for the rapid development of urbanization in Guangzhou, which leads to the land-use change from cultivated land to built-up area(Gong et al., 2009).



FD: food; RM: raw material; GR: gas regulation; CR: climate regulation; WS: water supply; WT: waste treatment; SR: soil formation and retention; BP: biodiversity protection; RC: recreation and culture

Figure 2. *The changes of ecosystem service values of the five landscape classes in Guangzhou in 1995 and 2005*

Table 6. *Ecosystem service values of each service type in 1995 and 2005 (10^6 Yuan·a^{-1})*

First class	**Ecosystem service types**	**1995**			**2005**		
		ESVs	**%**	**Rank**	**ESVs**	**%**	**Rank**
Supply	Food production	234.48	2.61	9	223.11	2.39	9
	Raw material	673.15	7.49	7	688.18	7.38	8
Regulation	Gas regulation	1054.30	11.73	6	1058.39	11.36	6
	Climate regulation	1098.57	12.23	5	1110.83	11.92	4
	Water supply	1716.38	19.10	1	1881.74	20.19	1
	Waste treatment	1160.75	12.92	3	1269.28	13.62	3
Support	Soil formation and retention	1128.04	12.56	4	1108.24	11.89	5
	Biodiversity protection	1263.18	14.06	2	1285.39	13.79	2
Culture	Recreation and culture	655.78	7.30	8	695.03	7.46	7
	Total	8984.63	100.0	−	9320.19	100.0	−

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Spatial distribution of Ecosystem service values

ESVs of Guangzhou decreased from the north and south to the central part in 1995 and 2005 respectively (*Fig.3*). ESVs in the north were higher mainly due to the higher vegetation coverage. The average ESVs was more than 15 000 Yuan/ha in the north, where the mountainous area is known as the ecological shelters of Guangzhou. Moreover, ESVs in the south were highest mainly due to the Pear River's estuary. The average ESVs were more than 20 000 Yuan/ha in the south for water body, which could provide many kinds of aquatic ecosystem services such as water storage, water supply, aquatic product, tourism, flood control, water purification, hydro-power, transportation and sand transport, etc. On the contrary, the average ESVs was lower than 5 000 Yuan/ha in the central part of Guangzhou mainly due to the widely scattered of built-up area for rapid urbanization, where is the city center of Guangzhou, including the Districts of Yuexiu, Liwan, Tianhe, Haizhu, Huangpu, Luogang and Baiyun.

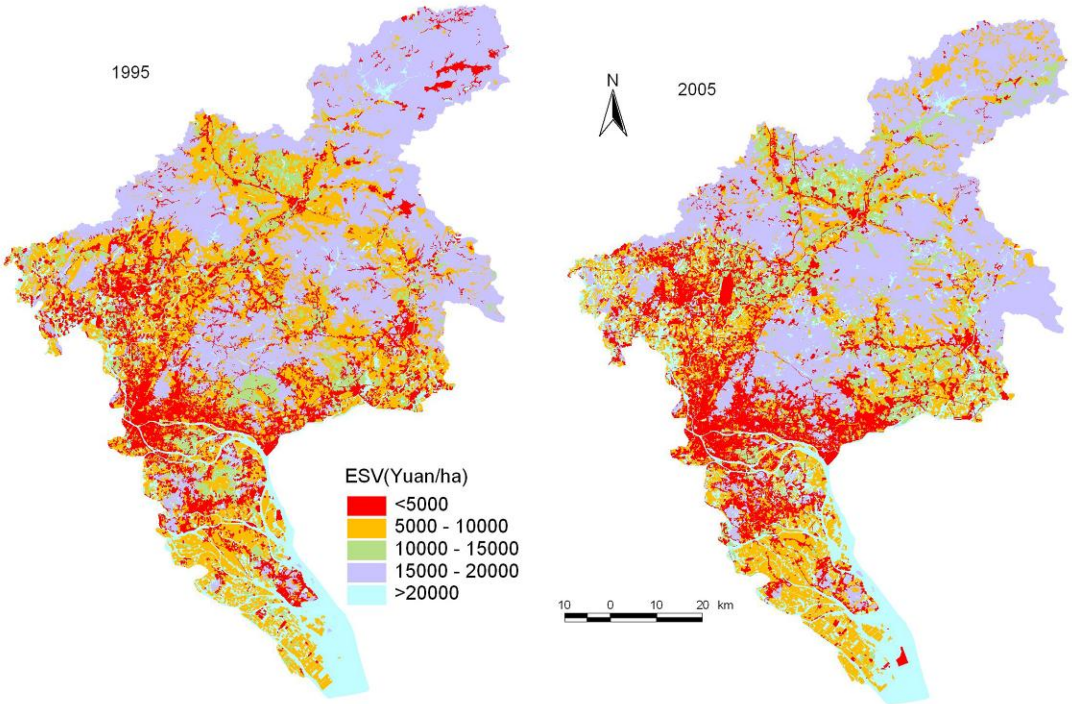

Figure 3. *Distribution of the ecosystem service values in Guangzhou in 1995 and 2005*

Discussions

The concept of ecosystem services from landscapes is rapidly gaining momentum as a language to communicate values and benefits to scientists. Landscape ecology has an enormous contribution to make to this field (Iverson et al., 2014) and responses of ecosystem services to landscape pattern changes are widely discussed(Estoque and

Murayama, 2013; Su et al., 2012; Zhang et al., 2011). ESVs are the benefits that human derive from ecosystem (Millennium Ecosystem Assessment, 2005). Evaluating ESVs in an economical way may draw sufficient public attention on one hand and build a green GDP accounting system and ecological compensation mechanism on the other hand (Feng et al., 2012). Landscape development changed the biogeochemical cycling, ecosystem structure, and ecosystem service values. The consequences have become the main difficulty of global sustainable development and the vital focus of public attention. Thus, evaluating its variations due to the landscape change is of profound significance. Our study indicated that the economic value of services of the landscape in Guangzhou is huge, which is 8984.63 million Yuan in 1995 and 9320.19 million Yuan in 2005. It cannot be imagined that the local people are able to make such huge quantity of investment per year.

Understanding the spatial distribution of the ecosystem service value is as important as the value itself, especially when the ecosystems are being faced with dramatic changes. Our study showed that remote sensing from satellites may be the only economically feasible way to regularly gather information with high spatial, spectral and temporal resolution over large areas (Seidl et al., 2000; Kreuter et al., 2001). And the satellite data are useful and inexpensive for analyzing changes in the value of ecosystem services and the spatial distribution at the local level for most available landscape data are based upon geopolitical boundaries and regional planning maps, neither of which relate well to the spatial arrangement changing landscape patterns (Zhao et al., 2004). Some studies on the relation between land use changes and the ecosystem service value indicate that it can only analyze the temporal change of the ecosystem service value for statistical data while the spatial distribution couldn’t be analyzed (Ye et al., 2008; Wang et al., 2009). So, our study also indicated that this comprehensive approach is feasible to study spatial distribution dynamics of ecosystem service values in response to landscape pattern change.

It is clearly shown that ecosystem services contribute substantially to human welfare on our Earth. In the decision-making process, we should give adequate weight to natural capital stock that produces these services and build up the mechanisms of economic compensation for the people who conserve ecosystem services (Li et al., 2011). In our study, forest lands in the north mountainous area, which is known as the ecological shelters and was called the “North Lung” of Guangzhou, provide huge ecosystem services (e.g., raw material, gas regulation, climate regulation, water supply, biodiversity protection, recreation) while the economic development is lag behind the southern area of Guangzhou. So, some economic compensation should be given to the north area. Furthermore, we should also pay specific attention to conserve this natural capital stock and find ways for its sustainable use. As natural capital and ecosystem services become more stressed and more limited in the future, we must determine how to use and protect them. The analyzing of ESVs is just a useful starting point.

Conclusions

Guangzhou City, a large central city in South China and China's Southern Gateway to the world, has enjoyed economic, political and social prominence for more than 2000 years, well known for its favorably subtropic climate and attractive natural landscape. This study has attempted to analyze the landscape pattern changes and the dynamics of the ESVs, which offer important insights to those in fast-urbanizing regions for achieving more successful landscape.

In this paper, not only the landscape pattern but also the spatiotemporal changes of ecosystem service values were analyzed in Guangzhou City from 1995 to 2005. The results showed that the forest and garden became less fragmented with the overall decrease in the number of patches (NP), patch density (PD) and landscape shape index (LSI) and the increase in mean patch of area (MPA), largest patch index (LPI). But the cultivated land, grassland, water body and built-up area became more fragmented for the overall increase in NP and PD, as well as the reduction in MPA, LPI, LSI and IJI. The total ESVs maintained sustaining growth of 335.55 million Yuan from 1995 to 2005 and the increasing rate was 3.73%. Two important landscape types (forest and water body) contribute to the majority of ESVs, which accounted for over 75% of the total ESVs. The ESVs tended to decline from the north and south to the central part of Guangzhou. The average ESVs was more than 15 000 Yuan/ha in the north and 20 000 Yuan/ha in the South in Guangzhou, but it was less than 5 000 Yuan/ha in the central part of the area due to low vegetation coverage and the widely scattered of built-up area for rapid urbanization.

Zhang et al.(2011) analyzed the relationships between spatio-temporal variation of ESVs and landscape pattern indices, the result of which indicated that ESVs tend to increase with the growth of patch area and decrease with the development of patch fragmentation and shrinking of patch sizes. So, in order to maintain the increase of ESVs in Guangzhou, it is high time to protect the landscape types such as forest, garden and water body and to increase patch size to avoid fragmentation.

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DIVERSITY AND ECOLOGY OF DIATOMS IN NORTHWEST OF ALGERIA: CASE OF EL-HAMMAM STREAM AND ESTUARY OF CHELIFF RIVER

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Abstract. Ecological research on algae from Algeria and in particular on freshwater and estuarine diatoms remains insufficient. The aim of our work is to contribute to the taxonomy and ecology of benthic diatoms from freshwaters and estuaries in Algeria. For this purpose, we studied two sites of water courses in north-western Algeria: El-Hammam stream at Mascara and the estuary of Cheliff river at Mostaganem. Our inventory of benthic diatoms during dry season at the middle of El-Hammam stream during April, June and August 2010 led to the identification of 44 cosmopolitan species, while 30 species occurred at the mouth of Cheliff river. 10 taxa were recorded for the first time in Algeria. Diversity values (Species richness, Shannon's index and Equitability index) for the two study sites are generally medium to low. The results of the 17 diatom-based metrics tested show that ROTT trophic index (TID) and the Trophic Diatom Index (TDI) best mirrored water quality, but a revision of taxa autecological values is required.

Introduction

The earliest studies on the algal flora of Algeria date back to 1799 with the work of Desfontaines (Baudrimont, 1971a), after that, first researches on Algerian diatom flora were performed by Montagne (1846), Ehrenberg (1854). The works of Baudrimont (1974) are remarkable contributions to the ecology of diatoms in Algeria. He has recorded 356 species, varieties and forms during his research on diatoms of inland waters in Algeria, estimating that this inventory represents only 17.5% of the world's known flora in 1968. Since then, the global number of described taxa has increased exponentially while the flora of Algerian diatoms has not changed apart from recent studies including the work of Al-Asadi et al. (2006), the monograph by Lange-Bertalot et al. (2009) identifying four new *Navigiolum* from samples collected in Algerian ephemeral rock pools ("Gueltas") and the papers of Chaïb et al. (2011) and Chaïb and Tison-Rosebery (2012) on samples collected from wadi (stream) Kebir-East, North-East Algeria.

Besides their floristic interest, several authors have studied diatoms as biological proxies for water quality, discussing their performance as surrogates for saprobity, trophic status, acidity, etc. Benthic diatoms are now considered key organisms for

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monitoring river ecological quality in several European countries (European Union, 2000). In Algeria apart from the study of Chaïb and Tison-Rosebery (2012), to our knowledge no attempt of diatom-based environmental monitoring has been performed to date in Algerian aquatic ecosystems, evidencing the lack of accurate ecological information linked to available diatom data.

This work presents two inventories of benthic diatoms in two different hydrosystems in northwestern Algeria: the estuary of Cheliff river (Brackish water) at Mostaganem and in the middle of El-Hammam stream (freshwater) at Mascara. The results of this study will contribute to the database of Algerian benthic diatoms. We also test the performance of 17 diatom indices in order to provide a scientific basis for the implementation of diatom-based monitoring programs in this region.

Material et methods

Study sites

Algeria includes many arid and semi-arid areas which suffer from long periods of drought and severe water shortages. Four stations at two sites have been chosen:

a) The site S1 in the middle of El-Hammam stream *(Fig. 1)*, located northwest of Algeria, municipality of Bouhanifia, region of Mascara. El-Hammam stream is subject to a semi-arid climate with an annual average temperature of 16.6 °C and a precipitation for the whole basin estimated at 385.3 mm. Its watershed is part of the great Makta's watershed; it is limited by the mountains of Beni-Chougrane north and massive of Siada south. Two stations were chosen at this site: H1 (35° 17' 58.70" N, 0° 2' 48.26" W, 231 m a.s.l.) and H2 (35° 18' 52.06" N, 0° 3' 4.45" W, 226 m a.s.l.) at a distance of about 2.26 km and 4.24 km respectively downstream of the dam of Bouhanifia.

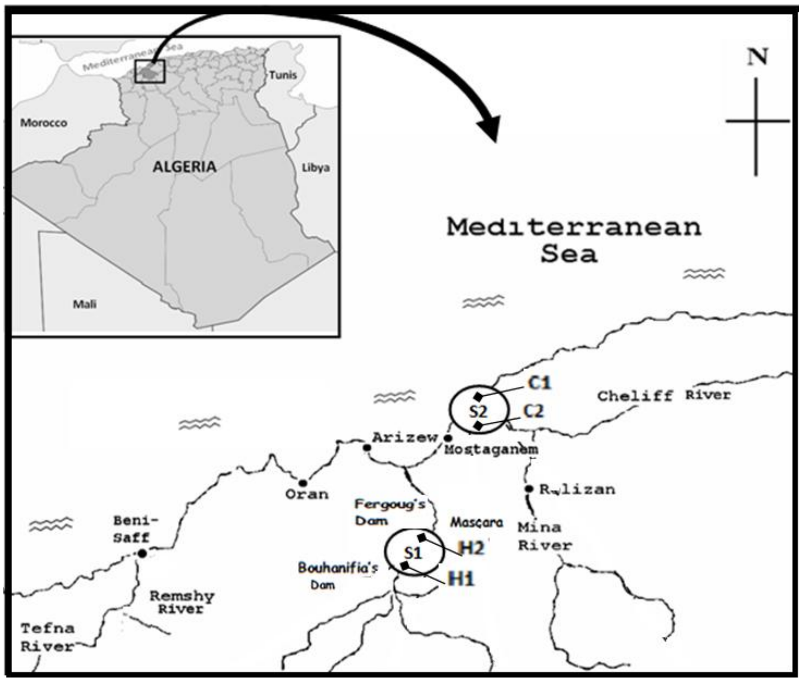

Figure 1. *Location of the four study sites H1, H2 at the site S1 at El-Hammam stream and C1, C2 at the site S2 at the mouth of Cheliff river.*

b) Site S2 belongs to the estuary of Cheliff river *(Fig. 1)*, the largest watercourse in Algeria, located northwest of the country, 700 km long, it passes through several wilayas including Mostaganem, Relizan, Ain-Aldeffla and Cheliff (Al-Asadi et al., 2006), it rises in the Tell atlas and flows 13 km east of the city of Mostaganem (Cheliff-beach). The zone is characterized by hot summers and cool winters, with temperatures ranging from 10.41 °C in January to 24.06 °C in August, while rainfall varies from 1.37 to 74.59 mm from July to December (Al-Asadi et al., 2006). Two stations were chosen near the mouth: C1 (36° 2' 14.47" N, 0° 8' 10.00", sea level) and C2 (36° 2' 0.17" N, 0° 8' 39.50", sea level) at a distance of about 360 m and 1.21 km respectively from the Mediterranean Sea.

Collection of benthic diatoms

Benthic diatoms were sampled during low flow periods during the months of April, June and August in the year 2010. Different natural substrates immersed in water were used for the collection of periphyton depending on sampling conditions, access and availability of substrates (Kelly et al., 1998; Prygiel and Coste, 2000; Taylor et al., 2007b). In total, 24 samples were collected. The surfaces of the natural substrates (pebbles, cobbles and stems) collected from watercourses were brushed using a toothbrush to remove periphyton containing diatoms, in the case of the filamentous algae; diatoms were collected after shaking algae in sachets containing clean medium water.

Identification of benthic diatoms

Benthic diatoms were collected in separate bottles and stored in the field with 10% of formalin neutralized the sample as treated with boiling hydrogen peroxide (H_2O_2 at 30%) for 10 minutes to degrade organic matter and for 5 minutes with boiling hydrochloric acid HCl 35%. Samples were then subjected to a series of centrifugations for 5 min at 2500 rpm and a succession of cleanings with distilled water. An aliquot of the cleaned sample was deposited on a coverslip, dehydrated by drying and mounted in the resin refractive Naphrax (Northern Biological Supplies Ltd., England, refractive index = 1.74), (Hasle and Fryxell, 1970; Prygiel and Coste, 2000). Microscopic examination of permanent slides were made using an optical microscope LEICA DM 5000 type B in differential interference contrast (DIC), diatom species were identified at a magnification X1000 with immersion oil, a minimum of 400 diatom valves were identified in each slide following usual references (Krammer and Lange-Bertalot, 1988, 1986, 1991a, 1991b, 2000 ; Prygiel and Coste, 2000; Bate et al., 2004; Taylor et al., 2007a; Lavoie et al., 2008; Blanco et al., 2010; etc.).

Physicochemical parameters

Main limnological parameters were provided by the National Agency of Water Resources (ANRH, 2010) at two stations: the dam of Bouhanifia (upstream of El-hammam) and station Hacine (downstream of El-hammam at around 8 km upstream of Fergoug's dam). Additionally, temperature, pH and conductivity were measured at the other locations. These three parameters were measured *in situ* by a thermometer (liquid in glass), digital pH-meter type PHT-01 ATC Voltcraft and conductimeter type LWT-01 Voltcraftwe.

Statistical analysis

Data were analysed for correlation and correspondence analyses (CA). A Correspondence Analysis (CA) was used to explore the distribution of diatom taxa in the different stations studied. The relationships between the different metrics calculated were assessed by means of Pearson's correlation coefficients. Both were calculated using XLStat software v. 2013 (Addinosoft, 2013). Diatom indices were calculated using Omnidia software (Lecointe and Coste, 1993).

Results and Discussion

The inventory of benthic diatoms at both sites consisted of a total of 56 taxa 44 of them found the middle of El-Hammam stream and 30 taxa at the mouth of Cheliff river, including 18 taxa in common to both sites. Ten taxa were recorded for the first time in Algeria *(Table 1)*.

During the low-flow periods in the months of April, June and August, the fresh water of station H1 at El-Hammam stream was characterized by the dominance (> 20%) of *Achnanthidium minutissimum*, *Cyclotella ocellata*, *C. meneghiniana*, *Nitzschia frustulum* and *N. palea*, while the station H2 was characterized by the dominance of *Achnanthidium saprophilum*, *Navicula cryptotenelloides*, *N. erifuga* and *Nitzschia palea*. Other frequent taxa (5-20%) at site H1 were *Achnanthidium saprophilum* and *Surirella brebissonii*, while at the site H2 *Achnanthidium exiguum*, *Cyclotella meneghiniana*, *Fragilaria nana*, *Gomophonema parvulum*, *Navicula frustulum*, *N. recens and*, *N. veneta* appeared as subdominant species.

Most species recorded at El-Hammam stream are known to be cosmopolitan, being common in European freshwaters with a slight salinity and alkaline content, as remarked by Baudrimont (1974) in his study of taxa from arid and semi-arid areas of Algeria inculding the region of Mascara. Most of these taxa were already recorded by some authors in north Algeria (Petit, 1895; Baudrimont, 1974; Al-Asadi et al., 2006; Chaïb et al., 2011; Chaïb and Tison-Rosebery, 2012). Baudrimont (1974) took samples at Hammam Bouhanifia from the thermal spring of El-Hammam stream with water above 50 °C, recording *Cocconeis pediculus* and *Cyclotella meneghiniana*, but no samples had been takes to date from El-Hammam stream where we record for the first time the presence of *Craticula buderi*, *Conticribra weissflogii, Cymbopleura incerta*, *Denticula kuetzingii*, *Fragilaria nana*, *Navicula phyllepta*, *Navicula simulata* and *Nitzschia perminuta.*

Concerning the saline waters, during the sampling period at the estuary of Cheliff river the dominant taxa (> 20%) in both stations C1 and C2 were *Amphora* sp. and *Berkeleya* sp.2. The most abundant taxa (5-20%) in both stations were *Achnanthes minutissimum* var. *gracillima*, *Berkeleya* sp.1 and *Navicula phyllepta*. *Cyclotella meneghiniana* was found abundant at station C1 and *Achnanthes minutissimum var gracillima*, *Cylindrotheca closterium, Halamphora coffeaeformis* and *Nitzschia incerta* at station C2. All the taxa recorded at the estuary of Cheliff river are known to be cosmopolitan. Except for a few refrences on marine diatoms from saline waters from Algeria like Chott and Sebkha (Petit, 1895; Amosse, 1941; Baudrimont, 1970, 1971a, 1974) to our knowledge no further papers study Algerian estuarine diatoms, thus we cite for the first time the following taxa: *Achnanthes minutissima var gracillima, Craticula buderi, Gyrosigma scalproides, Navicula phyllepta.*

Table 1. *Average frequency of diatoms recorded in different stations at two sites in the middle of El- Hammam stream and at the mouth of Cheliff river. **: Taxa preceded by asterisk were recorded for the first time in Algeria.*

☐ Absent ░ Rare (0.2-1 %) ▒ Common (1-5 %) ▓ Frequent (5-20 %) ■ Dominant (>20 %)

Sites	S1 El-Hammam stream						S2 Estuary of Cheliff river					
Dates	April		June		August		April		June		August	
Taxa / Stations	*H1*	*H2*	*H1*	*H2*	*H1*	*H2*	*C1*	*C2*	*C1*	*C2*	*C1*	*C2*
Achnanthes exigua Grunow in Cleve et Grunow	0.2		0.5		0.3							
***Achnanthes minutissimum var. gracillima* (Meister) Lange-Bertalot							5.2	0.3	9	5.8		
Achnanthidium minutissimum (Kützing) Czarnecki	54.5	0.5	9.2	3	8	5.4		5.3	1.7	4.8	4.8	1.6
Achnanthidium saprophilum (Kobayasi & Mayama) Round & Bukhtiyarova	10				2.3	61.6						
Aulacoseira ambigua (Grunow) Simonsen					0.3							
Halamphora coffeaeformis (Agardh) Z. Levkov							4.8		0.3	1	4.8	9.1
Amphora sp.							0.7	1.3	38	31.8	54.	78.9
Amphora pediculus (Kützing) Grunow			1.2									
Berkeleya sp. 1 *(rutilans ?)*							5.2		2.0	11.9	9.1	0.9
Berkeleya sp. 2 *(scopulorum ?)*							58.8	55	24	22.8	2.7	0.3
Cocconeis euglypta Ehrenberg	0.5		0.7	0.4	4.4	0.4						
Cocconeis pediculus Ehrenberg			0.2		1.3							
***Conticribra weissflogii* (Grunow) K.Stachura-Suchoples & D.M.Williams					0.5			0.3	0.3		3.2	
***Craticula buderi* (Hustedt) Lange-Bertalot			0.2			0.4	0.7	1.1		0.3		
Cyclotella meneghiniana Kützing	1.2	0.2	9	1.1	23.3	6.1		1.3	0.3	1.0	9.1	2.5
Cyclotella ocellata Pantocsek	3.2	1	23.9	1.3	1.3	1.3	3.8	3.2	2	1		0.3
Cylindrothica closterium (Ehrenberg) Reimann & J.C.Lewin							0.7	5.3		0.3	0.5	
** *Cymbopleura incerta* (Grunow) Krammer				0.6								
***Denticula kuetzinqii* Grunow		0.2										

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Sites	**S1 El-Hammam stream**						**S2 Estuary of Cheliff river**					
Dates	**April**		**June**		**August**		**April**		**June**		**August**	
Stations **Taxa**	***H1***	***H2***	***H1***	***H2***	***H1***	***H2***	***C1***	***C2***	***C1***	***C2***	***C1***	***C2***
Encyonema minutum (Hilse) D.G.Mann	0.2		0.2		0.5							
Entomoneis sp.								2.1	0.7			
Eolimna subminuscula (Manguin) G. Moser *et* al.		0.2							0.3	0.3		0.3
** *Fragilaria nana* F.Meister(Meister) Lange-Bertalot					4.4	10.6						
Gomphonema parvulum (Kützing) Kützing	0.2	13.4	0.5	1.1	0.3							
***Gyrosigma scalproides* (Rabenhorst) Cleve										0.3		0.3
Hippodonta hungarica (Grunow) Lange-Bertalot *et* al.					0.5							
Luticola mutica (Kützing) D.G.Mann			0.5									
Navicula cryptotenelloides Lange-Bertalot		34.2	0.2	3								
Navicula erifuga Lange-Bertalot *et* Krammer			0.2	28.4	0.3	0.2						0.6
Navicula gregaria Donkin	0.2	3.2	1	0.2	1.3	0.7						0.3
Navicula lanceolata Ehrenberg	0.5		1.2	2.0	4.9	0.5		0.3			3.7	0.9
***Navicula phyllepta* Kützing	0.2						12.7	11.3	9	5.8	4.8	0.3
Navicula reichardtiana Lange-Bertalot	1.2	3	0.5	1.9	0.5	1.4		0.3				0.3
Navicula recens Lange-Bertalot in Krammer *et* Lange-Bertalot	1	8.7	4.2					0.3		0.3		
***Navicula simulata* Manguin		0.7										
Navicula sp.			0.7									
Navicula viridula var. *rostellata* (Kützing) Cleve	0.5		1		0.3							
Navicula veneta Kützing	0.2	7.4		2.8	1.8							
Nitzschia acicularis (Kützing) W.Smith					0.3							

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Sites	S1 El-Hammam stream						S2 Estuary of Cheliff river					
Dates	April		June		August		April		June		August	
Taxa / Stations	*H1*	*H2*	*H1*	*H2*	*H1*	*H2*	*C1*	*C2*	*C1*	*C2*	*C1*	*C2*
Nitzschia dissipata (Kutzing) Grunow. var. *dissipata*					0.8	0.4				0.3		
Nitzschia capitellata Hustedt				0.4	0.5	0.4		4.2				
Nitzschia clausii Hantzsch									0.3		1.1	
Nitzschia clausii Hantzsch									0.3		1.1	
Nitzschia frustulum (Kützing) Grunow	24.2	18.6	15.2	17.5	6.5	1.1	0.3	0.3	1.3	0.3		0.9
Nitzschia filiformis (W.Smith) Hustedt			0.5				0.3		0.7			
Nitzschia linearis (C.Agardh) W.Smith			2.2	0.4	0.3							
Nitzschia palea (Kützing) W.Smith	1.2	6.5	17.5	35.3	22.5	7.6	1.4	1.8	2	1	0.5	0.3
***Nitzschia perminuta* (Grunow) M.Peragallo				0.2								
Nitzschia incerta (Grunow) M.Peragallo							4.8	6.3	4.0	8.7		
Nitzschia fonticola (Grunow) Grunow in Van Heurck				0.4	2.8	0.9						
Pleurosira laevis Ehrenberg *f. laevis*					0.3	0.5						
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	0.2		4.2	0.2	1.8	0.2						
Stauroneis sp.							0.7		1.7	0.6	0.5	
Surirella brebissonii Krammer et Lange-Bertalot	0.2	1.7	2.5		6.5							
Tabularia fasciculata (C.Agardh) D.M.Williams et Round			1			0.2						
Tryblionella apiculata Gregory		0.2	1.2		1.3	0.2			0.3	0.6	1.1	0.3
Ulnaria ulna (Nitzsch) P.Compère								0.3	2.0	1.0		1.6

Concerning diversity indices, the values of stand structure metrics (species richness S, Shannon's diversity H_{α} and evenness E) at both sites are generally low, with an average Shannon's diversity not exceeding 2.5 bits *(Table 2)*. At El-Hammam stream, the diatom communities at stations H1 April and H2 August are less balanced and diversified compared to others stations. Station H1 April was dominated by *Achnanthidium minutissimum* (55%) and *Nitzschia frustulum* (24%) wich influenced negatively Shannon's diversity "Hα" and evenness "E". Chaïb and Tison-Rosebery (2012) noted that *Achnanthidium minutissimum* represented up to 75% of the community abundance during the summer at a station of Kebir-East stream (North-est of Algeria). Mean calculated values of diversity index at Kebir-East stream in summer were around E= 0.7 and Hα= 3.7, being E= 0.6 and Hα= 1.9 at El-Hammam stream.

***Table 2**. Shannon-weaver diversity index and equitability of El-Hammam stream and Cheliff's estuary.*

El-Hammam Stations	**April**		**June**		**August**	
	H1	***H2***	***H1***	***H2***	***H1***	***H2***
Shannon -weaver diversity index (H_{α})	1.41	1.95	2.39	1.80	2.51	1.48
Equitability (E)	0.48	0.72	0.73	0.61	0.73	0.49
Species richness (S)	19	16	27	19	30	20
Estuary of Cheliff Stations	***C1***	***C2***	***C1***	***C2***	***C1***	***C2***
Shannon -weaver diversity index (H_{α})	1.53	1.72	1.96	1.85	1.71	0.95
Equitability (E)	0.58	0.59	0.65	0.60	0.65	0.33
Species richness (S)	14	19	20	21	14	18

At Cheliff river, station August C2 shows clearly the most unbalanced and less diversified assemblage, with E= 0.33 and Hα= 0.95. This was characterized by the abundance (78%) of *Amphora* sp. *(Table 1)*. In general, diatom diversity at the estaury of Cheliff river (around E= 0.5 and Hα= 1.6) was influenced by the presence of brackish taxa such *Berkeleya* sp. 2 and *Amphora* sp., ecological studies of most species of the genus *Amphora* recorded at Algeria were from saline and brackish waters such Chott (Baudrimont, 1970, 1971a, 1974). However, the ecology of the genus *Berkeleya* in Algerian saline waters is still uknown (Petit, 1895; Amosse, 1941). Rovira et al. (2009) studied the diversity of periphytic diatom community in a Mediterranean salt wedge estuary at the Ebro Estuary (Spain), noting the most abundant genera (considering all species) were *Cocconeis* (23%), followed by *Navicula* (21%), *Nitzschia* (17%) and *Tabularia* (11%), with a mean value of Hα= 3.4 and species richness S= 39, in clear contrast with our results. The diatom community at estuary of Cheliff river was less diversified, with a mean Hα= 1.6 and S= 18, these values probabli related to mineral and organic pollutants accumulated in this area (Kies, 2009). Al-Asadi et al. (2006) studied the Cheliff river, finding that diatoms showed less dominance than in the Mina river in northwestern Algeria.

Concerning organic pollution, ecoligical studies in Algerian waters have been focused only on inorganic components, with no previous mesurements of parameters such as biological or chemical oxygen demand. The saprobity status of Algerian waters has not been properly assessed either. We calculated the Organic Pollution Index or

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"Indice de Pollution Organique IPO" (Leclercq and Maquet, 1987) from physicochemical data given by National Agency of Water Ressources of Algeria *(Table 3)* in summer of 2010 at El-hammam stream upstream of fargoug's dam which value of "IPO" show that water quality was strongly polluted by organic materials (IPO: from 2.0 to 2.9). Also we calculated the same index at Kebir-East stream in summer 2007 from physicochemical data published by Chaïb and Tison-Rosebery (2012) which water was also strongly polluted by organic materials (IPO: from 2.0 to 2.9). Using this metric, Bahroun and Kherici Bousnoubra (2011) at the Kebir-East stream in 2004-2005, indicated that water was extremely polluted by organic materials.

According to the classification of Van Dam et al. (1994), the most frequent taxa at El-hammam stream were β-mesosaprobous (42.7%) and polysaprobous (30.7%), compared to Kebir-East stream were most taxa recorded as frequent by Chaïb and Tison-Rosebery (2012) were β-mesosaprobous to α-meso-/polysaprobous (*Achnanthidium minitissimum*, *Amphora pediculus*, *Gomphonema parvulum*, *Navicula gregaria*, *Nitzschia frustulum*, *Eolimna subminuscula*) except for *Cyclotella ocellata* (oligosaprobous) and *Nitzschia palea* (polysaprobous). While species abundance at El-Hammam stream was a bit different additional to *Achnanthidium minitissimum*, *Cyclotella ocellata*, *Nitzschia frustulum*, *Nitzschia palea* we recorded also *Achnanthidium saprophilum* (polysaprobous), *Cyclotella meneghiniana* and *Navicula cryptotenelloides (*β-mesosaprobous). The abundance of *Cyclotella ocellata* at El-Hammam stream reached up (23%) at H1 June and was not as stable as that of *Nitzschia frustulum* and *N. palea* during the summer at El-Hammam stream probably because high oxygen concentrations (128 % stauration) recorded near this station at dam of Bouhanifia *(Table 3)*. The abundance of *Nitzschia frustulum* and *N. palea* was also noted by Fawzi et al. (2001) at wadi Hassar from Morocco, under semi-arid climate with oceanic influence at, where these two species indicated an important degree of organic pollution.

With respect to the trophic status, the communities at stations of El-Hammam stream were generally dominated by eutrophentic (42.5%) and hypereutrophentic taxa (28 %), Dominant species (>20%) such as *Nitzschia frustulum* at H1 April, *Navicula erifuga* at H2 June and *Cyclotella meneghiniana* at H1 August were eutrophilous, but hypereutrophilous taxa such as *Achnanthidium saprophilum* at H2 August, or *Nitzschia* palea at H2 June and H1 August also occured, thereby indicating that these areas were impacted by nutrient inflows, presumably related to adjacent anthropogenic activities including agriculture. The same pattern was observed at Kebir-East stream by Chaïb and Tison-Rosebery (2012) with eutrophentic taxa representing 52.5% of the community.

Concerning conductivity and salinity levels, most dominant taxa at El-Hammam stream were fresh-brackish taxa (55.7%) with salinity optima under 0.825 PSU according to the classification of Van Dam et al. (1994) and brackish-fresh taxa (34.9%) with salinity optima of 0.825 - 1.65 PSU.

In general, the same dominant species were recorded at Kebir-East stream by Chaïb and Tison-Rosebery (2012), including *Achnanthidium minutissimum*, *Cyclotella ocellata*, *Nitzschia frustulum*, and *Navicula recens*, exept species such *Amphora pediculus*, *Navicula caterva* and *Eolimna subminuscula* that occurred at Kebir-East stream or *Achnanthidium saprophilum*, *Cyclotella meneghiniana*, *Fragilaria nana* and *Navícula cryptotenelloides* at El-Hammam stream. The high chloride concentration (325 mg l^{-1}) and conductivty levels (around 1575 µS cm^{-1}), together with wastewater discharges observed near station H2, favored the developement of taxa adapted to such conditions. Baudrimont (1974) concluded that 206 different species recorded in Algerian aquatic systems such as wadis, chotts and sebkha were related to salt waters, concluding that freshwater streams with oligohalobious species were absent in Algeria. In our case, we noted the ocasional abundance of freshwater taxa such *Cyclottela ocellata* in H1 June and H2 August, although oligohalobous species were indeed very rare. Chaïb and Tison-Rosebery (2012) showed comparable results at Kebir-East stream, with scarce occurrence of oligohalobous species.

Table 3. *Physicochemical parameters at dam of Bouhanifia (A: upstream of El-hammam) and at downstream of El-Hammam (B: upstream of Fergoug's dam). O_2 sat = saturation of oxygen; O_2 dis = dissolved oxygen; Cond = conductivity; BOD = Biological oxygen demand after 5days. Source: Nationa Agency for Water Resources, Algeria (ANRH, 2010).*

		April		May		June		July		August	
Parameters	**Stations** **Unit**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
T	C°	17	18	20	20	25	-	28	25	30	28
pH		8.1	7.9	7.9	7.7	8.2	-	7.9	7.8	8.1	7.8
Cond	µS cm-1	1270	3460	1330	1392	1204	-	1249	1281	1330	1363
O_2 sat.	%	82.2	111.	82.6	69	128.4	-	69.7	77.7	87.7	80
O_2 dis.	mg l-1	*7.7*	*0.2*	7.3	6.1	10.4	-	5.4	6.3	6.6	6.2
Cl	mg l-1	256	834	237	244	246	-	243	250	268	268
SO_4	mg l-1	208	798	241	245	184	-	208	203	227	227
SiO_2	mg l-1	2.3	2.5	2.2	2.1	4.5	-	3.1	3.4	3.9	4.2
BOD	mg l-1	8.7	13.5	9.7	11.9	7.1	-	8.7	13.3	10.3	13.5
NO_3	mg l-1	3	2	5	6	3	-	5	3	2	1
NO_2	mg l-1	0.32	0.05	0.45	0.32	0.24	-	0.17	0.25	0.1	0.12
PO_4	mg l-1	0.04	0.06	0.03	0.18	0.08	-	0.08	0.23	0.07	0.1

Regarding the acidity levels at El-Hammam stream, the diatom communities observed consist generally of a mixture of alkaliphilous (53.3%) and circumneutral (46.3%) taxa. Field pH measurements ranged from 7.5 to 7.9, with water temperatures betweenf 18 and 28 °C during the study period. Acidophilous species were absent. Most taxa observed by Chaïb and Tison-Rosebery (2012) at Kebir-East stream were also alkaliphilous (70 %), with pH ranging from 6.5 to 8.3 and scarce some acidophilous species. Baudrimont (1974) noted that species typical from European acid waters can adapt to alkaline waters in Algeria.

According to the classification of Van Dam et al. (1994), the dominant taxa at stations of El-Hammam stream were generally from moderately oxygenated environments -such as *Nitzschia frustulum*- to poorly oxygenated ones like *Achnanthidium saprophilum* or *Nitzschia palea*. Poly-oxibiontic taxa represented by *Achnanthidium minutissimum* were only occasional with abundance (54%) at station H1 April and *Cyclotella ocellata* (24%) at H1 June. A similar variation was observed by Chaïb and Tison-Rosebery (2012) at Kebir-East stream, with *Achnanthidium minutissimum* reaching 75% of the whole diatom community during summer.

Biological Diatom index BDI (Lenoir and Coste, 1996) had been already tested in north-eastern Algeria at Kebir-East stream by Chaïb and Tison-Rosebery (2012). BDI scores in their study were not contrasted enough, and did not appear to be in correspondence with the water quality gradient, where high conductivities and associated species tended to confuse the biological quality status; of the highest BDI value (18/20) was obtained at Ain Assel in summer 2007 by these authors, where

Achanthidium minutissimum was dominant, and did not reflect the actual poor water quality of that station, with $[NH_4^+] = 1$ mg l^{-1} and $[PO_4^{3-}] = 0.5$ mg l^{-1} (Chaïb and Tison-Rosebery, 2012).

In our case, firstly we calculated Pearson's correlations between 17 diatom indices and IPO for Kebir-East wadi by using diatom counts and physicochemical results provided by the workcounts of diatom species and physicochemical variables of Chaïb and Tison-Rosebery (2012) from seven stations at summer *(Table 4)*. The highest correlation for Kebir-East wadi at summer is for TDI: Trophic Diatom Index (Kelly and Whitton, 1995) with r= 0.62 and p=0.14. As a result we calculated the Pearson's correlations between 16 diatom indices and TDI for El-hammam stream (*Table 5)* and the results give the highest correlation are respectively for TID: ROTT trophic index (Rott et al., 1999) with r=-0.95 and p=0.004, IDAP: Artois-Picardie Diatom Index (Prygiel et al., 1996) with r=-0.92, p=0.011 and GDI: Generic Diatom Index (Rumeau and Coste, 1988) with r=-0.90 and p=0.013. Being based on a low taxonomic resolution, GDI does not give accurate results; usually GDI exhibits significant correlations against PO_4^{3}-P and Cl^- concentrations (Blanco et al., 2007). In which the mean concentration of Cl^- and PO_4^- exceed 316 and 0.1 mg l^{-1} respectively at El-Hammam stream. Fawzi et al. (2001) tested some diatoms indices at Hassar stream (Morocco), concluding that IDAP (Prygiel et al., 1996) is more suitable for the assessment of water quality in this stream. It has a fairly significant correlation with the Organic Pollution Index (IPO) and seems to incorporate saltwater taxa.

Table 4. *Pearson correlation coefficients between diatom indices and organic pollution index (IPO) applied for Kebir-Est stream [results are calculated by using Data counts of diatom species and physicochemical variables was trailed from work of Chaib & Tison-Rosebery (2012) of seven sampling stations on the Kebir-East stream in summer period].* ***SLA:*** *Sládeček's Index;* ***DESCY****:Descy's Pollution Index;* ***IDSE:*** *Leclerq;* ***SHE****: Schiefele and Schreiner's index;* ***WAT:*** *Watanabe index****;*** ***TDI:*** *Trophic Diatom Index;* ***%PT:*** *Pourcentage of Pollution Tolerant Taxa****;*** ***GDI****: Generic Diatom Index;* ***CEC****: Commission of Economic al Community Index;* ***SPI****: Specific Pollution Sensitivity Index;* ***BDI****: Biological Diatom Index;* ***IDAP****: Artois-Picardie Diatom Index;* ***EPI-D:*** *Eutrophication/Pollution Index;* ***DI_CH:*** *Hurlimann Suisse;* ***IDP:*** *Pampean Diatom Index;* ***LOBO:*** *LOBO index;* ***SID****: Saprobic Index and* ***TID:*** *Rott Trophic Index.* ***IPO:*** *Organic Pollution index "Indice de Pollution Organique".*

Indices	SLA	DESCY	IDSE/5	SHE	WAT	TDI	GDI	CEE	SPI	BDI	IDAP	EPI-D	DI_CH	IDP	LOBO	SID	TID
IPO	-0.37	0.31	-0.26	-0.21	-0.18	**0.62***	-0.12	-0.14	-0.15	-0.21	-0.14	-0.51	-0.28	-0.3	-0.54	-0.46	-0.57

Table 5. *Pearson correlation coefficients between diatom indices and Trophic Diatom Index (TID) applied for El-Hammam stream.*

Indices	SLA	DESCY	IDSE/5	SHE	WAT	GDI	CEE	SPI	BDI	IDAP	EPI-D	DI_CH	IDP	LOBO	SID	TID
Trophic Diatom Index (TDI)	-0.58	-0.44	-0.66	-0.55	-0.06	-0.9	-0.8	-0.87	-0.87	-0.92	-0.69	0.03	-0.68	-0.33	-0.24	**-0.95***

Tested indices at El-hammam stream *(Table 6)* show that water quality indicated by TDI and TID at station H1 in April reflect a water quality with a little degree of eutrophication, being eutrophic in June and August with some evidence of organic pollution (PT%= 21-40%). While station H2 in April and June show a high degree of eutrophication with organic pollution likely to contribute significantly to eutrophication of site (PT%=41-60), being fairly eutophic at August with free of significant organic pollution (PT%= <20). The station H2 was frequently influenced by fluctuating urban wastewater discharges from Bouhanifia while station H1 near to downstream of the dam of Bouhanifia (2.2 Km) was less influenced by urban discharges.

Table 6. *Values of diatom indices applied for stations at El-Hammam stream.*

Stations / **Indices**	**Avril**		**Juin**		**Aout**	
	H1	**H2**	**H1**	**H2**	**H1**	**H2**
SLA	14.3	9.3	10.9	9	9.2	9.4
DESCY	14.8	10.3	9.9	6.3	8.4	7.8
IDSE/5	4.24	2.83	3.16	2.72	2.88	2.96
SHE	13.4	6.7	9.2	5.4	8.5	6.6
WAT	14.7	10.2	9.5	7.6	7.8	4.2
TDI	36	85	71.8	82.5	68.8	35.4
%PT	26.4	42.2	38.9	57.4	40.4	11.4
GDI	12.7	9.3	8.5	5.9	9	14.6
CEE	13.9	4	6.7	2.7	6.3	7.1
SPI	13.9	7.5	7	4	6.8	10.5
BDI	14.2	10.1	10.3	7.6	10	12.1
IDAP	12.9	5.1	9.2	6.4	8.9	10.7
EPI-D	15.4	7.5	11.7	8	8.6	9.8
DI_CH	10.2	7.3	10	5.2	5.8	3.2
IDP	13.8	7.7	8.8	7.8	8.2	8.5
LOBO	19.7	14.5	17.2	19.3	14.7	16.5
SID	11.7	9.4	10.2	9.3	10.1	8.5
TID	7.8	4	5.9	4.8	5.2	7.1

Sites C1 and C2 at the estuary of wadi Cheliff river had in general the same taxa composition during April, June and August, including *Berkeleya* sp. 1, *Berkeleya* sp. 2, *Amphora* sp. *Navicula phyllepta*, *Achnanthidium minutissimum* var. *gracillimum* and *Nitzschia incerta.* These sites there are not affected by effluent discharges or wastewater inflows between sites C1 and C2. This zone being characterized by a high input of inorganic nitrogen and phosphorus (Kies, 2009).

Correspondence analysis (CA) applied to diatom abundance data in relation with the different sampled stations is represented on a factorial symmetrical plot of two axes *(Fig. 2 and 3)* with the maximum of inertia (57.91%) for El-Hammam stream analysis and 84.05% for estuary Cheliff river ($p < 0.001$). CA applied for species densities in El-Hammam stream (F1= 33.78%, F2 = 24.04%) show that the x-axis separated the stations H2 April and H2 August from the others *(Fig. 2)*. Conversely, the y-axis distinguishes stations H2 April, H1 June and H2 June from H1 April, H1 August and H2 August. Site H2 at El-hammam stream was characterized by taxa singular taxonomic composition in April, June and August, this maybe due to urban wastewater discharge and a unstable flow regime caused by water discharges from the dam of Bouhanifia followed by dry summer periods. All these factors were unstable during the study period, but site H1, near to the downstream of dam of Bouhanifia and not influenced by signficant urban waswater discharges, remained more stable, with similar diatom communities along the study period.

At the the estuary of Cheliff river, CA (F1=73.44%, F2 = 10.62%) shows that first two axes discriminate stations C1 and C2 monthly *(Fig. 3)*. The stations C1 and C2 June, and C1 and C2 August, appear very close, while C1 and C2 April are more distant, this can be related to the fact that diatom community in April C1, near to sea water, was more influenced by sea intrusions during this month.

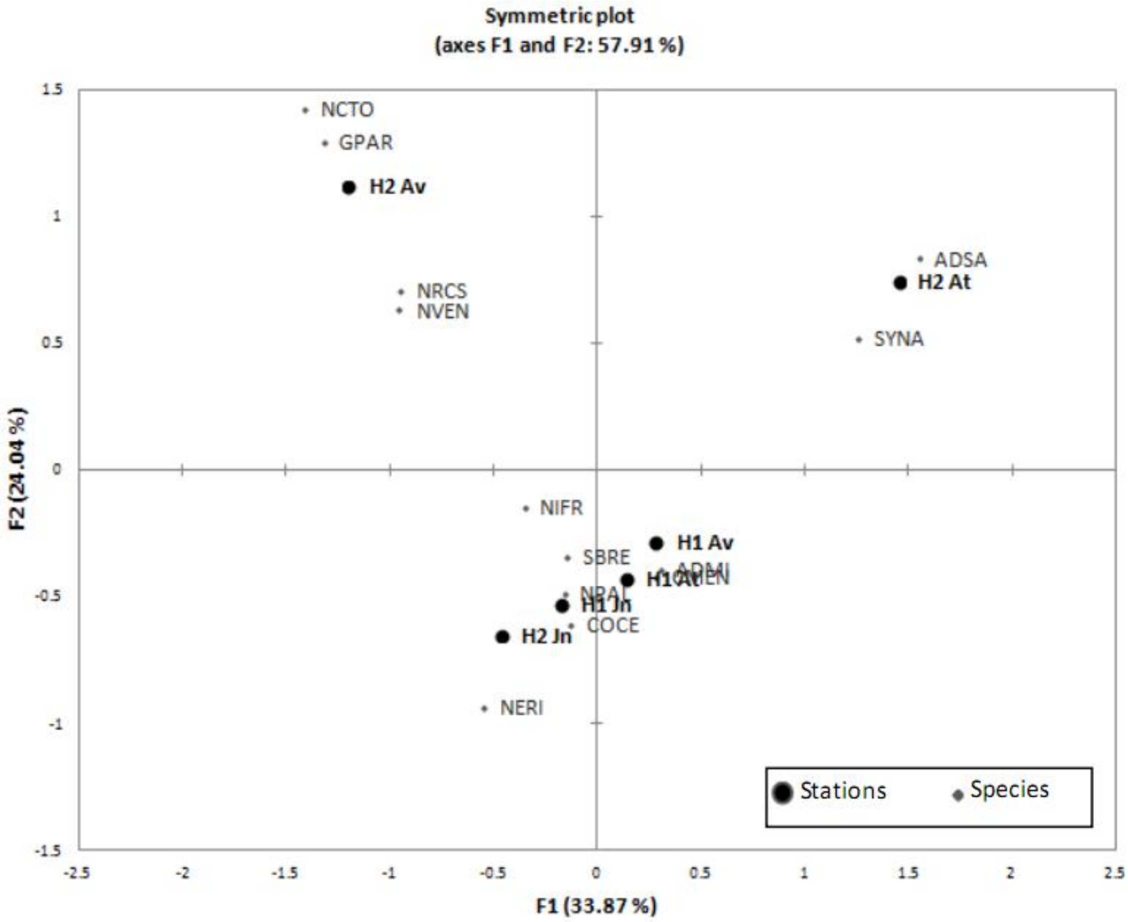

Figure 2*. Factorial plot of the Classification Analysis (CA) applied to the distributions of 13 species densities (abundance >5%) on sampled sites of El-Hammam stream H1 and H2: April (**H1 av**; **H2 av**), June (**H1 Jn**; **H2 Jn**), and H1 August (**H1 At**; **H2 At**). Each species was coded by four letters of scientific name of the species **ADMI:** Achnanthidium minutissimum. **ADSA:** Achnanthidium saprophilum. **CMEN:** Cyclotella meneghiniana. **COCE:** Cyclotella ocellata. **SYNA:** Fragilaria nana. **GPAR:** Gomphonema parvulum. **NCTO:** Navicula cryptotenelloides. **NERI:** Navicula erifuga. **NRCS:** Navicula recens. **NVEN:** Navicula veneta. **NIFR:** Nitzschia frustulum. **NPAL:** Nitzschia palea. **SBRE:** Surirella brebissonii. **AMGR:** Achnanthes minutissima var gracillima. **ACOF:** Amphora coffeaeformis. **A.sp:** Amphora sp, **B.sp1:** Berkeleya sp. 1 (rutilans?). **B.sp2:** Berkeleya sp. 2 (scopulorum?). **NPHY:** Navicula phyllepta. **NZCL:** Cylindrotheca closterium. **NREV:** Nitzschia incerta.*

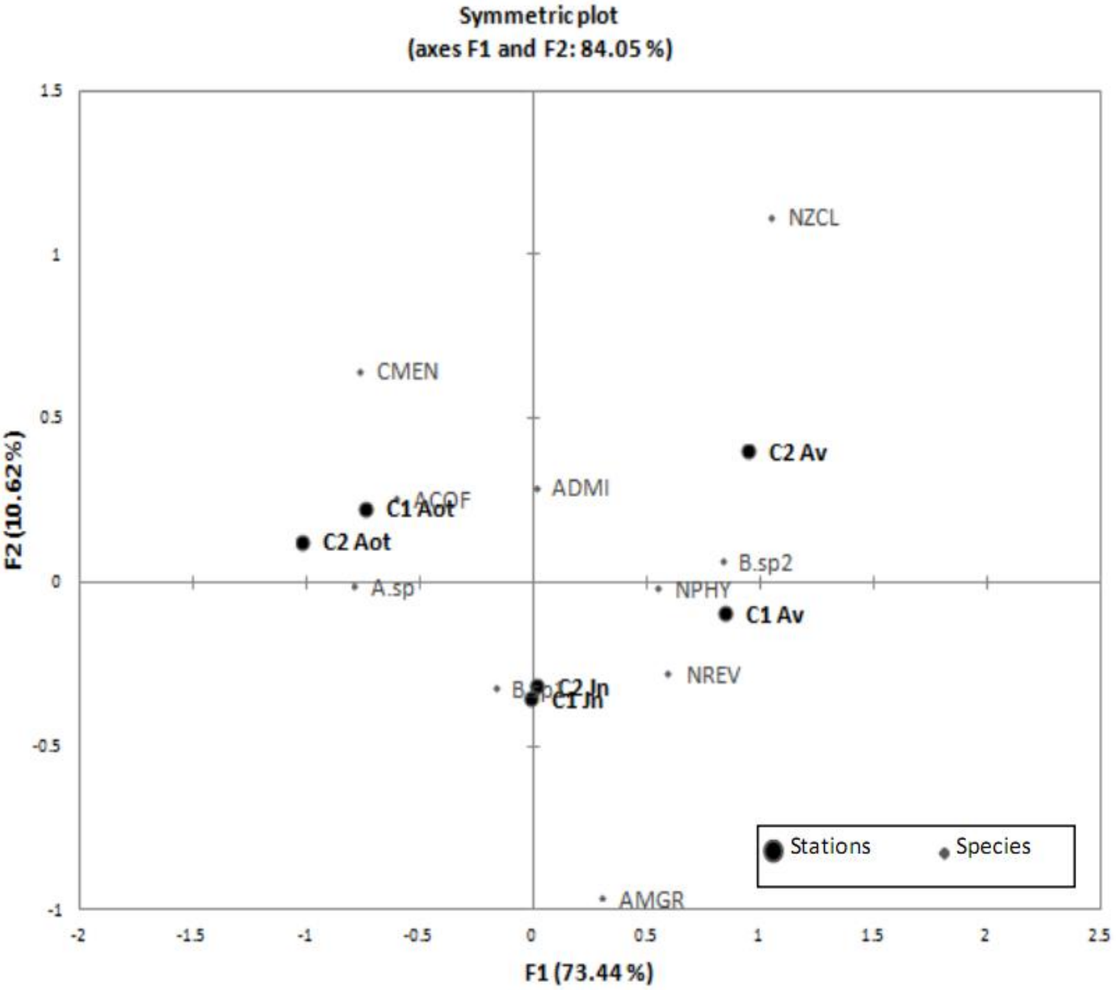

Figure 3. *Factorial plot of the Classification Analysis (CA) applied to the distributions of 10 taxa densities (abundance >5%) on sampled sites of estuary of Cheliff river C1 and C2: April (**C1 av**; **C2 av**), June (**C1 Jn**; **C2 Jn**), and C1 August (**C1 At**; **C2 At**). Each species was coded by four letters of scientific name of the species (Fig. 2).*

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EFFECTS OF RIPARIAN VEGETATION INTEGRITY ON FISH AND HETEROPTERA COMMUNITIES

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Abstract. Freshwater ecosystems are essential to human societies. However, many anthropogenic activities have been constantly modifying these ecosystems. Such modifications can be detected and measured with the use of environmental bioindicators. This work aims to identify the effect of physical integrity of streams, riparian vegetation coverage and physical and physicochemical variables on fish and Heteroptera faunas of streams, identifying which group is a better indicator of environmental disturbance. Predictor variables were sorted into three matrices: (1) physical and physicochemical parameters of the streams, (2) environmental variables and (3) structural variables of riparian vegetation. We tested the effects of physical and physicochemical descriptors on species richness and abundance through multiple linear regressions. The effects of environmental and structural variables of the riparian vegetation were tested using ridge regressions. Physical and physicochemical variables had no effect on Heteroptera and fish communities. Environmental variables showed effect only on the community of Heteroptera. Ichthyic assemblages were not influenced by any environmental variables. Selected structural variables had no relation with the Heteroptera assemblages, neither with fish community. Therefore, we conclude that, in small Amazonian streams, Heteroptera is more sensitive to human impacts than fish. This sensibility is demonstrated by the negative relation between Heteroptera fauna and the canopy opening over the river and positively related to environment integrity. These associations were not found for icthyofauna.

Keywords: *fish community; aquatic insects; habitat integrity; Normalized Difference Vegetation Index (NDVI); Enhanced Vegetation Index (EVI); bioindicator*

Introduction

Freshwater ecosystems are highly important to human societies, especially in respect tosupply of water to animal watering, fishing, agricultural and industrial production. Despite this recognition, various human activities have been leading to constant changes in aquatic environments (Greenwood et al., 2004; Holland-Clift et al., 2011; Richter, 2003), reducing the ecological quality of aquatic ecosystems (for example, by increasing water temperature). Consequently, ecosystem processes are also negatively affected by anthropogenic activities (e.g., water purification). Subsequently, the

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availability of social and economic usage of this resource is decreased. Therefore, a major goal of the management of water resources is to restore or maintain the ecological integrity of these ecosystems (Karr, 1993).

Rivers and streams can be described as continuous longitudinal gradients of physical and physical-chemical conditions, where the biota is directly related to this dynamic (Vannote et al., 1980). The degree of dependence on vegetation is inversely proportional to volume of water (Minshall et al., 1983). Montgomery (1999) suggests that in addition to environmental and physical variables, geomorphological processes represented by the topography, climate and geology, may also determine the quality, the availability and the distribution of habitats - and consequently, wildlife. Thus, physical and chemical monitoring of water is not sufficient to detect changes in habitat and microhabitat availability in aquatic ecosystems (e.g., De Marco et al., 2005). As a result, measuring environmental changes in physical, chemical and physicochemical parameters may not capture variations in the community structures of aquatic organisms (e.g., Callisto et al., 2005; Murray and Innes, 2009; Warren et al., 2010).

By contrast, disturbances or changes in aquatic ecosystems can be detected and measured with the assistance of environmental bioindicators (Oertli, 2008). Since biological communities are determined by local environmental conditions, they are able to reflect the ecological integrity of ecosystems (e.g., physical and chemical integrity and changes in the availability of food resources and substrates) in a more accurate way (e.g., Barbour et al., 1999). Since biomonitoring consists of the systematic use of biological responses to assess environmental changes, it can be considered as a way to assess the "health" of ecosystems (Buss, 1986).

The selection of adequate taxa to be used as bioindicators relies on a variety of factors, such as: (i) type of environment to be assessed, (ii) local community, (iii) costs of collection and identification and, especially, (iv) the community response to impacts on the environment. Since aquatic invertebrates and fish present high biodiversity and occur in almost all aquatic systems, having tolerant and susceptible species to different types of impacts (Callisto, 2001; Callisto et al., 2001; Resh et al., 1995; Rosenberg and Resh, 1993), they can be considered good bioindicators.

This study aims to identify the relationship between environmental (physical integrity of streams and riparian vegetation coverage), physical and physicochemical variables on the icthyofauna and on Heteroptera fauna of streams. We predict that Heteroptera fauna will respond to environmental variations whereas fish fauna will respond to physical and physicochemical variables.

Methods

This study was conducted in 21 streams of the headwaters of the Xingu River basin, in the municipalities of Canarana, Água Boa and Querência, state of Mato Grosso, Brazil, (*Fig. 1, Table 1*). These municipalities are situated in the Amazonian Deforestation Arc and have been submitted to high deforestation rates in the last decades. Samples were collected in a single step, between June and July 2011. In each stream, a stretch of 100 m was demarked and then divided into 20 segments of five meters. Fish were collected using the trawl method. In each segment, a trawl net was dragged once towards the longitudinal direction of the stream. Once collected, fish were fixed in formaldehyde 10% solution. In laboratory, fish were washed to remove any excess of formaldehyde, and then identified. Identification and systematic sorting followed current literature (e.g. Buckup, 1993; Vari and Williams, 1987; Vari, 1991, 1989) going until species level, whenever possible.

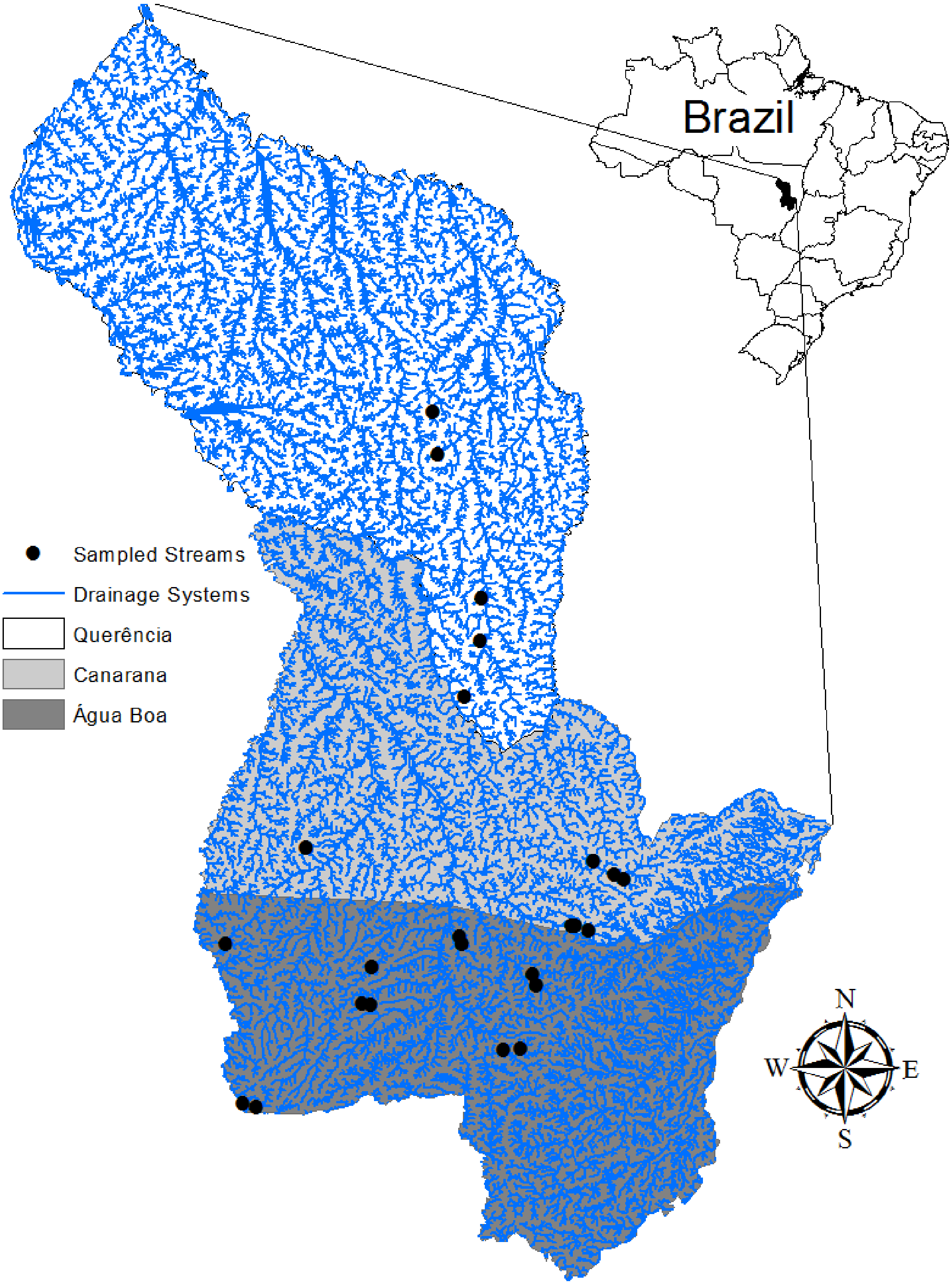

***Figure 1**. Location of the streams sampled (black circles) in the Xingu River basin, Central Brazil.*

Table 1. *Sampled streams at the Upper Xingu Basin, Mato Grosso, Brazil.*

Acronym	**Municipality**	**Use of Soil**	**Date**	**Coordinates**	
				Lat	**Long**
A1	Canarana	Pasture/Cerrado	04/jul/11	-13.55	-52.03
A3	Canarana	Pasture/Cerrado	09/jul/11	-13.59	-51.97
A4	Canarana	Pasture /Cerrado	09/jul/11	-13.6	-51.94
B1	Canarana	Sugarcane/Cerrado	08/jul/11	-13.73	-52.08
B2	Canarana	Pasture	08/jul/11	-13.73	-52.09
C1	Canarana	Recovery Area	17/jul/11	-13.51	-52.81
D1	Água Boa	Pasture	17/jul/11	-13.78	-53.03
E1	Água Boa	Pasture	13/jul/11	-13.94	-52.66
E2	Água Boa	Pasture	13/jul/11	-13.94	-52.63
F2	Água Boa	Pasture	14/jul/11	-13.84	-52.63
G1	Água Boa	Pasture	12/jul/11	-13.77	-52.38
G2	Água Boa	Pasture	12/jul/11	-13.75	-52.39
H1	Água Boa	Pasture	11/jul/11	-13.86	-52.19
H2	Água Boa	Pasture	11/jul/11	-13.89	-52.18
I1	Água Boa	Pasture /Cerrado	15/jul/11	-14.21	-52.98
I2	Água Boa	Cerrado	15/jul/11	-14.22	-52.94
K1	Querência	Soy	06/jul/11	-12.45	-52.45
K2	Querência	Forest	06/jul/11	-12.33	-52.47
L1	Querência	Forest	07/jul/11	-13.1	-52.38
L2	Querência	Forest	05/jul/11	-12.84	-52.33
L4	Querência	Soy	05/jul/11	-12.95	-52.34

Meanwhile, the semi-aquatic Heteroptera (Gerromorpha) were collected with the assistance of a strainer. In each of the 20 segments of five meters, we sieved the water three times (left, right and center segments), in downstream-upstream direction(Cabette et al., 2010). Individuals collected in each segment were separated and identified by segment and stream. Collected material was sorted and stored in commercial ethanol (concentration of 85%) and identified until genus level with the aid of the taxonomic keys of Nieser and Melo (1997) and Pereira and Melo (2007). All specimens of fish and Heteroptera are deposited in the Zoological Collection of the Federal University of Goiás (ZUFG).

Ecological integrity of streams was measured by species richness and abundance of individuals. Species richness of fish was estimated by Jackknife method (Heltshe and Forrester, 1983), using the software EstimateS version 8.0 (Colwell, 2005). The segments of each stream were classified as pseudo-samples and 1000 iterations were performed. For Heteroptera, we considered the observed richness of genera. Predictor variables were divided into three matrices: (i) physical and physicochemical parameters of the streams, (ii) environmental variables and (iii) structural variables of riparian vegetation.

Physicochemical and physical variables of the streams used in this study were: width, depth, slope, water temperature, dissolved oxygen, conductivity and pH. Width was measured every 20m. Depth was also measured three times per stream, distant 20 meters from each other. Slope was measured with the assistance of two rulers and a transparent tube (10m length) containing water inside. We estimated slope by submerging the whole tube and positioning the tips above the water layer, and

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calculating the difference between both measures of the air bubble height in each side of the tube. This procedure was repeated five times in each stream (i.e., a measurement was taken every 10 m). We used a multi-parameter probe YSI Professional Plus to measure water temperature, dissolved oxygen, conductivity and pH.

The environmental variables we have used were: (i) Index of Habitat Integrity (IHI; Nessimian et al., 2008), (ii) incident luminosity and (iii) canopy openness over the stream. They were extracted from 30 pictures in each stream (15 on each side of the stream) that were obtained parallel to the ground, at a height of 30 cm from the water blade immediately above from the stream margin. Structural variables were determined by two riparian vegetation indices: (i) Normalized Difference Vegetation Index (NDVI) and (ii) Enhanced Vegetation Index (EVI).

Vegetation indices (VI) employed here were obtained from Landsat TM images (May 2010, orbits 222 and 223, points 69 and 70) that present a spatial resolution of 30 x 30 m and have seven spectral bands. Chosen images minimized the amount of clouds that could generate noise in the data. We made a color composite based in the bands TM5, TM3 and TM4 of the images. We used the cubic convolution resampling of pixels of each scene, based on the georeferenced mosaic Geocover NASA. Then, we calculated two VI of the images: (i) Normalized Difference Vegetation Index (NDVI), (Rouse et al., 1974) and (ii) Enhanced Vegetation Index (EVI) (Huete et al., 1997). Each VI measure was taken the creation of nine buffers (30, 90, 150, 210, 270, 330, 390, 450 e 510 meters) around each sampled point and the obtention of the average (representing the density of vegetation coverage) and standard deviation (representing the heterogeneity of vegetation cover) of both VI.

First, we tested the effect of physical and physicochemical descriptors (pH, water temperature, dissolved oxygen, conductivity, slope, width and depth) on the descriptors of the assemblages (i.e., species richness and abundance) through multiple linear regressions. Prior to these analyses, we used Pearson correlations to exclude correlated descriptors (i.e., descriptors that presented a correlation equal to or greater than 0.75 were removed from the analyses).

The effect of environmental variables (canopy openness and integrity of habitat) on the descriptors of the assemblages (richness and abundance) was tested using ridge regressions. This approach was adopted due to collinearity found between the predictor variables (HIH, mean canopy openness (M), standard deviation of canopy openness (SD) and median canopy openness (MD).

We also used ridge regressions to teste the effect of structural variables of the riparian vegetation (complexity and heterogeneity of riparian vegetation) on the descriptors of the assemblages (species richness and abundance). Regressions were performed between complexity of riparian vegetation, NDVI and EVI, and the descriptors of biotic assemblages (species richness and abundance). Additionally, we tested the effect of heterogeneity of riparian vegetation, NDVI and EVI, and the descriptors of biotic assemblages, also using ridge regression. Adopted lambda for ridge regressions was of 0.01.

Results

We collected and identified 4,961 individuals of Heteroptera that were distributed into 4 families and 15 genera (*Table 2*). The most abundant genera were *Rhagovelia* (2,063), *Stridulivelia* (1,212), *Neogerris* (524), Brachymetra (443) and *Cylindrostethus*

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(419), representing, respectively, 42%, 24%, 11%, 9% and 8% of the total number of collected individuals. We obtained 1,333 fishes belonging to 37 species, divided between the orders Characiformes, Siluriformes, Gymnotiformes and Perciformes, 75%, 18%, 5%, 2% respectively, of the total number of collected individuals (*Table 3*).

***Table 2**. Genera of Heteroptera (Gerromorpha) collected in the Upper Xingu, Mato Grosso, Brazil. Genera are sorted by Family. The values represent the abundance of individuals of each genus.*

Family	Genus	A01	A03	A04	B01	B02	C01	D01	E01	E02	F02	G01	G02	H01	H02	I01	I02	K01	K02	L01	L02	L04	Total
Gerridae	*Brachymetra*	1	2	3	2	29	2	17	5	12	1	3	5	2	8		1	2	12	66	167	5	443
	Cylindrostethus	12	79	115	39	46		4	1	19	2	6	3	7	12			1		15	8	9	419
	Limnogonus		4	7	5	6		2	2	2			2	7	6	3		1			1		52
	Neogerris	53	74	97	59	43		2	17	18	1		1	51	38		1	8	2	4	1		524
	Ovatametra													1	3								4
	Rheumatobates	4						2	67				1			17	29				1		128
	Tachygerris	3	5		4	3							1					2	2	1	6		28
	Trepobates								2						1	2							14
Hydrometridae	*Hydrometra*	1	1						3										4				9
Mesoveliidae	*Mesovelia*								1						1					1	1	1	5
Veliidae	*Microvelia*	5							36		1				3				6	1			54
	Paravelia																		2				3
	Platyvelia																		2	1			3
	Rhagovelia	155	38	33	124	87	29	116	45	27	57	14	4	196	258	6	39	143	58	128	145	45	263
	Stridulivelia	8	54	364	312	159			8			56	11		7			59	28	16	2	77	1212
Total		242	257	619	545	373	31	143	187	78	62	79	28	264	337	28	70	216	116	233	332	137	3161

***Table 3**. Fish species collected in the Upper Xingu, Mato Grossom Brazil. Species are sorted by Order and by Family. The values represent the abundance of individuals of each species*

Order	Family	Species	A1	A3	A4	B1	B2	C1	D1	E1	E2	F2	G1	G2	H1	H2	I1	I2	K1	K2	L1	L2	L4	Total
Characiformes	Curimatidae	*Steindachnerina conspersa*	1														4							5
	Acestrorhinchidae	*Acestrorhincus falcatus*						1																1
	Anostomidae	*Leporinus megalepis*							8							1								9
	Characidae	*Astyanacinus cf. guianensis*														16								16
		Astyanax dnophos											9		2									11
		Bryconops alburnoids						21	7			22	1		6	1	20				1		4	83
		Gymnocorymbus cf. thayeri			1									1										2
		knodus victoriae				14	4	31	8	2	19	6		79		261	6		56		17	19	5	527
		Moenkhausia oligoleps				1				1	4		1	1										8
		Moenkhausia xinguensis	11	37	13		1	28	16	5		7	3		24	14	10	9	3		1	16		198
		Pseudochalceus sp			9											21								30
		Tetragonopterus chalceus															1							1
	Crenuchidae	*Characidium zebra*			3				4						1	12		1	7		1			29
	Erythrinidae	*Erythrinus erythrinus*			3	5	14	3					1	4							1			31
		Hoplerythrinus unitaeniatus			1												1							2
		Hoplias malabaricus			4	1														1	1	2		9
	Hemiodontidae	*Hemiodus cf. gracilis*															5							5
	Serrasalminae	*Myleus setiger*																					1	1
Gymnotiformes	Gymnotidae	*Gymnotus* sp1																	1	18		1		20
		Gymnotus sp2																		5	1			6
	Rhamphichthyidae	*Gymnorhamphichthys rosamariae*											2	4					7		22	8		43
	Sternopygidae	*Eigenmannia trilineata*												1						2				3
Perciformes	Cichlidae	*Aequidens pallidus*			6			1		1	2		2	3		2			1	2	1	7	1	29
		Crenicichla rosemariae	1			1								1	2		1		1				2	9
Siluriformes	Aspredinidae	*Bunocefalus coracoideus*													1									1
	Callichthyidae	*Aspidoras* sp	6	17	69	11	4		2			3				4								116
		Callichthys callichthys																		1				1
	Heptapteridae	*Goeldiella eques*		2		1	1								3		1		2	2	1	1		14
		Imparfinis sp		1						1		1	13	2		1			1		3	3		26
		Imparfinis sp2											1											1
		Imparfinis sp3		3	4		1							2					2					12
		Pimelodella gracilis			2		2						7		5	2					1			19
		Rhamdiopsis sp					3												7	6	10	6		32
	Loricariidae	*Hemiancistrus* sp										1			1									2
		Hypostomus sp1			1													2						3
		Otocinclus vitatus			4	2		1	1			1	2						3				1	15
	Trichomycteridae	*Trichomycterus* sp	1			10	1				1													13
Total			20	60	120	46	31	86	46	10	26	41	42	98	45	335	49	12	91	37	61	63	14	1333

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The most dominant species was *Knodus victoriae* that counted with 537 individuals collected, followed by *Moenkhausia xinguensis* and *Aspidoras* sp., with 243 and 116 specimens respectively.

Due to high correlation with other variables (equal or higher than 0.75) used in the regression models (*Table 4*), parameters of dissolved oxygen percentage, electric conductivity, slope and depth were not considered in regressions performed with physical and physicochemical variables. Physical and physicochemical variables had no effect on the communities of Heteroptera and fish (*Table 5*).

Table 4*. Pearson correlation between the physical and physicochemical data collected in 21 streams in the basin of the Upper Xingu, Mato Grosso, Brazil. Acronyms: pH - hydrogenionic potential; Water Temp. - water temperature; DO - percentage of dissolved oxygen; DOm - dissolved oxygen in milligrams per liter of water; SPC - specific conductivity; C – conductivity; Slope A - average slope of the stream; Slope SD - standard deviation of the slope of the stream; Width A - average width of the stream; Width SD - standard deviation of the stream channel width; Depth A - average depth of the stream, and; Depth SD - standard deviation of the stream depth.*

	pH	Water temp.	DO	DOm	SPC	C	Slope A	Slope SD	Width A	Width SD	Depth A	Depth SD
pH	**1.00**											
Water temp.	-0.66	**1.00**										
DO	0.19	-0.28	**1.00**									
Dom	0.34	-0.49	**0.97**	**1.00**								
SPC	0.51	-0.24	-0.45	-0.34	**1.00**							
C	0.49	-0.20	-0.47	-0.37	**1.00**	**1.00**						
Slope A	0.10	-0.01	0.28	0.26	-0.49	-0.49	**1.00**					
Slope SD	0.22	-0.23	0.24	0.28	-0.25	-0.27	**0.83**	**1.00**				
Width A	0.42	-0.31	0.14	0.21	0.47	0.46	-0.01	0.28	**1.00**			
Width SD	0.13	-0.24	0.08	0.13	0.31	0.30	0.01	0.28	0.62	**1.00**		
Depth A	-0.19	0.19	0.09	0.04	-0.08	-0.07	0.10	0.16	0.32	0.34	**1.00**	
Depth SD	-0.09	0.07	0.25	0.22	-0.04	-0.03	0.05	0.21	0.39	0.47	**0.92**	**1.00**

Table 5*. Results of multiple regressions between the physical and physicochemical variables of the streams and the descriptors of the assemblages.*

	Fish				Heteroptera			
	Abundance		**Estimated Richness**		**Abundance**		**Observed Richness**	
	B	p	B	p	B	p	B	p
pH	-0.14	0.81	0.48	0.37	1.03	0.03	0.74	0.09
Water temperature	0.42	0.31	0.52	0.19	-0.03	0.91	-0.47	0.13
Dissolved Oxygen	0.43	0.35	0.03	0.95	-0.4	0.25	-0.82	0.02
Electrical Conductivity	0.15	0.82	-0.66	0.29	-1.28	0.02	-1.31	0.01
Slope	0.11	0.77	-0.32	0.39	-0.62	0.05	-0.51	0.08
Width	0.18	0.66	-0.2	0.6	0.05	0.88	0.35	0.25
Depth	0.04	0.90	0.05	0.86	-0.12	0.61	-0.05	0.83
r^2	0.21		0.31		0.55		0.59	
P	0.90		0.70		0.17		0.11	

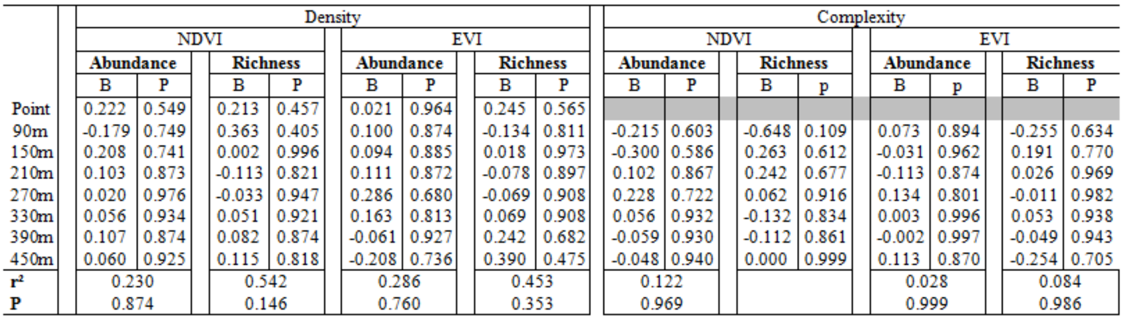
Environmental variables had effect only on the community of Heteroptera (*Table 6*). There was a positive relationship with abundance for HIH (r^2=0.21; p=0.04), modified HIH (r^2=0.22; p=0.03) and canopy openness (r^2=0.21; p=0.03). HIH (r^2=0.22; p=0.03) and canopy openness (r^2=0.21; p=0.03) also were positively related to genera richness of Heteroptera. Ichthyic assemblages were not affected by environmental variables.

Structural variables selected for analysis - riparian vegetation density (average NDVI and average EVI) and heterogeneity (standard deviation of NDVI and EVI) - did not correlate with Heteroptera (*Table 7*) or fish (*Table 8*) communities.

Table 6. *Regressions results between environmental variables and the descriptors of the assemblages; Mean – M, Standard Deviation – SD, Median MD.*

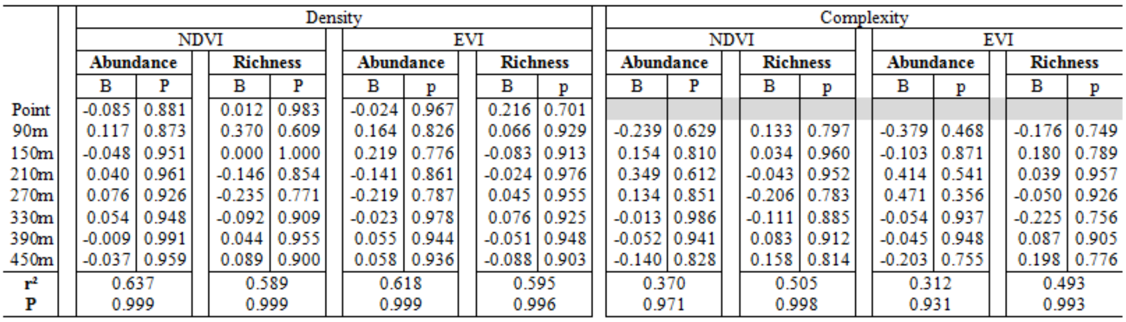
	Fish				Heteroptera			
	Abundance		**Richness**		**Abundance**		**Richness**	
	B	P	B	P	B	p	B	P
HIH	0.181	0.700	-0.077	0.880	0.21	**0.04**	0.22	**0.03**
Canopy openness (M)	-0.189	0.734	0.088	0.884	-0.21	**0.03**	-0.35	**0.01**
Canopy openness (SD)	0.257	0.431	0.051	0.884	-0.11	0.14	-0.11	0.14
Canopy openness (MD)	-0.084	0.876	-0.176	0.762	-0.24	**0.03**	-0.36	**0.00**
r²	0.088		0.437		0.426		0.367	
P	0.649		0.980		**0.047**		**0.029**	

Table 7. *Regressions results between density and vegetation complexity of Heteroptera assemblages; NDVI - Normalized Difference Vegetation Index; EVI - Enhanced Vegetation Index.*



	Density								Complexity							
	NDVI				EVI				NDVI				EVI			
	Abundance		**Richness**		**Abundance**		**Richness**		**Abundance**		**Richness**		**Abundance**		**Richness**	
	B	P	B	P	B	P	B	P	B	P	B	p	B	p	B	P
Point	0.222	0.549	0.213	0.457	0.021	0.964	0.245	0.565								
90m	-0.179	0.749	0.363	0.405	0.100	0.874	-0.134	0.811	-0.215	0.603	-0.648	0.109	0.073	0.894	-0.255	0.634
150m	0.208	0.741	0.002	0.996	0.094	0.885	0.018	0.973	-0.300	0.586	0.263	0.612	-0.031	0.962	0.191	0.770
210m	0.103	0.873	-0.113	0.821	0.111	0.872	-0.078	0.897	0.102	0.867	0.242	0.677	-0.113	0.874	0.026	0.969
270m	0.020	0.976	-0.033	0.947	0.286	0.680	-0.069	0.908	0.228	0.722	0.062	0.916	0.134	0.801	-0.011	0.982
330m	0.056	0.934	0.051	0.921	0.163	0.813	0.069	0.908	0.056	0.932	-0.132	0.834	0.003	0.996	0.053	0.938
390m	0.107	0.874	0.082	0.874	-0.061	0.927	0.242	0.682	-0.059	0.930	-0.112	0.861	-0.002	0.997	-0.049	0.943
450m	0.060	0.925	0.115	0.818	-0.208	0.736	0.390	0.475	-0.048	0.940	0.000	0.999	0.113	0.870	-0.254	0.705
r²	0.230		0.542		0.286		0.453		0.122				0.028		0.084	
P	0.874		0.146		0.760		0.353		0.969				0.999		0.986	

Table 8. *Regressions results between density and vegetation complexity of fish assemblages; NDVI - Normalized Difference Vegetation Index; EVI - Enhanced Vegetation Index.*



	Density								Complexity							
	NDVI				EVI				NDVI				EVI			
	Abundance		**Richness**		**Abundance**		**Richness**		**Abundance**		**Richness**		**Abundance**		**Richness**	
	B	P	B	P	B	p	B	p	B	P	B	p	B	p	B	p
Point	-0.085	0.881	0.012	0.983	-0.024	0.967	0.216	0.701								
90m	0.117	0.873	0.370	0.609	0.164	0.826	0.066	0.929	-0.239	0.629	0.133	0.797	-0.379	0.468	-0.176	0.749
150m	-0.048	0.951	0.000	1.000	0.219	0.776	-0.083	0.913	0.154	0.810	0.034	0.960	-0.103	0.871	0.180	0.789
210m	0.040	0.961	-0.146	0.854	-0.141	0.861	-0.024	0.976	0.349	0.612	-0.043	0.952	0.414	0.541	0.039	0.957
270m	0.076	0.926	-0.235	0.771	-0.219	0.787	0.045	0.955	0.134	0.851	-0.206	0.783	0.471	0.356	-0.050	0.926
330m	0.054	0.948	-0.092	0.909	-0.023	0.978	0.076	0.925	-0.013	0.986	-0.111	0.885	-0.054	0.937	-0.225	0.756
390m	-0.009	0.991	0.044	0.955	0.055	0.944	-0.051	0.948	-0.052	0.941	0.083	0.912	-0.045	0.948	0.087	0.905
450m	-0.037	0.959	0.089	0.900	0.058	0.936	-0.088	0.903	-0.140	0.828	0.158	0.814	-0.203	0.755	0.198	0.776
r²	0.637		0.589		0.618		0.595		0.370		0.505		0.312		0.493	
P	0.999		0.999		0.999		0.996		0.971		0.998		0.931		0.993	

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Discussion

There is a predominance of the genus *Rhagovelia* in the sampled Heteroptera communities. This pattern might be explained by the gregarious habits that individuals of the genus *Rhagovelia* present, agreeing with other studies performed at this region with the same group (Dias-Silva et al., 2010; Nieser and Melo, 1997). For fish populations, we observed the predominance of Characiformes and Siluriformes orders. This pattern is common for non-estuarine localities (Lowe-McConnell, 1987) and was already observed in other studies performed in streams (e.g., Araújo and Tejerina-Garro, 2007; Benedito-Cecilio et al., 2004; Melo et al., 2009; Pereira et al., 2008).

Generally, streams that exhibit dominance of benthic species (e.g., *Aspidoras* sp.) are classified as unsuitable places (Roth et al., 1996). However, many streams of the Amazon basin exhibit natural sandy substrate, which is a characteristic related to the geological formation of the basin. Therefore, the presence or abundance of species of the genus *Aspidoras* are not valid indicators of degradation in the sampled streams. This was confirmed when we found that streams that had the highest abundances of *Aspidoras* sp. (69 from a total of 116 collected specimens) presented integrity indices equal to 0.63, which considers that the habitat is submitted to intermediary levels of degradation.

We also observed high abundance of the omnivorous species *Knodus victoriae* and *Moenkhausia xinguensis*. These species are usually associated with integrate sites and low sedimentation (Casatti et al., 2008; Langeani, 2009; Oliveira and Bennemann, 2005). Since *Knodus victoriae* and *Moenkhausia xinguensis* are nektonic species, they need sites with relatively high water column height in order to acquire food in this portion (Langeani, 2009). This requirement causes these species to respond negatively to silted sites, reinforcing the existence of sites with no degradation or with an intermediate level of degradation.

In our study, we found no relationship between physicochemical/physical variables and descriptors of biotic assemblages. The intake of sediments prevenient from unstable soils that are next to the stream (Allan, 2004; Vondracek et al., 2005) can be considered one possible factor that influences the biota. We observed that the landscape matrix area of our study is predominantly composed of agriculture and grassland. This type of matrix is usually negatively correlated with the physical integrity and stability of the stream, which leads to the decrease of the richness and abundance of biotic groups (Pinto et al., 2006; Trimble, 1997).

Although only confirmed for Heteroptera, we conclude that the riparian coverage showed a negative effect on biota. The association found between canopy openness and Heteroptera indicates that Heteroptera is more sensitive to changes in vegetation than fish (which were not associated with canopy openness). Therefore, we conclude that, from the tested groups, only Heteroptera responds well to changes in landscape. This indicates that this group is a good indicator of preservation of vegetation coverage.

We observed that physical and physicochemical variables of the water did not show any effect on the Heteroptera community of this study, demonstrating either that the variation in these variables is not intense or that the group exhibits great plasticity to physicochemical variations. Thus, the group is affected only by environmental variables, such as shading, as described by Dias-Silva et al. (2010), in a study conducted in streams of eastern Mato Grosso.

Additionally, aquatic Heteroptera are recognized for being resistant to natural disturbances, such as flood pulses, inorganic sediment delivery to streams and changes in physical and physicochemical characteristics of the water (Gordon et al., 1992). This feature may imply that these communities are less effective bioindicators of water quality. However, changes of magnitude and frequency of occurrence of these disorders might be negatively correlated with the biota (Buss et al., 2003; Ferreira et al., 2006; Magner et al., 2004; Simon and Rinaldi, 2000). In this way, we can expect that Heteroptera is more sensitive to anthropogenic disturbances than fish.

The sensitivity of the Heteroptera fauna can also be justified by its the positive relationship with HIH and by the no relationship of this index with the icthyofauna. This index is easy to apply and summarizes a series of questions about the use of areas adjacent to the channel, the format and channel stability on availability, habitat complexity and others hydrological factors (even though these indices are measured visually).

The non-relation between fish fauna and metrics performed in the sites might still be a problem caused by the lack of reference streams. When we aim to relate biota to environmental disturbances, the choice of reference streams (i.e., sites that have stable and pristine characteristics of the channel; Harrelson et al., 1994) is of great importance (Barbour et al., 1999; Harrelson et al., 1994; Hughes, 1995). This argument reinforces the sensitivity feature of Heteroptera that, even without reference sites, was able to capture the variation in canopy openness and HIH through richness and abundance of genera.

Relationships between fish species and human disturbance are recurrent in literature (e.g., Casatti, 2005; Casatti et al., 2008; Langeani, 2009). Casatti et al. (2005) found that fish dependent on rocky substrates are more affected by habitat loss and deterioration. Additionally, richness and abundance of native species tend to be higher in reference sites than in impacted sites (Casatti et al., 2008b; Ferreira et al., 2006).

At last, we conclude that the fauna of Heteroptera is more sensitive to human impacts than icthyofauna. This sensitivity is demonstrated by the negative relationship of Heteroptera with the canopy openness over the stream and positively related to the integrity of the environment. These relationships were not found for the icthyofauna. This also characterizes Heteroptera as a good indicator of impacts on riparian vegetation. However, for monitoring purposes, we cannot simply discard fish as potential bioindicators, because the icthyofauna might be related to other variables associated to other human impacts (e.g., type of channel substrate or margin stability).

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MODELLING THE DISTRIBUTION OF WETLAND BIRDS ON THE RED SEA COAST IN THE KINGDOM OF SAUDI ARABIA

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Abstract. Species distribution modelling is useful for addressing knowledge gaps for poorly studied geographical areas by identifying potentially suitable habitats for species across landscapes. This study was undertaken to identify areas containing wetland habitats to support actions aimed at the conservation of wetland bird species on the Red Sea coast of the Kingdom of Saudi Arabia (KSA). We used a maximum entropy approach to build habitat suitability models for 22 wetland bird species (gulls, shorebirds, and terns). Ten variables relating to topography, habitat, latitude, coast complexity, man-made structures, and human settlements were used to produce individual habitat suitability models for each of the bird species. The areas under the curve (AUC) for the final model were 0.9013 and 0.879 for the training and testing data, respectively, and the jackknife analyses suggested that the models generally performed well. Using the modelled distributions of the 22 species along the Red Sea coast, the analysis suggested 17 core areas where the habitat and landscape configuration were suitable for supporting high species richness. Of these 17 sites, one is already protected, and there is a need to protect the remaining sites. The use of these models to inform conservation strategies in the Red Sea region of the KSA is discussed.

Keywords: *wetland birds, Red Sea coast, species distribution model, MaxEnt.*

Introduction

The Red Sea region has diverse coastal and marine habitats. These include mangroves, mudflats, marshes, sand dunes, sand plains, rocky shores, coastal reefs, and marine islands (PERSGA/GEF, 2003). The region is particularly important for corals, marine invertebrates, and turtles (AbuZinada et al., 2002).

However, the size of the region and limited opportunities for survey and census fieldwork, mean that the fauna of the Red Sea coast remains relatively poorly studied. Previous surveys have suggested that the region is important for a variety of bird taxa, including Palaearctic migrants and winter residents (PERSGA/GEF, 2004). On the Red

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Sea coast and the Gulf of Aden, 17 seabird species have been recorded as breeding, including endemic species such as White-eyed Gull (*Larus leucophthalmus*), a subspecies of the Red-billed Tropicbird (*Phaeton aethereus indicus*) and the Brown Noddy (*Anous stolidus plumbeigularus*). Furthermore, some species endemic to the northwest Indian Ocean and important sub-populations breed in the region such as Jouanin's Petrel (*Bulweria fallax*), Sooty Gull (*Larus hemprichii*), Swift Tern (*Sterna bergii velox*), and White-cheeked Tern (*Sterna repressa*) (PERSGA/GEF, 2004). This region includes Socotra Cormorant (*Phalacrocorax nigrogularis*) that is classified as 'threatened', with three further species classified as 'near threatened' on the IUCN Red List (IUCN, 2012): Jouanin's Petrel, Persian Shearwater (*Puffinus persicus*), and White-eyed Gull that are classified as being 'near threatened' on the IUCN Red List (PERSGA/GEF, 2004). Furthermore, AlRashidi et al. (2011) found that some sites of the Red Sea coast are highly suitable for breeding and wintering Kentish Plover (*Charadrius alexandrinus*).

Despite its relative isolation, the Red Sea coast is subject to growing human pressures. In particular there has been an issue with coastal development and pollution (PERSGA/GEF, 2004). Moreover, the taking of seabird eggs is a potential problem in the region and needs regulation and/or sustainable take agreements (Newton, 2006).

Within the Red Sea area, research and monitoring was one of seven priority 'enabling' actions identified in the 2004 PERSGA report (PERSGA/GEF, 2004). Understanding the complex ecological relationships between species distribution, environmental structure and human pressure is of high utility for the development of: (i) Integrated Coastal Zone Management (ICZM) frameworks, (ii) the identification of protected sites and networks, and (iii) effective evaluation of conservation activities. The present project is one of only a small number of research initiatives that have so far been undertaken in the region.

Despite the need for greater understanding species-habitat relationships, modelling wetland birds in the Red Sea region can be problematic because of the lack of range and gradients of the landscape's physical features AlRashidi et al. (2011).

Recent field monitoring efforts at key seabird sites and greater availability of high resolution data from the region now make it possible to develop species distribution models (SDMs). Recent testing of SDMs using biological data (including birds) has shown that robust biologically relevant models can be developed from the integration of 'presence only' observations of species occurrence with measurements of environmental characteristics. These models predict the actual or potential distribution of a species (Elith and Leathwick, 2009), and provide an understanding of the underlying species-habitat relationships (Guisan and Zimmermann, 2000; Franklin, 2009). SDMs have also been used for estimating the effects of climate change (Buckland et al., 1996; Austin et al., 1996; Thomas et al., 2004), estimating population size (Long et al., 2008), understanding the correlation between distribution and abundance (Milsom et al., 2000; Ledee et al., 2008), conservation planning and forecasting (Rushton et al., 2004; Phillips and Dudík, 2008), and species distribution monitoring (Rodriguez et al., 2007).

Species distribution models require detailed information about the environment of the study area, and species occurrence data. This can be either presence-only data or presence- absence data (Graham et al., 2008). In practice, 'true' absence data are rarely available, because they require a considerable amount of time and effort to collect and it can be difficult to ensure that there are no false-negatives in the data. The vast majority of datasets for species distribution modelling are therefore presence-only datasets (Phillips et al., 2006), and this is the case for the present study.

To address the current lack of understanding about factors driving the abundance and distribution of shorebirds along the Red Sea coast of the Kingdom of Saudi Arabia (KSA), we have utilised available data from an extensive coastal bird survey (AlRashidi et al*.,* 2011), to attempt the first species distribution modelling in the region. The core aim of this research was therefore to give guidance for the development of conservation strategies for the Red Sea coastal area of the KSA by providing a better understanding of the distribution of 22 wetland bird species along the Red Sea coast. Therefore, this will help guide future surveys and monitoring of wetland bird species in this region. We also targeted to quantify the relative importance of key sites by determining areas of high species richness.

Methods

Environmental variables for the model

The Red Sea coastline of the KSA extends 1,840 kilometres from the border with Jordan in the northern Gulf of Aqaba region (29° 30' N) to the border with Yemen in the southern Red Sea region (16° 22' N) (PERSGA/GEF, 2003). This coastline has an arid climate with temperatures reaching up to 50 °C in summer. The average rainfall is less than 70 mm per year (PERSGA/GEF, 2003).

We utilised the wetland bird species data collected by AlRashidi et al. (2011) between 2 July and 10 August 2008 (*Fig. 1*). The data were gathered from 98 randomly selected sites located within 1 km of the sea along the west coast of Saudi Arabia including the Farasan Islands. 35 bird species have been recorded along the Red Sea coast, and here we focus on 22 of these species, for which more than 14 records were available (*Table 1*).

Ten environmental variables were selected for the species distribution modelling: vegetation cover, soil moisture, distance to main cities (big cities), distance to cities (small cities), distance to roads, brightness, latitude, coast complexity, elevation, and slope. Information about vegetation cover, soil moisture, brightness, and elevation was derived from 21 Landsat 7 satellite images and rendered into GIS format (below) by online tools associated with the Global Land Cover Facility (AlRashidi et al., 2011).

The tasseled cap transformation was introduced by Kauth and Thomas (1976). It is an appropriate tool for improving spectral data and deriving important environmental information (Crist and Cicone, 1984). A tasselled cap transformation with coefficients for the Landsat ETM+ sensor was used (Huang et al., 1998) to produce three rasters: tasselled cap greenness shows the existence and density of green vegetation; tasselled cap moistness, which describes the amount of soil moisture; and tasseled cap brightness, shows variations in soil structures. Finally, all transformed images were rescaled such that pixels took digital number values from 0 to 255 (see AlRashidi et al., 2011; Long et al., 2008).

Elevation data were derived from the Shuttle Radar Topography Mission (SRTM). Tiles of SRTM data corresponding to the 21 WRS-2 scenes of Landsat data used were downloaded from the Global Landcover Facility (http://www.landcover.org). These were then mosaiced and clipped in the same way as the satellite images were. The resolution of this dataset was 90 m, but in order to overlay all layers of environmental data exactly, we resampled the SRTM to 30 m pixel size to produce the final elevation map (see AlRashidi et al., 2011; Long et al., 2008).

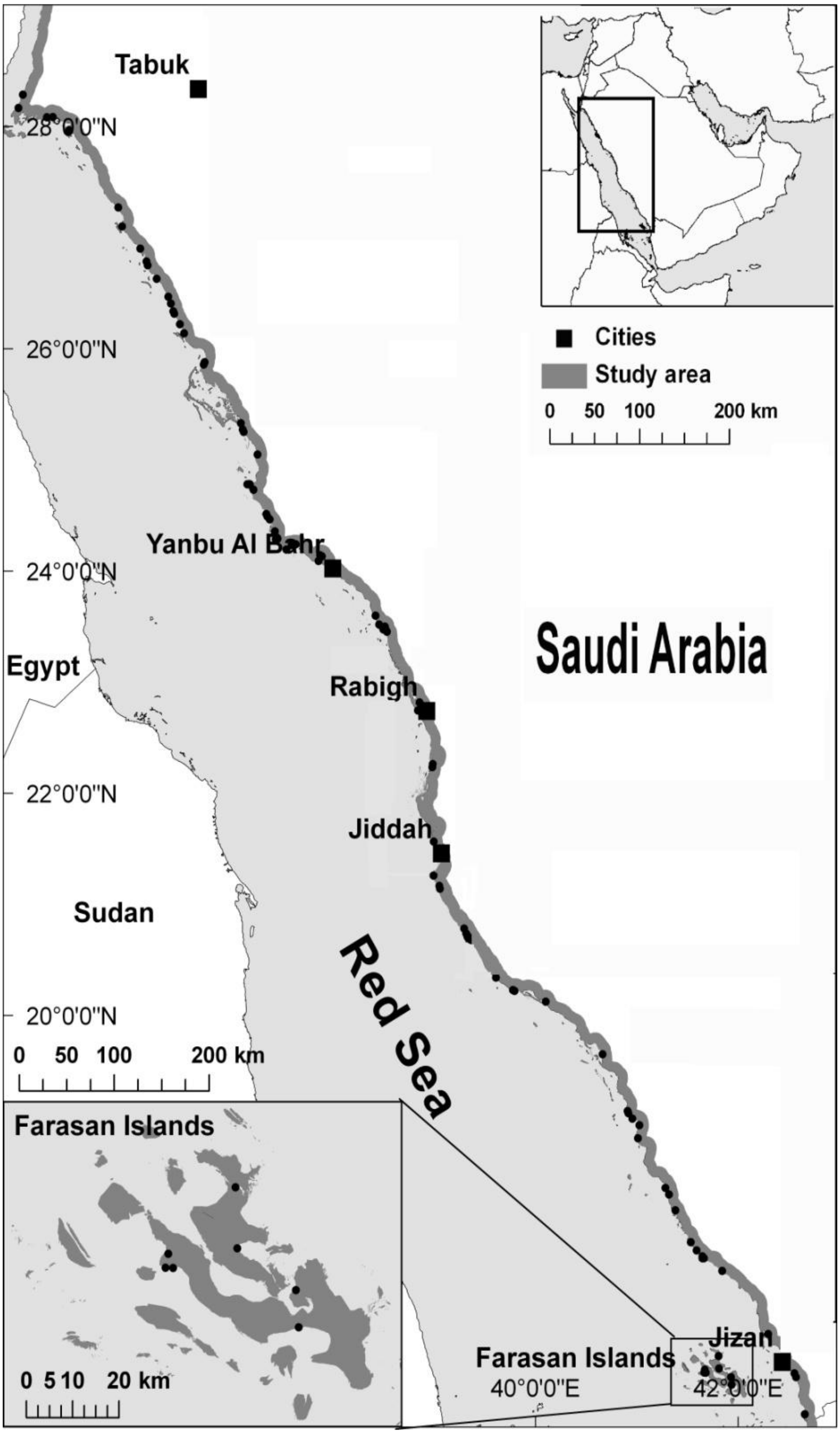

Figure 1. *Map showing the study area on the west coast of Saudi Arabia (shaded dark grey); black dots represent starting points of 98 randomly selected sites (AlRashidi et al., 2011)*

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Table 1. *The 22 wetland bird species included in distribution modelling*

No	Species	Scientific name	Threat status (IUCN 2012)	Population trend (IUCN 2012)
1	Eurasian Spoonbill	*Platalea leucorodia*	LC	unknown
2	Intermediate Egret	*Mesophoyx intermedia*	LC	decreasing
3	Western Reef Heron	*Egretta gularis*	LC	stable
4	Pink-backed Pelican	*Pelecanus rufescens*	LC	stable
5	Eurasian Oystercatcher	*Haematopus ostralegus*	LC	decreasing
6	Crab Plover	*Dromas ardeola*	LC	stable
7	Kentish Plover	*Charadrius alexandrinus*	LC	decreasing
8	Lesser Sand Plover	*Charadrius mongolus*	LC	unknown
9	Greater Sand Plover	*Charadrius leschenaultii*	LC	Unknown
10	Whimbrel	*Numenius phaeopus*	LC	decreasing
11	Eurasian Curlew	*Numenius arquata*	NT	decreasing
12	Redshank	*Tringa totanus*	LC	unknown
13	Marsh Sandpiper	*Tringa stagnatilis*	LC	decreasing
14	Terek Sandpiper	*Xenus cinereus*	LC	stable
15	Ruddy Turnstone	*Arenaria interpres*	LC	decreasing
16	White-eyed Gull	*Larus leucophthalmus*	NT	stable
17	Sooty Gull	*Larus hemprichii*	LC	decreasing
18	Caspian Tern	*Sterna caspia*	LC	increasing
19	Swift Tern	*Sterna bergii*	LC	stable
20	Lesser Crested Tern	*Sterna bengalensis*	LC	stable
21	Saunders's Tern	*Sterna saundersi*	LC	decreasing
22	White-cheeked Tern	*Sterna repressa*	LC	decreasing

As a proxy measure of human impact, we made a data layer showing the distance to the nearest main cities, cities and roads. A point shapefile containing all buildings on

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the west coast of Saudi Arabia was projected to UTM 37N and clipped to the study area. The source of these data was http://www.gospatial.com. Three distance-to-feature rasters were created to measure the euclidean distance between all cells within the study area to the nearest main cities, cities and roads. We then converted the data to raster format in which each cell took as its value the distance (km) to the nearest main cities, cities and roads (see AlRashidi et al., 2011).

To investigate the effect of latitude, we added latitude coordinate data in ArcGIS as a layer with decimal degrees format. We then converted the data to raster format. After that, raster was clipped to the study area to generate the latitude layer that matches all layers of environmental data exactly. We then converted raster to ASCII.

To investigate the effect of coastal complexity (the physical shape attributes of each coastal pixel), a fishnet layer was created to split the coastline into 1 km^2. Next, we calculated the geometric intersection of the feature classes and feature layers. We then converted the data to raster format in which the value of each cell was given an indication of how curvy the coastline is. Finally, this raster was clipped to the study area to generate the coast complexity map that matches all layers of environmental data exactly. We then converted raster to ASCII.

Species data

The species occurrence data were entered into ArcGIS version 10, re-projected to UTM in the same coordinate system, and then rasterised. All environmental variables (described above) were converted to raster layers within ArcGIS, and modified to fit the same geographical boundary and cell size. Finally, we converted the environmental layers from raster format to ASCII and exported them to the modelling program.

Species distribution modelling

All analyses were implemented using MaxEnt software version 3.3.3e (Phillips et al., 2006). MaxEnt uses presence-only data to predict the likelihood distributions of maximum entropy as the basis for forecasts of potential distributions of species (Young et al., 2009).

The models were estimates of the maximum and the most uniform spread of a species across a study area, based on environmental constraints. An internal regularisation technique is used to control overfitting data (Phillips et al., 2006). The output of MaxEnt was a raster map of the same resolution as the input data; each cell represented the relative suitability of an area for a species to reside there. The value of each cell is re-scaled from 0 to 1, with zero being the lowest and 1 being the highest probability of suitability (Phillips et al., 2006).

MaxEnt's jackknife options were used to investigate the relative importance of the environmental variables. The gain is a measure of the probability of the samples; which specifically maximizes the likelihood of the presence samples with reference to the background data. Thus, the higher gain value denoting a better fit of model. (Phillips et al., 2006). For each candidate model set/species, ten replicate models were run and model averages were taken across the set in order to display the distribution of wetland birds as a species group. Sufficient data were available to allow replicate models to be run with a split 50% training data and 50% test data.This permits an evaluation of the model's performance.

To produce an indication of the distribution of wetland birds as an assemblage of wetland bird species and to estimate the proportion of suitable habitats along the Red Sea coast, Maxent was run with a single model based on all of the 22 bird species to give "*All birds*" model, where presence equal the presence of any species. After that, the Maxent map of *"All birds*", was imported into Arc GIS and cells with a habitat suitability probability of <0.7 were given the value 0 (not suitable) and those with a probability >1 given a value of 1 (likely to be suitable habitat). The proportions of suitable and unsuitable habitat cells were then calculated.

Evaluation of the models

The predictive performance of the models was tested using receiver-operating characteristics (ROC). The area under the curve (AUC) was then used as a measure of model performance; the value of AUC was scaled from 0 to 1, where 1 is optimal performance, 0.5 is no better than random, and less than 0.5 is worse than random (Elith et al., 2006; Pearce and Ferrier, 2000).

22 species models were used to build the species richness map. Maximum training sensitivity plus specificity was used to determine the threshold value necessary to predict unsuitable and suitable habitats for each species model. According to Jiménez-Valverde and Lobo (2007), the resulting threshold generally achieves high prediction accuracy. In order to apply the maximum training sensitivity plus the specificity threshold to each species, the MaxEnt average ASCIIs were converted to rasters in ArcGIS. Then the threshold value was assigned to each model by reclassifying raster values to 0 for unsuitable habitat (< threshold) and 1 for potentially suitable habitat (≥ threshold).

Then, the threshold-validated distribution models of each species were combined in one model using raster calculator in ArcGIS. After that, raster appeared in the display looking with a different colour scheme allow us to determine the areas of high species richness across Saudi Arabia's Red Sea coast.

Results

Individual species models

The performance was generally high for all individual species models. The mean AUC ranged from 0.532 and 0.990. The key significant variables for the 22 wetland-bird species distribution models are summarised in *Table 2*.

All birds

The species distribution model generated when using all bird species occurrence data showed that the most suitable habitats for wetland birds were predicted to be in the middle and southern parts of the Red Sea coast (*Fig. 2*). The results of the jackknife test (*Fig. 3*) revealed that the coast complexity is the key variable in explaining the distribution of all birds. This variable introduced a higher gain compared to the other variables and would likely be highly influential if withdrawn from the model. Models using slope alone do not perform well and thus would be the least transferable. The next two variables that contributed the most to the model are distance to main cities and distance to roads.

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***Table 2**. Summary statistics of the 22 wetland bird species on the Red Sea coat of Saudi Arabia including species occurrence data, variable with highest gain (from jackknife test of variable importance), percent contribution of top two contributors to each model (from the table of relative contributions of the environmental variables), and the mean test AUC for each model*

Species	Occurrence records	Variable with highest gain	The first important variable	Percent contribution	The second important variable	Percent contribution	Mean test AUC
Eurasian Spoonbill	17	Distance to cities	Distance to cities	53.6	Latitude	23.1	0.895
Intermediate Egret	30	Distance to main cities	Distance to main cities	32	Elevation	23.4	0.774
Western Reef Heron	47	Coast complexity	Latitude	31.7	Distance to road	25.7	0.853
Pink-backed Pelican	14	Distance to cities	Coast complexity	36.6	Distance to cities	36.1	0.900
Eurasian Oystercatcher	14	Distance to cities	soil moisture	38.5	Distance to cities	35.8	0.619
Crab Plover	190	Coast complexity	Coast complexity	28.4	Distance to main cities	23.7	0.914
Kentish Plover	766	Distance to main cities	Distance to main cities	27.3	Coast complexity	26.5	0.896
Lesser Sand Plover	210	Coast complexity	Coast complexity	31.8	Distance to main cities	30.2	0.906
Greater sand plover	139	Coast complexity	Coast complexity	29.9	Distance to road	23.5	0.914
Whimbrel	50	Distance to road	Distance to road	54.2	Distance to cities	18.5	0.820
Eurasian Curlew	51	Distance to road	soil moisture	25.1	Coast complexity	19.8	0.881
Redshank	119	Coast complexity	Coast complexity	30.2	Distance to road	23	0.890
Marsh Sandpiper	33	Distance to road	Distance to road	30	Latitude	29.6	0.836
Terek Sandpiper	21	Distance to road	Distance to road	70.1	soil moisture	9.5	0.729
Ruddy Turnstone	59	Coast complexity	Coast complexity	30.9	Distance to cities	24.4	0.791
White-eyed Gull	20	Latitude	latitude	33.1	Distance to cities	21.6	0.990
Sooty Gull	73	Coast complexity	Distance to main cities	24.8	Distance to cities	18.9	0.819
Caspian Tern	26	Distance to road	Distance to road	49.3	Coast complexity	13.1	0.853
Swift Tern	15	Distance to cities	Distance to cities	37.2	Distance to road	34	0.723
Lesser Crested Tern	25	Slope	Slope	29.3	Latitude	25.2	0.532
Saunders's Tern	19	Distance to road	Distance to road	24.5	Distance to cities	22.3	0.878
White-cheeked Tern	16	vegetation cover	vegetation cover	33.1	soil moisture	21.6	0.612

An examination of the model response curves to key environmental variables indicated that the greater the value of the distance to main cities and soil moisture, the greater the value of the potential habitat suitability, while the greater the value of the coast complexity, distance to roads, distance to cities and elevation variables, the smaller the value of the potential habitat suitability.

The "*All birds*" model suggested that approximately 17% of the Red Sea coastal habitat is suitable for wetland bird species.

Model validation

The "*All birds*" model performed well in predicting the presence of all birds when evaluated using a ROC plot (AUC mean = 0.901 and 0.879 for the training and testing data, respectively). This result indicated that in the final model, a cell predicted as suitable habitat at any threshold of suitability would be more suitable than a randomly selected cell in the study area at least 87% of the time.

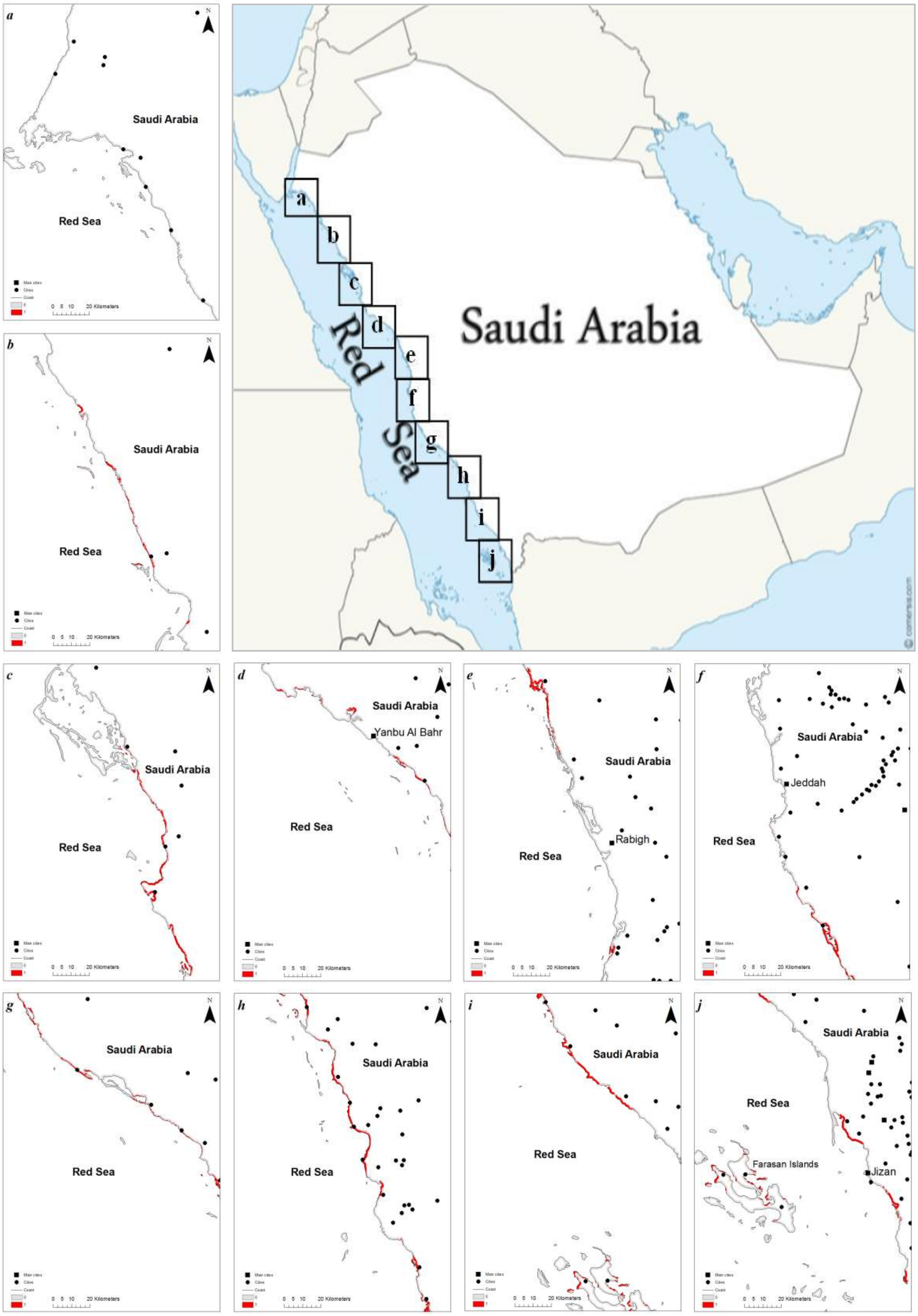
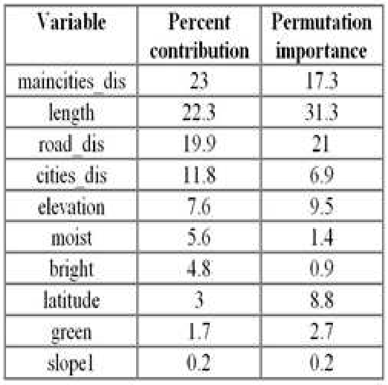


__Figure 2.__ Maps showing the predicted potential suitable habitats for "All birds" (50% training data and 50% test data); using Maximum training sensitivity plus specificity threshold; red line represent potential habitat suitability for "All birds". The square black dots represent the main cities and circle black dots represent the cities



Variable	Percent contribution	Permutation importance
maincities_dis	23	17.3
length	22.3	31.3
road_dis	19.9	21
cities_dis	11.8	6.9
elevation	7.6	9.5
moist	5.6	1.4
bright	4.8	0.9
latitude	3	8.8
green	1.7	2.7
slope1	0.2	0.2

Figure 3. *Results of jackknife evaluations of relative importance of predictor variables and table gives estimates of relative contributions of the environmental variables for "All birds" using Maxent model. Note: 'bright is brightness; 'cities_dis' is distance to cities; 'green' is vegetation cover, 'length' is coast complexity, 'maincities_dis' is distance to main cities, 'moist' is soil moisture, 'road_dis' is distance to road*

Species richness areas

The areas of high species richness for wetland birds in the Red Sea coastal area are illustrated in the map shown in *Figure 4*, which reveals that 17 areas may represent potentially suitable habitats for a large number of wetland bird species. Furthermore, the map illustrates that areas of the Red Sea coast were predicted as suitable habitat for up to 19 species, particularly in the middle and southern parts of the Red Sea coast. In contrast, the northern part of the Red Sea coast contains only two areas of high species richness. The areas of high species richness appear fragmented in places and cover broad distances along the study area. The majority of endemic and near-threatened species occurs in all 17 areas, except the White-eyed Gull, which occurs only in two areas located in the northern part of the Red Sea coast (*Table 3*).

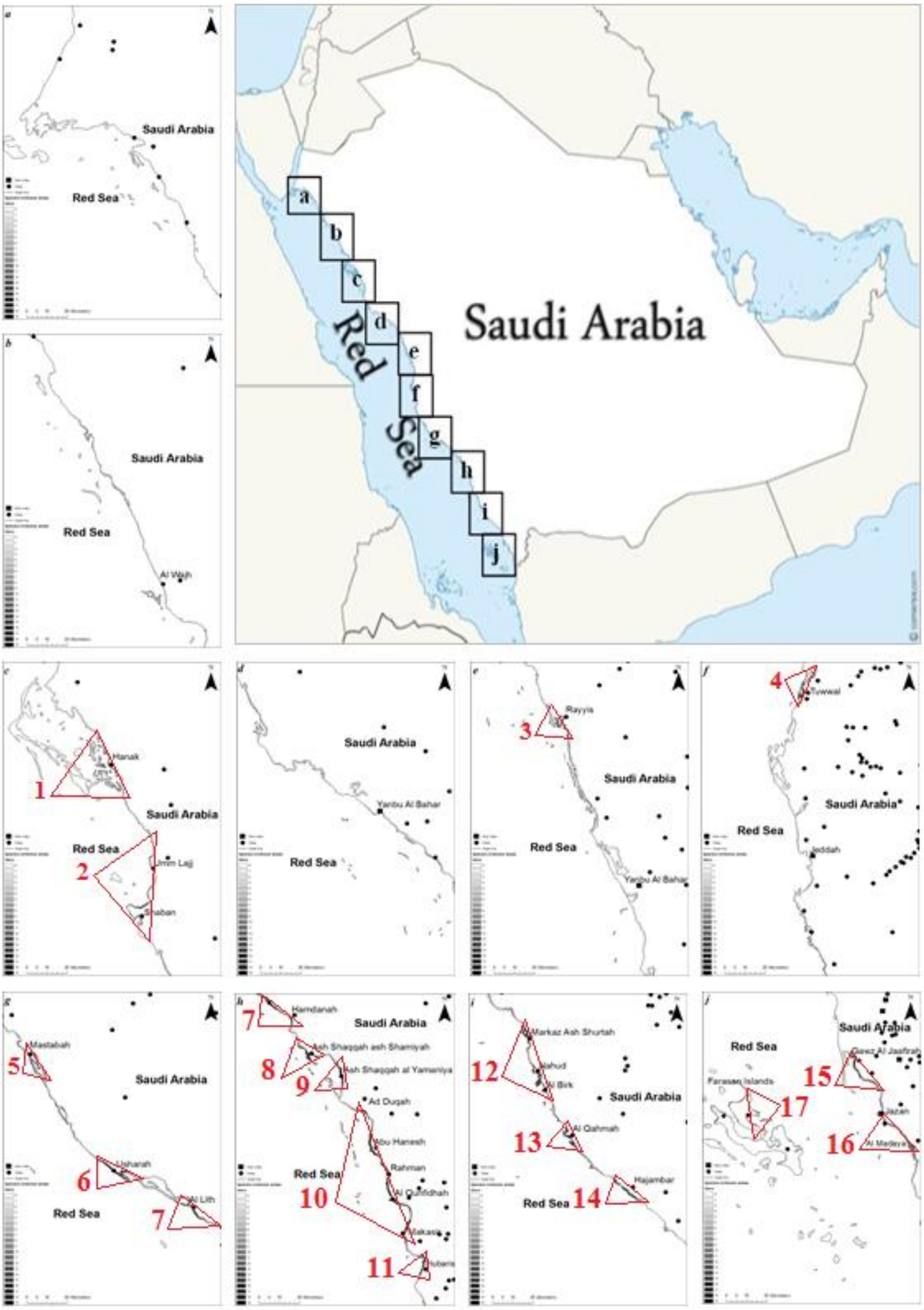


***Figure 4.** Maps showing the 17 final predicted areas of high species richness for wetland birds in the Red Sea coast of Saudi Arabia. The red triangles indicate the potential areas of high species richness. The square black dots represent the main cities and circle black dots represent the cities*

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Table 3. *Showing all 17 locations that are considered potentially suitable habitats for wetland birds along the Red Sea coast of Saudi Arabia, the locations of the endemic and near-threatened species that occurs in all 17 sites and the total species numbers that occur in each potential suitable habitat*

No.	Sites	Species						Number of species occur in potential suitable habitats
		Crab Plover	Eurasian Curlew	White-eyed Gull	Sooty Gull	Saunders's Tern	White-cheeked Tern	
		Endemic LC	NT	Endemic NT	Endemic LC	Endemic LC	Endemic LC	
1	Hanak and the islands around it	√	√	√	√	X	√	18
2	From Noth Umm Lujj to Shaban	√	√	√	√	X	√	16
3	Rayyis	√	√	√	√	√	√	16
4	Thuwwal	X	√	X	√	√	√	18
5	Mastabah		√	X	√	√	√	17
6	Usharah	X	√	X	√	√	√	18
7	From Alith to Hamdanah	√	√	X	√	√	X	19
8	Ash Shaqqah Ash Shammiyah	√	√	X	√	√	X	19
9	Ash Shaqqah Al Yamaniya	√	√	X	√	√	X	19
10	East Ad Duqah 7km to Makasir	√	√	X	√	√	X	19
11	Hubaris	√	√	X	√	√	X	18
12	From Markaz Ash Shurtah to Al Birk	√	√	X	√	√	√	17
13	Al Qahmah	√	√	X	√	√	X	17
14	West Hajambar 3 km	√	√	X	√	√	X	17
15	Qawz Al Jaafirah	√	√	X	√	√	X	16
16	From Jazan to At Tahiriyah	√	√	X	√	√	X	15
17	The East shoreline of Al Segied Island in Farasan Islands	√	X	X	X	X	√	11

Discussion

This study provides the first predicted potential habitat suitability maps for 22 wetland bird species along the Red Sea coast in the KSA. Although we built some models with occurrences data less than 20 locations, MaxEnt proved to give a robust performance in practice compared to other methods and remains effective despite a small sample size (Elith et al., 2006; Baldwin, 2009). The results demonstrated distance to cities, distance to roads and coast complexity variables influence negatively quite a few wetland bird species. These effect may be mediated through human activities (e.g disturbance, hunting and pollution), and the risk of predation by introduced cats, dogs and crows in the vicinity of settlements (AlRashidi et al., 2011). Furthermore, Findlay and Houlahan (1997) found that there is highly negative correlation between bird species richness and road within around 1 km of a wetland. Many studies have been indicated that some bird species avoid selecting habitats near the roads (Bollinger and Gavin 2004, Carrascal et al., 2006, Gavashelishil and McGrady, 2006). Roads have highly influence on birds in different directions such as, direct mortality, indirect mortality, habitat fragmentation, isolation and disturbance (Jacobson, 2005; Findlay and Bourdages, 2000).

Several wetland bird species that have particular importance in terms of conservation status are present on the Red Sea coast. These species include Crab Plovers (*Dromas ardeola)*, White-eyed Gulls, Sooty Gulls, and White-cheeked Terns (PERSGA/GEF, 2003). Due to their importance in regional conservation strategies, these specific species will be discussed below in light of the potential species distribution models.

Crab Plover. Crab Plovers are known to utilise areas of sandy and muddy coastline on islands, intertidal sandflats and mudflats, estuaries, lagoons, and bare coral reefs (Burton and Burton, 2002), and breed on sandy islands or extensive coastal sandy banks (BirdLife International, 2013). Examining the habitat suitability model the distance to main cities and soil moisture have a positive influence on the distribution of Crab Plovers, whereas, coast complexity, distance to cities and distance to roads have a negative influence on their distribution.

White-eyed Gull. White-eyed Gulls are known to utilise coastal areas and breed on island shorelines on exposed rock and sand flats (del Hoyo et al., 1996). Although some populations in Egypt have been shown to get their food from drifting litter and harbours (Baha El Din, 1999), the species mostly feeds at sea (PERSGA/GEF, 2003). Examining the habitat suitability model the latitude has a positive influence on the distribution of White-eyed Gulls, whereas the distance to cities, vegetation cover and slope have a negative influence on their distribution.

Sooty Gulls. Sooty Gulls which are known to utilise areas of along coast and at islands and breed on coastal and inshore islands near sea level which are sparsely vegetated, rocky and sandy (Cramp and Simmons, 1983, PERSGA/GEF, 2004). Furthermore, sometimes they feed in mudflat and rest in harbours and unusually seen inland (del Hoyo et al., 1996). Examining the habitat suitability model the distance to main cities, distance to roads and latitude have a positive influence on the distribution of Sooty Gulls, whereas the coast complexity and distance to cities have a negative influence on their distribution.

White-cheeked Tern. White-cheeked Terns are known to utilise coastal areas and along inshore bodies of water. They nest on rock, sand, gravel, and coral islands (del Hoyo et al., 1996), as well as on the exposed sandflats and sparsely vegetated open ground of sand dunes and above shorelines' high-water marks (Snow and Perrins, 1998). Examining the habitat suitability model the soil moisture and latitude have a positive influence on the distribution of White-cheeked Terns, whereas the vegetation cover and distance to main cities have a negative influence on their distribution. Saudi Arabia is generally an arid country with very high temperatures in summer. It receives about 70 mm of precipitation annually. Therefore, these factors led to decreased vegetation cover (Darfaoui and Al Assiri, 2011).

Kentish Plover. It is encouraging to compare our model results for the Kentish Plover with those reported by AlRashidi et al., (2011). He used a Generalised Linear Model (GLM) with four habitat variables elevation, distance to settlements, vegetation cover and soil moisture for predicting potential suitable habitat for Kentish Plover. In particular, AlRashidi et al., (2011) reported that the most suitable habitat for Kentish Plovers on the Farasan Islands is located on the northern and eastern shores; this result is consistent with the findings of the present study. Furthermore, AlRashidi et al., (2011) found the most suitable continental habitat for the Kentish Plover species to be located in two concentrated areas along the Red Sea coast. The first area is located between Yanbu Al Bahr and Jiddah, and lies approximately 100 km south of Jiddah. The second area is located near the city of Jazan. However, our study predicted the most suitable continental habitat patches for the Kentish Plover to be located along the Red Sea coast south of the cities Hanak and Jazan.

Mapping areas of high species richness

This study provided the first predicted potential habitat richness map for wetland bird species along the Red Sea coast of Saudi Arabia. Mapping species richness and distributions has become a key strategy in conservation planning (Cardillo, 1999; Graham and Hijmans, 2006). Identifying species richness areas that include endemic species will provide conservation agencies and practitioners with the information needed to develop and optimise conservation strategies and actions (Bojórquez-Tapia et al., 1995).

Each of the 17 areas of high wetland bird species richness that were identified in this study contains different levels of endemic species. We already know that wetland birds and their habitats along the Red Sea coast face many threats. Therefore, a survey of these 17 areas should be undertaken in the future in order to investigate the actual presence of wetland birds in these areas and to thereby determine the most significant of these 17 areas for conservation purposes.

Potential Sources of error

Several studies have indicated that environmental variables, such as habitat structure and composition, play important roles in improving the accuracy of bird habitat descriptions (Dettmers and Bart, 1999). The advantage of species modelling techniques is that they can be used to estimate the potential conservation value of both surveyed and under-surveyed areas (Maddock and Du Plessis, 1999). However, Osborne et al., (2001) pointed out two potential issues when building predictive models for species distribution over large regions. First, there may be problems related to the consistency of predictor variables over large geographic spaces, which is particularly apparent in variables derived from remote sensing because of the inherent variability of angular surface reflectance (Stoms, 1992). The second issue concerns the variations in habitat selection among individual birds.

Implications for conservation

The conservation of wetland bird habitats has become a global conservation priority (BirdLife International, 2011). The species distribution model presented in this study can be used to underpin future conservation planning in the Red Sea region (Guisan and Thuiller, 2005).The "*All birds*" model in this study indicated that approximately 17% of the Red Sea coastal habitat is suitable for wetland bird species.

In Saudi Arabia, there are 15 protected areas; only two of these (the Farasan Islands and the Umm Al Qamarie Islands) are located along the shores of the Red Sea (Saudi Wildlife Commission, 2011). Thus, we recommend that future monitoring and surveys should be carried out to cover all 17 areas of high wetland bird species richness along the Red Sea coast in order to determine the most significant coastal areas for wetland bird species and therefore, incorporate them into the protected areas system in the future. These areas should include both nesting and foraging sites for wetland bird species. Furthermore, we strongly recommend that establishing new protected areas along the Red Sea coast line are becoming priority to protect in particular the remain suitable habitat areas for threatened and endemic wetland birds.

Conclusions

This study has produced the first predicted potential habitat distribution maps for wetland bird species along the Red Sea coast of the Kingdom of Saudi Arabia. It has revealed that there are 17 important areas of species richness for wetland birds along the Red Sea coast of the KSA. Understanding the correlation between species occurrence and environmental variables will help both researchers and policy makers to implement appropriate conservation plans in terms of wetland bird species. The modelling presented here has the potential to form the basis for conservation strategy of wetland birds in the Red Sea coastal region of Saudi Arabia, and it is hoped that this study will guide future field surveys and conservation programs in the Red Sea coast areas of Saudi Arabia.

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IS THE PERIPHYTIC STRUCTURE OF TESTACEANS (PROTOZOA: RHIZOPODA) RELATED TO WATER QUALITY: A CASE STUDY IN THE CUIABÁ RIVER, BRAZIL

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Abstract. Presented study describes the periphytic community of Testaceans of the Cuiabá River, Central Brazil and evaluates its relationships to physical, chemical and biological water characteristics. Three sampling stations were established along an urban reach passing Cuiabá city, realizing four campaigns at the right and left river margins. Canonical Correspondence Analysis was conducted to study relationships of chemical and physical water characteristics with the colonization of the periphytic community of Testaceans on artificial substrata. Assemblage composition varies according to seasonal hydrological cycles and water quality, as well as spatially along the pollution gradient reflected by sampling sites along the river reach. Species richness and abundance showed highest values during the dry season, being *Cochlyopodium*, *Centropyxis* and *Cyclopyxis* the most common genera. The genus *Centropyxis* was frequent at all sampling stations throughout the year, being dominant at the highest impacted station. 27 of the 28 *Difflugia* species were only observed in the dry season under low flow velocities and discharge and have little potential as indicator of water quality parameters. On the other hand, species such as *Cyclopyxis plana microstoma* and *Euglypha acanthophora* only occured at impacted sampling stations, independently of the season, indicating a potential for bioindication.

Keywords: *testate amoeba, periphyton, artificial substrata, Central Brazil*

Introduction

Between the diverse groups of water quality indicators, periphyton communities have been widely applied for the study of pollution estate in lotic ecosystems. According to Ward (1989), lotic environments have primary heterotrophic functions and reflect therefore the characteristics of its contributing watersheds (Lampert and Sommer, 1997). Therefore, basins geoecology and land use reflect on spatial distribution and colonization processes of protozoaria communities. Most studies focused on the ecology on algae assemblages in freshwater ecosystems (Barinova et al., 2006; Gaiser et al., 2006; Carr et al., 2005; Vis et al., 1998). The group of sarcodian protozoae (Testacea),

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however, is supposed to have advantages for ecological approaches: they are abundant in many aquatic ecosystems, produce high amounts of biomass and its size and reproduction cycles allow investigations about demographic processes. As they are cosmopolitans, the regional auto-ecology of species can be compared with that of other regions.

Quantification of testate amoeba on natural substrata implicates in a series of methodological problems, particularly if quantitative results should be obtained. Therefore artificial substrates have been suggested for sampling (Cooke, 1956; Sládecková, 1962; Cairns et al., 1979; Schwarzbold, 1990). The studies of Castenholz (1961), Sladecek and Sládecková (1993) and Strüder-Kypke (1999) identified glass slides to be appropriate as artificial substrates to study periphyton fauna. Different incubation periods for glass slides had been proposed for the study of periphyton communities (Brown and Austin, 1971). For approaches, which use direct observation of species by microcopy, incubation periods of glass should not exceed one month. Godinho-Orlandi and Barbieri (1983) stated highest number of individuals of protozoa population after 21 days of colonization, after testing periods of 2, 3, 7, 14, 21 and 28 days.

Despite the significant amount of ecological studies on the testate amoebae communities in rivers of temperate climates (Jax, 1996; Opravilová, 1986), little is known about their distribution and its suitability as ecological indicators in sub-humid tropical, lotic ecosystems. The few available studies for South American freshwater ecosystems refer to plankton communities (Lansac-Tôha, 2000, Velho et al., 1999, Walker, 1982). Until the mid 70ties, studies on periphyton communities in the Central Western regions of Brazil were restricted to general, descriptive biogeographical approaches (Cunha, 1916, Green, 1975). Even the more recent studies carried out in Brazil (Lahr and Lopes, 2006; Lansac-Tôha et al., 2007, 2008) and the Mato Grosso state (Hardoim and Heckman, 1996, Lansac-Tôha et al., 2001, Hardoim, 1997, Silva Neto, 2001, Missawa, 2000) give only a very limited image of spatio-temporal distribution of Testacea fauna and its ecological determinants.

Many Testacea were supposed to be poor indicators for water quality, as they can tolerate a broad spectrum of environmental factors (Green, 1975; Ogden and Hedley, 1980; Opravilová, 1986). The Testacea are mostly anchored to the substrate with only a temporary adhesion by pseudopods, leading to a weak competitive ability and passive relocation and are therefore often restricted, in higher species diversity and density in competition poor habitats. At least in lentic environments, Testacea however have been related successfully to water quality (Patterson and Kumar, 2002; Kumar and Patterson, 2000). Roe et al. (2010) showed a strong association of thecamoebian assemblages with the trophic status of lake sediments. Escobar et al. (2008) related the structures of assemblages to pH in subtropical Florida lakes to organic matter content and pH and pointed out that diversity indices are higher in mesotrophic to eutrophic lakes than under dystrophic conditions.

In this context in present study we investigate, if Testacea periphyton communities have a potential as indicators to evaluate the pollution gradient along a urban reach of the Cuiaba river, which passes the most densely populated region of Mato Grosso state, Central Brazil. More specifically, we analyze the influence of pollution gradients and the climatic driven temporal variations of water quality on the assemblage composition.

Study Area

The upper Cuiabá River is one of the main tributaries of the northern Pantanal, the world greatest floodplain. Its watershed covers, river ahead the Pantanal, about 28.700 km^2 and is located between 14° 18’ and 17° 00 s.L. and 54° 40’ and 56° 55’ w.L in the southern part of the Mato Grosso state (*Fig. 1*).

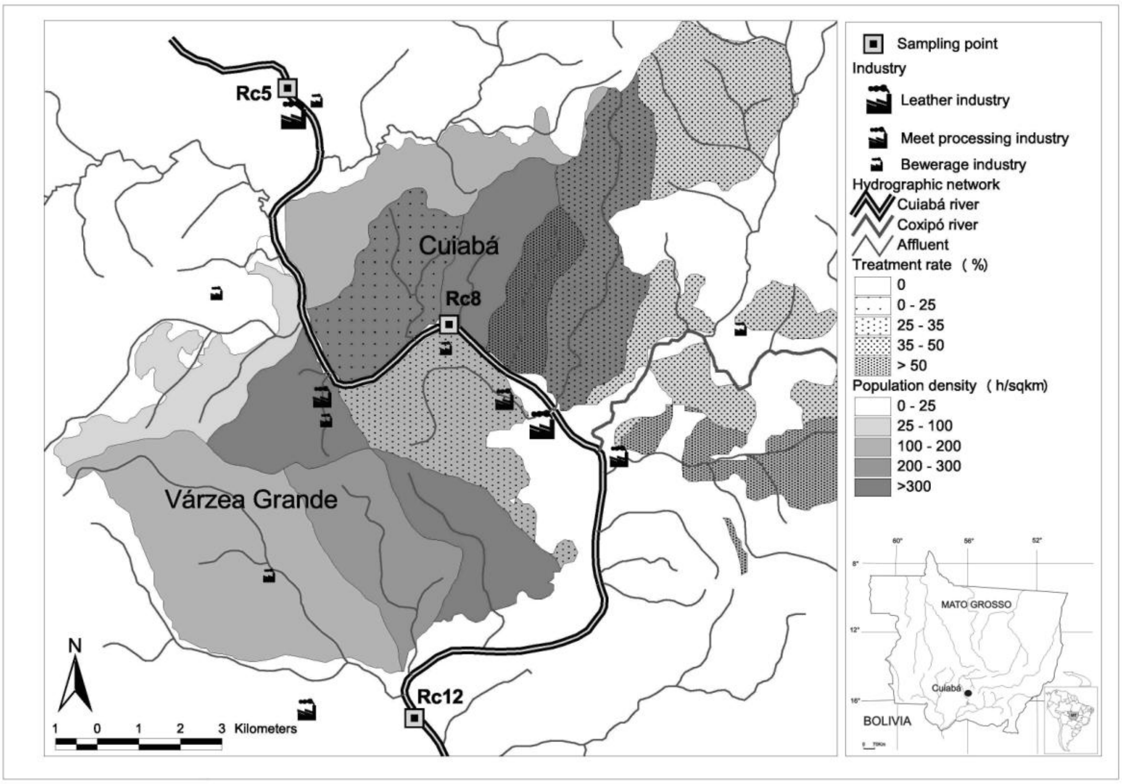

Figure 1. *Sampling stations in the urban agglomeration of Cuiabá and Várzea Grande. Sub-watersheds are colored according to population density and wastewater treatment taxes.*

The tropical sub-humid climate in the basin is characterized by mean annual temperatures between 22 and 25 degrees and a mean precipitation of 1342 mm (1960-1998) in the urban agglomeration of Cuiabá and Várzea Grande with maxima of up to 2000 mm in the headwater regions of the Cuiabá river. Precipitation and river runoff present an expressive seasonality. More than 80 % of rainfalls are concentrated in the months of November through April. The average monthly stream discharge at a gauging station in Cuiabá City varies between about 800 m^3/s in April and 100 m^3/s in September (1984-1999). The lower parts of the watershed are formed by Pre-Cambrian metasediments of the Cuiabá group, the Manso and Casca river headwaters are located in the mountain ranges of the Planalto of Guimarães, mainly formed by sandstones. The upper Cuiabá River crosses calcareous formations of the Pre-cambrian Alto Paraguai group. Cerrado savannahs, the main vegetation formation of the watershed are mainly occupied by extensive cattle farming, in the plateaus of the mountain ranges native vegetation had been widely substituted for the cultivation of soybeans and cotton.

In 2002, the urban agglomeration of Cuiabá and Várzea Grande city had a population of more than 720,000 inhabitants. It is estimated, that less than 30% of domestic effluents are treated and that about 95% of organic loads in the watershed are introduced in the Cuiabá River in the urban agglomeration (Zeilhofer et al., 2010). Non-published, population-based estimates for the year of 2006 indicate that more than 30 t of organic loads are daily introduced along river reaches in the urban agglomeration of Cuiabá.

Methods

Sampling

Artificial substrata were installed at three monitoring stations, each composed by two sampling points at both river margins, representing a pollution gradient along a 28 kilometers urban reach of the Cuiabá river (*Fig. 1*). First station (Rc5) is located river ahead of the urban perimeter, receiving only diffuse pollution loads from extensive agricultural activities and some aquacultures. The sampling point Rc8 is located about 300 m above the Gambá stream in the central area of the urban agglomeration. Rc12 is a sampling point river downstream the Cuiabá/Várzea Grande agglomeration, after the Cuiabá river receiving principal organic contributions from the urban watersheds. Chemical, physical and biological water quality samples were collected during the campaigns for installation and removal of the artificial substrata, at both river sides (sampling points of Testacean colonization) and for the main channel. Four sampling campaigns were conducted, two during the dry season (15/08/2000 and 30/08/2000) and two during the rainy season (08/03/2001 and 09/04/2001). Water quality analyses were conducted according to APHA - Standard Methods (*Tab. 1*).

Table 1. *Methods applied for physical, chemical and biological parameters of water quality monitoring.*

Parameter	Acronym	Analytical technique	Unit
Alkalinity	Alka	Titrometry	mg $CaCo_3$/l
Biological Oxygen Demand	BOD5	5-Day BOD test	mg O_2/l
Chemical Oxygen Demand	COD	Closed reflux, colorimetric	mg O_2/l
Fecal coliforms	FC	Multiple-tube fermentation	MPN/100ml
Total coliforms	TC	Multiple-tube fermentation	MPN/100ml
Color	Color	Visual comparison	Chloroplatinite
Condutivity	Cond	Conductometry	µS/cm
Dissolved Oxygen	DO	Potentiometry/O2 probe	mg O_2/l
Total Kjedahl Nitrogen	TKN	Block digestion, spectrophotometry	mg N/l
pH	pH	Electrometric method	pH units
Total phosphorous	TP	Ascorbic acid	mg P/l
Total solids	TS	Drying at 100 ºC/weighting	mg/l
Temperature	Temp	Temperature probe	ºC
Turbidity	Turb	Nephelometric method	NTU

Testacean communities were sampled using artificial substrates with replicas, composed by two previously sterilized glass slides. At each sampling point a total of six pairs of glass slides were disposed in a metallic grid. Fixed at the bottom of the water course at a distance of about 1 meter from the river border, the sampling modules were disposed parallel to the stream current in order to reduce selective effects (Kralik 1957/58). After 16 days, glass slide replicas were removed, fixed in a 4% formol solution and colored

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with an indicator of viability Bengal's Rose. Identification of testate amoeba was done by optical microscopy. Replicas were considered as a unique sampling unit, summing species counts of glass slides. During the wet season, inferior parts of glass slides were in some cases covered by sediments. Testate counts were therefore extrapolated proportionally to the non-populated glass slide area. Sampled material is available for consultation at the Laboratory of Taxonomy and Ecology of Aquatic Microorganisms – LATEMA, at the Bioscience Institute of the Federal University of Mato Grosso.

Data Analysis

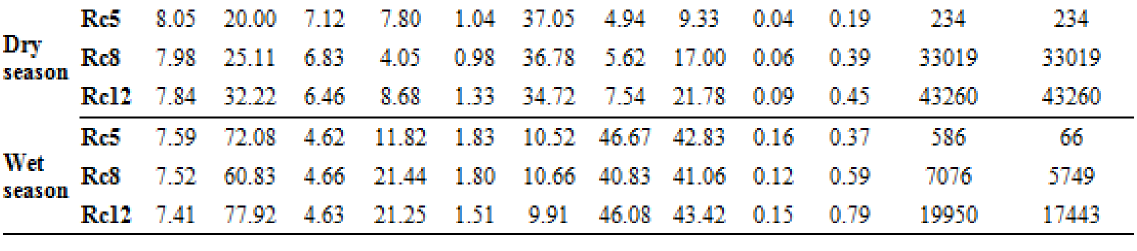
Direct gradient analysis

Canonical Correspondence Analysis (CCA) was conducted to evaluate relationships between water quality variables and community structure of Testacea protozoa. CCA is a direct gradient analysis (ter Braak and Prentice, 1988), where a matrix Y of dependent variables (species) is ordinated under the constraint of a matrix X of explanatory variables or predictors (water quality data). CCA is indicated for ordination, if dependant variables have a unimodal distribution along environmental gradients. In their comparison of CCA with General Linear Models (GLM), Guisan et al. (1999) pointed out that CCA has good performance in ordination of multiple species. General null hypothesis of CCA, that there is no relationship between the response and explanatory variables (independence of Y and X), or that the model is not significant, can be tested through a permutation test, where a pseudo-F statistic of the unpermuted data is repeatedly compared with the pseudo-F statistic of permuted data (Anderson and ter Braak, 2003). As CCA is supposed to be biased by rare species (Legendre and Gallagher, 2001), only species with relative frequencies above 20% were considered in ordination. Both dependent variables (species abundance) as well as explanatory variables were log-transformed for ordination. CCA was conducted with the RDACCA software (Makarenkov and Legendre, 2002). Significance of ordination axis was tested applying a Monte Carlo randomization test with 5000 permutations.

Results

A Mann-Whitney test for paired samples of the three sampling stations along the urban reach of the Cuiabá river showed significant differences ($p<0.05$) between dry and wet season for most water quality variables. Only TC and FC counts do not differ statistically due to strong dilution effects in the streams during the rainy season (*Tab. 2*).

Table 2. *Physical, chemical and biological water quality variables for the three sampling stations at each sampling point (main channel, left and right margin) and for the two sampling dates.*



		pH	Color uH	DO mg/l	COD mg/l	BOD5 mg/l	Alcal mg/l	Turb UNT	SST mg/l	TP mg/l	TN mg/l	TC NMP/100ml	FC NMP/100ml
Dry season	**Rc5**	8.05	20.00	7.12	7.80	1.04	37.05	4.94	9.33	0.04	0.19	234	234
	Rc8	7.98	25.11	6.83	4.05	0.98	36.78	5.62	17.00	0.06	0.39	33019	33019
	Rc12	7.84	32.22	6.46	8.68	1.33	34.72	7.54	21.78	0.09	0.45	43260	43260
Wet season	**Rc5**	7.59	72.08	4.62	11.82	1.83	10.52	46.67	42.83	0.16	0.37	586	66
	Rc8	7.52	60.83	4.66	21.44	1.80	10.66	40.83	41.06	0.12	0.59	7076	5749
	Rc12	7.41	77.92	4.63	21.25	1.51	9.91	46.08	43.42	0.15	0.79	19950	17443

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PH, DO and Alkalinity were higher during the dry season, when Color, COD, BOD, Turbidity, SST, TP and TN concentrations were higher during the wet season.

These results appoint for a cyclical deterioration of physical and chemical water quality during the months November through April (rainy season) due to the local geographic specifities. During this period first flush events drain dry deposition components to the streams. In addition, water treatment plants in the urban agglomeration of Cuiaba and Varzea Grande city have frequently an overloaded capacity during and after rain storms, causing massive inflow of untreated wastewater in the Cuiaba river (Zeilhofer et al., 2010).

A comparison between the stations Rc5 and Rc8 (Wilcoxon paired sample test) resulted in significant differences for the variables pH, Turbidity, CT and TC. Water quality alterations turn more expressive when the stations Rc5 and Rc12 are compared. Values obtained for pH, color, DO, COD, Alkalinity, TC and FC differ significantly ($p<0.05$). Tributaries of urban watersheds mainly reflect on indicators of organic pollution such as decreasing DO and increase of COD and coliform counts. TN concentrations show significant increase between the stations Rc5 and Rc12 for the samples of both seasons; TP concentrations significantly increase between Rc5 and Rc12 during the wet season.

Table 3 presents seasonal species densities, which are averages from the two samplings points (left and right river margin) at each river station (Rc5, Rc8, Rc12), as well as species abundance and richness. Total abundance was highest at station Rc8 at both seasons and species. Richness was higher at this station than at the little impacted station Rc5 and similar as at station Rc12. During sampling campaigns of both seasonal periods *Cochlyopodium*, *Centropyxis* and *Cyclopyxis* were the most common genus. The genus *Cochlyopodium*, represented only by one species (*Cochlyopodium vestitum*) was abundant at each sampling campaign. Specimen counts, however, were lower during the wet season. The genus *Centropyxis* (8 species) was frequent at all sampling stations throughout the year, being dominant at highly impacted station Rc12 during the dry season. The three species of the *Cyclopyxis* genus were more common at the impacted stations Rc8 and Rc12, during both seasons. The genus *Difflugia* is represented by 28 species, but none of them was abundant inside the assemblages. Specimen counts were generally much higher during the dry season. With exception of *Euglypha acanthophora*, species of the genus *Euglypha* (three species), *Pseudodifflugia* and *Pyxidicula* (one species each) are rare during both seasons.

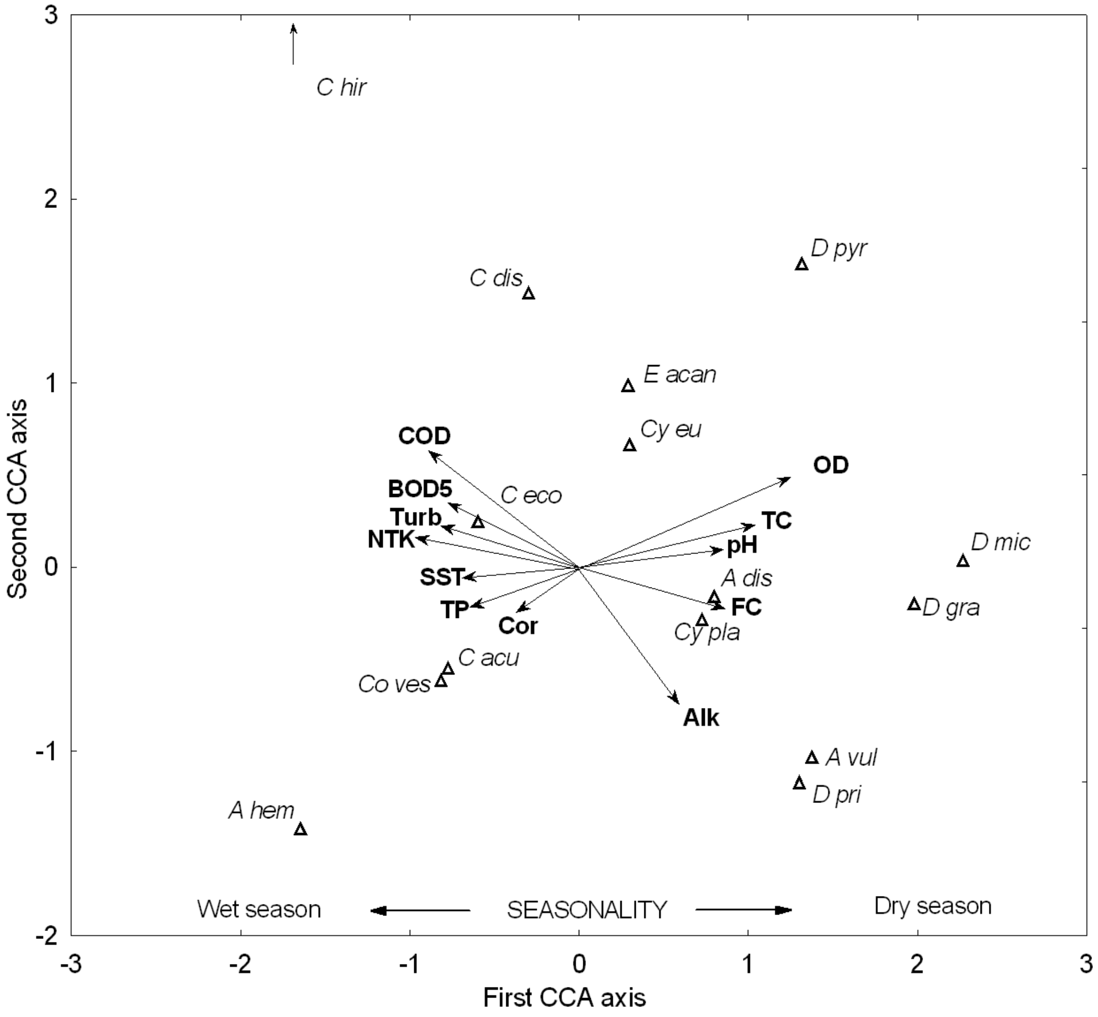
Table 3. *Individuals at sampling points, averaged for left and right river margins.*

	Dry season			Wet season		
Sampling station	Rc5	Rc8	Rc12	Rc5	Rc8	Rc12
Arcella dentata (Ehrenberg, 1838)	0	2	0	0	0	0
Arcella discoides (Ehrenberg, 1843)	11	94	90	1	1	45
Arcella hemisphaerica (Perty, 1852)	1	6	0	5	0	8
Arcella lobostoma (Deflandre)	0	0	0	4	0	0
Arcella vulgaris (Ehrenberg, 1830)	0	8	2	0	0	0
Centropyxis aculeata (Ehrenberg, 1830)	36	157	218	25	41	125
Centropyxis sp.	0	0	0	1	96	0
Centropyxis cassis (Wallich, 1864)	1	0	0	0	0	0
Centropyxis constricta (Ehrenberg, 1841)	1	1	2	0	0	0
Centropyxis discoides (Pénard, 1890)	3	10	19	0	12	12
Centropyxis ecornis (Ehrenberg, 1841)	19	82	268	10	122	20
Centropyxis platystoma (Pénard, 1890)	0	0	1	0	0	0

Centropyxys hirsuta (Deflandre, 1929)	1	1	6	1	4	7
Cochlipodium vestitum (Archer)	85	359	120	32	112	78
Cyclopyxis arcelloides (Penard, 1902)	2	0	0	0	0	0
Cyclopyxis eurystoma (Deflandre, 1929)	5	12	40	0	0	17
Cyclopyxis plana microstoma (Schönborn,1966)	0	216	133	0	184	136
Difflugia acuminata (Ehrenberg, 1838)	0	1	1	0	0	0
Difflugia ampulla (Ehrenberg, 1840)	1	0	1	0	0	0
Difflugia bicruris (Gauthier-Lievre and Thomas,	0	1	0	0	0	0
Difflugia brevicolla (Cash, 1909)	0	1	0	0	0	0
Difflugia bryophila (Penard)	0	0	1	0	0	0
Difflugia distenda (Ogden)	0	1	0	0	0	0
Diffludia elegans (Pénard, 1890)	1	3	3	0	0	0
Difflugia gassowskii (Ogden)	0	0	1	0	0	0
Difflugia glans,(Pénard, 1902)	0	2	1	0	0	0
Difflugia globularis (Wallich, 1864)	0	3	1	0	0	0
Difflugia gramen,(Pénard, 1902)	1	11	0	0	0	0
Difflugia lobostoma (Leidy, 1879)	0	1	0	0	0	0
Difflugia lucida (Pénard, 1890)	0	2	2	0	0	0
Difflugia manicata (Pénard, 1902)	1	0	0	0	0	0
Difflugia mamillaris (Pénard)	1	1	5	0	0	0
Difflugia mica (Frenzel, 1897)	0	23	30	0	0	0
Difflugia minuta (Rampi, 1950)	0	0	1	0	0	0
Difflugia mitriformis (Wallich)	0	0	1	0	0	0
Difflugia molesta (Pénard, 1902)	0	0	1	0	0	0
Difflugia oblonga (Ehrenberg, 1838)	0	1	0	0	0	0
Difflugia penardi (Cash)	0	0	1	0	0	0
Difflugia petricola (Cash, 1909)	0	1	0	0	0	0
Difflugia pristis (Penard, 1902)	0	3	7	1	6	2
Difflugia pyriformis (Perty, 1885)	1	3	3	0	0	0
Difflugia rubescens (Penard, 1891)	0	2	0	0	0	0
Difflugia tenuis (Penard, 1890)	0	0	2	0	0	0
Difflugia urceolata (Carter, 1864)	0	0	1	0	0	0
Difflugia venusta (Ogden, 1983)	0	0	1	0	0	0
Euglypha acanthophora (Ehrenberg, 1841)	0	8	11	0	5	4
Euglypha capciosa (Couteaux, 1978)	0	0	0	1	0	0
Euglypha laevis (Ehrenberg)	0	2	0	1	0	0
Euglypha sp 1	0	0	1	0	1	0
Netzelia sp (Ogden, 1979)	0	2	2	1	0	0
Pseudodifflugia gracilis (Schlumberger, 1845)	0	1	0	0	0	0
Pyxidicula SP	3	0	0	0	0	0
Total abundance	163	1008	964	77	581	450
Richness	9	13	14	5	7	7

First two axis of CCA ordination were found to be significant ($p < 0.01$) and had eigenvalues of 0.19 and 0.13, respectively. First ordination axis explains 21.7 % of canonical variance and synthesizes principally seasonal effects in species abundance (*Fig. 2*).

This axis is positively related with pH, DO, TC, FC and Alkalinity, variables with higher values during the dry season characterized by low river discharge. High abundances of *Arcella discoides, Difflugia pristis, Difflugia gramen e Difflugia mica* were related to these conditions. *Difflugia gramen* and *Difflugia mica* are hereby located on the extreme right side of ordination diagram. As further exposed in the discussion, preferences of this seems to be linked however to altering hydrodynamic conditions and the difficulties of Difflugiidae to adhere to substrate than to a reduced tolerance to physical and chemical pollution. *Arcella vulgaris* are related to higher values of alkalinity and present little tolerance to higher taxes of organic pollution during the wet season, presented by its opposite location to the COD and BOD vectors. First axis is negatively related to COD, BOD, Turbidity, Color, Total Solids, TP and TNK discriminating the wet season, when values of these variables rise. The species which mainly occur under these conditions were *Centropixys ecornis, Arcella hemisphaerica, Cochliopodium vestitum e Centropixys aculeata*.



A dis: Arcella discoides; A hem: Arcella hemisphaerica;
C acu: Centropixys aculeata; C dis: Centropixys discoides; C eco: Centropixys ecornis; C hir: Centropixys hirsuta
Co ves: Cochliopodium vestitum
Cy eu: Cycopyxis euristoma
Cy pla: Cyclopyxis plana microstoma
D gra: Difflugia gramen; D mic: Difflugia mica; D pri: Difflugia pristis; D pyr: Difflugia pyriformis
E acan: Euglypha acanthophora

Figure 2. *First two factorial axis of CCA. In the ordination, only species with a relative frequency above 20% were considered.*

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The two last species, in accordance to their location along the second CCA axis, seem to have preferences to higher TP concentrations. *Centropixys ecornis* seems to benefit by higher concentrations of organic material, evidenced by its location near the ordination vectors of COD, BOD and Turbidity. Alkalinity has the highest (negative) contribution to the second ordination axis, which explains 15.1 % of CA variance. *Arcella hemisphaerica* is ordered on the lower left side of the biplot, indicating a relation to higher Alkalinity, but coupled with higher nutrient levels found during the wet season. Species positively related to this axis are *Centropixys hirsuta, Centropixys discoides, Cycopyxis euristoma, Difflugia pyriformis* and *Euglypha acanthophora,* when *Arcella hemisphaerica* is ordered at the opposite site of the biplot.

Discussion

This study presents a characterization of periphyton testaceae assemblages in a tropical freshwater ecosystem in the Central-Western Brazilian Cerrado region and its relationships to physical-chemical and biological measurements of water quality. More specifically, exploratory analysis and CCA direct gradient analysis were applied to evaluate species distribution along a quality gradient of urban river reaches, and to compare frequencies and richness during the dry and wet seasons. Results of applied empirical methods to relate assemblages with environmental factors must not be misinterpreted that they are necessarily explaining causal relationships between structure and composition and measured external variables. In our case, water quality of the studied river reach is heavily influenced by hydrodynamics, which determine components of the periphython testatacea assemblages. This is further detailed and exemplified in following.

Total richness of periphyton tecamebiana fauna as found in this study (53 species) is similar to the 49 species reported by Hardoim (1997) on natural substrates in the Bento Gomes stream, an affluent of the northern Pantanal, about 100 km south of our study area. About 2/3 of sampled species were found to be rare. Rare species are in their majority are pertaining to *Difflugidae* family, similar as the results obtained by Silva Neto (2001) in planctonic samples of urban reaches of the Cuiabá river. *Cochliopodium vestitum*, *Cyclopyxis plana microstoma* and *Centropyxis aculeata* were found to be the three most abundant species, each of them dominant during one sampling date. In accordance, Missawa (2000) stated *Centropyxis aculeata* to be one of the three species most abundant species in planctonic communities in the main urban reaches of the Cuiabá river. Silva Neto (2001) found the family *Centropyxidae* to be the most abundant in an urban Cuiabá river reach, about 100 km river ahead of Cuiabá city. *Cochiopodium vestitum* has not been reported in none of the cited regional studies and *Cyclopyxis plana microstoma* was generally found to be rare in plankton assemblages. Abundant in present research, both species, which are characterized by flattened shell form, seem to be morphologically well adapted to the periphyton.

Generally, environmental preferences of studied periphyton organisms could be detected with more clarity for samplings from the dry, low water season. We suppose that this is due to a higher stability in environmental conditions, contrary to those of the wet season, when hydrodynamic and physical-chemical conditions oscillate frequently, caused by rainfalls and varying loads of aloctone material. Installation of assemblages seems to be more difficult under these conditions and total abundance and richness decrease. During the wet season some glass slides were partially covered by

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sedimentation, too, a shortcoming of applied periphyton sampling procedure on artificial substrates.

Our investigations did not conduct systematic work to evaluate, if it were limnological or hydrodynamic gradients, which cause variations in assemblages at artificial substrates between the dry and wet season. Nevertheless, richness of assemblages is impoverished during the wet season, when almost all *Difflugiidae* disappear. Studies such as those of Bobrov and Mazei (2004) and Wanner and Meisterfeld (1994) conclude that morphologic aspects of testate amebas influence abundance in different habitats. *Difflugiidae* are known to be widely unable to adhere to artificial surfaces, which may explain their absence at sampling campaigns from the wet season, with higher discharge and flow velocities. As examples, *Difflugia gramen* and *Difflugia lucida* have rounded shells, impeding permanence in the periphyton of reaches with higher runoff velocities. Similar, *Arcella vulgaris* which has previously been observed in the periphyton as well as in plankton assemblages of the study area (Hardoim and Heckman, 1996) was abundant during the dry season and disappeared during the wet season. Velho et al. (1999, 2004) pointed out that this species is more abundant in lentic than in lotic systems. The higher abundance during the period with lower runoff velocities suggests the that hemispheric form of shell may difficult permanence of *Arcella vulgaris* in the periphyton during the wet season.

There is however clear evidence in our data and previous studies that differences in assemblage structure and species abundance are not independent of water quality or exclusively determined by hydrodynamics of habitats. The genus *Difflugia* was found to be better adapted to environmental conditions during the dry season not only in the periphyton but as well in planktonic communities river ahead of Cuiabá city (Silva Neto, 2001). In our study, *Difflugia mica* was always absent at Rc5, but frequent at the impacted Rc8 and with 23 and 30 counts, respectively during the dry and wet season. Moreover *Centropyxis aculeate, Centropyxis ecornis, Centropyxis discoides, Cochlipodium vestitum* and *Cyclopyxis eurystoma* were always several times more frequent at the impacted stations (Rc8, Rc12) than at Rc5, river ahead the urban area, this independent of the climatic period of the year. Contrary, *Centropixys aculeata*, when reported in the Pantanal was abundant under different water conditions (Hardoim, 1997).

Euglypha acanthophora only occurred at Rc8 and Rc12, a species already reported for plankton communities of the Cuiabá River (Missawa, 2000; Silva Neto, 2001) and in periphyton of the Pantanal of Poconé (Hardoim, 1997). In this last study, species presence was observed in slightly acid waters with low or intermediate saturation of DO, conditions common as well at both impacted stations of our study.

The preference for elevated nutrients loads and organic pollution is seen even more clearly for *Cyclopyxis plana microstoma,* which was never observed at Rc5, but had counts higher than 100 during the dry and the wet season under deteriorated water quality conditions. The species revealed therefore potential to be used as indicator for mesotrophic environments.

Higher abundances of *Arcella hemisphaerica* were only observed during the wet season, or at the impacted Rc8 station during the dry season. *Arcella hemisphaerica* has been described as common in mesotrophic environments, tolerant to organic pollution (Opravilová and Zelinka, 1978). Hardoim (1997) observed higher abundance of this species at the beginning and during the rainy season, at an impacted sampling station of Bento Gomes river in the border region between the Cuiabá depression and the northern

Pantanal floodplain. Differing to our results, where *Arcella discoides* seem to prefer enriched environments the species has been described in the Pantanal region as common, tolerant to different ecological water conditions, and abundant during low and high water conditions (Hardoim, 1997). Similar findings were obtained by studies from temperate climates (Sládecekova, 1962; Opravilová and Zelinka, 1978), where *Arcella discoides* is tolerant to broad variations of saprobity.

Conclusions

In the urban reach of the Cuiabá river, Testacean communities alter as a function of hydrodynamics and changing water quality between dry and wet season and between sampling stations. Richness and abundance is higher during the dry season and in the downstream sampling points, both characterized by lower oxygenation levels and higher nutrient concentrations.

Difflugia species are almost absent during the wet seasons. As there is evidence that this occurs mainly due to differences in river discharge, this genus has little potential as water quality indicator. Contrary, species such as *Cyclopyxis plana microstoma* and *Euglypha acanthophora* only occur at impacted sampling stations at both seasons, indicating a potential to indicate deteriorated water quality conditions.

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INFLUENCE OF DIFFERENT LONGITUDINAL DUNE POSITIONS IN THE GURBANTUNGGUT DESERT ON THE REPRODUCTION OF *HALOXYLON AMMODENDRON* SEEDLINGS

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Abstract: Field observations have been conducted on the survival mechanism of Haloxylon ammodendron seedlings in different longitudinal dune positions in the Gurbantunggut Desert for six consecutive years. In addition, the relationship between seedling settlement and environmental factors has been analyzed. *Haloxylon ammodendron* seedlings on windward slopes exhibit good natural reproduction, which is significantly higher than those on dune crests and leeward slopes (P=0.002). Wind erosion and sand burial impose restrictions on the natural production of *Haloxylon ammodendron* seedlings on dune crests and leeward slopes. Soil water content is the most important factor that influences the survival of seedlings. The other factors are herbaceous coverage, accumulated temperature at 30 cm aboveground from April to June, and vapor pressure deficit. Seedlings planted artificially and through afforestation can better adapt to wind erosion and sand burial. The average preserving rates of seedlings on windward slopes, leeward slopes, and dune crests are 35.67%, 29.6%, and 32.44%, respectively, which are significantly higher than that of naturally reproduced *Haloxylon ammodendron* seedlings (P<0.01). In afforestation, snow entropy combined with a water-retaining agent is applied during spring on 2-year-old seedlings artificially planted on dune crests and leeward slopes to increase the survival rate of seedlings.

Key words: *Gurbantunggut Desert; Haloxylon ammodendron; seedling reproduction;longitudinal dune position; soil water content*

Introduction

Haloxylon ammodendron, which belongs to genus *Haloxylon*, is an important component of the Tethys flora. This plant formation, which has the widest distribution of vegetation in the Asian desert region, consists mainly of *Haloxylon ammodendron* (Wu, 1995; West, 1983). In the Xinjiang Province in China, the distribution area of *Haloxylon ammodendron* is approximately 81.44 million km^2, occupying 68% of the total area with *Haloxylon ammodendron* in China. The distribution area of *Haloxylon ammodendron* in the Gurbantunggut Desert is approximately 71.95 million km^2, occupying 94% of Xinjiang. This area represents the most centralized distribution of *Haloxylon ammodendron* in China. However, the area of natural *Haloxylon ammodendron* forest is decreasing because of land development, groundwater mining,

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excessive deforestation, and overgrazing. Hence, *Haloxylon ammodendron* has been listed as a national grade 3 endangered plant in China (Guo et al., 2005).

Wind power, terrain, hydrology, vegetation, and sand dune types have various influences in the Gurbantunggut Desert (Wu, 1997). Along the prevailing wind direction, sand dunes can be divided into different longitudinal positions as windward slopes, leeward slopes, and dune crests. Windward and leeward slopes are stable or semi-stable sand dunes, respectively, whereas dune crests are moving at a width of 10 m to 40 m (Wang et al., 2005). As the dominant vegetation species in the Gurbantunggut Desert, *Haloxylon ammodendron* has high drought endurance and saline–alkaline tolerance (Zhou and Xu, 2002). This plant is commonly used as a pioneer greening tree species in desert regions (Bedunah and Schmidt, 2000), and has a significant role in maintaining the stability of desert sand dunes. Investigation on permanent sample plots in the Gurbantunggut Desert for six consecutive years (2006 to 2011) showed that the reproduction methods of *Haloxylon ammodendron* seedlings in different longitudinal dune positions exhibit significant differences. The reproduction rate of *Haloxylon ammodendron* seedling on windward slopes is higher than those on leeward slopes and dune crests. Vegetation on leeward slopes and dune crests is sparse, whereas the bare areas of sand dunes have high vegetation proportion, which causes wind erosion.

The established study indicates that different longitudinal positions of desert dunes exhibit significant differences in parameters such as soil moisture (Wang et al., 2006), ephemeral plant coverage (Wang et al., 2004), activity intensity of the sand surface (Guo et al., 2010), wind erosion, and sand burial (Wang and Lei, 1998), among others. Wind erosion in desert areas is not favorable to capturing seeds on the ground surface, whereas extreme sand burial thickness is not beneficial to seedling emergence (Wang and Lei, 1998; Li et al., 2011; Al-khalifah and Shanavaskhan, 2007). *Haloxylon ammodendron* has winged fruits and disperses mainly by wind. The ephemeral plant coverage on windward slopes is well-developed, thus creating conditions that allow the ground surface to capture *Haloxylon ammodendron* seeds. Appropriate sand burial thickness guarantees smooth emergence of *Haloxylon ammodendron* seedlings (Wang and Lei, 1998; Li et al., 2011), thus resulting in more *Haloxylon ammodendron* plants growing through natural reproduction on windward slopes. Wind deposition leads to excessively deep sand burial on leeward slopes (Wang and Lei, 1998), thus inhibiting the germination of seeds and the emergence of seedlings. Wind erosion on dune crests is excessive, and vegetation is sparse, which makes it difficult for the ground surface to capture seeds. Therefore, few seedlings grow on leeward slopes with deep sand burial and on dune crests with excessive wind erosion. The combined actions of biotic (e.g., ephemeral plant coverage) and abiotic (e.g., sand burial, wind erosion, and soil moisture) factors in different longitudinal dune positions influence capturing of *Haloxylon ammodendron* seeds. The successful settlement link of seedlings is also important in natural plant reproduction. However, reports on the influence of biotic and abiotic factors on different longitudinal dune positions on the settlement process of *Haloxylon ammodendron* seedlings are few.

Sand burial and wind erosion inhibit the natural reproduction of *Haloxylon* *ammodendron*. Vegetation on leeward slopes and dune crests in desert regions is particularly sparse, and thus, these areas become sources of dust. The established study demonstrates that because of insufficient "seedling banks" on leeward slopes and dune crests, the natural reproduction of *Haloxylon ammodendron* in microhabitats becomes challenging. Single prohibition measures have difficulty realizing the natural reproduction of seedlings on leeward slopes and dune crests. Thus, whether such measures can promote seedling reproduction in regions with wind erosion and sand burial by creating "seedling banks" of *Haloxylon ammodendron* through artificial recovery remains unanswered.

The distribution area of natural *Haloxylon ammodendron* and its coverage degree are decreasing because of excessive development and increasing environmental degradation. Some areas of moving sand dunes are gradually extending, whereas several fixed dunes tend to become moving sand dunes. Therefore, specifying the reproduction structure and formation mechanism of *Haloxylon ammodendron* in different longitudinal dune positions as well as investigating the feasibility of artificial recovery of *Haloxylon ammodendron* on dune crests and leeward slopes have significant implications in recovering *Haloxylon ammodendron* population. By focusing on the aforementioned phenomena, this study addresses the following issues: (1) exploring the reproduction mechanism of *Haloxylon ammodendron* in different longitudinal dune positions; (2) specifying the influences of biotic and abiotic factors in different longitudinal dune positions on the reproduction of *Haloxylon ammodendron* seedlings; (3) probing into the recovery feasibility of artificial vegetation in areas with wind erosion and sand burial (i.e., leeward slopes and dune crests), and providing a reference to recover *Haloxylon ammodendron* population and to practice wind erosion prevention and sand fixation.

Materials and methods

Study site

The Gurbantunggut Desert is located in the hinterland of the Junggar Basin in northwest China (44°11′ to 46°20′ N, 84°31′ to 90°00′ E), with an area that reaches 48,800 km^2. The Gurbantunggut Desert is the second largest desert in China. Located far from the ocean, it has a temperate arid desert climate. The annual precipitation is 80 mm to 190 mm. The annual average temperature is 5.0 °C to 5.7 °C. The annual evaporation capacity is 2,000 mm to 2,800 mm. The annual average wind velocity is 2.0 m.s^{-1} to 3.6 m.s^{-1}. The annual windy days are 174, and the prevailing wind direction is northwest. Compared with other arid regions, the Gurbantunggut Desert has 95 d to 110 d of stable snow-covered period each year, with a snow depth of 10 cm to 30 cm. *Haloxylon ammodendron* is the dominant species in this desert, and the ephemeral plant layer is well-developed. The vegetation coverage rate in the internal desert is 20% to 30%. Approximately 80% of the study area remains stable under good vegetation conditions (Wang et al., 2005). *Table 1* lists the characteristics of the longitudinal

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positions of sand dunes in different locations. These characteristics include gradient, aspect, soil water content, vegetation coverage, organic content, soil total salt, wind velocity, and precipitation.

Table 1. *Data on environmental factors in each location*

Location	Ter	Slope gradient (°)	Slope aspect	SWC (%)	Cov (%)	Org (%)	TSC (%)	Win ($m.s^{-1}$)	Pre (mm)	EVA (mm)
Kuitun	Windward slope	13	West	8.10	38.8	0.69	0.58	3.07	192	2096
	Leeward slope	27	East	8.20	15.3	0.65	0.46	2.15		
	Dune crest	/	/	6.50	12.2	0.72	0.53	3.62		
Shihezi	Windward slope	14	Northwest or North	6.50	24.4	0.85	0.64	3.21	117	2042
	Leeward slope	29	South or Southeast	6.1	10.3	0.88	0.55	2.21		
	Dune crest	/	/	4.9	7.7	0.73	0.56	3.75		
Jinghe	Windward slope	9	South	3.9	7.0	0.64	0.72	4.74	144	2100
	Leeward slope	25	North	4.3	5.7	0.48	0.65	3.29		
	Dune crest	/	/	3.8	3.9	0.54	0.63	3.94		

Note: Annual precipitation was based on the years 1999 to 2008. Climate data (Pre and EVA) come from weather station sites of state-owned farms. The farms where the sampling sites were located ran deeply into the desert. Each sampling site was 10 km to 20 km from the weather station.

Experimental design

Investigating the survival dynamic condition of Haloxylon ammodendron through natural reproduction

Sample regions with representative *Haloxylon ammodendron* growth and climate in the Gurbantunggut Desert, such as Jinghe (44°34.747′ N, 83°16.162′ E), Kuitun (44°47.739′ N, 84°59.897′ E), and Shihezi (45°1.681′ N, 86°18.294′ E), were selected. Three longitudinal dune positions, namely, dune crests, windward slopes, and leeward slopes, were also chosen in each location. The soil in all areas was sandy soil. Sample regions measuring 10 m × 10 m for each longitudinal dune position were randomly set, thus giving a total of 90 sample regions. The first investigation was conducted in September 2006. The survival conditions in each sample region of the three microhabitats of one-year-old *Haloxylon ammodendron* grown under the shrub crown, under the herb layer, and on open space were investigated. (In general, *Haloxylon ammodendron* blossoms and yields fruits after 5 years to 6 years of being planted. This study defines the growth of *Haloxylon ammodendron* seedlings from 1 year to 6 years). During the investigation, *Haloxylon ammodendron* appeared under the projection of the shrub crown, with renewed seedlings after falling under shrub crown. *Haloxylon ammodendron* appeared within the projection range of the herbaceous layer, with renewed seedlings after falling under the layer. The remaining seedlings were renewed after falling on open space. The one-year old seedlings grown on the sample regions were marked and their survival condition was recorded. During the same period, the survival condition of the marked *Haloxylon ammodendron* seedlings was tracked and investigated from 2007 to 2011.

Investigating the survival condition of Haloxylon ammodendron *seedlings through artificial reproduction*

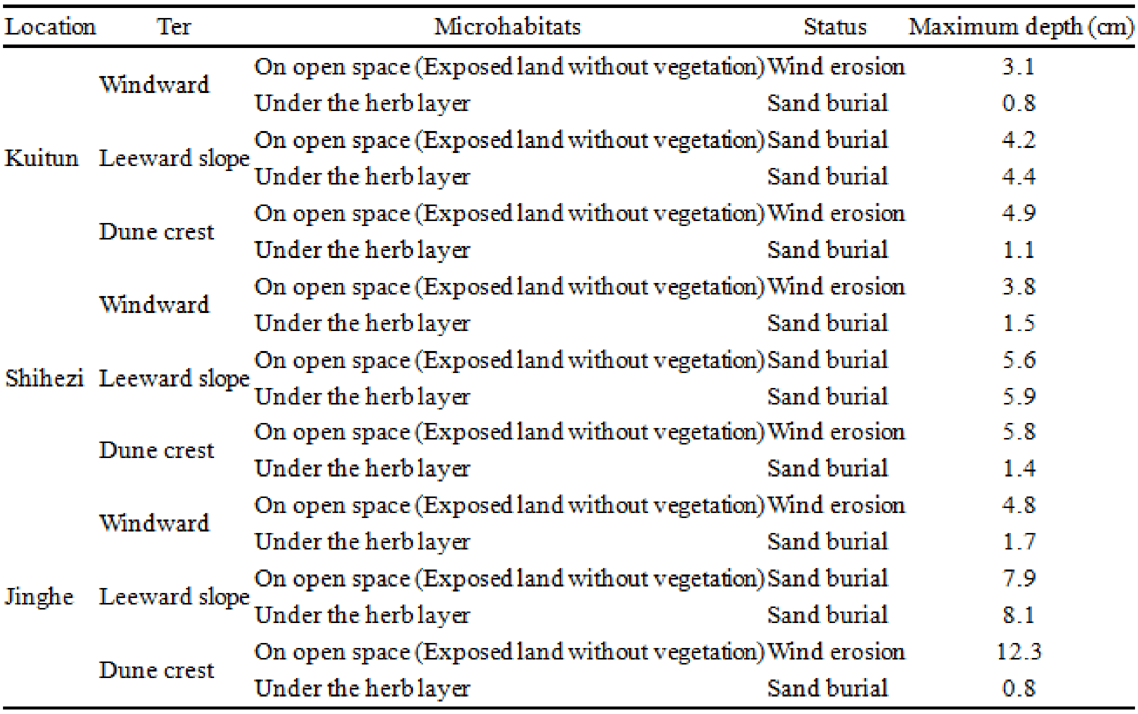
To explore the artificial reproduction potential of *Haloxylon ammodendron* on windward slopes, leeward slopes, and dune crests, other longitudinal dune positions in the spring of 2007 were set in Jinghe, Kuitun, and Shihezi. Snow entropy was combined with a water-retaining agent to plant two-year-old *Haloxylon ammodendron* seedlings. Ten sample regions measuring 10 m × 10 m were randomly set in each longitudinal dune position, thus giving a total of 90 sample regions. Trenching specification was 0.3 m × 0.3 m × 0.3 m. Transplant density was 2 m × 2 m, and the dosage of the water-retaining agent was 60 g per plant. Water was fully absorbed by the water-retaining agent (polyacrylamide and granulate; the highest water-absorbent rate was 312.5 times) to a completely saturated state (water was absorbed for 1 h) before planting and emphatically act on the bottom. A minimum distance of 2 m was maintained from mature *Haloxylon ammodendron* trees when planting. The investigation on the survival rate of *Haloxylon ammodendron* and its growth height was conducted for five consecutive years from September 2007 to September 2011.

Investigating the sample region and determining the indicators

It conducted in the artificially planted sample region. In June 2007, the first survey on indicators, such as the individual number of shrub and crown width [coverage was calculated by using the formula of elliptical areas, i.e., $C = \pi XY/4$, where X and Y refer to the major and minor axis lengths of each crown, respectively (Phillips and MacMahon, 1981)], was made. Three small quadrats measuring 1 m × 1 m were set in each quadrat. The coverage degree of the herbaceous plant in each small quadrat (Causton, 1988) was determined. The coverage degree in each 10 m × 10 m quadrat was also calculated. The indicators in each sample region, such as soil water content (SWC), wind velocity (Win), accumulated temperature at 30 cm aboveground from April to June (ATA), and vapor pressure deficit (VPD), were successively observed in 7 d intervals from April to September 2007 (actual observations were conducted 24 times). Meanwhile, automatic weather stations (Campbell® Scientific, Australia) observed the meteorological factors of Win, ATA, and VPD from 8:00 to 20:00 on the aforementioned days. The numerical values of the meteorological factors were recorded in 30 min intervals.

Soil samples from the four corners and the center were collected from depths of 0 cm to 10 cm and 10 cm to 30 cm to perform blending analysis in each sample region. The wet and dry weights of all soil samples were used to calculate SWC. pH was determined in a 1.0:2.5 (w:v) suspension of soil in water by using a pH/ORP/EC/°C field meter (Hanna® Instruments, USA). Total salt content (TSC) was determined by using an EC/TDS/°C meter (Hanna® Instruments, USA). Organic matter content (Org) was determined through the Tyurin method. The thickness of sand burial (SBD) was determined by adopting a drilling method, in which 9 iron bars were evenly inserted in each sample region. Wind erosion and SBD were measured from April to September. The measurement results are shown as *Table 2*.

Table 2. *Sand burial or wind erosion characteristics in each location*



Location	Ter	Microhabitats	Status	Maximum depth (cm)
Kuitun	Windward	On open space (Exposed land without vegetation)	Wind erosion	3.1
		Under the herb layer	Sand burial	0.8
	Leeward slope	On open space (Exposed land without vegetation)	Sand burial	4.2
		Under the herb layer	Sand burial	4.4
	Dune crest	On open space (Exposed land without vegetation)	Wind erosion	4.9
		Under the herb layer	Sand burial	1.1
Shihezi	Windward	On open space (Exposed land without vegetation)	Wind erosion	3.8
		Under the herb layer	Sand burial	1.5
	Leeward slope	On open space (Exposed land without vegetation)	Sand burial	5.6
		Under the herb layer	Sand burial	5.9
	Dune crest	On open space (Exposed land without vegetation)	Wind erosion	5.8
		Under the herb layer	Sand burial	1.4
Jinghe	Windward	On open space (Exposed land without vegetation)	Wind erosion	4.8
		Under the herb layer	Sand burial	1.7
	Leeward slope	On open space (Exposed land without vegetation)	Sand burial	7.9
		Under the herb layer	Sand burial	8.1
	Dune crest	On open space (Exposed land without vegetation)	Wind erosion	12.3
		Under the herb layer	Sand burial	0.8

Redundant variables analysis (RDA)

Monte Carlo testing was performed in the sample region to select the significant variables ($P<0.05$). The environmental factors that significantly affects *Haloxylon ammodendron* density are SWC, pH value (pH), Org, TSC, ATA, VPD, SBD, soil evaporation (EVA), annual precipitation (Pre), terrain (Ter), herbaceous layer cover degree (Cov), and Win. Other environmental factors are not significant, thus RDA is not analyzed (*Table 3*). The density of artificially planted *Haloxylon ammodendron* seedlings was determined for each sample region in September 2011. The matrix of [seedling density × sample region]$_{10\times9}$ was established; the selected 12 environmental factors were SWC, pH, Org, TSC, ATA, VPD, SBD, EVA, Pre, Ter, Cov, and Win. These factors constituted the matrix of [environment × sample region]$_{12\times9}$ and were used in the multiple quantity analysis of the relationship between vegetation and environment (ter Braak and Šmilauer, 2002) (*Table 3*).

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Table 3. *Forward selection analysis and Monte Carlo test of the environmental factors that affect seedling density of Haloxylon ammodendron*

Environmental factor	Seedling density	
	P-value	*R*-value
SWC (%)	0.001	0.51
pH	—	
Org (g/kg)	0.04	0.02
TSC (g/kg)	0.02	0.03
ATA (°C)	0.002	0.08
VPD	0.002	0.06
SBD (mm)	0.47	
EVA (mm)	—	
Pre (mm)	0.02	0.01
Ter	0.08	
Cov (%)	0.002	0.25
Win (m/s)	0.17	

$P<0.01$ indicates that the influence of environmental factors on the seedling density of *Haloxylon ammodendron* is significant at the 0.01 level. $P<0.05$ indicates that the influence of environmental factors on the seedling density of *Haloxylon ammodendron* is significant at the 0.05 level. "—" indicates that a collinearity relationship exists between environmental factors and other environmental factors. The *R*-value represents relative percentage. Terrain is evaluated by an artificial method: windward slope: 0, leeward slope: 0.5, dune crest: 1.

Data analysis

Data analysis was conducted via SPSS 15.0 (SPSS Inc., USA). The quantity variance of *Haloxylon ammodendron* with respect to different longitudinal dune positions, microhabitats, and reproduction manner (natural reproduction and artificial reproduction) adopted one-way ANOVA.

Results

Natural reproduction of Haloxylon ammodendron seedlings in different longitudinal dune positions

The survey results of the natural reproduction of *Haloxylon ammodendron* seedlings from 2006 to 2011 shows that the windward slopes in the three areas has the largest number of *Haloxylon ammodendron* seedlings, accounting for 72.39% ± 2.24% of the total number of seedlings and obviously exceeding those on dune crests and leeward slopes ($P=0.002$, $F=8.07$ $F_{crit}=5.61$). The difference between dune crests and leeward slopes is not significant, i.e., 13.70% ± 1.06% and 13.91% ± 1.29%, respectively (*Fig.*

1). In Kuitun and Shihezi, the distribution patterns exhibit that the seedlings under the herb layer has the largest number, followed by the seedlings on open space, and then the seedlings under the shrub crown (*Table 4*). In Kuitun, the number of seedlings under the herb layer, on open space, and under the shrub crown accounts for 66.49%, 21.40%, and 12.11%, respectively. In Shihezi, the number of seedlings under the herb layer, on open space, and under the shrub crown accounts for 66.49%, 21.40%, and 12.11%, respectively. In Jinghe, the seedlings under the shrub crown have the largest number (72.75%), followed by the seedlings under the herb layer (19.14%), and then the seedlings on open space (8.12%) (*Table 4*).

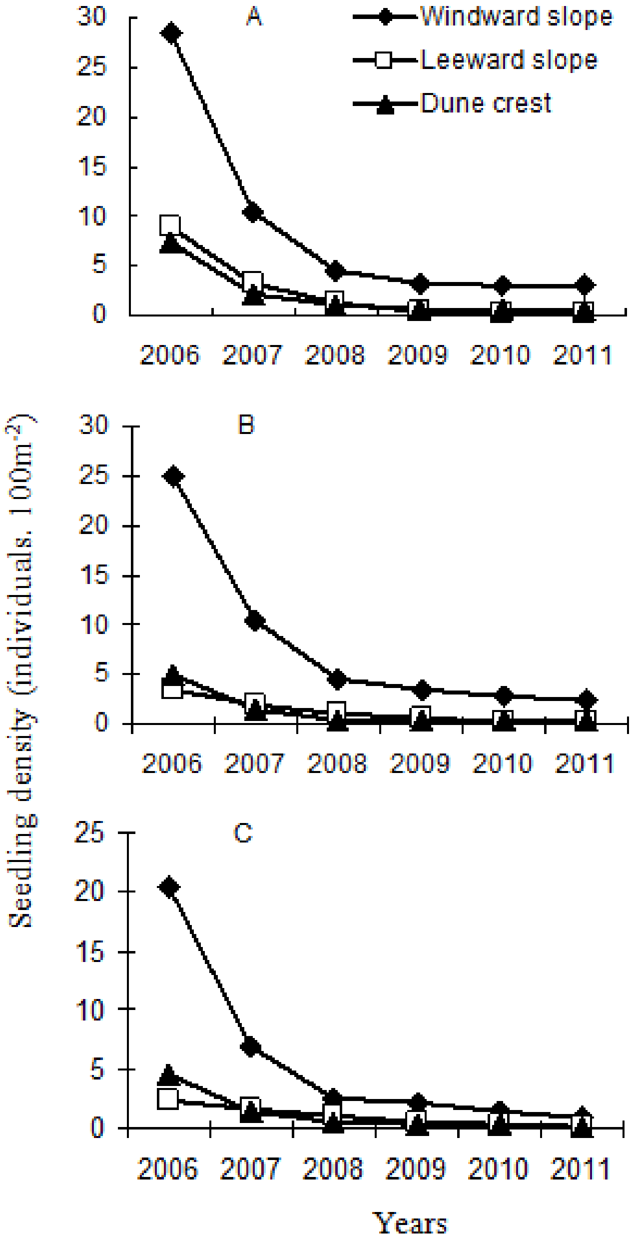

Figure 1. *The survival of naturally regenerated seedlings of Haloxylon ammodendron. Seedling density in A: Kuitun, B: Shihezi, and C: Jinghe*

Table 4. *The ratio of seedling numbers in each microhabitat*

Microhabitats	Kuitun	Shihezi	Jinghe
On open space (Exposed land without vegetation)	30.64%	21.40%	8.12%
Under the herb layer	59.41%	66.49%	19.14%
Under the shrub crown	9.95%	12.11%	72.75%

The death rate of *Haloxylon ammodendron* seedlings is higher during the first stage than in the latter stages. The average death rate in 2007 reached 58.9% ± 4.48%. The root system becomes stronger and deeper with the increasing age of *Haloxylon ammodendron* seedlings. Consequently, the growth condition and stress resistance of the plants also become stronger, and thus, the death rate gradually decreases. The average death rate in 2011 was only 22.08% ± 8.54%. Based on the statistics on the final survival rate of the seedlings in 2011, each sample region present the pattern in which windward slopes has the highest survival rate (8.31% ± 1.96%), followed by dune crests (6.81% ± 1.19%), and then leeward slopes (4.46% ± 0.69%).

Artificial reproduction of Haloxylon ammodendron seedlings in different longitudinal dune positions

For the 2-year-old seedlings of *Haloxylon ammodendron* which were artificially planted on different locations, i.e., windward slopes, leeward slopes, and dune crests, the 5-year consecutive surveys show that the average survival rate of the seedlings during the fifth year on windward slopes, leeward slopes, and dune crests are 24.4% ± 9.04%, 17.47 ± 6.9%, and 14.67 ± 6.08%, respectively (*Fig. 2*). The seedling survival rate for artificial reproduction is obviously higher than that for natural reproduction (P=0.008, F=9.06, F_{crit}=4.49).

The relationship between the survival of Haloxylon ammodendron seedlings and environmental factors

Among the environmental factors that affect the density of *Haloxylon ammodendron* seedlings, SWC is the most important, followed by Cov, ATA, and VPD. Other factors do not have a significant influence (*Fig. 3*). Kuitun has the highest SWC, followed by Shihezi, and then, Jinghe. Thus, the density of *Haloxylon ammodendron* seedlings presents a corresponding varying pattern.

Discussion

Restoring vegetation cover is significant in recovering ecosystem function (O'Brien and Zedler, 2006). However, vegetation cannot thrive through natural reproduction under harsh environmental conditions (Lindig–Cisneros and Zedler, 2002). Our research shows that wind erosion and sand burial are serious problems on leeward slopes and dune crests, which make these areas not suitable for *Haloxylon ammodendron* reproduction. *Haloxylon ammodendron* have winged fruits and they mainly rely on the wind for dispersal. Hence, surface substrate characteristics determine the distribution pattern of the seeds. The investigated sample regions in the Gurbantunggut Desert are not advantageous for surface capturing of *Haloxylon ammodendron* seeds because of low SWC, strong wind, unstable substrate characteristics, and other conditions on dune crests. Moreover, among the three longitudinal dune positions, uncovered dune crests result in wind-blown sand movements under the action of wind and sand (Wang et al., 2004), thus exhibiting wind erosion. After crossing a dune crest, wind-drift sand is obstructed by the dual effects of vegetation block and whirly vortex, and thus, wind velocity decreases sharply on leeward slopes, which causes eolian deposits (sand burial).

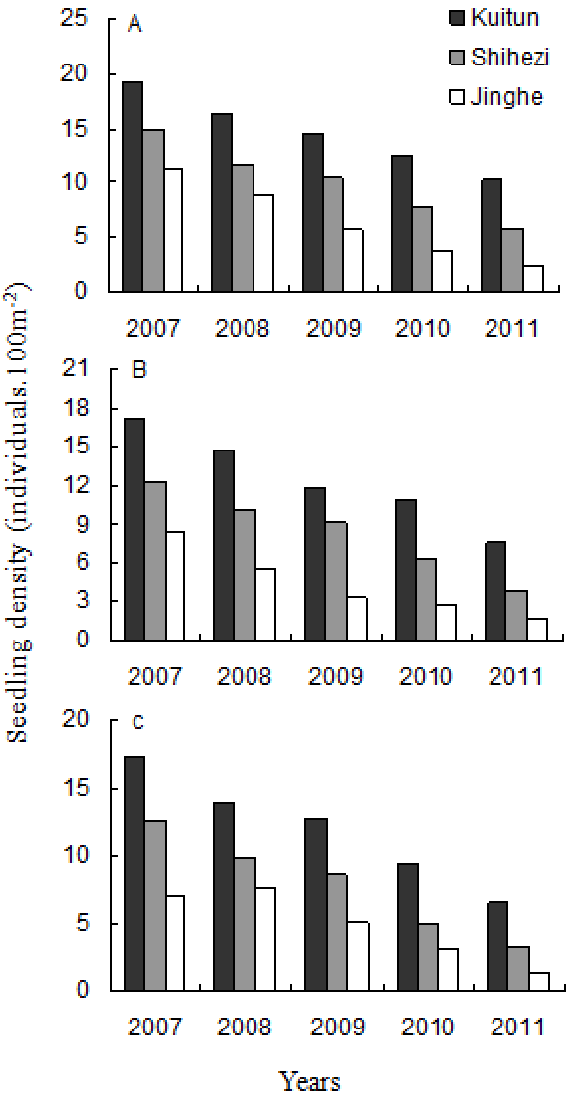

Figure 2. *The survival rate of planted Haloxylon ammodendron seedlings. A: Windward slope, B: Leeward slope, and C: Dune crest*

Among the three positions, the SBD on leeward slopes is the highest, exceeding 4.2 cm (*Table 4*), thus leading to the inhibition of *Haloxylon ammodendron* seed germination and seedling emergence (Li et al., 2011). This result indicates that longitudinal dunes affect natural reproduction by influencing ephemeral coverage, wind erosion, and sand burial (Maun, 1996; Huang and Gutterman, 1998), which is consistent with the research results of Jansen and Ison (1995) and Huang et al. (2004).

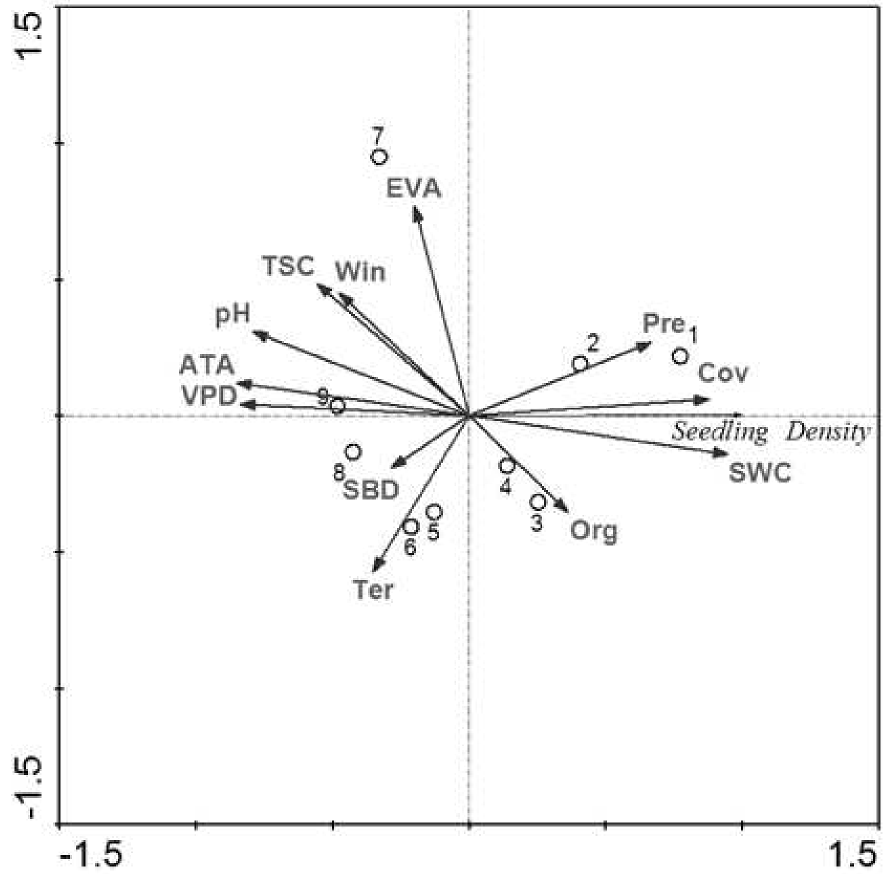

Figure 3. *RDA of the seedling density with environmental factors. The Hindu-Arabic numerals refer to the plots.*

Therefore, natural reproduction of *Haloxylon ammodendron* seedlings on dune crests and leeward slopes will be slow. Through afforestation, "seedlings banks" of *Haloxylon ammodendron* can be built, thus significantly improving the success rate of *Haloxylon ammodendron* reproduction. The results of our research show that artificially planting *Haloxylon ammodendron* seedlings have growth potentials, with full-grown root systems enabling the plants to absorb deep soil water (Marushia and Holt, 2008), thus allowing them to survive on sand dunes. The survival rate of artificially planted seedlings on windward slopes, dune crests, and leeward slopes are respectively 1.94, 2.91, and 1.15 times higher than that of naturally reproduced seedlings (*Figs. 1* and *2*), which further confirms that promoting the stable recovery of flowing sand surface by artificially building “seedlings banks” of *Haloxylon ammodendron* on dune crests and leeward slopes where natural reproduction is difficult is feasible.

Meanwhile, the harsh environmental conditions in deserts, such as drought, high temperature, and so on, result in high death rates. For example, the survival rate of *Eremosparton songoricum* (Fabaceae) in the Gurbantunggut Desert is only 2% (Liu et al., 2011). *Haloxylon ammodendron* is the dominant species in the Gurbantunggut Desert. However, its seedling death rate during the first year is still higher than 80% (Huang et al., 2009). The results of the research on seedling natural reproduction indicate that the final survival rates of the seedlings in their sixth year on the longitudinal dune positions of windward slopes, leeward slopes, and dune crests are 8.31%, 4.46%, and 6.81%, respectively, which are higher than the reproduction success rates of perennial plants in the Sonoran Desert (Ackerman, 1979) and the Mojave Desert (Bowers et al., 2004). Meanwhile, the 2-year-old artificially planted seedlings effectively avoid the high death rate during the initial growth stage. The final survival rate of artificially planted *Haloxylon ammodendron* seedlings in the first year is higher than 49.07%. During the fifth year, the final survival rates of the seedlings on windward slopes, leeward slopes, and dune crests are 24.40%, 17.47%, and 19.40%, respectively, thus exhibiting the advantages of artificially planting seedlings.

This research adopts RDA to investigate the influences of environmental factors on the survival of *Haloxylon ammodendron* seedlings. In the wilderness (desert) ecosystem, water is the most important factor that affects the survival of plants. Rainfall is the only source of SWC in the Gurbantunggut Desert. The high SWC that corresponds to more rainfall in Kuitun compared with in Jinghe and Shihezi also proves this statement. However, the SWC in Jinghe is generally lower than that in Shihezi even if rainfall in Jinghe is more abundant in Shihezi because the wind is strong in Jinghe and the soil, which is mainly coarse sand, has poor water-retaining property. At different longitudinal dune positions, soil water exhibit sideway movements along the slopes during vertical infiltration because of the short-term concentrated ablation of winter snow cover in early spring as well as the presence and downward movement of confining beds (tjaele). As a result, soil water in the sample plots forms a spatial distribution pattern in which windward and leeward slopes have higher SWCs than dune crests (Wang et al., 2006). A pattern in which the density of *Haloxylon ammodendron* seedlings increases with increasing SWC is also observed (Fig. 3). As the direct driving force of water evaporation, atmospheric drought can cause the death of plants as frequently reported (Yarranton and Yarranton, 1975). VPD indicates that the degree of atmospheric drought also controls the evaporation of surface soil water and the transpiration rate of vegetation. When atmospheric drought is severe, water evaporation loss on the surface soil of longitudinal dunes and water consumption of vegetation transpiration are higher (Liu et al., 2011). Consequently, the death rate of *Haloxylon ammodendron* seedlings will also be higher (Fig. 3). In addition to drought, high temperature also inhibits seedling survival (Callaway, 2007). On one hand, high temperature directly causes an increase in surface temperature that leads to burning the part of the stem closest to the ground. On the other hand, high temperature aggravates soil water stress and then reduces the vitality of *Haloxylon ammodendron*. Assimilating shoots wilt, thus significantly affecting the survival of *Haloxylon ammodendron* seedlings. Our study found that the density of *Haloxylon ammodendron* seedlings at each sample plot has a significant negative correlation with ATA (*Fig. 3*). By contrast, the presence of the herb layer relieves high temperature and reduces soil evaporation on microhabitats (Gong et al., 2008), thus promoting the survival of the seedlings. In the present research, coverage has a significant positive correlation with the density of *Haloxylon ammodendron* seedlings (*Fig. 3*). However, its specific mechanism still needs further study.

Conclusion

(1) The most important environmental factor that influence the survival rate of *Haloxylon ammodendron* seedlings is SWC, followed by Cov, ATA, and VPD.

(2) The main factors that cause poor reproduction of *Haloxylon ammodendron* seedlings on dune crests and leeward slopes are wind erosion and sand burial, whereas artificial seedling afforestation can promote reproduction success rate of *Haloxylon ammodendron* seedlings on dune crests and leeward slopes.

(3) In the Gurbantunggut Desert, water is the most significant factor that affects the survival of *Haloxylon ammodendron* seedlings (*Fig. 3*). Therefore, in some key areas such as the edges of an oasis, dune crests, and the longitudinal dunes on the leeward slopes on both sides of a desert road, water can effectively improve the survival rate of *Haloxylon ammodendron* seedlings by conducting seedling afforestation during early spring, which is the best period of SWC, and by taking advantage of water-retaining agents with excellent water-retention capacity.

(4) With better conditions on windward slopes, *Haloxylon ammodendron* seedlings have high survival rate on these positions. These plants can be protected by closing hillsides for afforestation to promote natural reproduction.

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AN EVALUATION OF PHYTOPLANKTON ASSEMBLAGE IN RELATION TO ENVIRONMENTAL VARIABLES OF NARMADA ESTUARINE REGION OF GULF OF KHAMBHAT, GUJARAT, INDIA

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Abstract. A study on the relationship between physicochemical parameters and phytoplankton assemblage in Narmada estuarine region (21◦40’05.19”N and 72◦34’26.90”E) of the Gulf of Khambhat, Gujarat, India was carried out from July 2009- June 2010. Principal Component Analysis with environmental variables like pH, dissolved oxygen, salinity, ammonia, phosphate, nitrate, silicate and chlorophyll-a reveled two factors influencing variability in the water nutrient composition up to 72% during the whole study period. Canonical Correlation Analysis between environmental variables and 31 dominant taxa of phytoplankton showed the freshwater influence on phytoplankton distribution in the estuarine zone. The maximum diversity in phytoplankton assemblage was observed during the post-monsoon (November, December, January, February) and pre-monsoon (March, April, May, June) period. The same period reported a high load of inorganic nutrients at the middle and upper reaches of the estuary due to anthropogenic influence and low freshwater flow in this zone.

Keywords: e*stuary; environmental variable; PCA; CC; phytoplankton*

Introduction

Estuaries are biologically dynamic zones with intensive exchange of matter and energy occurring between terrestrial and marine ecosystems (Prandle, 2009; Ahel, et al., 1996). Physicochemical properties play a major role in determining the density, diversity and occurrence of phytoplankton in marine and freshwater ecosystem. The quality and quantity of phytoplankton and their seasonal patterns have been successfully implicated in the quality of water and its capacity to sustain heterotrophic communities (White et al., 2004). Dynamic changes in; pH, trace metal speciation, concentrations of dissolved gases like oxygen, carbon dioxide, methane, inorganic nutrients (nitrate, phosphate, silicate) and organic compounds such as amino acids, organo-sulfur compounds are all closely associated with fluctuations in phytoplankton composition (George et al., 2012). Trophic linkages also exist, between phytoplankton who are primary producers and populations of consumer organisms including zooplankton, benthic invertebrates and fish. The phytoplankton communities are also useful indicators of estuarine water quality (Paerl et al., 2007).

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The variation of phytoplankton succession is strongly linked to meteorological and water stratification mixing processes in a tropical estuary (Devassy and Goes, 1988). The complex dynamism in physico-chemical characteristics of coastal waters is related to riverine flow, up welling, atmospheric deposition, vertical mixing and other anthropogenic sources. The coastal Gulf of Khambhat, Gujarat State, India, is a unique marine environment in the tropical belt with marked continental influence due to the drainage by 16 major and minor rivers in to the Gulf. In the present study involving one such riverine system, the Narmada estuarine region, hydro-chemical variables were evaluated to determine their role in phytoplankton distribution using various statistical methods.

Materials and Methods

Narmada estuarine region is located at geographical coordinates 21◦40’05.19”N, 72◦34’26.90”E. Three study sites in this region were selected Zadeshwar, Bhadbhut and Ambata (10-15 km between each sites) on the basis of upcoming industrial set ups and anthropogenic pressure at these regions (*Figure 1*).

Surface water samples were drawn at monthly intervals during the period from July 2009 to June 2010 at high tide. For nutrient analysis, water samples were collected in 1 L clean polythene bottles and kept in an ice box at 4°C and transported immediately to the laboratory. Physico-chemical parameters such as pH, dissolved oxygen (DO), phosphate (PO_4-P), nitrate (NO_3-N), ammonia (NH_4) and silicate (SiO_4-Si) were measured according to the standard procedures (APHA, 1998; Strickland and Parsons,1979). The data quality was ensured through careful standardization, procedural blank measurements, and using spike and duplicate samples. Measurements of *in situ* temperature (°C) and salinity (ppt) were made using probes, while DO was measured using Winkler’s method. The chlorophyll-a estimation was carried out in a spectrophotometer after filtering the samples using glass fiber filter papers and extraction in 90% acetone (Maiti, 2003).

Plankton samples were collected by filtering 10 liters of water through planktonic net of 20µ mesh size and were preserved in 4% formalin for future use. Plankton identification was carried out with help of literatures and books (Desikachary, 1959; Newell and Newell, 1977; Thomas, 1997). The enumerations of phytoplankton were carried out with the aid of light microscope by Lackey’s drop method (Lackey, 1938).

Results and Discussion

Principal component analysis (PCA) is one of the best statistical techniques for extracting relationships among a set of variables. Principal component analysis aims to transform the observed variables to a new set of variables of principal components (PC) which are arranged in decreasing order of importance. . Principal components are the linear combinations of original variables and are the eigenvectors (Reingner, 2008). The data obtained from the laboratory analysis were used as variable inputs for principal components analysis (PCA), for water samples described using the SPSS 17 package. Prior to the analysis, the data was standardized by transforming all data to have a zero mean and a unit standard deviation $(X_i-\mu)/\delta$, µ and δ are standard deviations of X_i’s. Rotation of data will ensure that the variability explained is more or less evenly distributed between the factors. From the standardized covariance or correlation matrix of the data, the initial factor solution was extracted by the multivariate principal components extraction, then a number of PC were selected only those with eigenvalues >1.0.

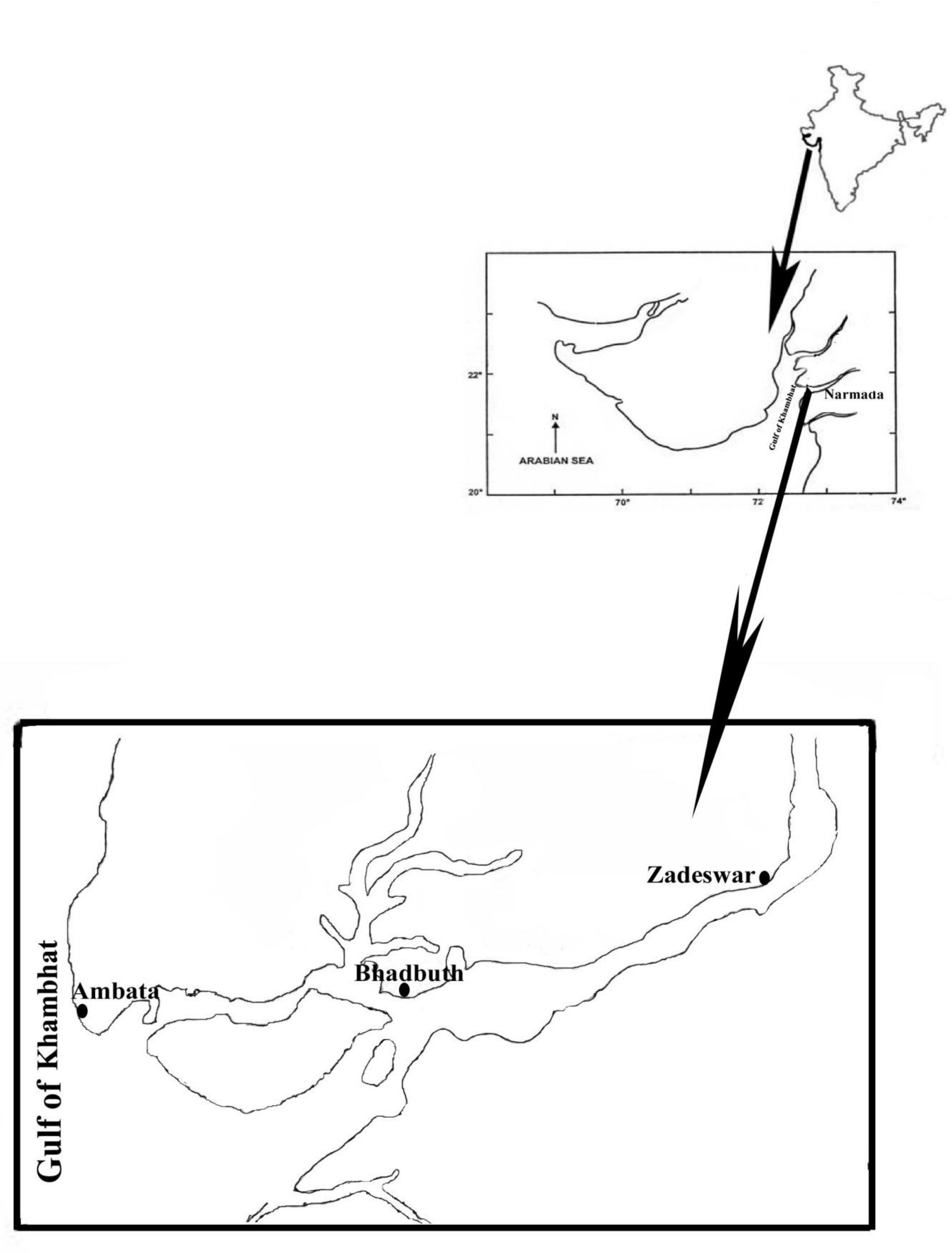

Figure 1. *Selected study sites on Narmada estuarine zone.*

Initial eigenvalues, percentage of variance and cumulative percentage obtained through principal component analysis is represented in table 1. Eigenvalues above 1.0 account for the first two axis and represent more than 76% of the variance. Components loading, which measure the degree of closeness between the variables and the PC, the largest loading either positive or negative, suggest the meaning of the dimensions; positive loading indicates that the contribution of the variables increases with the increasing loading in dimension and negative loading indicates a decrease. Temperature variation in Narmada estuarine region followed a similar trend observed in other tropical regions (Newall et al., 2011).

Table 1.*Total Variance Explained Narmada estuary*

	Initial Eigenvalues			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.582	62.021	62.021	4.658	51.754	51.754
2	1.309	14.541	76.562	2.233	24.808	76.562
3	.892	9.915	86.478			
4	.746	8.293	94.771			
5	.155	1.722	96.492			
6	.128	1.423	97.915			
7	.099	1.096	99.012			
8	.054	.595	99.607			
9	.035	.393	100.000			

PC1 accounts for 62.02% of the total variance, which is due to strong positive load of silicates (0.950), chlorophyll-*a* (0.870) and nitrate (0.794), ammonia (0.801) , dissolved oxygen (0.880) and a strong negative load of salinity (–0.842) and pH(-0.820). There is a significant positive correlation ($p \leq 0.05$) of pH with salinity (r = 0.81), where as silicates and chlorophyll showed a positive correlation with DO (r = 0.75 and 0.76 respectively). Ammonia showed a significant positive correlation with silicate (r=0.84) and chlorophyll-*a* (r=0.85) and silicate also found to be positively correlated with chlorophyll-a ((r=0.87). Silicate (r= -0.86) and chlorophyll-a (r= -0.65) showed a significant negative correlation with salinity (*Table 2*).

The highest value of ammonia is associated with freshwater inflow, and positive correlation with silicate indicates the freshwater origin of nutrients (Martin et al., 2008).The highest amount was reported in the upper reaches during the post monsoon period. In an estuarine environment, the primary productivity depends upon phytoplankton, which along with macrophytes contributes more than 90% of the total estuarine primary productivity (Bally et al., 1985). Thus, chl-*a*, which constitutes the chief photosynthetic pigment of phytoplankton, is an index that indicates the primary production potential of that system's biodiversity, biomass and carrying capacity (Nirmal, et al, 2009). Chlorophyll-*a* (3.5mg/L- 8.9 mg/L) having a significant positive correlation with DO and inorganic nutrients were observed to be high during the post monsoon period. The range of inorganic nutrient concentration during the study period is represented as box plot in *Figure 2*. The concentration of nitrate (0.489 mg/L- 1.84

mg/L) and phosphate (0.058 mg/L – 0.377mg/L) were observed to be high at the middle reaches, mostly during the post- monsoon and pre-monsoon because of surface run off, increased pollution load, low freshwater inflow and tidal influence (Pradhan et al., 2009).

Table 2. *Correlation between physicochemical parameters of Narmada estuary* ($P<0.05$).

	Temp	pH	DO	Salinity	Ammonia	Phosphate	Nitrate	Silicate	Chlorophyll
Temperature	1.000								
pH	.470	1.000							
DO	-.706	-.674	1.000						
Salinity	.238	.815	-.603	1.000					
Ammonia	-.253	-.581	.729	-.687	1.000				
Phosphate	-.302	-.067	.197	-.164	.015	1.000			
Nitrate	-.620	-.696	.638	-.658	.342	.504	1.000		
Silicate	-.431	-.739	.754	-.860	.841	.325	.692	1.000	
Chlorophyll	-.409	-.519	.765	-.654	.853	.422	.536	.871	1.000

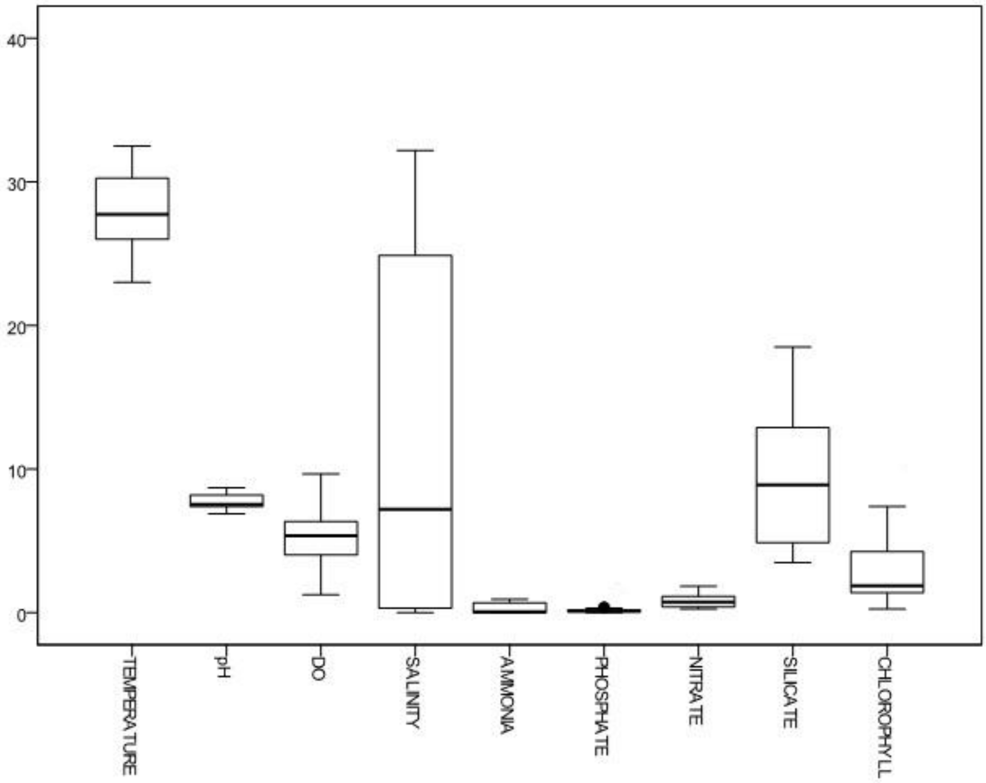

Figure 2. *Box plot showing variation of hydro-chemical parameters. All parameters are in mg/L except temperature and pH.*

PC2 explains 14.5% of the total variance with strong positive loading of NO_3-N (0.74) (*Table 3*). There is a strong positive correlation ($p<0.05$) of nitrate with silicate (*r*

= 0.69) and negative correlation with temperature (*r*=0.62), pH (*r*=0.69) and salinity (*r*=0.44). pH **showed** a clear variation from fresh water receiving sites to the highly saline area. Comparatively, the strong negative correlation between salinity and NO_3-N (*r* = -0.63) indicates the addition of NO_3-N from the riverine freshwater direction. The amount of nitrates (0.489 to 1.84mg/L) was observed higher in post-monsoon season followed by pre-monsoon season in the upper and middle reaches. The high amount of inorganic nutrients observed at upper reaches may be contributed by freshwater inflow (Satpathy et al., 2009).

Table 3. *Principle component loadings in Narmada estuary*

	Component	
	1	2
Temperature	-.606	-.499
PH	-.820	.145
DO	.880	.016
Salinity	-.842	.266
Ammonia	.801	-.475
Phosphate	.357	.742
Nitrate	.794	.411
Silicate	.950	-.133
Chlorophyll a	.870	-.067

Relationships between phytoplankton species composition and environmental factors were calculated by Canonical Correlation Analysis (Ariyadej, 2004) using *Statistica 9 software (Statsoft)***.** CCA is a direct ordination that selects the combination of environmental variables that maximize the dispersion of the scores of species (Palmer, 2006). Results of environmental variables are shown by lines radiating from the center of the graph along with the points for samples. The line representing environmental variable indicates the direction of maximum change of that variable across the diagram. The position of the species point represents the environmental preference of the species.

In Zadeshwar, a total of 35 species and 16 environmental variables (which may have contributed to phytoplankton distribution) were selected for CCA analysis. Eigenvalue of axis 1 (λ= 0.2027), 2 (λ= 0.1694), 3 (λ= 0.1065) and 4 (λ= 0.087) explained 72.88% of the relation between species and environmental data. Species having significant correlation (>0.5 to <-0.5) with axis were marked in bold. The length of environmental arrows and their orientation on the biplot indicates their relative importance to each axis. Environmental arrows represent a gradient where the mean value is located at the origin and the arrow points in the direction of its increase (*Fig.3*). In CCA analysis, the first axis can be interpreted as the marine water influence and the main contributors include pH, salinity, chloride, chlorinity, alkalinity, sodium, potassium, sulphate and EC. Chlorophyll-a, phosphate, DO, temperature and silicate concentration were significantly correlated to each other in one side of axis 1 whereas silicate and nitrate shows a significant correlation with axis 1 on another side (*Table 4*).

Oscillatoria accuminata, Anabena anomala, Closterium gracile, Cladophora glomerata showed positive correlation with axis 1 which signify the effect of nitrate and silicate on its distribution. Temperature was found to have a positive relation with

Scenedesmus quadricaudata. Cladophora glomerata. Phacus accuminatus, Navicula amphirhynclius, Gymnodium, Merismopedium punctata, Pinnularia elongatum showed a positive correlation with pH, salinity, chloride, chlorinity, alkalinity, sodium, potassium, sulphate and EC which indicate the significant role of these parameters in phytoplankton distribution. The effect of chemical factors, especially salinity and nutrient composition on phytoplankton distribution in Mahanadi estuary of India was studied by Naik et al. (2009) and they have reported higher phytoplankton count, chlorophyll-a concentration and nutrients during post-monsoon season which corroborated with our studies. Moreover, *Oscillatoria curviceps, O.subbrevis* and *Nostoc sp.* showed a positive correlation with chlorophyll-a and phosphate. While *Nitzschia plea, Merismopedium glauca, Anabena anomala, Pinnularia elongatum* and *Ankistrodesmus hantzschii* showed a positive correlation with silicate and nitrate. The values of each canonical variable of phytoplankton with the axis and the range of occurrence along with abbreviation are given in *Table 5*.

Table 4. *Correlation of environmental variables with axes in Zadeshwar*

Variables	**CV 1** (λ= **0.** 2027)	**CV 2** (λ= **0.** 1694)	**CV 3** (λ= **0.** 1065)	**CV 4** (λ= **0.** 087)
Temperature	**-0.500**	**0.741**	-0.006	0.074
pH	**-0.563**	0.309	-0.121	0.182
DO	-0.156	**-0.746**	0.095	-0.142
TS	0.175	0.378	-0.024	0.031
Salinity	**-0.612**	**0.450**	-0.365	0.154
Chloride	**-0.601**	**0.457**	-0.359	0.149
Alkalinity	**-0.703**	0.199	**-0.403**	0.231
Sodium	**-0.627**	0.295	-0.315	0.042
Potassium	**-0.651**	**0.421**	-0.247	0.229
Ammonia	-0.166	0.049	0.176	0.273
Phosphate	**-0.538**	-0.349	-0.265	0.225
Nitrate	0.011	**-0.458**	0.212	-0.256
Sulphate	**-0.583**	0.158	-0.324	0.362
Silicate	0.064	**-0.808**	0.094	0.111
Chlorophyll-a	-0.152	**-0.715**	-0.254	0.105
EC	**-0.534**	0.237	-0.124	0.226
% of Total variation	**26.13**	**21.83**	**13.73**	**11.19**

In Bhadbhut, a total of 31 species were reported and analyzed with 16 hydro-chemical parameters for CCA analysis. Eigenvalue of axis 1 (λ= 0.187), 2 (λ= 0.173), 3 (λ= 0.102) and 4 (λ= 0.064) explained 73.32% of the relation between species and environmental data (*Table 6*). Chlorophyll-*a* and silicate concentration showed a negative correlation with chloride, alkalinity, sodium, pH, potassium, sulphate and temperature. Phosphate, ammonia and nitrate showed positive correlation with axis 2. *Nitzschia obtusa, Chroococcus gigantium, Pediastrum simplex, Amphiprora alata, Oscillatoria subbrevis, Navicula amphirhynclius* and *Fragilaria crotonensis* did not explain much variation in distribution with change in physicochemical properties (Fig. 4). *Navicula viridis, Amphora ovalis* and *Chaetoceros tenuissimus* illustated close relationship with nitrate and phosphate. *Chlorella pyrenoidosa, Ankistrodesmus hantzschii, Ankistrodesmus flactus, Ceratium, Microcystis aeruginosa* and *Navicula amphirhynclius* found to have close relationship between DO, chlorophyll-*a* and silicate. Panigrahi et al. (2009) also reported the effect of these nutrients in phytoplankton distribution from their studies on brackish water ecosystem (Chilika Lagoon, India). *Closterium acerosum* and *Pleurosigma elongetum* found to be correlated with potassium, sulphate and temperature variation in the estuary. The correlation of phytoplankton with axis is given in the (*Table 7*) and the values having significant correlation are marked in bold.

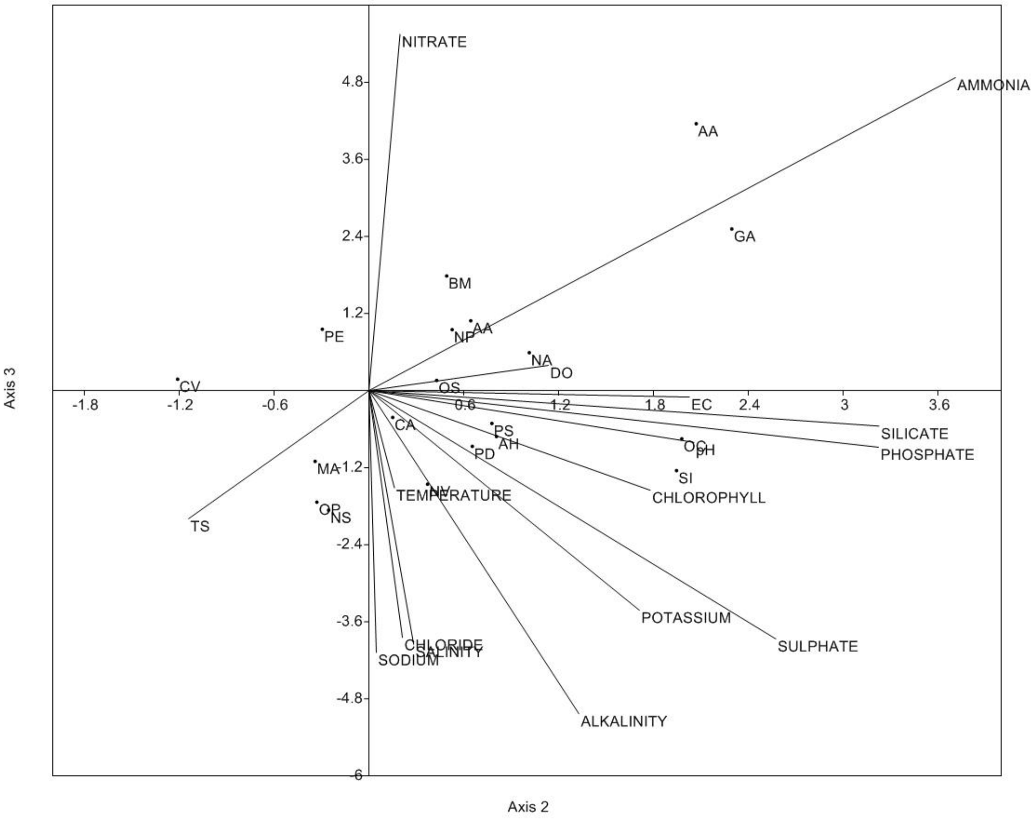

Figure 3. *CCA plot showing relationship between environmental variables and phytoplankton of Zadeshwar.*

Table 5. *List of phytoplankton taxa included in the canonical correlation analysis of Zadeshwar*

Variables	Abbreviation	CV 1	CV 2	CV 3	CV 4
Oscillatoria perornata	OP	-0.007	-0.070	**-0.559**	0.118
O. subbrevis	OS	0.217	-0.295	-0.130	0.165
O. acuminata	OA	**0.679**	-0.125	**-0.744**	0.072
O. curviceps	OC	-0.178	-0.395	-0.153	**0.549**
Spirulina subtilissima	SS	0.112	-0.307	**-0.724**	**0.519**

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Microcystis aeruginosa	MA	-0.137	0.118	-0.096	-0.065
Anaebena anomala	AA	**0.768**	-0.082	0.326	0.310
Merismopedium glauca	MG	0.100	-0.261	0.073	0.255
M. punctata	MP	0.021	-0.273	-1.211	0.443
Nostoc sp	NS	-0.061	-0.436	-0.318	-0.067
Ankistrodesmus hantzschii	AH	-0.092	-0.263	0.035	0.195
Clorella vulgaris	CV	-0.118	0.309	-0.113	-0.332
Closterium acerosum	CA	0.218	**-0.635**	-0.200	0.031
C. gracile	CG	**0.699**	0.390	**-0.572**	0.285
Cladophora glomerata	CGL	**0.662**	**0.964**	0.405	0.492
Scenedesmus quadricauda	SQ	0.477	**0.890**	0.227	0.120
Spirogyra indica	SI	-0.481	**0.774**	0.038	**0.796**
Pediastrum simplex	PS	0.014	-0.276	-0.045	0.262
P. duplex	PD	0.109	-0.286	-0.284	0.337
Amphiprora alata	AA	-0.157	-0.364	1.461	-0.193
Biddulphia mobiliensis	BM	0.192	0.008	0.528	-0.044
Coscinodiscus marginatus	CM	-0.176	**-0.800**	0.268	**-0.507**
Cymbellacistula	CC	0.039	-0.397	-0.337	0.383
C. tumida	CT	-0.226	**-0.573**	**0.862**	0.142
Gyrosigma acuminatum	GA	-0.110	**-0.481**	1.003	0.121
G. scalproides	GS	-0.187	-0.420	**0.931**	0.256
Navicula amphirhynclius	NA	-0.340	0.229	0.142	0.208
N. viridis	NV	-0.057	**-0.596**	-0.248	0.039
Pinnularia elongetum	PE	0.402	-0.331	0.041	-0.128
Nitzschia palea	NP	-0.158	-0.163	0.193	-0.044
Euglena gracilis	EG	**-0.573**	**0.534**	**-0.724**	**0.955**
E. ehrenbergii	EE	0.106	**-0.933**	-0.015	**0.539**
Phacus acuminatus	PA	**-0.521**	0.265	0.230	0.372
Peridinium	Pe	0.399	0.390	**-0.572**	0.285
Gymnodium	Gy	**-0.741**	**0.579**	0.313	-0.243

Table 6. *Correlation of environmental variables with axes of Bhadbhut*

Variables	**CV 1** (**λ= 0.** 187)	**CV 2** (**λ= 0.**173)	**CV 3** (**λ= 0.** 102)	**CV 4** (**λ= 0.** 064)
Temperature	**0.57**	**0.17**	0.23	-0.62
pH	0.40	**0.52**	0.31	-0.29
DO	-0.39	-0.23	-0.14	**0.65**
TS	-0.44	**0.58**	0.10	0.17
Salinity	0.05	**0.60**	0.31	-0.33
Chloride	0.05	**0.60**	0.31	-0.33
Alkalinity	0.14	**0.51**	**0.63**	-0.11
Sodium	0.15	0.29	0.39	-0.40
Potassium	0.44	**0.77**	0.14	-0.11

Ammonia	0.47	**0.49**	**0.62**	0.31
Phosphate	-0.39	0.45	0.05	0.25
Nitrate	-0.23	**0.57**	0.04	0.56
Sulphate	0.44	**0.50**	0.41	-0.40
Silicate	**-0.78**	0.00	**-0.57**	-0.08
Chlorophyll-a	**-0.62**	0.22	0.14	0.42
EC	0.11	**0.58**	0.44	0.07
% Total Variation	**26.07**	**24.13**	**14.25**	**8.87**

Table 7. *List of phytoplankton taxa included in the canonical correlation analysis of Bhadbhut*

Variables	**Abbreviation**	**CV 1**	**CV 2**	**CV 3**	**CV 4**
Oscillatoria perornata	OP	**0.58**	-0.21	0.03	0.29
O. subbrevis	OS	-0.03	-0.02	0.51	**-0.66**
Microcystis aeruginosa	MA	0.17	-0.18	-0.19	-0.03
Anaebena circularis	AC	**-0.58**	-0.65	0.18	-0.42
Merismopedium glauca	MG	**-0.56**	-0.16	-0.65	-0.39
Ankistrodesmus hantzschii	AH	**-0.79**	-0.27	0.14	-0.01
A. flactus	AF	**-0.63**	0.29	**0.75**	0.36
Chlorella pyrenoidosa	CP	**-0.82**	0.18	0.02	0.25
Closterium acerosum	CA	**0.88**	0.27	-0.17	**0.82**
Pediastrum simplex	PS	-0.04	0.23	**0.69**	-0.15
Spirogyra indica	SI	0.19	-0.41	0.14	-0.32
Amphiprora alata	AA	0.02	0.23	-1.09	-0.13
Amphora ovalis	AO	-0.26	0.21	-0.01	0.45
Biddulphia mobiliensis	BM	0.13	-0.22	-0.19	-0.22
Chaetoceros tenuissimus	CT	-0.30	**0.48**	-0.35	-0.29
Chroococcus gigantium	CG	-0.09	0.18	-0.17	0.44
Coscinodiscus marginatus	CM	**-0.62**	0.21	-1.15	-0.46
Cymbella cistula	CC	0.20	0.35	**-0.70**	-0.18
Fragilaria crotonensis	FC	0.02	-0.32	0.04	-0.16
Gyrosigma acuminatum	GA	0.25	-1.02	-0.76	-0.02
G. scalproides	GS	**0.72**	-3.31	0.33	0.03
Navicula amphirhynclius	NA	-0.42	-0.22	0.02	0.22
N. sphaerophora	NS	-0.03	0.27	0.01	0.33
N. viridis	NV	-0.32	0.28	-0.07	0.10
Nitzschia amphibia	NA	0.04	-0.24	**0.57**	-0.26
N. obtusa	NO	-0.03	0.27	0.01	0.33
Surirella nervosa	SN	**-0.55**	0.18	**-0.68**	**-0.69**
Pleurosigma elongetum	PE	**0.56**	**0.42**	0.12	-0.06
Euglena gracilis	EG	**0.72**	-0.31	0.33	0.03
Ceratium	Ce	**-0.66**	0.18	-0.78	-0.31
Gymnodium	Gy	-0.37	-0.92	**0.60**	**0.53**

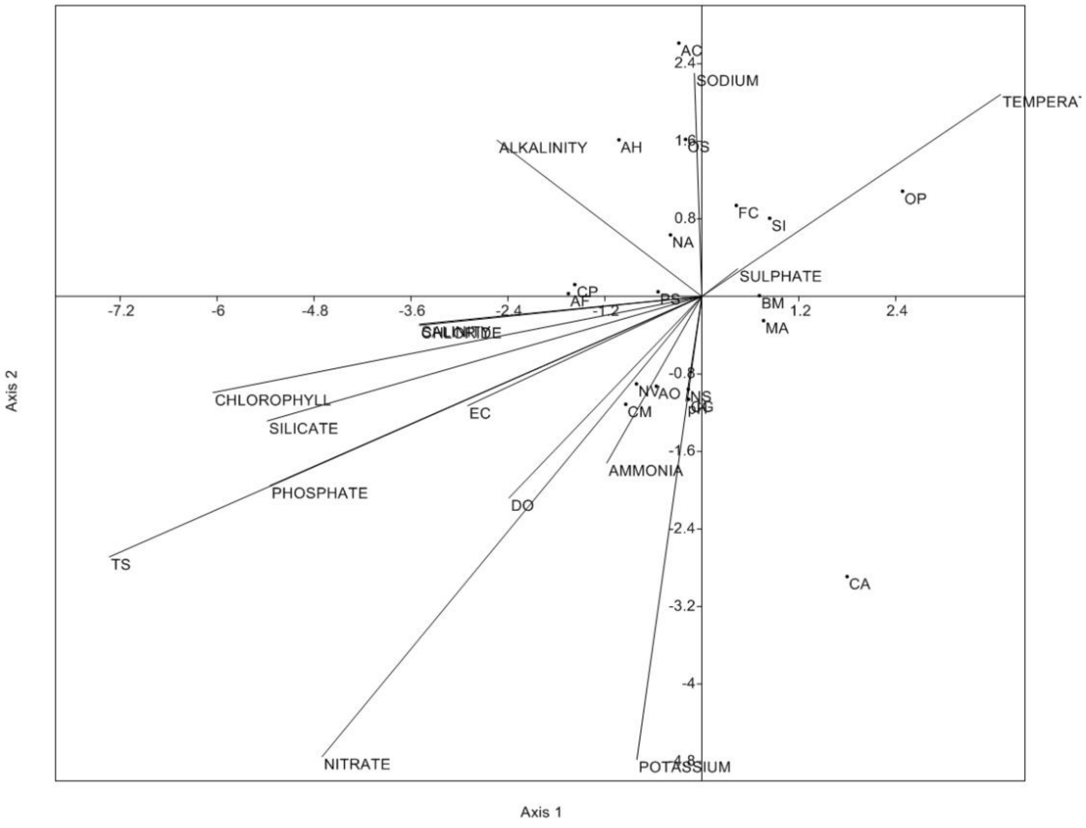

Figure 4. *CCA plot showing relationship between environmental variables and phytoplankton of Bhadbhut.*

Ambata site reported the least number of phytoplankton (26 species) dominated by Bacillariophycean members. Eigenvalue of axis 1 (λ= 0.361), 2 (λ= 0.271), 3 (λ= 0.234) and 4 (λ= 0.177) explained 70.44% of the relation between species and environmental data (*Table 8*). DO, nitrate, ammonia, chlorophyll-a, and silicate found to be correlated with each other and showed a negative correlation with potassium, temperature and salinity. These indicated the freshwater and marine water source of these nutrients in to the estuarine environment. *Peridinium, Ceratium, Amphora elliptica, Surirella nervosa* and *Gymnodium* showed a positive correlation with potassium and temperature. *Thalassionema nitzschioides, Chaetoceros tenuissimus, Merismopedium punctata, Fragilaria oceanic* and *Leptocylindrus danicus* found to be closely associated with concentration of nitrate, chlorophyll, ammonia and silicate (*Fig. 5*).Correlation of environmental variables with phytoplankton species were given in *Table 9*. Similar results were also obtained from shallow coastal station of Bay of Bengal by Choudhury and Pal, (2010) in which they observed prominent effect of temperature, pH, dissolved oxygen, salinity and nutrient contents-including nitrate, phosphate and silicate on Bacillariophycean distribution.

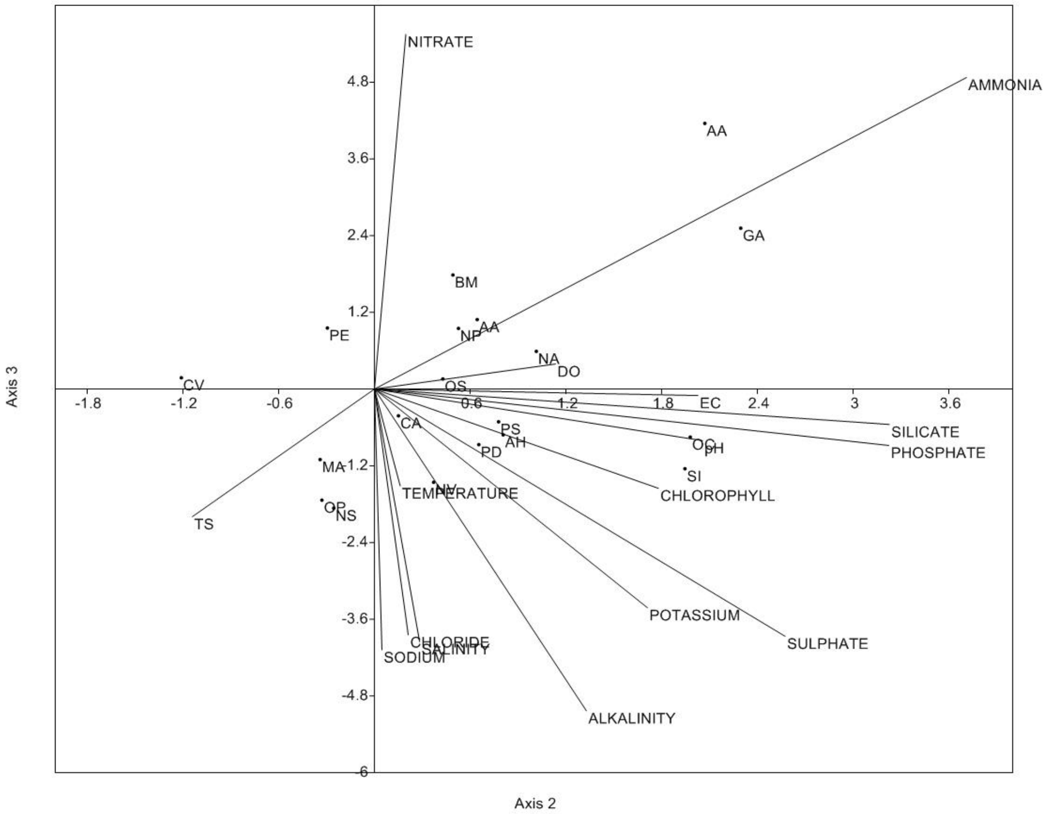

Figure 5.*CCA plot showing relationship between environmental variables and phytoplankton of Ambata.*

Table 8. *Correlation of environmental variables with axes in Ambata*

Variables	**CV 1** **(λ= 0.** .361)	**CV 2** **(λ= 0.** 271)	**CV 3** **(λ= 0.** 234)	**CV 4** **(λ= 0.** 176)
Temperature	0.11	0.42	0.18	-0.32
pH	-0.41	0.68	-0.14	-0.40
DO	-0.07	-0.49	-0.38	0.29
TS	-0.49	0.51	-0.34	0.05
Salinity	-0.16	0.57	-0.49	-0.42
Chloride	-0.16	0.57	-0.49	-0.42
Alkalinity	-0.17	0.56	-0.05	-0.25
Sodium	-0.66	0.47	-0.20	-0.36
Potassium	-0.05	0.65	-0.01	-0.38
Ammonia	-0.16	-0.62	-0.18	-0.21
Phosphate	-0.45	0.16	-0.42	-0.30
Nitrate	-0.48	-0.35	-0.13	0.24
Sulphate	-0.18	0.53	-0.32	-0.22

Silicate	-0.06	-0.68	-0.31	-0.04
Chlorophyll-a	-0.54	-0.48	-0.15	0.16
EC	-0.34	0.20	-0.72	-0.42
% Total variation	**21.41**	**16.08**	**13.87**	**10.47**

Table 9. *List of phytoplankton taxa included in the canonical correlation analysis of Ambata*

Variables	Abbreviation	CV 1	CV 2	CV 3	CV 4
Oscillatoria perornata	OP	**-0.50**	0.41	2.38	**0.98**
Microcystis aeruginosa	MA	-0.27	0.43	-0.34	**-0.44**
Merismopedium punctata	MP	-0.32	-0.24	1.65	0.07
Ankistrodesmus flactus	AF	-0.20	**0.87**	-0.29	0.21
Closterium gracile	CG	0.08	-0.04	**0.54**	-0.20
Amphiprora alata	AA	0.43	-0.07	-0.49	0.16
Amphora elliptica	AE	0.07	**0.96**	-0.14	-0.28
A. ovalis	AO	-0.07	0.54	0.09	-0.48
Chaetoceros tenuissimus	CT	**-0.45**	-0.14	**-0.55**	0.14
Coscinodiscus marginatus	CM	-0.32	0.22	0.21	0.27
Fragilaria crotonensis	FC	0.22	-0.14	-0.07	-0.32
F. oceanica	FO	**-0.53**	**-0.89**	0.09	**-0.66**
Gyrosigma acuminatum	GA	0.10	-0.03	**0.58**	0.11
G. scalproides	GS	3.34	-1.05	-0.32	0.47
Leptocylindrus danicus	LD	-0.13	**-0.57**	**0.86**	**-0.50**
Navicula cuspidata	NC	**-0.55**	-0.49	**-0.49**	0.01
N. radiosa	NR	-0.04	0.16	0.33	-0.08
Nitzschia amphibia	NA	0.27	-0.23	-0.11	0.13
Thalassionema nitzschioides	TN	-0.26	-0.20	-0.07	0.02
Pinnularia elongetum	PE	0.20	0.07	-0.13	-0.46
Surirella nervosa	SN	0.33	**0.81**	0.12	-0.18
Peridinium	Pe	-0.64	-0.38	-0.43	0.10
Ceratium	Ce	0.10	0.11	-0.09	-0.19
Gymnodium	Gy	0.31	**0.92**	**-0.65**	-0.14

In the present study phytoplankton have shown a positive correlation with salinity value at all sampling stations because estuarine regions are subjected to considerable fluctuations and these micro floras were well adapted to such dynamic environment (Lionard et al., 2005). Phytoplankton need a wide variety of chemical elements with the critical ones being nitrogen and phosphorous (Dawes, 1981). In the present study it was registered that phytoplankton showed positive correlation with phosphate and inorganic nitrogenous nutrients but the relationship was not very significant. This could be due to lower concentration or rapid recycling of these nutrients. Similar positive correlation between phytoplankton and nitrogenous organic nutrients were observed by Steinhart et al. (2002) on southern Chilean lakes and Hergenrader (1980) in salt valley reservoirs (California). Dawes (1981) reported a negative relationship of phytoplankton with temperature and turbidity which supports our present observed results. Studies carried out by Ye and Cai (2011) suggested that the occurrence of Cyanophycean and Chlorophycean members were directly proportional to the concentration of dissolved inorganic nitrogen and phosphate which also corroborated with our studies.

Most of the species abundance were found to be correlated with environmental variables, and this might be due to cosmopolitan characteristic of the species indicating the species tolerance to a wide range in water quality (Bonilla et al., 2005). A negative correlation was observed for Cyanophycean members like *Oscillatoria perornata* and *Merismopedium glauca* with environmental variables like chlorophyll-*a* and silicate. Most of the species belonging to Bacillariophyceae showed a positive correlation with environmental parameters like chlorophyll-a, silicate and phosphate. Chlorophycean members like *Ankistrodesmus flactus, Closterium acerosum* and *Spirogyra indica* showed a positive correlation with nitrate, DO and ammonia. Similar results were also obtained by Ye and Cai (2011) for their assessment on spring phytoplankton bloom of Xiangi Bay.

Navicula amphirhynclius and *Navicula radiosa* showed a positive correlation with pH and salinity which may have a major effect on its distribution. The close association of salinity and pH revealed the effect of tidal influence in the estuarine area. Temperature was found to have a positive relation with *Clorella vulgaris* and *Merismopedium punctata*. However, the negative correlation of temperature with ammonia and silicate showed the freshwater influence on these nutrients during post-monsoon season. *Navicula cuspidate, Surirella nervosa, Thalassionema nitzschioides, Amphiprora alata, Amphora ovalis, Coscinodiscus marginatus* and *Amphora elliptica* showed a positive correlation with chloroplyll-*a*, silicate and phosphate which indicates the significant role of these parameters in phytoplankton distribution (Harnstrom et al., 2009). *Anaebena anomala, Nitzschia amphibian* and *Gymnodium sp.* showed a negative correlation with inorganic nutrients, which showed their adaptability to a wide range of variations in physicochemical properties (Varis, 1991).

Conclusion

The present study summarizes the seasonal fluctuations of various physico-chemical parameters and plankton diversity in the coastal waters of the Narmada estuary as exploratory statistical data output. Freshwater discharges through the river and rivulets include additions of nitrate, phosphate and silicate to the coastal water mainly during the monsoon season (Martin et al., 2008). The addition of nitrogenous compounds and phosphorus compounds from anthropogenic sources such as fertilizer output, as an effect of industrialization and from agricultural runoff in the northern region of the Narmada estuary, has been observed during the monsoon in the water near the upper and middle reaches. Principal component analysis extracted two components (PC1 and PC2) responsible for the 76% variation observed in the estuary. The high load of nutrients like phosphate, nitrate and silicate during the monsoon contributes to the growth of phytoplankton community which is evident from the canonical correlation analysis. Hydrology and nutrients factors were found to be the main determining factors of phytoplankton distribution among the estuary (Costa et al., 2009). In the canonical correlation analysis the maximum correlation of phytoplankton with inorganic nutrients is linked to the abundance of these nutrients mostly entering during the monsoon season. The present study suggests that, hydrochemical variables play an important role in determining the phytoplankton distribution along estuarine gradient.

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ASSESSMENT OF ABOVEGROUND BIOMASS STOCK IN THE PACHAIMALAI FOREST OF EASTERN GHATS IN INDIA

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Abstract. Forest biomass plays a vital role in the global carbon cycle. The aim of the present study was to determine aboveground biomass of trees in the Pachaimalai forest of the Eastern Ghats in India. A sum of 5388 trees representing 131 species were recorded in twenty four transects (0.5 ha each) sampled from Pachaimalai. The total aboveground biomass stock for the 12 ha sampled was 608.2 tonnes. The average biomass value per transect was 25.3±5.6 t/0.5 ha, and it ranged from 4.2 to 103.5 t/0.5 ha. The average species richness, density and basal area per transect were 22±1.7 (species/0.5 ha), 225±9.5 (trees/0.5 ha) and 6.3±1.0 (m^2/0.5 ha) respectively. Among the 131 species, *Nothopegia heyneana* (Anacardiaceae) contributed the maximum (19.1%) to the total forest biomass followed by *Canthium dicoccum* var. *dicoccum, Albizia amara, Gyrocarpus asiaticus* and *Schleichera oleosa*. Linear regression analysis showed that forest biomass increased with the increase in basal area ($R^2 = 0.979$) and species richness ($R^2 = 0.702$), but not with density ($R^2 = 0.320$). The aboveground biomass of Pachaimalai forest falls within the range reported for other tropical forests of the world. The present study provides valuable data for the estimation of carbon stock of Pachaimalai forest, which would facilitate carbon stock modelling at national as well as at global level.

Keywords: *biomass; Eastern Ghats; India; Pachaimalai forest; regression analysis*

Introduction

Global climate is changing day by day as a result of natural variability and anthropogenic activities. Changes in the atmosphere, water cycle, solar inputs and land surface are evident (Negi et al., 2003; Alamgir and Al-Amin, 2008). Among the few global issues that have received more attention from scientists, resource managers, policy makers and public is climate change (Tiwari and Singh, 1987). Increase in carbon emission has become a major concern, which was well addressed in the Kyoto Protocol because it is the main causal factor in global warming (Alamgir and Al-Amim, 2008).

Tropical forests represent 30-40% of the terrestrial net primary productivity (Mitra et al., 2011). The rapid conversion of tropical forests was a major source of greenhouse gases such as carbon dioxide, methane, and nitrous oxide (Nascimento et al., 2002). The trees act as a major CO_2 sink which captures carbon from the atmosphere and store the same in the form of fixed biomass during the growth process (Chavan and Rasal, 2010).

Carbon stock includes aboveground biomass measurements as an essential aspect (Ketterings et al., 2001). Forest biomass can be determined either by direct methods or by indirect methods. Direct methods or destructive methods involve felling of trees to determine biomass (Salazar et al., 2010). Destructive techniques for biomass estimation are time consuming and expensive due to the large dimensions and the amounts of biomass that have to be processed and also against conservation (Verwijst and Telenius, 1999). Indirect means of estimation of forest biomass are based on allometric equations,

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using tree inventory data (Brown et al., 1989). Allometric relationship is the most preferred method since it yields a non-destructive and indirect measurement of biomass components and it also consumes less time and less expensive (Nath et al., 2009).

Allometric equation is a crucial step in the estimation of tree biomass, yet it is seldom directly tested (Crow, 1978; Brown et al., 1989; Houghton et al., 2001; Chave et al., 2005). Since, a hectare of tropical forest may be home to as many as 300 different tree species and one cannot use species-specific regression models, as in the temperate zone (Shepashenko et al., 1998; Brown and Schroeder, 1999).

With regard to the estimation of tree biomass, allometric equations using the diameter of the tree are a strong predictor of biomass for practical purposes (Salazar et al., 2010), owing to the ease and precision with which it can be obtained and because it relates the total volume of biomass to functional processes such as transport and the age of the tree (Satto and Madgwick, 1982). In the other hand, allometric equations using tree height along with tree diameter is also used, but it does not improve the results, besides it represents an additional hindrance in data compilation (Salazar et al., 2010). Further, it is often difficult to measure the height in dense forests (Segura, 2005). Hence, tree diameter alone was used as the independent variable for the estimation of forest biomass in most of the earlier studies.

Forest biomass data are available for different regions of the world (Murphy and Lugo 1986; Brown et al., 1989; Laurance et al., 1997; Clark and Clark, 2000; Mani and Parthasarathy, 2007; Salazar et al., 2010), but the biomass stock of the Pachaimalai forest of the Eastern Ghats in India was not estimated. Hence, the present study was undertaken to determine the biomass stock in Pachaimalai forest.

Materials and methods

Study area

The present study was undertaken in the Pachaimalai forest of the Eastern Ghats in India (Latitude-11°08.5'-11°28.5' N; Longitude-78°29.0'-78°48.5' E) (*Fig. 1*). Pachaimalai forest covered an area of about 586 km^2, altitudes ranged from 200 m to 1021 m above mean sea level. The study area composed of masses of Charnockite associated with gneisses and varied metamorphic rocks, and soil was red, loamy and rich in aluminum and iron oxides (Pragasan and Parthasarathy, 2010). Climate data such as the mean annual temperature and rainfall during the 1988-2007 (20 years) for Salem, the nearest station to Pachaimalai forest were 28.3°C and 1058 mm, respectively. The bulk of the rainfall was received from August to October, and the mean annual rainy days were 61 days. Tribal settlements were common in Pachaimalai forest. The hilly terrain and the surrounding plains of Pachaimalai forest were densely populated. Increasing human population, encroachment of forest land, expanding agriculture, poor harvesting methods and over-exploitation of wild species besides the invasion of the weed species such as Lantana camara constitute major threats to the study area.

Methods

The Pachaimalai forest was divided into twenty four grids of size 6.25 km × 6.25 km. Within each grid, a transect of 0.5 ha (5 m × 1000 m) area was laid, and all the trees with ≥ 30 cm girth at breast height (1.37 m from the ground level) were inventoried (Pragasan and Parthasarathy, 2010). The inventory data was used for determination of

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biomass stock in Pachaimalai forest. The aboveground biomass of trees were estimated through allometric equation using tree diameter following Brown et al. (1989), $Y = 34.4703 - 8.0671D + 0.6589D^2$, where ‘Y’ represents biomass per tree in kg, and ‘D’ is diameter in cm. Regression analysis was performed to find the relationships of forest biomass with species richness, density and basal area.

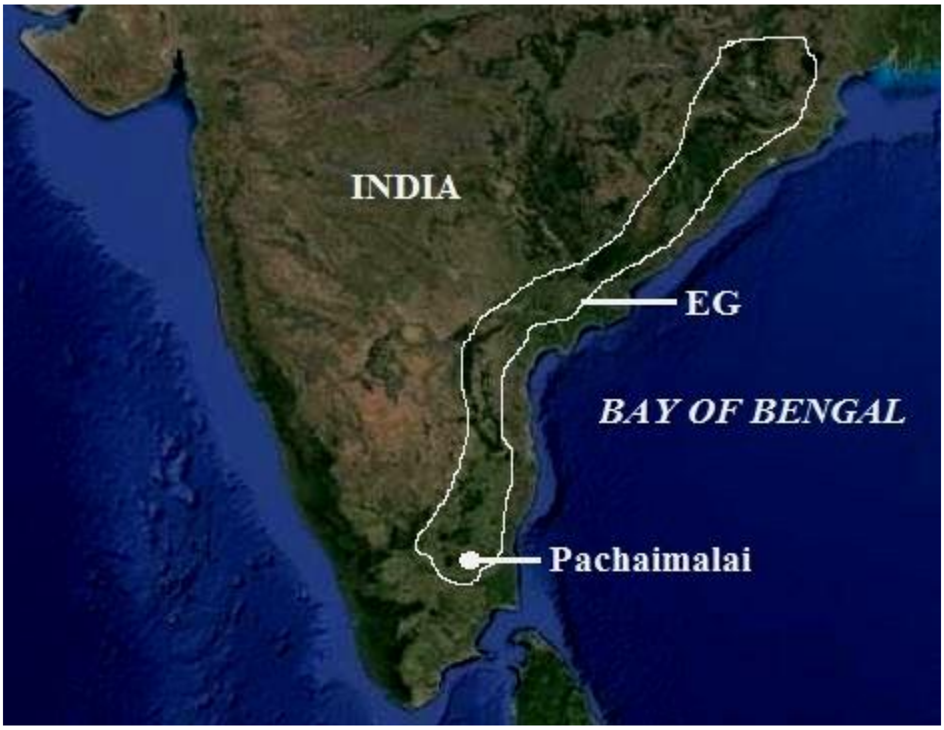

***Figure 1**. Location of the study area, Pachaimalai forest of the Eastern Ghats in India*

Results

Vegetation structure

A sum of 5388 trees representing 131 species in 43 families were recorded in twenty-four 0.5 ha transects sampled from the Pachaimalai forest in India. The average species richness per transect was 22±1.7 species (±S.E.), and it ranged from 12 to 38 species per transect. The tree density per transect was 225±9.5 trees/0.5 ha. The density among the 24 transects was varied from a low of 130 trees/0.5 ha to a high of 348 trees/0.5 ha. The average standing basal area of trees per transect was 6.3±1.0 m^2/0.5 ha, and it varied from 1.6 to 20.0 m^2/0.5 ha among the 24 transects in Pachaimalai forest.

Forest biomass

The total aboveground biomass estimated for the 12 ha (24 0.5 ha transects) sampled in Pachaimalai forest was 608.2 tonnes. The average biomass of trees per transect was 25.3±5.6 t/0.5 ha, and it ranged from 4.2 to 103.5 t/0.5 ha. Among the 131 species recorded, *Nothopegia heyneana* (Anacardiaceae) shared a maximum of 116.39 tonnes (19.1%) to total biomass followed by *Canthium dicoccum* var. *dicoccum* (Rubiaceae) (5.9%), *Albizia amara* (Mimosaceae) (5.7%), *Gyrocarpus asiaticus* (Hernandiaceae) (4.2%), and *Schleichera oleosa* (Sapindaceae) (3.8%) *(Table 1)*. The top five species

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together shared 38.7% of total aboveground biomass estimated for the 12 ha sampled in Pachaimalai.

***Table 1**. The aboveground biomass (AGB, in tonnes) of 131 tree species recorded from the twenty four transects (covering 12 ha area) sampled from the Pachaimalai forest*

Species (Family)	**AGB**	**(%)**
Nothopegia heyneana (Hook.f.) Gamble (Anacardiaceae)	116.39	19.1
Canthium dicoccum (Gaertn.) Teijsm. and Binn. var. *dicoccum* (Rubiaceae)	35.95	5.9
Albizia amara (Roxb.) Boivin (Mimosaceae)	34.92	5.7
Gyrocarpus asiaticus Willd. (Hernandiaceae)	25.31	4.2
Schleichera oleosa (Lour.) Oken (Sapindaceae)	23.01	3.8
Vitex altissima L.f. (Verbenaceae)	22.32	3.7
Euphorbia antiquorum L. (Euphorbiaceae)	20.77	3.4
Drypetes roxburghii (Wall.) Hurusawa (Euphorbiaceae)	20.77	3.4
Commiphora caudata (Wight and Arn.) Engler (Burseraceae)	16.46	2.7
Chukrasia tabularis A.Juss. (Meliaceae)	16.44	2.7
Memecylon edule Roxb. (Melastomataceae)	16.15	2.7
Scolopia crenata (Wight and Arn.) Clos (Flacourtiaceae)	15.60	2.6
Diospyros ebenum Koen. (Ebenaceae)	15.39	2.5
Mangifera indica L. (Anacardiaceae)	14.26	2.3
Acacia leucophloea (Roxb.) Willd. (Mimosaceae)	12.67	2.1
Commiphora berryi (Arn.) Engler (Burseraceae)	9.56	1.6
Anogeissus latifolia (Roxb. ex DC.) Wall. ex Guill. and Perr. (Combretaceae)	9.52	1.6
Acacia planifrons Wight and Arn. (Mimosaceae)	9.11	1.5
Celtis philippensis Blanco (Ulmaceae)	8.13	1.3
Ficus nervosa Heyne ex Roth (Moraceae)	8.12	1.3
Pterospermum xylocarpum (Gaertn.) Sant. and Wagh (Sterculiaceae)	7.53	1.2
Terminalia paniculata Roth (Combretaceae)	7.43	1.2
Chloroxylon swietenia DC. (Flindersiaceae)	7.37	1.2
Pleiospermium alatum (Wall. ex Wight and Arn.) Swingle (Rutaceae)	6.10	1.0
Syzygium cumini (L.) Skeels (Myrtaceae)	5.47	0.9
Terminalia bellirica (Gaertn.) Roxb. (Combretaceae)	5.31	0.9
Givotia rottleriformis Griff. (Euphorbiaceae)	5.19	0.9
Lannea coromandelica (Houtt.) Merr. (Anacardiaceae)	4.91	0.8
Drypetes sepiaria (Wight and Arn.) Pax and Hoffm. (Euphorbiaceae)	4.55	0.7
Margaritaria indica (Dalz.) Airy Shaw (Euphorbiaceae)	4.38	0.7
Bridelia crenulata Roxb. (Euphorbiaceae)	4.37	0.7
Dolichandrone arcuata (Wight) Clarke (Bignoniaceae)	3.98	0.7
Ficus mollis Vahl (Moraceae)	3.71	0.6

Atalantia monophylla (L.) Correa (Rutaceae)	3.67	0.6
Pongamia pinnata (L.) Pierre (Papilionaceae)	3.64	0.6
Phyllanthus polyphyllus Willd. (Euphorbiaceae)	3.58	0.6
Acacia farnesiana (L.) Willd. (Mimosaceae)	3.45	0.6
Diospyros ferrea (Willd.) Bakh. var. *buxifolia* (Rottb.) Bakh. (Ebenaceae)	3.39	0.6
Strychnos nux-vomica L. (Loganiaceae)	3.25	0.5
Wrightia tinctoria (Roxb.) R.Br. (Apocynaceae)	3.03	0.5
Azadirachta indica A.Juss. (Meliaceae)	2.97	0.5
Mimusops elengi L. (Sapotaceae)	2.77	0.5
Clausena dentata (Willd.) M.Roem. (Rutaceae)	2.50	0.4
Cinnamomum malabatrum (Burm.f.) Blume (Lauraceae)	2.26	0.4
Euodia lunu-ankenda (Gaertn.) Merr. (Rutaceae)	2.21	0.4
Terminalia chebula Retz. (Combretaceae)	2.15	0.4
Cassine glauca (Rottb.) Kuntze (Celastraceae)	2.10	0.3
Manilkara hexandra (Roxb.) Dubard (Sapotaceae)	2.10	0.3
Acacia horrida (L.f.) Willd. (Mimosaceae)	2.08	0.3
Acacia chundra (Roxb. ex Rottl.) Willd. (Mimosaceae)	2.07	0.3
Lepisanthes tetraphylla (Vahl.) Radlk. (Sapindaceae)	2.03	0.3
Aglaia jainii M.V.Viswan. and K.Ramach. (Meliaceae)	1.98	0.3
Ficus drupacea Thunb. var. *pubescens* (Roth) Corner (Moraceae)	1.90	0.3
Strychnos potatorum L.f. (Loganiaceae)	1.75	0.3
Holoptelea integrifolia (Roxb.) Planch. (Ulmaceae)	1.71	0.3
Diospyros ovalifolia Wight (Ebenaceae)	1.70	0.3
Nothopegia racemosa (Dalz.) Ramam. (Anacardiaceae)	1.61	0.3
Hardwickia binata Roxb. (Caesalpiniaceae	1.34	0.2
Albizia odoratissima (L.f.) Benth. (Mimosaceae)	1.23	0.2
Premna tomentosa Roxb. (Verbenaceae)	1.22	0.2
Pterocarpus marsupium Roxb. (Papilionaceae)	1.15	0.2
Moringa concanensis Nimmo ex Gibs. (Moringaceae)	1.14	0.2
Ziziphus mauritiana Lam. (Rhamnaceae)	1.01	0.2
Diospyros montana Roxb. (Ebenaceae)	0.97	0.2
Chionanthus zeylanica L. (Oleaceae)	0.93	0.2
Canthium dicoccum (Gaertn.) Teijsm. and Binn. var. *umbellata* (Wight) Sant. and Merch. (Rubiaceae)	0.91	0.2
Ixora pavetta Andr. (Rubiaceae)	0.87	0.1
Beilschmiedia bourdilloni Brandis (Lauraceae)	0.82	0.1
Citrus medica L. (Rutaceae)	0.77	0.1
Litsea deccanensis Gamble (Lauraceae)	0.74	0.1
Dalbergia latifolia Roxb. (Papilionaceae)	0.72	0.1
Erythroxylum monogynum Roxb. (Erythroxylaceae)	0.70	0.1
Casearia rubescens Dalz. (Flacourtiaceae)	0.68	0.1

Tarenna asiatica (L.) Kuntze (Rubiaceae)	0.67	0.1
Ficus beddomei King (Moraceae)	0.64	0.1
Grewia tenax (Forssk.) Fiori (Tiliaceae)	0.60	0.1
Catunaregam spinosa (Thumb.) Tiruvengadum (Rubiaceae)	0.60	0.1
Alstonia scholaris (L.) R.Br. (Apocynaceae)	0.59	0.1
Erythrina stricta Roxb. (Papilionaceae)	0.55	0.1
Careya arborea Roxb. (Lecythidaceae)	0.49	0.1
Terminalia crenulata Roth (Combretaceae)	0.49	0.1
Bauhinia racemosa Lam. (Caesalpiniaceae)	0.48	0.1
Sapindus emarginatus Vahl. (Sapindaceae)	0.48	0.1
Alseodaphne semicarpifolia Nees var. *semecarpifolia* (Lauraceae)	0.47	0.1
Antiaris toxicaria (Pers.) Lesch. (Moraceae)	0.45	0.1
Atalantia racemosa Wight and Arn. (Rutaceae)	0.44	0.1
Limonia acidissima L. (Rutaceae)	0.41	0.1
Filicium decipiens (Wight and Arn.) Thw. (Sapindaceae)	0.39	0.1
Spondias pinnata (L.f.) Kurz (Anacardiaceae)	0.38	0.1
Mallotus philippensis (Lam.) Muell.-Arg. (Euphorbiaceae)	0.36	0.1
Dolichandrone atrovirens (Heyne ex Roth) Spangue (Bignoniaceae)	0.35	0.1
Mallotus stenanthus Muell.Arg. (Euphorbiaceae)	0.33	0.1
Dichrostachys cinerea (L.) Wight and Arn. (Mimosaceae)	0.23	<0.1
Bischofia javanica Blume (Bischofiaceae)	0.21	<0.1
Ficus microcarpa L.f. (Moraceae)	0.21	<0.1
Madhuca longifolia (Koen.) Macbr. var. *longifolia* (Sapotaceae)	0.20	<0.1
Ehretia pubescens Benth. (Cordiaceae)	0.17	<0.1
Streblus asper Lour. (Moraceae)	0.16	<0.1
Morinda pubescens J.E. Smith (Rubiaceae)	0.15	<0.1
Albizia lebbeck (L.) Benth. (Mimosaceae)	0.11	<0.1
Cipadessa baccifera Miq. (Meliaceae)	0.10	<0.1
Benkara malabarica (Lam.) Tirvengadum (Rubiaceae)	0.09	<0.1
Cassia fistula L. (Caesalpiniaceae)	0.09	<0.1
Murraya koenigii (L.) Spreng. (Rutaceae)	0.08	<0.1
Diospyros barberi Ramaswami (Ebenaceae)	0.08	<0.1
Flacourtia indica (Burm.f.) Merr. (Flacourtiaceae)	0.07	<0.1
Ochna obtusata DC. (Ochnaceae)	0.07	<0.1
Rhus mysorensis G. Don (Anacardiaceae)	0.07	<0.1
Alangium salviifolium (L.f.) Wang. (Alangiaceae)	0.07	<0.1
Streblus taxoides (Heyne ex Roth) Kurz (Moraceae)	0.06	<0.1
Capparis grandis L. (Capparaceae)	0.05	<0.1
Polyalthia cerasoides (Roxb.) Bedd. Nakulsi (Annonaceae)	0.04	<0.1
Ziziphus xylopyrus (Retz.) Willd. (Rhamnaceae)	0.04	<0.1
Santalum album L. (Santalaceae)	0.04	<0.1
Maytenus emarginata (Willd.) Ding Hou (Celastraceae)	0.04	<0.1

Ficus benghalensis L. (Moraceae)	0.04	<0.1
Symplocos cochinchinensis (Lour.) Moore (Symplocaceae)	0.04	<0.1
Diospyros melanoxylon Roxb. (Ebenaceae)	0.04	<0.1
Sterculia foetida L. (Sterculiaceae)	0.03	<0.1
Buchanania axillaris (Desr.) Ramam. (Anacardiaceae)	0.03	<0.1
Cleistanthus collinus Benth. ex Hook.f. (Euphorbiaceae)	0.03	<0.1
Euphorbia nivulia Buch.-Ham. (Euphorbiaceae)	0.03	<0.1
Gardenia resinifera Roth (Rubiaceae)	0.03	<0.1
Firmiana colorata (Roxb.) R.Br. (Sterculiaceae)	0.02	<0.1
Solanum erianthum D.Don (Solanaceae)	0.02	<0.1
Murraya paniculata (L.) Jack (Rutaceae)	0.02	<0.1
Chionanthus mala-elengi (Dennst.) P.S.Green (Oleaceae)	0.02	<0.1
Rapanea wightiana (Wall. ex DC.) Mez (Myrsinaceae)	0.02	<0.1
Diospyros humilis Bourd. (Ebenaceae)	0.02	<0.1
Euonymus indicus Heyne ex Roxb. (Celastraceae)	0.02	<0.1
Antidesma zeylanicum Lam. (Euphorbiaceae)	0.02	<0.1
Total	608.24	100

Size class distribution

All the trees sampled in Pachaimalai forest were categorized into eight size classes viz. 30-60cm class, 60-90cm, 90-120cm, 120-150cm, 150-180cm, 180-210cm, 210-240cm and >240cm class, based on their girth measurement at breast height. Contribution of aboveground biomass was maximum (31.1%) for 60-90cm class followed by 30-60cm (22.2%), 90-120cm and 120-150cm (*Fig. 2*).

The stand basal area was dominated by 30-60cm size class (35.9%), followed by 60-90cm (33.8%), 90-120cm and 120-150cm class (*Fig. 3*). In case of density, the smallest class 30-60cm alone contributed more than seventy six per cent of the total trees recorded from 12 ha area sampled from the Pachaimalai forest (*Fig. 4*).

Regression analysis

The relationships of aboveground biomass with species richness, density and basal area of trees were analyzed through linear regression (*Fig. 5*). The analysis showed that tree biomass were positively correlated with species richness (R^2 = 0.702) and basal area (R^2 = 0.979), for the 24 transects sampled in Pachaimalai forest (*Fig. 5*). Whereas, no significant relationship was predicted between biomass and density of trees (R^2 = 0.320) in the Pachaimalai forest of Eastern Ghats (*Fig. 5*).

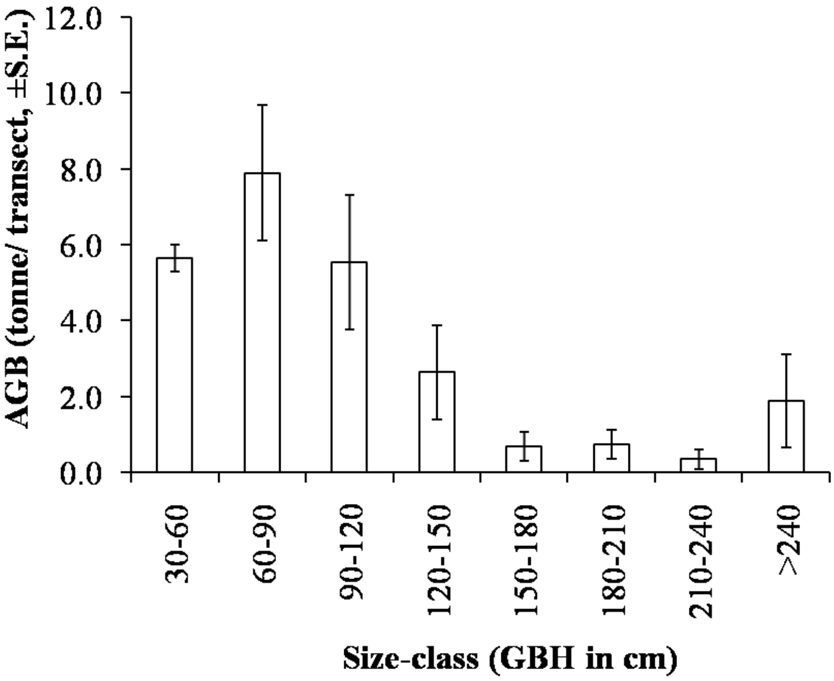

***Figure 2**. The average aboveground biomass (AGB, in tonnes) of trees categorized into eight size classes for the twenty four 0.5 ha transects sampled at the Pachaimalai forest*

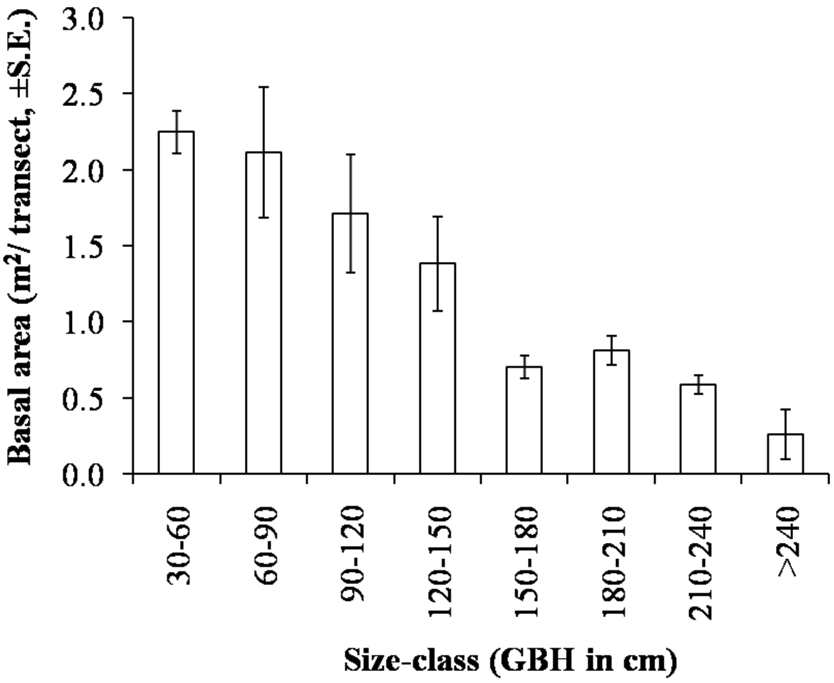

***Figure 3.** The average basal area of trees categorized into eight size classes for the twenty four 0.5 ha transects sampled at the Pachaimalai forest*

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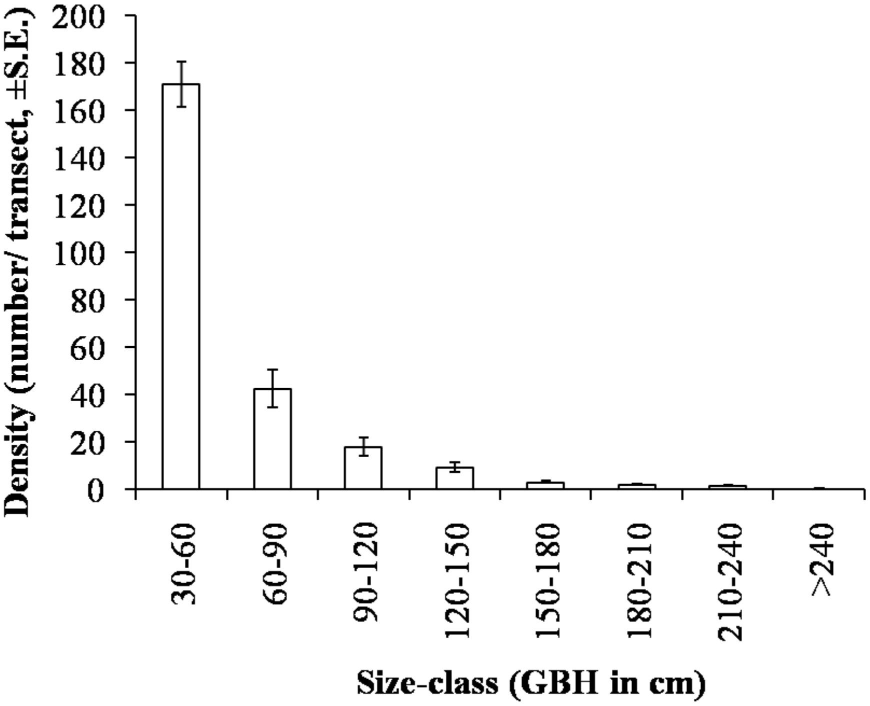

Figure 4. *The average density of trees categorized into eight size classes for the twenty four 0.5 ha transects sampled at the Pachaimalai forest*

Discussion

During the last decade, climate change has increased the need of quantification on the amount of forest biomass in different regions of the globe for climate policy definition (Djomo et al., 2010). The results of this study reveal that the aboveground biomass of the Pachaimalai forest of the Eastern Ghats in India (50.7 t/ha) was moderate and falls within the range reported for other tropical forests of the world (*Table 2*). It was almost five folds greater than the biomass value reported for rainforest at Bajo Calima, Choco, Columbia (Fabre-Langendoen and Gentry, 1991), but ten folds lesser than the value reported for the rain forests in New Guinea (Edwards and Grubb, 1977).

The order of aboveground biomass contribution of trees categorized into eight size classes in Pachaimalai forest were 60-90cm>30-60cm>90-120cm>120-150cm>above 240cm>180-210cm> 150-180cm>210-240cm class. The smaller size classes (30-60cm to 90-120cm class) contributed seventy five percent of the total aboveground biomass. This reveals that the standing biomass stock of the Pachaimalai forest was dominated by the young trees.

The regression analysis revealed a strong positive relationship that the biomass of trees increased with an increase in basal area and species richness, but not with density (*Fig. 5*). This shows that basal area and species richness of forest stands were good indicators for the stand biomass rather than stand density of Pachaimalai forest, and a similar trend was reported in Murali et al. (2005).

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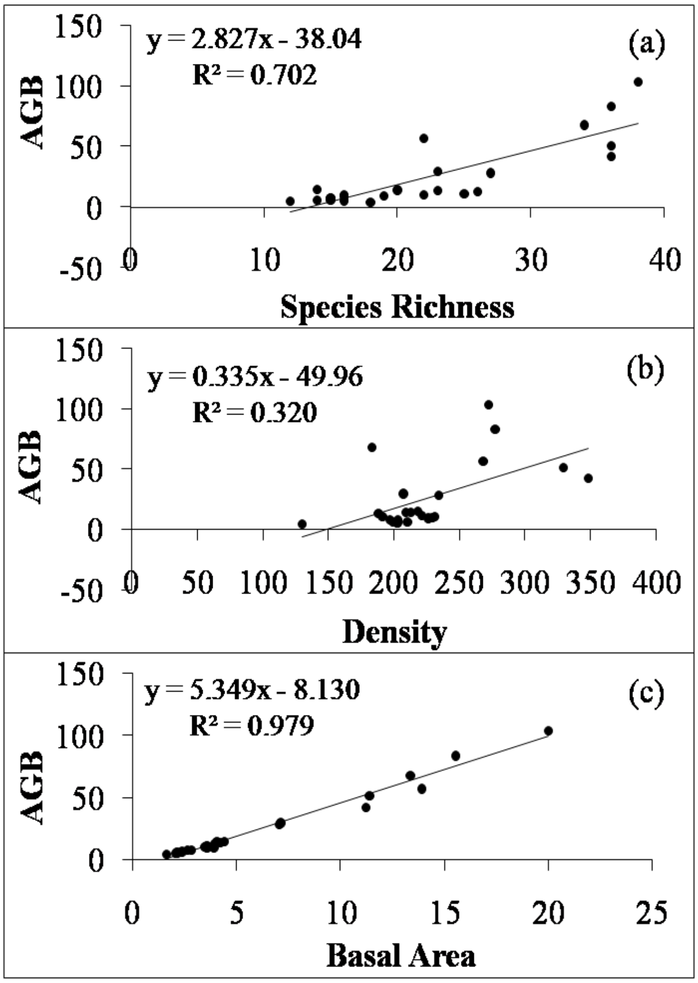

Figure 5. *The relationships between aboveground biomass (AGB) and species richness (a), density (b) and basal area (c) for the twenty four transects sampled at Pachaimalai forest*

Table 2. *Comparison of aboveground biomass (AGB, t/ha) of Pachaimalai forest with other forests of the world.*

Forest type and location	**AGB**	**BA**	**DN**	**RS**
Tropical forests, Pachaimalai forest, India	57.7	12.5	449	PS
Rainforest, Bajo Calima, Choco, Columbia	193.29	25.01	664	A
Rainforest, Bajo Calima, Choco, Columbia	168.71	22.48	704	A
Rainforest, Bajo Calima, Choco, Columbia	17.63	5.4	2820	A
Rainforest, Bajo Calima, Choco, Columbia	11.09	4.9	2000	A
Tropical dry evergreen forest, Rayapatti, India	73.06	12.4	889	B
Tropical moist forests, Bangladesh	85-206	-	-	C
Tropical dry evergreen forest, Arasadikuppam, India	90.25	17.6	2813	B
Tropical dry evergreen forest, Kuzhanthaikuppam, India	90.25	16.9	1349	B
Tropical dry evergreen forest, Maramadakki,	91.06	15.5	724	B

India				
Tropical dry evergreen forest, Araiyapatti, India	103.68	19.1	807	B
Tropical dry evergreen forest, Oorani, India	113.46	27.3	1284	B
Tropical dry evergreen forest, Shanmuganathapuram, India	127.91	22.1	1663	B
Tropical dry evergreen forest, Thirumanikkuzhi, India	134.13	29.3	1077	B
Tropical dry evergreen forest, Karisakkadu, India	138.73	21.6	596	B
Tropical moist forests, Bangladesh	150-210	-	-	D
Tropical dry evergreen forest, Puthupet, India	173.1	36.5	1567	B
Montane rain forests, Jamaica	229-312	-	-	E
Tropical rain forests, Malaysia	230-290	-	-	F
Lower Montane forests, El Verde, Puerto Rico	233.4	-	-	G
Tropical seasonal rain forest, Xishuangbanna, China	241.27	31.28	730	H
Tropical rain forests, Khade, Ghana	265.8	-	-	I
Tropical rain forests, New Guinea	286	-	-	J
Tropical rain forests, Khado Chang, Thailand	290.6	-	-	K
Tropical rain forest, Cambodia	348-415	-	-	L
Tropical rain forests, Western Ghats, India	458.2	-	-	M
Montane rain forests, New Guinea	505	-	-	N

BA-Basal area (m^2/ha), DN-density (stems/ha); RS-References; PS-Present study; A-Fabre-Langendoen and Gentry, 1991; B-Mani and Parthasarathy, 2007; C- Drigo et al., 1988; D- Milde et al., 1985; E-Tanner, 1975; F-Whitmore, 1975; G-Jordon, 1981; H-Shanmughavel et al., 2001; I-Greenland and Gowel, 1970; J-Enright, 1979; K-Kira et al., 1974; L-Hozumi et al., 1979; M-Rai, 1984; N-Edwards and Grubb, 1977

Estimation of forest biomass is an essential aspect of studies for quantification of forest carbon stocks and their effects on the global carbon balance (Ketterings et al., 2001). Hence, the present study provides valuable base line biomass data helpful for quantification of carbon stocks of Pachaimalai forest of Eastern Ghats which in turn would help for modeling forest carbon stocks at national as well as global level.

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SOIL ENZYMES AS BIOINDICATORS OF SOIL ECOSYSTEM STATUS

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Abstract. A variety of methods were developed to measure soil biological activity. All these methods are not suited to produce generally accepted results, but they give relative information about the ecological status of soil. Soil enzymatic activity assays is only one way to measure the ecosystem status of soils. The technique is quite simple and produces reproducible results, and is nowadays of practical importance because the influence of agro-chemicals, industrial waste, heavy metals, as well as soil fertility management can be measured. Especially the search for urease inhibitor is of particular interest in order to reduce ammonia losses from soils. Soil enzymes have been reported as useful soil quality indicators due to their relationship to soil biology, being operationally practical, sensitive, integrative, ease to measure and described as "biological fingerprints" of past soil management, and relate to soil tillage and structure. The focus of this article is to provide a review of soil enzyme activity as a biological, process-level indicator for impacts of natural and anthropogenic activities on soils. This knowledge of soil enzymology can be applicable as bioindicator to human endeavour of ecosystem perturbation, agricultural practices and xenobiotic pollution.

Keywords: *soil fertility management, ecosystem perturbation, agricultural practices, xenobiotics*

Introduction

Soil is a living-dynamic, non-renewable resource and its conditions influence food production, environmental efficiency and global balance (Dick, 1997; Doran and Zeiss, 2000). The quality of soil depends in part on its natural composition, and also on the changes caused by human use and management (Pierce and Larson, 1993). Human factors influencing the environment of the soil can be divided into two categories: those resulting in soil pollution and those devoted to improve the productivity of soil (Gianfreda and Bollag, 1996). A soil is biologically active, when biological processes proceed rapidly, i.e. in a distinct span of time a lot of metabolites are produced (Schaller, 2009). A variety of methods were developed to measure soil biological activity. All these methods are not suited to produce generally accepted results, but they give relative information about the ecological status of soil ecosystem (Burns, 1982; Frankenberger and Dick, 1983). The soil enzymatic activity assay is only one way to measure the ecosystem status of soils. Baldrian (2009) proposed a variety of methods for measuring enzymatic activities in soils. These techniques are quite simple and produce reproducible results, but they often differ in the mode of detection (spectrophotometry, fluorescence, radiolabelling), the reaction conditions (temperature,

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use of buffers, time of reaction), and/or in the use of a variety of reaction substrates of measuring the enzyme activity, even for a single enzyme (Tabatabai 1994; Alef and Nannipieri 1995; Gianfreda and Bollag 1996; Schinner et al., 1996, Burns and Dick, 2002). Unfortunately, generally accepted standard procedures still do not exist (Baldrian, 2009).

The role of soil enzymes and their activities are defined by their relationships with soil and other environmental factors (e.g., acid rain, heavy metals, pesticides, and other industrial chemicals) that affect their activities (Burns, 1982; Hussain et al., 2009). Soil enzymes are the mediators and catalysts of important soil functions that include: decomposition of organic inputs; transformation of native soil organic matter; release of inorganic nutrients for plant growth; N_2 fixation; nitrification; denitrification; and detoxification of xenobiotics (Dick, 1997). In addition, soil enzymes have a crucial role in C (β-glucosidase and β-galactosidase), N (urease), P (phosphatase), and S (sulphatase) cycle (Karaca et al., 2011). Soil enzymology is nowadays of practical importance because the influence of agro-chemicals, industrial waste, heavy metals, as well as soil fertility management can be measured. Especially the search for urease inhibitor is of practical interest in order to reduce ammonia losses from soils (Schaller, 2009). The importance of soil enzymes has been explained as useful in describing and making prediction about ecosystem's function, quality and interactions among sub-systems (Dick and Tabatabai, 1992). The focus of this article is to provide a review of soil enzyme activity as a biological, process-level indicator for impacts of natural and anthropogenic activities on soils. This knowledge of soil enzymology can be applicable as bioindicator to human endeavour of ecosystem perturbation, agricultural practices and xenobiotic pollution.

What is bioindicator?

A bioindicator is defined as an organism, part of an organism, the product of an organism (e.g., enzyme), collection of organisms or biological process which can be used to obtain information on the quality of all or part of the environment (Killham, 2002). A number of bioindicators have been suggested for monitoring soil health and they include: soil microbial biomass, carbon and nutrient cycling, community structure and biodiversity, soil animals, plants, and soil enzymes (Killham, 2002). Bioindicators are very important for resource managers in order to understand ecological changes within the soil ecosystem (Dale et al., 2008). Dale and Beyeler (2001) and Dale et al. (2008) summarized the criteria for ecological indicators: (i) easy to measure, (ii) sensitive to system stresses, (iii) respond to stress, (iv) anticipation of change in ecological system, (v) predicts changes, (vi) being integrative, (vii) ability to respond to natural disturbances, anthropogenic stresses and changes over time, (viii) variable with response and (ix) having the attention of measured parameters of spatial and temporal change.

Soil enzymes have been reported as useful soil quality biological indicators due to their relationship to soil biology, being operationally practical, sensitive, integrative, ease to measure and described as "biological fingerprints" of past soil management, and relate to soil tillage and structure (Bandick and Dick, 1999). They are also indicative of biological equilibrium (Frankenberger and Tabatabai, 1991), fertility (Nannipieri, 1994; Antonious, 2003), quality (Dick, 1997; Bucket and Dick, 1998), and changes in the

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biological status of soil due to pollution (Nannipieri and Bollag, 1991; Schaffer, 1993; Trasar-Cepeda et al., 2000).

Some Selected Soil Enzymes used as Biological indicators

Dehydrogenase

Dehydrogenase is an enzyme that oxidizes soil organic matter by transferring protons and electrons from substrates to acceptors. This enzyme is considered to exist as an integral part of intact cells but does not accumulate extra-cellularly in the soil (Das and Varma, 2011). Dehydrogenase activities in soil are biological indicators of overall microbial respiratory activity of soils and are used by microorganisms in the soil to break down organic matter; metabolic processes that occur in abundance in healthy microorganisms (Bolton et al., 1985). Burns (1978) reported that dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils. This enzyme occurs only within soil bacteria (e.g. genus *Pseudomonas, with Pseudomonas entomophila* as most abundant). They do not act on their own without a bacterial host. Therefore, when dehydrogenase is present in the soil, you can reasonably conclude that bacteria are present (Walls-Thumma, 2000).

Testing for dehydrogenase activity in soil bacteria involves adding a substance (triphenyltetrazolium chloride) to the soil that serves two purposes. First, it makes organic materials more available to microorganisms. At the same time, the bacteria convert it to a chemical called formazan, which can be extracted from the soil and analysed (Alef and Nannipieri, 1995; Walls-Thumma, 2000). Higher levels of formazan indicate that bacteria are present and healthy, undertaking the metabolic processes that boost soil fertility (Walls-Thumma, 2000). Measuring dehydrogenase levels allow researchers to better understand the effect of agricultural practices, such as pesticide use, or other management practices on the health of soil, as well as a direct measure of soil microbial activity. It can also indicate the type and significance of pollution in soils (Walls-Thumma, 2000). For example, higher activities of dehydrogenases have been reported at low doses of pesticides, and, lower activities of the enzyme at higher doses of pesticides (Baruah and Mishra, 1986). Similarly, dehydrogenase enzyme is higher in soils polluted with pulp and paper mill effluents (McCarthy et al., 1994) but low in soils polluted with fly ash (Pitchel and Hayes, 1990).

β-Glucosidase

β-glucosidase is a common and predominant enzyme in soils (Eivazi and Tabatabai, 1988; Tabatabai 1994). The enzyme plays an important role because it is involved in catalysing the hydrolysis of various β-glucosides present in plant debris decomposing in the soil ecosystem. Thus, it is named according to the type of bond that it hydrolyses (Ajwa and Tabatabai 1994; Martinez and Tabatabai 1997). This enzyme is included in the category of glucosidases that hydrolyse disaccharides. α-Glucosidase, which catalyses the hydrolysis of α-D-glucopyranoside, is also included among glucosidases. Other glucosidases are α-galactosidase and β-galactosidase (also called lactase). β-glucosidase is more prominent in soil than α-glucosidase and α and β galactosidases. It is a rate limiting enzymes in microbial degradation of cellulose to glucose, an important C energy source of life for microorganisms in the soil (Esen, 1993; Tabatabai, 1994), according to the following reaction:

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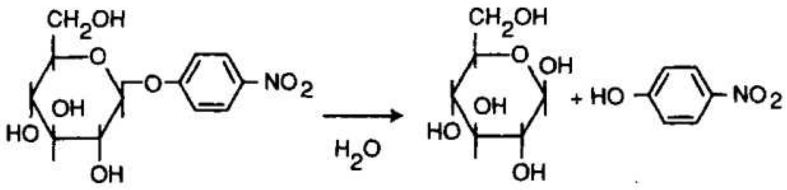


p-nitrophenyl-β-D-glucoside glucose *p*-nitrophenol

β-glucosidases are known to be widely distributed among plants, animals, fungi, bacteria and yeasts (Veena et al., 2011). Glucosidase activity has been observed in various plant species such as maize and sorghum (Verdoucq et al., 2003), roots of *Panax ginseng* plant (Zhang et al., 2001), and microbes like *Penicillium purpurogenum* (Dhake and Patil, 2005), *Ceriporiopsis subvermispora* (Magalhaes et al., 2006), *Flavobacterium johnsonae* (Okamota et al., 2000), *Trichoderma* spp. (Yun et al., 2001; Pragya et al., 2013), *Lactobacillus plantarum* (Spano et al., 2005) and *Dyella koreensis* spp. (An et al., 2005). There is considerable evidence suggesting that a significant fraction of β-glucosidase enzymatic activity measured in soil originates from abiontic enzymes (enzymes of biological origin no longer associated with living cells) excreted into the soil solution or immobilized enzymes of microbial origin sorbed to clays or humus colloids ((Hayano and Tubaki, 1985; Busto and Perez-Mateos, 1995; 2000).

The enzyme is characteristically useful as a soil quality bioindicator, and may give a reflection of past biological activity, the capacity of soil to stabilize the soil organic matter (SOM), and can be used to detect management effect on soils (Bandick and Dick, 1999; Ndiaye et al., 2000). This has greatly facilitated its adoption for soil quality testing (Bandick and Dick, 1999). Generally, β-glucosidase activities can provide advanced evidence of changes in organic carbon long before it can be accurately measured by other routine methods (Joachim and Patrick, 2008). The ß - glucosidase enzyme is very sensitive to changes in pH, and soil management practices. Acosta-Martinez and Tabatabai (2000) reported β-glucosidase as sensitive to pH changes, with its activity significantly ($P < 0.001$) and positively correlated with soil pH. This property can be used as a good biochemical indicator for measuring ecological changes resulting from soil acidification in situations involving activities of this enzyme. The ß - glucosidase enzyme is also known to be inhibited by heavy metal contamination such as Cu and several others (Joachim and Patrick, 2008). For instance, studies have shown that plant debris did not decompose or show β-glucosidase activities when exposed to heavy metal polluted soils (Joachim and Patrick, 2008). More understanding of the β-glucosidase enzyme activities and factors influencing them may contribute significantly to the studies of soil ecosystem status (Das and Varma, 2011).

Cellulase

Cellulase catalyses hydrolysis of cellulose to D-glucose (Hussain et al., 2009). Cellulose is the most abundant structural polysaccharide of plant cell walls with β-1, 4 - glucosidic linkages and represents almost 50% of the biomass synthesized by photosynthetic fixation of CO_2 (Eriksson et al., 1990). The cellulolytic enzyme consists of at least three enzymes (Joachim and Patrick, 2008). They include: endo-1,4-β-glucanase which attacks the cellulose chains at random, exo-1,4-β-glucanase which removes glucose or cellobiose from the non-reducing end of the cellulose chains, and β-D-glucosidase which hydrolyses cellobiose and other water soluble cellodextrins to glucose.

The cellulolytic enzyme systems in fungi can be divided into three groups. The soft-rot fungi (*Aspergillus niger*, *A. oryzae, Fusarium solani, T. harzianum, Trichoderma reesei*, *Trichoderma atroviride*, *Mucor circinelloides*), brown rot fungi (*Poria placenta, Coniophora puteana, Lanzites trabeum, Tyromyces palustris, Fomitopsis sp.*) and white-rot fungi (*Phanerochaete chrysosporium, Agaricus arvensis, Sporotrichum thermophile, Pleurotus ostreatus*) (Kleman-Leyer et al., 1996; Nutt, 2006; Sukumaran et al., 2005; Kuhad et al., 2011). Cellulase enzymes in bacteria are produced by aerobic (*Acinetobacter junii, Bacillus subtilis, Cellulomonas biazotea, Pseudomonas cellulose*) and anaerobic (*Acetivibrio cellulolyticus, Butyrivibrio fibrisolvens, Clostridium thermocellum*) microbes (Sukumaran et al., 2005; Sadhu et al., 2013). Also actinomycetes (*Cellulomonas fimi, Streptomyces drozdowiczii, Thermomonospora fusca*) produce cellulolytic enzyme (Sukumaran et al., 2005; Kuhad et al., 2011). Most cellulolytic microbiota produces, in addition to cellulases that hydrolyse the β (1-4) glucosidic bonds, a number of other cell-wall-degrading enzymes such as ligninases, xylanases, pectinases, etc. (Sukumaran et al., 2005). The production of cellulases is also documented in plants and in a number of invertebrate taxa that includes insects, crustaceans, annelids, molluscs, mussels and nematodes (Sadhu et al., 2013).

Activities of cellulases in agricultural soils are affected by several factors. These include temperature, soil pH, water and oxygen contents (abiotic conditions), the chemical structure of organic matter and its location in the soil profile horizon (Deng and Tabatabai, 1994; Alf and Nannipieri, 1995), quality of organic matter/plant debris and soil mineral elements (Sinsabaugh and Linkins, 1989; Deng and Tabatabai 1994) and the trace elements from fungicides (Deng and Tabatabai 1994; Arinze and Yubedee 2000).

Demonstrating the effects of increasing concentrations of fungicides on cellulases activities, Arinze and Yubedee (2000) showed that fungicides benlate, calixin and captan inhibited cellulase activity in *Fusarium monoliforme* isolates. Captatol inhibited the cellulose activity in the sandy loam soil, and chlorothalonil showed a clear reduction in cellulase activity under flooded or non-flooded conditions (Joachim and Patrick, 2008). Several mechanisms have been proposed in the degradation of cellulose by cellulases. For instance, chitin in the presence of cellulose induces the synthesis of chitinase and other cell wall lytic enzymes which promote the release of the intramural β-glucosidase into the medium . All these findings suggest that the activities of cellulases can be used to give preliminary indication of some of the physical and chemical properties of soil, thus, easing agricultural soil management strategies (Joachim and Patrick, 2008). Since cellulases enzymes play an important role in global recycling of the most abundant polymer, cellulose in nature, it would be of critical importance to understand this enzyme better so that it may be used more regularly as a predictive tool in our soil fertility programmes (Das and Varma, 2011).

Urease

Urease is an enzyme that catalyses the hydrolysis of urea into CO2 and NH3 with a reaction mechanism based on the formation of carbamate as an intermediate (Tabatabai, 1982).

$$H_2NCONH_2 + H_2O \rightarrow 2NH_3 + CO_2$$

It also catalyses the hydrolysis of hydroyurea, dihydroxyurea and semicarbazid and contains nickel as a co-factor with its molecular weight may range from 151,000 to 480,000 Da (Alef and Nannipieri, 1995). The urease is widely distributed in nature, being present mainly from plants (Polacco, 1977) and microorganisms found as both intra- and extra-cellular enzymes (Burns, 1986; Mobley and Hausinger 1989). On the other hand, urease extracted from plants or microorganisms rapidly degraded in soil by proteolytic enzymes (Pettit et al. 1976; Zantua and Bremner 1977). This suggests that a significant fraction of pureolytic activity in the soil is carried out by extracellular urease, which is stabilized by immobilization on organic and mineral soil colloids.

The enzyme urease has been widely used to evaluate changes in soil quality related to management, since its activity increases with organic fertilization and decreases with soil tillage (Saviozzi et al., 2001). This enzyme, mostly the cases are an extra-cellular enzyme representing up to 63% of total activity in the soil (Martinez-Salgado et al., 2010). It has been shown that its activity depends on microbial community, physical, and chemical properties of soil (Corstanje et al., 2007), and its stability is affected by several factors: organo-mineral complexes and humic substances make them resistant to denaturing agents such as heat and proteolytic attack (Makoi and Ndakidemi, 2008). Urease activity is used as a soil biological indicator because it is influenced by soil factors such as cropping history, organic matter content, soil depth, management practices, heavy metals and environmental factors like temperature and pH (Yang et al., 2006). The effect of temperature on urea hydrolysis has received considerable research attention. Generally, urease activity increases with increasing temperature. Consequently the understanding of urease activity should provide better ways to manage urea fertilizer, especially in warm high rainfall areas, flooded soils and irrigated conditions (Makoi and Ndakidemi, 2008).

Phosphatases

Organic phosphorus (P_o) is abundant in soils and can contribute to the P nutrition of plants and microbes following hydrolysis and the release of free phosphate (Condron et al., 2005). This process is catalysed by phosphatase enzymes, which are actively secreted into the soil by many plants and microbes in response to a demand for P, or passively released from decaying cells (Quiquampoix and Mousain, 2005). Of the phosphatases present in soil, phosphomonoesterases are the most studied. This group of enzymes act on a range of low molecular weight P compounds with monoester bonds, including mononucleotides, sugar phosphates, and polyphosphates (Reid and Wilson, 1971). They cannot initiate the cleavage of phosphate from phytic acid (myo-inositolhexakisphosphate), although they can catalyse the hydrolysis of lower-order inositol phosphates (Cosgrove, 1980). Phosphodiesterases are far less studied in both soils and soil organisms. This seems a significant oversight, because phosphodiesterase is involved in the degradation of phospholipids and nucleic acids, which constitute the majority of the fresh organic P inputs to soil (Cosgrove, 1967). Phosphomonoesterase and phosphodiesterase are both necessary to release free phosphate from a phosphate diester (Turner and Haygarth, 2005). Initial hydrolysis by phosphodiesterase releases a phosphate monoester, which must then by hydrolysed by phosphomonoesterase to release free phosphate for biological uptake (*Fig. 1*).

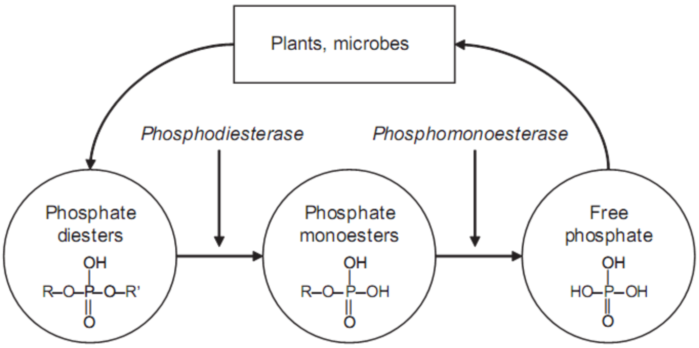


Figure 1. *A simplified conceptual model of the turnover of organic phosphorus inputs from plants and microbes in soil. Organic phosphorus inputs to soil from plants and microbes are mainly phosphate diesters, which must be hydrolyzed by phosphodiesterase and phosphomonoesterase prior to the release of free phosphate for biological uptake. R and R′ represent organic moieties (culled from Benjamin and Philip, 2005).*

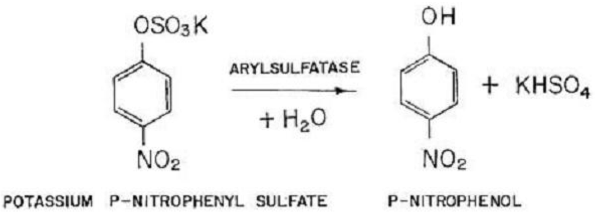
Microorganisms that produce phosphates in soil includes soil fungi, particularly those belonging to the genera *Aspergillus* and *Penicillium*, along with *Pseudomonas* and *Bacillus* bacteria that produce mostly neutral phosphatase, while Actinomycetes produced only negligible quantities of phosphatases (Tarafdar and Chhonkar, 1979). In soil ecosystems, these enzymes are believed to play critical roles in P cycles as evidence shows that they are correlated to P stress and plant growth (Speir and Ross, 1978). Land plants have evolved many morphological and enzymatic adaptations to tolerate low phosphate availability. This includes the transcription activity of acid phosphatases, which tend to increase with high P stress (Miller et al., 2001; Li et al., 2002). For example, when there is a signal indicating P deficiency in the soil, acid phosphatase secretion from plant roots is increased to enhance the solubilisation and remobilisation of phosphate, thus influencing the ability of the plant to cope with P-stressed conditions (Hayes et al., 1999; Karthikeyan et al., 2002; Versaw and Harrison, 2002).

In soil, phosphomonoesterases have been the most studied enzymes probably because they have activity both under acidic and alkaline conditions, according to its optimal pH, and because they act on low molecular P-compounds, including nucleotides, sugar phosphates and polyphosphates (Makoi and Ndakidemi, 2008); thus they can be used as soil quality bioindicators. Turner and Haygarth (2005), evaluated phosphatase activity in temperate grassland, and found a strong correlation between enzyme activity and soil properties such as pH, total N, organic P and clay content.

The amount of acid phosphatase exuded by plant roots has been shown to differ between crop species and varieties (Izaguirre-Mayoral et al., 2002; Ndakidemi, 2006), as well as crop management practices (Wright and Reddy, 2001; Ndakidemi, 2006). For instance, research has shown that legumes secrete more phosphatase enzymes than cereal (Yadav and Tarafdar, 2001; Li et al., 2004). This may probably be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals (Joachim and Patrick, 2008). The ability to solubilize soil mineral elements by these phosphomonoesteraces is expected to be a higher in biologically-managed systems because of a higher quantity of organic C found in those systems. In fact, the activity of acid and alkaline phosphatases was found to correlate with organic matter in various studies (Guan, 1989; Jordan and Kremer, 1994; Aon and Colaneri, 2001). It is, therefore, anticipated that management practices that induce P stress in the rhizosphere may also affect the secretion of these enzymes in the ecosystem ((Ndakidemi, 2006). Joachim and Patrick (2008) observed that there have been few studies examining the influence of management options in the ecosystem on phosphatases activity in soil where most crops are grown. Understanding the dynamics of phosphatase activities in the soil ecosystems, according to Das and Varma (2011) is crucial for predicting their interactions as their activities may, in turn, regulate nutrient uptake and plant growth.

Arylsulphatase

Arylsulfatase is the enzyme that catalyses the hydrolysis of organic sulfate ester (Kertesz and Mirleau, 2004) and is typically widespread in the soils (Tabatabai and Bremner, 1970; Gupta et al., 1993; Ganeshamurthy et al., 1995). They are classified according to the type of the ester in arylsulphatases: akylsulphatases, steroid sulphatases, glucosulphatases, chondrosulphatases and myrosulphatases (Tabatabai, 1982). The enzyme also catalyses the hydrolysis of p-nitrophenyl sulfate, potassium phenyl sulphate, potassium nitrocatechol sulphate and potassium phenolphthalein sulphate (Alef and Nannipieri, 1995).The role of this enzyme in the hydrolysis of aromatic sulphate esters to phenols and sulphate, or sulphate sulphur is shown in the following chemical equation (Tabatabai, 1994):



The enzyme has been detected in strains of bacteria (*Actinobacteria* sp., *Pseudomonas* sp., *Klebsiella* sp. and *Raoultella* sp.), fungi (*Trichoderma* sp. and *Eupenicillium* sp.), plants and animals (Nicholls and Roy, 1971), and was first detected in soils by Tabatabai and Bremner (1970). Arylsulphatases are secreted mainly by bacteria into the external environment as a response to sulphur limitation (McGill and Colle, 1981). Its occurrence in different soil systems is often correlated with microbial biomass (Klose and Tabatabai, 1999) and rate of S immobilization (Vong et al., 2003). The release of sulphate from soluble and insoluble sulphate esters in the soil is affected by various environmental factors such as heavy metal pollution (Tyler, 1981; Kucharski, et al., 2011), pH changes in the soil solution (Acosta-Martinez and Tabatabai, 2000), organic matter content and its type (Sarathchandra and Perrott, 1981; Dalal, 1982), the concentration of organic sulphate esters (Dodgson and Rose, 1976), the extent to which organic sulphate esters are protected against enzymatic hydrolysis such as sorption to particle surfaces in soils, and the activity persistence of extracellular arylsulphatases in the soil (Joachim and Patrick, 2008).

Soil Enzyme Activity as a Biological Indicator of Soil Ecosystem Status

Soil Enzymes as Bioindicators of Ecosystem Perturbation

Changing land use from one type to another generally affects the soil ecosystem status. Sicardi et al. (2004) found that land use conversion from natural grazed pastures to commercial *Eucalyptus grandis* plantations, showed that no significant effect was found on the number of cellulolytic aerobes, P-solubilizer and *Azotobacter* spp. communities, whereas significant effect was observed on soil respiration, C-mineralization coefficient, dehydrogenase, fluorescien diacetate hydrolysis and acid and alkaline phosphatase activity. Sicardi et al. (2004) also reported that land use and management practices alter the total amount and composition of soil organic matter and significantly change the enzyme activities. Natural systems changed to agricultural systems not only affect vegetation, but also biological properties are altered in soil ecosystems (Acosta-Martinez et al., 2003). They observed higher enzymatic activities (β-glucosidase, β-glucosaminidase, arylamidase, alkaline and acid phosphatase, phosphodiesterase and arylsulphatase) in conservation reserves programme, native grassland and rotation with other crops (wheat or sorghum) when compared with continuous cotton (Acosta-Martinez et al., 2003).

Devegetation and revegetation influence soil quality when compared with undisturbed soils. In the study carried out by Bastida et al. (2006), they found that dehydrogenase and protease were lower in devegetated soils (devegetation of *Pinus halepenis* and natural shrubs) than undisturbed soil. Izquierdo et al. (2005) found that elimination of vegetation caused a long-term negative influence on biochemical and microbial activity of the soil. Soil quality has not been recovered even after 15 years of deforestation. They also observed that protease and β-glucosidase activities were in revegetation with *Casuarina equisetifolia* than with *Anacardium occidentale*; however, urease, protease, acid phosphatase and β-glucosidase activities were significantly greater in revegetated soil (soil restoration: after revegetation of mining area) than in bare soil 4 years after planting (Izquierdo et al., 2005).

Forest fires are considered as natural disturbances and caused the most dramatic changes in forest ecosystems (Karaca et al., 2011). Due to the low volatilization temperature of N, most of the nitrogen found in biomass and soil is lost to the atmosphere when forest fires occur. Only some enzyme activities discriminate the fire effect on the ecosystem as bioindicators. Different soil enzymes were investigated by different researchers for discriminating fire stress on soil quality. Some activities were decreased and others were increased (Karaca et al., 2011). Invertase and proteinase activities were declined by burning, but acid phosphatase, polyphenoloxidase and peroxidase activities were increased (Zhang et al., 2005). Urease activity declined by the fire effect and this negative effect on urease activity was time dependent and recovered after 12 years of burning (Cetin et al., 2009). Sardans et al. (2005) reported that climatic conditions influence all living things as well as soil quality. The reduction of 10% of soil moisture decreased urease (10-67%), protease (15-66%) and β-glucosidase (10-80%) activities while decreasing of 21% of soil moisture decline urease (42-60%), protease (35-54%) and β-glucosidase (35-83%) and acid phosphatase (31-40%) activities and no significant influences were found on alkaline phosphatase activities. N-cycling enzymes (protease and urease) were the most influenced by drought (Sardans et al., 2005).

Soil Enzymes as Bioindicators of Change in Agricultural Practices

Fertilization of soils is conducted in soils by using different fertilizers such as mineral, manure, green manure, compost, and vermicompost. Kandeler et al. (1999) showed that farmyard manure enhanced microbial biomass, urease, deaminase and alkaline phophatase activities in soils compared with other treatments (mineral fertilizers) under rotations. They observed that stage of plant growth should be the cause of concern on enzyme activities in soils in terms of evaluating the impact of fertilizers. Similarly, soil enzyme activities (phosphatase, invertase, catalase and urease) under different fertilizers (no fertilization, organic manure, organic manure + N, organic manure + NP, organic manure + NK, organic manure + NPK) were lower in the early growth stage of cucumber, but enhanced in the late stages (Yang et al., 2008). Also, the type of mineral fertilizer used influence soil enzyme activity depending on the soil enzymes involved, in which there is, nutrient cycling (N, P, C, and S). Soil enzyme activities were inhibited with N fertilizer while they were promoted by P and K fertilizers. A decrease of urease activity could be explained by the activation of nitrification and denitrification causing suppression in urease production (Aon et al., 2001).

Organic fertilizers are used in agricultural systems, especially organic farming. Compost application is important in establishing and maintaining soil organic matter to a certain level in organic farming. Chang et al. (2007) found that soil enzyme activities (dehydrogenase, cellulase, protease, arylsulphatase, β-glucosidase, urease, arylsulphatase, and acid and alkaline phosphatases), as well as other microbial properties increased significantly in compost-treated soil compared with chemical-fertilizer soils; however, no significant evaluation was observed in studied enzyme activities after the compost dose of 540 Kg N ha^{-1} yr^{-1}. Saha et al. (2008) observed that dehydrogenase activity is higher in composted cattle manure (44-200%) and vermi-compost (22-108%) than in control. They concluded that: (i) organic applications, enhanced organic matter contents and microbial biomass and thus provide better potential for higher enzyme production and greater enzyme activities. (ii) additions of organic amendments showed different responses on soil enzyme activities depending on the organic matter type, and (iii) addition of organic amendments (cattle manure, compost or vermicompost) improve soil quality, increase soil organic matter content and stimulate biological and biochemical properties (Saha et al., 2008).

Organic amendments influence soil microbes and biochemical properties in different ways depending on the nutrient content. Rajashekhararao and Siddaramappa (2008) found that application of higher rates of organic amendments (rice residue and tree litters, high C content) was favourable to soil quality parameters (microbial biomass, microbial quotients, urease and acid phosphatase activities). Although they evaluated the other soil health parameters (extraction yield of humus and composition of humus), and observed that microbial quotient was the most sensitive indicator for reflecting the decline in soil quality. The authors also reported that microbial biomass; microbial quotient and soil enzyme activities are used for measuring biological soil quality as parameters. Addition of different carbon sources enhanced urease activity at different levels of elevation depending on N levels. Higher level of N stimulated urease activity in different C sources (Rajashekhararao and Siddaramappa, 2008).

Organic amendments can be used for suppression of plant diseases. Root rot severity was strong, adversely correlated with total C, arylsulfatase, β-glucosidase activities (Leon et al., 2006). *β*-glucosidase was not accepted as a useful indicator of disease suppression because it varied over time. Arylsulfatase was the best bioindicator for reflecting disease suppression. They indicated that applying organic amendments to soil can cause disease suppression by enhancing antagonist micro-organisms and microbial biomass and activity can be related to microbial competition with pathogens (Leon et al., 2006).

Tillage application may change soil quality through altering soil physico-chemical, hydrological, microbiological and biochemical properties and thus influences soil microbial community diversity and the production of soil enzymes. Tillage also affects soil nutrient levels and its availability, distribution of organic matter in the soil profile, soil water and oxygen content and soil fertility (Karaca et al., 2011). Tillage especially influences soil organic matter by exposing more soil organic matter to microbial attack and finally rapid loss of soil organic matter. Losing soil organic matter causes a decline of crop productivity, increase soil erosion and reduction in soil biological activity that negatively affects soil enzymes. To sum, tillage causes a great perturbation in soil environments (Madejon et al., 2007; Karaca et al., 2011). Many researchers conducted the impact of tillage on soil quality parameters as well as soil enzymes. Dehydrogenase activity increased under continuous zero-tillage practices and alkaline phosphatase and protease activities were higher in the zero-tillage systems over conventional practice; however, cellulase activity was greater in conventional practice compared to other management (Mina et al., 2008). No-till systems provide better enzyme activities in soils. Deng and Tabatabai (1997) showed that acid phosphates, alkaline phosphates, phosphodiesterase, inorganic pyrophosphatase, and arylsulfatase were significantly higher in no-till/double mulch than in other treatments (no-till/bare, no-till/normal, chisel/normal, chisel/mulch, mouldboard/normal, mouldboard/mulch).

Soil enzyme activities are accepted early and are more reliable bioindicators than soil physico-chemical properties under different tillage systems. Curci et al. (1997) evaluated the influence of conventional tillage systems (shallowing plowing: 20cm, deep plowing: 40 cm and scarification: 50 cm) at different depth (0-20, 20-40, 40-50, 50-70 cm) on soil enzyme activities (acid phosphatase, alkaline phosphatase, phosphodiesterase, pyrophosphatase, arylsulfatase, dehydrogenase, α-and β-glucosidase, α-and β-galactosidase, urea and nitrate reductase). The result showed that: (i) glucosidase, galactosidase, nitrate reductase and dehydrogenase activities were influenced by tillage systems, (ii) their activities were greater in shallow plowing and scarification than deep plowing plots in the upper layer (0-20 cm) of soil and (iii) no significant differences were found in the physical-chemical properties of the soil under different tillage systems (Curci et al., 1997).

Irrigation as one of the agricultural practices that provides adequate moisture level in the soil for plant growth affects the soil enzymatic activities. Zhang and Wang (2006) investigated the impact of subsurface irrigation (-10 -16 -25 -40 -63KPa) on phosphatase, urease and catalase under tomato cultivated in greenhouse experiment. They found that phosphatase and catalase activities increased in more frequent irrigation (-10 and -16 KPa) and urease activity decreased under irrigation.

Soil Enzymes as Bioindicators of Xenobiotic Pollution

Xenobiotics are by definition unnatural compounds (e.g. pesticides, industrial wastes) but the wider definition include naturally occurring compounds (e.g. heavy metals) that are synthesized or are present in unnaturally high concentrations in the environment (Skladany and Metting, 1993). Such compounds are of crucial concern in the soil environment as they could affect many biological and biochemical reactions in soils (Dick, 1997). Pesticides, which include herbicides, fungicides, and insecticides etc., introduced into the environment, have potential to affect non-target organisms and soil biochemical processes.

Pesticides reaching the soil may disturb local metabolism or enzymatic activities (Engelen et al., 1998; Liu et al., 2008), and its applications have been shown to both negative and positive effects on enzyme activity in soils (Ladd, 1985). The negative impact of pesticides on soil enzymes (hydrolases, oxidoreductases, and dehydrogenase) activities has been widely reported in the literature (Ismail et al., 1998; Monkiedje et al., 2002; Menon et al., 2005). There is also evidence that soil enzyme activities and ATP contents are increased by some pesticides (Shukla, 1997; Megharaj et al., 1999). ATP contents correlate with specific soil enzyme activities and may provide valuable information on trends in transformation of pesticides in soils (Kanazawa and Filip, 1986). A number of factors, for example, chemical nature of pesticides, the concentration used, microbial community structure, type of soil, and soil conditions can contribute to divergent research findings (Hussain et al., 2009). Malkomes (1997) attributed such differences to the dual behaviour of pesticides (both harmful and beneficial for soil enzymes), diversity and various stages of the processes taking place in soil that are frequently overlapped.

When pesticides are applied at recommended field rates, short-term studies often show an initial stimulatory, but small, effect on dehydrogenase activity: this may or may not occur with other enzymes (Dick, 1997). In relation to the effects of pesticides in soils, the two most widely-studied enzymes other than deydrogenase are phosphatase and urease. Again, short-term studies involving applications of pesticides to soils at recommended dosages for periods ranging from a few days up to 8 weeks have shown slight increases or no significant effect on the activity of these two enzymes (Baruah and Mishra, 1986; Tu, 1993). These results might be expected because these enzymes are known to exist as abiontic enzymes and thus, unless there was a direct effect on the enzyme reaction, there should be little effect on an abiontic enzyme (Dick, 1997).

To overcome the confounding effect of the multiple sources of isoenzymes in soils, studies have been conducted on the effect of pesticides on pure enzymes. Gianfreda et al. (1993) studied the effect of three herbicides and one insecticide on pure enzymes in free solution and found that responses could not be generalized were enzyme and pesticide are specific. For example, glyphosate and paraquat showed a marked activation of invertase activity, but urease and phosphatase activities were unaffected by these pesticides. Carbaryl inhibited urease and invertase activities, but had no effect on the activity or the kinetics of acid phosphatase. Atrazine did not affect the kinetics of urease, phosphatase or invertase except at very high concentrations. Further work showed that the 'state of an enzyme' can affect its activity in response to the presence of a pesticide. The purified urease was unaffected by paraquat or glyphosate, but urease activity complexes on montmorillonite increased in the presence of paraquat or glyphosate (Gianfreda et al., 1994). It was hypothesized that the pesticide displaced some inactive/immobilized urease from the clay surface which regained catalytic capabilities upon release into solution. These simplified systems show the complexity of the mechanisms involved in pesticide-enzyme interactions (Gianfreda et al., 1994).

When pesticides are applied to soils at very high concentrations such as when there is an accidental spill, enzyme activities have been significantly affected. Alachlor (10,000 mg kg^{-1}) alone or in mixture with atrazine and metolachor severely depressed dehydrogenase activity for 125 days, whereas esterase was only affected by the herbicide mixture (Dzantor and Felsot, 1991). Although bacterial numbers recovered, fungal numbers were still inhibited 90 days after the pesticide was applied. Adding the herbicide imaxethapyr at 100 times the recommended rate to soil showed decreases in microbial biomass -C and dehydrogenase activity, whereas hydrolytic enzyme activities (protease and 3,6'- diacetylfluorescein hydrolysis, FDA; a broad spectrum enzyme assay) showed corresponding increases up to 15 weeks after application of the herbicide (Perucci and Scarponi, 1994). In this case, Perucci and Scarponi (1994) hypothesized that the hydrolytic enzymes were released during lysis of microbial cells killed by imaxethapyr.

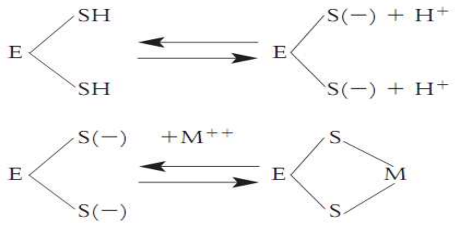
The difference between the enzymatic response at low and high pesticide concentration is due to the persistence of the pesticide which results in the inability of microbial populations to degrade or flourish in its presence (Junk et al., 1984; Schoen and Winterlin, 1987). For example, Junk et al. (1984) found that alachlor and atrazine applied alone or in combination at rates of 15 or 300 mg kg^{-1} soil showed no degradation after 68 weeks. Dzantor and Felsot (1991) reported that an emulsifiable concentrate of alachlor (10,000 mg a.i. kg $soil^{-1}$) was stable for 337 days, which caused depressed bioactivity (dehydrogenase and esterase activity) and reduced bacterial and fungal numbers. Felsot and Dzantor (1995) suggested that high pesticide concentrations cause biotoxicity and that the addition of nutrients in the form of organic amendments to pesticide-contaminated soils appeared to stimulate pesticide degradation. This further indicated that some specific microbial species could survive these high concentrations and that readily-available nutrients may be limiting in pesticide-contaminated soil.

Although single applications of pesticides have shown minimal effects on soil biological properties, it may be more important to consider the effects of repeated applications over many years. Voets et al. (1974) showed that long- term atrazine applications significantly reduced the activity of phosphatase, invertase, P-glucosidase, and urease in soils. They hypothesized that this was due to reduction of biological activity rather than a direct effect on the catalytic behaviour of these enzymes. Rai (1992) found that the effect of long-term (15 years) applications of 2, 4-D on dehydrogenase and urease activity depended on application formulation. The water-based dimethylamine salt formulation plus 2,4-D showed little effect on the activity of these enzymes over the control, whereas the 2,4-D oil-based isoctyl ester formulation significantly depressed activity of these enzymes. This was thought to be due to toxic metabolite(s) formed during degradation of the ester (Rai, 1992).

Alternatively, the amine may stimulate microbial degradation of 2, 4-D as evidenced by increased soil respiration.

Environmental pollution of soils with heavy metals and trace elements has been reported to have toxic effects on soil biology and to affect soil biochemical processes (Dick, 1997). The sources of these contaminants can come from repeated applications of sewage sludge, municipal wastes, smelting wastes, electroplating industry wastes, impurities in fertilizers, and deposition from air pollutants such as burning of fossil fuels and various industrial activities. Dick (1997) reported that enzyme reactions are inhibited by metals: (i) through complexation of the substrate; (ii) by combining with the protein-active groups of the enzymes; or (iii) by reacting with the enzyme-substrate complex. The mode of action of metals varies with enzyme and little is known about the exact mechanisms of interactions of metals and the multitude of enzymes that can exist in soils (Dick, 1997). Some ions can act as co-factors or activators and at certain concentrations can increase the activity of some enzymes (e.g. Mg, Ca, Ba, CO, Ni, Zn and Mn for pyrophosphatase; Dick and Tabatabai, 1983). Sulphydral groups of enzymes serve as catalytic sites or as groups involved in maintaining the correct conformation of the protein. Metals can react with sulphydral groups causing inactivation or inhibition of enzyme activity. In studies where a wide range of trace elements has been tested, Hg, Ag, Cr and Cd have generally caused the greatest inhibition of sulphatase, L-glutaminase, cellulase, L-asparginase, and P-glucosidase (Deng and Tabatabai, 1995).

Kucharski, et al. (2011) in their study of changes in the enzymatic activity in sandy loam soil exposed to Zn pressure found that soil contamination with zinc in doses from 70 to 10,000 mg kg^{-1} d.m. of soil causes highly significant inhibition of the activity of arysulphatase, dehydrogenases, acid phosphatase, urease and β-glucosidase activity. In respect to their sensitivity to soil contamination with zinc, the enzymes can be ordered as follows: arysulphatase > dehydrogenases > acid phosphatase > urease > β-glucosidase. They hypothesized that the increased inhibition by Zn of enzymes was likely caused by the larger deprotonation of the sulfhydryl groups in enzyme proteins which enhance the interactions between the enzyme molecules (Eq.1). Zinc contamination causes persistent changes in the soil environment, but according to an index of resilience (RL), dehydrogenases are the first to return to the normal state of equilibrium (RL = 0.276), while arysulphatase takes longer (RL = 0.173) and acid phosphatase is the least resilient (RL = 0.064). Urease, instead of having its activity improved in time, becomes increasingly disturbed (RL = -0.350). Kucharski, et al. (2011) also reported that soil acidification reinforces the negative effect of zinc contamination most evidently in respect to the activity of β-glucosidase and arysulphatase.



(1)

Another important factor associated with the toxicity of heavy metals is the oxidation state of an element. For example, Speir et al. (1995) observed that Cr (III) is significantly less toxic to soil biological properties than Cr (V1) because of Cr (III)'s tendency to undergo precipitation, complexation or fixation reactions in soils. Cr (VI) is a powerful oxidizing agent which can cause enzyme degradation by oxidation of structural linkages and has been shown to cause irreversible inhibition of urease in soils (Speir et al., 1995).

Conclusion

Soil enzymes have been reported as useful soil quality bioindicators due to their relationship to soil biology, being operationally practical, sensitive, integrative, ease to measure and described as "biological fingerprints" of past soil management, and relate to soil tillage and structure. They are also indicative of biological equilibrium, fertility, quality and changes in the biological status of soil due to pollution. Their activities may, however, be influenced by unknown natural and anthropogenic activities either in a major or minor amount. Studies focusing the discovery of new enzymes from microbial diversity in the soil might be the most suitable practices that may positively influence their activities for improved soil ecosystem status.

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THE EFFECT OF CHANGES IN TRAFFIC FLOW ON MAMMAL ROAD KILL COUNTS

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Abstract. It is estimated that one million wild animals are killed on UK roads each year and these casualties are potentially a valuable source of data relating to the size and distribution of populations. Road kill surveys have the potential to form the basis of a nationwide mammal monitoring scheme with multi-species coverage and excellent area sampling. However it has been argued that road casualty counts cannot be used to monitor population change because variation in the number of road deaths is related more to variation in traffic flow than to population density. We used rabbit and hedgehog as study species to examine if there was a relationship between traffic flow and road kill counts. We found no correlation between changes in traffic flow and road counts of hedgehog or rabbit. We conclude that a decrease in road kill counts of these species is highly likely to represent a decrease in absolute abundance and would therefore be a good index with which to monitor population decline.

Keywords: *traffic mortality, road kill, mammal monitoring*

Introduction

It is estimated that one million animals are killed annually on UK roads (Slater, 1994), and these casualties represent a potentially very valuable source of data relating to the size and distribution of populations. Important advantages of using road casualty data to monitor mammals include the fact that road-kill surveys can simultaneously collect data on multiple species across broad geographic areas. While it would be impossible to design a single index of abundance or monitoring scheme capable of assessing numbers of all UK mammal species simultaneously (since some have very specific habits and/or locations), the broader the species range and area covered the better in terms of cost, efficiency and comparability. Furthermore, the fact that the most common mammalian casualties belong to easily identifiable species such as rabbit, *Oryctolagus cuniculus,* and hedgehog, *Erinaceus europaeus*, (Davies, 1957) means that there is no need for skilled experts to conduct surveys. Roads effectively form linear sampling devices across large areas of countryside, they are freely accessible to the public, and they are unlikely to suffer from problems associated with changing land use during or between surveys.

One of the most frequently voiced arguments against the use of casualty counts to monitor population change is that variation in the number of road deaths is related more to variation in traffic flow than to population density. Indeed, some studies have suggested that traffic flow is the single most important explanatory variable when considering variation in road casualty figures for certain taxa (Driessen et al., 1996; Fahrig et al., 1995). However, there are also several examples of studies suggesting that, although traffic flow has a role to play, it is less important than population density (e.g.

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Morris and Morris, 1988; Brockie et al, 2009). Between 2001 and 2004 traffic flow in Great Britain increased by an estimated 5.09%, or 1.69% per year (DTLR, 2005). Increased traffic flow could, irrespective of mammal population density, lead to an increase in mammal road counts, if the probability of a species crossing roads remains constant (or increases) but the probability of death while crossing roads increases in relation to traffic flow. Alternatively it could result in a decrease in mammal road kill counts, if a species is more inhibited from crossing roads as a result of greater traffic flow, or if road traffic mortality *per se* is having a biologically significant impact on a species population. A third possibility is that there would be no change in mammal road kill counts, if the increase in traffic flow is too small to influence road crossing behaviour or mortality. Of crucial importance may be whether traffic flow at times when mammals are active has increased sufficiently to influence road counts. Therefore, it is important to clarify the relationship between traffic flow and road casualty counts in order to determine the reliability of the latter as an index of mammal abundance.

A number of studies have included attempts to calibrate road casualty counts to estimates of abundance (Inbar and Mayer, 1999; Jahn, 1959; Mallick et al., 1998; McCaffery, 1973; Rolley and Lehman, 1992). Case (1978) showed that annual road-kill counts for pheasants (*Phasianus colchicus*) in Nebraska correlated well with a sightings survey carried out by rural postal delivery workers, and suggested that road casualty data may be a substitute or supplement to this census technique. The Illinois Department of Conservation compared racoon (*Procyon lotor*) road casualty numbers to spotlight surveys along roads and found a strong correlation, suggesting that numbers of road casualties recorded were generally accurate reflections of the numbers living in the surrounding area (Hicks, 1993). Studies of white-tailed deer populations have also shown correlations between spotlight surveys (Bellis and Graves, 1971), buck harvests (Arnold, 1978; McCaffery, 1973) and road casualties. In the UK, George et al (2011) found a direct relationship between numbers of rabbit road casualties and numbers living in the wider landscape.

Studies such as these reinforce the idea that road casualty figures could be accurately and reliably used in the UK to monitor mammal populations. Road-kill surveys have the potential to form the basis of a nationwide mammal monitoring scheme with multi-species coverage and excellent area sampling. Furthermore, the fact that unskilled volunteers could be employed as surveyors means that the necessary project effort and cost would be minimal.

Methods

Between August and September 2001 to 2004, 105 road transects were surveyed by passengers travelling by car. There were 15 transects in each of the seven landclass groups classified by the Centre for Ecology and Hydrology (Bunce et al., 1996). Each transect was approximately 97km (60 miles) in length and included only single-carriageway, non-urban roads (i.e., only those that did not pass through ≥3.2km of built up land). Each covered ‘A’, ’B’ and ‘Minor’ roads in the ratio 4:3:1, respectively. The nearest town, the odometer reading and the road number were recorded at the start and end of each transect, and at approximate 16km intervals along each. In addition, at each of these points the National Grid Reference (NGR) was recorded (to the nearest 10m) using a Garmin global positioning system (GPS) receiver.

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Whilst travelling each transect, whenever the surveyor turned onto a new road the odometer reading and new road number were recorded, so that the route could be accurately plotted. Each individual road was classified as a segment of the respective transect. Traffic flow was recorded as the count of all oncoming vehicles encountered during a journey.

During each transect survey, every mammal road casualty encountered was noted and identified to species level (or recorded as unidentifiable if this was impossible). The time of each casualty sighting was recorded, along with the NGR of the casualty site.

As it was not possible to ensure that all transects were identical in length, counts of mammal road casualties were transformed into the number recorded per 100km driven. The proportion of UK road area surveyed was calculated using data provided by the Department for Transport, Local Government and the Regions (DTLR; 2000).

Hedgehogs cross roads at a mean speed of 0.5m/second (S. Carter unpubl. data). We were unable to measure road crossing speeds of rabbits, but Garland (1983) suggests that their maximum running speed will be 15.5m/second. Hedgehogs, under experimental conditions which could have reduced risk-aversion, perceived a threat from a vehicle, on average, when it was 70m away. Rabbits, from the safety of the road verge, exhibited alarm responses to traffic that was, on average, 162m from them (George, 2004). From these findings and the Department of Transport statistics referenced above, we can estimate how changes in traffic flow may have affected the probability that a road is crossed, the probability that a mammal once on a road will collide with a vehicle and thus the probability of mammal road traffic accidents. We assume a worse case scenario where mammals are crossing an A road 10m wide, traffic is moving at 60mph (96kph) and that hedgehogs cross roads during the night time period defined previously, whilst rabbits may cross at any time of the day or night. We assume that mammals do not cross roads when vehicles are within the distance that they show risk-aversion to traffic and that they cross roads at the speeds suggested above.

The traffic flow-dependent probability of road crossing (P_c) is given by equation 1.

$$P_c = \frac{DV_tL_t}{L_r} \qquad \text{(Eq. 1)}$$

D is the distance at which a mammal detects approaching traffic from the road verge, V_t is traffic speed, L_t the length of rural principal A roads and rural minor roads with traffic at any one instant (second) and L_r is the length of rural principal A roads and rural minor roads.

The probability that a mammal, once on a road, will be hit by a vehicle (P_h) is given by equation 2, where T is the length of time taken by a mammal to cross a road.

$$P_h = \frac{TL_t}{L_r} \qquad \text{(Eq. 2)}$$

The probability of traffic-dependent mammal-vehicle collision (P_{ch}) is the product of the two forgoing estimates.

Results

Our dataset was derived from standardised journeys covering different landscape types and 10,202km of roads driven (approximately 3% of the total length of all UK roads: 14% of the length of all 'A' roads, 11% of 'B' roads and 0.4% of 'Minor' roads). In total 893 dead rabbits and 164 dead hedgehogs were counted.

Traffic flow was recorded as the count of all oncoming vehicles during a journey.

Both rabbit counts and traffic flow were related to road width (*Figure 1*).

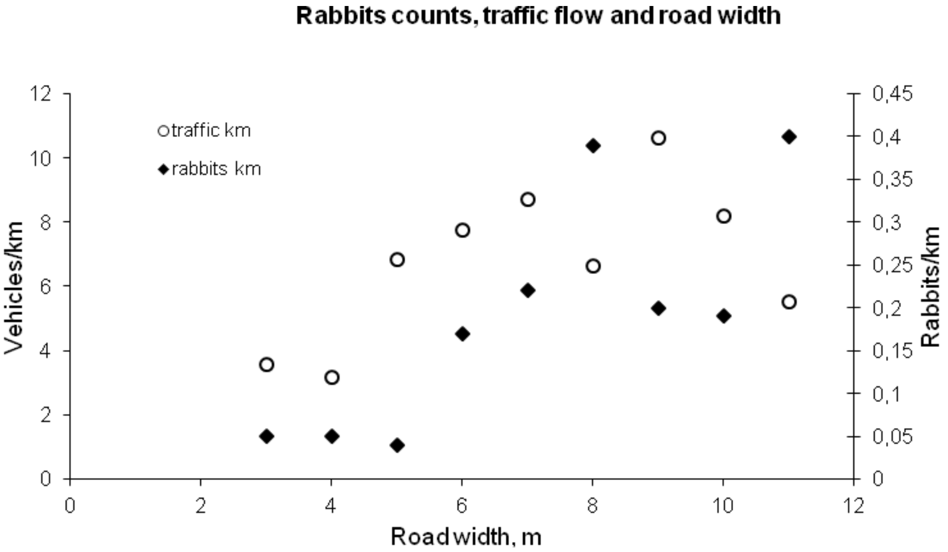

***Figure 1.** The relationships between road width, traffic flow and rabbit road counts.*

However road width, but not traffic flow was a (strong) predictor of rabbit road kill counts (F=20.86, d.f. 1,41, P<0.001, r^2=33%). Including the (non-significant) traffic flow term in the above model increased r^2 by only 2%. The situation was very similar for hedgehog (*Figure 2*).

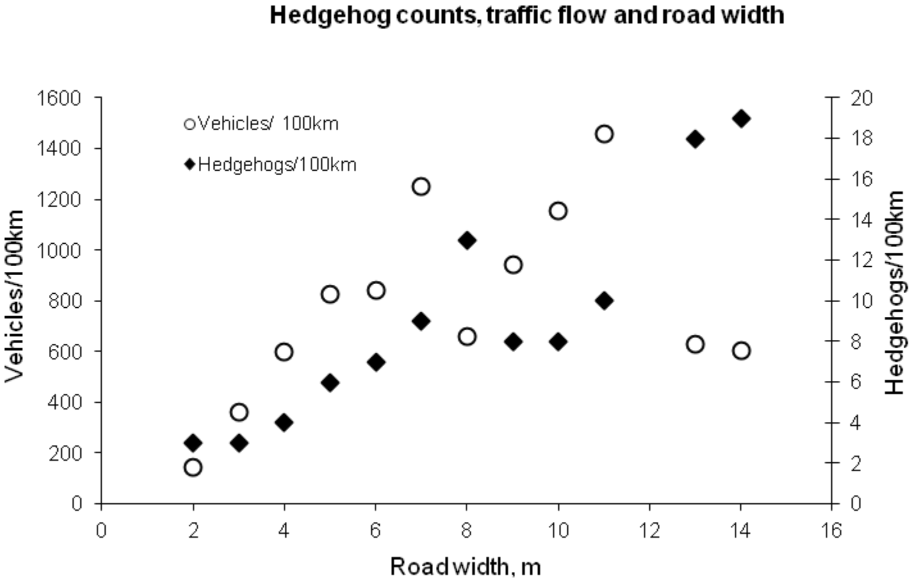

***Figure 2.** The relationships between road width, traffic flow and hedgehog road counts.*

Road width, but not traffic flow, was a strong predictor of hedgehog road kill counts (F=27.73, d.f. 1,63, p<0.001, r^2=30%). Including the (non-significant) traffic flow term in the model did not increase r^2 at all. These data suggest that it is road width, not traffic flow that influences road kill counts. However, traffic flow and road width are, inevitably, autocorrelated and we cannot rule out the possibility that the effect of one masks that of the other.

It is highly instructive to consider how the increase in traffic flow has affected what we here term the ‘safety window’ i.e. the period of time when a section of road will be free from traffic and a mammal will be able to cross without risk of colliding with vehicles. We used 2004 traffic flow data (vehicle kilometres driven per year) for rural principal A roads and rural minor roads (Table 1.3 in Transport Statistics Bulletin 2004 (DTLR, 2005)). This we divided by the length of the same road types (Table 4.1b in Transport Statistics Bulletin 2004) to give a measure of the mean length of time between vehicles during day and night (the safety window). Then, using the ratio of day time to night time (here defined as 19:00 to 05:00) traffic flow (Table 3.3, Transport Statistics Bulletin 2004; day time traffic flow is 3.86 times that of night time traffic) we estimated the night time safety window.

During day and night a safety window of 10 seconds was available on 89% of the rural road network in Scotland and 76% and 66% of the network in England and South East England respectively (*Figure 3*).

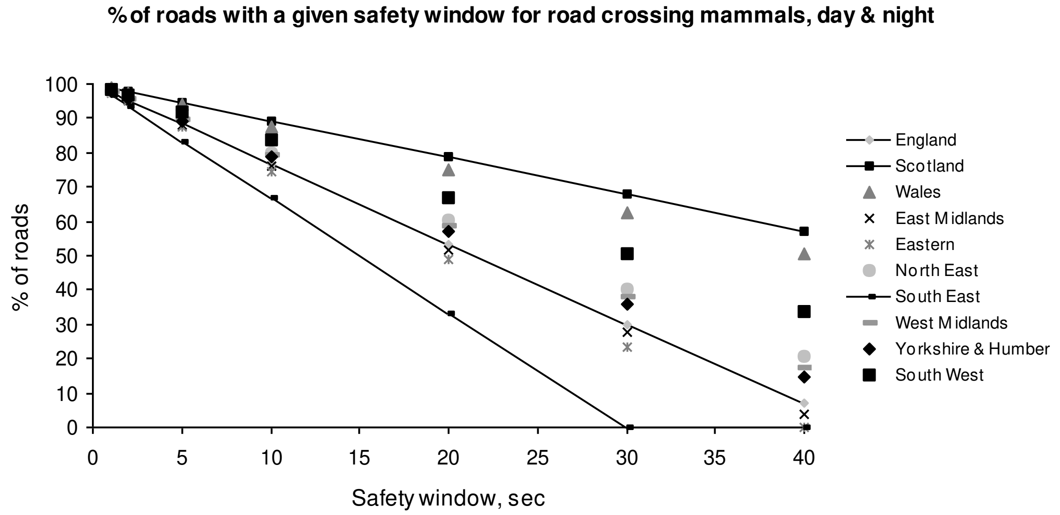

Figure 3. *The percentage of the road network in rural areas without vehicular traffic for a given length of time (safety window, seconds). Data for day and night time. The lines, top to bottom, represent Scotland, England and South East England.*

During the night a safety window of 10 seconds was available on 97%, 93% and 91% of the road network in Scotland, England and South East England. A night time safety window of 40 seconds was available on 85%, 75% and 65% of the road network in the same regions (*Figure 4*).

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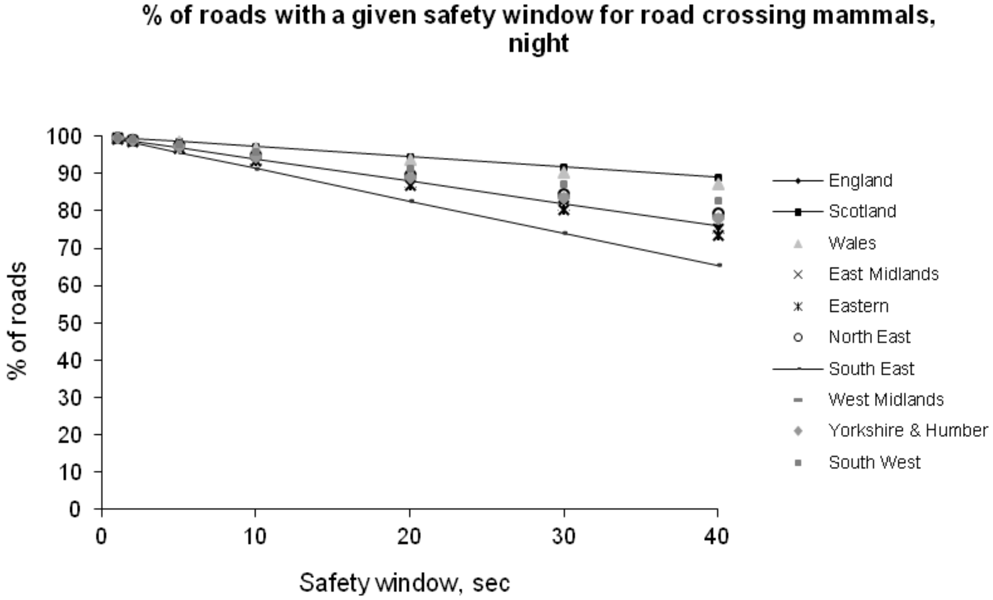

Figure 4. *The percentage of the road network in rural areas without vehicular traffic for a given length of time (safety window, seconds). Data for night time only (19:00 to 05:00). The lines, top to bottom, represent Scotland, England and South East England.*

These calculations suggest that most of the road network will offer ample time for hedgehogs and rabbits to cross even wide rural roads (time to cross a 10m wide road: hedgehogs 20 seconds, rabbits 0.64 seconds).

It is clear that for both rabbits and hedgehogs the vast majority of the rural road network is likely to be perceived as free from risk of oncoming vehicles and the traffic-dependent probability of road crossing is thus very high (*Figure 5*).

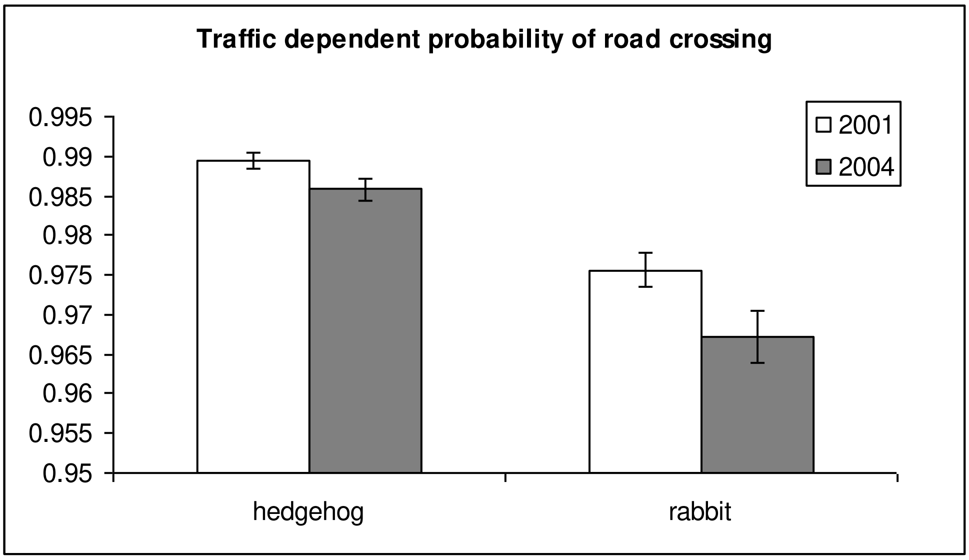

Figure 5. *The influence of traffic flow on the probability of animals (hedgehog and rabbit) crossing roads (UK mean ±s.e.).*

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However because traffic speeds are high compared with speeds of mammal road crossing (hedgehogs $0.5m^{-1}s^{-1}$; rabbits $15.5m^{-1}s^{-1}$; vehicle travelling at 60mph, $26.6m^{-1}s^{-1}$), even risk-averse road-crossing mammals are at risk from collisions with vehicles.

The probability of rabbits being hit by a vehicle when on a road was, due to their much greater speed, two orders of magnitude less than that for hedgehogs (*Figure 6*).

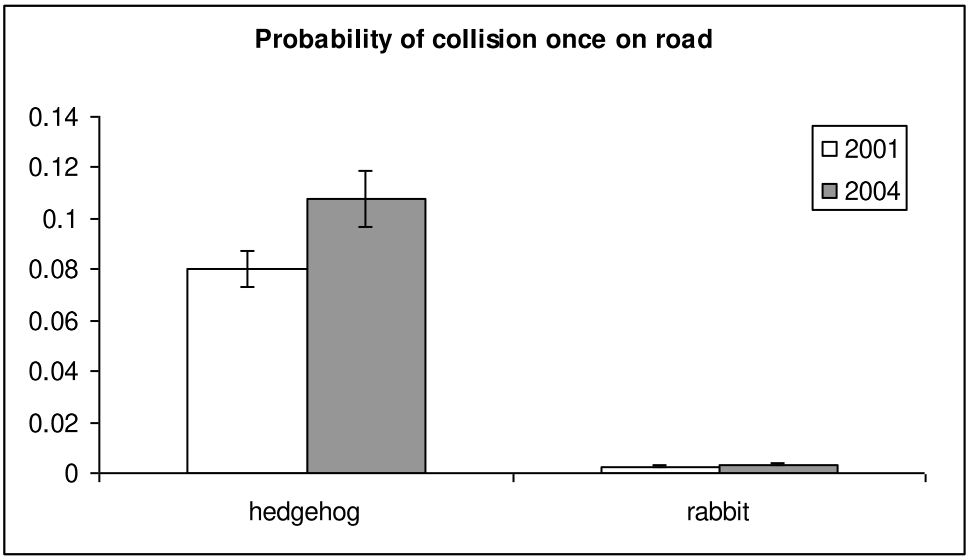

Figure 6. *The influence of traffic flow on the probability of animals (hedgehog and rabbit) colliding with a vehicle once on road.*

The overall traffic-dependent probability of rabbits colliding with vehicles was very low. It increased from 0.0025 in 2001 to only 0.0033 in 2004. We suggest that the small order of magnitude of this effect would not have been likely to influence rabbit road counts – rabbits will generally be too fast and too risk-averse. For hedgehogs, the same measure increased from 0.079 in 2001 to 0.10 in 2004. This could, perhaps, have influenced hedgehog road counts.

In view of the foregoing findings it is not surprising that there is no correlation between changes in traffic flow and road counts of hedgehog or rabbit (2001 to 2004, counts by country and region, for hedgehog: Spearman correlation coefficient 0.4, n=10, p=0.286; for rabbit: Spearman correlation coefficient –0.075, n=9, p=0.849). Clearly this a fairly crude test of association and traffic data at a finer spatial scale (10-km grid squares) are desirable.

Discussion

With reference to the potential effects of increasing traffic flow on road-kill counts, our results suggest that increased risk aversion resulting from higher traffic flow *per se* will not have been nearly sufficient to account for the 2001 to 2004 reduction in hedgehog road kill counts in England reported from the annual Mammals on Roads survey (Mammals Trust UK, 2005).

This conclusion holds provided the primary cue of risk perceived by hedgehogs is a direct one: the sight and sound of oncoming vehicles. It is of course possible that

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hedgehogs perceive greater risk from roads with more traffic (irrespective of road width) by using indirect olfactory cues. However, levels of pollutants (potential cues) next to rural roads are not likely to have increased sufficiently in three years to provide a reliably discriminatory cue of higher traffic flow. Furthermore, roads, even without traffic, are likely to be a very noisy environment to the olfactory sense of even an insectivore. We conclude that hedgehogs are not likely to have become more inhibited from crossing roads during the survey period. It is suggested that further research is required into the behavioural responses of rabbits and hedgehogs (the two species most commonly seen dead on UK roads) to roads and traffic, with the aim of determining how these behavioural responses affect the probability of an individual animal crossing a road, and dying as it does so. This is relevant because it has important bearing on the relationship between population density and road-kill counts. For example, given a certain population density, road casualty numbers are likely to be higher for those species that respond to oncoming traffic by freezing in the road, compared to those whose members run for the verge.

The decrease in hedgehog road kill counts could be the direct or partial result of increased road traffic mortality reducing hedgehog abundance. Huijser (2000) found that traffic flow had no significant impact on hedgehog population density, though his study was only capable of detecting changes of more than 35% in hedgehog density. Clearly, however, if road mortality is directly affecting hedgehog abundance and hedgehog road counts are decreasing, we can be certain that hedgehog abundance is declining. If road mortality is affecting abundance then an increase in road counts might underestimate increases in absolute abundance. A decrease in road kill counts, however, will still be a good index of a decrease in absolute abundance.

It is crucial to understand what these calculations can and cannot show. They are used here only to explore the relations between changes in traffic flow and the likelihood of mammal road casualties. They do not provide absolute estimates of the probabilities of mammal road crossing or of mammal-vehicle collisions occurring. Rather, they show only how traffic flow may impact on these probabilities. Our calculations are based on very extensive traffic monitoring by the Department of Transport, but this is designed to show changes in traffic flow in the order of hundreds of thousands of vehicle-kilometres driven per year. It may thus not sort well with extrapolation to a scale of metres which is needed in understanding risks to mammals from road crossing. Nevertheless our estimates of the risks of road crossing are likely to be overestimates because traffic is clumped in space, not evenly distributed over the rural road network as our calculations assume and especially because we assume wide (10m) roads.

In conclusion, our results suggest that a decrease in road counts of hedgehog, and in general, is highly likely to represent a decrease in absolute abundance (at a high rate, albeit over only four years) and thus be a good index with which to monitor population decline. Increases in road counts coincident with increases in traffic flow need to be viewed with more caution.

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HONEYBEE (*APIS MELLIFERA*) MEDIATED INCREASED REPRODUCTIVE SUCCESS OF A RARE DECEPTIVE ORCHID

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Abstract. Honeybees are the most numerous and significant among insect pollinators, but despite their significance their potential role in increasing the reproductive success of threatened European orchids remains largely unexplored. In this study we aim to evaluate the effect of apiary proximity on the fructification rate of the rare and endangered Adriatic Lizard Orchid (*Himantoglossum adriaticum* H. Baumann). To do so, first we quantified the reproductive success (fruit set) of the Adriatic Lizard Orchids in a Hungarian population in close proximity to a honeybee apiary at Nagytevel. Then we compared the reproductive success in this population to the reproductive success in 3 other Hungarian, 4 Croatian populations (observed during the same year, 2013) and further 4 populations from Hungary (studied in previous years, 1992–2011) with no apiaries in the close proximity of these. The realised 61.7 % fruit set at Nagytevel is the highest reproductive success ever recorded for this species. The importance of apiaries is further emphasised by the fact that the reproductive success of Lizard Orchid individuals decreased significantly with increasing distance from the apiary. Our results provide empirical support for the conservation importance of apiculture and suggest promising results for practical application of apiaries in the conservation of of deceptively pollinated orchids characterised by lower fruit set (e.g. *Orchis, Anacamptis, Himantoglossum*).

Keywords: *apiculture, fruit set, Himantoglossum adriaticum, pollination crisis, Orchidaceae*

Introduction

The family *Orchidaceae*, with its more than 25,000 species, is one of the largest in the plant kingdom (Dressler, 1993). Despite their world-wide dispersion and extreme plasticity, the majority of these species are of key conservation importance (Jacquemyn et al., 2005; Kull and Hutchings, 2006; Swarts and Dixon; 2009). Causes of their decline are complex, but – independently of the conservation status – the long-term persistence of plant populations in every case partly depends on seed production.

The reproductive success of entomophilous orchids depends on the effectiveness of pollination processes, namely; pollinator attraction, pollen sack removal and its deposition on conspecific flower. Such interactions have immediate consequences for

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endangered *Spiranthes* species, as increases in both conspecific and heterospecific coflowering density may ameliorate the negative effects of rarity on pollination, hence overall reproductive success (Duffy and Stout, 2011). Lack of suitable insects to pollinate the flowers of *Orchis militaris* has been characteristic of British populations since the beginning of 19th century according to herbaria data (Farrell, 1985). Similar low fruit set were detected at the beginning of 20th century in Germany (Sprengel, reported by Godfrey 1933).

The flowers of deceptive (i.e. non-rewarding) orchids usually experience very low pollinator visitation rates that will result in low levels of fruit set compared to rewarding orchids (Dafni and Ivri, 1979; Gill, 1989; Neiland and Wilcock, 1999; Harder and Johnson, 2008). Deception is not rare among the orchids, it is estimated that as much as one-third of orchid species use various forms of floral deception to attract pollinators (Dafni, 1984; Ackerman, 1986; Jersáková et al., 2006).

In temperate climate regions the most important pollinators are insects, among which bees predominate. It was the finding of the last decades that bees in parallel with insect-pollinated plants are declining on both western European (Biesmeijer et al., 2006) and global scales (Allen-Wardell et al., 1998; Kearns et al., 1998; Potts et al., 2010). The major threat to bee diversity is habitat loss, but competitive invasive species, newly emerging diseases, extensive use of pesticides, and climate change also have the potential to negatively impact bee population sizes (Brown and Paxton, 2009; Brittain and Potts, 2011). Bees require a large number of flowers to gather enough nectar and pollen to support the hive, and also need a large variety of flowering plants with different flowering times to provide them continuous food supplies throughout the growing season (Kearns and Inoyue, 1997). However, due to the relatively recent change in land-use practices, the nectar sources for bees, characteristic to fragmented landscapes have greatly diminished in recent years (Feon et al., 2013).

Despite the dominant individual number of honeybee in the bee assemblages and their importance in pollination, the potential role in the reproductive success of European orchids remains largely unclear.

The aim of the present study was to evaluate the role of apiculture in increasing the reproductive success of the rare and endangered Adriatic Lizard Orchid (*Himantoglossum adriaticum* Baumann). Red Data Books of most countries where it occurs (Conti et al., 1997; Király 2007; Maglocky and Feráková, 1993; Grulich, 2012). The conservation status of the species is Critically Endangered in the Czech Republic and in Slovakia, Endangered in Austria and in Hungary, Vulnerable in Slovenia and Near Threatened in Croatia (Dostalova et al., 2011). Additionally *H. adriaticum* is suffering ongoing population declines and is listed in the Annex II of Council Directive 92/43/EEC (the ‘Habitats Directive’).

The reproductive success of *Himantoglossum adriaticum* is generally low. Previously published fruit set data varied between 4.5 and 44 % in Austria (Vöth, 1990), and between 5.4% and 23.3% in Hungary (Bódis and Molnár, 2009).

We measured reproductive success (fruit set) in a population of Adriatic Lizard Orchid in close proximity to a honeybee colony and compared it with other populations observed during the same year without bees and four populations studied in previous years.

An apiary, composed of 174 hives hosting *Apis mellifera* colonies in 4 containers was located on the study site at Nagytevel (Hungary) between 25th May and 05th July 2013 (coinciding with the flowering time of *Himatoglossum* orchids). The distance of the hives from flowering *Himantoglossum* plants varied between 196–1455 meters. All studied *H. adriaticum* flowering specimens were situated within the effective foraging radius (1.5–3 km, Frisch, 1965; Bagella et al., 2013) of honeybees in Europe. To the best of our knowledge, no apiaries were present in the vicinity of this population in other years, nor in the rest of populations studied here.

We specifically stated the following study questions: (i) Is there significant effect of the apiary on the reproductive success of *H. adriaticum* population, living within the foraging distance of honeybee? (ii) Is there correlation between the individual reproductive success (fruit set) and the distances from the apiary?

Materials and methods

Study species

Himantoglossum adriaticum H. Baumann has a central submediterranean distribution, currently known to occur in Croatia, Slovenia, northern and central Italy, eastern Austria, western Slovakia and Hungary (Pecoraro et al., 2013, Rybka et al., 2005).

The characteristic habitat of the species is full sun to mid-shade and dry calcareous substrates. It prefers poor grassland, banks, thickets, woodland edges and open woodlands, up to 1600 meters of altitude above sea level (Delforge, 2006), relatively often occuring in secondary habitats, e.g. abandoned vine-yards, extensively used orchards and mown grassy verges on the sides of public roads (Molnár, 2011). The Adriatic Lizard Orchid is a terrestrial, tuberous photoautotrophic orchid with overwintering rosette, which consists of 2–7 large, lanceolate, pale green basal leaves. The generative shoots are 30–80 cm tall, the inflorescence is elongated and lax, composed of 15–40 brownish red, typically malodorous flowers. The lip is deeply 3-lobed, the median lobe is long, ribbon-like, incised (Delforge, 2006; Molnár, 2011). The flowers have been observed to be pollinated by the following bee species: *Andrena haemorrhoa, A. carbonaria, A. nigroaenea, A. potentillae*, *Apis mellifera* (Claessens and Kleynen, 2011), *Colletes similis* (Vöth, 1990; Sulyok et al., 1998), *Bombus* spp. (Teschner, 1980), *Osmia caerulescens*, *Lasioglossum* (*Evylaeus*) *morio*, *Lasioglossum* (*Evylaeus*) *lucidulum*, *Megachile melanopyga* (Bódis, 2010).

Measuring reproductive success

Eight populations of *Himantoglossum adriaticum* were studied in Hungary and Croatia in 2013 (*Table 1*).

Table 1. *Adriatic Lizard Orchid populations studied in 2013. n: total number of undamaged fruiting shoots observed; Date of observation: flowering and fruiting sampling.*

No.	Country	Locality	n	Date of observation	Geocoordinates
1	Hungary	Nagytevel	39	20th June; 31st July	N 47.264°, E 17.598°
2	Hungary	Kőszeg	36	19th June.; 13rd July	N 47.375°, E 16.526°
3	Hungary	Keszthely	33	15th June; 14th July.	N 46.794°, E 17.277°
4	Hungary	Sümeg	47	17 th June; 13rd July	N 46.957°, E 17.351°
5	Croatia	Učka	88	2nd–3rd June; 8th–9th July	N 45.317°, E 14.175°
6	Croatia	Paz	7	4 th June; 9th July	N 45.277°, E 14.104°
7	Croatia	Letaj I.	14	4 th June; 9th July	N 45.255°, E 14.121°
8	Croatia	Letaj II.	12	4 th June; 9th July	N 45.255°, E 14.132°

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All generative shoots were individually marked with labelled nail. The number of flowers on each specimen was recorded during the first visits on each site (between 2th and 20th June), while the number of developed fruits was counted 3–5 weeks later (between 8th and 31st July). Shoots which were damaged during the flowering period were excluded from the analyses. Reproductive success was defined as fructification rate: total number of fruits in the population divided by the total number of flowers in the given population on a given specimen; in other terms fruit set. The reproductive success of populations were compared to data on the fructification rate of the same species collected in earlier years using the same methodology (*Table 2*). The reproductive success means in every case population level.

Table 2. *Adriatic Lizard Orchid populations studied between 1992–2011. n: total number of undamaged fruiting shoots observed in a given population.*

No.	Country	Locality	Study period	n
1	Hungary	Nagytevel	2010, 2011	11, 41
2	Hungary	Kőszeg	2010, 2011	33, 18
3	Hungary	Keszthely	1992–2011	17–73
4	Hungary	Sümeg	2002, 2003, 2008–2011	76, 19, 25–49

Data analyses

To compare the reproductive success of different populations we employed a generalized linear model (GLM) with quasibinomial error structure. This approach was necessary because fruit set (the measure of reproductive success) is a binomial variable (it takes values between 0 and 1) and samples were not uniformly distributed within this interval (in several populations most plants had fruit sets close to 0); quasibinomial models take into account such overdispersion in the data. The relationship between distance to the apiary and fruit set was also evaluated using a quasibinomial GLM. GLMs were implemented in the R Statistical Environment (R Core Team, 2013).

Results

Reproductive success varied considerably both among sites and among years within specific sites (*Table 3*). Average fructification rate was under 10% in Učka and Keszthely, between 10–20% in Letaj, little above 20% in Sümeg and Paz, little above 30% in Kőszeg and outstandingly high (61.7%) at Nagytevel in 2013. The number of flowering individuals was not associated with the measured reproductive success, but the largest population (Učka) had the lowest fruit set.

The average reproductive success of the *H. adriaticum* population at Nagytevel in 2013 was significantly higher than in other population studied by us (both 2013 and earlier years), with three exceptions: Nagytevel 2010, Keszthely 2004 and Sümeg 2003 (*Table 4*).

The importance of apiaries in the pollination of Lizard Orchids is also shown by our observation that individual reproductive success in the population at Nagytevel decreased significantly with increasing distance from the apiary (quasibinomial GLM $F_{1,39}=18.52$, $p<0.001$, *Fig. 1.*).

Table 3. *Reproductive success of populations studied in 2013 and in earlier years. n: total number of undamaged fruiting shoots observed in a given population.*

Locality	Year	n	No. of flowers	No. of fruits	Fructification rate (%)
Nagytevel	2013	41	1477	911	61.7
Kőszeg	2013	36	1256	396	31.5
Sümeg	2013	54	1657	356	21.5
Keszthely	2013	32	1105	105	9.5
Učka	2013	100	2965	259	8.7
Letaj I.	2013	16	462	64	13.9
Letaj II.	2013	12	422	77	18.2
Paz	2013	7	210	44	21
Nagytevel	2010	11	227	118	52
Nagytevel	2011	41	1101	166	15.1
Sümeg	2002	76	2326	633	27.2
Sümeg	2003	19	486	256	52.7
Sümeg	2008	32	1009	242	24
Sümeg	2009	29	849	358	42.2
Sümeg	2010	25	640	179	28
Sümeg	2011	49	1337	281	21
Kőszeg	2010	33	1300	549	42.2
Kőszeg	2011	18	406	92	22.7
Keszthely	1992	25	909	171	18.8
Keszthely	1993	17	544	51	9.4
Keszthely	1994	19	671	36	5.4
Keszthely	1995	73	2758	219	7.9
Keszthely	1996	67	2130	342	16.1
Keszthely	1997	23	686	58	8.5
Keszthely	1998	30	975	197	20.2
Keszthely	1999	31	971	96	9.9
Keszthely	2000	12	333	19	5.7
Keszthely	2001	10	315	21	6.7
Keszthely	2002	32	912	156	17.1
Keszthely	2003	19	642	161	25.1
Keszthely	2004	20	524	286	54.6
Keszthely	2005	55	1736	297	17.1
Keszthely	2006	21	601	207	34.4
Keszthely	2007	21	530	187	35.3
Keszthely	2008	33	1040	128	12.3
Keszthely	2009	23	579	61	10.5
Keszthely	2010	12	267	10	3.7
Keszthely	2011	29	738	61	8.3

Discussion

The average reproductive success of the studied Lizard orchid populations was independent of the size of the population. A similar phenomenon was detected in the case of another nectarless orchid, *Orchis mascula*, where fruit set was low even in large populations in highly fragmented calcareous grassland in southern Belgium (Meekers and Honnay, 2011).I In that area the percentage fruit set of same species was independent from population size in undisturbed woodland but strongly related to population size in coppiced woodland (Jacquemyn et al., 2008). Fruit set was positively related to population size in the nectar producing *Gymnadenia conopsea* too (Meekers and Honnay, 2011). In contrast definitely reduced fecundity was observed in large populations of a nectar-rewarding species, *Spiranthes spiralis* (Petanidou et al., 2013). Fruit set in small populations of non-rewarding *Orchis purpurea* increased with population size (and decreased with increasing nearest-neighbour distance) (Jacquemyn et al., 2002).

Table 4. *Parameter estimates, their standard errors, t-values and significance levels from the quasibinomial GLM comparing reproductive success of the Nagytevel 2013 population (the intercept of the model) to other samples. Reproductive success was significantly lower in other populations / years, except for the Keszthely 2004, Nagytevel 2010 and Sümeg 2003 samples.*

Locality / Year	Estimate	Standard Error	t value	p value
Letaj II.-2013	-1.976	0.353	-5.603	0.000
Paz-2013	-1.804	0.458	-3.940	0.000
Letaj I.-2013	-2.304	0.373	-6.173	0.000
Učka-2013	-2.303	0.194	-11.857	0.000
Keszthely-2013	-2.730	0.298	-9.162	0.000
Sümeg-2013	-1.779	0.198	-9.008	0.000
Kőszeg-2013	-0.429	0.215	-2.000	0.046
Keszthely-1992	-1.938	0.258	-7.502	0.000
Keszthely-1993	-2.745	0.403	-6.810	0.000
Keszthely-1994	-3.346	0.462	-7.240	0.000
Keszthely-1995	-2.685	0.214	-12.524	0.000
Keszthely-1996	-1.931	0.198	-9.743	0.000
Keszthely-1997	-2.858	0.379	-7.535	0.000
Keszthely-1998	-1.849	0.247	-7.478	0.000
Keszthely-1999	-2.686	0.309	-8.685	0.000
Keszthely-2000	-3.281	0.624	-5.260	0.000
Keszthely-2001	-3.115	0.598	-5.212	0.000
Keszthely-2002	-2.054	0.265	-7.749	0.000
Keszthely-2003	-1.570	0.272	-5.775	0.000
Keszthely-2004	-0.292	0.265	-1.104	0.270
Keszthely-2005	-2.054	0.214	-9.584	0.000
Keszthely-2006	-1.120	0.260	-4.298	0.000
Keszthely-2007	-1.083	0.272	-3.986	0.000
Keszthely-2008	-2.440	0.279	-8.732	0.000
Keszthely-2009	-2.615	0.375	-6.977	0.000
Keszthely-2010	-3.722	0.841	-4.425	0.000
Keszthely-2011	-2.883	0.371	-7.775	0.000
Kőszeg-2010	-0.789	0.200	-3.951	0.000
Kőszeg-2011	-1.704	0.335	-5.086	0.000
Nagytevel-2010	-0.397	0.369	-1.075	0.282
Nagytevel-2011	-2.205	0.257	-8.579	0.000
Sümeg-2002	-1.024	0.177	-5.791	0.000
Sümeg-2003	-0.369	0.272	-1.359	0.175
Sümeg-2008	-1.629	0.235	-6.946	0.000
Sümeg-2009	-0.792	0.226	-3.506	0.000
Sümeg-2010	-1.422	0.265	-5.359	0.000
Sümeg-2011	-1.800	0.221	-8.142	0.000

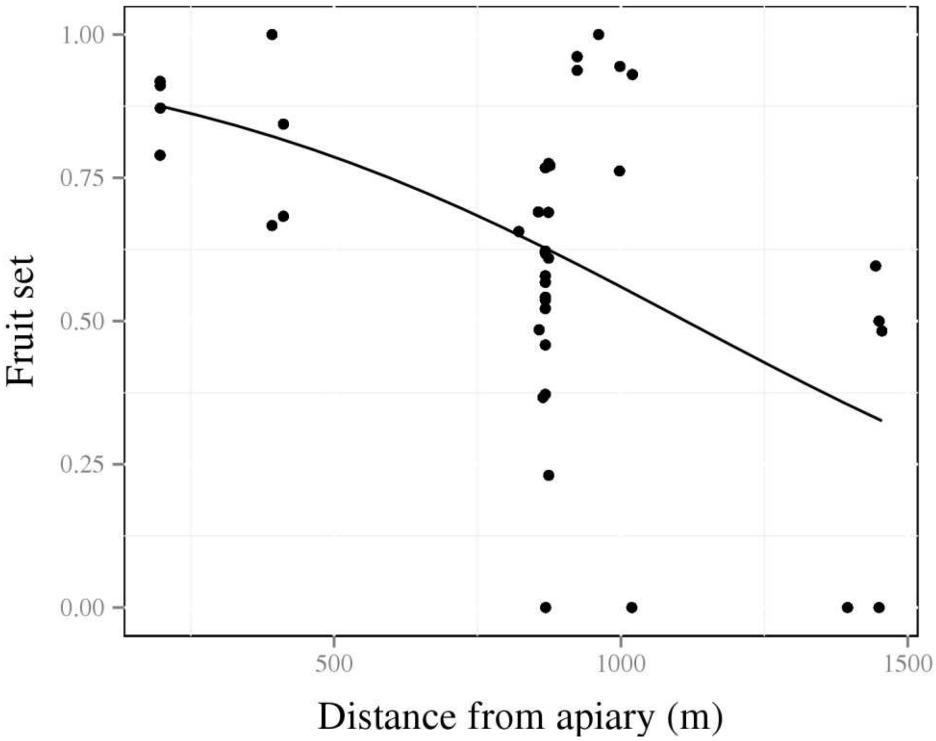

Figure 1. *Relationship between individual distance from apiary and fruit set in Adriatic Lizard Orchids at Nagytevel (2013).*

The realised 61.7% fructification rate in Nagytevel is the highest ever published population-level average reproductive success of *H. adriaticum*, although this was not significantly higher than the 52–54.6% reproductive success observed in three other populations in Hungary during earlier years. Overall average fruit set values of nectarless and nectariferous European orchids are 28% and 63%, respectively (Neiland and Wilcock, 1999) implying that the *Himantoglossum* population at Nagytevel reached the average fructification level of rewarding orchids.

Our results also show that the reproductive success of individual flowers in this population was negatively related to their distance from the beehives. Together, these results strongly suggest that honeybees were causally implicated in the high reproductive success of this population in 2013. Abundance of flower visiting honeybees depends on the distance from the nearest apiary in agricultural landscapes (Steffan-Dewenter and Tscharntke, 1999). Because the diversity of pollinators declines rapidly, the role of honeybees in pollination increases strongly (Brown and Paxton, 2009; Potts et al., 2010).

Frequent pollinators of *H. adriaticum* include both social (*Bombus* spp.) and solitary bees (*Andrena* spp., *Colletes similis*, *Osmia caerulescens*, *Megachile melanopyga*, *Lasioglossum* spp.). These are common species and forage on a wide variety of plant species. In arable field-margins in western France the most common pollinator species were *Andrena* and *Lasioglossum* (Féon et al., 2013), while near Göttingen in Germany, in an intensively managed agricultural landscape with islands of semi-natural calcareous grasslands *Osmia* and *Megachile* bee species were common pollinators (Steffan-Dewenter and Tscharntke, 2000).

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Bee and insect-pollinated plant species richness was highest in Hungary when compared to Switzerland and the Netherlands. The richness of insect-pollinated plants is a good predictor of bee species richness across large geographic scales (Batáry et al., 2010).

Despite of relatively high species richness of bees and flowering plants in Hungary the pollination effect of honeybees was significant in Nagytevel. We can not exclude, however, that the earlier high fruit set data from Hungary were caused by honeybees. On the one hand average bee density is continuously increasing in Hungary: there were 7.78 honeybee colonies/km^2 in 1992 and 12.18 in 2012. On the other hand average bee density can strongly vary regionally (Tóth, 2013).

According to published data, honeybees are common pollinators of 21 food-deceptive and 12 nectar rewarding European terrestrial orchids (*Table 5*), mostly from the genera *Anacamptis* and *Orchis* (6–6 species), *Dactylorhiza* (5 species), *Epipactis*, *Gymnadenia* and *Himantoglossum* (3–3 species).

Table 5. *Literature data about pollination European orchids by honeybees.*

Species	Pollination	Source
Anacamptis boryi	Food-deceptive	Gumbert and Kunze 2001
Anacamptis coriophora	Nectar rewarding	Berger 2004
Anacamptis coriophora subsp. *fragrans*	Nectar rewarding	Peisl and Forster 1975
Anacamptis morio	Food-deceptive	Darwin 1877
Anacamptis palustris	Food-deceptive	Sulyok in Molnár 2011
Anacamptis papilionacea	Food-deceptive	Vöth 1989a
Anacamptis pyramidalis	Food-deceptive	Vöth 1999
Dactylorhiza fuchsii	Food-deceptive	Reinhard et al.1991
Dactylorhiza ochroleuca	Food-deceptive	Bournérias and Prat 2005
Dactylorhiza sambucina	Food-deceptive	Nilsson 1980
Dactylorhiza sphagnicola	Food-deceptive	Claessens and Kleynen 2011
Dactylorhiza sudetica	Food-deceptive	Berger 2005
Epipactis atrorubens	Nectar rewarding	Wiefelspütz 1970
Epipactis helleborine	Nectar rewarding	Müller 1988
Epipactis palustris	Nectar rewarding	Darwin 1877
Goodyera repens	Nectar rewarding	Claessens and Kleynen 2013
Gymnadenia corneliana	Nectar rewarding	Berger 2009
Gymnadenia lithopolitanica	Nectar rewarding	Vöth 2000
Gymnadenia nigra s.l.	Nectar rewarding	Godfrey 1933
Himantoglossum jankae	Food-deceptive	Teschner 1980
Himantoglossum adriaticum	Food-deceptive	Vöth 1990
Neotinea tridentata	Food-deceptive	Vöth 1989b
Neottia ovata	Nectar rewarding	Nilsson 1981
Orchis italica	Food-deceptive	Vöth 1998
Orchis mascula	Food-deceptive	Darwin 1877
Orchis militaris	Food-deceptive	Vöth 1999
Orchis ovalis	Food-deceptive	Vöth 1999
Orchis purpurea	Food-deceptive	Godfrey 1933
Orchis simia	Food-deceptive	Godfrey 1933
Spiranthes romanzoffiana	Nectar rewarding	Larson and Larson 1987
Spiranthes spiralis	Nectar rewarding	Petanidou et al. 2013
Traunsteinera globosa	Food-deceptive	Vöth 1994

Based on our findings the practical application of apiaries in orchid conservation (for increasing reproductive success) seems to be promising, especially in the case of deceptive orchids, which are characterized by a low flower visitation rate by pollinators and therefore by a low fruit set.

On the one hand, honeybees have been assumed to be superior competitors because of the high nectar and pollen requirements of their large perennial colonies compared to solitary bees. Furthermore they are believed to rapidly exploit rich patches of flowering plants using their dance language to communicate direction and distance of food resources to other foragers (Frisch, 1965; Visscher and Seeley, 1982; Beekman and Ratnieks, 2000). On the other hand, apiculture does not appear to have a negative impact on wild bees: neither species richness nor abundance of wild bees was negatively correlated with the density of honeybee colonies (within a radius of 2 km) or the density of flower-visiting honeybees per site despite the assumed competition between them (Steffan-Dewenter and Tscharntke, 2000). The positive impact of increased honeybee amounts to the reproductive success of wild plants is not always well supported by empirical data (Klein et al., 2007). Our results based on reproductive success of deceptively pollinated orchid are confirming those opinions, that honeybees apart from being among the most important pollinators of agricultural crops, they also provide important pollination services to wild plants.

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ARBUSCULAR MYCORRHIZAL FUNGI COLONIZING THE PLANT COMMUNITIES IN ERITREA, NORTHEAST AFRICA

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Abstract. The colonization and diversity of arbuscular mycorrhizal (AM) fungi associated with some common plants were investigated in Eritrea, northeast Africa. Of the 37 plant species belonging to 14 families collected, 26 were colonized by AM fungi. The mean percentage root length colonized was 21.34%, ranging from 0-91%. Nineteen plant species formed Paris-type and 10 Arum-type AM. The average spore density was 43 per 50 ml air-dried soil and the average species richness was 2.4 AM species per sample. There was no correlation between percentage root length colonized by AM fungi, intensity of infection and spore density. Of the 21 AM fungal taxa belonging to four genera isolated from the root region soils, two belonged to Archaeospora, two to Diversispora fifteen to Glomus and two to Scutellospora.

Key words: *common plants; AM colonization; species diversity; Eritrea*

Introduction

Arbuscular mycorrhizas (AM) fungi represent a monophyletic fungal lineage (Glomeromycota) that establishes an intimate association with most land plants of the universe. This symbiotic association is known to determine the function and biodiversity of the terrestrial ecosystems by producing extensive underground networks, composed of hyphae and spores; that interconnect a number of unrelated individual plant species (Bonfante and Genre, 2010). This symbiosis acts in important processes in the soil-plant interface, such as improving plant nutrition by increased nutrient and water uptake (Harley and Smith, 1983; Smith et al., 2011), enhancing establishment, growth and survival of seedlings through improved stress tolerance (Allen, 1991; Koske and Gemma, 1996; Giri et al., 2007); binding of sand grains into large aggregates (Tisdall and Oades, 1982); and improving soil structure that can influence plant succession (Koske, 1975).

The fungal structures characteristic of AM colonization i.e. vesicles, arbuscules, hyphal coils and non-septate hyphae in the roots of plants, may have various functions related to both the fungus and the host (Smith and Smith, 1996, 1997). AM fungi develop two morphological types of colonization (Gallaud, 1905). These are: 1. the Arum-type, defined by intercellular hyphal growth in the root cortex; and 2. Paris-type, defined by cell-to-cell growth of intracellular hyphal coils (Smith and Smith, 1997). A prominent feature of the Arum-type morphology is the intercellular growth of hyphae in a longitudinal manner through the root. Arbuscules arise from the short side branches of these intercellular hyphae, typically at right angles to the main root axis (Smith and Smith, 1997). Coils of Paris-type mycorrhiza often, but not invariably become arbusculate i.e., they develop arbuscule branches from one or more loci on the coil (Gallaud, 1905; Smith and Read, 1997). The Arum-type morphology is abundant in field crops (Smith and Smith, 1997), whereas the Paris-type morphology has been more often seen in plants of natural ecosystems such as those occurring in herbaceous layers in temperate broad leaf forests (Brundrett and Kendrick, 1990) and plants of semi-arid systems (McGee, 1989).

Native plants play an important role in diversifying the land. Despite the low nutrient status, the sub-Saharan soils of Eritrea allow the growth of a number of native and exotic plant species which could act as hosts for AM fungi. However, this region is virgin as far as mycorrhiza research is concerned and even a preliminary study has not been conducted yet. The present study being the first, reports the incidence and diversity of AM fungi associated with some common plants of Eritrea.

Materials and methods

Study site and sampling procedure

The investigation was conducted in and around Asmara (N 15^0 28’, E 38^0 92’), Eritrea, located in the horn of Africa (*Figure 1*). The region is 2325 m above sea level and has an yearly average temperature of 16.89^0C and precipitation of 519.5 mm measured by Department of Meteorology, Ministry of Civil Aviation, State of Eritrea. Thirty seven common plant species were collected in August (Summer) 2005 (*Table 1*) and identified according to Flora of Ethiopia and Eritrea (Edwards et al., 1995, 1997, 2000; Hedberg and Edwards, 1989, 1995; Hedberg et al., 2003). Roots were traced back to their point of emergence to ensure that they originally belonged to the plants selected for sampling. Roots from three replicates of each plant species were taken by pulling out the entire plant with utmost care to get the roots intact. The roots were gently tapped to remove the soil particles and the adherent debris, and pooled to get one composite sample, rinsed in tap water and processed in 50% ethanol.

Soil samples of ca 100 ml were collected from the root region of replicate plant species to a depth of 20 cm. One composite soil sample from each plant species was obtained by pooling the replicate samples. Soil samples were kept in fresh polythene bags, labeled and air-dried for 48 h at room temperature (22^0C) and ground to pass a 2-mm sieve, stored at 4^0C and processed within 2 months. A total of 37 soil samples were used in this study.

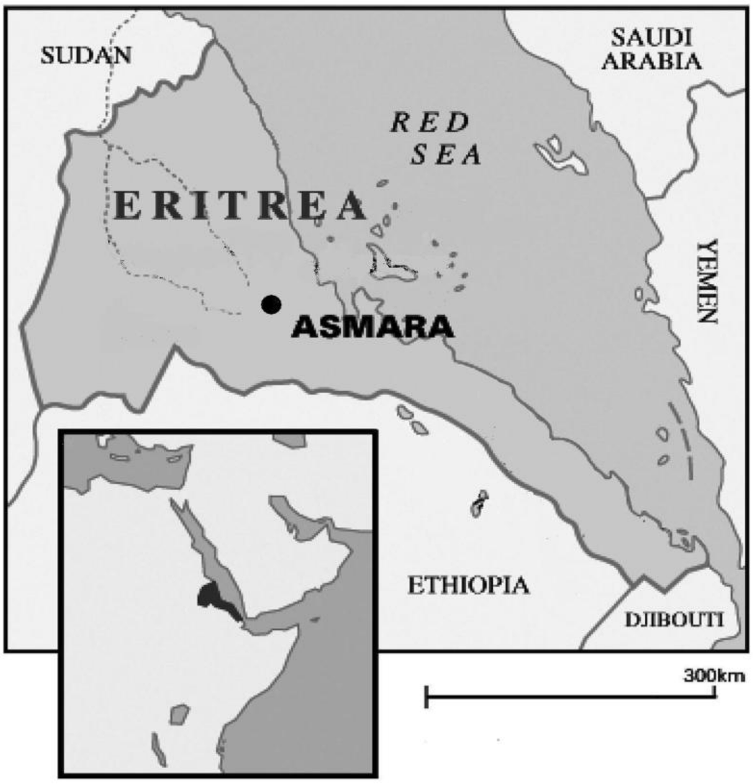

Figure 1. *Map showing location of sample collection*

Root staining

`Root samples were rinsed in tap water and stained with 0.05% cotton blue in acidic glycerol (50% glycerol in 0.01 % HCl) for 3 min at 121^0C after tissue clearing in 2.5% KOH and bleaching in alkaline H_20_2 (2% NH_40H in 3% H_20_2) as described by Koske and Gemma (1989) and examined with a compound microscope (Olympus BX50F4) at ×100 - 450 for the presence of fungal structures. AM types were classified according to Smith and Smith (1997). The incidence of mycorrhizal colonization per sample was estimated as the percentage of root segments with hyphae, vesicles and arbuscules of AM fungi. Intensity of infection (%) was calculated based on the abundance of hyphae, vesicles and arbuscules in each mycorrhizal root (Plenchette and Morel, 1996).

Spore extraction and analysis

Spores were isolated from 50 ml air-dried soil taken from each composite sample by wet-sieving followed by floatation-centrifugation in 50% sucrose (Dalpé, 1993). The finest sieve used was 45 µm. Spores were collected on a grid patterned (4×4) filter paper, washed three times with distilled water to spread them evenly over the entire grid and counted using a stereo microscope (Olympus SZX-ILLK200) at ×40 magnification.

Healthy spores (based on shape, surface condition, spore contents and no evidence of parasitism) of each distinct morphotype (as distinguished by color, shape, and size) were directly counted. Morphological properties of spores and their wall structures were determined based on observation of at least 100 spores mounted in polyvinyl alcohol/lactic acid/glycerol (PVLG; Omar et al., 1979) and a mixture of PVLG and

Melzer's reagent (1:1, v/v). Spores were crushed to varying degrees by applying pressure to the cover slip and then stored at 65^0C for 24 h to clear their contents from oil droplets. These were examined under a compound microscope (Olympus BX50F4) equipped with Nomarski differential interference contrast optics.

Terminology of the spore structures is as suggested by Stürmer and Morton (1997) and Walker (1983). Spore color was examined under a dissecting microscope on fresh specimens immersed in water. Color names are from Kornerup and Wanscher (1993). The authors of the fungal names are according to the Fungorum index (www.indexfungorum.org/AuthorsOfFungalNames.htm).

Data analysis

AM fungal composition was evaluated from the isolation frequency (IF), spore density, and species richness associated with each plant species. IF was expressed as the percentage of samples from which spores of a particular genus or species were isolated. Spore density (spores per 50 ml air-dried soil) was calculated from direct counts of spores. Species richness was defined as the number of AM fungal species per soil sample (Koske, 1987). The correlation between percentage of root length colonized, intensity of infection and spore density was analyzed by linear regression analysis. Goodness of fit was assessed by simple correlation coefficients (*r)* and degrees of freedom.

Results

AM colonization

Non-septate hyphae characteristic of AM fungi were present in 25 (68%) of 37 plant species screened. The mean percentage of root length colonized was 21.34%, ranging from 0 to 90%. The mean intensity of root infection was 6.73%, ranging from 0 to 50%. Among the plant species, *Amaranthus hybridus* and *Brassica rapa* belonged to Amaranthaceae and Brassicaceae (families often considered to be non-mycorrhizal) respectively, were typically colonized by AM fungi. Twelve plant species belonging to 9 angiosperm families remained non-mycorrhizal (*Table 1*).

AM structures

AM fungal structures, i.e. vesicles and arbuscules were present only in 11% of the plant species. Majority of plant species (51%) formed Paris-type and only 16% of plants formed Arum-type of colonization and in 12 plants the AM type could not be detected owing to the rare incidence of intercellular non-septate hyphae or vesicles in the roots.

Composition of AM fungi in soil

The average spore density of AM fungi was 43 spores per 50 ml air-dried soil, ranging from 10 to 160, and the average species richness was 2.4 species per sample, ranging from 1 to 5 (*Table 1*). There was no correlation among percentage root length colonized by AM fungi, intensity of infection (r=0.923, P>0.05) and spore density (r=0.311, P>0.05), as well as between intensity of infection and spore density (r=0.237, P>0.05) (results not shown).

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Table 1. *Arbuscular mycorrhizal (AM) association with roots and diversity in root region soils of common plants in Eritrea.Relative development of structures shown as: + present, - not detected, ND not determined (Length AM root length %, intensity intensity of root infection by AM %, V vesicles, A arbuscules, type Arum, Paris, density spore density per 50 ml air-dried soil, number species number per soil sample)*

Plant species	Family	Length	Intensity	Vesicles	Arbuscules	Type	Density	Number
Acanthospermum hispidum DC.	Asteraceae	10.50	1.05	-	-	P	30	2
Achyranthes aspera L.	Amaranthaceae	0.00	0.00	-	-	ND	15	2
Aerva javanica (Brum. F.) Schultes	Amaranthaceae	0.00	0.00	-	-	ND	10	1
Amaranthus hybridus L.	Amaranthaceae	65.00	32.50	+	+	A	25	2
Bidens biternata (Lour.) Merril & Sherff	Asteraceae	9.52	1.90	-	-	P	20	2
Brassica rapa L.	Brassicaceae	33.33	5.83	-	-	P	26	3
Calotropis procera (Ait.) Ait. f.	Asclepiadaceae	0.00	0.00	-	-	ND	20	1
Chenopodium schraderianum Schultes	Chenopodiaceae	0.00	0.00	-	-	ND	24	2
Commicarpus plumbagineus (Cav.) Standley	Nyctaginaceae	0.00	0.00	-	-	ND	95	3
Cosmos bipinnatus Cav.	Asteraceae	26.66	4.66	-	-	P	10	2
Crotalaria sp.	Fabaceae	15.38	2.30	-	-	P	60	1
Datura stramonium L.	Solanaceae	58.82	14.11	-	-	P	35	3
Echium plantagineum L.	Boraginaceae	70.00	32.00	+	+	A	55	2
Emex spinosa (L.) Campol.	Polygonaceae	37.50	3.75	-	-	P	75	1
Eruca sativa Mill.	Brassicaceae	0.00	0.00	-	-	ND	45	2
Erucastrum arabicum Fisch. & Mey.	Brassicaceae	0.00	0.00	-	-	ND	62	2
Heliotropium cinerascens DC.	Boraginaceae	33.33	5.55	-	-	P	160	5
Hypoestes forskaolii (Vahl.) R. Sch.	Acanthaceae	35.00	7.50	-	-	P	60	3
Leocas martinicensis (Jacq.) R. Br.	Lamiaceae	28.57	5.00	-	-	P	35	3
Osteospermum vaillanthii (Decne) T. Nordl.	Asteraceae	9.09	0.09	-	-	P	30	1
Pennisetum villosum Fresen.	Poaceae	38.88	20.27	-	-	A	34	4
Pennisetum sphacelatum (Nees) Th. Dur. & Schinz	Poaceae	30.00	9.50	-	-	A	73	5
Plantago lanceolata L.	Plantaginaceae	0.00	0.00	-	-	ND	56	3
Prosopis juliflora (Sw.) DC.	Fabaceae	10.00	2.00	-	-	P	19	2
Pulicaria crispa (Forssk.)Oliv.	Asteraceae	10.00	1.00	-	-	P	26	2
Reichardia tingitana (L.) Roth	Asteraceae	0.00	0.00	-	-	ND	30	1
Rumex nervosus Vahl.	Polygonaceae	0.00	0.00	-	-	ND	15	1
Rumex nepalensis Spreng.	Polygonaceae	90.90	50.00	+	+	A	85	4
Salvia shimperi Benth.	Lamiaceae	5.00	0.50	-	-	P	70	4
Schoenefeldia gracilis Kunth	Poaceae	78.94	35.78	+	+	A	60	1
Scolymus maculatus L.	Asteraceae	29.30	5.86	-	-	P	90	4
Senna italica Mill.	Fabaceae	6.25	0.06	-	-	P	32	3
Solanum dubium Fresen.	Solanaceae	28.57	5.00	-	-	P	28	2
Solanum incanum L.	Solanaceae	0.00	0.00	-	-	ND	21	2
Solanum nigrum L.	Solanaceae	22.22	2.22	-	-	P	15	3
Tagetes minuta L	Asteraceae	6.66	0.66	-	-	P	25	2
Xanthium strumatium L.	Asteraceae	0.00	0.00	-	-	ND	20	2
Average	-	21.34	6.73	-	-	-	43	2.38

The plant species sampled were associated with 18 described species and three undescribed morphotypes forming glomoid spores. The fungi represented five genera belonging to three families of Glomeromycota. The fungi most frequently found were *Glomus spp.* (12 described species and five undescribed morphotypes). Additionally, species of *A. spinosa*, *D. celata*, *D. spurca*, *S. dipurpurescens*, *S. fulgida* (Diversisporaceae) and *Ar. trappei* (Archeosporaceae) were isolated. The species most frequently co-occurring with roots of plants considered were *G. mosseae* (found in

42.31% of root region soils). *G. claroideum* (23.08%), *Ar. trappei* (15.38%), *G. Constrictum*, *G. intraradices* and *G. irragulare* (9.61% each) (*Table 2*).

***Table 2**. Frequency of occurrence of the AM fungi found in the root region of common plants in Eritrea*

Fungal species and morphotypes	**Frequency of occurrence (%)**
Acaulospora spinosa C. Walker & Trappe	1.92
Archaeospora trappei (R.N. Ames & Linderman) J.B. Morton & D. Redecker emend. Spain	15.38
Diversispora celata C. Walker, Gamper & A. Schüßler, in Gamper, Walker & Schüßler	3.85
Diversispora spurca (C.M. Pfeiff., C. Walker & Bloss) C. Walker & Schuessler	7.69
Glomus aurantium Błaszk., Blanke, Renker & Buscot	1.92
G. caledonium (T.H. Nicolson & Gerd.) Trappe & Gerd.	1.92
G. claroideum N.C. Schenck & G.S. Sm.	23.1
G. clavisporum (Trappe) R.T. Almeida & N.C. Schenck	1.92
G. constrictum Trappe	9.61
G. etunicatum W.N. Becker & Gerd.	5.77
G. indicum Blaszk., Wubet & Harikumar	3.85
G. intraradices N.C. Schenck & G.S. Sm.	9.61
G. irregulare Błaszk., Wubet, Renker & Buscot	9.61
G. macrocarpum Tul. & C. Tul.	1.92
G. mosseae (T.H. Nicolson & Gerd.) Gerd. & Trappe	42.31
G. versiforme (P. Karsten) S.M. Berch	3.85
Glomus 178	3.85
Glomus 212	1.92
Glomus 213	1.92
Scutellospora dipurpurescens J.B. Morton & Koske	1.92
S. fulgida Koske & C. Walker	1.92

Discussion

AM status of plant species

Our results, in general, confirm previous reports indicating that AM fungi are ubiquitous in terrestrial plant communities, associating with the majority of plant species (Smith and Read, 1997; Schüßler et al., 2001; Blaszkowski and Czerniawska, 2011) as 68% of the common plants of Eritrea screened in the present study were mycotrophic. Furthermore, the presence of intraradical non-septate mycelium, vesicles and arbuscules is considered elsewhere to be the evidence of colonization by AM fungi (Duckett and Ligrone, 1991; Gemma et al., 1992). As far as we are aware many reports of mycorrhiza in the present study are the first published reports of some of these plant species.

In general, the extent of mycotrophy in terms of percentage colonization by AM fungi and the intensity of root infection in common plants of Eritrea were comparatively low except in some plants where the colonization increased up to 91%, for example, *Rumex nepalensis* (Fam. Polygonaceae). In the present study, the plant species belonging to non-mycotrophic families were found to be mycotrophic. This is in accordance with Norman et al. (1995) who also indicated that some of the representatives of the non-mycotrophic families viz. Amaranthaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Cyperaceae and Juncaceae could also form mycorrhizal associations. However, our findings contradict the earlier reports of Tao and Zhiwei (2005) and Shi et al. (2006) who reported that members of family Asclepiadaceae, Nictaginaceae and Plantaginaceae are responsive to arbuscular mycorrhizas, as none of the plant species examined in the present study showed mycorrhizal association.

Many researchers have reported mycorrhizal association in native plants of varied ecosystems e.g. Wubet et al. (2003) reported 11 indigenous trees in the dry Afromontane forests of Ethiopia as typically being arbuscular. Similar situations have been observed in other drought prone ecosystems (Mukerji and Kapoor, 1986; Stutz et al., 2000; Shi et al., 2006) where up to 89% of the plant species exhibited mycorrhizal association. On the contrary, only 79% of the plants were found to be mycorrhizal in the rain forest of south Cameroon (Onguene and Kuyper, 2001). Comparison of the AM fungal colonization rate and intensity of infection in roots from different ecosystems suggested that plants grown in the semi arid and arid habitats might be more dependent on AM fungi.

AM colonization pattern

The AM in the majority of common plants of Eritrea corresponds to the Paris-type, characterized by intracellular hyphal coils, and arbuscules originated as intercalary structures on the coils in contrast to the Arum-type with extensive intercellular hyphal growth and intracellular arbuscule formation described elsewhere (Yamato, 2004). Imhof and Weber (1997) suggested that Paris-type mycorrhizas are more advantageous to plants than Arum-type under extreme conditions. As the coiled hyphae could be a sign of major control by the plant over fungal development, they considered it to be more advanced than the Arum-type (Weber et al., 1995). The different AM structures may have specialized roles in transfer of inorganic nutrients and organic carbon between the symbiotic partners (Smith and Read, 1997; Smith and Smith, 1997), however paucity of information on the functions or relationship between the diversity and roles in varied ecosystems still exists.

Spore density and its relationship with other fungal variables

Our study reports a spore density up to 95 spores per 50 ml air-dried soil, even in the root region soils of non mycorrhizal plants, presumably because of interwoven roots of other plants in the same field sample. Mycorrhizal plants may influence AM fungal spore number in the root region of non mycorrhizal plants (Zhao et al., 2001). It has been reported that both spore production and species richness of AM fungi are lower in arid climates than other ecosystems (Rose, 1981; Pond et al., 1984) and it decrease further as aridity increases (Stahl and Christensen, 1982; Stutz and Morton, 1996). The mean spore density of 43 spores per 50 ml of soil in our study was lower than earlier reports from other ecosystem (Zhao et al., 2001; Li et al., 2004). However, our results agree with those from certain desert ecosystem such as the Mojave (Titus et al., 2002) and Negev (He et al., 2002) deserts.

Contrasting to the report in other investigations (Muthukumar and Udaiyan, 2002), our results demonstrated no correlation between mycorrhizal variables such as percentage root length colonized by AM fungi, intensity of infection and spore density. The relationship between spore number, percentage colonization by AM fungi and intensity of infection is complicated as it is influenced by many environmental and biological factors (Smith and Smith, 1996; Liu and Wang, 2003). There are two possible explanations for our results: 1. the roots associated with AM fungi may have decayed prior to sampling, and 2. it is likely that some spores included in the counts were not viable, or were present in clusters functioning as a single infective propagule in field soil (Jasper et al., 1991; Jansa et al., 2002).

AM diversity in soil

The total number of AM fugal taxa (21) isolated in the present study coincides with the number reported by previous investigators in varied habitats. For example, 23 AM fungal taxa associated with 25 plant species were found in a grassland ecosystem using trap culture (Bever et al., 1996). Far fewer AM fungi (17 species) were found in samples from agricultural fields by both direct spore isolation and trap culture with different plants (Jansa et al., 2002) and even less in semiarid Sahelian areas of Africa (Dalpé et al., 2000) and hot arid ecosystems of southwest China (Tao and Zhiwei, 2005). It is likely that the site preference of AM species could be due to an interaction of factors pertaining to the host plants and to edaphic and climatic conditions (Brundrett, 1991). The present study reveals that *Glomus* is the dominant AM fungal genus which seems to be dominant in the root region of common plants of Eritrea. These findings are in agreement with the results of other researchers who reported the predominance of *Glomus sp.* in arid and semi-arid areas (e.g. Lamnot, 1982; Stutz et al., 2000; Chen et al., 2001; Pande and Tarafdar, 2004).

Conclusion

It can be concluded that the common plants of Eritrea host a variety of AM species in their roots and root region soils. The potential of these plants as alternate hosts of AM fungi and their role in the build up of indigenous AM inoculum in the soil for the benefit of cultivated plants have to be examined in detail.

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A BLEND OF BIOASH AND GYPSUM UTILIZED FOR AGRO-ENVIRONMENTAL PURPOSES IN A SOIL INCUBATION EXPERIMENT

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Abstract. Soil improver, nutrient supplier and heavy metal stabilizer effects of ash residue remaining after the process for K recovery was examined in a soil incubation model experiment using two soil types (acid sandy soil and neutral loam soil) at 4 ash residue dose levels (0, 1, 3, 5%). Possible environmental risks posed by its application were also studied by ecotoxicological assays. As the result of ash residue treatment alkalinity of the soils has been raised in all treatments. In the case of acid soil even the use of 1% of ash residue proved to be enough for neutralising the soil pH. Ash residue is principally suitable for K and P supply and mostly in soils with poor nutrient content, where ash residue applied in 1% dose is able to increase the concentration of plant available K and P significantly. According to the results, the ash residue proved to be able to decrease the mobility of Pb and Zn which means that it can be an effective stabilizer in acid and neutral soils. Stabilization has probably occurred via adsorption. Based on the findings of the ecotoxicological assays, the use of 1% ash residue does not present substantial environmental risk.

Keywords: *biomass ash, soil amelioration, fertilization, heavy metal stabilization, ecotoxicology*

Introduction

Increasing importance of biomass combustion has raised problems related to the resulting ash as a by-product. Every year tens of thousands of tonnes of biomass ash (bioash) are produced in biomass power plants and heating plants. Handling and disposal of bioash as waste is costly and presents environmental risks. However, product manufacturing from bioash means an alternative enabling the utilization of this special waste. According to several studies the high $CaCO_3$ content of bioash makes it suitable for the improvement of acid soils (Ulery et al., 1993; Muse and Mitchell, 1995; Meiwes 1995; Vance, 1996; Zang et al., 2002; Deydier et al., 2005; Vassilev et al., 2013). Because of its high nutrient content (firstly K and P) it can be utilized as a nutrient source (Ohno and Erich, 1993; Fransman and Nihlgard, 1995; Vance, 1996; Demeyer et al., 2001; Bungart et al., 2001; Park et al., 2005; Ahmaruzzaman, 2010; Tan and Lagerkvist, 2011; Huotari et al., 2008) as well. Furthermore, due to their adsorption

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capacity combustion products could be applied for soil remediation purposes by decreasing heavy metal mobility in contaminated soils (Feng et al., 2004; Srivastava et al., 2006; Gruiz et al., 2009; Ahmaruzzaman, 2010; Vassilev et al., 2013).

Biomass ash is composed of porous carbon-containing particles and irregularly shaped mineral particles. More than 80% of bioash is below 1 mm in size (Etiegni and Campbell, 1991). Its main component is $CaCO_3$. Density of bioash varies between 0.27 and 0.51 g/cm^3 (Muse and Mitchell, 1995). Alkalinity, acid neutralizing capacity of bioash is also significant (Demeyer et al., 2001).

Macroelement content of bioash varies over a wide range with the following median values: 0.06% N, 0.42% P, 18% Ca, 0.97% Mg and 2.27% K (Vance, 1996). Still, it is the applicability of bioash to be used as K and P supplier that might be of huge significance in plant production.

Similarly to macroelements, microelement concentration of bioash also shows great variation. According to the analyses of several bioashes with different origins the highest mean concentration among microelements was that of Mn (4370 mg/kg), followed by Zn (443 mg/kg), Cu (75 mg/kg), B (110 mg/kg), and Mo (15 mg/kg). Concentration of As, Ni, Cr and Pb varied between 23 and 65 mg/kg, whereas regarding non-essential trace elements the concentrations of Se and Hg (< 0,5 mg/kg), as well as Cd and Co (< 10 mg/kg) proved to be low (Someshwar, 1996).

In spite of being reasonable, application of bioash in plant production should be carried out cautiously due to its composition that might include organic and mineral pollutants. Besides contaminating both soils and groundwaters it can also affect their acidity, chemical composition and biodiversity in a disadvantageous way (Vassilev et al., 2013).

The possibilities of the re-utilisation of biomass ashes have been studied worldwide including industrial and agricultural purposes (Lima et al., 2012; Huang et al., 2011; Huang et al., 1992). One of the latest potential options is to use it as a raw material for K fertilizers. According to the method that is now being improved potassium is extracted from herbaceous biomass ash with 80-90°C water, thereafter solution and residue (ash residue) are separated by filtration. Following the addition of sulphuric acid the solution is condensed and potassium-sulphate is gained as the end product. Through processing the residue with gypsum and carbon-dioxide, a soil improver with high Ca and K content is produced. This resulting blend of ash residue and gypsum (hereafter "ash residue") enriched with Ca contains significant amounts of macro- and microelements. Regarding its basic properties, ash residue has similar qualities to that of untreated biomass ash (*Table 1*). It should be noticed that data from literature show great variances. Concentration values of several components of the ash residue analysed in our experiment were found to be of similar order of magnitude to those published before with the exception of Mn and Na for which we obtained lower values.

Blends of ash and gypsum have already been tested as construction materials (Sadique et al., 2013). In present experiment the soil improver, nutrient supplier and heavy metal stabilizer effects of the ash residue described previously were studied in a soil incubation model experiment. Possible environmental risk posed by its application was examined as well, using ecotoxicological methods. According to literature data favourable overall effects of the material could be expected. However, it should be considered that by containing significant amounts of potentially toxic elements, ash residue can cause environmental risk. These negative effects have already been demonstrated on different aquatic test organisms (Barbosa et al., 2013). On the grounds of these, principal questions to be answered are the following: (i) whether the ash residue is applicable for all of the three aforementioned purposes and (ii) whether its utilization is limited by the potential toxicity caused by its composition. Based on the literature, toxicological knowledge on the application of ash originating from the combustion of herbaceous plants as a soil improver is limited. This work might also provide new findings to this topic.

Table 1. *pH and element composition (mg/kg) of different biomass ash and ash-residue*

Parameter	**Wood ash** (Etiegni and Campbell 1991)*	**Wood ash** (Huang et al., 1992)*	**Reed canary-grass ash** (Paulrud et al., 2001)	**Switchgrass ash** (Vassilev et al., 2013)	**Ash residue**
Particle size distribution	80 %: <1 mm				18 %: 2-0.05 mm 82%: 0.05-0.002 mm
Density	0.27 – 0.51 g/cm^3				0.41 g/cm^3
pH	9-13.5				9.5
N	600	900			0
P	14000	6900	11800		44050
S	4455	6800			28570
Ca	317400	109400	18200		154600
Mg	22500	16200	7400		37390
K	41300	28600	28300		91510
Na	3400	1600	1800		101
Al	23650	13000	3200		1222
Fe	19500	3300			1563
Mn	6693	3470		872	222
Zn	700	794		103	576
Cu	145	78		66	388
Ba	8	127			64
Pb	130	66			1.2
Ni	47	12		37	42
Cr	86	14		49	6.1
Cd	21	3		9.8	3.6

*In: Demeyer et al., 2001

Materials and methods

Experimental design

Topsoil (0-0.2 m depth) samples were used in order to examine the effects of ash residue on soil properties and on heavy metal mobility. The samples were taken from two soil types, one from an acid sandy soil (Nyírlugos, EOV coordinates: 874919, 265291) and the other from a neutral loam soil (Gyöngyös, EOV coordinates: 716000, 271195). These types were chosen with the purpose of studying the effects of ash residue on two soils that differ from each other basically regarding both their alkalinity and colloid content. Soils were contaminated artificially except for one case in which it was left in its natural state, as follows:

1. uncontaminated soil sample: acid soil (Nyírlugos) (USS),
2. acid soil (Nyírlugos) contaminated with Pb and Zn salts ($Pb(NO_3)_2$ and $ZnSO_4(7H_2O)$) + 400 mg Pb and 800 mg Zn/kg of soil (LZSS),
3. loam soil (Gyöngyös) contaminated with mine refuse (11%) (Gyöngyösoroszi) (MRLS),
4. loam soil (Gyöngyös) contaminated with communal sewage sludge (Gödöllő) (SSLS).

Ash residue was mixed to the soil in the following ratios: control, 1, 3 and 5 m/m%. Each treatment was performed in three replicates. Total element contents of the soil mixtures are summarized in *Table 2*.

***Table 2**. Total (cc. HNO_3 + HCl soluble) element concentrations of the four soils (mg/kg)*

Element	USS	LZSS	MRLS	SSLS	limit values*
Ca	576	507	13683	18153	-
K	1543	1513	10644	11713	-
P	383	365	2925	5520	-
Mg	1012	1012	5432	6095	-
S	117	380	1607	1008	-
Zn	17.4	**599**	**235**	**591**	200
Cu	5	5	**85**	**195**	75
Ba	32.0	37.1	**306**	**407**	250
Ni	7.08	6.85	**51.1**	**46.6**	40
Cr	10.2	9.8	**75**	**198**	75
As	1.23	0.99	**43.2**	11.0	15
Cd	0.032	0.042	**1.14**	**3.40**	1
Co	3.14	2.98	16.0	13.2	30
Pb	5.12	**418**	**164**	48.1	100
Mo	0.127	0.186	2.25	1.13	7
Se	<dl	<dl	<dl	0.953	1

<dl: under detection limit. dl Hg: 0.12 mg/kg; dl Se: 0.6; *contamination limit values for soils in joint decree No. 6/2009; Bold characters indicate values above limit.

Each pot was filled with 1 kg of soil. In the case of the second soil mixture concentrations of Pb and Zn in the soil were four times higher than the limit values of the Hungarian regulations (Government Joint Decree No. 6/2009. (IV. 14.) KvVM-EüM-FVM on limit values established for the protection of groundwater and the geological medium). The levels of sewage sludge and mine refuse dosage were calculated in a way that the concentrations of some elements in the soil exceed the contamination limit values (according to the Joint Decree No. 6/2009. (IV. 14.) KvVM-EüM-FVM) many times over (*Table 2*). Different contaminants were applied in order to have a better understanding of heavy metal stabilization processes these materials are supposed to take part in.

Element composition and some properties of the ash residue are presented in table 1. It can be seen that it had a pH (H_2O) value of 9.5, contained 28.3% $CaCO_3$ and no nitrogen, whereas its ammonium lactate (AL) soluble K_2O and P_2O_5 contents were

10.5% and 3.7%, respectively. Some parameters of the soil mixtures can be found in *Table 3*.

***Table 3**. Selected properties of the soil samples*

Soil	pH (H_2O)	$CaCO_3$%	OM %	Upper limit of plasticity	CEC meq/100g
USS	5.04	0.00	0.92	33	1.76
LZSS	4.19	0.00	0.97	33	1.67
MRLS	7.04	0.26	4.44	45	27.9
SSLS	7.05	0.56	6.05	53	34.1

All contaminants (Pb and Zn solution, mine refuse and sewage sludge) and the different doses of ash residue were added to the soils at the same time. After homogenisation, the mixtures were filled into pots and incubated for 1 month. The mixtures were wetted to 70% of field capacity of the soils.

Methods

Analyses of soil parameters and soil chemical properties

The soil was analysed according to the following standards: pH(H_2O) – ISO 10390:2005, humus content – ISO 14235:1998, $CaCO_3$ content – ISO 10693:1995, cation exchange capacity (CEC) - ISO 11260:1994; aqua regia extract, distilled water 1:10 extract, NH_4-acetate + EDTA-soluble element fraction – MSZ 21470-50:2006, ammonium acetate buffer extract – MSZE 21420-31;2006, ammonium-lactate soluble element content - MSZ 20135:1999, particle size distribution - MSZ-08-0205:1978.

Ecotoxicological tests

All microbiological and ecotoxicological assays were performed in three replications. The number of aerobic heterotrophic colony forming units was determined in wet soil samples. The soil samples for the Sinapis alba root and shoot growth inhibition test were dried, ground and passed through a 2 mm sieve.

Aerobic heterotrophic colony-forming units. The soil microbial biomass and the number of viable microorganisms can be used to assess soil quality and are relatively easy to determine using routine methods such as plate count tests (Benedetti and Dilly, 2006). In this experiment different soil dilutions in sterile distilled water were inoculated into an appropriate media ensuring that after the incubation period one colony is formed from one living cell. The method was described by Lorch et al. (1995). The number of aerobic heterotrophic microorganisms was expressed as colony forming units (CFUs) per g of soil.

Sinapis alba *root and shoot growth inhibition test.* In the presence of toxic substances the germination rate and the root and shoot growth of *Sinapis alba* (white mustard) plants are inhibited (OECD, 2006). The test was performed as described by Leitgib et al. (2007). Five grams of soil were measured out into a 10 cm diameter Petri-dish, wetted to its water holding capacity and 20 seeds with over 90% germination ability were placed on the soil surface. The samples were incubated at 23° C in the dark for three days. The length of roots and shoots were measured manually with a ruler. The growth inhibition was expressed as follows: I(%) = (C-L)/C*100, where I means the inhibition %, C means the length of shoot or root in the control (OECD soil), while L means the length of shoot or root of *Sinapis alba* in the sample. The artificial soil recommended by the OECD Guideline 207, (OECD, 1984) (content: 70% sand, 20% clay, 10% peat) was used as the uncontaminated control.

Statistical Analysis

The data were analysed for treatment effects using one-way analysis of variance (ANOVA) on the main treatments (time period, different layers). Significant differences between the treatment means were calculated by the LSD (least significant difference) test at $p < 0.05$. In the correlation analysis the asterisks, *, ** and *** indicate significance at $p < 0.05$, 0.01 and 0.001, respectively. Statistica v.9 (StatSoft Inc.) software was used for all the statistical evaluations.

Results

Soil amelioration and nutrient supply

As presented in *Table 4*, the pH value has increased by more than 3 units in the case of the light-textured acid sandy soil. Correspondingly the application of 3% of ash residue made the sandy soils alkaline, whereas 1% of it (equivalent to 10t/ha $CaCO_3$)

Table 4*. Some soil properties in function of ash residue treatment*

Soil	Ash-residue %	pH (H_2O)	$CaCO_3$	AL-K_2O	AL-P_2O_5	CEC
			w/w%	mg/kg	mg/kg	meq/100g
USS	**0**	5.00	-	65	138	1.76
	1	7.64	0.09	793	952	3.83
	3	8.48	0.40	2504	2521	4.72
	5	8.69	0.82	3972	3680	5.84
	$LSD_{5\%}$	0.1	0.05	198	312	1.29
LZSS	**0**	4.19	-	70	151	1.67
	1	6.93	-	852	960	3.15
	3	8.43	0.36	2620	2286	4.06
	5	8.70	0.69	4200	3565	4.72
	$LSD_{5\%}$	0.08	0.04	438	186	0.96
MRLS	**0**	7.04	0.26	884	3748	27.9
	1	7.62	0.51	1680	4461	29.7
	3	8.05	0.90	3477	6111	25.6
	5	8.20	1.52	5140	7340	25.5
	$LSD_{5\%}$	0.05	0.11	169	373	n.s.
SSLS	**0**	7.05	0.56	936	7070	34.1
	1	7.50	0.75	1800	7869	35.3
	3	7.94	1.13	3633	8727	33.5
	5	8.13	1.56	5526	9873	30.7
	$LSD_{5\%}$	0.06	0.19	221	762	2.9

n.s. = not significant

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proved to be enough to neutralize them. It can be stated that regarding the experimental acid sandy soils, the pH value of 6.8 being desired when liming can be reached by using an ash residue dose of 15 t/ha. $CaCO_3$ contents were also found to increase significantly in both soils and following all treatments. The acid sandy soil had the smallest CEC, while the largest was that of the loam soil contaminated with sewage sludge. Regarding the acid sandy soil, ash residue treatment has increased CEC compared to the control in all of the cases, whereas no significant change was observed for loam soil contaminated with mine refuse. As for the loam soil contaminated with sewage sludge, 5% ash residue treatment has lowered the CEC statistically in comparison with the no ash treatment. This might be originated in the dilution effect (*Table 4*).

In the case of present experiment addition of 1% ash residue resulted in the 12-fold increase of AL-soluble K and the 7-fold increase of P in the acid sandy soil compared to the control (*Table 4*). In the case of the 3% ash residue treatment this surplus was 38-fold for AL soluble K and 18-fold for AL soluble P, while with the addition of 5% ash residue it reached 61-fold and 27-fold AL-K_2O and AL-P_2O_5 content of the control soil, respectively. With regards to the neutral loamy soil a 2-fold increase was observed in the case of AL soluble K with the use of 1% ash residue, whereas P was not found to increase considerably compared to the control. By adding 3% of ash residue this surplus proved to be 4-fold regarding AL-K_2O and about 1.5-fold for AL-P_2O_5. The addition of 5% ash residue led to a fold change of 6 in the case of AL-K_2O content, whereas in the case of AL-P_2O_5 content the difference was almost two-fold only in the soil contaminated with mine refuse. The changes in K and P concentrations were significant. Based on these results the ash residue may be suitable primarily for K and P supply.

Heavy metal stabilisation

Outstanding heavy metal stabilization effect was only observed in the Pb and Zn contaminated acid sandy soil (*Table 5*) of what the most probable cause is its low organic matter content and low metal binding capacity. In the uncontaminated sandy soil the plant available Pb and Zn concentrations increased significantly due to the element content of ash residue. However, even in this soil the water soluble concentrations decreased. Regarding the neutral loam soil the decrease in the solubility of Zn was proven statistically in the acetate and water soluble fractions but the ash increased the plant available NH_4-acetate + EDTA soluble Pb and Zn concentrations.

***Table 5**. Soluble fractions of Pb and Zn extracted by different solvents in an acid sandy soil*

Soil	**Ash-residue %**	**NH_4-acetate + EDTA soluble**	**NH_4-acetate soluble**	**Water soluble**	**NH_4-acetate + EDTA soluble**	**NH_4-acetate soluble**	**Water soluble**
		Pb mg/kg			**Zn mg/kg**		
USS	**0**	1.90	<dl	0.100	1.6	1.84	0.899
	1	2.43	<dl	0.081	5.7	3.53	0.519
	3	2.54	<dl	0.072	14.7	5.94	0.231
	5	2.79	<dl	0.060	21.9	7.09	0.060
	$LSD_{5\%}$	0.55		n.s.	1.7	1.31	0.276
	0	323	125	4.35	649	452	287
	1	307	25	2.64	627	413	11

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LZSS	**3**	263	9	1.45	590	386	2
	5	278	6	0.47	580	353	1
	LSD$_{5\%}$	n.s.	19	0.82	60	34	56
MRLS	**0**	26.7	<dl	<dl	73	11.8	<dl
	1	27.3	<dl	<dl	85	8.8	<dl
	3	24.1	<dl	<dl	87	7.0	<dl
	5	19.7	<dl	<dl	90	3.8	<dl
	LSD$_{5\%}$	1.5			7	1.5	
SSLS	**0**	19.8	<dl	<dl	314	16.3	0.256
	1	19.4	<dl	<dl	314	16.7	0.801
	3	17.4	<dl	<dl	306	10.9	0.107
	5	15.7	<dl	<dl	312	7.7	0.147
	LSD$_{5\%}$	1.7			n.s.	2.3	0.395

n.s. = not significant; <dl = under detection limit

Toxicology

Aerobic heterotrophic colony-forming units (CFU)

Aerobic heterotrophic cell number proved to be the lowest in the case of the uncontaminated acid sandy soil and it was the highest in the loam soil contaminated with sewage sludge (*Fig. 1*).

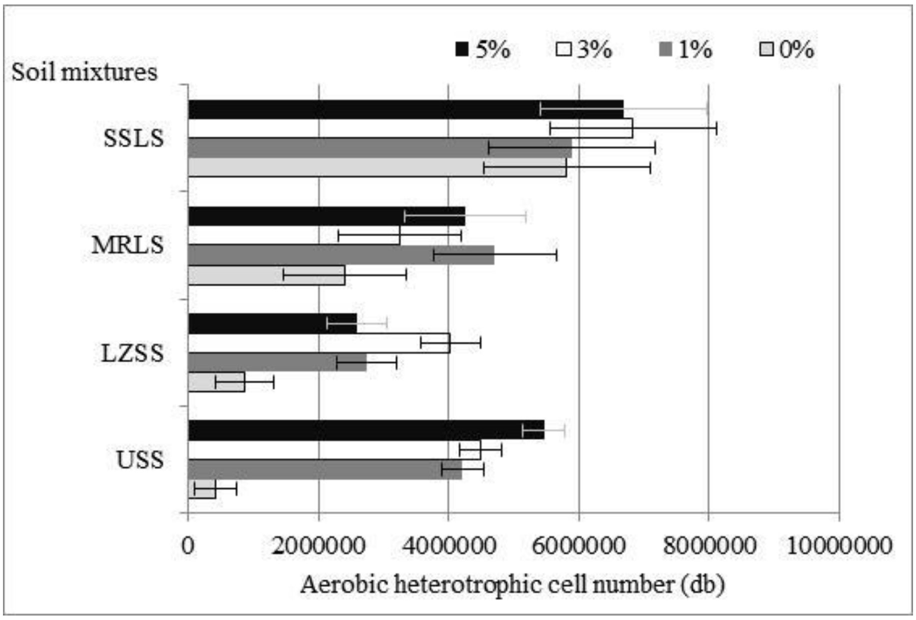

***Figure 1**. Aerobic heterotrophic cell number changes of soil mixtures in function of ash residue treatments*

It was also found that compared to the soil without ash treatment, cell number has increased significantly as a result of ash residue treatment both in the contaminated and in the uncontaminated acid sandy soils as well as in the neutral loam soil contaminated with mine refuse. Increase of ash residue dose to 3% and further to 5% was able to raise the aerobic heterotrophic cell number only in some cases.

Sinapis alba root and shoot growth inhibition test

The results in *Table 6* show that the ash residue had both stimulating and inhibiting effects on the growth of *Sinapis alba* root and shoot growth depending on the soil type and contamination. Mentioned effects were definitely positive only on the lead and zinc contaminated sandy soil where each load level resulted in growth stimulation. Inhibition occurred in the case of other soils on both shoot and root elongation but in most cases only above a load of 1 %.

***Table 6**. Results of the* Sinapis alba *growth inhibition test*

Soil	Ash-residue %	Root length (mm)	Root growth inhibition %	Shoot length (mm)	Shoot growth inhibition %
USS	**0**	24.0	0.0	13.1	0
	1	17.2	28.4	14.5	-10.8
	3	11.7	51.7	10.7	20.1
	5	7.4	68.3	5.9	53.5
	$LSD_{5\%}$	5.5	16.4	4.2	25
LZSS	**0**	4.1	0	4.1	0
	1	13.7	-237	15.4	-276
	3	11.9	-189	10.0	-144
	5	7.1	-74	5.4	-36
	$LSD_{5\%}$	2.7	81.1	2.4	46
MRLS	**0**	12.2	0	12.7	0.0
	1	11.6	3.3	15.0	-18.1
	3	10.2	14.9	14.5	-18.9
	5	7.3	39.4	9.6	26.0
	$LSD_{5\%}$	2.4	19.4	4.4	n.s.
SSLS	**0**	14.2	0.0	16.6	0.0
	1	14.6	-3.5	18.3	-10.2
	3	9.8	30.8	14.3	14.1
	5	9.3	33.9	13.4	18.8
	$LSD_{5\%}$	2.3	16.9	4.0	24.5

n.s. = not significant

Discussion

Based on the results ash residue treatment might be suitable to replace high-dose liming in the case of soils similar to those included in this experiment, in accordance with earlier findings (Ulery et al., 1993; Muse and Mitchell, 1995; Park et al., 2012). Because of the adsorption capacity of bioash, it was important to investigate what effect the ash residue treatment has on the CEC of the soils (Feng et al, 2004; Srivastava et al., 2006; Vassilev and Vassileva, 2007; Umamaheswaran and Batra, 2008). The ash residue treatment increased the adsorption capacity of the sandy soil, thus may improve the nutrient balance of similar soils. However, the higher (5%) dose levels of ash residue may decrease the CEC of heavier textured soils due to the dilution effect (*Table 4*).

Based on literature data and regarding macroelements, biomass ash contains significant amounts of potassium and also has a relatively high phosphorous content with a mean of almost 0.5% (Vance, 1996; Fransman and Nihlgard, 1995). It contains large concentrations of microelements as well. Contrarily, according to some previous findings, with the addition of bioash to soil the solubility and availability of Fe, Mn, Zn and Cu decreased owing to the increased pH (Clapham and Zibliske, 1992; Krejsl and Scanlon, 1996). The biomass ashes proved to be suitable P and K suppliers for different cultivars (Schiemenz and Eichler-Löbermann, 2010; Kikamägi et al., 2013).

The effect of stabilisation is only presented by the concentration values of Pb and Zn in the four soil mixtures. Outstanding heavy metal stabilisation effect was only observed in the Pb and Zn contaminated acid sandy soil (*Table 5*) which might be explained by its low organic matter content and metal binding capacity. Chemical stabilizers/immobilizers can reduce the mobility of metals through adsorption, the formation of complexes or precipitation (Kumpiene et al., 2008). As shown in table 3, ash residue has increased the CEC of the acid sandy soil.

In the contaminated acid sandy soil the stabilizing effect of ash residue was the most observable on distilled water and ammonium-acetate buffer extractions, the former is representative for the easily mobilisable fraction while the latter for exchangeable cations (Tessier, 1979). The combination of NH_4-acetate and EDTA is a much stronger extractant than the two mentioned due to the complex forming agent of EDTA which is able to make coordinated metal ions extractable as well (Schnitzer and Khan, 1978; Stevenson, 1982; Ram and Raman, 1984; Peijnenburg et al., 2007). Regarding this extraction stabilization effect of fly ash was not or only very slightly detected, therefore it can be assumed that (owing to the properties of ash residue as well) immobilization occurred via adsorption.

In the case of sewage sludge contaminated loam soil the CEC decreased in function of the amount of ash residue added, thus the observed stabilisation in the case of water and acetate soluble Zn concentration can be traced back to the precipitation of zinc-phosphate due to the P content of ash residue (Crannell et al., 2000; Theodoratos et al., 2002; Diyab et al., 2003).

Based on the above information the ash residue may reduce the element solubility by two ways: increasing the CEC and precipitating the metals into less soluble phosphorus salts. Thus, it is a possible stabilisation agent.

Although microbial count is not a direct measure of activity in soils, it is indicative of soil microbial viability (Bossert and Kosson, 1997) which is in relation with the organic matter and nutrient content of the soils (*Table 2 and Table 3*) (Kanazawa and Filip, 1986; Torsvik and Øvreås, 2002; Maron et al., 2006). Despite that favourable effects of bioash on soil microflora were described previously (Fritze et al., 2000), they were not observable in the soil contaminated with sewage sludge. The positive impacts of ash residue (*Fig.1*) can be explained by its following features: its capacity to provide good air and moisture supply, the fact that it increases the adsorption surface due to its particle composition, and that it decreases the biological availability of heavy metals by their stabilization (Nielsen et al., 2002; Dombos and Szalkai, 2004; Gillert et al., 2009).

The results of the *Sinapis alba* ecotoxicologcal test on uncontaminated sandy and contaminated loam soils are partly in accordance with results of similar experiments in literature. Rikala and Jozefek (1990) found that wood ash application in lover rates may increase the germination but higher doses (over 5 t/ha) have negative effect on it. Similarly, Singh et al. (1997) demonstrated that fly ash application in low rates insignificantly increased the germination of bean (*Vicia faba*) which was inhibited at the rate of 30 % . In our experiment the slightly positive effect was insignificant in contrast to the inhibition on higher ash residue application rates. Thus, the nutrient content of ash residue did not promote the shoot and root growth but in higher doses its potentially toxic element content inhibited these processes. In contrary, on the contaminated sandy soil the ash residue successfully promoted the shoot end root growth due to the stabilisation of Pb and Zn contaminants.

Conclusions

Due to its alkaline pH and high (nearly 30%) $CaCO_3$-content, ash residue as a soil improver has significantly raised the pH of the studied soils. Even 1% dose of ash residue proved to be enough to turn the acid soil into neutral. The ash residue is mainly suitable for nutrient supply, primarily in the soils with poor nutrient content where ash residue applied in 1% dose is able to increase the concentration of plant available K and P significantly. We successfully demonstrated the capacity of ash residue to decrease the solubility of Pb and Zn in both the acid sandy and the neutral loam soils, which means that it is an appropriate stabilizer in the acidic environment. Stabilization has probably occurred via adsorption and precipitation. Based on the findings of the ecotoxicological assays, the use of 1% ash residue does not present substantial environmental risk.

For the establishment of real ways of utilization of ash residue pilot-plant and large-scale field experiments are needed with the application of complex technology evaluation methods (material balance; cost-effectiveness analysis; description of risks).

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RESPONSE OF HORNWORT (*CERATOPHYLLUM DEMERSUM* L.) TO WATER LEVEL DRAWDOWN IN A TURBID WATER RESERVOIR

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Abstract. The research focuses on the effects of seasonal water level drawdown on the submerged vegetation in a turbid water reservoir measured through the biomass, the maximum depth of colonization and the occurrence frequency of hornwort *Ceratophyllum demersum* L. Furthermore, the underwater light intensity, water turbidity and biovolume of planktonic algae were determined to evaluate the environmental factors affecting the development of macrophytes. Because of low global radiation in late summer, the irradiance in the period following the water level drawdown was lower compared to the preceding period, but still above the species tolerance level. Nonetheless, the water level regulation improved the underwater light conditions for submerged macrophytes through the water level reduction. Plant biomass, the incidence of *C. demersum* and the maximum depth of macrophyte colonization increased after the water level drawdown in deep phytolittoral, whereas no effects were observed in shallows. Our research showed that the water level changes could be a useful tool in treatments aimed at maintaining the aquatic vegetation and improving the water quality in regulated water bodies.

Keywords: *macrophytes, phytolittoral, water level drawdown, hornwort*

Introduction

Submersed macrophytes play a key role in freshwater shallow ecosystems. They provide habitats and refuges for predatory fish and zooplankton, which may indirectly inhibit phytoplankton abundance (Jepessen et al., 1998). Macrophytes reduce the resuspension processes and increase the sedimentation rate, which improves the water transparency (Madsen et al, 2001; Søndergaard et al., 2003). Some macrophyte species may inhibit the growth of algae by effective competition for nutrients and allelopathic activity (Scheffer et al., 1993; Nakai et al., 1999). Hence, changes in macrophyte biomass are likely to have an important effect on the lake ecosystem.

Hornwort *Ceratophyllum demersum* L. may play an important structuring role in eutrophic lakes and reservoirs. It is a submerged, perennial plant with no roots, which often grows with the base of the stem buried in sediments, attached to the lake bottom by modified leaves. Hornwort can hamper the phytoplankton growth in shallow eutrophic lakes, which contributes to the stabilization of the macrophyte-dominated state characterized by clear water (Gross et al., 1993; Mjelde and Faafeng, 1997). The species is very common in turbid, disturbed shallow water bodies, such as man-made reservoirs, due to its high tolerance to shade and disturbance (Ali et al., 1995, 2011).

A specific, annual water-level cycle in man-made reservoirs is related to water management for hydropower, agricultural use, fishery, flood prevention or recreation

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(Geraldes et al., 2005). This contributes to an increase in the annual and inter-annual amplitude of the water level fluctuations, which are usually much higher than in natural lakes affected only by climatic and hydrological factors (Noges et al., 2003; Haldna et al., 2008). Changes in the water level strongly affect the aquatic vegetation abundance in water bodies (Hellsten, 2002; Coops et al., 2008). A relatively large number of studies dealt with effects of annual water-level fluctuations on macrophytes, however data on the role of intra-annual variability are scanty and limited to subtropical freshwaters (Thomaz et al., 2006).

Shallow man-made reservoirs may undergo rapid eutrophication due to the nutrient load from rivers and canals (Solis et al., 2012). High water turbidity related to over-fertilization and high algal biomass causes deterioration of the underwater light climate, thus affecting the structure and abundance of macrophytes in such disturbed lakes (Scheffer et al., 1993).

It seems, however, that the water level drawdown can stimulate the re-development of submerged macrophytes (Beklioglu et al., 2006; Bucak et al., 2012), thus making such operations a potentially useful tool in restoration of reservoirs. The aim of the study was to investigate the effect of water level drawdown in a shallow, turbid water reservoir on short-term dynamics of hornwort (*C. demersum*). Additionally, we aimed at evaluating the effect of light conditions on the plant biomass after the water level lowering.

Material and methods

Lake Domaszne (51°28’14” N, 23°00’07” E) is a small (95 ha), shallow (max. depth 3.1 m) and polymictic reservoir situated in the West Polesie region in Eastern Poland. The reservoir originates from natural Lake Domaszne, which was embanked and included in the hydrological system of the Wieprz-Krzna Canal. The water level in the reservoir is regulated to ensure the water supply for irrigation management and fish farming. Nutrient-rich water from the canal contributed the most to the present eutrophic/hypertrophic conditions of the water body (Solis, 2012).

Based on the data obtained from the local water management authorities, the water level in Lake Domaszne fluctuated in the hydrological year of 2007. The reservoir was filled up with water from the Canal in November-December 2006, and then again in April 2007 in order to replenish the water resources before the summer season. Water from the reservoir was discharged twice: in March and in June-July 2007. Thus, the study season 2007 was divided into two periods in terms of water level fluctuations and hydrological stages: the first one between the 1st of April and the 12th of July and the second one between the 12th of July and the 30th of September, hereafter referred to as the “early period” and the “late period”, respectively (*Fig. 1*). Based on the data from the watermark, the mean water level in the successive periods was on average of 2.56 and 2.06 m, respectively. The mean volume of the reservoir changed from 1.98 10^6 m^3 to 1.52 10^6 m^3, respectively.

The macrophyte community was studied in a monospecific bed of *Ceratophyllum demersum* situated in large shallows (ca. 0.2 km^2) in the northern part of the reservoir. Hornwort samples were collected on the 12th of July and the 30th of September 2007 with a rake sampler from 0.4×0.4 quadrates. A total of 160 samples were collected randomly at two locations: in the shallow phytolittoral (Zone I, depth of 1.1-1.3 m) and in the deep phytolittoral (Zone II, depth of 1.5-1.8 m). Based on sampling depths, the sampling isobaths were calculated and they were expressed in terms of elevation above watermark zero (150.31 m a.s.l.). Plant samples were collected in September from the same isobaths as in July, regardless of the current water level. After sampling, plants were cleaned, dried at a temperature of 105 ºC to constant weight and then weighed.

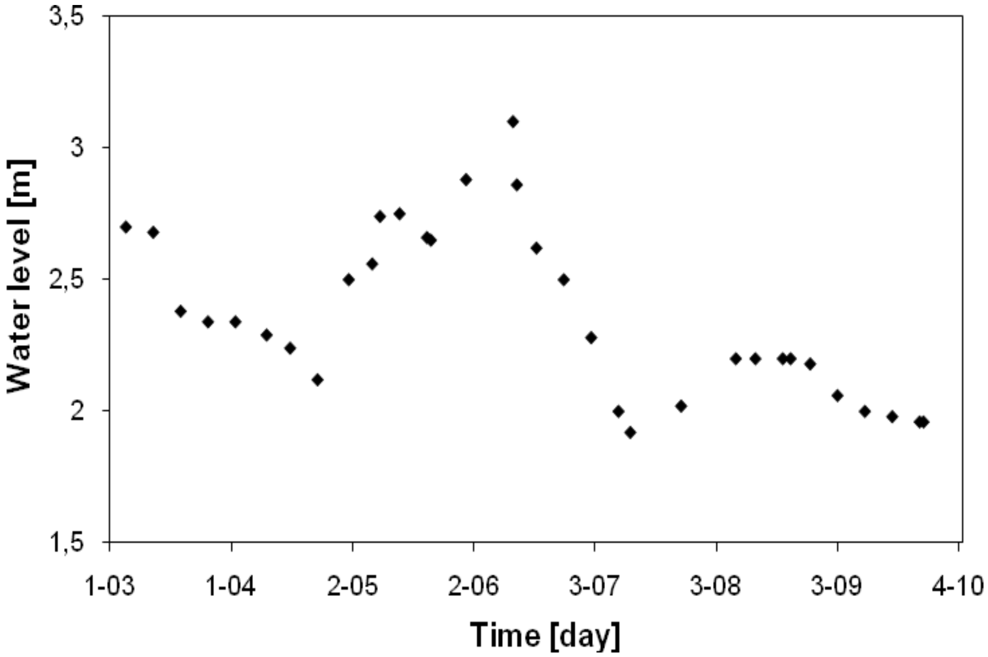

Figure 1. *Relative water levels in Lake Domaszne based on the watermark readings in 2007. Arrows indicate sampling dates.*

The maximum depth of macrophyte colonization (Z_c) was determined on the same dates at additional 70 sites.

Water transparency was considered as an attenuation coefficient, calculated from the measurements of PAR performed in situ using a Li-Cor meter (Li-250A) with an underwater quantum sensor (Li-192SA). The mean PAR above the bottom was calculated according to the following formula (Behrendt and Nixdorf, 1993):

$$I_{PAR} = 0.45\, I_0 \left(\frac{1 - e^{-K_d Z_b}}{K_d\ Z_b}\right) \qquad \text{(Eq.1),}$$

where Z_b is a water depth, K_d is an attenuation coefficient (m^{-1}) and I_0 is an average daily global radiation (J cm^{-2} d^{-1}). The contribution of PAR was calculated by a correction factor of 0.45 (Eq. 1). Data on global radiation were obtained from the meteorological station in Włodawa situated ca. 40 km from the reservoir.

Phytoplankton was sampled every two weeks between April and September. The abundance was estimated after fixation with Lugol's solution in sediment chambers according to Wetzel and Likens (2000). Biovolume was calculated using the geometric formulas after prior measurements of corresponding dimensions (Hillebrand et al., 1999).

The data were log-transformed prior to statistical analysis or non-parametric tests were used if data did not meet assumptions of parametric tests. The effects of the water level drawdown on the plant biomass was analysed by two-way ANOVA. The normality of the data was tested by the Lilleforst test and the homogeneity of variance was tested by the Levene test. Post-hoc Tukey's test was used to compare particular groups in ANOVA. Other differences were analysed by the Mann-Whitney test. The relationship between water transparency and phytoplankton was evaluated by Spearman's rank correlation test. All calculations were performed with Statistica 9.1 software (Sokal and Rohlf, 1995).

Results

The abundance of *Ceratophyllum demersum* in Lake Domaszne was significantly correlated with the depth of phytolittoral in terms of elevation above zero of the watermark, irrespective of the water level fluctuations (*Table 1*). Dry biomass was on average 151.6 g m^{-2} in the shallow phytolittoral (Zone I) and 21.4 g m^{-2} in the deep phytolittoral (Zone II).

Table 1. *Effects of location in phytolittoral (Zone), water level (Drawdown) and their interaction (Drawdown × Zone) on dry mass of Ceratophyllum demersum in the regulated lake; df – degrees of freedom, F – F-test, p – significance level*

Effect	**df**	**F**	**p**
Zone	1	212.58	<0.001
Drawdown	1	0.42	>0.5
Drawdown × Zone	1	12.31	<0.001

As expressed by the significance of Drawdown × Zone interaction, the response of *C. demersum* to drawdown was significant, with different effects on the biomass both in the shallow and deep phytolittoral (*Table 1*). The biomass in Zone I declined by 22%, however, the effect was not significant (Tukey's test, $p > 0.5$) (*Fig. 2*).

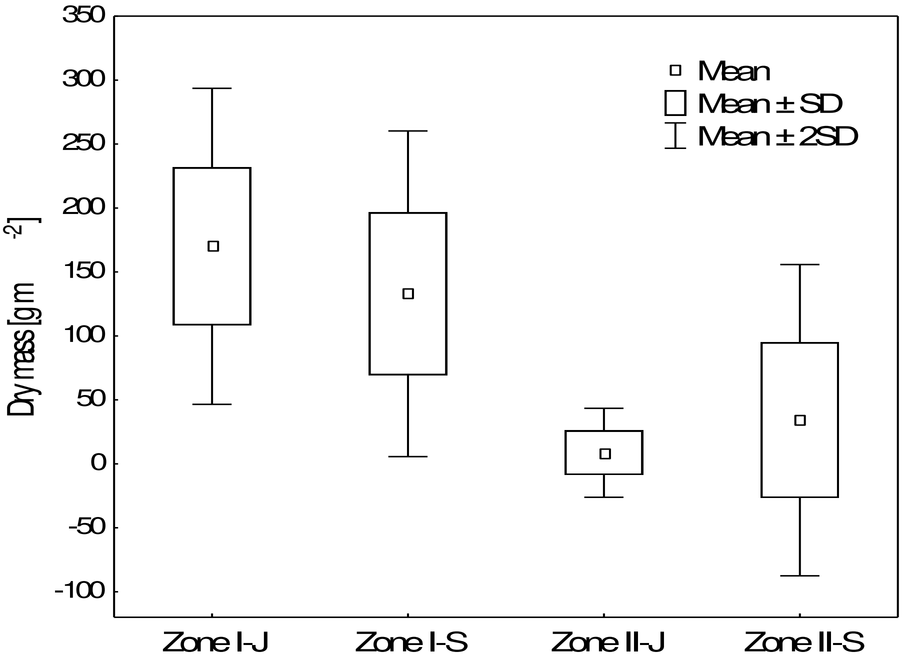

Figure 2. *Biomass of C. demersum in Lake Domaszne after water level drawdown at different depths; Zone I – shallow phytolittoral, Zone II – deep phytolittoral, E – early period, L – late period*

The impact of the water level lowering on *C. demersum* was strongly significant in Zone II (Tukey's test, $p < 0.001$), where the biomass increased from 8.6 g m^{-2} in the early period to 34.2 g m^{-2} in the late period (*Fig. 2*).

Following the increased biomass, the frequency of *C. demersum* occurrence slightly increased after water level drawdown, i.e. from 45% (the early period) to 55% (the late period) in Zone II, whereas it was constant in Zone I (100% in both periods).

The maximum depth of macrophyte colonization (Z_c) reflected strong light limitation. The depth Z_c, measured in relation to the watermark zero, slightly but significantly decreased from 0.93 m in the early period to 0.87 m in the late period (U-test, Z = 2.72, $p < 0.01$). The depth in relation to water surface was on average 1.26 m (max. 1.7 m) in the early period and 1.23 m (max. 1.4 m) in the late period. The results indicate that *C. demersum* occurred in both periods nearly at the same depth, but it shifted to deeper isobaths not colonized before water drawdown.

The positive response of the submerged vegetation occurred despite the high water turbidity related to high algal abundance. Phytoplankton biovolume ranged from 11.7 mm^3 dm^{-3} in June to 29.2 mm^3 dm^{-3} in July (*Fig. 3*). The abundant occurrence of *Limnothrix redekei* (van Goor) Meff. and *Planktothrix agardhii* (Gom.) Anag. et Kom. was observed; on average the two species accounted for 38% and 24% of the phytoplankton biovolume, respectively. The accompanying taxa included other Cyanoprokaryota: *Limnothrix planctonica* (Wołosz.) Meff., *Aphanizomenon gracile* (Lemm.) Lemm., *Microcystis* spp. and *Chroococcus* spp. Apart from blue-greens, diatoms and chlorophytes contributed the most to the total phytoplankton biovolume. The water level drawdown did not significantly affect the amount of phytoplankton. No differences were found between the early and the late period, neither in total phytoplankton (U-test, Z =-0.82, $p > 0.05$) nor in Cyanoprokaryota biovolume (U-test, Z =-0.64, $p > 0.05$).

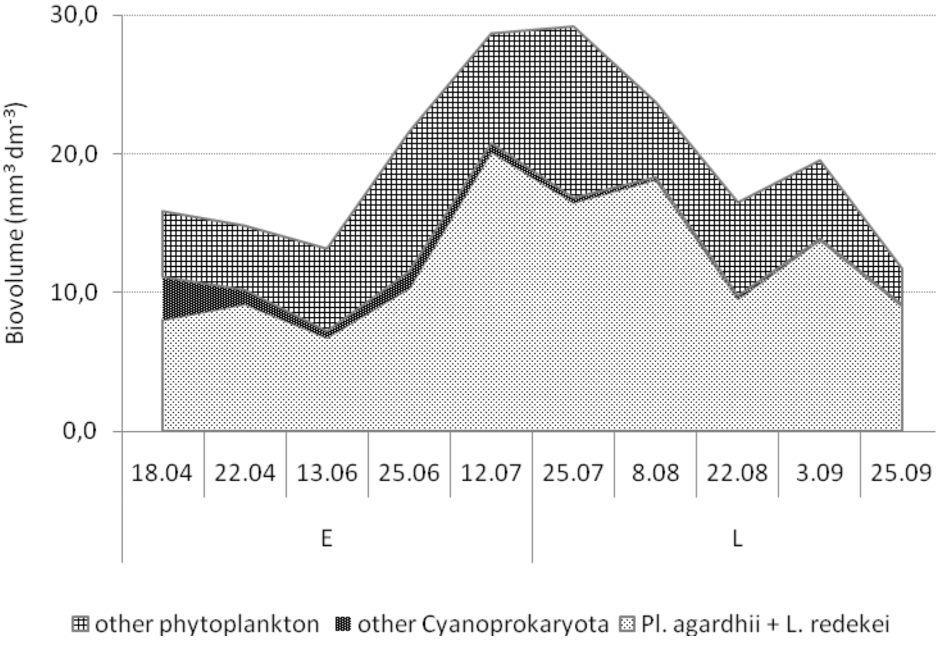

Figure 3. *Total phytoplankton biovolume in Lake Domaszne from April to September 2007; E – early period, L – late period*

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Water transparency in Lake Domaszne was similar in both periods (U-test, Z =-1.55, $p > 0.05$), while the attenuation coefficient (K_d) was 3.0 and 3.8 in the early and late period, respectively. Water turbidity was significantly correlated with the biovolume of phytoplankton (Spearman's test, R = 0.92, $p < 0.05$), especially the growth of blue-green algae (Spearman's test, R = 0.87, $p < 0.05$). It means that the growth of the latter affected the light conditions under the water surface in both periods.

The effects on *C. demersum* were observed despite the fact that light conditions were generally less favourable in the late period compared to the early one. Subsurface underwater PAR intensity (mean I_0) was 39.8 E m^{-2} d^{-1} in the early period and 27.4 E m^{-2} d^{-1} in the late period. A decrease in the light intensity above the bottom in deep phytolittoral ranged from 0.8 to 0.5 E m^{-2} d^{-1} for the early and late period, respectively. In the shallow phytolittoral it ranged from 1.3 to 1.1 E m^{-2} d^{-1}, respectively (*Fig. 4*). However, in terms of average percentage values of subsurface light, the irradiance at the bottom in Zone II was comparable in the early (2%) and in the late period (2.3%). Subsurface light intensity at the bottom in Zone I declined between the early and late period from 5.6% to 4.3%.

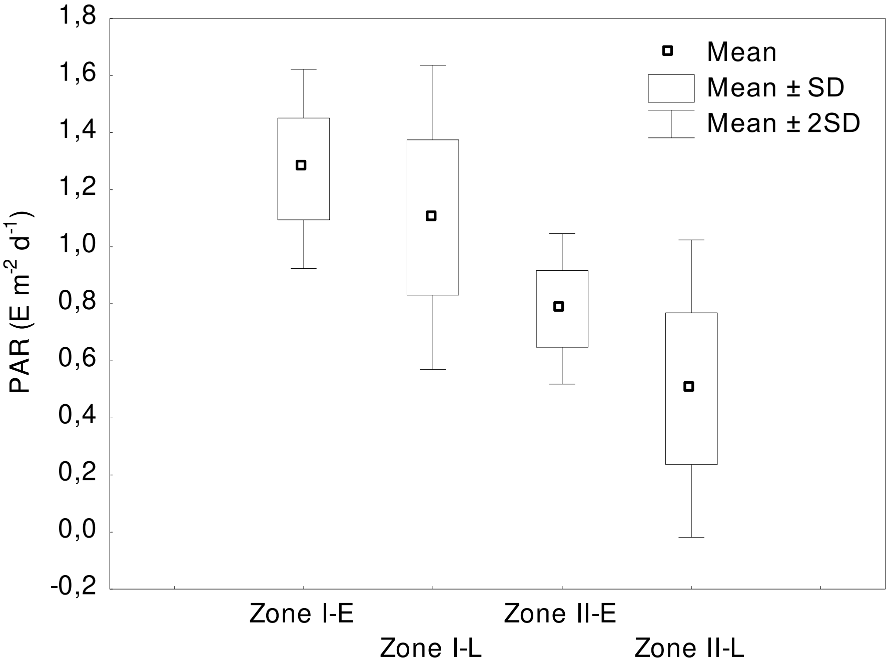

Figure 4. *Light intensity after water level drawdown in Lake Domaszne at different depths; Zone I – shallow phytolittoral, Zone II – deep phytolittoral, E – early period, L – late period*

Discussion

Water level fluctuations in shallow water bodies can affect the composition, diversity and abundance of macrophytes (Hellsten, 2002; Riis and Hawes, 2002). The effect of the water level changes on the submerged vegetation is, however, not straightforward. Several studies showed that a low water level during a vegetation season has a positive effect on submerged plant biomass (Blindow, 1992; Beklioglu et al., 2006; Bucak et al., 2012). On the other hand, macrophytes could be negatively affected by increased water turbidity after the water level lowering. Then, the increased resuspension caused by the wave action could cause a deterioration of water transparency and light conditions (Pieczyńska, 2008).

Extreme water drawdown in regulated lakes may be catastrophic for submerged macrophytes due to freezing-up of the exposed parts of the bottom in winter or their desiccation in summer. Thus, the water level manipulation may be used as a measure to control nuisance macrophytes in reservoirs (Rørslett and Johansen, 1996).

Submerged macrophytes in temperate lakes begin to senesce and die after reproduction in summer (Westlake, 1965). During the seasonal life cycle, the biomass of *Ceratophyllum demersum* declines from the seasonal maximum in June-July to the minimum in September or October (Asaeda and Bon, 1997; Nikolić et al., 2007) due to decreasing solar radiation and low temperatures (Wetzel, 2001). Contrary to previous studies from natural lakes, our research showed~~,~~ that~~,~~ the abundance of *C. demersum* in regulated Lake Domaszne tends to increase in late summer after the water level drawdown.

The effect of water level drawdown on *Ceratophyllum demersum* was correlated with the location in phytolittoral, similarly to a sub-tropical reservoir described by Thomaz et al. (2006). Plant biomass of *C. demersum* slightly decreased at shallow sites most likely because of self-shading, which is an important regulating mechanism of production and biomass growth in aquatic macrophytes (Westlake, 1965; Calado and Duarte, 2000). After the water level and the reservoir volume decreased the biomass of *C. demersum* in the shallow phytolittoral became more concentrated. The increased shoot density reduced the light in the macrophyte beds especially at shallow sites where *C. demersum* was very abundant. Organic matter deposited on plant organs (because of the increased sedimentation rate in dense macrophyte beds) could also negatively affect the vegetative growth of the submerged plants (Barko and Smart, 1986). Desiccation of the lake bottom after the water level drawdown was rather of little importance in the reservoir due to the shoreline regulation and inclination of lake shores.

In contrast to shallow phytolittoral, the biomass of *C. demersum* increased after the drawdown in deep phytolittoral and the vegetation shifted to a deeper, previously non-colonized area of the reservoir. As a consequence of the water level lowering, larger parts of the bottom have became exposed to more favourable light intensity in the water column, which affects the spread of *C. demersum* in late summer.

The effect of annual water level regime on the submerged macrophyte was observed despite the light conditions – in the late summer they were generally worse than during the preceding period. The shade tolerance of *C. demersum* seems to be the major factor responsible for the species relatively high abundance and common occurrence in turbid waters (Ali et al., 1995, 2011). Submersed macrophytes need, however, approximately 1-3% of the full sunlight spectrum (Wetzel, 2001). Hornwort could benefit from the water level regulation in the reservoir because light intensity in the water column was still above the species tolerance level and comparable to the light compensation point of the species (Van Nes et al., 2007).

Phytoplankton abundance is the major factor limiting the development of submerged macrophytes (Scheffer et al., 1993; Asaeda and Bon, 1997). Shallow reservoirs can be devoid of macrophytes due to a large amount of algae (Van Nes et al., 2007). High biomass of cyanobacteria caused high water turbidity and negatively affected the distribution of submerged vegetation in the lake (Scheffer and Van Nes 2007). On the other hand, sediment resuspension in shallow lakes can also affect the water turbidity (Jackson, 2003).

The phytoplankton community in the studied reservoir was dominated by filamentous blue-green algae *Planktothrix agardhii* and *Limnothrix redekei*. Their abundant occurrence was observed mostly in shallow, polymictic and nutrient-rich water bodies, sometimes throughout the year (Solis et al., 2009; Kokociński et al., 2010). Both taxa are water-mixing and shade tolerant. According to Scheffer and Van Nes (2007), the presence of cyanobacteria from the Oscillatoria group can negatively affect the light conditions.

An increased abundance of submerged macrophytes affects the lake functioning and improves the water transparency, e.g. by negative effects on phytoplankton, increased sedimentation and by reduction of the resuspension rate (Scheffer et al., 1993; Horppila and Nurminen, 1997; Mjelde and Faafeng, 1997; Pęczuła et al., 2012). Moreover, the development of macrophyte beds stimulates other food-web components such as zooplankton and predatory fish, which are important for stabilization of clear-water conditions in a lake (Jeppesen et al., 1998). Several studies (e.g. Blindow, 1992; Bucak et al., 2012) highlighted the impact of inter-annual fluctuations in the water level on submerged macrophytes in shallow lakes as a shift between two alternative states: the vegetation-dominated one with clear-water conditions and the phytoplankton-dominated one with turbid water (Scheffer et al., 1993).

In conclusion, water level regulation within the annual cycle can improve the conditions for *C. demersum* development in a shallow, turbid water reservoir. Hence, it could be a helpful tool in treatments aimed at maintaining the aquatic vegetation and improving the water quality in such water bodies.

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ARBUSCULAR MYCORRHIZAL SYMBIOSIS, ECOSYSTEM PROCESSES AND ENVIRONMENTAL CHANGES IN TROPICAL SOILS

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Abstract. Arbuscular mycorrhizal fungi (AMF) are more widely distributed and associate with a wide range of plant species. AMF are keystone organisms that form an interface between soils and plant roots, and are sensitive to changes in soil and plant conditions. They are important microbial symbioses for plants and under conditions of P-limitation and are significant in the maintenance of soil health and fertility. AMF are crucial for the functioning of terrestrial ecosystems and plants form symbiotic interactions with AMF and colonize more than 80% of plant roots. Mycorrhizal fungi are known to influence plant diversity patterns in a variety of ecosystems globally. AMF hyphae form an extensive network in soil and length is a common parameter used to quantifying fungal hyphae. The mycelial network of AM fungi extends into the soil volume and greatly increases the surface area for uptake of immobile nutrients. Also, AM symbioses improve drought tolerance of plants and enhance tolerance of or resistance to root pathogens. Furthermore, networks of AM hyphae play a role in the formation of stable soil aggregates, building up a macroporous structure of soil that allows penetration of water and air and prevents erosion. The functioning of AMF symbiosis is mediated by direct and indirect effects of biotic and abiotic factors of the surrounding rhizosphere, community, and ecosystem. They have great potential in the restoration of disturbed land and low fertility soil. Little is known about the effects of environmental changes on AMF abundance, activity and the impact on ecosystem services. Also, we still have much to learn regarding the extent of mycorrhizal fungal diversity. It is critical to gain a clearer understanding of functional variation among AM fungal species to guide conservation and restoration efforts.

Keywords: *arbuscular; mycorrhizal; symbiosis; mycelium; hyphae; soil; terrestrial ecosystem.*

Introduction

Arbuscular mycorrhiza is the most ancient and widespread form (Smith and Read, 2008). Paleobotanical and molecular sequence data suggest that the first land plants formed associations with Glomalean fungi from the Glomeromycota about 460 million years ago (Redecker et al., 2000). This is estimated to be about 400 million years before the appearance of root nodule symbioses with nitrogen-fixing bacteria. Arbuscular mycorrhizal (AM) symbioses can be formed with a very wide range of plant species, as many as 250,000 (Smith and Read, 2008). Only 150-200 species of AM fungi have so far been distinguished on the basis of morphology, but DNA-based studies suggest the true diversity of these symbionts may be very much higher (Fitter, 2005; Santos-González et al., 2006). The symbiosis is characterized by highly branched fungal structures, *arbuscules*, which grow intracellularly without penetrating the host plasmalemma (Brundrett, 2004).

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AMF are more widely distributed than other types of mycorrhizal associations (Smith and Read, 2008). They are keystone organisms that form an interface between soils and plant roots, and are sensitive to changes in soil and plant conditions (Power and Mills, 1995). They associate with a wide range of plant species (Atayese et al., 1993) and can infect most species of flowering plants in most habitats (Sieverding, 1991). AMF are important microbial symbioses for plants and under conditions of P-limitation and are significant in the maintenance of soil health and fertility, plant community development, nutrient uptake and above-ground productivity (Smith and Read, 1997). For example, Borowicz (2001) showed that plants generally grow better when they are mycorrhizal. van der Heijden et al. (1998) found that plant biodiversity, nutrient capture, and productivity in macrocosms increase significantly with increasing AM hyphal length and AMF species richness. Plants acquire nutrients and water through mycorrhizal symbioses. Numerous studies indicate that the mycorrhizal symbiosis is most important to plants when soil nutrients are limiting (Marschner and Dell, 1994; Johnson et al., 2010). Plants exchange carbon (C) for fungal phosphorus (P) and nitrogen (N) (Smith et al., 2009). AM fungi release signaling molecules, which trigger a series of symbiotic plant genes; this actively prepares the intracellular root environment for colonization and arbuscule formation inside the root cortex (Reinhardt, 2007; Bonfante and Genre, 2008). Studies have been undertaken on the distribution and diversity of AMF species in relation to individual plant species and plant communities in farming systems (Jefwa et al., 2004), and recently there is emerging interest in the role of mycorrhizae in ecosystem processes (Rilling, 2004). Also, a few studies have tracked individual fungi through time as environments change (Hirose et al., 2004; Hu et al., 2013). Yet, these kinds of studies are necessary to understanding of the dynamics of mycorrhizal symbioses.

AMF functioning and the ecosystem services

AMF are crucial for the functioning of terrestrial ecosystems and terrestrial plants form symbiotic interactions with AMF and colonize more than 80% of plant roots (Brundrett, 2004). Mycorrhizal fungi are known to influence plant diversity patterns in a variety of ecosystems globally (Klironomos, 2002). However, the contribution of mycorrhizal fungi to the maintenance of plant diversity in the tropics is poorly known (Mcguire et al., 2008). It is well recognized that humans are changing global environments at an unprecedented rate. These changes are known to impact global climate and biota, however, the implications for communities and ecosystems are not known (IPCC, 2001). Understanding mycorrhizal responses to anthropogenic environmental changes can help predict the trajectories of future communities and ecosystems in a changing world (Yang et al., 2013; Hu et al., 2013). There is limited work undertaken in AMF at landscape level in relation to land use changes in the tropics. With increasing interference of landscape by human beings, it is vital to establish how land use changes influence AMF abundance, activity and the impact on ecosystem services. Loss of propagules of AMF will result in a decrease in the capacity of plants to take up nutrients, thus lowering soil fertility as a result the stability of the ecosystem then becomes threatened (Jeffries et al., 2003). There are a number of situations where management of the mycorrhizal symbiosis is necessary to restore plant cover, improve plant health or increase plant productivity.

Studies carried out in agricultural systems both in tropical and temperate regions have suggested that AMF abundance may decline upon agricultural intensification (Oehl et al., 2003). Whilst reports of AM hyphal lengths in agricultural soils are becoming increasingly common (Leake et al., 2004), our knowledge of them in natural ecosystems remains scant. Knowledge about mycelial biomass is important to understand the potential roles of fungi in decomposition and nutrient cycling, and plant symbionts. For example, a study by Langley and Hungate (2003) showed that the presence of mycorrhizal fungi can alter rates of above- and below-ground litter decomposition due to chemical changes in roots and interactions with decomposer fungi. Also, at present, little is known about the control of diversity of AM fungal communities in tropical soils and, given the increasing importance attached to mycorrhizal fungal diversity for maintenance of ecosystem functioning, a better understanding of the causes of AMF diversity and its loss is clearly required.

The mycelial network of AM fungi extends into the soil volume and greatly increases the surface area for uptake of immobile nutrients, particularly P, N and Cu (Brundrett, 2004; Johnson et al., 2010). Also, AM symbioses improve drought tolerance of plants and enhance tolerance of or resistance to root pathogens (Smith and Read, 1997; Auge, 2001). Furthermore, networks of AM hyphae play a role in the formation of stable soil aggregates (Cavagnaro et al., 2005), building up a macroporous structure of soil that allows penetration of water and air and prevents erosion (Rillig et al., 2002). AMF may stabilize soils up to 5 months after their host’s death (Tisdall and Oades, 1980). It is also accepted that AMF receive all their carbohydrate from the host plant (Zhu and Miller, 2003; Le Tacon et al., 2013) and that the association of AMF with roots could create a sink demand for carbohydrate, which could result in up to 20% drain of carbon from the host plant and could indirectly influence carbon storage in soils (Graham, 2000). Carbon obtained by host plants can be allocated to fungal structures of functional importance to plants, AMF, or both members of the symbiosis (Olsson et al., 2002; Le Tacon et al., 2013). Allocation to fungal arbuscles and extraradical hyphae can increase plant acquisition of soil resources (Johnson et al., 2003). Fungal allocation to spores and vesicles (C storage structures) is associated with C accumulation by AMF (Bever et al., 2001). AM fungal diversity and activity in tropical soils have not been adequately studied and understood (Hawksworth, 2001). AMF play an important role in many ecosystems, relatively little is known about the effects of land use changes on AMF abundance and activity in tropical soils. A better understanding of the influence of land use changes on AMF abundance will facilitate their management to improve plant productivity in the poor soils. Determining the affects of land use changes on the abundance, and activity of beneficial AMF in tropical soils will be helpful in designing more sustainable management practices.

Marshner and Dell (1994) reported that the external hyphae of AMF can deliver up to 80% of a plant’s P requirements. Understanding of the factors that enhance nutrient uptake by AMF is especially important for poor countries where the use of mineral fertilizers is not economically feasible (Johnson et al., 2010). Mycorrhizal fungi are of high value for ecosystem functioning and sustainability (Sanders, 2010). Land degradation and soil fertility depletion are considered the major threats to food security and natural resource conservation in sub-Saharan Africa (Cardoso and Kuyper, 2006). Mycorrhizal associations are complex hierarchical systems (O'Neill et al., 1991). At the core of every association is a fungus and a plant living symbiotically (Johnson et al., 1997). The functioning of this symbiosis is mediated by direct and indirect effects of biotic and abiotic factors of the surrounding rhizosphere, community, and ecosystem (Linderman, 1988). Also, AMF abundance may be influenced directly or indirectly by biotic interactions (Reinhard et al., 1994). Subsequently soil-borne microorganisms (i.e. fungal symbionts) may directly compete for host C; on the other hand, they may develop a beneficial partnership in which the plant supplies C and the fungus supplies the catalytic elements (Wellings et al., 1991). The benefit of mycorrhiza formation is considered to depend on the balance between the fungal demand for energy and the plant's needs for nutrients (Corrêa et al., 2011). Negative effects of mycorrhizal colonization on the host plant are expected when the net C costs for fungal maintenance and growth exceed the net benefits obtained from improved nutrient supply (Tuomi et al., 2001). Several studies have indicated that the effect of mycorrhiza on the host plant productivity depends on the amount of nutrients available, and on the host plant nutrient status (Cavagnaro et al., 2005; Janos, 2007).

Importance of mycorrhizal mycelial networks

AM hyphal networks impact soil structure and plant community composition and are important belowground carbon sinks (Rillig, 2004; Le Tacon et al., 2013). AMF hyphae form an extensive network in soil and length is a common parameter used to quantifying fungal hyphae (Hynes et al., 2008). Giasson et al. (2008) found that hyphae of AMF may extend 8 cm from the root surface. For example, in rhizosphere of Ryegrass roots, Tisdall and Oades (1979) measured about 55 m of hypahe per cubic centimetre of soil. Olsson et al. (1999) suggested that the mycelial network of AMF accounts for approximately half of the microbial biomass in grassland soils. For instance, it has been estimated that one gram of soil contains up to 200 m fungal hyphae (Leake et al., 2004). Read et al. (1976) found that it is the root-based hyphal network in soil, rather than resting spores that is responsible for infecting seedlings that become established in a natural grassland sward. Soil densities of AMF hyphae in temperate grasslands have been shown to vary with precipitation, soil fertility (Johnson et al. 2003), and plant productivity (Rillig, 2004). Hunt and Fogel (1983) reported that length of hyphae decreases with increasing soil depths. Also, there is an exponential decline in both infection and spore numbers with depth (Abbott and Robson, 1991).

Extraradical hyphal densities are important with respect to potential ecological selection for different life-history strategies under contrasting environmental conditions (Brito et al., 2011). This is because the extent of the extraradical mycelium is an important trait of AMF, affecting nutrient supply to host plants and thus probably their fitness and survival (Dodd, 2000). External AM hyphae also produce recalcitrant forms of C, such as chitin and glomalin (Zhu and Miller, 2003), and therefore might be important contributors to the structural stability of the soil and C sequestration (Zhu and Miller, 2003). Hyphal length is an important consideration that needs to be integrated into any planning for plant conservation because of the potential contribution. Despite their ubiquity and potential importance for ecosystem structure and functions, surprisingly little is known about the abundance of AM networks in tropical soils. AM hyphal length could make to plant community production and stability (Utobo et al., 2011). AMF have great potential in the restoration of disturbed land and low fertility soil (Quilambo, 2003). A more appropriate management of mycorrhizae in poor agricultural soils is expected to allow substantial reduction in the amount of mineral used without losses in productivity, whereas permitting a more sustainable production management.

AM fungal species composition, host range and infectivity

AM fungi vary considerably in their life histories and their affects on soil structure and plant health (Miller et al., 1995; Klironomos et al., 2000). AMF are also known to vary in their response to the mineral environment of the soil (Bever et al., 2001). For instance, it has been shown that differences in AMF species distributions are caused by habitat preferences of taxa, for example differences in tolerance to high nutrient availability (Egerton-Warburton et al., 2007; Porras-Alfaro et al., 2007), pH and soil type (Lekberg et al., 2007; Oehl et al., 2010) and mechanical disturbance (Schnoor et al., 2011). Some species of mycorrhizal fungi decline with nitrogen enrichment while others proliferate (e.g. Lilleskov et al., 2001). Wallenda and Kottke (1998) predicted that AM fungal species with a narrow host range (e.g. conifer specialists) are more adversely affected than species with a broad range of host plants. When soil phosphorus is not limiting, members of the AM fungal family Gigasporaceae are often dramatically reduced by nitrogen enrichment (Egerton-Warburton and Allen, 2000). On the other hand, when soil phosphorus is in limiting supply, nitrogen enrichment increases populations of Gigasporaceae (Eom et al., 1999). This suggests that nitrogen enrichment of phosphorus deficient soils exacerbates phosphorus limitation and increases the net benefits of mycorrhizas.

Taxa of AM fungi vary in growth rate, biomass allocation and symbiotic effects (Abbott and Robson, 1985; Miller et al., 1995). For example, Glomaceae and Acaulosporaceae allocate more biomass inside roots, and benefit their host plants through increasing pathogen resistance while Gigasporaceae allocate more biomass outside roots and are more beneficial for plant phosphorus acquisition (Klironomos et al., 2004). Brundrett et al. (1984) reported that members of the Gigasporaceae never form vesicles in plant roots, instead they form clusters of auxiliary cells in the surrounding soil (extraradical). Furthermore, the genus Gigaspora produces intra- and extraradical hyphae that are much thicker than other genera (Klironomos et al., 2000). Mycorrhizal roots on intact plants or germinated spores are best understood as sources of infective hyphae for initiating new sites of colonization of roots (Abbott and Robson, 1991). Vesicles formed within roots have been shown to act as propagules for some AM fungi (Biermann and Linderman, 1983). Extra-matrical vesicles of *Gigaspora spp.* represent potential propagules (Jabaji-Hare et al., 1986). For each species of fungus, types of propagules may differ widely in their tolerance of some conditions (Abbott and Robson, 1991). For example, Jasper et al. (1989) showed that hyphae of *Acaulospora laevis* in soil completely lost their infectivity with disturbance. Teasing apart the relationships between the environment and community composition is essential for our understanding of AMF diversity in tropical ecosystems.

Influence of grazing on AMF abundance

The abiotic environment, particularly soil fertility, water and sunlight may structure the balance of trade among symbionts (Azcon et al., 1991). The influence of grazing on soil nutrient availability and host plant productivity (Frank and McNaughton, 1993) may cause variable effects on AMF community composition and structure (Eom et al., 2001; Bai et al., 2013). Grazing of pasture grasses in the field has been found to affect the proportion of root length infected by decreasing root length per unit volume of soil (Trent et al., 1988; Yang et al., 2013). Grazing intensity might change the level of mycorrhizal infection in a community by altering the plant composition (Abbott and Robson, 1991). It is likely that the activity of AMF is an important factor regulating the cycling of nutrients in undisturbed ecosystems (Abbott and Robson, 1991). Because symbiotic AMF depend so heavily upon living plants for C, they will be impacted by any process which alters belowground C allocation (Frank et al., 2002). Grazing can influence the dynamics of nutrient exchange between host plants and AMF (Gehring and Whitham, 2002). Herbivore grazing can alter leaf photosynthetic rates (McNaughton 1979), aboveground production (Frank and McNaughton, 1993), and C allocation belowground (Frank et al. 2002). Allocation to AMF morphological structures can increase or decrease depending on the timing and severity of herbivory (Gange, 2007). Grazers also influence allocation to AMF morphological structures by altering soil nutrient status through direct inputs of N and P in dung and urine deposition (Schnyder et al., 2010; van der Waal et al., 2011).

Effect of soil disturbance on AMF abundance and their distribution

Disturbance can affect the occurrence of AM fungi in both agricultural and natural ecosystems. It may change the abundance and distribution of mycorrhizal fungi in several ways (Abbott and Robson, 1991). First, it may change the physical, chemical or biological environment of soil leading to either direct effects on AM fungi or indirect effects operating via effects of disturbance on plant growth. Second, disturbance may change the plant composition of the stand or eliminate host plants leading to changes in the distribution and abundance of AM fungi (Abbott and Robson, 1991). Removal of surface soil layers mainly by water erosion decreased markedly both the number of propagules of AM fungi and the extent of mycorrhiza formation (Powell, 1981; Habte, 1989). Disturbance of soil can decrease mycorrhizal infection and several factors may be responsible (Jasper et al., 1989). There may be effects of tillage on root growth affecting the extent of root colonization by mycorrhizal fungi (Borie et al., 2006). Increasing the intensity of cultivation decreased mycorrhiza formation in dry beans *(Phaseolus vulgaris* L.) and was apparently associated with increased soil compaction and decreased root growth (Mulligan et al., 1985). The effects of soil disturbance on the formation of mycorrhizas may be associated with decreased phosphate uptake after ploughing compared with the uptake of plants grown without tillage (O'Halloran et al., 1986).

Responses of AMF abundance to environmental changes

In the mid-1970s, it was recognized that land cover change modifies surface albedo and thus surface atmosphere energy exchanges, which have an impact on regional climate (Sagan et al., 1979). A much broader range of impacts of land use and cover change on ecosystem goods and services include impacts on biotic diversity worldwide (Sala et al., 2000), soil degradation (Trimble, 2000), and the ability of biological systems to support human needs (Vitousek et al., 1997). Land use and cover changes also determine the vulnerability of places and people to climate change (Vitousek et al., 1997; Odada et al., 2009). When aggregated globally, land use and cover changes significantly affect central aspects of earth system functioning (Lambin et al., 2003). Predicting how land use changes affect land degradation, the feedback on livelihood strategies from land degradation, and the vulnerability of places and people in the face of land use and cover changes requires a good understanding of the dynamic human-environment interactions associated with land use change (Ramankutty and Foley, 1999).

During the last century, land use and cover have changed drastically in the tropics due to changing economy and growing population (Meyer and Turner, 1992). Natural vegetation covers have given way not only to cropland but also to pasture. Globally, concerns about the changes in land use and cover emerged due to the realization that land surface processes influence climate and that change in these processes impact ecosystem goods and services (Lambin et al., 2003). The impacts of primary concern are the negative effects of land use change on biological diversity, soil degradation and the ability of biological systems to support human needs. One way by which plants can potentially increase ecosystem productivity and stability is by forming mycorrhizal associations (van der Heijden et al., 1998; Eriksson, 2001; Hartnett and Wilson, 2002). Plants are most likely to form associations with and benefit from mycorrhizal fungi under conditions in which availability of one or more soil nutrients, including water, is low (Auge, 2001; Hoeksema and Schwartz, 2003; Jones and Smith, 2004). Tropical savanna soils have been eroded and poor in nutrients leading to reduced plant productivity (Pimentel, 2006). AMF are of particular importance to the plant in soils that are nutrient-poor (Jeffries et al., 2003; Johnson et al., 2010). Moreover, AMF may be used as sensitive indicators of ecological soil quality if they respond to environmental variation in a predictable way (Verbruggen et al., 2012).

Land use practices have placed new pressures on plant-mycorrhizal symbiosis and are evidently a threat for AMF (Siddiqui and Pichtel, 2008). Agricultural management practices might affect AMF communities both qualitatively and quantitatively (Miller et al., 1995; Cavagnaro et al., 2005; Barber et al., 2013). Studies have shown that crop rotation, fertilization, and tillage affect the composition and diversity of AMF communities as well as spore and mycelium densities in temperate and tropical agroecosystems (Jansa et al., 2002; Oehl et al., 2003). Tillage physically disrupt soil aggregates and AM hyphal networks which deteriorates soil structure, lessens fertility and nutrient cycling, and results in more C allocation within fungal hyphae to re-establishing these networks and less C to glomalin formations (Nichols and Wright, 2004). Disturbance resulting from agricultural activities has been shown to decrease AMF species richness and infectivity (Douds and Millner, 1999). In some environments, cultivation through tillage and fertilizer application has led to fewer species of AM fungi (Schenck and Kinloch, 1980). Continuous cropping with inadequate external inputs has caused depletion of nutrients such as phosphorus (P) and nitrogen (N) in tropical soils (Smithson and Giller, 2002). In no-till and reduced-tillage systems, maintenance of the integrity of the hyphal network contributes to rapid AMF infectivity and efficient nutrient uptake (Habte and Osorio, 2001; Johnson et al., 2010). Non-tillage practices along with continuous cropping system using mycorrhizal host crops, and reducing mineral fertilizers, enhance the plant-mycorrhizal symbiotic relationship (Rilling, 2004). The AMF diversity occurring over a broad range of the tropical natural systems has not yet been investigated. Studies have been done in temperate and agricultural settings (Daniell et al., 2001) but little is known about the effects of land use and cover changes in natural systems in the tropics. Information about species composition of AMF community appears important to understand mycorrhizal function in the ecosystems (Johnson and Pfleger, 1992). It is evident that AMF are crucial for the functioning of terrestrial ecosystems. Therefore, understanding the impact of land use and cover changes on AMF abundance in tropical soils is crucial.

Effect of soil nutrients on mycorrhizal infection

The relationships between the level of mycorrhizal colonization and soil chemical and physical properties are variable (Newman et al., 1981). High levels of infection have been observed over a wide range of soil pH and soil phosphate levels and (Read et al., 1976; Jeffries et al., 1988). It seems that changes in soil pH in the field will affect the proportion of colonization associated with particular fungal species but are unlikely to change the total extent of colonization (Abbott and Robson, 1991). There are, however, marked differences among species of AM fungi in the effects of soil properties on their distribution and abundance (Abbott and Robson, 1991). For example, some species of AM fungi are restricted to either acid or alkaline soils; whereas others occur in both acid and alkaline soils (Porter et al., 1987). Negative association has been found between the amounts of extractable phosphate in soils and the abundance of AM fungi as assessed by infection (Bolgiano et al., 1983; Morita and Konishi, 1989). It has been shown that some species of AM fungi differed in the extent to which phosphate decreased mycorrhiza formation (Thomson et al., 1986). AMF are also known to vary in their response to the mineral environment of the soil (Bever et al., 2001). There are critical ranges of soil-solution P concentration at which the host-fungus association is truly mutualistic, i.e., where the benefit each partner derives from the association outweighs the costs (Habte and Osorio, 2001; Brundret, 2004). Habte and Osorio (2001) suggested that if P concentration in the soil is suboptimal for mycorrhizal function, AMF symbiotic effectiveness is reduced, and the fungus and the host may compete for scarce P. When solution P concentration is much above the optimum for a given host-fungus combination, mycorrhizal colonization will be suppressed (Habte and Osorio, 2001). There is also considerable information on the negative effects of nitrogen fertilizer on mycorrhizal formation (Mosse et al., 1976). Hyphal growth tends to decrease under fertilization (Treseder, 2004).

Studies of agricultural systems have shown that high levels of fertilization can select for AMF that are less beneficial or even parasitic on their host plants (Johnson, 1993; Johnson et al., 1997). Additionally, high levels of nitrogen fertilization can decrease colonization by mycorrhizal fungi (Smith and Read, 1997) and lead to significant change in mycorrhizal community structure (Parrent and Vilgalys, 2007). The adverse effect of high soil P levels on AM formation is well documented and is mainly caused by higher P concentrations in the roots (Jasper at el., 1979; Abbott et al., 1984; Abbott and Robson, 1991). It has also been shown that high P levels in the soil can reduce not only spore germination and hyphal growth from the germinated spores (Miranda and Harris, 1994a) but also early colonization of the roots and growth of the extraradical mycelium (Miranda and Harris, 1994b). A comparative study across North American grasslands showed that nitrogen fertilization reduces AM hyphal densities in phosphorus rich soil, but increases AM hyphal densities when phosphorus is in limited supply (Johnson et al., 2003). Adding phosphate fertilizers has been shown either to decrease the level of mycorrhizal infection in a range of agricultural crops (George et al., 1995). For example, Nitrogen applications to wheat decreased spore numbers and mycorrhizal infection (Abbott and Robson, 1991). However, few studies have been made of the effects of nutrients, other than phosphorus, on the abundance and distribution of AMF in the field (Abbott and Robson, 1991).

Investigating how AMF abundance vary with changing levels of soil N and P in tropical grasslands will further advance our understanding of the factors controlling mycorrhizas because, unlike temperate grasslands, there is virtually no seasonal temperature fluctuation in tropical grasslands. Decomposition and mineralization processes are more likely to be controlled by land use, grazing pressures and seasonal moisture deficit in tropical grasslands than in temperate grasslands. There are only a few studies, however, concerning the effects of P on the extraradical mycelium of AM fungi, and no attempts have been made to investigate its effects on the root-soil partitioning in these fungi. Howeler et al. (1987) suggested that AM hyphae have a lower threshold for uptake of phosphorus than that of non-colonized plant roots. In soil with a high capacity to immobilize phosphorus and low available phosphate, such as many tropical soils, AM can be of great benefit to plants (George et al., 1995; Johnson et al., 2010). High phosphorus can inhibit AM colonization of plant roots, reduce formation of entry points and vesicles (Amijee et al., 1989), and decrease the length of external hyphae associated with AM (Abbott et al., 1984), consequently diminishing nutrient uptake and host benefit from AM (Schroeder and Janos, 2004).

Conservation implications

It should be apparent from the preceding discussion that Arbuscular mycorrhizal symbioses play fundamental roles in shaping plant communities and terrestrial ecosystems. The main significance of mycorrhizal fungi is that they connect the primary producers of ecosystems, plants, to the heterogeneously distributed (N and P) nutrients required for their growth. Mycorrhizal fungi are of high value for the ecosystem functioning and sustainability. A more appropriate management of mycorrhizae in poor soils would allow substantial reduction in the amount of mineral used without losses in productivity, whereas permitting a more sustainable production management. Studies on AMF species diversity and their functions across land use types are crucial in understanding the impact of land use changes on ecosystem services. For example, ecologists conducting field studies of the impacts of land use changes on mycorrhizal colonization and community composition could benefit from collaboration with plant physiologists to provide mechanistic insights. Currently, most studies of mycorrhizal mediation of below ground processes have examined individual plant-fungus pairs or interactions among individual mycorrhizas and biota or abiotic conditions. Although this scale of inquiry provides precise understanding of specific plant-fungal systems, it cannot provide meaningful information about mycorrhizal function within communities and ecosystems (Read and Perez-Moreno, 2003). Also, we still have much to learn regarding the extent of mycorrhizal fungal diversity. Among species of mycorrhizal fungi, there is very little knowledge of functional attributes such as stress tolerance and nutrient uptake efficiency. Comparative analysis of natural systems will improve our understanding of responses to environmental and climatic perturbations. This new knowledge is an important prerequisite for future, sustainable management of terrestrial ecosystems. It is critical to gain a clearer understanding of functional variation among AM fungal species to guide conservation and restoration efforts.

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UNDERSTANDING THE BEHAVIOUR OF OIL-DEGRADING MICRO-ORGANISMS TO ENHANCE THE MICROBIAL REMEDIATION OF SPILLED PETROLEUM

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Abstract: Petroleum-contamination of both terrestrial and marine environments have persisted as a result of the increasing demand on liquid petroleum globally which has led to the need to clean up spilled petroleum using eco-friendly methods. Of all the petroleum-cleaning techniques explored, the use of petroleum-degrading microbes has received most attention. The microbial remediation of spilled petroleum has been proved to be cost-effective, eco-friendly and sustainable. However, these microbes have been found to thrive under certain environmental/nutritional conditions which influence their behaviour towards spilled petroleum. This study aims to identify the factors responsible for the change in behaviour of oil-degrading microbes which might help facilitate better petroleum spill management. Some of these factors include: the physical nature of the spilled petroleum; chemical nature of the spilled petroleum; availability of nutrients; water temperature; concentration of oxygen; soil region/soil particle size; competition from other micro-organisms. Petroleum-degrading microbes were also found to degrade specific hydrocarbon components in liquid petroleum due to the specific metabolic pathway utilized by individual microbes. This makes the use of a microbial consortium a more aggressive option for the microbial degradation of spilled petroleum than the use of microbial isolates. However, more research on the factors influencing theabundance and productivity of oil-degrading anaerobes may need to be carried out. Also, how oil-degrading microbes can be aided to break down asphalthenes should be investigated.

Keywords: *microbial remediation, biodegradation, oil-degrading microbes, spilled petroleum, oil spillclean-up.*

Introduction

Oil spill incidence and accidents

Every year, an average of about 35 million barrels of petroleum is transported across the seas around the world and this renders the marine ecosystem vulnerable to pollution (Anisuddin et al., 2005). Between 1996 and 1999, an average of 407 oil spills occurred annually in Alaska (Poland et al., 2003). A UNDP (2006) study reports that approximately 273 oil spills have occurred annually in Nigeria between 1976 and 2001. The National Oil Spill Detection and Response Agency (NOSDRA) in Nigeria estimated that the crude oil spilled into the Niger Delta between 1976 and 1996 was more than 2.4 million barrels (The Guardian, 2010), of which 1.82 million (77%) of the total spill was not recovered (Kadafa, 2012). The crude oil was spilled offshore, in swamps and on land (Uyigue and Agho, 2007). Some of the spills were caused by oil pipeline sabotage but the majority of oil spills were due to poor maintenance and monitoring by the oil companies (Aljazeera, 2012). In addition, Egwu (2012) also pointed out that some of the drilling facilities used by the oil companies are out-dated and the procedures employed for preventing oil spills are ineffective.

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The incidence of oil spill accidents is higher on land than on water due to the extremely lengthy (thousands of kilometres long) pipelines which transport crude oil into storage tanks, rail tanks, road tanks and other facilities utilised in the export of liquid petroleum both locally and internationally (Owens, 2000). However, oil spill events on land are confined and often readily containable, while the infrequent oil spill accidents on water are often massive and devastating (Freedman, 1989). This is probably as a result of the greater difficulty in controlling the oil dispersion and spread.

Oil spill incidents are not only caused by pipeline vandalisation and accidents such as collision or explosion of supertankers or oil well blowout. There have been cases of oil spills initiated by natural disasters. For example, in 2005, Hurricane Katrina accounted for the spilling of a total of about 134,300 barrels of crude oil held by Bass Enterprises, Shell, Chevron and Murphy Oil Company in the United States (Llanos, 2005). The deliberate spill of liquid petroleum hydrocarbon into the environment may also occur due to war. Two examples were seen in 1991 during the Gulf war and in 2006 during the Israel-Lebanon crisis, releasing a total of 2-4 million barrels (Khordagui and Al-Ajmi, 1993) and 210,000 barrels (BBC, 2006) of crude oil respectively into the environment. However, the largest and most damaging pollution events usually involve a spill of petroleum from a disabled tanker or drill platform at sea or to a lesser extent from a blowout or a broken pipeline on land (Freedman, 1989).

It is imperative to note that the increasing demand for liquid petroleum may likely not reduce the number of oil spill occurrences. Therefore, oil spill accidents are prone to happen considering the enormous pressure on oil companies/drilling firms to make the petroleum product readily available for global consumption. Whenever there is an oil spill, shorelines, marine waters, groundwater, soils (including farmlands), lakes, rivers and creeks, stand the risk of being severely polluted, and if not controlled within a short time frame, may lead to long-term ecological devastation. Several oil spill remediation techniques for the clean-up of polluted terrestrial and marine environments have been established. However, most of them have been proven to be cost-ineffective and environmentally-unfriendly and hence, unsustainable. As a result, a control measure that will be swift, efficient and sustainable is a necessity. Therefore, attention has been shifted to the use of micro-organisms in cleaning-up both land and water oil spills. Liquid petroleum is composed of numerous hydrocarbon types and it has been observed that individual oil-degrading microbes have special preference for certain hydrocarbon types. Oil-degrading microbes also thrive best under certain environmental/nutritional conditions. This review is aimed at understanding the behaviour of oil-degrading microbes so as to improve the efficacy of microbial remediation of spilled petroleum.

The rate of biodegradation of petroleum hydrocarbonsvariesdepending on the composition and chemical nature of thepetroleum constituents. Crude oil is a liquid petroleum having thousands of hydrocarbon components. Each component has a unique chemical behaviour which makes it eithereasily biodegradable, quite difficult to digest or not degradable at all (The American Academy of Microbiology, 2011).

The chemical composition and structure of Petroleum

Petroleum hydrocarbon molecules can be grouped into four broad categories:

(a) Saturates (branched, unbranched and cyclic alkanes)

(b) Aromatics – ringed hydrocarbon molecules such as monocyclic aromatic hydrocarbons (MAHs) and polycyclic aromatic hydrocarbons (PAHs)

(c) Resins (polar oil-surface structures dissolved in saturates and aromatics) (Speight, 1999)
(d) Asphalthenes (dark-brown amorphous solids colloidally dispersed in saturates and aromatics) (Balba et al., 1998; Speight, 1999; The American Academy of Microbiology, 2011).

In the structural arrangement of the four main hydrocarbon components of crude oil, saturates make up the outermost layer of the oil whilst asphalthenes constitute the innermost portion of the oil due to their heavier molecular weights.

PAH consist of about sixteen types that are priority pollutants (US EPA, 1993; Othman et al., 2011) but the following three have been the most studied: naphthalene, phenanthrene and pyrene. Alkene, another hydrocarbon component is mainly found in refined petroleum and not in crude petroleum because it is obtained by catalytic cracking (Matar, 1992). Light petroleum hydrocarbons such as gasoline, kerosene and diesel fuel are volatile and easily biodegradable (within days or weeks), so that they rarely persist in marine environments, although they may remain longer in terrestrial environments if buried in sediments, soils, groundwater, or marshes where oxygen levels are very low. However, heavy petroleum hydrocarbons are biodegraded slowly (The American Academy of Microbiology, 2011).Aromatics are more difficult to degrade than alkanes whilst asphalthenes may persist in the environment (The American Academy of Microbiology, 2011).

In general, lighter fractions of petroleum are more soluble in water than heavier ones; aromatics are much heavier than alkanes. Benzene, the lightest MAH has a solubility of 1780g/m^3 whilst naphthalene, the lightest PAH has a solubility of 31g/m^3 (Parker et al., 1971; Clark and MacLoed, 1977). Although alkanes are the most biodegradable petroleum hydrocarbons, those with 5-10 carbon atoms are toxic to most micro-organisms by disrupting their lipid membranes (Bartha, 1986). Furthermore, petroleum hydrocarbons with 20-40 carbon atoms are hydrophobic at room temperature, which probably explains their slow biodegradation (Bartha and Atlas, 1977). Some bacteria, remarkably, produce waxes after degrading crude oil (Ishige et al., 2003).

According to van Hamme et al. (2003),the susceptibility of crude oil components to microbial degradation are in the following order: alkanes >light aromatics (MAHs such as benzene) >cycloalkanes > heavy aromatics (PAHs such as Phenanthrene)> asphalthenes. Resins are easily degraded naturally because they are light polar molecules (Spiecker et al., 2003).

PAHs are made up of more than one benzene ring and those that are made up of two or three cyclic rings such as naphthalene (two-ringed), phenanthrene (three-ringed) and anthracene (three-ringed) with molecular weights of 128g/mol, 178g/mol and 178g/mol respectively, are referred to as low molecular weight or light PAHs (Flores and Mestahoward, 2001). PAHs made up of four rings and above such as pyrene (four-ringed), chrysenes (four-ringed), fluorenthene (five-ringed), benzo [a] pyrene (five-ringed) and coronenes (seven-ringed) with molecular weights of 202g/mol, 228g/mol, 202g/mol, 252g/mol and 300g/mol respectively, are referred to as high molecular weight or heavy PAHs (Niederer et al., 1995; Kanaly and Harayama, 2000; Flores and Mestahoward, 2001; Schulz et al., 2012; MOE, 2013).

PAHs are common petroleum contaminants in the environment considered to be potentially mutagenic and carcinogenic (Boonchan et al., 2000; Mao et al., 2012). BCF (2013) reported that heavy PAHs such as Benzo [a] pyrene damage the DNA of living organisms (i.e. they are genotoxic) and are implicated in human breast cancer. This accounts to a high number of studies on the biodegradation of PAHs in order to safeguard the environment and biodiversity from severe long-term ecological and medical damage by oil spills. However, the focus has been on the biodegradation of light PAHs whilst very little research has been carried out on the biodegradation of heavy PAHs which have been found to be of medical importance.

Asphalthenes are considered to be highly resistant to biodegradation due to their heavy and viscous nature (The American Academy of Microbiology, 2011). Asphalthenes are very complex chemical structures made up of sulphur (0.3 - 10.3%), nitrogen (0.6 - 3.3%), oxygen (0.3 - 4.8%) and trace amounts of metals such as iron, nickel and vanadium (Tavassoli et al., 2012). In addition, asphalthenes have the highest molecular weight of all hydrocarbon compounds in crude oil with values ranging from 600 to $3x10^5$ g/mol and from 1000 to $2x10^6$ g/mol (Speight and Moschopedis, 1981; Kawanaka et al., 1989; Flores and Mestahoward, 2001). This chemical complexity has rendered asphalthenes resistant to microbial attack and unfortunately few studies have been carried out to enhance the potential of biodegradation of asphalthenes.

Factors influencing the microbial remediation of petroleum hydrocarbon

Although it had been established that petroleum hydrocarbons were degraded by oil-eating micro-organisms since the 1940s (Zobell, 1946), the degree to which these processes occurred and the conditions that limited or enhanced biodegradation were not well understood until the 1960s (Le Petit and Barthelemy, 1968).

Oil-degrading microbes are able to break downcrude oil naturally (known as natural attenuation), however, this process is too slow to meet the demands of the environment due to the nutritional imbalance in the ecosystem (Le Petit and Barthelemy, 1968); hence, the need to optimise environmental/nutritional conditions in order to facilitate increasedmicrobial degradation of petroleum hydrocarbon.

Over the years, scientists have manipulated environmental indices in order to understand the factors that encourage microbial metabolism and hydrocarbon degradation, a knowledge which could help restore the environment to a pre-pollution state as early as possible. The main environmental/nutritional factors include the physical nature of the crude oil, the chemical nature of the crude oil, availability of nutrients, concentration of oxygen, water temperature, soil region and soil particle size, and competition from other micro-organisms (Boopathy, 2000; Fountoulakis et al., 2009; The American Academy of Microbiology, 2011).

The physical nature of the spilled petroleum

The physical nature of the spilled petroleum is a factor that affects both terrestrial and marine oil-spill bioremediation. A single large oil slick has a lesser surface area for oil-eating microbes to access compared to numerous small-sized oil slicks. As a result, the physical nature of the hydrocarbon pollutant can either slow down or hasten biodegradation. Also, heavy and viscous hydrocarbon compounds may prove to be recalcitrant as lighter hydrocarbons are quite easier for microbes to digest due to the higher rate of diffusion through the oil-water interface (The American Academy of Microbiology, 2011).

The chemical nature of the spilled petroleum

The chemical nature of the spilled petroleum is determined by the particular hydrocarbon components of the liquid petroleum. Not all hydrocarbon components are biodegradable; some are degradable; some are degraded quickly while others are degraded slowly or not at all. In terrestrial and marine environments, the unbranched alkanes can be degraded within weeks but the branched alkanes and the multiple-ringed aromatic hydrocarbons are resistant to microbial degradation. Asphalthenes, on the other hand, are considered to be the most recalcitrant, thus, could accumulate in the environment (The American Academy of Microbiology, 2011).

Availability of nutrients

Similar to the nutritional needs of other organisms, oil-eating microbes also require nutrients for optimal growth and development. These nutrients are available in the natural environment but occur in low quantities. When there is an oil spill, the petroleum hydrocarbon provides carbon nutrients for the oil-eating bacteria to utilize, but the rate of degradation depends on the availability of other nutrients. The two most limiting nutrients observed are nitrogen and phosphorus which are incorporated into the cellular biomass and stimulate hydrocarbon metabolism (Prince, 1997; McKew et al., 2007a; Calvo et al., 2009). Other nutrients include sulphur and potassium (Evans et al*.,* 2004). These nutrients are limiting factors for both land and water oil spill bioremediation.

Water temperature

The temperature of the surrounding water in which the oil is spilled determines the rate of hydrocarbon degradation whether it is fresh water or marine. Crude oil degradation is faster in warm water because the heat generated within the water body will further encourage the breakdown of the spilled petroleum through natural processes such as evaporation. This leaves the oil-degrading microbes with a smaller size of hydrocarbon pollutant to clean up (The American Academy of Microbiology, 2011). On the other hand, in cold environments such as the Arctic, oil degradation via natural processes is very slow and puts the microbes under more pressure to clean up the spilled petroleum.

The sub-zero temperature of water in this region causes the transport channels within the microbial cells to shut down or may even freeze the entire cytoplasm, thus, rendering most oleophilic microbes metabolically-inactive (Yang et al*.,* 2009). However, some oleophilic microbes are cold-tolerant but are faced with the problem of freeze-thaw seasonal cycle thereby fluctuating between winter and summer, which limits the bioavailability of the spilled petroleum (Yang et al*.,* 2009).

The concentration of oxygen

Oxygen is a gaseous requirement for most living organisms. Similarly, most oleophilic microbes are aerobes (such as *Pseudomonas* and *Proteus*) and a few others are anaerobes (such as *Geobacter*). As a result, environments with low oxygen concentration such as groundwater, deep soils and ocean sediments, have limited rates of hydrocarbon biodegradation compared to the open surfaces where oxygen is readily available for numerous oil-degrading aerobes to digest the pollutants. The presence of oxygen generally accelerates hydrocarbon metabolism (The American Academy of Microbiology, 2011).

The soil region and soil particle size

The soil region and soil particle size are crucial factors limiting the biodegradation of petroleum hydrocarbon in the terrestrial environment. The subsurface soil regions include the vadose zone (unsaturated zone) and the groundwater zone (saturated zone). The surface soil region has the highest microbial population due to the regular input of organic material from plants and animals at the surface whilst the groundwater sediment zone has the lowest microbial population due to the anoxic condition (insufficient oxygen) which increases with soil depth (Adriaens and Hickey, 1993). Many anaerobes and few aerobes have been found to survive in the saturated zone (Boopathy, 2000).

In addition, the size of the soil particle determines the rate of soil permeability, which indirectly affects the rate of petroleum biodegradation. Fine soil particles such as clay have small interstitial spaces which make the soil impermeable, thus, retaining the spilled petroleum at the surface and reducing the bioavailability of microbial nutrients and oxygen. Coarse soil particles such as sand have large interstitial spaces which heavily drain hydrocarbon pollutants through the soil to the unsaturated and saturated zones, oxygen-deficient regions where the rate of biodegradation is very slow.

Moderately drained soils are the optimum requirements for the rapid bioremediation of oil-polluted soils. Soil pH has also been pointed out by the US EPA (2006) to influence bioremediation as it inhibits microbial activity and affects nutrient availability. The generally acceptable soil pH range for optimum bioremediation is 6-8 (US EPA, 2006).

Competition from other micro-organisms

The natural microbial community is composed of oil-degrading microbes that either cooperate together or compete with one another. Competition influences the rate of bioremediation and it could be inter-specific (between members of different species) or intra-specific (between members of the same species). Oil-degrading fungi could compete with oil-eating bacteria not only for the petroleum hydrocarbon but also for the limited nutrients available. As mentioned earlier, some antagonistic oleophilic bacteria have also been found to release metabolites that inhibit the growth and development of other oleophilic bacteria. The interdependence of microbial populations is a requirement for the successful application of bioremediation, which makes competition a limiting factor (The American Academy of Microbiology, 2011).

The appropriate manipulation of the aforementioned environmental and nutritional factors affecting the rate of bioremediation which could be helpful in improving the success of crude oil bioremediation strategies.

Microbial remediation of petroleum hydrocarbons

Microbial remediationhas been argued to be an efficient and sustainable oil spill clean-up technique by many authors (Trindade et al., 2005; Okoh and Trejo-Hernandez, 2006; Tyagi et al., 2011). It is a technology that utilizes the metabolic potential of micro-organisms such as bacteria, fungi and few protozoans (Watanabe, 2001) in degrading liquid petroleum spilled on terrestrial and marine environments, into harmless compounds. These oil-degrading microbes are not living in isolation, hence, referred to as microbial consortia. Of all the known oleophilic micro-organisms, bacteria are the most predominant in the environment (Leahy and Colwell, 1990). These oil-degrading bacteria are sometimes, referred to in literature, as hydrocarbonoclastic bacteria (HCB)

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(McKew et al., 2007a). Bacteria's reproductive potential and ubiquity may have made them the most preferred agent of hydrocarbon degradation for bioremediation.

In an oil remediation experiment using oil-utilising bacteria and protozoa as agents of degradation, it was observed that the population of the two agents initially increased which was then followed by a considerable decline in the population of bacteria (Accola, 1994). Acea and Alexander (1988) suggested that the sudden decline in bacterial population may have been "chiefly attributed to predation by protozoa"; although Huang et al. (1981) pointed out that the protozoans may be playing a useful role in stabilising the ecosystem by limiting the possible over-proliferation of bacteria. However, the presence of protozoa tends to disallow the establishment of oleophilic microbial consortia which may explain why not much research has been carried out on protozoa as agents of bioremediation.

The number of micro-organisms capable of utilizing the carbon in crude oil as their sole source of energy is relatively few compared to the general number of microbes available in the environment (Lebkowska et al., 1995). This is the reason it is important to have a good understanding of the available oil-degrading micro-organisms found both on land and water. Oil-eating micro-organisms produce enzymes which break down hydrocarbon compounds through single or multiple metabolic pathways.

The enzymes produced are incapable of breaking down all forms of hydrocarbon compounds; as a result, most oleophilic microbes are hydrocarbon-specific, although a few are physiologically-versatile and can degrade a wide-range of hydrocarbons (see *Table 1*). For example, toluene, an aromatic hydrocarbon, can be broken down via multiple metabolic pathways by the enzyme, toluene 2-monoxygenase (Ramos et al., 2011), produced by a toluene-degrading microbe (such as *Rhodococcus* spp.). The metabolic pathways include five aerobic pathways and at least one anaerobic pathway (Pazos et al., 2004). This biochemical diversity and hydrocarbon-specificity displayed by oleophilic microbes, if expressed in synergy by microbial consortia, will enhance bioremediation (Roling et al., 2002).

***Table 1**.The habitat and hydrocarbon-specificity of40 oleophilic micro-organisms*

Oleophilic micro-organisms	**Bacteria/ Fungi**	**Habitat**	**Hydrocarbon-specificity**
Pseudomonas spp. (Kanaly and Harayama, 2000)	Bacterium	Soil, river and marine	Benzene, toluene, ethylbenzene, xylene, naphthalene, phenanthrene, kerosene and diesel (Watanabe, 2001; Joshi and Pandey, 2011)
Cycloclasticus spp.	Bacterium	Marine	PAH (not specific) (Watanabe, 2001)
Alcanivorax spp. (Watanabe, 2001)	Bacterium	Soil, river and marine	Alkanes (Yakimov et al., 1998; Harayama et al., 1999; Chang et al., 2000)

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Geobacter spp.	Bacterium	Groundwater, deep soil and ocean sediments	Anaerobic breakdown of benzene (Watanabe, 2001)
Syntrophus spp.	Bacterium	Groundwater, deep soil and ocean sediments	Anaerobic breakdown of hexadecane (Zengler et al., 1999)
Burkholderias spp.	Bacterium	Soil	PAH (not specific) (Watanabe, 2001) and xylene (Bacosa et al., 2012)
Sphingomonas spp.	Bacterium	Soil	Pyrene (Ho et al., 2000)
Mycobacterium spp.	Bacterium	Soil	Benzo [a] pyrene and pyrene (Watanabe, 2001)
Rhodococcus spp. (de Carvalho et al., 2005)	Bacterium	Soil, river and marine	Benzene, toluene, ethylbenzene, xylene, anthracene and Benzo [a] pyrene (Farhadian et al., 2008)
Ralstonia spp. (de Carvalho et al., 2005)	Bacterium	Soil, river and marine	Benzene, toluene, ethylbenzene and xylene (Farhadian et al., 2008)
Haemophilus spp.	Bacterium	Soil, river and marine	Phenanthrene and pyrene (McKew et al., 2007a)
Thalassolituus oleivorans	Bacterium	Marine	Phenanthrene and pyrene (McKew et al., 2007a)
Proteus spp.	Bacterium	Soil	Xylene and diesel (Joshi and Pandey, 2011)
Bacillus spp.	Bacterium	Soil	Toluene and diesel (Joshi and Pandey, 2011)
Mesorhizobium spp.	Bacterium	Soil	Most PAH (not specific) (Mao et al., 2012)
Alcaligenes spp.	Bacterium	Soil	Most PAH (not specific) (Mao et al., 2012)
Nocardia nova	Bacterium	Soil	A wide spectrum of petroleum hydrocarbon (Trindade,2002)

Rhodotorula glutinis var. dairenesis	Fungus	Soil	A wide spectrum of petroleum hydrocarbon (Trindade,2002)
Planococcus spp.	Bacterium	Soil	Light Arabian oil (Engelhardt et al., 2001; Evans et al., 2004)
Stenotrophomonas spp.	Bacterium	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Ochrobactrum spp.	Bacterium	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Pandorea spp.	Bacterium	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Labrys spp.	Bacterium	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Fusarium spp.	Fungus	Soil	PAH (not specific) (Vinas et al., 2002; Vinas et al., 2005)
Fundibacter spp.	Bacterium	Soil, river and marine	Alkanes (not specific) (Bruns and Berthe-Corti, 1999)
Pseudomonas paucimobilis	Bacterium	Soil, river and marine	Fluoranthene (Mueller et al., 1990)
Micrococcus spp.	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Corynebacterium spp.	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Pediococcus spp.	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Sphingobacterium spp	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Tsukamurella spp.	Bacterium	Soil/sludge	Low molecular weight PAH (Othman et al., 2011)
Phanerochaete spp.	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Chrysosporium spp.	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Cuuninghamella spp.	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Alternaria alternate	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Penicillium chrysogenum	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Aspergillus niger	Fungus	Soil	PAH (not specific) (Li et al., 2009)
Zoogloea spp.	Bacterium	Soil	PAH (not specific) (Li et al., 2009)

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Flavobacterium spp.	Bacterium	Soil	PAH (not specific) (Li et al., 2009)
Cupriavidus spp.	Bacterium	Soil; marine	Decane (Bacosa et al., 2012)

Besides the catabolic potential of oil-degrading microbes, other strategies have been adopted by these microbes to enhance the detoxification of pollutants, such as the modification of their cell membrane to preserve their structural integrity when in contact with pollutants (de Carvalho et al., 2005); the production of surface active compounds known as biosurfactants which helps to partially weather the pollutant, thereby, rendering it vulnerable to microbial degradation (Ron and Rosenberg, 2002) and the release of toxic pollutants from within the microbe's protoplasm via efflux pumps, active transporters located in the cytoplasmic membrane (van Hamme et al., 2003).

Competition amongst oil-degrading micro-organisms has also been observed. Some oleophilic microbial consortia develop synergistic relationships whilst few others show competitive relationships. For example, *Cycloclasticus* and *Alcanivorax borkumensis* show a synergistic association because *A. borkumensis* produces an extracellular lipid biosurfactant which render PAHs readily available for digestion by *Cycloclasticus* and the competition for hydrocarbon resources is absent because *A. borkumensis* feeds on another hydrocarbon (alkanes) (Yakimov et al., 1998; McKew et al., 2007a). However, *Thalassolituus* exhibits an antagonistic relationship with other oil-eating bacteria by producing a metabolite which inhibits their growth and development (Yakimov et al., 2005).

It has been well established that the presence of microbial consortia rather than individual microbes will enhance bioremediation; therefore, the antagonistic potential of some oil-degrading microbes could limit the efficiency of microbial remediation. The activities of these antagonistic oil-degrading bacteria have to be controlled in order to encourage the continued development of viable oil-degrading microbial consortia.

Conclusion

The behaviour of oil-degrading micro-organisms towards spilled petroleum has beeninvestigated. Oil-degrading microbes modify their cell membranes, produce biosurfactants and eject toxic pollutants from their protoplasm in order to survive petroleum-contaminated areas. The hydrocarbon-specificity displayed by oil-degrading micro-organisms is as a result of the metabolic pathway individually utilized. Microbes capable of utilizing multiple biochemical pathways find it easy to degrade a wider range of hydrocarbon compounds.Of all the four basic components of petroleum, asphalthene is the most recalcitrant to microbial degradation due to its chemical complexity. Also, it appears that there are more oil-degrading aerobes than anaerobes, and most of these aerobes are terrestrial in habit. The study of the behaviour and factors influencing the microbial metabolism of hydrocarbon compounds will ultimately aid the understanding of how best these factors and elements can be manipulated to achieve an accelerated microbial degradation of spilled petroleum world-wide.

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A REVIEW OF THE INVASIVE, BIOLOGICAL AND BENEFICIAL CHARACTERISTICS OF AQUATIC SPECIES EICHHORNIA CRASSIPES AND SALVINIA MOLESTA

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Abstract. The aquatic invasive plant species *Eichhornia crassipes* and *Salvinia molesta* originated in the Neotropics (Bolenz et al., 1990; Tipping and Center, 2005). Based on environmental, economic and human problems, *E. crassipes* and *S. molesta* are ranked first and second places respectively on a list of the world's most problematic aquatic weeds (Holm et al., 1977; Barrett, 1989). This review paper presents the impacts of these two species on the environment, mainly in aquatic ecosystems of the tropics and subtropics, and also their use in societies and agro-industry with particular reference to their distribution and environmental impacts in ecosystems.

Keywords: *Eichhornia crassipes, Salvinia molesta, biological control, chemical control, mechanical control.*

Introduction

This paper is reviewing the positive and negative impacts of *Eichhornia crasspes* and *Salvinia molesta*, two of the world's worst aquatic tropical weeds to strengthen and improve the knowledge and use of these species in the areas they invaded. This review provides an overview of agricultural and ecological uses of the two species based on the findings made during the last decades.

The worst tropical aquatic weeds are mainly native to a vast biogeographic unit comprising South and Central America, the Caribbean and southern Mexico, amongst them *Eichhornia crassipes* and *Salvinia molesta* (Barreto et al., 2000). Due to their aggressive invasive characteristics much research has been focused on identifying biological control agents in their native area (Holm et al., 1977; Barreto et al., 2000).

Even though, both species have induced several negative impacts on the environment (Room, 1988), their positive impacts have been also evaluated (Vandecasteele et al., 2005). *E. crassipes* is the neotropical aquatic weed that has received the greatest attention from plant pathologists (Barreto et al., 2000), and is amongst seven aquatic weeds (i.e., *Eichhornia polystachya*, *E. crassipes*, *E. azurea*, *Paspalum repens*, *P. stratiotes*, *P. spectabile* and *Typha domingensis*) for which a survey of mycobiota has been made in Brazil.

The review of Villamagna and Murphy (2009) gives details of the ecological and socio-economic negatives changes resulting from invasion by *E. crassipes*. This review gives interesting details on impacts on water quality, the community composition of zooplankton, macroinverterbrates, fish and birds, and socio-economic conditions.

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Henry-Silva and Camargo (2006) evaluated the efficiency of *E. crassipes* and *S. molesta* to threaten effluents from fishing farming from Nile tilapia culture ponds.

Moreover, both species have positive impacts. E. *crassipes* can also be used for phytoremediation and biogas (Singhal and Rai, 2003; Zimmels et al., 2006). Oliver (1993) and more recently McFarland et al. (2004) reviewed many aspects of the ecological and socio-economic effects of invasion by *S. molesta*. Even though *S. molesta* is not widely used for phytoremediation and biogas compared to *E. crassipes*, and regardless of their invasive characteristics, *E. crassipes* and *S. molesta* may have beneficial agricultural and ecological uses (Oliver, 1993; McFarland et al., 2004).

Vandecasteele et al. (2005) argued that aquatic plant based treatment systems are low cost technologies which may be adopted by developing countries for recycling and treating wastewater mainly contaminated by heavy toxic metals. *E. crassipes* was more efficient in total phosphorus removal (82%) and total nitrogen removal (46.1%) than *S. molesta* which removed only 72.1% of total phosphorus and 42.7% of total nitrogen, while the control only removed 50.3% (total phosphorus) and total nitrogen (22.8%). The authors concluded that the water of treated effluents may be reused in aquaculture activity. The authors also concluded that considering the potential for using the biomass of aquatic macrophytes as plant compost, biogas production and animal feed, *E. crassipes* should be preferred as treatment for aquaculture effluents. From a synthesis of 192 lakes of tropical and subtropical aquatic systems, Huszar et al. (2006) revealed P as a better predictor of phytoplankton biomass (Huszar et al., 2006).

Biomass size and the concentration of nutrients in plant tissues both influenced the potential absorption of nutrients by aquatic macrophytes. *S. molesta* is less efficient in the removal of nutrients than the larger *E. crassipes* because of its lower biomass. *S. molesta* biomass reached the maximum of its carrying capacity in the first month of experiment while *E. crassipes* increased biomass for a number of months showing its greater ability of absorption. Thus, after macrophyte treatment it is possible to reuse the water recovered from effluent from fish farms and to release it into aquatic ecosystems thus avoiding eutrophication (Henry-Silva and Camargo, 2006).

Eichhornia crassipes

Origin, morphology and dispersal

E. crassipes (Martius) Solms (*Pongtederiaceae*), commonly called water hyacinth, is a floating plant native to the Neotropics originating in the Amazon basin in Brazil (Bolenz et al., 1990; *Table 1*). Center et al. (1987) described *E. crassipes* as a plant having an individual rosette with an erect, free floating, stoloniferous and perennial herb bearing a whorl of 6-10 sequentially produced succulent leaves on short vertical stem. The species has become a widespread pantropical weed and is recognized as the world's worst aquatic weed (Holm et al., 1977). *E. crassipes* spread from its native South America and occurs in lakes, slow moving rivers and swamps of the world between 40°N and 40°S (Center, 1994).

The species gained attention as an ornamental plant because of its attractive purple flower and more than a century ago, *E. crassipes* was first distributed by gardeners and horticulturists and introduced from South America into North America during the late 19th century (Center et al., 2005). Thereafter spread to different tropical and subtropical areas of the world. According to Twongo and Balirwa (1995) *E. crassipes* was introduced in Africa to the River Nile in the 1870s and it is believed to have been

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present in Lake Victoria since the early 1980s. It has become widespread across the lake since this date and 80% of the Ugandan coast line of Lake Victoria was covered in the 1995. In West Africa, it was first observed in the late 1970s and became a major problem in the late 1980s (De Groote et al., 2003). The species is widespread present in Mali, Burkina Faso, Niger, Côte d'Ivoire, Togo, Benin and Nigeria, and has negatively affected the highly productive coastal creek and lagoon systems, from which many people derived their livelihoods (Julien et al., 1999). According to Mbati and Neuenschwander (2005), *E. crassipes* invaded the Congo basin much earlier than western Africa in the 1950s. There are even reports that *E. crassipes* was deliberately introduced to the upper reaches of the Likouala-aux-Herbes River, Congo-Brazzaville to prevent access by boats from warring villages approaching other villages.

Reproduction

E. crassipes produces huge numbers of seeds (Barrett, 1980) and its expansion throughout the tropics has been favoured by its extremely fast and uncontrolled plant growth and the robustness of its seeds (Aweke, 1993; Malik, 2007). Its average annual production is of 50 dry (ash-free) tons per hectare per year, which qualifies the species as one of the most productive plants in the world (Abbasi and Ramasamy, 1999) and it has become the major floating waterweed of tropical and subtropical regions of the world (Aweke, 1993; Gunnarson and Petersen, 2007; Malik, 2007). The species reproduces sexually by seeds and asexually by budding and stolen production; for rapid expansion asexual reproduction is more important (Watson and Cook, 1987; Verma et al., 2003). The optimum pH for its growth is 6-8 and the species persists over a wide range of temperature 1 to 40°C with optimum growth at 25-27.5°C. Its growth rate increases with the increase of water nitrogen contents, but salinity levels of 6-8% are lethal to the species (Malik, 2007).

E. crassipes causes several environmental and socio-economic problems (*Table 1*): (i) its dense floating mass hinder fishing and transport; (ii) it interferes with the use of water for drinking purposes, irrigation and electric power generation; and (iii) its negative effects on biodiversity. This has caused a reduction of fish biomass, obstruction of shipping routes, losses of water in irrigation systems due to higher evaporation and increased sedimentation by trapping silt particles. Invasion of water hyacinth has had great environmental and socio-economic impacts in Lake Victoria (Williams et al., 2005; Twongo et al., 2005; Katerrega and Sterner, 2007) and many other tropical lakes (Mironga, 2004). Keterrega and Sterner (2007) reliably estimated the extent of water hyacinth coverage for the period 1990-2001 and showed the greatest coverage of Lake Victoria by *E. crassipes* occurred after 1995 and its reduction after 1997.

Nutrient contents

E. crassipes can be *rich* in nitrogen (up to 3.2% of dry matter) and has a C/N ratio of around 15 (Gunnarson and Petersen, 2007). *E. crassipes* is a rich source of mineral contents and can serve as suitable economic feed (Lata and Veenapani, 2010). The authors found that dry matter of *E. crassipes* consisted of 5.2% N, 0.22% P, 2.3% K, 0.36% Ca, 280 ppm of Fe, 45 ppm of Zn, 2 ppm of Cu and 332 ppm of Mn.

Positive use

Besides its fast-growing invasive characteristics, *E. crassipes* may also have beneficial purposes (*Table 1*). Ebel et al. (2007) determined the sodium cyanide phytotoxicity and removal capacity of *E. crassipes*: The authors found that this species can remove cyanide from water because of its high biomass production, wide distribution and tolerance to cyanide and metals. In fact, 2 g of plant material needed about 20 h to degrade 50% of the cyanide applied and > 50 h to eliminate the cyanide completely at 2 mg l^{-1}. Ebel et al. (2007) found that *E. crassipes* may be useful in treating effluents from small-scale gold mines. Similarly, its enormous biomass production rate, its high tolerance to pollution and its heavy-metal and nutrient absorption capacities permit its use in wastewater treatment ponds in mining areas of its native continent, South America (Ebel et al., 2007). The experiments conducted by Caldelas et al. (2009) of the combined exposure to excess nutrients and Hg, have confirmed the ability of the species in the heavy metal phytoremediation of eutrophic water.

E. crassipes may be used in composting or vermicomposting. Gajalakshmi et al. (2002) demonstrated the feasibility of high-rate composting or worm-composting systems. Gunnarson and Petersen (2007) identified various beneficial effects of *E. crassipes* in composting since the species is rich in nitrogen (up to 3.2% of dry matter). *E. crassipes* can be used as a substrate for compost or biogas production and the by-product of this can be used as fertilizer, since the sludge from the biogas contains almost all of nutrients of the substrate. The use of water hyacinth compost on different crops has resulted in improved yields. Its abundant growth and high concentrations of nutrients give *E. crassipes* great potential as a fertilizer, which is particularly pertinent for the nutrient deficient soils of the tropics. In addition, its high protein content makes it possible to be used as fodder for livestock. In fact, Dada (2002) argued that the use of sundried *E crassipes* for raising goats at levels up to 40% of diet is beneficial. The authors found *E. crassipes* (leaves and stalk) contained 80% of dry matter of which 10.8 % of crude protein, 2.3 % of ether extract, 14.06% of ash and 18% of crude fibre.

The *species* has a valuable role in the treatment of wastewater (Brix, 1997). Zimmels et al. (2006) showed the effectiveness of sewage purification by *E. crassipes* in the laboratory. It reduced indicators of sewage such as the biochemical oxygen demand, the chemical oxygen demand, the total suspended solids and turbidity to low levels which permitted the use of purified water for irrigation of tree crops. Their results have shown that the use of this free water surface flow system and its low maintenance system for treatment of urban and agriculture sewage is a viable option. Kutty et al. (2009) argued that *E. crassipes* is capable of removing up 49% of chemical oxygen demand, 81% of ammonia, 67% of phosphorus and 92% of nitrate from the municipal wastewater treatment effluent.

E. crassipes may be also be used in agro-industry and industry (Verma et al., 2007). Xia and Ma (2006) investigated the potential of water hyacinth to remove a phosphorus pesticide ethion from water. Accumulated ethion in live water hyacinth plant was lower in shoots and roots, suggesting that plant uptake and phytodegradation might be the dominant process for ethion degradation of agro-industrial wastewater polluted with ethion.

Negative impacts

The growth of floating macrophytes is a well-known undesirable consequence of eutrophication (Brendonck et al., 2003). Shallow lakes can be very clear with abundant submerged plants or very turbid because of the high concentration of phytoplankton and suspended particles (Scheffer et al., 2003). Cooke et al. (1993) have shown that in small aquatic ecosystems, the main control measure used mainly in tropical areas is the mechanical removal of free-floating macrophytes. However, macrophyte control often induces unwanted side-effects; the removal of one nuisance species, either native or exotic, may enhance the successful colonisation by another more aggressive species as was observed in aquatic zones by Cooke et al. (1993).

Control

Until recently, attempts to control or destroy *E. crassipes* by chemical, biological, mechanical or hybrid methods had had limited success (Abbasi *et* al., 1997), in part because it is one of the most productive and hardly of all weeds (Abbasi and Ramasamy, 1999; Malik, 2007). However, it is very likely that almost all alien invasive plant species will have natural enemies or predators in their original ecosystem (e.g. Lee, 1979). Malik (2007) identified the agents and limitations of three main method of control of water hyacinth: (i) biological control by insects such as *Neochetina eichhorniae*, *N. bruchi* etc…; allopathic plants, lantana, fungal pathogens (*Alternaria eichhorniae*): these may not cause a sufficient reduction in the area affected by *E. crassipes* and could allow a possible resurgence in growth by *E. crassipes* (Julien and Griffiths, 1998; Saxena, 2000; Kauraw and Bhan, 1994; Babu et al., 2004); (ii) various chemical products (including 2,4 dichlorophenoxyacetic acid and complexed copper, 2,4 D Amine spray or Endothall dipotassium salts): these have limitations such as cost; are not suitable for controlling large infestations and will affect the environment (Westerdahl and Getsinger, 1988; Olaleye and Akinyemiju, 1996); and (iii) physical control such as manual and mechanical removal which have physical limitations and are labour intensive which could involve health risks (Smith et al., 1984) or might be expensive and energy intensive (Harley et al., 1997). These three main methods are considered in more detail below.

Efforts have been made to collect arthropods for the biological control of *E. crassipes* (Harley, 1990), although it has proved difficult to assess the effects of these species on the plant. Ten species were selected for biological control of *E. crassipes* (Charudattan, 1996) of which six were considered to be of potential practical use. One species believed to have outstanding potential for mycoherbicide development is alternative *eichhorniae Nag Raj* and *Ponnappa*, a species widely spread in Asia and Oceania, but not known from the Neotropics (Charudattan, 1996).

Julien and Griffths (1998) selected 7 arthropods from the Neotropics which attack *E. crassipes* in its native region and released them in 33 countries to assess the effectiveness of each for biocontrol purposes. Among these, two weevils *Neochetina eichhorniae* Warner and *N. bruchi* Hustache (*Coleoptera, Curculionidae*), appeared to cause a substantial reduction of the water hyacinth in countries where they have been, including African countries such as Sudan, Benin, Côte d'Ivoire, Ghana, Nigeria, Burkina Faso, the countries around Lake Victoria and in Southern Africa (De Groote et al., 2003).

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Between 1991 and 1993, three natural enemies (two weevil species and one moth) that feed exclusively on water hyacinth, were released in southern Benin. Six years later, i.e., in 1999, the survey showed that *E. crassipes*, although not eliminated, had been reduced from a serious pest to one of minor or moderate importance (De Groote et al., 2003). The authors reported that at the peak of the investigation *E. crassipes* had reduced national annual income by approximately US$84 million, and biological control using these species increased income by US$30 million per year and the total cost of the control program was of US$ 2.09 million.

The South American *Neochetina spp.* weevil has a positive impact on water hyacinth. They have been used successfully throughout the world in the countries such as the USA, Australia, India (Julien et al., 1999). Williams et al. (2005) reviewed the effects of *Neochetina spp* on reduction of *E. crassipes* in Lake Victoria and argued that the wet and cloudy weather of 1997/1998 played a major part by accelerating the decline of *E. crassipes* through reduced light on its growth throughout the lake basin. Williams *et al.* (2005) argued that the improvement of light climate since 1997/1998 and the availability of nutrients may induce the resurgence and proliferation of *E. crassipes* in the Lake Victoria. However, the weed remains sparse on the lake.

Biological control of *E. crassipes* has been conducted in many part of the world. Center et al. (1999) showed that sustained herbivory of *E. crassipes* reduced proportionately biomass and floral structures. Normally *E. crassipes* will out-compete other floating aquatic plants; Center et al. (2005) measured *E. crassipes* biomass production and compared to *Pistia stratiotes* and found that *E. crassipes* biomass yield was 41 times that of *Pistia stratiotes*. However when controlled the species with weevils, Center et al. (2005) argued that herbivory directly and indirectly affected plant performance by altering competition between the two invasive plant species. The competitive response depended upon the herbivore species and availability of nutrients. In addition, Center and Dray (2010) argued that improved nutritional quality of the host plant could lead to more effective biological.

Julien et al. (1999) argued that the use of chemical control might be effective but it had negative side effects on the environment. Cadmium (Cd), a widespread non essential toxic heavy metal, emitted in the environment in different ways (power station, heating systems, metal-working industries etc…), di Toppi et al. (2007) was an effective chemical control of *E. crassipes.* The authors found that *E. crassipes* accumulated Cd in leaves. Many countries such as Thailand, South Africa and Kenya have used mechanical control of *E. crassipes* either by hand or machine, although it is considered expensive and not very effective (Cilliers, 1991).

Salvinia molesta

Origin, morphology and dispersal

Salvinia molesta D.S. Mitchell (Salviniaceae) is a sterile rhizomatous plant, very suitable for demographic studies because of its free-floating habit which enables non destructive access to 'rhizomes' and 'roots' (Room, 1988). *S. molesta* also called giant salvinia, is a problematic aquatic weed indigenous to south-eastern Brazil and occurs between latitudes 24 and 32 degrees south (Forno and Harley, 1979, *Table 1*). This species, which is a free-floating fern, quickly covers and dominates stagnant and slow flowing water systems and can choke waterways. The species floats on still and slow-moving water and can grew rapidly to cover the entire water surface with a thick mat of vegetation. The vegetation shades out any submerged plant life and impedes oxygen exchange making unsuitable for animals lives and reducing the natural beauty and biodiversity of wetlands. The species is now widespread across the world, in Africa, Australia, Asia and the southern part of North America (Jacano, 1999; McFarland et al., 2004; Owens et al., 2005; Tipping and Center, 2005).

Reproduction

S. molesta spreads at rapid rate by vegetative reproduction. *S. molesta* floats with wind or water currents to uninfected waters. It can grow and propagate vegetatively. It reproduces solely from production and loss of ramets on branched rhizomes. The ramets can survive independently but they are held together in colonies until rhizomes break (Room, 1988). When the species is introduced to new habitats, it produces colonizing stage plants which have thin stems and fragment easily, to produce further new plants. The species is a highly aggressive and competitive species. Kammathy (1968) argued that the species successfully competes with and even replaces water hyacinth and water lettuce. According to Owens et al. (2004), temperature is probably the greatest factor limiting *S. molesta* growth, survival and spread. In fact, the species appears to be unable to survive in locations where ice forms for extended periods (Owens et al., 2004). Owens et al. (2005) also found that *S. molesta* biomass was produced over 2-fold increase at the lower pH.

In the northern part of the Republic of the Congo, Mbati and Neuenschwander (2005) also observed that *S. molesta* was more aggressive than *E. crassipes*. *S. molesta* invaded the Congo basin 10 years later than *E. crassipes* in the 1960s. *S. molesta* was found in the main river in unshaded waters of the country, not apparently found in shaded forested areas. Despite this, it does not form large monospecific mats, indeed it was rarely seen in groups of more than 2-3 'individuals'. It is possible that it was also able to grow in the small affluent, but here it was not greatly evident because of the abundance of *E. crassipes*. Again, the ecological characteristics of the waters of the swamps may prevent this species becoming a widespread pest.

Nutrient contents

According to Leterme et al. (2010), dry matter of *S. molesta* contained 132g kg^{-1} of crude protein, 130g kg^{-1} of ash, 42 g kg^{-1} of ether extract and 135g kg^{-1} of lignin.

Positive use

Compared to *E. crassipes,* which is more often used for phytoremediation (Malik et al., 2007), *S. molesta* presents less of an environmental challenge (*Table 1*). *S. molesta* has been used as a compost and mulch and as supplement to folder for livestock in some Asian countries (Oliver, 1993). The species could be used as feed ingredients for sows (Leterme et al., 2010). However the authors argued that *S. molesta* does not seem to present any additional advantage from existing feed ingredients because of its low crude protein (132g kg^{-1}) compared to *Azolla filiculoides* (>220 kg^{-1}), which is floating ferns with symbiotic N_2-fixing cyanobacteria that are used in Asia to fertilise rice fields.

Chantiratikul et al. (2009) found that *S. molesta* had higher contents of total phenolic and antioxidant than *E. crassipes* with naringinin as the main phenolic compounds while myricetin, vanillin, kaempferol and quercetin were present in low concentrations.

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Therefore, the authors argued that *S. molesta* can be utilized as a source of aquatic weeds antioxidant with a potential use in feed animals.

Some studies have also reported *S. molesta* suitability in papermaking and generation of biogas (Thomas and Room, 1986) and in treating sewage effluent (Finlayson et al., 1982). However McFarland et al. (2004) reported that none of these efforts has led to large scale utilization, probably due to high costs associated with labour and machinery.

Negative impacts

S. molesta combines a high growth rate with a slow rate of decomposition to reduce nutrients available for absorption by other plants (*Table 1*). Thus *S. molesta* potentially alters the natural nutrient dynamics of water bodies which it colonizes (Shrama and Goel, 1986). *S. molesta* is an important host of *Mansonia* mosquitoes, which serve as one of the main vectors of rural elephantiasis (Pandro and Soerjani, 1978). Other diseases including encephalitis, malaria and dengue fever are caused by mosquitoes sheltering in *S. molesta* (Creagh, 1992). *S. molesta* can also have other detrimental effects on the ecology of aquatic systems by restricting light penetration and exchange of gases between the water and atmosphere (McFarden et al., 2004).

Control

There is a possibility of biological control using the species *Cyrtobagous salvinae*, the insect attacks buds and young ramets of the plant. Julien and Griffiths (1998) have showed that *Cyrtobagous salviniae* successful controlled *S. molesta* in 13 countries. The stochastic simulation model accurately mimicked branching growth of plants in the field and was predicted to achieve biological control of infestations of S. *molesta* with a population density of 300 adults and 900 larvae of *Cyrtobagous salviniaceae* per square metre. Two fungal species *Phoma glomerata* and *Nigrospora sphaerica* were tested at Bangalore, India (Sreerama Kumar et al., 2005). The species were found to be the cause of the sudden decline in *S. molesta* in Bangalore.

Conclusions

In this review on *E. crassipes* and *S. molesta*, we have shown that both species have serious environmental and socio-economic impacts on equatic ecosystems of the tropics and subtropics (Center, 1987; Room, 1988; Malik, 2007; Villamagna and Murphy, 2009). However the two species and mainly *E. crassipes* may have a beneficial use in phytoremediation, animal feed and biogas activities (Brix, 1997; Ebel et al., 1997; Zimmels et al., 2006; Xia and Ma, 2006). Improved and large scale utilization of the species could serve as a positive approach to control *E. crassipes*, especially in the developing countries.

Control mechanisms have had an important impact in controlling the spread of *E. crassipes*, but have been little studied in Africa, especially in the central part. We would agree with Malik (2007), which from the review literature concluded that a holistic approach of the combination of watershed management controlling nutrient supply and integrated biological and herbicidal control seems to offer a viable control option. The ecology of these two species in central Africa remains poorly understood and ecological studies would prove of benefit to fisheries and other industries, particularly as large scale hydroelectric schemes are planned for the Congo basin.

Table 1. *Impacts of E. crassipes and S. molesta on environmemt and human health*

Species	***E. Crassipes***	***S. molesta***
Origin	Amazon (Brazil)	South-eastern Brazil
Reproduction	Sexually/Asexually	Asexually
Negative effects on human health	+	+++
Negative effects on environment	+++++	++++
Beneficial effects	++	+
Sewage	+++	--
Phytoremediation and Biogas	+++	++
Compost	++	+
Animal feed	++	+

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TREE RINGS FOR THE ASSESSMENT OF THE POTENTIAL IMPACT OF CLIMATE CHANGE ON FOREST GROWTH

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Abstract.The Earth's climate is changing rapidly and while human influence on the same is an inconvenient truth, it is important to assess the likely impacts of climate change on vulnerable ecosystems across the globe. Forests contain the world's largest terrestrial carbon pool and constitute a major sink that reduces the build-up of atmospheric carbon dioxide. Climate change is likely to result in substantial changes in the structure and function of forests. The effects of climate variability on forests can be best estimated from tree rings which are highly sensitive to changes in environmental conditions. However, there is conflicting evidence as regards the response of forests to climate change. Will climate change accelerate tree growth and mitigate CO2 release from fossil fuels and land-use change? Or would the outbreak of forest fires, insect attacks and storms negate any increase in forest carbon storage from faster growth, the 'divergence problem', as propounded by some researchers? Climatic variations in recent decades and in the years to come may pose grave threats to the world's forests and their role as potential carbon sinks, hence concerted research is needed in the future to validate the claims of forest growth dynamics in relation to climate change.

Keywords: *climate, forests, tree rings, growth, carbon*

Introduction

The earth's climate is a complicated, multi-component system which has experienced many changes and variations throughout its history. The 20th Century was a pivotal period as it marked the onset of a rapid climatic warming brought on by major anthropogenic changes in global atmospheric composition. Climate change has been one of the most engaging environmental subjects of debate in recent times. Intergovernmental Panel on Climate Change (IPCC, 2007) states that "Climate change is a change in the state of the climate that can be identified by changes in the mean and / or the variability of its properties and that persists for an extended period typically decades or longer". Nigeria's First National Communication on climate change under United Nations Framework Convention on Climate Change (UNFCCC) defines climate change as a change of climate which is attributed directly or indirectly to human activities that alter the composition of the global atmosphere and which are in addition to natural climate variability, observed over comparable time period.

The fourth assessment report of the IPCC specifies the linear trend of global mean surface temperatures over the last century – from 1906 to 2005 – with 0.74°C ±0.18°C. Climate warming is by now unequivocal, as it is evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea level (IPCC, 2007).

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Forests are particularly sensitive to climate change, because the long life-span of trees does not allow for rapid adaptation to environmental changes. Unlike in agriculture, adaptation measures for forestry need to be planned well in advance of expected changes in growing conditions because the forests regenerated today will have to cope with the future climate conditions of at least several decades, often even more than 100 years. Thus it is important to understand the response of unmanaged natural forests to changing climate because it is possible to adapt forest management practices to a changing environment (Lopatin et al., 2008).

Dendrochronology (the study of tree rings) provides important information for reconstructing past climate and assessing the impacts of climate change and disturbance events on tree growth (Abrams and Orwig, 1995; Mann et al., 1999; Abrams, 2003). Tree rings, when viewed as time series of annual increments, present a valuable, long-term record of tree growth across many forest environments. Tree species are unique in their response to environmental conditions and can be used as a fingerprint of recent climate change (Root et al., 2003) and a natural archive of past climate (Fritts, 1976).

The impact of climate change on the world's forests is a debatable issue. There is uncertainty as to whether the rising temperatures and the changing precipitation patterns may enhance or decline the growth and productivity of forests. Different workers have different inferences concerning this question. Large-scale greening in some northern regions observed in satellite data since the early 1980s (Myneni et al., 1997; Nemani et al., 2003; Brown et al., 2004) is thought to be due to enhanced warming; however in some areas it could be replaced by decreased growth, browning (Bunn and Goetz, 2006) and weakened sensitivity of temperature response in trees (D'Arrigo et al., 2004a). Evidence of improving vegetation productivity in the coastal Arctic tundra initiated by climatic warming during the late 20th to early 21st Centuries are cumulating in analyses of satellite data and in situ field measurements. In contrast, areas of declining vegetation productivity were documented in the southern adjacent boreal ecosystems across a belt stretching from Interior Alaska to northwestern Quebec (Girardin, 2014). Herein we provide an overview of key studies on the impact of climatic changes on the growth of forests as estimated through tree ring analysis.

Tree rings as natural archives of climate

Climatic changes have been recorded by different natural systems such as pollen records, ice cores, corals, caves, varves and tree-rings which can be utilized as archival records of past climates (Bradley, 1992). The use of tree-ring series has also recently spread to climate model validation in the context of global warming assessment. The most important premises for applying the tree-ring studies to forest dynamics are to find out the existence of critically good and bad years for tree growth, to define them objectively and quantitatively and to find out the common factors that are responsible for the growth fluctuation.

Dendrochronology is the science of tree-ring dating. Andrew E. Douglass is considered the founder of the discipline of Dendrochronology. According to Fritts, 1987, the term dendro is from dendron, the Greek word for tree, and chronology means the assignment of dates to particular events in a time series. The study of past and present climate from tree rings is known as dendroclimatology (Fritts, 1976). The fundamental scientific basis of dendrochronology, established by Douglass in the early 20th century, is that climate affects all trees in a given area consistently. This means that the pattern of relative ring width variation between different trees from close locations should be very similar for a given time period, even though their absolute ring widths may be quite different. This could be, for example, due to local soil conditions at a particular tree. Dendrochronology works on the principle that over a long period of time (especially 100 years or more) there is a pattern of tree-rings of wider and narrower rings which respectively reflect years favourable for the tree versus bad years and that the period during which any tree lived will be uniquely represented in its tree-ring pattern (considering all features, including ring-widths).

Matching of relatively fast and slow growth ring patterns among trees is called cross-dating (Swetnam et al., 1985). By coupling this to climate data, it is possible to establish a connection between critical climatic variables and tree growth. When tree rings are dated to the exact calendar year through the technique of crossdating (Stokes and Smiley, 1968), the science of dendroclimatology extracts the maximum amount of climatic information contained in the tree-ring record.

The major strengths of tree rings as climate change indicators are (i) their annual resolution, (ii) the existence of large geographic-scale patterns of synchronic interannual variability, (iii) the increasing availability of extensive networks of tree-ring chronologies, and (iv) the possibility of using simple linear models of climate–growth relationships that can be easily verified and calibrated (Hughes, 2002). Their weaknesses include (i) an intrinsic sampling bias, given that tree-ring information is available only for terrestrial regions of the globe, (ii) the fact that methods used to extract growth signals from tree-ring series retain only certain wavelengths of climate variability (IPCC, 2001) (iii) the complexity of biological responses to climate forcing, and (iv) the presence of nonclimatic variability in the series attributable to intrinsic growth trends and other nonclimatic disturbances (Fritts, 1976).

Anticipated impacts of climate change on forest growth

Rising atmospheric CO2 concentration, higher temperatures, changes in precipitation, flooding, drought duration and frequency will have significant effects on tree growth. Changes in species dominance, species distribution, species survival, fecundity and phenological phases are likely to occur. Tree-line fluctuations and species migration are another major anticipated impact. Rowe (1967) reported a northward migration rate of 150 m per year for the boreal forests in Canada and Bruce and Hengeveld (1985), suggested that tree lines in Canada would gradually migrate 100 km northward for every Celcius degree of warming.

Among these possible impacts, the impact of elevated CO2 on tree physiology and growth has been the subject of an enormous amount of scientific investigation over the last 50 years (Bazzaz, 1990). The lengthening of the growing season due to changes in the temperature and precipitation regime coupled with increasing CO_2 and nitrogen deposition are assumed to cause increased forest growth (Makinen et al., 2003). Recent studies suggest that the turnover and growth rates of tropical forests have increased in the last few decades due to stimulation of forest growth caused by increase in CO_2 concentrations in the atmosphere (Baker, 2004; Lewis, 2006; Phillips, 2009). Many CO2 enrichment experiments, conducted at the plant or stand scale, have consistently reported positive effects of elevated CO2 on tree growth (Tissue et al., 1997; Telewski et al., 1999). However, it is suggested that forest trees may now be CO2 saturated and further increases in atmospheric CO2 will not lead to further enhancements in photosynthesis and growth (Korner, 2003). On the contrary, a decline in forest growth and productivity with the changing climate is anticipated. As opposed to the enhancement effect of elevated CO2, the increase in tropospheric ozone in many parts of the world typically has detrimental impacts on trees (Karnosky et al., 2007). These negative impacts on tree physiology and growth typically counteract the positive impacts of elevated CO2 although the impacts of ozone are much more localized than those of elevated CO2. Recent results from an outdoor field experiment in the northern U.S. showed that the expected benefits of elevated CO_2 on aspen growth were negated when ground-level ozone was raised to the levels occurring in more polluted areas (Isebrands et al., 2001). Besides, predicted temperature increases in arid environments may exacerbate the effects of water stress even if soil water availability is not directly affected. While small increases in temperature often result in increased photosynthesis and tree growth (Way and Oren, 2010), large increases in temperature are inhibitory.

An anomalous reduction in forest growth indices and temperature sensitivity has been detected in tree-ring width and density records from many circumpolar northern latitude sites since around the middle 20th century. This phenomenon is known as the ‘divergence problem’. The possible causes include temperature-induced drought stress, nonlinear thresholds or time-dependent responses to recent warming, delayed snowmelt and related changes in seasonality, and differential growth/climate relationships inferred for maximum, minimum and mean temperatures. Another possible cause of the divergence is ‘global dimming’, a phenomenon that has appeared, in recent decades, to decrease the amount of solar radiation available for photosynthesis and plant growth on a large scale (D'Arrigo et al., 2008).

Tree ring studies supporting enhanced forest growth in response to climate change

Several studies have been conducted to study the impact of climate change on forest growth through tree ring analysis. Climatic warming may be expected to benefit the productivity of forests, especially in cold climatic regions where the length of the growing season is limited by low temperatures. Further benefits may be expected owing to the role of CO_2 in the photosynthesis of trees and other plants: as CO_2 levels increase, forest growth will tend to increase due to CO_2 fertilization effect, if other factors are not limiting. It has also been suggested that large-scale forest productivity has been significantly enhanced by human-induced increases in nitrogen (N) deposition (Chen et al., 2000). The inclusion of these positive effects in global models has led to projections of increasing forest productivity and carbon sequestration over the next century, especially in northern forests (Cao and Woodward, 1998; White et al., 1999). In a survey of 11 leading ecologists, most believed that standing biomass of northern forests is likely to increase under future climate change with a doubling of CO_2 levels, although soil carbon was generally expected to decrease (Morgan et al., 2001). A recent global analysis of remote sensing measurements from 1981 to 1999 suggests that the recent warming trend has led to an overall greening of most northern areas, especially in Eurasia (Zhou et al., 2001).

Increasing ring widths in recent decades in subalpine pines in New Mexico, Colorado and California (LaMarche et al., 1984) in *Pinus uncinata* at the tree line in the Pyrenees (Badeau et al., 1996) and in subalpine *Pinus cembra* in the central Alps (Nicolussi et al., 1995) have been cited as evidence of possible CO2 enhanced growth. Finally, strong evidence for CO2 growth enhancement comes from tree ring analyses of *Quercus ilex* continuously exposed to about 650 ppm CO2 near natural CO2 vents in Italy. These trees have grown about 12% faster than those growing in ambient CO2 nearby, especially when the trees were young and during dry seasons (Hattenschwiler et al., 1997). Across northern latitudes, ring width analyses suggest that forests started growing faster after 1850, well before the warming that occurred in the first half of the 20th century, but when CO2 levels first started to increase (Briffa et al., 1998b). Another intriguing observation is that the NPP (as measured by rates of tree mortality and recruitment – the turnover rates) of humid tropical forests throughout the world seem to have increased in recent decades (Phillips and Gentry, 1994).

One study in central Nevada found that the widths of annual rings of bristlecone (*Pinus longaeva*) and limber pine (*Pinus flexilis*) trees near timberline were 106 percent greater in the decade ending in 1983 than in the years 1850 to 1859 at one site and 73 percent greater at another. They explained that the observed increase in tree-ring widths was due to an increase in biomass due to elevated CO2. The CO2 fertilization hypothesis proposes that plants will grow faster under elevated atmospheric CO2 concentrations; that accelerated growth might take many forms like increase in underground root mass, increase in foliage and increase in cambial activity and thus tree-ring width (LaMarche et al., 1984).

In the mixed-grass prairie of southwestern Manitoba, Canada the evidence of an atmospheric CO2 fertilization effect on radial growth rates for open-grown white spruce was found. About 61% of the total variation in radial growth index was explained by climate for both young and old trees, residuals from young trees for the period of 1955–1999 demonstrated a stronger upward trend than old trees for the period of 1900–1996. The results suggested that (1) open-grown white spruce trees improved their growth with time at the early developmental stage, and (2) at the same developmental stage, a greater growth response occurred in the late period when atmospheric CO2 concentration, and the rate of atmospheric CO2 increase were both relatively high (Wang et al., 2006).

The tree-ring records of Siberian larch in the Egiin Davaa region in Hangay mountains, Mongolia, reaching altitudes in excess of 3500 m showed sustained overall increase in growth despite aging of the trees, suggesting that growth conditions were considerably more favorable in the 20th Century (Stratton, 2007).

In Komi Republic of northwestern Russia both Siberian spruce (*Picea obovata*) and Scots pine (*Pinus sylvestris*) showed increased radial growth in response to increasing temperature and precipitation in the region. Thus, climate change could partly explain the increased site productivity (Lopatin et al., 2008).

Another study found that the Great Basin bristlecone pine (*Pinus longaeva*) in western North America near the upper elevation limit of tree growth showed ring growth in the second half of the 20th century that was greater than during any other 50-year period in the last 3,700 years. The accelerated growth is suggestive of an environmental change unprecedented in the millennia. Both an independent proxy record of temperature and high-elevation meteorological temperature data were positively and significantly correlated with the ring width both before and during the high-growth interval. The study concluded that increasing temperature at high elevations is likely a prominent factor in the modern unprecedented level of growth for *Pinus longaeva* at these sites (Salzer et al., 2009).

Analysis of instrumental period surface air temperature data over the region indicated significant increasing trend over the last century with a noticeable warming during the recent four decades. A study of Himalayan conifers (*Cedrus deodara, Picea smithiana*) observed an anomalous higher growth during the last few decades in the multi century tree-ring width chronologies near the glacier areas of Kinnor and Gangotri in Western Himalayas. These chronologies indicated strong relationship to the mean annual and winter temperatures of concurrent year (Borgaonkar et al., 2009).

Increasing growth in European forests was also found by Laubhann et al. (2009) who studied the effects of climate change on four species namely common beech (*Fagus sylvatica*), oak (*Quercus petraea* and *Q. robur*), Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) in 382 plots in 18 European countries. Increasing temperature showed a positive effect on growth for all species except Norway spruce. Nitrogen deposition showed a positive impact on growth for all four species.

Tree ring studies supporting reduced forest growth in response to climate change

Contradictory results have also been recorded by several researchers who negate the phenomenon of enhanced forest growth particularly in the northern hemisphere due to climate change. The divergence problem in dendroclimatological studies as mentioned earlier has two premises, one that it is more pronounced in the northern latitudes and two that it may be anthropogenic in origin. A recent analysis by Cook et al. (2004a) suggests that the divergence is restricted to the recent period and is unique over the past thousand years. It is thus likely to be anthropogenic in origin. Cook et al. (2004a) utilized a fourteen chronology ring width data set used previously to model low-frequency temperature variability for the past millennium (Esper et al., 2002). The data from these fourteen sites were split into northern (eight boreal sites, 55°–70° N), and southern (six temperate sites, 30°–55° N) groups. While the northern group, which broadly corresponds to the region considered most sensitive to divergence by Briffa et al. (1998a,b), shows a significant recent downturn, the southern group does not and is more consistent with recent warming trends. Cook et al. (2004a) and Briffa et al. (1998a) discovered less divergence in the more southern regions, with declines in common variance with temperature of 5–12% vs. over 30% for some northern regions. Considerably more such research is needed, however, before we can conclude unequivocally that the recently observed divergence phenomenon is unique over the past thousand years and is more pronounced in the northern hemisphere.

Under global climate change, future increases in the severity of extreme climatic events may be expected to impact forests in a wide variety of ways (IPCC, 2001b). In some areas, the projected warming poses a risk for much drier conditions in northern continental regions, potentially leading to large-scale impacts on forests either directly (Hogg and Hurdle, 1995), or in combination with drought-induced increases in fire (Flannigan et al., 1998). There is evidence that increased drought stress associated with recent climatic warming has already led to reduced tree growth in some high-latitude areas such as Alaska (Barber et al., 2000). Potentially, climate change may also lead to losses of boreal forest cover following disturbance in areas that become too dry for regeneration to occur (Hogg and Schwarz, 1997). Furthermore, climate change poses a risk of increasing forest damage from outbreaks of pests (Volney and Fleming, 2000), including both native and exotic species (Krcmar-Nozic et al., 2000). The concerns for large-scale impacts also extend to the tropics, where large areas of rain forests have been recently devastated by the combined impacts of drought, selective logging and fire (Siegert et al., 2001). In the northern hardwood forests of the eastern U.S. and Canada, thaw-freeze events in winter and early spring have also been implicated as a major cause of forest dieback episodes during the past century (Auclair et al., 1996), but such events are rarely considered in large-scale models.

Increasing evidence indicates that warmer and earlier springs lead to earlier snow melt and stronger evaporation in temperate continental semi-arid mountain forests in the northern hemisphere (Ye et al., 1999; Barnett et al., 2005; Xu et al., 2008), where the water supply in the early growing season is dominated by the melting of winter snow (Barnett et al., 2005, Adam et al., 2009). Earlier snow melt leads to faster and earlier runoff in spring (Barnett et al., 2005; Xu et al., 2008) and a possible reduction of water storage available for active tree growth.

Barber et al. (2000) investigated ring widths, density and isotopic records from closed canopy, productive upland white spruce stands in the interior boreal forest zone of Alaska. They concluded that temperature-induced drought stress was the cause of divergence at their sites, with the greatest declines in temperature sensitivity found in the faster-growing trees.

Lloyd and Fastie (2002) found that growth declines were widespread in an analysis of tree-ring records from eight alpine and latitudinal treeline sites in Alaska. After 1950, warmer temperatures were associated with decreased tree growth in all but the wettest region, the Alaska Range. Negative responses to temperature were found that were widespread across Alaska's boreal forests. Growth declines were more common at the warmer and drier locations, leading these authors to conclude that drought stress may have accompanied the increased warming of these forests in recent decades.

Davi et al. (2003) detected a decline in temperature sensitivity and tree growth after 1970 in ring width data from elevational treeline sites in the Wrangell mountain region of southeastern Alaska. This decline coincided with warming in the instrumental temperature data, and was attributed to probable drought stress for at least one of the sites studied.

Tree-ring analysis of aspen (*Populus tremuloides* Michx) in western Canada showed that during the period 1950-2000 these forests have undergone major oscillations in growth. These cycles were mainly driven by drought and insect defoliation, which caused reduced growth. Besides, the influence of thaw-freeze events in late winter or spring, as well as damage by fungal pathogens and wood-boring insects, which tend to increase in aspen stands following drought or defoliation events were other causal factors (Hogg et al., 2003).

Another study in northwest China found that temperate continental semi-arid forests on the Tianshan Mountains are suffering from prolonged growth limitation in recent years accompanying spring warming. This prolonged limitation of tree growth was attributed to the effects of soil water limitation in early spring caused by the rapid spring warming. Warming-induced prolonged drought stress contributed, to a large part, to the marked reduction of regional basal area increment (BAI) in recent years and a much slower growth rate in young trees (Wu et al., 2013).

A dendroclimatic study on eastern white cedar (*Thuja occidentalis*) at its northern limit in North America found that radial growth was limited by short growing seasons and by summer moisture availability in the marginal zone. Growth was also limited by an excess of water before or after the growing season in some part of the gradient, especially in poorly drained soils (Housset et al., 2014).

Climate change and the Pandora’s Box

At this stage it is still inconclusive to state whether global climate change will actually enhance or reduce forest growth. The highly divergent results of researchers do not yield any consistent pattern that could shed light on the response of the world’s forested biomes to the changing climate. Despite recent scientific and technological advances, a high degree of uncertainty remains in predicting the direction of change in the potential productivity of the world’s forests. This uncertainty arises from the complexity in ascertaining how the effects of positive and negative factors will eventually balance out in a given geographical area. While a low to moderate increase in the concentration of greenhouse gases like carbon dioxide coupled with an increase in temperature may augment tree growth however, an uncontrolled increase in these parameters may either have adverse impacts on tree physiology and forest growth or the growth enhancement may reach a point of saturation.

A study by Andreu-Hayles et al. (2011) analysed the response of boreal forests to anthropogenic climate change using tree-ring data from the Firth River site at treeline in northeastern Alaska, in a tundra–forest transition region where pronounced warming has already occurred. Both tree-ring width (TRW) and maximum latewood density (MXD) chronologies were developed in white spruce (*Picea glauca*), a dominant Arctic treeline species. While the MXD chronology maintained a significant positive relationship with summer mean temperatures throughout the 20th century, for the TRW chronology good agreement with summer temperatures was found only from 1901 to 1950, whereas no significant relationships were found from 1951 to 2001, an indication of the divergence phenomenon. Forests where 'divergence' is detected may have reached a threshold beyond which tree growth is no longer positively influenced by summer temperature. This could be linked to the unusually rapid warming of the late 20th century due to recent anthropogenic change (ACIA, 2005, Trenberth et al., 2007), as well as potentially greater moisture stress due to an increase in evapotranspiration demand (Angert et al., 2005, Bunn and Goetz, 2006, Beck et al., 2011). However, wider and denser rings were more frequent during the 20th century, particularly after 1950, than in previous centuries. Their study concluded that there was indeed an increase in forest productivity at this forest–tundra transition zone, though TRW may be more influenced than MXD by changes in phenological patterns linked to ongoing warming (Menzel and Fabian, 1999) or variations in snowfall and timing of snowmelt (Vaganov et al., 1999).

With global climate change and its cascading impacts on the biosphere, the pandora’s box is indeed open but hope stills lies at the bottom of the box with a scope to adapt to or mitigate the foreseen climatic calamities. A deeper understanding of forest climate sensitivity is crucial for their conservation and management. Forest management practices should focus on the adaptive capacity of forests in light of their imminent vulnerability to the ravages of climatic change. This would only be possible if we have a clear understanding of what we are heading towards, with respect to forest growth and productivity in the coming years, when the ill effects of anthropogenic disturbances to global ecology are likely to manifest in one form or the other. As existing records are updated and new ones developed, we will be able to derive more accurate and reliable inferences concerning the pertinent question of forest growth dynamics in relation to the changing climate.

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