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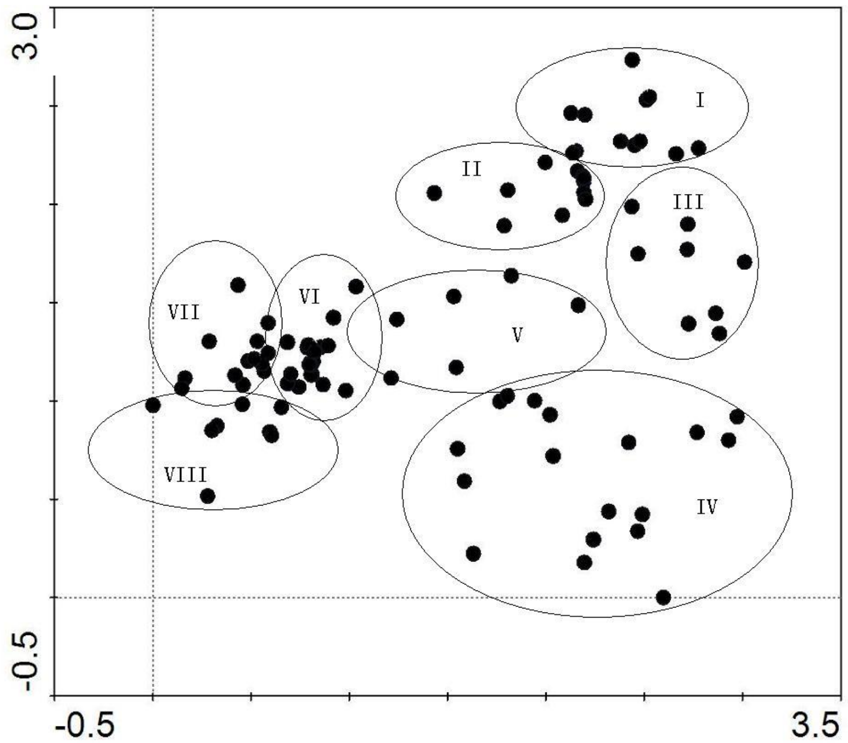

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GIS-BASED ESTIMATION OF WETLAND CONSERVATION POTENTIALS IN EUROPE

SCHLEUPNER, C.* – SCHNEIDER, U.A.

Research Unit Sustainability and Global Change, Center for Marine and Atmospheric Science
Bundesstrasse 55, 20146 Hamburg, Germany
(phone: +49-40-42838-7071; fax: +49-40-42838-7009)

**Corresponding author*
e-mail: christine.schleupner@zmaw.de

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Abstract. In the EU different utilization demands compete with each other and space is limited. As a consequence, socio-economic considerations and economic activities play therefore an important part in land use management and conservation planning. Often the integration of conservation concerns in agricultural as well as forestry production land use models has been neglected. One reason is a lack of accurate and consistent basis data. Therefore, conservation studies that offer high-accuracy landscape information at the European level are often recommended by policy makers, but rarely realized. This study contributes to this problem by creating and preparing a wetland distribution model (SWEDI) for integration into the mathematical bottom-up land use assessment model EUFASOM, which studies synergies and tradeoffs between biodiversity conservation efforts, greenhouse gas mitigation options, as well as traditional agriculture and forestry. The basis of SWEDI is the optimal combination of existing spatial datasets to obtain the spatial distribution of wetlands by definition of flexible knowledge rules. The model distinguishes between existing wetlands and sites suitable for wetland restoration at 1 km resolution. It differentiates several wetland types and covers the whole EU-25 area. The results of the model may help to locate sites suitable for restoration programs, or for the introduction of faunistic corridors.

Keywords: *land use planning, restoration ecology, spatial analysis*

Introduction

Because of its settlement history, land utilization in Europe is diverse and complex. As a consequence, many natural ecosystems as wetlands are altered and experience degradation, fragmentation and loss. It is estimated that more than two thirds of all wetlands in Europe have been lost since beginning of the 20th century (LIFE, 2007) because economically profitable land utilization requires drainage of the wetlands. Today, wetlands are considered to be among the world’s most threatened ecosystems. Besides biotope reduction and habitat fragmentation wetland loss has been also made responsible for unprecedented flooding events and species declines (Dahl, 2006). Because of natural habitat shortage latest conservation efforts include the option of restoration and creation of wetlands to meet the targets set out by the European Commission to halt biodiversity decline. But changing land use for food and, increasingly, biofuel production constitutes a great challenge to nature conservation. Conservationists are concerned that the promotion of bioenergy plantations in the context of climate change mitigation policies could threaten nature reserves and would lead to further biotope loss.

In the past the integration of conservation concerns in agricultural as well as forestry production land use models has often been neglected (Franklin and Swanson, 2007). One reason is often a lack of accurate and consistent basis data. Therefore, conservation studies that offer high-accuracy landscape information at the European level are often recommended by policy makers, but rarely realized by researchers (Wascher, 2000; Klijn, 2002; Scott and Tear, 2007; Wiens, 2007).

Usually, economic land use models refer to country statistics as base data. These data differ in spatial accuracy, reliability, acquisition data and class definition. Aggregating statistical and spatial data from many sources into one database often causes low spatial accuracy and complicates comparability, especially between eastern and western European countries. So is the spatial distribution of wetlands in Europe not well known except for large wetland areas or for wetlands of special ecological interest (Merot et al., 2003). Even those wetland areas, which have been identified on the behalf of European Environment Agency (*EEA*), correspond to wetland areas of ecological interest and represent only a rather small part of all wetland areas (Bernard, 1994). Some wetland studies modeled wetland distribution at global scales (Matthews and Fung, 1987; Aselmann and Crutzen, 1989; Stillwell-Soller et al., 1995; Joint Research Centre, 2000; Lehner and Döll, 2004). Due to its global perspective, the spatial resolution is coarse and wetlands are seldom differentiated in detail what makes the use of these data for conservation studies in European scale inappropriate. The most detailed consistent information about wetland habitats in Europe offers the EUNIS (European Nature Information System) Database with the distinction of over 2600 terrestrial habitat classes at the fourth level (Moss and Davies, 2002 a,b). However, the corresponding EUNIS habitat type map (European Topic Centre on Biological Diversity (ed.)) that has been created using mainly aggregated CORINE data refers only to the first level (= 10 major habitats) of the EUNIS habitat classification. Also, the CORINE biotopes data (European Commission, 1991; Moss and Wyatt, 1994; Moss et al., 1996; EEA, 2000a) that are based on reported NATURA2000 sites do not represent the existing wetlands completely and are only available in terms of spots on the map without area size statements. At present, the CORINE data (EEA, 2000b) is the most detailed land cover database covering the European Union. One disadvantage is the heterogeneity of the classes determined by functional land use and not by land cover itself. The digital map of the potential natural vegetation of Europe (Bohn and Neuhäusel, 2003) shows a detailed classification and potential distribution of wetland vegetation types across Europe. However, this distribution is irrespective of human influences and therefore only conditionally suitable because river regulation, peat extraction or urbanization on former wetland areas often lead to changed wetland restoration potentials. In all it becomes clear that there are no digital land cover or vegetation maps of the EU that show detailed wetland distribution.

This study contributes to this problem by compiling spatially consistent information on wetlands differentiated by wetland types and characteristics using GIS-based techniques. Not only existing wetland habitats should be documented but also potential wetland restoration sites by considering actual land use options. This way a new methodology on broad scale distribution modeling is developed. The study presented here is an attempt to extend the distribution modeling process to a broad continental scale by keeping the spatial accuracy as high as possible. This is important because European wetlands are often fragmented ecosystems of small extent. Many wetlands are smaller than one $1km^2$. But improvements in data quality and availability as well as simplifications in earth observation techniques make the more detailed studies feasible. As a result the narrow stripes of alluvial forests or small isolated bogs may be better represented in broad-scale analyses of wetlands. Besides the importance of regional case studies, decision makers of European land use policies demand spatial ecosystem information at holistic scales. The resulting wetland distribution model is finally aimed to be integrated into the mathematical bottom-up land use assessment model EUFASOM (European Forest and Agricultural Sector Optimization Model), which is used to study synergies and trade-offs between wetland conservation efforts, greenhouse gas mitigation options including carbon sinks and bioenergy, and agriculture and forestry of Europe (Schneider et al., 2008). Through EUFASOM, economic wetland potentials for optimal land use options are determined under certain policy scenarios.

Methods

Definition of wetlands

Often wetland terms and definitions are not standardized. The RAMSAR Convention (Article 1.1) defines wetlands as "areas of marsh, fen, peat land or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six meters". In wetlands water is present at or near the surface of the land also if only for varying periods of the year. Wetlands vary widely in soil, topography, climate, hydrology, water chemistry, vegetation, and other factors, also because of human disturbance (Pott and Remy, 2000; Dierssen and Dierssen, 2001; Blume et al., 2002). In this study we concentrate on the natural freshwater or inland wetlands whose types are further defined in *Table 1*.

Table 1. *Used wetland terms and their definitions, based on Cowardin et al.(1979), Mitsch (1994), Sanderson (2001)*

Common Wetland Names	Definition
Peatland	generic term of any wetland that accumulates partially decayed plant matter.
a. Bog	peat-accumulating wetland that has no significant inflows or outflows. Water and nutrient input entirely through precipitation; characterized by acid water, low alkalinity, and low nutrients. Peat accumulation usually dominated by acidophilic mosses, particularly sphagnum.
b. Fen	peat-accumulating wetland that receives some drainage from surrounding mineral soil. Usually dominated by sedge, reed, shrub or forest (→ swamp forest). Surface runoff and/or ground water have neutral pH and moderate to high nutrients.
Wet grassland (Marsh)	in European terminology a marsh has a mineral soil substrate and does not accumulate peat. Permanently or periodically inundated site characterized by nutrient-rich water and emergent herbaceous vegetation (grasses, sedges, reed) adapted to saturated soil conditions.
Wet forest	any wetland that is dominated by forest.
a. Swamp forest	wetland dominated by trees, most often forested fen. Depends on nutrient-rich ground water derived from mineral soils.
b. Alluvial forest	Periodically inundated forest areas next to river courses.

The definition of inland wetlands also includes marshes and wet meadows dominated by herbaceous plants that are most often human made as well as shrub- or tree-dominated swamps. In Europe, inland wetlands are most common on floodplains along rivers and streams, along the margins of lakes and ponds, and in other low-lying areas where the groundwater intercepts the soil surface or where precipitation sufficiently saturates the soil (vernal pools and bogs). Many of these wetlands are seasonal and may be wet only periodically. The freshwater wetlands denote the most relevant areas for land use options implemented in EUFASOM. Open waters are considered separately.

Locating wetland potentials

The spatial wetland distribution model "Swedi" is developed as extraction tool to denote wetland allocations in Europe. In this respect GIS and spatial modeling are used as instrument to locate existing wetland areas as well as to identify the most suitable areas for wetland regeneration measures. This GIS model aims to depict the distribution of wetland areas at regional level and also at coarse geographic scale. This involves the integration of a variety of GIS datasets and multiple iterations of expert review and interpretation to delineate the potential wetland areas of Europe. We used the GIS tool ArcGIS9 for analysis.

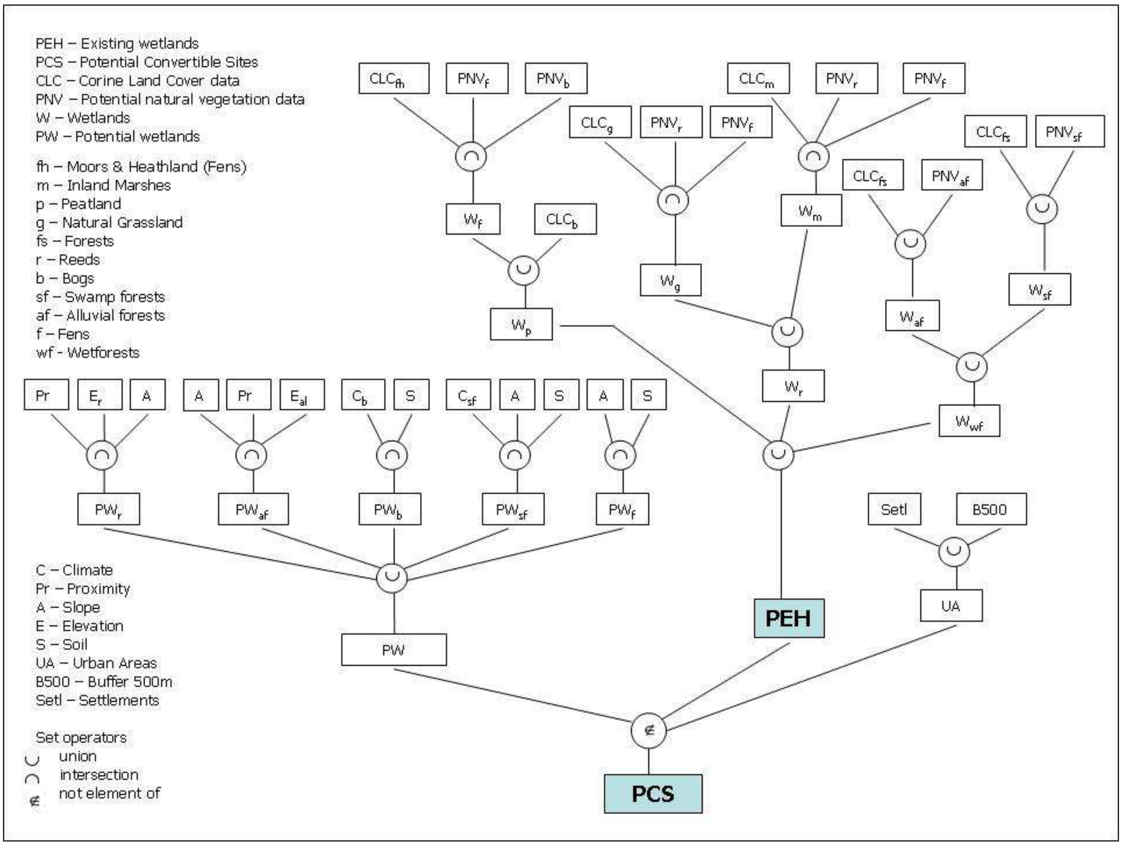

Figure 1. *The spatial wetland distribution model "Swedi"*

Figure 1 gives an overview of the Swedi (*Spatial wetland distribution*) model structure and its core input data. It is described in more detail in the following methodological section that is subdivided into two parts. The first deals with the evaluation of existing wetlands in Europe, and the second with the modeling of potential convertible sites for wetland restoration management.

Existing wetland habitats (PEH)

Existing wetland biotopes are defined as areas where wetlands with state close to nature actually appear within Europe. The analysis is executed with Model Builder and the Spatial Analyst Extension of ArcGIS9. The Corine land cover map 2000 with spatial resolution of 100 m serves as core base map (EEA 2000b). From the CORINE data, the following land cover classes have been extracted: moors & heathland (3.2.2.), inland marshes (4.1.1.), peat bogs (4.1.2.), inland waters and estuaries (5.1. and 5.2.2.), natural grassland (3.2.1.) and forests (3.1.). The EEA (1995) gives detailed definitions of each class. Within the spatial model the land cover class “peat bogs” serves as the only one that does not need to be altered to show existing “bog” wetlands, whereas all other selected land cover classes have to be split up separately: out of the generalized forest classes, only the wet forests, namely alluvial forests next to river courses and fen or swamp forests, are extracted through rule based statements using set operators. The CORINE classes “natural grassland” as well as “inland marshes” serve as base data for the model parameter “natural wet grasslands”. In addition, moors, wet heaths and riverine and fen scrubs are extracted from the general class “moors and heathland”. The map of the potential natural vegetation (PNV) of Europe (Bohn and Neuhäusel, 2003) has been selected as source to locate the wetland sites within these CORINE land cover classes. The PNV map in general distinguishes following wetland types: a. tall reed vegetation and tall sedge swamps, aquatic vegetation (PNV class R), b. mires (S), c. swamp and fen forests (T), d. vegetation of flood plains, estuaries and fresh-water polders and other moist or wet sites (U). These types can then be further subdivided. We extracted these wetland types and intersected them with the corresponding CORINE data. Only those sites matching both attributes were considered as present existing wetland site. The remaining sites were assumed to be non-wetland. However, this does not exclude the probability of the non-wetland areas to be potential wetland restoration sites as is explained in more detail below. *Figure 2* gives examples of the intersection and extraction procedure.

In order to verify the accuracy of the distribution of existing wetlands in Swedi, resulting outputs must be compared with an independent data set (Verbyla and Litaitis, 1989; Araujo et al., 2005). A description of general CORINE Land Cover data accuracy is found in the EEA Technical Report 7 (2006). In this study we use the CORINE biotopes database and parts of the RAMSAR list of wetlands of international importance (2008) for comparative analyses. The Corine biotopes (Version 2000) database is an inventory of major nature sites. The aim of the database was to enhance reliable and accessible information about vulnerable ecosystems, habitats and species of importance as background information for community environmental assessment. The wetland sites of the database are - among others - attributed with the size of the wetland. Site coordinates are included for easy localization of the biotopes within a GIS. We selected 50 freshwater wetlands from the database and compared their occurrence in the Swedi model considering spatial accuracy and wetland size. The same procedure has been applied to 50 selected RAMSAR sites. Additionally, the spatial extends of denoted NATURA2000 wetland sites as well as available biotope maps of individual sites are compared to the existing wetlands of Swedi.

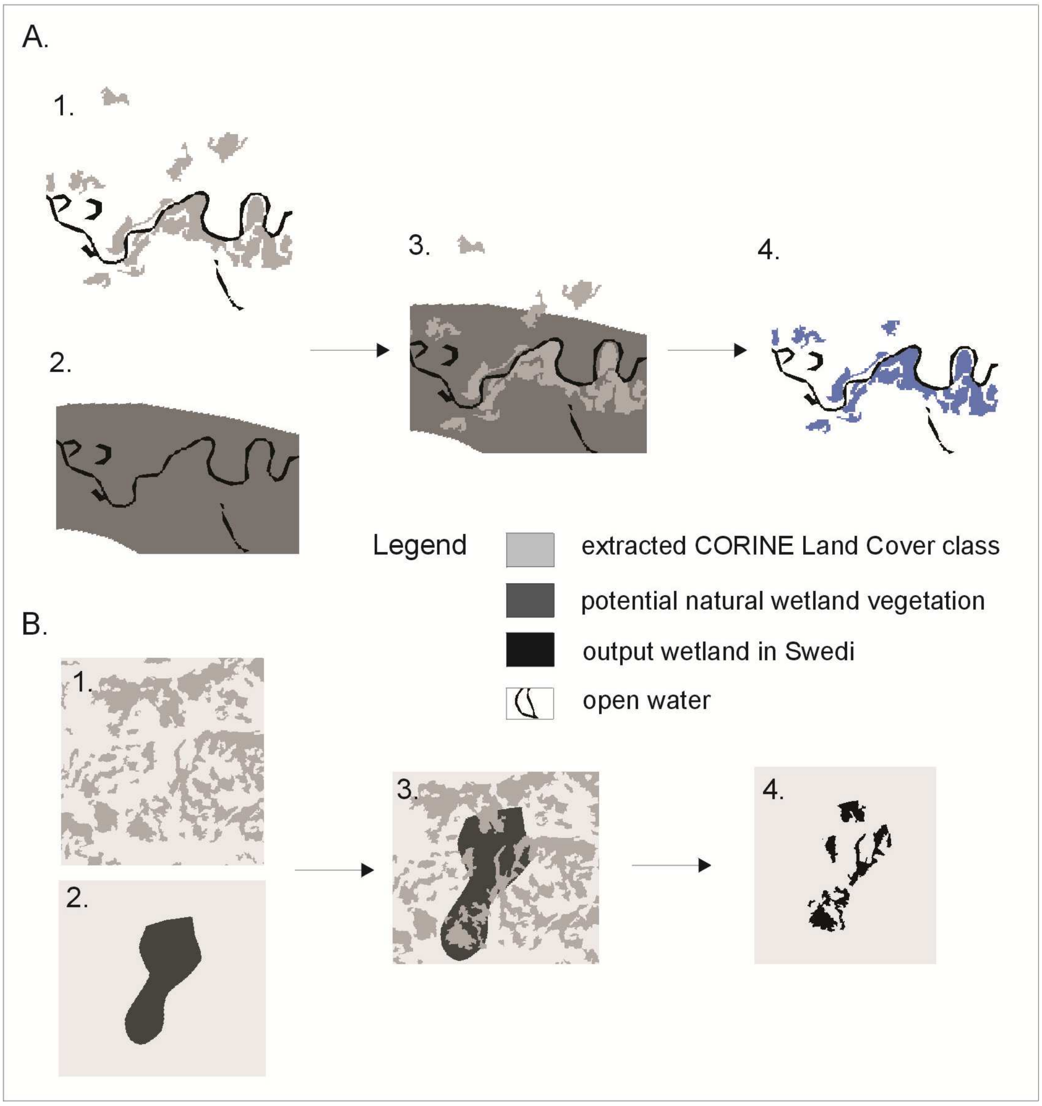

Figure 2. *two regional examples showing the methodology of the extraction procedure A. for wetforests by intersection of extracted CORINE Land cover class "broad-leafed forest" with extracted "swamp and fen forests" (PNV class T) and "vegetation of flood plains, estuaries and fresh-water polders and other moist or wet sites" (U) classes of the potential natural vegetation map of Europe. Shown is a section of the Elbe river between Dessau and Wittenberg / Germany. B. for moors, wet heaths and riverine and fen scrubs by intersection of extracted CORINE Land cover class "moors and heathland" with extracted "tall reed vegetation and tall sedge swamps, aquatic vegetation" (R) and "mires" (S) classes of the potential natural vegetation map of Europe. Shown is a region in the Grampian Mountains / Scotland*

Potential convertible sites (PCS)

The second part of the GIS assessment evaluates potential convertible wetland sites. These areas may be used for location of restoration programs or habitat creational measures. The distribution of wetlands is explained by many dependent and explanatory variables. Important factors are the climatic, hydrological, geological, ecological and socio-economic conditions of the area. The classification of wetland distribution is therefore preferably based on analysis of these independent variables (Guisan and Zimmermann, 2000). The connection between the respective information of the database and the probable appearance of the wetlands is determined by assuming that there is a relationship between environmental gradients such as soil, climate, or slope and wetland distribution (Franklin, 1995). We use traditional statistical methods based on observed correlation as well as geographically weighted regression analysis to analyze environment-wetland relationships. This proved to be useful concerning European scale analyses, because it allows for regional differences in relationships by estimating regression parameters that vary across space (Miller et al., 2007). Characteristic soil parameters, climate conditions, slope angles, and elevations are worked out for every wetland type on the basis of several literature resources (Brinson, 1993; Kuntze et al., 1994; Ellenberg, 1996; Succow and Joosten, 2001; BfN, 2004). Through this, rule-based statements are derived about the potential appearance of the target wetland types. In combination with geographical data these statements allow the identification and localization of potential wetland sites within a GIS. *Table 2* illustrates the resulting factors that characterize each wetland type.

Table 2. *Rating factors that characterize each wetland type (after Brinson 1993, Kuntze et al. 1994, Ellenberg 1996, Succow and Joosten 2001, BfN 2004)*

	Soil	**Slope Angle**	**Climate**	**Proximity to open waters**	**Elevation**
Fen	X	X			
Bog	X		X		
Swamp Forest	X	X	X		
Alluvial Forest		X		X	X
Reeds		X		X	X

Former wetland areas are considered as most suitable for wetland recreation (Ellenberg et al., 1991; Wheeler et al., 1995; Schultlink and Van Vliet, 1997). These might be arable fields, pasture lands, fallow or forested areas on sites of former wetlands that have been intensely changed. Actual soil conditions might give hints for potential wetland biotopes. We use the European soil database (Joint Research Centre, 2004) of 1 km grid resolution and extract following potential wet- and peatsoil-classes: gleysols, fluvisols, gleyic luvisols, histosols, gleyic podzol. The wetland types bogs, swamp forests and fens are considered to be soil dependent (cf. *table 2*).

The climate parameter is only applied for the parameters bogs and swamp forests; all other wetland types are rated as azonal and therefore relatively climate independent (Succow and Jeschke, 1990; Ellenberg, 1996; Walter and Breckle, 1999). The climate variables of the wetland types shown in *table 3* are extracted from the explanatory text of the map of the Natural Vegetation of Europe (BfN 2004) and are mainly based on Walter and Lieth (1967). We use the attributes temperature (max temp of warmest month, min temp of coldest month, average annual) and precipitation (average annual) of the Bioclim and Worldclim data at spatial grid resolution of 30 arc-seconds (~ 1 km²).

Table 3. *Wetland type characteristics concerning their climate ranges of occurrence (after BfN 2004, Walter and Lieth 1967)*

Wetland type	Average annual temperature (in °C)	Average precipitation (mm/year)	Max temp av. warmest month (°C)	Min temp av. coldest month (°C)
Bogs	3 - 6	300 – 1 000	12 - 17	-15 – (-2)
	9 - 11	1 200 – 2 000	13 - 15	5 – 7
	3 - 8	1 400 – 2 400	10 - 12	-2 - 0
	5 – 9,5	550 – 1 500	14 - 19	-3 - 5
	4 – 5,5	900 – 1 400	11 - 12	-3 – 0
	3.5 – 7.8	530 - 630	17.5 - 19	-10 – (-2)
	- 10 - 1	200 - 500	8 - 13	-25 – (-10)
Aapa mires (fens)	- 3 - 5	250 - 700	8 - 15	-17 – (-5)
transitional mires (fens)	0 - 5	500 - 870	8 - 14	-12 – (-3)
degraded bogs, now wet forests	8 - 9	600 – 1 200	15 - 16	0 – 4
wet forests	6 - 11	450 – 1 000	16 - 21	-5 – 0
	14 - 15	> 1 000	20 - 22	6 – 8
	9 - 10	550 – 1 000	15 - 16	4 - 5

The analyses of elevation dependent wetland types might also refer to climate or relief conditions (Merot et al., 2003). However, we are confined to the statements of highest occurrences of respective wetland types by the explanatory text of the PNV map of Europe (BfN 2004). The base elevation data for Europe are taken from GTOPO30 data a global digital elevation model at spatial resolution of 30 arc-seconds (sheets: W020N90, E020N90, W020N40, E020N40) (USGS 1996). In addition to that the bio-geographical regions map of Europe (EEA 2002) contribute to the elevation parameter by dividing the height variables into several bioclimatic regions that better reflect the height-limits than country based distinctions of regions. *Table 4* shows the wetland type characteristics concerning their maximum elevation occurrence range.

Table 4. *Wetland type characteristics concerning their maximum elevation occurrence range (after Brinson 1993, Kuntze et al. 1994, Ellenberg 1996, Succow and Joosten 2001, BfN 2004)*

Wetland type	Biogeographical Region	Elevation (m)
Reeds	Boreal, alpine (scand.)	<= 500
Reeds	Alpine (other), all others	<= 800
Alluvial forests	Boreal, alpine (scand.)	<= 500
Alluvial forests	Alpine (other), all others	<= 1 200

The map of bio-geographical regions is based on the PNV map (Bohn and Neuhäusel, 2003). It distinguishes between six bio-geographical regions in the EU-25, namely Alpine, Boreal, Continental, Atlantic, Mediterranean, and Pannonian. Alluvial forests and reeds are considered elevation dependent, less because of climatic conditions, but more due to loss of suitable ground conditions (Ellenberg, 1996; Mulamoottil et al., 1996; BfN, 2004). The climate dependent wetlands are assumed to limit their height occurrence by this parameter itself. An elevation constraint is therefore not necessary. Only the fens are assumed neither climate nor elevation dependent. They solely refer to soil conditions and the slope parameter.

The slope parameter is evaluated based on the elevation data using the Spatial Analyst extension of Arc GIS9. Only those areas with a slope angle below 1° are assumed suitable for the wetland types reeds, alluvial forests, swamp forests and fens (Mulamoottil et al., 1996; Lyon, 2001). Due to scale reasons the slope angle is set to this maximum extension and does not distinguish slope angles below that point as has been done in case studies of larger scale (Tsihrintzis et al., 1998; Helmschrot and Flügel, 2002).

Also the proximity to inland waters or to existing inland peatland is an important criterion for localization of target areas if other parameters are fulfilled. The proximity criterion has been initially set to 500 meters. But this border may be handled flexible. For implementation in the GIS-based model we establish multiple ring buffers around inland waters and other bog areas with radius of the defined proximity. The extension of potential water surrounding wetland sites like alluvial forests can be detected by a combination of the proximity with other parameters.

Highly populated areas as are towns and cities provide very limited space for wetland restoration or construction. For this reason, potential convertible sites are only modeled for agriculturally used areas, grasslands and forests by using pseudo-absences for urban areas (cf. Chefaoui and Lobo, 2008). Urban areas including a buffer zone of 800 meters are omitted by the model. We use the Corine Land Cover 2000 data for determination of these sites.

For accuracy assessment the potential wetland sites are correlated with the spatial distribution of the existing wetlands. And in a last step, the existing wetland sites are subtracted from the preliminary results to obtain only data on potential convertible areas. All data encompass the whole EU-25 states boundaries with exception of Malta and Cyprus that are not included in the analysis.

Results

The comparison of existing wetlands with samples of the independent data sets (RAMSAR wetlands and CORINE biotopes) shows that all selected wetlands of the databases are also represented in Swedi. The differences lie in the area extent of the respective wetlands: In over 70% of the cases the model overestimates the size of an existing wetland. One reason is the fact that the existing wetlands module of the Swedi model accepts uncertainties about the state of the wetland ecosystem also due to scale reasons. We are not able to distinguish between afforestations or natural alluvial forests in a floodplain, for example, what also might lead to overestimation errors of the results. 26% of the sites are underestimated in size. The difficulty is the accurate demarcation of wetlands and its types from open waters and terrestrial land due to their dynamic characteristics and their fluctuating and undefined borders. Often open waters are integrated into the wetland definitions of the databases whereas these wetland types are considered separately in the Swedi model. However, more than 85% of the selected wetlands stay within the defined uncertainty range of 15% deviation. No significant differences in accuracy are found between northern and southern or eastern and western European wetlands. *Figure 3* exemplarily shows a comparison of available spatial wetland data of Natura2000 with Swedi data as used for the accuracy assessment. Not all wetlands are implemented in the Natura2000 network and therefore it is reasonable that some existing wetlands of Swedi are not represented in the Natura2000 data. Also the spatial extend of the wetlands differ due to the fact that the wetland dimensions are difficult to define and that often the Natura2000 data include other biotopes combined in biotope complexes or buffer zones as well. However despite its inconsistencies in extent, more than 91% of the Natura 2000 wetlands data are also represented in Swedi.

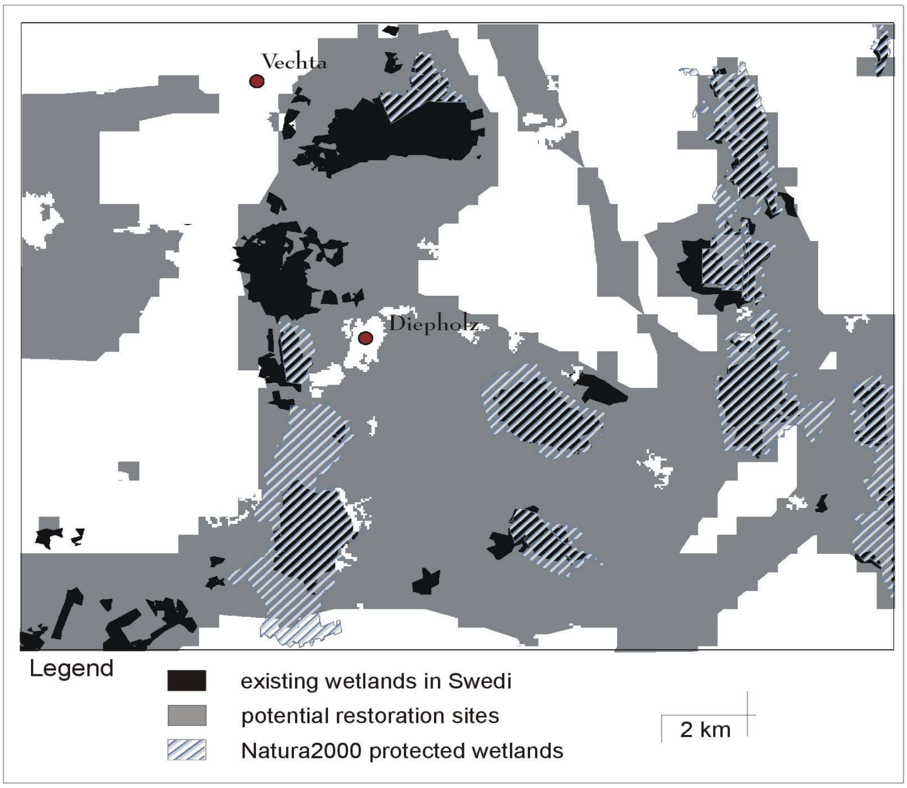

Figure 3. *Example of the accuracy assessment in the case of the Dümmer Region in Lower Saxony / Germany. We used spatial information of wetlands implemented in the Natura2000 sites and correlated them to the wetland distribution of Swedi*

Other comparisons with individual biotope maps on regional scale revealed more uncertainties and weaknesses of Swedi. This is due to the fact that wetlands below a certain extent are not well represented in Swedi and therefore some present wetlands may be underestimated or indicated as restoration site. At the same time the restoration sites overestimate the extent of potential wetland sites because of coarse base data in elevation or soil, for example, that do not respect geographic diversity at landscape scale. Under consideration of these limitations Swedi is only useful for general European scale studies.

Problems in comparability arose by drawing comparisons with a simple per-country aggregation of Swedi wetland areas to results of the Pan European Wetland Inventory (PEWI) (Nivet & Frazier, 2004). This may simply be due to the fact that both datasets apply to different wetland type determinations and basis data. Whereas the Swedi data are spatially explicit and rely on other spatial and geophysical data, the PEWI data are on country scale and rely on different kinds of national wetland inventories or statistics. For this reason an accuracy assessment with PEWI data has been refused.

The outcomes of Swedi are spatially explicit data on wetland distribution in Europe. The results may be illustrated through wetland distribution maps. *Figure 4* shows the spatial distribution of existing habitats (dark grey) and potential convertible sites (light grey) exemplarily for selected areas. The whole illustrated data set is available for download at http://www.fnu.zmaw.de/Dipl-Geogr-Christine-Schleupner.5728.0.html.

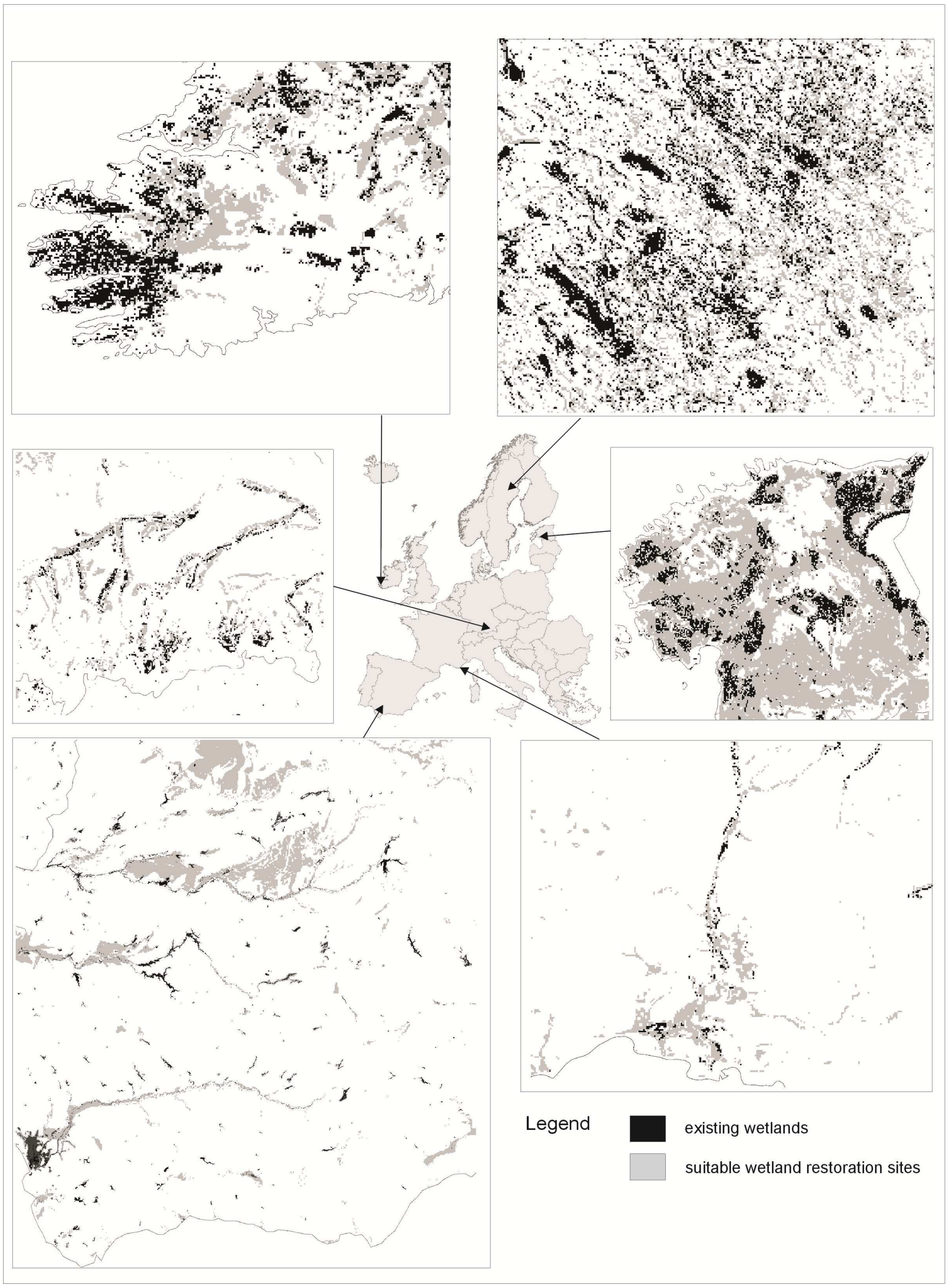

Figure 4. *Detailed examples of the spatial distribution of potential existing habitats (dark grey) and potential convertible sites (light grey) of Swedi*

An analysis of the Swedi map reveals that the majority of existing wetland areas (PEH) is situated in the northern and western European countries, while the potential convertible sites (PCS) are well distributed over the EU. In total, about 4% of the EU-25 land area consists of potentially existing wetlands and an additional 21% of the land areas are potential convertible to wetland sites. This constitutes a maximum share of wetlands of one fourth of the total land area of the EU-25. *Figure 5* gives an general overview of the total area (in 1 000 ha) of existing and the potential convertible wetland sites per country derived from Swedi.

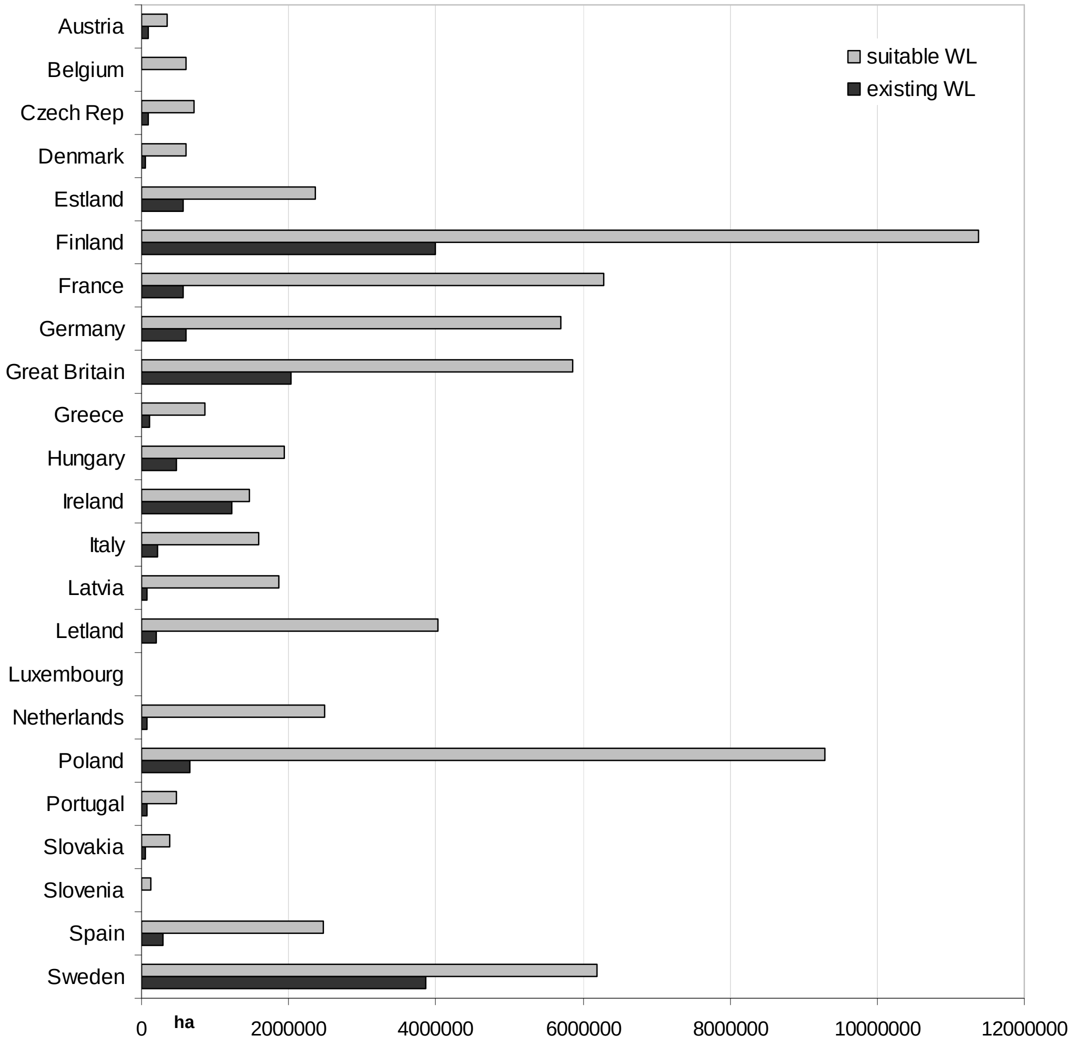

Figure 5. *Total wetland area (in 1000 ha) per country distinguished after existing wetlands and suitable restoration sites*

Even if not only restricted to formerly existing wetland areas the extent of potential wetland sites also give an impression of wetland loss. Open waters are excluded from the evaluation. Finland and Sweden own by far the most extending existing wetland areas with about 3.8 million ha wetlands. Also Ireland has great amounts of existing wetland areas (about 1.3 million hectares) but less in comparison to the Scandinavian countries. Finland and Sweden also lead in the amount of potential convertible wetland sites. In this category Poland, Great Britain as well as France and to a certain extent Germany as well show high amounts of land suitable for wetland restoration.

The relationship between wetland areas and country size (see *Figure 6*) displays a different picture: Now Ireland shows the highest wetland rate (PEH) with about 19% of its country area, followed by Estonia (13%) and Finland (12%). Also Sweden and the UK with 8.7 % and 9.25 % of their country size own a high existing wetland rates in comparison to other countries whose amounts lie between 0.03 % (Luxembourg), 0.6 % (Spain), and 5.1 % (Hungary), or 3.2 % PEH of the country area in the case of Latvia, for example.

Concerning the PCS per country area, Latvia (68%), the Netherlands (75.6 %), and Estonia (66%) have the highest relative potentials. The PCS rate of Finland, Poland, Great Britain, and Ireland amounts to between 31 and 49% per country area. In this case Denmark, Sweden and Germany have potentials of about 16 to 20% and the PCS rate of all other countries amount between 5.1 % as lowest rate in Austria and 12.5 % in France.

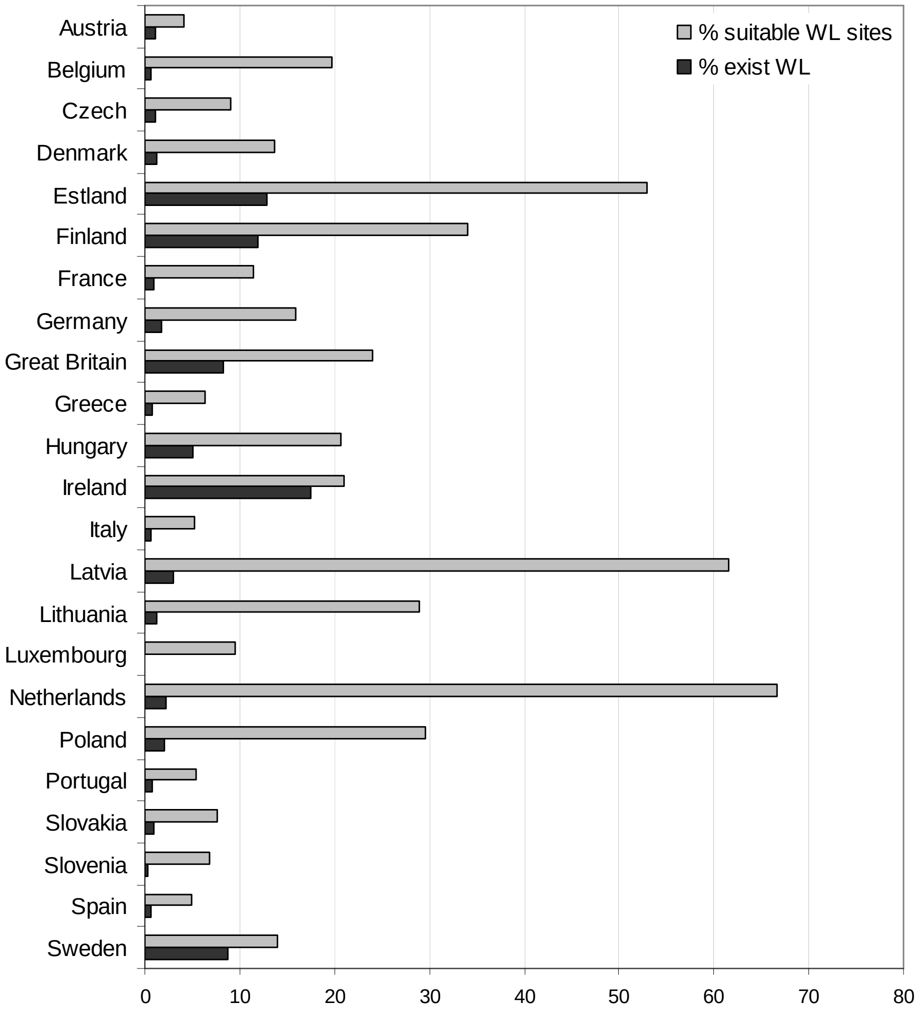

Figure 6. *Relative wetland area (%) per country*

Through Swedi the main wetland types peatland (fen/bog), wet forests (alluvial forest/ swamp forest) and wet grassland and its spatial potentials are visualized. Its results make evident that the potential wetland restoration sites are often overlapping.

This is especially true for peatlands. Moreover, some wetland types might be temporarily successional vegetation states of others within the wetland biotope complexes. Main areas of existing peatland are the Scandinavian countries and Ireland. Here, as well as in Scotland, Eastern Poland and Estland also highest amounts of potential bog areas are found. All other illustrated potential peatland areas may be favorable for fen restoration. Fens can also be created on potential bog areas, but this constraint does not work vice versa. It is remarkable that the formerly extending bog areas of North-Western Germany, that have been mainly drained and exploited during the last centuries, show fen instead of expected bog potentials in Swedi. This might be due to model uncertainties or errors, but can as well be a hint that the bogs have developed under different climatic conditions from the end of the last ice age and are relicts only. The destruction of these bog areas possibly means an unrecoverable demise of the ecosystem. Like wet grasslands also wet forests are found along water courses and in the proximity of other open waters. Especially the swamp forests are constricted to wet soils and to specific climate conditions. Main areas of potential swamp forest sites are therefore found in Central and Eastern Europe but also in the UK. In northern and western European countries the wet forested area does not exceed the peatland areas whereas in Germany and Poland and further south wet forests are the most extending wetland types. Extending areas of potential wet grassland sites are shown in Scandinavia, Estland, and Ireland, but also in Hungary. Wetland areas need to have at least a size of one hectare to be included into the spatial model. Therefore, often reeds along lakeshores are not shown in the results and Finland even counts no wet grasslands even though there are reeds growing along many waters.

Summarizing the total PCS areas distinguished after the main wetland types for the European countries one gets following results: a maximum area of respectively 1 329 200 km², 643 300 km² and 305 700 km² could be potentially used for additional bog, additional fen as well as additional wet forest creation. It must be noted that these data are no absolute numbers, but moreover provide an informative basis of potential wetland restoration area.

Discussion

There is a growing demand of policy makers and researchers for high-accuracy landscape information at the European level. Despite numerous data on land use in Europe, a detailed analysis of the distribution of wetlands and potential restoration sites has been lacking so far. We developed a detailed wetland distribution map in European scale with high spatial resolution. Not only does it distinguish between different wetland types but also between existing and potential convertible wetland restoration sites; information that has not been available before. Whereas the evaluation of existing wetlands relies on a cross-compilation of existing spatial datasets, the potential wetland restoration sites are determined by definition of flexible knowledge rules in combination with geographical data. The orientation towards physical parameters and the allowance of overlapping wetland types characterizes the Swedi model. The detailed spatially explicit wetland classification of Swedi allows connections to other habitat databases, for example EUNIS, as well.

The accuracy of Swedi is strongly restricted by the availability and quality of geographical data. For example, the soil information is generally poor and often misleading from the standpoint of wetland functionality. The same holds for the elevation and slope data. Also the water factor is only indirectly integrated into the model through climate and soil data. As long as these detailed Pan-European data are unavailable provides Swedi a static estimation of wetland potentials suitable for broad scale studies that may be analyzed at landscape scale in more detail. The utilization of GIS makes the methodology highly applicable and easily to improve concerning data sources.

The accuracy assessment showed uncertainties in the wetland size and extent that can be explained through base data uncertainties but also through differences in wetland definition and its assignment. Varying analogies in accuracy of different wetland types is more a reason of scarce and inhomogeneous reference data and less of dissimilar modeling precision. Due to the fact that with Swedi only those wetlands with an area extent of more than 1 ha are displayed, the total wetland distribution may be underestimated. Many wetlands, especially those in central and southern Europe, are very small-sized and its implementation in broad scale maps is still not realized. However, we found no differences in accuracy between northern and southern wetlands. Another uncertainty is the state of the ecosystem of the existing wetlands. In Swedi we are not able to make statements about the naturalness of the site. Nevertheless, the comparison of Swedi with independent datasets of wetland biotopes proved high accuracy of the existing wetland sites in the Swedi model and the area extent is mainly reproduced within the uncertainty range.

The direct use of country aggregated Swedi data by simple polygon measurement as shown in the results section should be regarded with caution. The data give useful results but the European scale should be used with care for area estimation because it can give strongly biased results. In principle, the direct use of such data for estimation is only acceptable when no other data are available (Gallego & Bamps, 2008). And for the spatially explicit estimation of wetland distribution no other homogeneous data exist in Europe.

The knowledge of the extent and distribution of wetlands is important for a variety of applications. This study applies an empirical distribution model to wetland ecosystems in European scale. These data can be used as ground information for further studies, for example helping to locate sites suitable for renaturation programs, or for the introduction of faunistic corridors respecting the Natura 2000 network of sites. The application of the model in nature conservation issues favours the success in regional conservation planning. The Swedi model on the other hand is meant to be integrated into the economic optimization EUFASOM model to evaluate the economic wetland potentials per EU-country (Schneider et al., 2008). The promotion of bioenergy plantations in the context of climate change mitigation policies constitutes a great challenge to nature conservation, because land use changes could threaten the availability of land for nature reserves and would lead to further biotope loss. By integrating the spatially explicit biotope information of Swedi into the economic land use model EUFASOM, the costs and potentials of different land utilizations as well as optimal conservation opportunities are evaluated.

Furthermore, so far Swedi builds the base data for European biodiversity studies of endangered wetland species that are analyzed for systematic conservation planning options. It is of utmost importance to provide accurate base data for the management and planning of conservation areas. This study offers some first guidelines but is also intended for an impulse of discussion on improvements of such data. The next enhancing step of Swedi besides base data refinements is to make this static extraction tool dynamic through the integration of hydrologic parameters for questions concerning climate change, conservation and land use planning options.

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AFFORESTATION OF DOLOMITE GRASSLANDS WITH NON-NATIVE *PINUS NIGRA* IN HUNGARY AND ITS EFFECT ON SOIL TRACE ELEMENTS

CSONTOS, P.[1]*– HALBRITTER, A.[2] – TAMÁS, J.[3] – SZILI-KOVÁCS, T.[1] – KALAPOS, T.[4] – UZINGER, N.[1] – ANTON, A.[1]

[1]*Agricultural Research Centre of the Hungarian Academy of Sciences, Institute for Soil Science and Agricultural Chemistry, H-1022 Budapest, Herman O. út 15, Hungary*
(phone: +36-1-2122265; fax: +36-1-2122265)

[2]*University of West Hungary, Apáczai Csere János Faculty,*
H-9002 Győr, P.O. Box 204, Hungary
(phone: +36-96-516-763; fax:+36-96-329-934)

[3]*Hungarian Natural History Museum, Department of Botany*
H-1476 Budapest, P.O. Box 222, Hungary
(phone: +36-1-210-1330; fax: 36-1-314-1483)

[4]*Eötvös Loránd University, Institute of Biology, Department of Plant Systematics, Ecology and Theoretical Biology, H-1117 Budapest, Pázmány P. stny. 1/C, Hungary*
(phone: +36-1-381-2187; fax: +36-1-381-2188)

** Corresponding author*
e-mail: cspeter@rissac.hu

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Abstract. The non-native *Pinus nigra* has been widely planted on natural dolomite grasslands in Hungary, yet little is known on its influence on soil properties. We compared soil micro-element concentrations in rock grassland (RG) and under *P. nigra* plantation (PP), both grown on north facing slopes of dolomite bedrock. At PP sites, the original vegetation was RG prior to afforestation. For both vegetation types, five sampling sites were selected, and at each site soil samples were taken from three depths (0-5, 5-10 and 10-15 cm). Micro-element concentrations of corresponding soil layers in the two vegetation types were compared. Under the pine plantation, the concentration of a number of soil trace elements was altered compared to the original rock grassland soil, and this effect increased with soil depth. At the deepest layer, significant differences were found for 10 microelements (Al, Fe, Mn, Ba, Cd, Co, Ni, Pb, Sr, Zn), and in each case the concentration was higher in PP than in RG soil. In contrast, the concentration of Cr, Cu, Hg, Mo and Se was not different between the two habitats throughout the soil layers sampled. Values exceeding the Hungarian environmental limits of background concentrations were detected for lead and cadmium. In the RG soil, concentrations of Pb and Cd were highest in the topmost layer, while for the PP sites these elements showed concentrations higher in the 10-15 cm layer than in the 5-10 cm depth. Concentrations of some other micro-elements showed similar inversion at PP sites. The depth inversion of these, often airborne pollutant trace elements was explained as a consequence of afforestation with *P. nigra* and such phytostabilization effect of the pine stands is assumed to increase with stand age.

Keywords: dolomite, heavy metals, *Pinus nigra*, rock grassland, soil pollution

Introduction

Austrian pine (*Pinus nigra* Arn.) has long been used in afforestation within and beyond its native range in the Mediterranean areas of Europe to prevent soil erosion (Barčić et al., 2006; Topić et al., 2008). Recently, *Pinus nigra* was involved in afforestation in other continents as well, owing to its wide ecological tolerance (Liu et al., 2005; Meamarian et al., 2007). In Hungary, where *P. nigra* is not native, its plantations were first established for soil erosion control and landscape protection purposes on steep dolomite slopes of the Hungarian Central Range in the beginning of the 20th century (Tamás, 2003). Later on, the primary goal of afforestations shifted towards wood production.

Recently, *P. nigra* stands cover 67,168 hectares that is 3.75 % of the total forested land of the country (Kottek, 2008). Foresters consider these afforestations as places of previously useless areas that were successfully integrated into wood production. Indeed, most of the stands are found on nutrient poor, low quality soils of either the steep slopes of dolomite hills (intensively grazed during last centuries), or sand dunes of the Danube-Tisza interfluve. Furthermore, *P. nigra* has not become an invasive species, in contrast to other exotic trees applied for afforestation of low quality habitats in Hungary, like *Robinia pseudoacacia* and *Ailanthus altissima* (Keresztesi, 1983; Udvardy, 1998). It was also suspected that *P. nigra* is able to improve soil quality, thus later on its stands could be followed by planting native forests, mainly oak stands (Tamás, 2003; Barčić et al., 2011).

In spite of arguments from the side of foresters, nature conservationists intensively criticize *P. nigra* plantations from two points of view. 1) These plantations destroy biodiversity of the former species rich grasslands that were supporting populations of several relict and endemic species (Zólyomi, 1942, 1987). 2) In practice, *P. nigra* stands were almost never followed by native trees in the rotation cycle, but instead were re-grown as non-native tree stands (Tamás, 2003). These points indeed highlight conflicts, but a society must decide what to prefer: conserving low productivity grasslands or producing wood, and if the latter the case, then should it be made via pine plantations or managing native deciduous forests instead. The decision should not be based merely on scientific arguments, of course. However, if wood production is the goal then it poses a scientific question: how do pine plantations affect the soil?

Surprisingly few works have been published on the soils of the Hungarian dolomite hills. Kovácsné Láng (1966) studied soils of rock grasslands, and Járó (1996) investigated forests of dolomite slopes, including pine plantations. Járó's work reported data on pH, humus- and macro-element contents of soils, but because of the lack of statistical approach his work was inappropriate to judge soil improvement under pine stands. Therefore, our group made a detailed study of soil properties examined by Járó, and concluded that pine plantations did not improve soil quality (Anton et al., 2008).

However, questions about the micro-element contents still remained open, because rock grasslands and pine stands were never studied from this point of view earlier. Since the afforestation of grassland habitats is obviously a major change, we hypothesized that the micro-element contents of the soil is altered during this vegetation transformation. To test this, we compared micro-element contents of soils of *P. nigra* stands and rock grasslands based on standardized soil sampling followed by statistical evaluation. Such comparisons can reveal important effects that should be encountered in the evaluation of the success of pine plantations establishment.

Materials and methods

The study region was the Buda-Hills (near Budapest, Hungary) where rock grasslands and *P. nigra* plantations are found over large areas. The Buda-Hills are formed by Triassic dolomite bedrock that are fragmented by surface erosion into several hills elevating 250-600 m above see level (Pécsi, 1958). Hill-slopes are steep (25°-50°) and are covered by shallow rendzina ("Leptosols" group according to the system of WRB 2006). The climate of the region is semiarid temperate, annual mean temperature is 9.9 °C, yearly precipitation is 575 mm (Marosi & Somogyi, 1990). A dry period of one or two months is common in summer and causes particularly arid conditions on the hill slopes of shallow soil. On the north facing slopes the typical vegetation is closed rock grassland (RG) dominated by *Festuca pallens* and *Bromus erectus*. *Pinus nigra* stands (PP), created by afforestation after World War II, are also found on north facing slopes. The stands selected for sampling were even-aged, 50 years old plantations with canopy cover of 80-95% and tree height of 14-18 m. For each sampling site, the presence of rock grassland prior to afforestation was ascertained based on historical photographs.

For both vegetation types (RG and PP), five stands were selected for sampling (*Table 1*). In each stand, four soil samples were taken at least 4 m apart from each other on 19th March (RG stands) and on 24th April (PP stands) in 2002. Soil samples were taken with soil corer from three depths: 0-5 cm, 5-10 cm and 10-15 cm. Soil samples were air-dried in the laboratory, then skeleton (d >2 mm), roots, twigs and other plant fragments were removed by sieving. The corresponding layers of the four soil cores taken from a stand were combined.

Table 1. *Basic geographical data of the 5-5 sampling sites for closed rock grassland (RG) and for Pinus nigra plantation (PP) in the Buda Hills, Hungary*

Short name	Slope aspect	Slope angle	Altitude a.s.l. (m)	GPS-coordinates Latitude	GPS-coordinates Longitude
RG	NW(320°)-N(20°)	25-30°	385-400	1. 47° 35.695’	18° 52.778’
				2. 47° 35.699’	18° 52.779’
				3. 47° 35.709’	18° 52.803’
				4. 47° 35.767’	18° 52.739’
				5. 47° 35.767’	18° 52.727’
PP	N(350°)-N(20°)	20-30°	390-405	1. 47° 35.452’	18° 53.928’
				2. 47° 35.445’	18° 53.915’
				3. 47° 35.440’	18° 53.903’
				4. 47° 35.455’	18° 53.934’
				5. 47° 35.452’	18° 53.918’

The following methods were used for soil analysis: a) pH: standard pH measurements were made in a suspension of 1:5 soil:water; b) Ca^{2+}, Mg^{2+}, Na^+ and K^+ concentrations were also determined in 1:5 soil:water suspension using flame photometer; c) organic matter content (expressed in weight ratio), according to Tyurin (1951); d) K_2O: plant available-K was determined with Lakanen-Erviö extraction (NH_4-acetate + EDTA, Lakanen & Erviö, 1971); e) total As-, Ba-, Ca-, Cd-, Co-, Cr-, Cu-, Hg-, Mg-, Mo-, Na-, Ni-, Se-, Sn-, Pb- and Zn-content were analysed by digestion with Aqua regia (1.0 g soil + 4.5 cm^3 HCl + 1.5 cm^3 65 m/m% HNO_3 + 1.0 cm^3 30 m/m% H_2O_2) for 15 minutes in microwave chamber, then the samples were allowed to cool down, transferred quantitatively to a volumetric flask and filled to 50 cm^3 with doubled deionised water. Before measurements, the samples were allowed to settle down. Jobin-Yvon Ultrace 2000 plasma spectrometer was used to determine quantities of trace elements.

In the statistical analyses, values of soil properties were compared by Student's *t*-tests between corresponding soil layers of rock grassland and pine plantation sites. Gaussian distributions (normality) of data was tested using the method of Kolmogorov and Smirnov. The F-test was used to determine if the standard deviations of the compared data sets were equal. Differences were considered significant at $p<0.05$ probability level. For the analyses the SAS ver. 6.4 (SAS, 1989) and the Graphpad Instat (InStat, 2003) softwares were used.

Table 2. *Comparisons of trace element contents in the 0-5 cm soil layers of the closed dolomite rock grassland (RG) and the Pinus nigra plantation (PP), based on t-test (n= 5)*

Digestion method	**Soil properties**	**RG**		**PP**		
		mean	**S.d.**	**mean**	**S.d.**	**sign.**
1:5 soil: water extract	Organic matter, w%	24.9	6.5	20.5	0.7	n.s.
	pH (H_2O)	7.4	0.1	7.1	0.1	++
	Ca^{2+}, mg/kg	271	31.1	161	14.8	++
	Mg^{2+}, mg/kg	105	13.7	70.4	8.5	++
	Na^{+}, mg/kg	5.7	1.1	6.0	2.4	n.s.
	K^{+}, mg/kg	19.5	2.7	2.8	3.8	++
LE-method	K_2O, mg/kg	186	41.5	185	18.0	n.s.
extracted by aqua regia	Ba, mg/kg	9.6	0.9	11.1	1.2	+
	As, mg/kg	0.4	0.2	0.4	0.1	n.s.
	Hg, mg/kg	0.1	0.1	0.0	0.0	n.s.
	Se, mg/kg	0.0	0.0	0.0	0.1	n.s.
	Mo, mg/kg	0.0	0.0	0.0	0.0	n.s.
	B, mg/kg	4.6	0.6	3.8	0.5	+
	Zn, mg/kg	54.3	15.8	30.7	4.6	+
	Pb, mg/kg	46.2(!)	17.3	30.3(!)	4.9	n.s.
	Co, mg/kg	0.7	0.1	1.4	0.4	+
	Cd, mg/kg	0.8(!)	0.2	0.8(!)	0.0	n.s.
	Ni, mg/kg	1.5	0.3	2.5	0.3	++
	Fe, mg/kg	529.2	100.6	474.0	39.9	n.s.
	Cr, mg/kg	0.4	0.6	0.2	0.0	n.s.
	Mn, mg/kg	218.6	39.5	304.6	88.3	+
	Cu, mg/kg	10.1	10.3	4.8	0.3	n.s.
	Al, mg/kg	153.0	38.9	192.8	35.9	n.s.
	Sr, mg/kg	6.9	0.8	6.2	0.4	n.s.
	<2mm fraction, w%	55.3	16.4	100.0	0.0	

Abbreviations: ++ = significant differences at p< 0.01 level; + = significant difference at p< 0.05 level; n.s. = not significant (p> 0.05); sign.= level of significance; * = components showing considerable differences, but their significance were not tested (because some "0" values in the data set); ! = concentrations above the limit of Hungarian environment protection rules; LE = Lakanen and Erviö; w% = weight percentage. Concentrations below the lower detection limit were uniformly replaced by zero values.

Table 3. *Comparisons of trace element contents in the 5-10 cm soil layers of the closed dolomite rock grassland (RG) and the Pinus nigra plantation (PP), based on t-test (n= 5). Abbreviations are the same as listed for Table 2.*

Digestion method	**Soil properties**	**RG mean**	**RG S.d.**	**PP mean**	**PP S.d.**	**sign.**
Tyurin	Organic matter, w%	18.3	2.5	11.2	1.9	++
1:5 soil: water extract	pH (H_2O)	7.4	0.0	7.4	0.1	n.s.
	Ca^{2+}, mg/kg	226	8.2	169	10.9	++
	Mg^{2+}, mg/kg	87.1	3.9	62.4	7.3	++
	Na^{+}, mg/kg	5.6	0.7	0.5	0.9	++
	K^{+}, mg/kg	14.9	2.6	0.4	0.8	++
LE-method	K_2O, mg/kg	124	8.7	171	14.4	++
extracted by aqua regia	Ba, mg/kg	7.3	1.0	9.8	1.6	++
	As, mg/kg	0.3	0.2	0.1	0.1	+
	Hg, mg/kg	0.1	0.1	0.0	0.0	n.s.
	Se, mg/kg	0.0	0.0	0.0	0.0	n.s.
	Mo, mg/kg	0.0	0.0	0.0	0.0	n.s.
	B, mg/kg	4.8	0.4	4.3	0.5	+
	Zn, mg/kg	38.3	37.0	10.1	2.5	n.s.
	Pb, mg/kg	29.7(!)	9.7	12.4	1.3	++
	Co, mg/kg	0.6	0.2	1.5	0.4	++
	Cd, mg/kg	0.5	0.1	0.6(!)	0.1	+
	Ni, mg/kg	0.9	0.1	1.5	0.4	++
	Fe, mg/kg	383.2	53.9	280.6	38.2	++
	Cr, mg/kg	0.1	0.0	0.1	0.0	n.s.
	Mn, mg/kg	186.8	41.0	354.4	94.5	++
	Cu, mg/kg	3.1	0.5	2.8	0.4	n.s.
	Al, mg/kg	88.2	16.9	89.7	24.9	n.s.
	Sr, mg/kg	6.1	0.4	5.8	0.6	n.s.
	<2mm fraction, w%	48.3	13.8	100.0	0.0	

Results

Results are focused on remarkably significant differences of micro-element contents of the studied soils. Special attention is paid to pollutant trace elements, considering the pollution threshold limits in Hungary (KÖM, 2000). Tables also contain data on pH, organic matter content and the main macro-element concentrations of the soil to better inform readers, although a detailed evaluation of these data was given earlier (Anton et al., 2008).

In the upper 5 cm soil layer, concentrations differed significantly for six trace elements between samples taken from the rock grasslands and the pine plantation (Table 2). Concentrations of barium (Ba), cobalt (Co), nickel (Ni) and manganese (Mn) were higher in the soils of pine plantations, whereas that of boron (B) and zinc (Zn) were higher in the rock grasslands' soils. Actual concentrations of lead (Pb) and cadmium (Cd), two toxic heavy metals, exceeded the reference limits of background concentrations at both sites with more or less the same extent.

In the 5-10 cm soil layer, differences of trace element concentrations increased between the two vegetation types, and were significant for nine elements (*Table 3*). In the soils of pine plantations, similarly to the 0-5 cm layer, higher concentrations were detected for Ba, Co, Ni and Mn. Furthermore, the concentration of cadmium (Cd) was also significantly greater here than in the rock grasslands' soils. At the same time, concentrations of arsenic (As), boron (B), lead (Pb) and iron (Fe) were significantly higher in the soil of closed rock grasslands than that of pine plantations. Concentrations exceeding the limit of corresponding background values were found in this depth for two micro-elements only: lead (Pb) was above limits in the rock grasslands, while cadmium (Cd) was so in the pine plantations.

Table 4. *Comparisons of trace element contents in the 10-15 cm soil layers of the closed dolomite rock grassland (RG) and the Pinus nigra plantation (PP), based on t-test (n= 5). Abbreviations are the same as listed for Table 2.*

Digestion method	**Soil properties**	**RG**		**PP**		
		mean	**S.d.**	**mean**	**S.d.**	**sign.**
	Organic matter, w%	12.0	4.8	12.9	2.7	n.s.
1:5 soil: water extract	pH (H_2O)	7.5	0.1	7.3	0.1	n.s.
	Ca^{2+}, mg/kg	200	14.4	165	9.8	++
	Mg^{2+}, mg/kg	65.7	25.2	64.1	6.3	n.s.
	Na^+, mg/kg	4.4	2.7	0	0	*
	K^+, mg/kg	12.4	2.8	0	0	*
LE-method	K_2O, mg/kg	91.2	19.1	218	6.5	++
extracted by aqua regia	Ba, mg/kg	5.0	1.6	10.8	1.2	++
	As, mg/kg	0.2	0.1	0.2	0.1	n.s.
	Hg, mg/kg	0.1	0.1	0.0	0.0	n.s.
	Se, mg/kg	0.0	0.0	0.0	0.0	n.s.
	Mo, mg/kg	0.0	0.1	0.0	0.0	n.s.
	B, mg/kg	3.9	0.6	4.3	0.6	n.s.
	Zn, mg/kg	11.7	6.9	19.2	3.7	+
	Pb, mg/kg	12.7	1.5	18.6	2.1	++
	Co, mg/kg	0.5	0.2	1.6	0.4	++
	Cd, mg/kg	0.3	0.1	0.7(!)	0.1	++
	Ni, mg/kg	0.5	0.1	1.9	0.3	++
	Fe, mg/kg	261.2	44.6	339.2	41.9	+
	Cr, mg/kg	0.4	0.7	0.2	0.0	n.s.
	Mn, mg/kg	135.2	57.3	350.0	90.9	++
	Cu, mg/kg	6.6	11.0	3.7	0.4	n.s.
	Al, mg/kg	68.0	34.0	122.7	27.3	+
	Sr, mg/kg	5.0	0.3	6.3	0.6	++
	<2mm fraction, w%	37.8	17.1	>90		

In the 10-15 cm soil layer, further increase was observed in the number of trace elements expressing significant concentration differences between RG and PP sites. Noteworthy, that the 10 trace elements showing significant differences (Ba, Zn, Pb, Co, Cd, Ni, Fe, Mn, Al and Sr) always reached higher concentrations in the soils of PP than that of RG (Table 4). In this depth, cadmium (Cd) was the only trace element that surpassed the corresponding limits of background concentrations, as 0.7 (+/-0.1) mg/kg was measured in the pine plantations. It was also remarkable that lead (Pb) concentration was significantly higher under pine stands than at the grassland sites (as opposed to the two upper layers), although it remained within the permissible background limits. In the soils of pine stands, an additional feature of lead concentration was observed: it reached higher value in the 10-15 cm layer than in the 5-10 cm depth. Further two trace elements, zinc and cadmium, displayed a similar concentration inversion: both had significantly higher concentrations in the lowermost soil layer than in the intermediate soil layer of pine stands. In contrast to pine stand soils, lead-, zinc- and cadmium concentrations in the rock grassland soils always showed monotonic decrease from upper to lower layers.

Discussion

Our results showed that under the pine plantation the concentration of a number of soil micro-elements is altered compared to the original rock grassland soil, and this effect increased with soil depth. While in the 0-5 cm and 5-10 cm depths the direction of change varied depending on the microelement, in the deepest soil layer (10-15 cm) the pine stand soil exceeded the rock grassland soil in the concentration of each trace element which differed between the two habitats. Only few trace elements displayed differences consistent across all soil layers studied: Ba, Co, Mn and Ni concentrations were higher in the PP than in the RG throughout the 0-15 cm soil depth. The lack of difference between the PP and RG soils were consistent in each soil layer for the elements Cr, Cu, Hg, Mo and Se. The rest of the micro-elements investigated (Al, As, B, Cd, Fe, Pb, Sr and Zn) displayed depth-specific patterns between the two habitats. For the pine plantation, it was also documented that the concentration of certain trace elements were significantly higher in the 10-15 cm than in the 5-10 cm soil layer.

In our study, the two trace elements (Pb and Cd) that were detected in concentrations above the permissible background limits are typically known as airborne pollutants (Migon et al., 1991; Cizmecioglu & Muezzinoglu, 2008). Today, the study area is a nature reserve with no inhabitants, and historically it was used for sheep grazing. Therefore, there is certainly no local source of metal pollution. Instead, the Pb and Cd detected in the soil should originate from anthropogenic activities in the neighborhood.

In this respect, three different emission sources can be considered. The first one is a main road that connects Budapest with Dorog Industrial Region. Traffic roads are well known as heavy metal pollution sources, especially during the middle and late 20th century, prior to the introduction of led-free gasoline in the 1990’s (Harrison & Laxen, 1981; Zupančič, 1999; Enayatzamir, 2008).

The second possible source is the open air dolomite quarry at Pilisvörösvár, which was first mined at the end of the 19th century and operates continuously since then. According to literature data, limestone and dolomite quarries, together with related stone crushers can be responsible for heavy-metal emission (Connor & Shacklette, 1975; Durn et al., 1993).

Both the main road and the quarry are about 3-3.5 km distance from the sampling sites located under the main wind direction (N-NW) of the region. Therefore, these pollution sources most probably contribute to the trace element content of the studied soils.

The third pollutant factor is likely the capital, Budapest. Its population of about 2 million inhabitants with the associated traffic and other anthropogenic activities make it a serious source of heavy metals. Although Budapest is situated 10 km South-East from the study area, under certain weather conditions its emission can reach the region. Other big cities have been reported as sources of considerable heavy metal pollution on their whole surroundings (McDonnell et al., 1997; Li et al., 2011).

The detection of inverse depth distribution for Cd, Pb, and Zn is an unexpected finding of our study, thus it requires discussion. These trace elements are typically airborne pollutants, thus their concentration is usually highest in the uppermost soil layer and then decreases with depth (Adriano, 1986; Davies, 1990; Sipos et al., 2011). Such typical depth distribution was found in the soil of the studied rock grasslands (*Tables 2-4*). However, in the soil of the pine plantation concentrations of Zn, Pb and Cd were significantly higher at depth of 10-15 cm than at depth of 5-10 cm (Tables 3 and 4). In addition, several other trace elements showed similar inversion in concentration distribution although these differences were not significant.

A possible cause of the detected inversion phenomenon in the pine plantation could be associated with the biological effect of *P. nigra* itself. Pine species – as well as other conifers – are known to reduce soil pH beneath their stands (Pallant and Riha, 1990; Thelin et al., 1998). Also for the studied stands, a moderate shift of pH from 7.4 to 7.1 was detected in the upper soil layer (*Table 2*). It is known that soil acidification enhances solubility of metal compounds, thus increases concentration and mobility of metal ions in the soil solution (Strom, 1997; Szalai et al., 2010).

Plant roots (by root exuded organic acids) and also their rhizosphere play active role in soil acidification, therefore, mobility of heavy metal ions can be supported by pine roots towards deeper soil layers. This process could partly be responsible for the accumulation of certain trace elements in deeper soil layers of the pine stands, since pine roots reach much deeper soil horizons than the root system of perennial grasses and herbs that forms the vegetation of the rock grasslands. However, dissolubility studies showed that Pb appears to be strongly bound to the soil matrix, due to complexation and specific adsorption processes (Abumaizar and Smith, 1999; Sipos et al., 2008). Sauve et al. (1998) reported that the solubility of Pb shows a linear decrease from pH 3 to 6.5 and is independent of soil organic matter in that pH range. From pH 6.5 to 8, higher pH promotes the formation and dissolution of organo-Pb complexes, which increase Pb solubility. In this pH range, higher organic matter content results in higher concentrations of dissolved and labile Pb.

In general, cadmium expresses higher mobility than lead, and Cd should be fairly mobile in soils of pH 4.6 to 6.6. However, above pH 7 cadmium shows reduced mobility and in case of soils with high calcium carbonate concentration its co-precipitation with $CaCO_3$ can occur (Filep, 1999). Conclusively, soil acidification due to pine plantation might have a role in the accumulation of polluting trace elements in deeper soil layers, but these movements of metals could be considerable only adjacent to pine roots where the calcareous soil particles are unable to compensate pH decrease caused by the root exuded acids.

A further anthropogenic factor may also be involved in the detected concentration inversion in the pine stands' soil. It is known that the studied pine stands were planted around 1950, within the frame of the so called "barren land afforestation" programme (Tamás, 2003). Prior to bedding out the pine seedlings, narrow terraces (for strip cultivation) were prepared on the dolomite hill-slopes using traditional hand-tools (spade, hoe and pickaxe). This operation obviously resulted in turning over the soil layers, and it could contribute to the movement of polluting heavy metals to deeper layers.

Our results indicate that the establishment of *P. nigra* plantations can be considered as a possible way for reducing concentrations of heavy metals in the upper soil layer, because during this operation soil surface contaminants are placed to deeper soil layers either by immediate turning of soil layers, or later transportation processes along roots. Consequently, uptake of toxic heavy metals by ground layer vegetation of the polluted soils is limited, thus their further passage to food-web is practically prevented. The phytostabilization effect of these plantations increase further with aging (more closed) stands by slowing down near-surface wind velocity, thus reducing concentrations of heavy metal contaminants in the wind-blown dust almost to zero.

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PLANT DIVERSITY AND COMMUNITY ATTRIBUTES OF WOODY PLANTS IN TWO CLIMAX SUBTROPICAL HUMID FORESTS OF MEGHALAYA, NORTHEAST INDIA

MISHRA, B.P.[1,2] – JEEVA, S.[1,3]*

[1]*Ecology Laboratory, Centre for Advanced Studies in Botany, School of Life Sciences, North-Eastern Hill University, Umshing - Mawkynroh, Shillong – 793 022, Meghalaya, India*

[2]*Department of Environmental Science, Mizoram University, Post Box- 190, Aizawl- 796 001, Mizoram, India*

[3]*Department of Botany, Scott Christian College (Autonomous), Nagercoil - 629 003, Kanyakumari, Tamil Nadu, India*
(phone: + 91–364-2722227)

**Corresponding author*
e-mail: solomonjeeva@gmail.com

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Abstract. The present study was conducted in two sacred forests, representing subtropical humid forest of the state of Meghalaya, India. Measurement of canopy cover, light interception and disturbance index revealed that these two sacred forests are comparable with respect to plant diversity and community attributes. The disturbance index was slightly higher in Mairang sacred forests, which had supported high species richness, better recruitment of seedlings leading to high regeneration potential. This was due to formation of gaps within forest. On other hand, gaps facilitated expansion of crown of trees, which inhibited growth and survival of saplings, the retarded growth of saplings had resulted in low conversion of saplings to trees. This condition could also be a reason for high basal area and low tree density in Mairang sacred forest. Endemic, rare and rare endemics were also reported from both the forests. Altitude had impacted composition, and dominance of species and families. Both the forests were highly dissimilar with respect to species composition. Contagious distribution was prevalent in both the forests, clumping is a characteristic feature of natural forest in tropical and subtropical region. Wide-girth structure and log-normal dominance-distribution curves justify complexity and stability of communities that lead to climax vegetation.

Keywords: *Population attributes, regeneration potential, sacred forest, subtropical humid forest, woody species diversity*

Introduction

Biodiversity refers to the quality, range or extent of variation between the biological entities in a given set. Plant community dynamics and vegetation management is intricately interrelated, and an understanding of the basic processes involved in vegetation change is essential for the sound manipulation of plant communities (Niering, 1987). Larger environmental variation within a small geographical area makes altitudinal gradients ideal also for several ecological and biogeographical hypotheses (Wilson *et al.,* 1990; Korner, 2000; Ohlemuller & Wilson, 2000), therefore, altitudinal gradient has become increasingly popular for investigating patterns in species richness (Rohde, 1992; Rahbek, 1995; Odland & Birks, 1999; Kessler, 2000; Srinivas & Parthasarathy, 2000; Grytnes, 2003).

In India, northeast region is an extension of eastern Himalaya. It has stable geological history, equable climate, heavy and uniform rainfall, which support a wide range of subtropical forest ecosystem (Champion & Seth, 1968) and considered as hot spot of plant diversity (Myers, 1988; Groombridge, 1992; Myers *et al.,* 2000; Behera *et al.,* 2002). Phytogeographically, forests of this region are species rich, and harbor a number of phylogenetically primitive plant species and regarded as a "treasure trove" of ancient and unique vegetation (Champion & Seth, 1968). Takhtajan (1969) has considered these forests as the cradle of flowering plants.

As per an estimate of Forest Survey of India (1997), the actual forest cover in Meghalaya is about 41.6 % (9330 km^2) of the total geographical area (22429 km^2) of the state. The subtropical semi-evergreen forests topped the list with coverage of 21.4 % land and is followed by subtropical semi-evergreen forest (11.9 %), subtropical pine forest (7.6 %) and sal forest 0.1 % (Mishra *et al.,* 2005a). That the subtropical humid forests are found in tropical and subtropical belts, and inhabit major population of plant species, act as reservoirs of biodiversity (Whitmore, 1984; Whitmore & Sidiyasa, 1986; WCMC, 1992; Richards, 1996; Whitmore, 1998; Fashing & Gathua, 2004; Fashing *et al.,* 2004).

The sacred forests of Meghalaya are the best example of subtropical humid forests. Indigenous people of Meghalaya protect some forest patches traditionally since time immemorial due to strong religious beliefs anointed with groves (sacred forests), such tracts of forests are species rich and regarded as virgin forests, and are popularly known as sacred forests or sacred groves (Gadgil & Vartak, 1975, 1976). The forefathers of indigenous people made a simple way for conservation of sacred forests by attaching various orthodox religious laws, myths and taboos attached with them. Over the years, these forests have become a part of the cultural life of the indigenous people of the Meghalaya. These forests often act as a gene bank, as they are rich in genetic resources (UNEP, 1995; Mc.Neely, 1996; Edwards & Cyrus, 1998). Moreover, these forests are considered as a repository of plant diversity and refugia for rarity and endemism (Jeeva & Anusuya, 2005; Jeeva *et al.,* 2005, 2006a; Mishra *et al.,* 2005b, 2005c). Previously, the sacred forests were found near each and every village. Ongoing struggle between a popular belief and need of people has resulted in extinction of several sacred forests, many are on verge of extinction, some are facing different degrees of disturbance and few forests are still protected and they harbor climax vegetation.

Change in landscape due to human interference has been identified as the major threat to biodiversity in terms of loss of species and change in community structure (Daniels *et al.,* 1991; Daniels *et al.,* 1995; Gadgil, 1996; Daniels, 1997; Menon & Bawa, 1997; Pramod *et al.,* 1997*;* Nagendra & Gadgil, 1998*;* Nagendra & Gadgil, 1999a*,* 1999b*;* Nagendra, 2001; Nagendra & Utkarsh, 2003). Oldfield and his coworkers (1998) have reported 10% of the world's tree species under threat. Anthropogenic disturbance causes maximum loss to trees layer than other plant groups such as shrubs, climbers and herbs. Thus, conservation of plant resources has become a global issue owing to habitat destruction, deforestation, climate change and environmental degradation (Phillips, 1997; Myking, 2002).

The conservation and forest management operation requires quantitative information of biodiversity inventories such as species diversity, population structure, and distribution pattern of species and other community attributes (Andel, 2001; Singh, 2002). Such biodiversity inventories are best integrated with the timber resources in order that forest management operations can be planned (Rennolls & Laumonier, 2000). Being a dominant life form, trees are easy to locate precisely and to count (Condit *et al.*, 1996) and are also relatively better known, taxonomically (Gentry, 1992; Sagar *et al.,* 2003). Quantification of tree species diversity is an important aspect as it provides resources and habitat for many species (Cannon *et al.,* 1998).

In the state of Meghalaya hilly terrain and undulating topography have resulted in marked variation in altitude, irrespective of distance. The similar kind of forests situated in nearby area having differed altitude, the species composition varied greatly. The ecologists have paid little attention on researches to gather information, that how altitudinal variation affects species composition, plant diversity, community attributes and population structure in similar kinds of forests. Pooling the data from such kind of studies could be an effective tool for development of appropriate measures for rehabilitation of degraded forests land using plant species regenerating efficiently in respective edapho-climatic conditions.

In view of the above, the present study was carried out to determine extent of change in species composition, plant diversity, community attributes and population structure in Mairang and Mawphlang sacred forests of Meghalaya, representing of subtropical humid forest.

Materials and methods

Study area

The present study was conducted in two sacred forests of Meghalaya namely, Mawphlang and Mairang sacred forests, representing subtropical humid forests. (*Fig.1*). The Mawphlang sacred forest (25^{0}34'N, 91^{0}56E, altitude 1430m asl) is located about 28 km southwest of Shillong in the East Khasi Hills district of Meghalaya. It occupies an area of about 75 hectare and is surrounded by a landscape covering grassland vegetation. Another study site, the Mairang sacred forest (125^{0}33'N, 91^{0}38'E, 1748 m asl) is situated near Mawnai village at Mairang town in the West Khasi Hills district of Meghalaya and it has an area of about 80 ha. The distance between the two forests is about 30 km, but they are situated at an altitudinal variation of about 300 m asl.

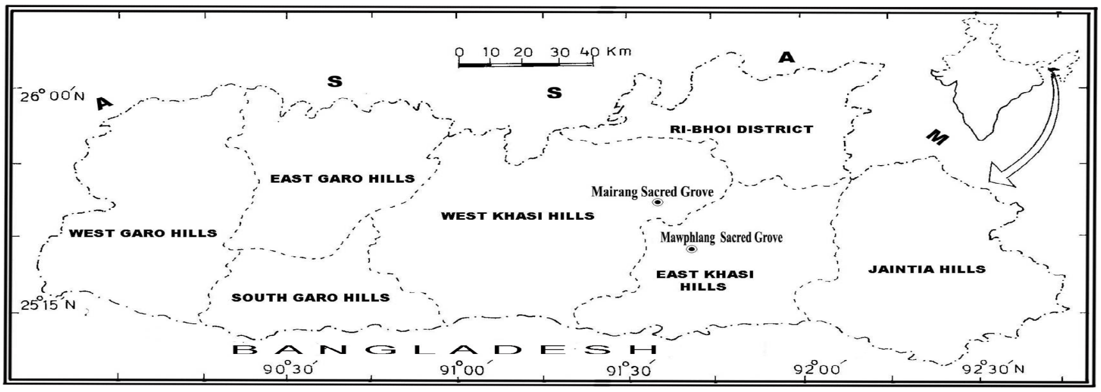

***Figure 1.** Geographical location of study area*

Vegetation profile

The vegetation of both sacred forests falls under semi-evergreen subtropical broad-leaved wet-hill forests (Champion & Seth, 1968). The canopy tree species are *Aporusa dioica, Castanopsis kurzii*, *Camellia caduca*, *Corylopsis himalayana*, *Engelhardtia spicata*, *Exbucklandia populnea*, *Ficus nerifolia*, *Garcinia cowa*, *Ficus nerifolia*, *Myrica esculenta*, *Pyrus pashia*, *Quercus griffithi*, *Quercus dealbata*, *Quercus glauca*, *Rhododendron arboreum and Taxus baccata* in Mawphlang sacred forest. However, canopy layer of Mairang sacred forest is composed of *Aesculus assamica*, *Aporusa roxburghii*, *Beilschmiedia brandisii*, *Beilschmiedia roxburghiana*, *Citrus medica*, *Cleidion javanicum*, *Cryptocarya amygdalina*, *Cryptocarya andersonii*, *Dysoxylum binecteriferum*, *Echinocarpus assamicus*, *Echinocarpus dasycarpus*, *Echinocarpus murex*, *Fissistigma wallichii*, *Macropanax undulatus*, *Ostodes paniculata*, *Phoebe* sp., *Quercus griffithii*, *Quercus smiserrata, Rhus acuminata*, *Sapium baccatum Schima wallichii* and *Trevesia palmata*. The tree species composition suggests that these forests are semi-evergreen types (NRSA, 1995; FSI, 1997). The canopy cover, light interception, and disturbance index justify that there is no marked variation in status of two sacred forests, in terms of disturbance. The disturbance index was very low and it was slightly higher in the Mairang sacred forest than Mawphlang sacred forest. The value of disturbance index depicts that both the forests are undisturbed/mildly disturbed (*Table 1*).

Table 1. *Canopy cover, light interception and disturbance index in the two sacred forests of Meghalaya*

Parameters	**Sacred forests**	
	Mairang	**Mawphlang**
Canopy cover (%)	50 – 100	60 – 100
Light interception (%)	40 – 100	50 – 100
Disturbance index (%)	0 – 3	0 – 1

Climate and soil

The monsoon season is characteristics of the climate of Meghalaya. The state receives rainfall throughout the year and about 85% of the annual rainfall is received during the wet season (June to September). The mean annual rainfall is accounted as 2500mm (Jeeva et al., 2006b). The soil is mainly lateritic and derived from gneisses, schists and granites of Archean age (Gansser, 1964).

Methodology

The field study was conducted during 2000-2001 following the methods as outlined by Misra (1968), Kershaw (1973), Muller-Dombois and Ellenberg (1974). The vegetation analysis was done by quadrat method. In each forest, 50 quadrats were laid randomly for trees (gbh > 20cm) and shrubs (gbh 5cm to 20cm and/or individuals more than 1m height), using quadrats of size 10m × 10m and 5m × 5m, respectively. For seedlings (gbh < 5cm and/or individuals up to 1m height), 100 quadrats (1m × 1m size) were laid in each forest. The density, frequency, basal area and importance value index (IVI) were computed. The distribution pattern of species was determined by computing Whitford index (Whitford, 1948). The dominance-distribution pattern was determined at both the species and family levels. The disturbance index was calculated by using the formula used by Mishra *et al.* (2003). The species richness index (Margalef, 1958), Shannon diversity index (Shannon and Weaver, 1949) and Simpson dominance index (Simpson, 1949) were determined.

Species richness index (Margalef, 1958) = S – 1/ ln N
where, S is the total number of species, N is total number of individuals and ln is log2.

Diversity index (Shannon and Weaver, 1949)

$$H' = -\sum_{i=1}^{s} pi \ln pi$$

where, *H'* is the Shannon–Weiner diversity index, pi is the proportion of IVI of a species i.e. (*ni* / *N*).

Dominance index (Simpson, 1949)

$$\mathrm{Cd} = \sum_{i=1}^{s} (pi)^2$$

$$\text{Disturbance index (\%)} = \frac{\text{Number of tree stumps}}{\text{Total number of trees including tree stumps} \times 100} \qquad \text{(Eq.1)}$$

Plants species were identified using regional floras (Balakrishnan, 1981-83, Haridasan and Rao, 1985-87, Kanjilal *et al.,* 1934-40). Plant specimens were counter-checked with the reference material available at the Botanical Survey of India, Eastern Circle Shillong and herbarium of the Department of Botany, North-Eastern Hill University, Shillong. The Red Data Book (Nayar & Sastry, 1987, 1988, 1990) and Balakrishnan & Vasudeva (1983) were consulted to ascertain rarity and endemicity.

Results

Species composition, distribution pattern and similarity

Altogether, 186 woody species belonging to 124 genera and 59 families of angiosperms were recorded from one ha area of two forests (i.e., 0.5 ha area of each forest). Result showed that contagious distribution was predominant in both the forests. Of 133 species in Mairang sacred forest, 132 species showed contagious and one species namely *Citrus medica* was distributed randomly. On other hand, 62 species showed contagious, 9 species random and 10 species regular distribution in Mawphlang sacred forest. The Sϕrensen index of similarity between two forests was calculated very low (27%), only 29 species were common in both the forests (*Appendix 1*).

Endemicity and rarity

From both the forests, 11 endemic, 7 rare and 5 rare as well as endemic species were recorded. The rare endemics were *Baliospermum micranthum, Cinnamomum pauciflorum, Ilex embelioides, Ilex khasiana* and *Lindera latifolia.* Among rare species, *Anomospermum excelsum, Antidesma roxburghii, Baliospermum micranthum, Beilshmiedia fagifolia, Cinnamomum pauciflorum, Cordia fragrantissima, Fagraea ceilanica, Helicia excelsa* and *Psychotria symplocifolia* and *Camellia caduca* were noticed. The endemic species were *Carpinus viminca, Daphniphyllum himalayense, Elaeocarpus acuminatus, Erythroxylum kunthianum, Michelia punduana, Neillia thyrsiflora, Persea kingii, Quercus glauca, Schima khasiana* and *Zanthoxylum khasianum* (*Appendix 1*).

Floristic richness, diversity and dominance

Woody species content was markedly high in Mairang sacred forest (133 species) than Mawphlang sacred forest (81 species). A similar trend of result was also obtained with respect to generic composition, a total of 92 genera in former and 65 genera in later case were recorded. Similarly, family richness was also higher in Mairang sacred forest (48 families) than Mawphlang (40 families) sacred forest. The number of species per 100 m^2 was reported very high in Mairang sacred forest (26) than Mawphlang sacred forest (12). Species richness index and Shannon diversity index were high in Mairang sacred forest. The Simpson dominance index was contrary to the diversity index. Both the forests had high diversity and low dominance indices (*Table 2*).

Table 2. *Plant diversity and other community attributes in the two sacred forests of Meghalaya*

Parameters	**Sacred forests**	
	Mairang sacred forest	**Mawphlang sacred forest**
Number of families	48	40
Number of genera	92	65
Number of species	133	81
Species richness (species per 100 m^2)	26 ± 1.5	12 ± 1.2
Tree density (individuals ha^{-1})	1256 ± 64	1490 ± 59
Sapling density (individuals ha^{-1})	5690 ± 114	4230 ± 80
Seedling density (individuals ha^{-1})	41700 ± 417	37900 ± 379
Tree basal area (m^2 ha^{-1})	42.8 ± 3.9	21.7 ± 2.3
Margalef species richness index	18.5	12. 4
Shannon diversity index	4.5	3.9
Simpson dominance index	0.014	0.01

In the Mairang sacred forest, *Citrus medica* was the dominant (IVI 14. 3) species, however, *Rhododendron arboreum* was the dominant (IVI 39. 65) species in Mawphlang sacred forest. *Alangium chinense* (IVI 0.25) and *Ardisia undulata* (IVI 0.24) were least dominant species in Mairang and Mawphlang sacred forests, respectively. The distribution of IVI among species was more uniform among the species in Mairang sacred forest (*Appendix 1*). The dominance-distribution curve followed a log-normal distribution pattern in both the forests, with short curve in Mawphlang sacred forest (*Fig. 2*).

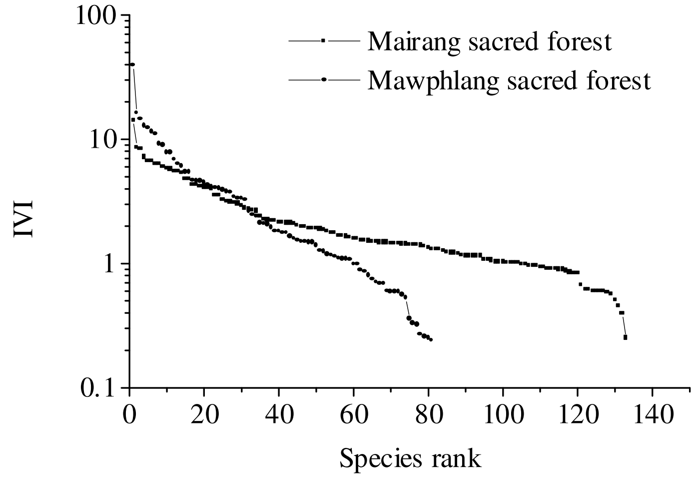

***Figure 2**. Dominance-distribution pattern of species in two sacred forests of Meghalaya*

Density and tree basal area

Result showed that seedling recruitment was markedly high in both the forests, seedling density was high in Mairang sacred forest (41700 individuals ha^{-1}) than Mawphlang sacred forest (37900 individuals ha^{-1}). Sapling density also followed a similar trend. On the contrary, Mawphlang sacred forest had more tree density (1490 individuals ha^{-1}) than Mairang sacred forest (1256 individuals ha^{-1}). Irrespective of tree density, the basal area was about two folds higher in Mairang sacred forest (42.8 ± 3.9 m^2 ha^{-1}) than the Mawphlang sacred forest (21.7 ± 2.3 m^2 ha^{-1}) (*Table 2*).

Girth class distribution of individuals declined sharply from lower to higher girth classes in both the forests and it showed a pyramidal structure. Both the forests had a wide-range girth structure. The Mawphlang sacred forest was represented by individuals up to girth class 250-300cm. However, Mairang sacred forest had a few individuals having girth more than 300cm. Adults (gbh 20-50cm) were predominantly present in both the forests, i.e., 62% and 80% in Mairang and Mawphlang sacred forests, respectively (*Fig. 3*).

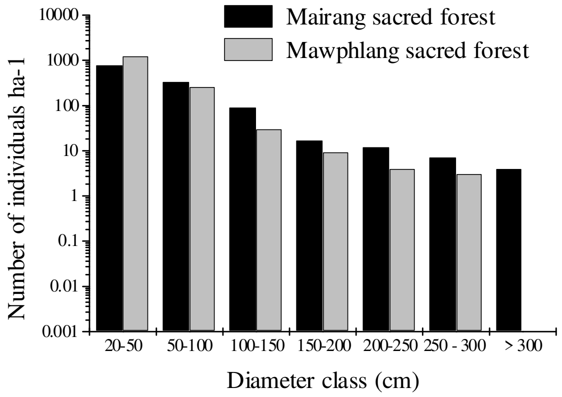

Figure 3. *Girth-distribution of trees (gbh >20 cm) in the Mairang and Mawphlang sacred forests Girth-distribution*

Distribution and dominance of families of angiosperms

Altogether, 59 families of angiosperms were reported from both the forests. The Mairang sacred forest was represented by 48 families, however, 40 families were recorded from Mawphlang sacred forest. In Mairang sacred forests, Lauraceae (17 species) was the dominant family. Euphorbiaceae, the codominant family was represented by 11 species. Euphorbiaceae and Lauraceae with 12 and 10 species respectively, were dominant and co-dominant families in the Mawphlang sacred forest. The number of families represented by a single species was higher in Mawphlang sacred forest (26) than Mairang sacred forest (23). Generic composition had depicted that Euphorbiaceae dominating in both the forests as it had highest number of genera (*Table 3*). Dominance distribution of families has resulted in log-normal distribution of families in both the forests (*Fig. 4*).

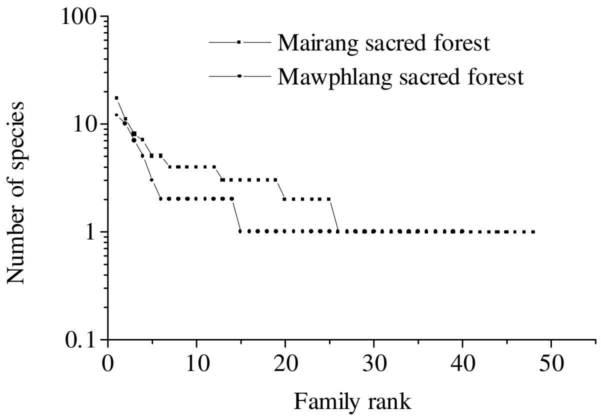

Figure 4. *Dominance-distribution pattern of families in two sacred forests of Meghalaya*

Table 3. *Genera and species composition of different families of angiosperms recorded from the two sacred forests of Meghalaya. The families are arranged with respect to family rank based on number of species in a particular family*

Family Rank	**Family**	***Mairang sacred forest***		**Family**	***Mawphlang sacred forest***	
		Genera	**Species**		**Genera**	**Species**
1	Lauraceae	7	17	Euphorbiaceae	10	12
2	Euphorbiaceae	9	11	Lauraceae	7	10
3	Araliaceae	6	8	Fagaceae	2	7
4	Rubiaceae	7	7	Theaceae	4	5
5	Elaeocarpaceae	2	5	Lamiaceae	3	3
6	Theaceae	3	5	Clusiaceae	1	2
7	Anacardiaceae	2	4	Elaeocarpaceae	2	2
8	Verbenaceae	3	4	Ericaceae	2	2
9	Symplocaceae	1	4	Hamamelidaceae	2	2
10	Magnoliaceae	1	4	Magnoliaceae	1	2
11	Oleaceae	2	4	Moraceae	1	2
12	Rosaceae	4	4	Myricaceae	1	2
13	Rutaceae	3	3	Proteaceae	1	2
14	Annonaceae	3	3	Rosaceae	2	2
15	Fagaceae	1	3	Actinidiaceae	1	1
16	Buxaceae	1	3	Apocynaceae	1	1
17	Ulmaceae	2	3	Aquifoliaceae	1	1
18	Myrsinaceae	2	3	Araliaceae	1	1
19	Sterculiaceae	1	3	Asteraceae	1	1
20	Sapindaceae	2	2	Berberidaceae	1	1
21	Daphniphyllaceae	1	2	Betulaceae	1	1
22	Boraginaceae	1	2	Caprifoliaceae	1	1
23	Myrtaceae	2	2	Corylaceae	1	1
24	Aquifoliaceae	1	2	Daphniphyllaceae	1	1
25	Fabaceae	2	2	Elaeagnaceae	1	1
26	Juglandaceae	1	1	Erythroxylaceae	1	1
27	Pittosporaceae	1	1	Juglandaceae	1	1
28	Myricaceae	1	1	Meliaceae	1	1
29	Meliaceae	1	1	Menispermaceae	1	1
30	Asteraceae	1	1	Myrsinaceae	1	1
31	Berberidaceae	1	1	Myrtaceae	1	1
32	Simaroubaceae	1	1	Olacaceae	1	1
33	Erythroxylaceae	1	1	Oleaceae	1	1
34	Salicaceae	1	1	Polygalaceae	1	1
35	Cannaceae	1	1	Rubiaceae	1	1
36	Combretaceae	1	1	Rutaceae	1	1
37	Betulaceae	1	1	Symplocaceae	1	1
38	Moraceae	1	1	Ulmaceae	1	1
39	Thymeliaceae	1	1	Urticaceae	1	1
40	Tiliaceae	1	1	Verbenaceae	1	1
41	Caprifoliaceae	1	1			
42	Loganiaceae	1	1			
43	Cappaceae	1	1			
44	Sabiaceae	1	1			
45	Urticaceae	1	1			
46	Clusiaceae	1	1			
47	Cornaceae	1	1			
48	Caesalpiniaceae	1	1			

Discussion

The findings of present study revealed that both the forests inhabit climax vegetation and are best representative of subtropical humid forests of Meghalaya. During the investigation, 133 and 81 species were recorded from 0.5 ha area of Mairang and Mawphlang sacred forests, respectively. High species richness in former case may be due to formation of gaps due to felling of few mature trees, which had facilitated introduction of more species from neighboring forests. Mishra *et al.* (2003) have reported that mild disturbance supports species richness and have recorded high species richness in mildly/moderately disturbed stand of Swer sacred grove in Meghalaya. A similar result was also noticed by Upadhaya *et al.* (2003) and they have recorded 123 woody species from 1 ha area (0.5 ha each of Ialong and Raliang sacred groves in Jaintia hills of Meghalaya). High species richness in Mairang sacred forest showed that mild disturbance is linked with species turnover, colonization and high species richness (Whittaker, 1975; Connell, 1979). Mairang sacred forest had high species richness per unit area, which is due to presence of synuisae in the forest (Richards, 1996). Predominance of contagious distribution indicates interaction of abiotic and biotic factors acting together as population and it was more conspicuous in case of Mairang sacred forest. Insufficient mode of seed dispersal had resulted in clumping (Richards, 1996). Although clumping is the characteristic feature of natural forests (Armesto *et al.,* 1986). Jamir (2000) has also reported a similar result in sacred forests of Meghalaya.

Change in edapho-climatic conditions due to variation in the altitude has altered species composition in two similar kinds of forests. The result showed that change in species composition is highly linked with variation in altitude, as both the forests are highly dissimilar (dissimilarity index 73%) in terms of species composition. The present finding is in conformity with the work of Liberman *et al.* (1996) and Kadavul & Parthasarathy (1999). The change in the position of dominant and codominant species and families in these two forests is also associated with variation in altitude. Despite high family richness in Mairang sacred forest, the number of monospecific families were low. This could be attributed to elimination of some families, which are very sensitive to low level of disturbance. Moreover, there was chance of inclusion of more species in the families with increased dominance. These sacred forests had a number of endemic and rare plants. Haridasan & Rao (1985-87) have also pointed out that sacred forests of Meghalaya harbor a large number of such species, which are confined to sacred forests only.

Wide range girth-distribution in two forests indicates stability and complexity of community. Better recruitment of seedlings and predominance of individuals in lower girth classes (adults) showed high regeneration efficacy at forest stand level (Mishra *et al.,* 2003; Laloo *et al.,* 2006). Conversion of seedlings into saplings follows a similar pattern in two forests. Low conversion of saplings into trees in Mairang sacred forests could be due to presence of high canopy tree species. Gaps also facilitated expansion of crown of trees, and subsequently suppression of growth and survival of saplings growing beneath these trees (Mishra *et al.,* 2003). Population structure at forest stand level indicates that these forests harbor a growing population (Mishra, 2004). Girth-distribution follows reverse J shaped curve, which suggests that both the forests are climax and stable (Mishra *et al.,* 2005a).

The dominance-distribution pattern at the levels of species and family justifies mature, stable and complex nature of vegetation. High species content and more even distribution of IVI among the species in Mairang sacred forest depict high degree of stability and complexity of community (Mishra *et al.,* 2004; Mishra et al, 2005a). It has been argued that the ecosystem with high species diversity is more stable and resilient to environmental disturbances than those having low species, diversity (Hurd *et al.*, 1971; McNaughton, 1977, 1985; Tilman, 1988; Frank & McNaughton, 1991; Tilman & Downing, 1994).

Conclusion

From the present study it can be concluded that altitudinal variation leads to change in species composition and taxonomic position even in similar kind of forest. This could be due to altered edapho-climatic conditions of the area. Low level of disturbance in terms of felling of few trees can favour natural regeneration of woody species, it involves increased percent conversion of seedlings to saplings, and saplings to trees. Form earlier studies it is evident that majority of sacred forests are facing different degrees of disturbance, few forests (*ca.* 10%) are still intact. In view of this, it is recommended that such kind of studies in addition to regeneration behaviour of dominant and important species need much attention of ecologists to find out appropriate strategy for *in situ* conservation of genetic resources on sustained basis. Strengthening researches and pooling data generated through extensive studies could be a tool for rehabilitation of degraded sacred forests, by planting suitable species.

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Appendix 1. *Community attributes of woody plant species in the two sacred forests of Meghalaya. Species rank was determined o the basis of IVI of a species*

Plant species	**Family**	**Mairang sacred forest**			**Mawphlang sacred forest**		
		IVI	**Specie s rank**	**Whitford index**	**IVI**	**Species rank**	**Whitford index**
Acanthopanax aculeatum Seem.	Araliaceae	1.3	81	0.17	-	-	-
Actinidia callosa Lindl.	Actinidiaceae	-	-	-	0.26	23	0.15
Aesculus assamica Griff.	Sapindaceae	6.73	5	0.07	-	-	-
Alangium chinense (Lour.) Harms.	Cornaceae	0.25	133	0.83	-	-	-
Alnus nepalensis D. Don.	Betulaceae	-	-	-	4.07	10	0.21
Alphonsea ventricosa Hk.f. & Thunb.	Annonaceae	2.89	30	0.16	-	-	-
Anomospermum excelsum Dalz.	Euphorbiaceae	-	-	-	0.8	45	0.09
Antidesma khasianum Hk. f.	Euphorbiaceae	-	-	-	3.47	62	0.05
Antidesma diandrum (Roxb.) Roth.	Euphorbiaceae	1.63	59	0.17	-	-	-
Antidesma roxburghii Wall.	Euphorbiaceae	-	-	-	0.32	43	0.1
Aporusa dioica (Roxb.) Muell.-Arg.	Euphorbiaceae	-	-	-	4.17	9	0.22
Aporusa roxburghii Baill.	Euphorbiaceae	5.75	11	0.14	-	-	-
Aralia armata (G.Don) Seem.	Araliaceae	1.23	85	0.17	-	-	-
Ardisia nerifolia DC.	Myrsinaceae	1.01	103	0.38	-	-	-
Ardisia undulata Cl.	Myrsinaceae	0.6	124	1	0.24	51	0.08
Baliospermum micranthum Muell.-Arg.	Euphorbiaceae	-	-	-	0.25	44	0.1
Bauhinia variegata Linn.	Caesalpiniaceae	0.6	125	1	-	-	-
Beilshmiedia brandishii Hk.f.	Lauraceae	5.56	12	0.12	1.95	56	0.06
Beilshmiedia fagifolia Nees.	Lauraceae	1.51	66	0.22	-	-	-
Beilshmiedia roxburghiana Nees.	Lauraceae	1.96	47	0.17	-	-	-
Berberis wallichiana DC.	Berberidaceae	-	-	-	6.3	57	0.06
Betula alnoides Buch.-Ham. ex D.Don	Betulaceae	1.26	84	0.5	-	-	-
Bidens spinosa Linn	Asteraceae	-	-	-	0.27	58	0.06
Boehmeria platyphylla D.Don.	Urticaceae	0.62	123	1	-	-	-
Boehmeria sidaefolia Wedd.	Urticaceae	-	-	-	1.17	39	0.11
Brassiopsis aculeata Seem	Araliaceae	1.39	79	0.22	0	0	0
Breynia retusa (Dennst.) Alst.	Euphorbiaceae	-	-	-	0.59	46	0.09
Callicarpa arborea Roxb.	Verbenaceae	2.24	37	0.16	-	-	-
Callistemon citrinus (Curt) Skeels	Myrtaceae	1.44	74	0.22	-	-	-
Caloxylon leucocarpum Kurz.	Euphorbiaceae	-	-	-	1.82	52	0.07
Camellia caduca Cl. ex Brandis.	Theaceae	0.83	119	0.25	4.49	53	0.07
Camphora glandulifera Nees.	Lauraceae	-	-	-	1.77	36	0.12
Capparis assamica Hk.f. & Th.	Cappaceae	0.99	106	0.38	-	-	-
Carallia brachiata (Lour.) Merr.	Cannaceae	1.56	62	0.17	-	-	-
Carpinus viminca Wall. ex Lindl.	Corylaceae	-	-	-	1.19	32	0.13
Castanopsis armata Spach.	Fagaceae	-	-	-	1.77	47	0.09
Castanopsis kurzii (Hance).	Fagaceae	-	-	-	4.67	37	0.12
Castanopsis sp.	Fagaceae	-	-	-	3.77	40	0.11
Celtis cinnamomea Lindl. ex Planch.	Ulmaceae	0.99	105	0.25	-	-	-

Plant species	Family	Mairang sacred forest			Mawphlang sacred forest		
		IVI	**Species rank**	**Whitford index**	**IVI**	**Species rank**	**Whitford index**
Celtis tetranda Roxb.	Ulmaceae	3.22	25	0.16	0.69	12	0.19
Cinnamomum glanduliferum (Wall.) Meissn.	Lauraceae	0.58	128	0.5	11.03	16	0.17
Cinnamomum pauciflorum Nees	Lauraceae	3.12	28	0.22	3.99	48	0.09
Cinnamomum sp.	Lauraceae	-	-	-	1.58	54	0.07
Cissampelos pareira Linn.	Menispermaceae	-	-	-	1.47	41	0.11
Citrus medica Linn.	Rutaceae	14.34	1	0.04	-	-	-
Cleidion javanicum Bl.	Euphorbiaceae	5.82	10	0.13	2.67	33	0.13
Clerodendrum infortunatum auct. non Linn.	Verbenaceae	0.6	126	1	-	-	-
Cleyera grandiflora Hk.f. & Th. ex Dyer	Theaceae	-	-	-	1.64	59	0.06
Coffea khasiana Hk.f.	Rubiaceae	1.93	48	0.39	-	-	-
Combretum acuminatum Roxb.	Combretaceae	1.48	67	0.22	-	-	-
Cordia fragrantissima Kurz.	Boraginaceae	2.17	40	0.18	-	-	-
Cordia grandis Roxb.	Boraginaceae	1.55	63	0.22	-	-	-
Corylopsis himalayans Griff.	Hamamelidaceae	-	-	-	0.33	20	0.16
Croton caudatus Geisel.	Euphorbiaceae	0.85	118	0.25	-	-	-
Cryptocarya amygdalina Nees.	Lauraceae	8.68	2	0.08	-	-	-
Cryptocarya andersonii King ex Hk. f.	Lauraceae	3.96	22	0.13	5.45	71	0.03
Cryptocarya sp.	Lauraceae	-	-	-	3.78	67	0.04
Cyclostemon assamicus Hk.f.	Euphorbiaceae	4.13	20	0.16	-	-	-
Daphne cannabina Wall.	Thymeliaceae	1.15	93	0.5	-	-	-
Daphniphyllum himalayense (Benth.) Muell.-Arg.	Daphniphyllaceae	1.86	52	0.22	0.59	76	0.01
Daphniphyllum sp.	Daphniphyllaceae	3.53	23	0.14	-	-	-
Dysoxylum binectariferum Hk.f. & Bedd.	Meliaceae	3.51	24	0.24	1.1	63	0.05
Echinocarpus dasycarpus Benth.	Elaeocarpaceae	4.18	19	0.13	0.59	60	0.06
Echinocarpus murex Benth.	Elaeocarpaceae	6.39	7	0.08	-	-	-
Elaeagnus latifolia Linn.	Elaeagnaceae	-	-	-	1.07	34	0.13
Elaeocarpus acuminatus Wall ex Mast.	Elaeocarpaceae	3.13	27	0.11	-	-	-
Elaeocarpus floribundus Bl.	Elaeocarpaceae	0.94	110	0.25	-	-	-
Elaeocarpus lancifolius Roxb.	Elaeocarpaceae	2.26	36	0.16	-	-	-
Elaeocarpus sikkimensis Mast.	Elaeocarpaceae	-	-	-	9.21	29	0.14
Elsholtzia blanda Benth.	Lamiaceae	-	-	-	2.08	24	0.15
Embelia ribes Burm. f.	Myrsinaceae	0.87	117	1.5	-	-	-
Engelhardtia spicata Leschn ex Bl.	Juglandaceae	4.81	15	1	6.06	17	0.17
Eriobotrya dubia Decne.	Rosaceae	1.45	72	0.38	1.14	30	0.14
Erythrina stricta Roxb.	Fabaceae	1.01	104	0.38	-	-	-
Erythroxylum kunthianum Wall. ex. Kurz.	Erythroxylaceae	1.65	58	0.22	12.27	6	0.23
Eugenia lanceolaria Roxb.	Myrtaceae	-	-	-	0.99	18	0.17
Eupatorium odoratum Linn.	Asteraceae	1.3	83	0.63	-	-	-
Eurya acuminata DC.	Theaceae	3.05	29	0.18	16.3	74	0.02
Eurya japonica Thunb.	Theaceae	1.82	53	0.16	12.85	77	0.01
Exbucklandia populnea (R. Br. ex Griff) R. W. Br.	Hamamelidaceae	-	-	-	1.27	64	0.05
Fagraea ceilanica Thunb.	Loganiaceae	1.03	98	0.38	-	-	-
Ficus hispida Linn. f.	Moraceae	1.44	73	0.17	-	-	-
Ficus silhetensis Miq.	Moraceae	-	-	-	0.56	19	0.17
Ficus sp.	Moraceae	-	-	-	8.91	11	0.2

Plant species	Family	Mairang sacred forest			Mawphlang sacred forest		
		IVI	**Species rank**	**Whitford index**	**IVI**	**Species rank**	**Whitford index**
Fissistigma wallichii (Hk.f. & Th.) Merr.	Annonaceae	7.19	4	0.16	-	-	-
Garcinia cowa Roxb. ex DC.	Clusiaceae	0.83	120	0.25	0.99	13	0.19
Garcinia lancifolia (G. Don) Roxb.	Clusiaceae	-	-	-	1.25	25	0.15
Gaultheria ovalifolia Wall.	Ericaceae	-	-	-	0.53	21	0.16
Glochidion assamicum Hk.f.	Euphorbiaceae	1.41	75	0.13	4.61	7	0.23
Glochidion khasicum Hk.f.	Euphorbiaceae	0.9	115	0.25	0.71	68	0.04
Grewia multiflora Juss.	Tiliaceae	1.35	80	0.17	-	-	-
Helicia excelsa Bl.	Proteaceae	-	-	-	2.06	49	0.09
Helicia nilagirica Bedd.	Proteaceae	-	-	-	4.28	26	0.15
Ilex embelioides Hk.f.	Aquifoliaceae	1.15	94	0.5	-	-	-
Ilex khasiana Purk.	Aquifoliaceae	0.96	109	0.38	3.34	2	0.29
Ixora acuminata Roxb.	Rubiaceae	0.6	127	1	-	-	-
Leptodermis griffithii Hk.f.	Rubiaceae	1.03	99	0.38	-	-	-
Ligustrum indicum (Lour) Merr.	Oleaceae	1.48	68	0.22	-	-	-
Ligustrum robustum (Roxb.) Bl.	Oleaceae	0.57	129	1	-	-	-
Lindera caudata (Nees) Hook. f.	Lauraceae	-	-	-	1.4	42	0.11
Lindera latifolia Hk.f.	Lauraceae	0.97	107	0.25	-	-	-
Lindera pulcherrima (Nees) Benth.	Lauraceae	4.11	21	0.28	-	-	-
Litsea citrata Bl.	Lauraceae	1.78	54	0.13	-	-	-
Litsea salicifolia (Roxb. ex Nees.) Hk.f.	Lauraceae	1.08	95	0.38	-	-	-
Machilus bombycina King ex Hk. f.	Lauraceae	-	-	-	0.6	72	0.03
Macropanax undulatus (wall. ex G.Don) Seem.	Araliaceae	6.37	8	0.08	-	-	-
Magnolia excelsa Wall.	Magnoliaceae	-	-	-	0.86	50	0.09
Magnolia insignis (Wall.) Bl.	Magnoliaceae	-	-	-	1.83	55	0.07
Mahonia pycnophylla (Fedde) Takeda	Berberidaceae	2.21	39	0.28	-	-	-
Meliosma wallichii Planch. ex Hk.f.	Sabiaceae	0.62	122	0.5	-	-	-
Melodinus khasianus Hook. f.	Apocynaceae	-	-	-	3.24	31	0.14
Michelia champaca Linn.	Magnoliaceae	1.52	64	0.17	-	-	-
Michelia lanuginosa Wall	Magnoliaceae	0.92	112	0.25	-	-	-
Michelia oblonga Wall. ex Hk.f.	Magnoliaceae	1.01	101	0.25	-	-	-
Michelia punduana Hk.f.	Magnoliaceae	2.8	31	0.16	-	-	-
Micromelum pubescens (non Bl.)	Rutaceae	1.76	55	0.22	-	-	-
Millettia pulchra (Benth.) Kurz.	Fabaceae	1.01	102	0.25	-	-	-
Mussaenda roxburghii Hk.f.	Rubiaceae	1.22	87	0.5	-	-	-
Myrica esculenta Buch-Ham. ex D.Don.	Myricaceae	-	-	-	2.12	65	0.05
Myrica nagi Hk.f	Myricaceae	1.6	61	0.22	1.11	69	0.04
Nauclea griffithii Hav.	Rubiaceae	1.22	86	0.38	-	-	-
Neillia thyrsiflora D.Don.	Rosaceae	0.4	132	0.5	-	-	-
Neolitsea cassia (Linn.) Kosterm.	Lauraceae	-	-	-	11.6	35	0.13
Olax acuminata Benth.	Olacaceae	-	-	-	0.75	3	0.28
Olea dentata Wall ex DC.	Oleaceae	2.21	38	0.16	-	-	-
Olea dioica Roxb.	Oleaceae	0.92	113	0.25	-	-	-
Olea salicifolia Wall. ex. Cl.	Oleaceae	-	-	-	3.92	5	0.25

Plant species	Family	Mairang sacred forest			Mawphlang sacred forest		
		IVI	Species rank	Whitford index	IVI	Species rank	Whitford index
Ostodes paniculata Bl.	Euphorbiaceae	5.51	13	0.16	-	-	-
Panax armatus G. Don	Araliaceae	-	-	-	1.1	14	0.19
Persea bombycina (King ex Hk.f.) Kosterm.	Lauraceae	1.9	51	0.13	-	-	-
Persea duthiei (King ex Hk.f.) Kosterm.	Lauraceae	2.1	44	0.16	-	-	-
Persea gamblei (King ex Hk.f.) Kosterm.	Lauraceae	2.12	43	0.19	-	-	-
Persea Khasyana Missn.	Lauraceae	0.46	131	0.5	-	-	-
Persea kingii (Hk.f.) Kosterm.	Lauraceae	1.15	90	0.25	-	-	-
Phoebe attenuata (Nees) Nees.	Lauraceae	8.43	3	0.17	-	-	-
Phyllanthus retusus Dennst.	Euphorbiaceae	1.17	89	0.5	1.47	27	0.15
Picrasma javanica Bl.	Simaroubaceae	2.12	42	0.16	-	-	-
Pittosporum glabratum Lindl.	Pittosporaceae	4.32	18	0.28	-	-	-
Plectranthus striatus Benth. in Wall.	Lamiaceae	-	-	-	1.5	1	0.31
Pogostemon strigosus Benth.	Lamiaceae	-	-	-	1.55	4	0.27
Polyalthia jenkinsii Benth. & Hk.f.	Annonaceae	1.91	50	0.17	-	-	-
Polygala arillata Buch.-Ham in D. Don	Polygalaceae	-	-	-	2.4	22	0.16
Premna racemosa Schauer in DC.	Verbenaceae	-	-	-	0.89	8	0.23
Prunus cerasoides D. Don. Prodr.	Rosaceae	-	-	-	1.49	38	0.12
Prunus nepaulensis (Ser.) Steud.	Rosaceae	1.15	92	0.38	-	-	-
Psychotria symplocifolia Kurz.	Rubiaceae	1.19	88	0.5	5.42	78	0.01
Pyrus pashia D.Don.	Rosaceae	0.67	121	1	-	-	-
Quercus dealbata Hk. f. & Th. ex Miq	Fagaceae	-	-	-	4.61	73	0.03
Quercus glauca Thunb.	Fagaceae	1.98	46	0.28	7.75	79	0.01
Quercus griffithii Hk.f. & Th. ex DC. Prodr.	Fagaceae	4.76	16	0.15	14.55	70	0.04
Quercus kamroopii D. Don.	Fagaceae	-	-	-	6.89	66	0.05
Quercus semiserrata Roxb.	Fagaceae	5.39	14	0.08	-	-	-
Randia longiflora Lamk.	Rubiaceae	1.41	76	0.22	-	-	-
Rhododendron arboreum Sm.	Ericaceae	-	-	-	39.65	80	0.01
Rhus acuminata DC.	Anacardiaceae	2.03	45	0.17	-	-	-
Rhus insignis Hk.f.	Anacardiaceae	1.69	56	0.22	-	-	-
Rhus javanica Linn.	Anacardiaceae	4.34	17	0.14	-	-	-
Salix psilostigma Anders.	Salicaceae	1.4	78	0.5	-	-	-
Sapindus attenuatus Wall. Ex Hiern.Rark DC.	Sapindaceae	0.97	108	1	-	-	-
Sapium baccatum Roxb.	Euphorbiaceae	5.97	9	0.07	0.69	15	0.18
Sapium eugeniaefolium Ham. ex. Hk.f.	Euphorbiaceae	1.15	91	0.25	-	-	-
Sarcococca pruniformis Lindl.	Buxaceae	1.08	96	0.38	-	-	-
Sarcococca saligna (D.Don) Muell-Arg.	Buxaceae	1.46	71	0.25	-	-	-
Sarcococca sp.	Buxaceae	1.41	77	0.22	-	-	-
Schefflera hypoleuca (Kurz.) Harms.	Araliaceae	2.65	34	0.25	-	-	-
Schefflera venulosa (W&A) Harms.	Araliaceae	1.46	70	0.22	-	-	-
Schefflera wallichiana (W & A) Harms.	Araliaceae	2.75	32	0.16	-	-	-
Schima khasiana Dyer.	Theaceae	1.6	60	0.17	-	-	-

Plant species	Family	Mairang sacred forest			Mawphlang sacred forest		
		IVI	Species rank	Whitford index	IVI	Species rank	Whitford index
Schima wallichii (DC.) Korth.	Theaceae	6.72	6	0.09	3.36	81	0.01
Skimmia laureola (DC.) Sieb. & Zucc.	Rutaceae	1.48	69	0.38	-	-	-
Sterculia hamiltonii (O. Ktze.) Adelb.	Sterculiaceae	1.67	57	0.22	-	-	-
Sterculia roxburghii Wall.	Sterculiaceae	0.9	116	0.25	-	-	-
Sterculia villosa Roxb.	Sterculiaceae	0.51	130	0.5	-	-	-
Symplocos racemosa Roxb.	Symplocaceae	1.06	97	0.38	-	-	-
Symplocos crataegoides D.Don.	Symplocaceae	1.93	49	0.39	-	-	-
Symplocos spicata Roxb.	Symplocaceae	2.66	33	0.16	7.8	75	0.02
Symplocos theaefolia D.Don.	Symplocaceae	0.92	114	0.25	-	-	-
Syzygium balsameum (Wt.) Wall ex AM. & SM. Cowan.	Myrtaceae	1.3	82	0.17	-	-	-
Tapiria hirsuta Hk.f.	Anacardiaceae	3.17	26	0.16	-	-	-
Trevesia palmata (Roxb.) Vis.	Araliaceae	2.16	41	0.38	-	-	-
Vernonia vulkamerifolia DC.	Asteraceae	0.94	111	0.25	-	-	-
Viburnum foetidum Wall.	Caprifoliaceae	1.03	100	0.38	2.48	61	0.06
Vitex negundo Linn.	Verbenaceae	1.51	65	0.17	-	-	-
Vitex vestita Roxb.	Verbenaceae	2.4	35	0.22	-	-	-
Zanthoxylum khasianum Hk. f.	Rutaceae	-	-	-	0.36	28	0.15

Abbreviation: -, absent.

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THE IMPACT OF PERCEIVED CORPORATE ENVIRONMENTAL PERFORMANCE ON THE BEHAVIOR OF CAPITAL MARKET DECISION MAKERS: ANALYSIS OF FOOD INDUSTRY COMPANIES

DEAK, ZS.[1]* – HUFNAGEL, L.[2] – HAJDU, I.-NÉ [1]

[1] *Department of Food Economy*

[2] *Department of Mathematics and Informatics*

Corvinus University of Budapest
1118 Budapest, Villányi út 29-43., Hungary
(phone: +36-1-482-6200; fax: +36-1-209-0961)

**Corresponding author*
e-mail: zsuzsanna.deak@uni-corvinus.hu

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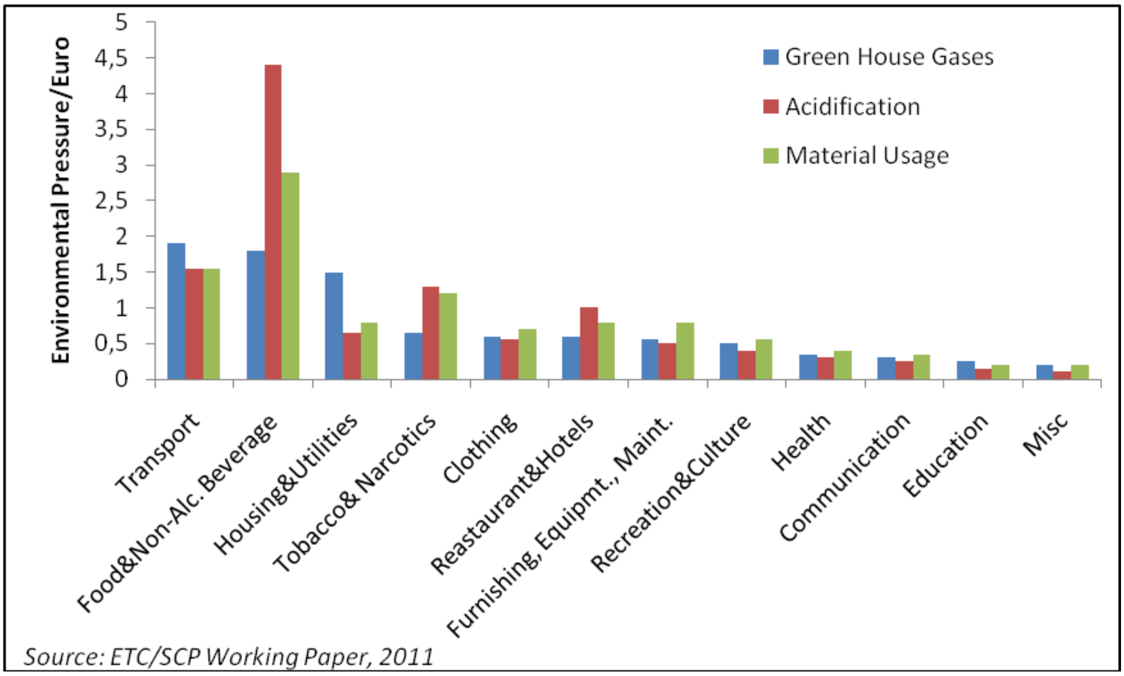
Abstract. Environmental protectionism and sustainable development has been gaining increased attention among governments, investors and consumers alike. As a result, firms are facing growing pressure from the various stakeholders to improve their environmental performance. This study is focusing on the food industry, which in recent years has been a subject of increased scrutiny due to their role in resource consumption, waste generation and unsustainable production practices. Our research is aiming to examine how the financial community evaluates the environmental stewardship of food industry companies as proxied by market reactions in response to environmental news. Are all company related environmental news items evaluated equally, and which financial and non-financial firm-specific attributes can influence market responses? Have there been changes in reactions on the stock exchange in the past two decades?

Keywords: *environmental performance, food industry, news impact, stock markets, firm-level variables*

Introduction

In the nineteenth century the basic focus was on the most efficient and fastest utilization of our natural resources in order to increase profitability. By the twentieth century, however, it became clear that the current rate of utilization will result in unsustainable social and economic development. As a result, the role of socially responsible management and their effects on profitability has become the topic of discussion. The central concern under discussion is how an individual firm's environmental performance influences its financial performance. Does a firm that endeavors to improve its environmental performance gain advantages over its competitors, or does better environmental performance only represent extra costs?

According to a recent study commissioned by the European Commission, the biggest contributors to environmental pressures are food production and consumption, transportation and housing (*Fig. 1*).



Source: ETC/SCP Working Paper, 2011

Figure 1. *Environmental Pressures per Euro of Spending of Household Consumption Categories*

Specifically, the food and drink sector contributes to some 23% of global resource use, 18% of greenhouse gas emissions and 31% of acidifying emissions (ETC/SCP, 2009). The numbers include all resource use and pollution emitted during the production of food from the farm to the supermarket shelf, including the production and application of fertilizers, fuels in agricultural machinery, electricity consumed in food processing plants etc. The United Nation reports similar figures. Of global emissions in 2005, agriculture accounted for an estimated 10-12% of carbon-dioxide, 60% of nitrous oxide and about 50% of methane (excluding emissions from electricity and fuel use). Globally, agricultural CH_4 and N_2O emissions have increased by nearly 17% from 1990 to 2005 (IPPC, 2007).

In recent international surveys reviewed, perhaps unexpectedly, some of the traditionally polluting industries fared better than food companies (*Table 1*).

Table 1. *Recent Environmental Performance Rankings by Industry Issued by Media Outlets*

Industry	**Avg. Score (out of 100)**
Panel A	
Chemicals	51.9
Electric Power	48.8
Automotive	47.9
Industrial Equipment	42.5
Metals & Mining	42.2
Forest Products	37.2
Oil & Gas	34.8
Coal	21.4
Food & Beverage	17.6
Airlines	16.6
Source: Report on Corporate Governance and Climate Change, CERES, 2006	
Panel B	
Technology	79.3
Pharmaceuticals	78.1
Banks, Insurance, Finance	72.8
Retail (other than food)	72.1
Consumer Products, Cars	71.6
Transport, Aerospace	71.3
General Industrials	71.1
Industrial Goods	69.1
Oil and Gas	68.4
Health Care	67.7
Basic Materials	64.9
Food & Beverage	62.1
Utilities	60.1
Source: Newsweek Green Rankings, 2010	

Based on stakeholder theory, expectation would be that the various concerned parties force companies to improve their environmental performance. If information based regulations works, we should see the effect of environmental news, positive or negative, in the firm’s security prices.

Review of literature

Results obtained in earlier research seeking to uncover the link between firm level social responsibility – of which environmental behavior is a subset – and financial performance have been mixed. Even though, in general, it has been found that companies experience a drop in market value following adverse environmental news, while they experience the opposite effect following good news, the findings are by no means homogenous.

Researchers have utilized various methods to investigate the relationship between environmental and financial performance. They sometimes explored the effects of specific positive (ISO certification) or negative (oil spill) events or actions on firms' financial variables (company stock prices or balance sheet items such as return on equity – ROE, return on assets – ROA or Tobin's q). Often they compared portfolios of polluting companies with more environmentally conscious ones (this is basically the same method that socially responsible investment (SRI) fund managers have adopted).

Dowell, Hart and Yeung (2000) in their study categorized US companies based on whether or not they operate at US environmental standards worldwide or adopt lower standards outside of the US where this is permitted. They found a positive correlation between Tobin's q (the ratio of the stock market value of the company to the cost of its tangible assets) and firm environmental performance. In a later study Konar and Cohen (2001), found that firms that are emitting fewer toxic chemicals, or are threatened with fewer environmental lawsuits, are also likely to have a higher Tobin's q. In a 2002 paper, King and Lenox posit that it is actually pollution prevention and not pollution remediation that results in better return on assets (ROA). Hamilton (1995) and Konar and Cohen (1997) investigated the effect of the release of the Toxics Release Inventory (TRI) data on the market value of firms, while Lanoie and Laplante (1994) and Klassen and McLaughlin (1996) looked at stock market reactions of companies to environmental news in the media. Klassen and McLaughlin documented significantly positive market reactions to independent third-party awards for environmental performance. In contrast, Gilley et al. (2000) who examined stock market reactions to environmental process improvements found negative results. Muoghalu et al. (1990) examined the impacts of hazardous waste mismanagement lawsuits on capital markets and found that the firms suffer significant losses. These varying results suggest that perhaps the market does not value all types of environmental accomplishments or misconducts equally. Additionally, King and Baerwald (1998) argue that unique firm characteristics influence how events are reported and interpreted when comparing environmental performance. Recent studies (Cormier and Magnan, 2007; Wagner, 2010; Horváthová, 2010) also confirm that these diverging outcomes received could be because of specific firm-level characteristics (such as size, R&D expenditure, advertising intensity, riskiness, leverage, industry, country etc.). This would support the resource-based view of strategic management (see Barney, 1986; Wernerfelt, 1984) based on which a firm's superior ability to manage their environmental problems and reputation compared to others in the industry could lead to higher returns.

There are fewer articles dedicated to the research of how market reactions developed over time. Dasgupta et al (2005) concludes that the average market reaction to negative events is indeed changing over time. They have examined the period between 1992 and 2000. Blancard and Laguna (2009), when looking at the effects of chemical disasters between 1995 and 2005, however found no significant differences among their selected periods.

Besides the various measurements used to evaluate environmental performance and the question of the specific setting in which the firms operate an additional area of contention is an econometric one. Researchers have observed that stock markets have a certain way of operating. For example, Jegadeesh and Titman (1993) observed that stocks that perform well/poorly over a three- to twelve-month period tend to continue their tendency in the next three to twelve months. This tendency, called the momentum, is an anomaly and has largely been attributed to the cognitive bias of „irrational investors" who under/overreact to the release of firm-specific information. Furthermore, they have noticed that the error terms do not follow a normal distribution with constant variance. To mitigate these effects researchers have proposed various econometric models (see more in detail in the Methodology section).

The four research questions we seek answers to are therefore the following: how are results influenced

Q1: by the econometric model used,
Q2: by the type of environmental events reported,
Q3: by company level characteristics (both financial and non-financial), and
Q4: by the time elapsed?

Methodology

The event study methodology developed by Fama, Fisher, Jensen, and Roll (1969) has become the standard method of measuring stock price reaction to some announcement or event in the financial economics literature. Event studies have been used to test the null hypothesis that markets efficiently incorporate new information and that under the maintained hypothesis of market efficiency, to examine the impact of an event on the wealth of a firm's shareholders (Binder, 1998).

An event study starts with identification of the event of interest and the event window, which is the time period over which the stock prices of firms will be examined. Assessment of an event's impact requires a measure of abnormal return. The abnormal return is the difference between the ex post return and the normal return of a firm's stock over the event window. Consistent with most event studies, here the "market model" is used to estimate abnormal returns. This model assumes a linear relationship between the return on a stock and the market return (in our case the Standard and Poor's 500 (S&P500) is used as a proxy for the market portfolio) over a given time period as:

$$R_{it} = \alpha_i + \beta_i R_{mt} + \varepsilon_{it} \qquad \text{(Eq.1)}$$

where R_{mt} is the return of the market portfolio for the period t, ε_{it} is the zero mean disturbance term, and α_i and β_i are the parameters to be estimated. In the standard event study framework abnormal returns for firm *i* on day *t* (AR_{it}) are modeled as prediction errors from the market model:

$$AR_{it} = R_{it} - \left(\widehat{\alpha}_i + \widehat{\beta}_i R_{mt}\right) \qquad \text{(Eq.2)}$$

where $\widehat{\alpha}_i$ and $\widehat{\beta}_i$ are the firm's estimated parameters over the estimation period. See MacKinlay (1997) and Binder (1998) for a detailed review of event study methodologies.

One can simply estimate the above equation with ordinary least squares (OLS). However, OLS estimation is based on several assumptions. The first assumption is that the error term ε_{it} is serially uncorrelated. However, Lo and MacKinlay (1988) showed that successive returns on individual stocks are indeed correlated; where large returns tend to be followed by further large returns. The second assumption is that the error term follows a normal distribution with constant variance, that is, it is homoskedastic. Giaccoto and Ali (1982) documented that if the assumption of homoskedasticity is not met, the parameter estimates are inefficient and thus any inferences based upon them are potentially misleading. Therefore, to measure the effect of a specific event on stock prices one must account for time-varying variance (heteroskedasticity). Engle (1982) developed a model, in which current conditional variance depends on the past values of squared random disturbances called autoregressive conditional heteroskedasticity (ARCH). The ARCH model is modified by Bollerslev (1986, 1987) to allow current conditional variance to depend on past conditional variances as well as the past squared random disturbances. The advantage of the generalized autoregressive conditional heteroskedasticity (GARCH) model is that not only does it model the mean of the returns (R_{it}), but at the same time allows for time-varying volatility. Since then GARCH models have been widely used in the literature and are found to be suitable in explaining stock price distributions (Bollerslev, 1987; Bollerslev, Engle and Wooldridge, 1988; French, Schwert and Stambaugh, 1987; Baillie and DeGennaro, 1990).

It has been found (see de Jong et al., 1991; Corhay and Tourani, 1996; Hansen and Lunde, 2001) that the modest gains obtained do not justify the use of more complicated GARCH models and GARCH (1, 1) provides a parsimonious but adequate model specification. The market model corrected for GARCH is:

$$R_{it} = \alpha_i + \beta_i R_{mt} + \varepsilon_{it} \quad \text{(Eq.3a)}$$

With variance equation of:

$$\varepsilon_{it} \sim t(0, \boldsymbol{h}_{it}, \nu), \quad \text{(Eq.3b)}$$

$$\boldsymbol{h}_{it} = \omega + \theta \varepsilon^{\boldsymbol{2}}_{i,t-\boldsymbol{1}} + \gamma \boldsymbol{h}_{i,t-\boldsymbol{1}} \quad \text{(Eq.3c)}$$

where ε_{it} is the error term with mean zero and variance h_{it}. R_{it} represents daily stock return of firm i on day t and R_{mt} represents daily return of the S&P 500. Returns are computed as $R_t = 100 \times (\ln P_t - \ln[P_{t-1})]$, where P_t is either the stock price for a company on day t or the S&P 500 index.

For our empirical analysis we calculate abnormal returns both with OLS and GARCH specifications. Since we are not only interested in the average abnormal return per event type but also the median return we utilize the standard event study method. We consider a three day event window with one day added before and after the event day to capture the full effect. From the abnormal returns thus received we calculate a three day cumulated average return (CAR):

$$CAR_i(t_1, t_2) = \sum_{t=t_1}^{t_2} AR_{it} \quad \text{(Eq.4)}$$

Thereafter, we want to test if the groups we have created (as described below) are significantly different from each other that is whether the various company characteristics or the passing of time influences the median CAR.

Sample and data description

For our study stock prices of food industry companies that were traded on the New York Stock Exchange (NYSE) and NASDAQ between the periods of January 1990 and December 2010 were collected. Only companies that were continuously traded and have sales greater than $1 million are included in the sample. With a list of keywords and phrases generally used in the environmental news, a search string is created in the Wall Street Journal Factiva database and all announcements that meet the search criteria are downloaded. In the case of news that appeared in more than one publication or multiple times in the same publication, only the news with the earliest publication date is retained. Additionally, days with multiple announcements or days where event windows overlapped are excluded, as in these cases it would be impossible to determine which environmental announcement is responsible for any market reaction. Only days with no additional confounding events, such as dividend and earnings announcements are used.

For our research questions we needed to create two sets of data sets. For the development of abnormal returns over time we have used the entire dataset of twenty years. This includes 880 environmental events. For the firm-level variables, due to the availability and volatility of data, only a subset of the last four years was used. Therefore, for the cross-sectional analysis we are left with 526 environmental events.

Our sample includes 23 unique firms from 17 primary Standard Industrial Classification (SIC) codes. The study excludes all alcoholic and tobacco related products as they would skew the results due to reputational preconceptions. *Table 2* provides descriptive statistics of the sample.

Table 2. *Descriptive Statistics of the 23 Sample Firms for Selected Firm-level Financial Variables: Size (assets), Profitability (Return on Equity, ROE and Price Earnings Ratio, P/E), Riskiness (beta), and Leverage (Long-term Debt to Equity Ratio, LEV)*

	Market Capitalization (Mill. USD)	**Sales (Mill. USD)**	**Total Assets (Mill. USD)**	**P/E Ratio**	**ROE**	**Beta**	**Debt/ Equity Ratio**
Mean	24,046	18,698	16,541	19.07	25.81	0.88	0.92
Median	10,477	11,158	8,840	14.88	17.49	0.73	0.75
Std. Error	7,649	3,800	3,862	3.43	4.86	0.14	0.14
Minimum	744	917	1,200	-0.06	4.09	0.21	0.00
Maximum	145,170	61,682	66,710	85.47	83.95	3.08	2.60

The food production industry is highly concentrated with the top four players (Nestlé, Unilever, Kraft and Danone) constituting more than 50% of the global market capitalization of the top thirty food companies (Eurosif, 2010). In our study, the average size of companies in terms of market capitalization is over $24 billion, while the mean profitability expressed in the P/E ratio is around 19, which is in line with the industry average.

Given that the environmental news collected consist of different types of events, both positive and negative, it is possible that market reaction varies across different event categories. For example, markets may react to negative news by a larger amount than they do to positive news. By aggregating news items of different types without knowing the sign of the news, could cancel out market reactions and result in an average reaction that is not statistically different from zero. To distinguish the effect of specific events, the news sample is first divided into positive and negative events and then to external and internal events to form the following four subcategories:

Event type 1: News relating to penalties, government action, lawsuits etc. against the companies
Key words: accident, clean, cleanup, "Department of Justice," "Environmental Protection Agency," fine, lawsuit, notice, order, penalty, settle, spill, superfund, tort, toxic, violation.

Event type 2: Actions taken by the companies to improve environmental performance or perception
Key words: carbon, certification, climate, conservation, donation, eco, EMS, endow, energy, environment, footprint, green, "ISO 14001," LEED, nature, recycling, renewable, reusable, "SA 8000," stewardship, support, sustainability.

Event type 3: Awards, rankings issued by an outside source about the company
Key words: admire, award, celebrate, certificate, honor, index, prize, rank, recognition, scorecard, tribute, win, won.

Event type 4: Boycotts, external company reports and studies, other external non-classifiable
Key words: accuse, action, activists, analysis, boycott, contamination, disaster, dump, emission, environment, Greenpeace, incident, rally, report, research, pollution, study.

Event types 1 and 2 focus on events that are direct results of specific internal actions of the companies while event types 3 and 4 are opinions of external parties. Further, event types 2 and 3 represent positive news items while event types 1 and 4 represent negative news (*Table 3*).

Table 3. *Breakdown of Events by Event Types: E1(negative internal), E2(positive internal), E3(positive external) and E4(negative external) and by Years for Cross-sectional Analysis*

By Event Type		**By Year**	
E1	51	2010	186
E2	264	2009	138
E3	167	2008	118
E4	44	2007	84

The financial variables we consider include size (assets), profitability (return on equity, ROE and Price Earnings Ratio, P/E), riskiness (beta), and leverage (long-term debt to equity, LEV). Non-financial variables include media coverage and green reputation. For company media coverage, we looked at the number of articles related to environmental issues published in the printed media. For environmental reputation we computed an average environmental score based on rankings publish in the media (Newsweek, CRO Magazine etc.), investment fund analyst companies (Maplecroft, KLD) and by NGOs (CERES, CDP). We broke down the companies into two groups for each of the financial and non-financial variables as shown in *Table 4*. Accordingly, group 1 of each variable consists of companies with the highest value and group 2 consists of companies with the lowest value of that variable.

Table 4. *Breakdown of the Selected Financial and Non-Financial Firm-level Category Ranges and the Number of Events and Companies in Each Group between the Years of 2007-10*

Category	**Range**	**No. of Companies**	**No. of Events**
Asset1 (A1)	>10	11	328
Asset2 (A2)	10>	12	198
PE1	>15	11	261
PE2	15>	12	265
ROE1	>30	9	219
ROE2	30>	14	307
Beta1 (B1)	>0.60	12	259
Beta2 (B2)	0.60<	11	267
LEV1	>0.80	11	215
LEV2	0.80<	12	311
Coverage1 (COV1)	>30	13	394
Coverage2 (COV2)	30>	10	132
Reputation1 (REP1)	>60	14	352
Reputation2 (REP2)	60>	9	174

With the two different econometrics models, we have thus created a total of 14 scenarios to investigate.

For the longitudinal analysis we have created a graphical representation of the development of the CARs for each event type for the twenty-year span examined to visualize the various trends and thus create suitable time periods to be compared.

Results and discussion

Cross-sectional analysis

The purpose of our testing was to determine how the event types and various company characteristics influence abnormal returns. Therefore, we wanted to perform a pair wise comparison of the medians for each possible combination. The medians for each group can be seen in *Table 5*.

***Table 5.** Median CARs gained per Grouped Firm-Level Variables and Event Types from OLS and GARCH testing*

	OLS				**GARCH**			
Event Category	**E1**	**E2**	**E3**	**E4**	**E1**	**E2**	**E3**	**E4**
A1	-0.2617	0.3958	0.1170	-0.0186	-0.3590	0.3426	0.0514	-0.1401
A2	-0.2198	1.2380	0.0504	0.4241	-0.4512	1.0917	-0.0300	0.4574
PE1	-2.0676	0.4235	0.0754	0.3131	-2.0730	0.4041	0.0013	0.2170
PE2	0.2294	0.7509	0.0578	-0.0186	0.1613	0.7006	0.0356	-0.1401
ROE1	-0.6071	0.2911	0.0575	-1.1048	-0.6604	0.2584	0.0013	-1.0496
ROE2	-0.1791	1.1461	0.0684	0.3165	-0.4512	1.0595	0.0356	0.1466
B1	-0.0371	1.0006	0.0615	0.1247	-0.1399	0.7809	0.0197	-0.0576
B2	-2.0676	0.3871	0.0664	0.1109	-2.0730	0.3593	0.0151	-0.0202
LEV1	-0.0299	0.5328	0.1740	-0.9953	-0.0072	0.5120	0.0151	-1.0462
LEV2	-0.9382	0.6070	0.0541	0.2845	-0.6635	0.6213	0.0197	0.0280
COV1	-0.1791	0.5193	0.1170	0.1247	-0.4512	0.4825	0.0514	-0.0552
COV2	-0.4596	0.7614	-0.0406	-0.8522	-0.4124	0.7712	-0.0749	-0.7774
REP1	-1.3458	0.4963	0.0615	-0.4794	-1.4372	0.4474	0.0288	-0.4626
REP2	-0.0371	1.1321	0.0789	0.2878	-0.1399	1.0595	0.0105	0.0093

Between the two econometric approaches used, negative events have become more negative and positive ones less positive. In fact, some lower positive results have even switched signs. However, the choice of statistical approach did not change the results drastically.

In the first step, we had to test whether the groups thus created have any significant effect on the results. Based on the Kruskal-Wallis test performed the grouping was relevant for both the OLS and GARCH type testing ($p<0.05$) (*Table 6*).

***Table 6.** Results of the Kruskal-Wallis Test for the Seven Firm-Level Categories for OLS and GARCH*

	OLS	**GARCH 1,1**
Asset	2.246E-05	2.874E-05
PE	1.206E-05	1.631E-05
ROE	5.251E-05	7.074E-05
Beta	1.878E-05	3.074E-05
Lev	0.0005604	0.0005848
Cover	0.001449	0.00167
Rep	8.846E-05	9.926E-05

Consequently, a post-hoc analysis was performed on the CARs gained from our tests. Since we are performing a number of pair wise comparisons there is an increased chance of committing a Type I error. Therefore, we have run the analysis in a Bonferroni corrected and uncorrected form (*Table 7*).

Table 7. *Results of the Mann-Whitney post-hoc Test (Bonferroni Corrected/Uncorrected) for GARCH prediction errors in the Seven Groups Created from Firm-level Characteristics for the Four Event Types*

ROE	ROE1E1	ROE1E2	ROE1E3	ROE1E4	ROE2E1	ROE2E2	ROE2E3	ROE2E4
ROE1E1		***0.0177***	0.0580	0.9698	0.7131	***0.0021***	0.0750	0.1073
ROE1E2	0.4953		0.1846	***0.0138***	***0.0410***	***0.0005***	0.4872	0.6539
ROE1E3	1	1		***0.0447***	0.1617	***1.16E-04***	0.7215	0.7506
ROE1E4	1	0.3849	1		0.7668	***0.0026***	0.0750	0.1269
ROE2E1	1	1	1	1		***0.0012***	0.1532	0.2661
ROE2E2	0.0575	***0.0132***	***0.0032***	0.0723	***0.0324***		***0.0010***	***0.0363***
ROE2E3	1	1	1	1	1	***0.0284***		0.9034
ROE2E4	1	1	1	1	1	1	1	0

PE	PE1E1	PE1E2	PE1E3	PE1E4	PE2E1	PE2E2	PE2E3	PE2E4
PE1E1		***4.25E-06***	***0.0001***	***0.0050***	***0.0179***	***6.0E-06***	***0.0003***	***0.0269***
PE1E2	***0.0001***		***0.0327***	0.3308	0.4036	0.1396	0.1235	0.0502
PE1E3	***0.0031***	0.9166		0.9484	0.8028	***1.85E-03***	0.7472	0.4917
PE1E4	0.1390	1	1		0.9909	0.2124	0.9376	0.5860
PE2E1	0.5020	1	1	1		0.1327	0.9974	0.5981
PE2E2	***0.0002***	1	0.0519	1	1		***0.0137***	***0.0144***
PE2E3	***0.0082***	1	1	1	1	0.3824		0.4470
PE2E4	0.7519	1	1	1	1	0.4024	1	

Asset	A1E1	A1E2	A1E3	A1E4	A2E1	A2E2	A2E3	A2E4
A1E1		***0.004402***	***0.0409***	0.6267	0.8183	***6.84E-05***	0.0671	0.0804
A1E2	0.1233		0.0937	***0.0224***	0.1570	***0.0020***	0.2106	0.9278
A1E3	1	1		0.1748	0.3812	***5.01E-05***	0.9035	0.4655
A1E4	1	0.6260	1		0.9808	***0.0005***	0.2465	0.2190
A2E1	1	1	1	1		***0.0364***	0.4555	0.3705
A2E2	***0.0019***	0.0567	***0.0014***	***0.0144***	1		***0.0012***	0.2440
A2E3	1	1	1	1	1	***0.0336***		0.6573
A2E4	1	1	1	1	1	1	1	

Beta	B1E1	B1E2	B1E3	B1E4	B2E1	B2E2	B2E3	B2E4
B1E1		0.05131	0.8079	0.9698	0.0705	0.3081	0.8633	0.6912
B1E2	1		***0.0146***	***0.0391***	***4.3E-05***	***0.0154***	***0.0002***	***0.0228***
B1E3	1	0.4099		0.8239	***0.0055***	2.85E-01	0.7827	0.4243
B1E4	1	1	1		***0.0306***	0.2456	0.8792	0.6031
B2E1	1	***0.0012***	0.1533	0.8569		***0.000101***	***0.0008***	0.0890
B2E2	1	0.4309	1	1	***0.0028***		0.0597	0.0917
B2E3	1	***0.0046***	1	1	***0.0222***	1		0.3534
B2E4	1	0.6388	1	1	1	1	1	

Debt	LEV1E1	LEV1E2	LEV1E3	LEV1E4	LEV2E1	LEV2E2	LEV2E3	LEV2E4
LEV1E1		0.475	0.9195	0.4642	0.2804	0.3582	0.9501	0.9626
LEV1E2	1		0.0787	***0.0191***	***0.0004***	0.8664	***0.0098***	0.1884
LEV1E3	1	1		0.0875	***0.0126***	5.89E-02	0.3971	0.8252
LEV1E4	1	0.5349	1		0.7499	***0.0262***	0.2889	0.3196
LEV2E1	1	***0.0106***	0.354	1		***0.0005***	0.0521	0.0948
LEV2E2	1	1	1	0.7323	***0.0128***		***0.0042***	0.1345
LEV2E3	1	0.2751	1	1	1	0.1182		0.7031
LEV2E4	1	1	1	1	1	1	1	

Coverage	COV1E1	COV1E2	COV1E3	COV1E4	COV2E1	COV2E2	COV2E3	COV2E4
COV1E1		***0.0007***	***0.0411***	0.2767	0.8156	***0.0016***	0.0990	0.4856
COV1E2	***0.0187***		***0.0089***	***0.0473***	0.1856	0.6285	***0.0483***	0.1679
COV1E3	1	0.2488		0.6208	0.4292	***1.92E-02***	0.9875	0.6639
COV1E4	1	1	1		0.6356	***0.0368***	0.6939	0.9795
COV2E1	1	1	1	1		0.1519	0.5155	0.7648
COV2E2	***0.0437***	1	0.5388	1	1		***0.0340***	0.1889
COV2E3	1	1	1	1	1	0.9526		0.5926
COV2E4	1	1	1	1	1	1	1	

Reputation	REP1E1	REP1E2	REP1E3	REP1E4	REP2E1	REP2E2	REP2E3	REP2E4
REP1E1		***6.67E-05***	***0.0027***	0.3625	0.2710	***0.0004***	***0.0198***	***0.0365***
REP1E2	***0.0019***		***0.0056***	***0.0163***	0.1130	0.0750	0.2429	0.2591
REP1E3	0.0764	0.1571		0.168	0.5708	***1.52E-03***	0.7303	0.8339
REP1E4	1	0.4553	1		0.7346	***0.0157***	0.2676	0.2998
REP2E1	1	1	1	1		0.0548	0.5857	0.5484
REP2E2	***0.0117***	1	***0.0425***	0.4391	1		0.0766	0.1025
REP2E3	0.5546	1	1	1	1	1		0.9785
REP2E4	1	1	1	1	1	1	1	

Note: Firm-level Categories: Return on Equity (ROE1, ROE2), Price/Earnings Ratio (PE1, PE2), Asset (A1, A2), Leverage-Debt Equity Ratio (LEV1, LEV2), Riskiness-Beta (B1, B2), Media Coverage (COV1, COV2), and Environmental Reputation (REP1, REP2); Event Types: E1(negative internal), E2(positive internal), E3(positive external) and E4(negative external)

Coverage and indebtedness has the least effect, while ROE, PE, beta and reputation produce about the same amount of significant results. Company size (asset), while also on the low end, seems to be the most persistent influence as even after Bonferroni correction half of the significant results remain. For all other categories this is only between 25-36%.

When looking at events within the individual groups, Type E4 events in most cases seem to be too homogeneous to sufficiently differentiate them from any other category (could possibly be because this group was not adequately specified or had the lowest sample count). Less than 20% of the possible pairings produced results and most of these were in the E2-E4 pairing (clearly due to the very strong significance of the positive internal events). With Bonferroni correction this becomes even more pronounced (with only one interpretable result remaining). Type E2 events on the other hand are the most clearly demarcated (above 55%). Median abnormal returns between E1 and E2 (positive and negative internal events) and between E2 and E3 (the two positive event types) are almost always clearly distinct for all categories (68% and 61%). Even after Bonferroni correction we are left with several significant differences.

In case of the E2-E3 pairing, companies with lower ROE and smaller size (group 2), are producing a difference of over 1% in positive CAR in response to positive internal news when compared to positive external news (within their own or the other group). However, while smaller asset size influences the difference downward (0.03 %), lower return on equity increases it by 0.08 %.

For the E1-E2 pairing, both lower risk and lower indebtedness influences the amount of penalty incurred for environmental infringements. Within the lower risk group companies that implement positive environmental actions gain 2.43% vs. their piers that incur penalties. In the higher risk group positive actions bring an additional 0.42 % abnormal return. For low debt companies positive actions result in an increase of 1.28 % vs. companies with negative internal news. For the high debt ratio companies this is only 1.18 %. Seems that investors do not appreciate additional capital outlays for already indebted companies. Companies with high profitability expectations are penalized by 2.48 % for environmental transgressions, while their less fortunate piers, with already lower PE, lose 2.77 % vs. firms that implement environmental measures. In this case the better financial situation shelters companies from the full effect of negative news. The dynamics between companies with good and bad reputation is similar, but even more pronounced. Here firms with better green image are shielded from negative effects, as they lose 0.61% less when bad news breaks versus companies with an already bad reputation. In the company media coverage category, the reaction is somewhat unexpected. Here companies that appear in the media less frequently have to face a more pronounced response by the market (by 0.28 %).

Additionally, it’s worth pointing out that for the E1-E3 pairing we can see similar, but less pronounced correlation. While it is true that companies with higher profitability expectations are facing stiffer penalties for infringements they also lose 0.03% less of their CAR compared to firms in worse financial situation, as these firms benefit more from positive outside opinions.

Especially interesting for our study was to see whether the firm level characteristics by themselves contribute to the development of abnormal returns. Therefore, we have highlighted the comparison of groups of similar event types with differing characteristics in *Table 8:*

Table 8. *Significant Differences in Median CAR Between Similar Event Types Based on Company Characteristics**

	Asset	PE	ROE	Beta	LEV	COV	REP
E1	O	X	O	O	O	O	O
E2	X	O	X	X	O	O	O
E3	O	O	O	O	O	O	O
E4	O	O	O	O	O	O	O

** Both for OLS and GARCH*
Note: x marks $p < 0.05$ or less

In company profitability, ROE impacts E2 while P/E ratio E1 type events. This difference in reaction to the two profitability ratios is understandable as E1 type events have clear monetary consequences that affect the bottom line directly while E2 type events affect the bottom line through investment outlays. Company perceived riskiness (beta) and company size influences E2 type events. Smaller, less profitable companies are rewarded to a greater extent for internal company initiatives (a difference of 0.84 % and 0.86 % respectively). Company indebtedness (LEV), media coverage and reputation do not seem to influence abnormal returns at all between the two groups. Again, after Bonferroni correction only ROE and Asset results remained significant.

When looking at environmental news in the media, there is a growing trend in the amount of news published in this topic (*Fig. 2*).

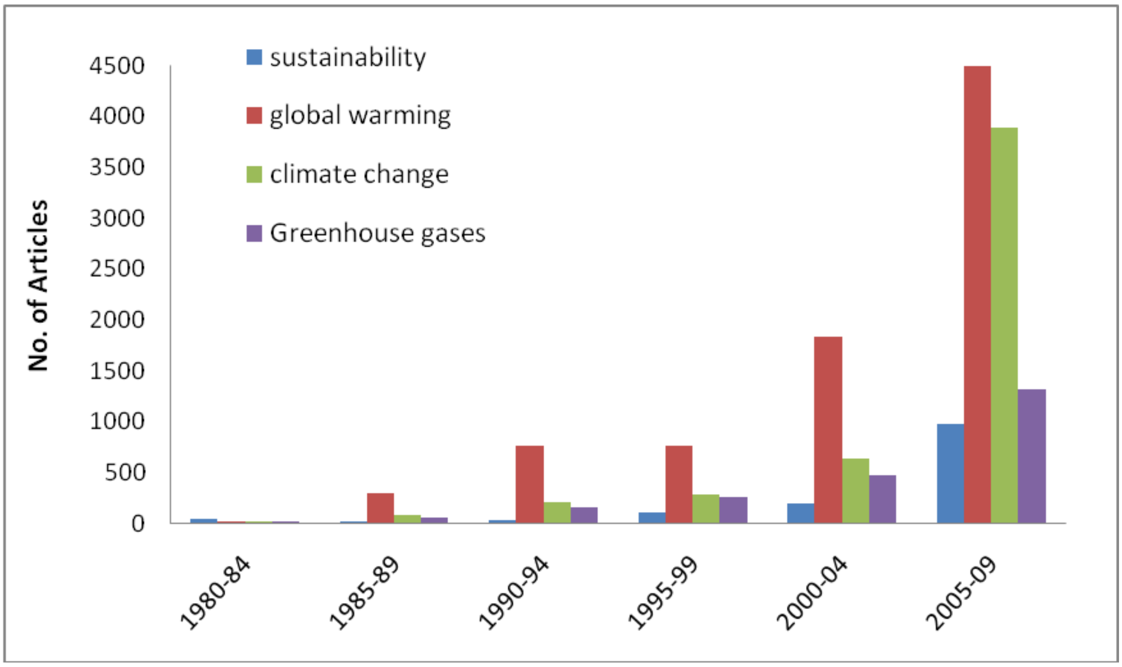


Figure 2. *News Articles by Topic in The New York Times between 1980 and 2010*

There is also a clear delineation between event types. While the number of negative news items relating to environmental penalties published leveled off over time, reports relating to positive company initiatives were growing exponentially. At the same time, news originating from outside evaluations, both positive and negative, have only appeared regularly in the media after the year 2000. Here, the number of awards and rankings was increasing steadily, while third party negative reports, after an initial spurt, have also stabilized (*Fig. 3*). This reflects two distinct trends: first the companies have realized the importance of the role of media and are placing greater emphasis on managing their image, and second the non-governmental organizations and investing communities have become increasingly active.

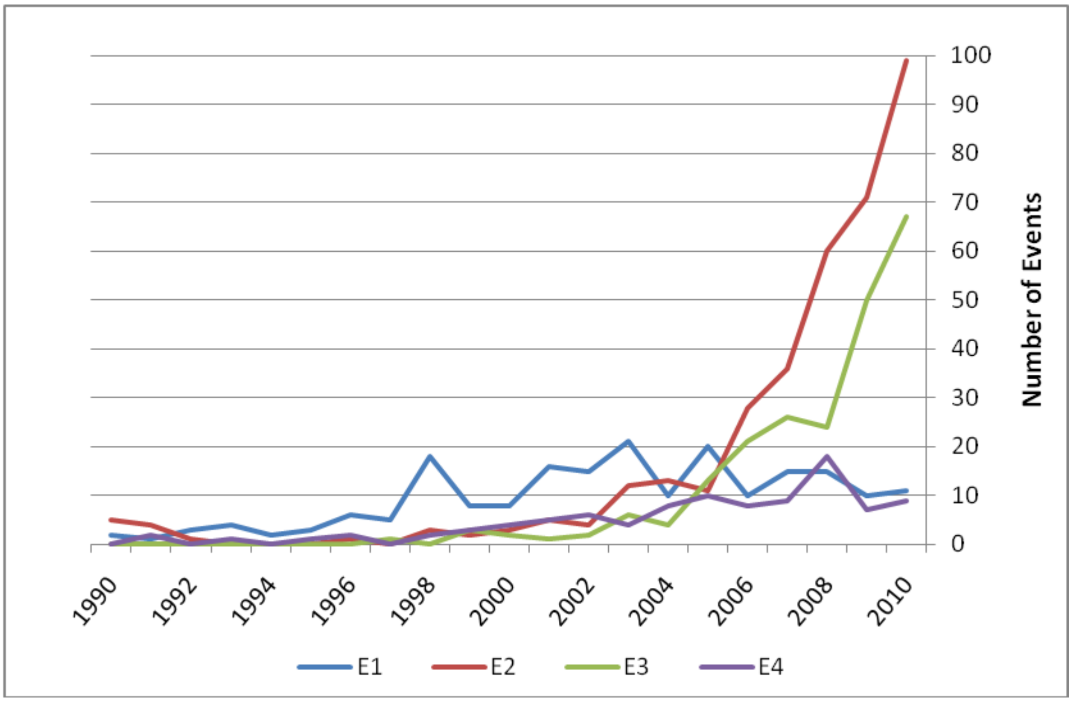


Figure 3. *Development of News by Event Types between 1990-2010*

We have examined our four distinct event types and the relating cumulative abnormal returns produced over time. The initial idea of splitting the twenty-year span into equal portions for all events was not feasible as the trends per event type did not overlap and such divisions produced no useable results. Therefore we have looked at each event type separately by creating four portfolios and calculating the cumulative gain/loss over time. For a graphical representation, please see Figures 4-7. Generally speaking the tipping points per event type do not coincide, with some interesting exceptions. In April of 2007, for example, due to a landmark judicial decision in the United States and a crucial IPCC (Intergovernmental Panel on Climate Change) report sentiments toward climate change and the environment improved significantly. As a result, we can see an upward movement in CARs for E2 events and a corresponding dip in CARs for E4 and E1 type events. Shifts in government policy, such as George Bush's climate plan announcement in February 2003, or Barack Obama's election in November 2008, were also important events that influenced attitudes of investors.

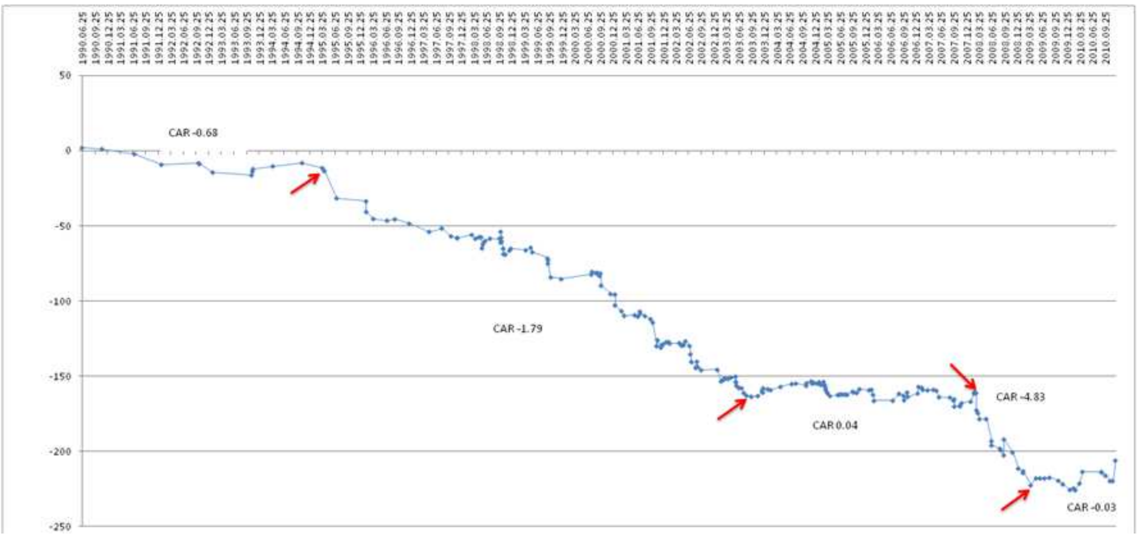


Figure 4. *Cumulative Average Returns for E1 Type Negative Internal Events*

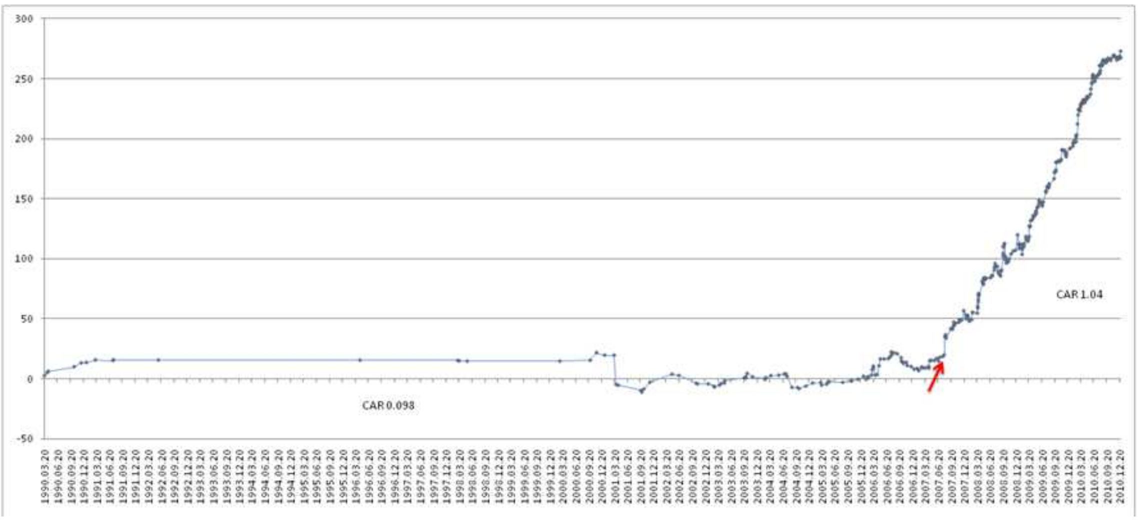


Figure 5. *Cumulative Average Returns for E2 Type Positive Internal Events*

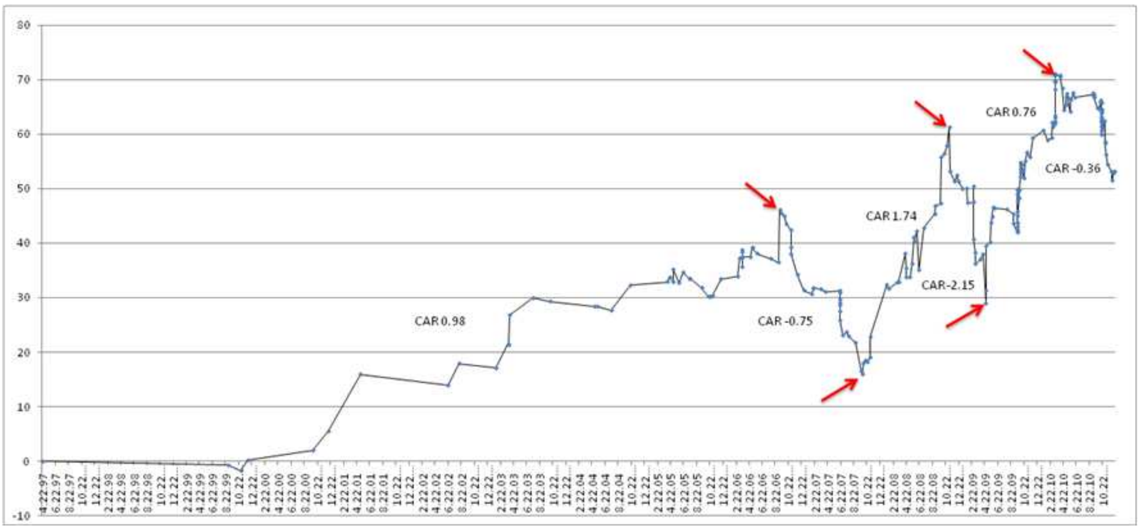


Figure 6. *Cumulative Average Returns for E3 Type Positive External Events*

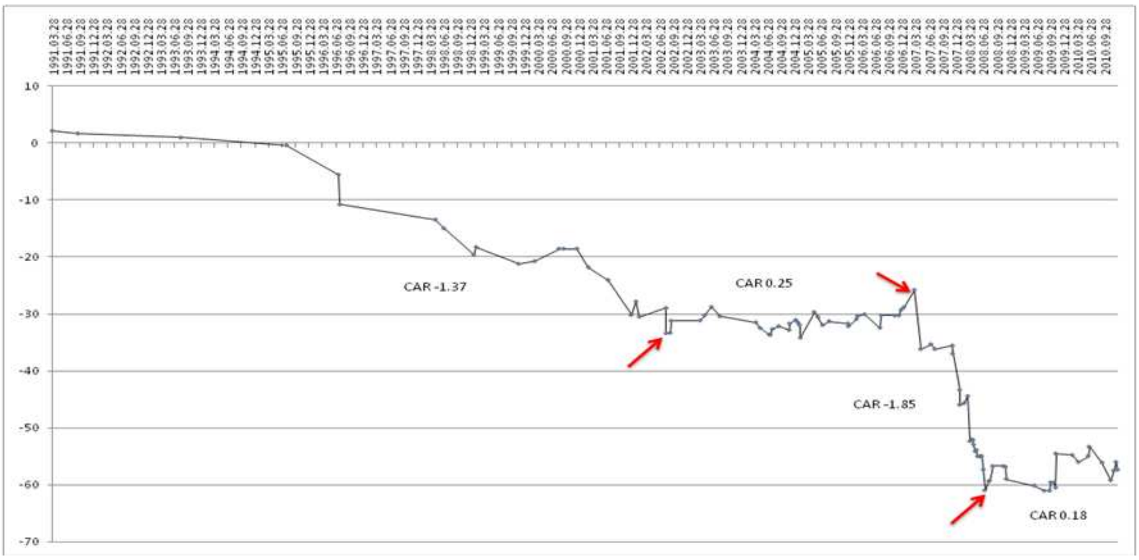


***Figure 7.** Cumulative Average Returns for E4 Type Negative External Events*

While all event types show a trend in the generally expected direction, E1 and E2 type events are more homogeneous while E3 and E4 are more cyclical. To verify that our selected periods are well defined we again looked at the pair wise comparison of medians (Table 9).

***Table 9.** Results of the Mann-Whitney post-hoc Test (Bonferroni Corrected/Uncorrected) for GARCH Prediction Errors for the Selected Time Periods of the Four Event Types*

E1 Type Events

H	21.24
p	0.0002832

	11/2/1994	2/18/2003	2/28/2008	4/6/2009	12/31/2010
11/2/1994		0.2688	0.9757	0.01572	0.8421
2/18/2003	1		0.002596	0.02318	0.07465
2/28/2008	1	0.02596		0.00006465	0.9327
4/6/2009	0.1572	0.2318	0.0006465		0.003224
12/31/2010	1	0.7465	1	0.03224	

E4 Type Events

H	9.871
p	0.0197

	8/13/2002	3/21/2007	7/10/2008	12/31/2010
7/20/2004		0.01698	0.9137	0.0520
3/21/2007	0.1019		0.01114	0.8546
7/10/2008	1	0.06683		0.09456
12/31/2010	0.312	1	0.5674	

E2 Type Events

H	5.042
p	0.02475

	4/23/2007	12/31/2010
4/23/2007		0.02478
12/31/2010	0.02478	

E3 Type Events

H	32.54
p	4.639E-06

	5/8/2006	10/2/2007	11/10/2008	4/27/2009	3/17/2010	12/31/2010
5/8/2006	0	0.00124	0.1583	0.00886	0.913	0.04802
10/2/2007	0.01865	0	6.85E-05	0.5739	1.64E-04	0.0264
11/10/2008	1	0.00103	0	0.00278	0.09813	0.00047
4/27/2009	0.1329	1	0.04173	0	0.00481	0.1543
3/17/2010	1	0.00246	1	0.07211	0	0.00945
12/31/2010	0.7203	0.396	0.007026	1	0.1418	0

The groupings were relevant for all four event types ($p<0.05$), and with the exception of E4 type events results remained significant even after the Bonferroni correction.

Summary

In conclusion, we can state that testing type does not significantly affect the results obtained. Our expectation phrased in Q1, that using GARCH type testing will smooth out volatility and clustering which is so typical for stock market returns and seemingly significant results gained through OLS tests will disappear, did not materialize.

When comparing event types it is clear that the stock market does not value all types of events equally (Q2). Our second question therefore is answered in the positive. From the groupings used in this study, event type 4 (outside negative events) is not well specified and abnormal returns in response to these types of events usually do not differ significantly from other types of events. Company initiated internal actions, with either positive or negative consequences, carry greater weight with investors. Negative events that are direct results of company actions (E1) produce more negative CARs than E4 types (outside evaluations) similarly E2 type positive environmental steps bring higher positive gains than do E3 type external assessments.

Company specific variables also influence results to varying degrees (Q3). The Bonferroni corrected GARCH results produced the most findings for size and future and expected profitability. In case of asset size smaller companies achieved significantly more favorable abnormal returns in E2 event types. The difference, for example, for small companies between positive internal and external events (E2-E3) is 1.12 % while the same difference for larger companies is only 0.29 %. Asset size influences reactions to environmental friendly steps, with 0.75 higher median CAR for companies in group A2, which clearly shows that the market puts greater value on these types of efforts of smaller companies. When comparing negative and positive internal events (E1-E2) however, smaller companies are penalized by 0.09 % more than larger companies.

Positive internal actions benefit companies with lower ROE more (CAR +1.06) while for lower PE it is only +0.70. Environmental measures currently undertaken by these firms lower future earnings expectations. Similarly, it is investors' expectation that influences results in case of penalties, where high PE companies lose the most (CAR -2.07).

Negative events (penalties, lawsuits) result in a more negative median CAR for companies with lower coverage, which might seem counter intuitive, but not when we consider that in this case we are not measuring coverage of the event itself but general coverage of the company. Companies that are not constantly in the public eye will have to face a more violent response to any negative news.

If a company has high reputation and simultaneously it institutes positive environmental measures, this differentiates it significantly from companies that have similar reputation but have experienced some kind of penalty (+1.88 %). The difference is even more pronounced for low reputation companies (+2.50). At the same time, companies with low reputation that receive praise for their environmental stewardship form an outside source benefit more (median CAR 1.06) than companies that already have high reputation and they bring about some positive environmental change internally (median CAR 0.45).

It is interesting to point out that reputation does not, by and in itself, seem to influence results. This finding is contradictory to previous research findings (see Bansal and Clelland, 2000 and Orlitzky et al., 2003). However, it is plausible that reputation indirectly influences reactions in conjunction with other variables.

Finally, in our review of trends for the last two decades (Q4) we can see a jump in company efforts to build a positive environmental image. In the case of company environmental improvement actions this clearly pays off as there is a 0.02 % continued upward trend in CARs. For both negative event types CARs are increasingly less negative (E1 by 0.7 % and E4 by 1%). However, outside rankings, awards and evaluations seem to have lost some of their appeal, as we can see a decreasing trend of 0.4% in cumulated abnormal returns. This perhaps signifies a certain maturation of the SRI market, where in recent years we can see a consolidation in ethical fund management companies and efforts at standardization of company evaluations.

In this research we examined company level characteristics independent of each other. Further suggested research could focus on the interaction between the various categories, that is, to see whether certain combinations of effects together might influence results differently. Additionally, since here groupings were arbitrary, it would be interesting to see what the actual tipping points are for certain categories. At what level of the firm-level variables does the behavior of the stock market changes?

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ECOLOGICAL STUDY OF FORESTS DOMINATED BY ENDANGERED SPECIES, TAXUS CHINENSIS VAR. MAIREI, IN SHANXI OF CHINA

RU, W.[1] – ZHANG, J-T.[2]*

[1]*Department of Biology and Chemistry, Changzhi University, Changzhi 046011, China*

[2]*College of Life Sciences, Beijing Normal University, Beijing 100875, China*

**Corresponding author*
e-mail: zhangjt@bnu.edu.cn

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Abstract. *Taxus chinensis* var. *mairei* is an endemic, endangered and first-class protected tree species with great medicinal values in China. Its forest distributes in very limited region and its area is reducing during the last 30 years. Conservation of this species and its communities is urgent. This study aimed to reveal relationships of *T. chinensis* var. *mairei* forests with soil variables and topographic variables in Shanxi of China. Floristic data and environmental data from 95 samples of 10 m × 10 m in temperate region were analyzed by TWINSPAN, DCA and CCA, and species diversity indices. Eight forest associations were recognized by TWINSPAN and testified by DCA. These associations with special characteristics were main forest types of *T. chinensis* var. *mairei*. The results of CCA showed that the vegetation patterns are related to both soil variables and topographic variables. Among the soil variables, soil organic matter, water content, N, P, Zn and Mn were the most important factors explaining the spatial patterns of forest communities. The topographic variables, elevation, slope and aspect were also significantly correlated with the vegetation. Interactions between the environmental variables were apparent. Species richness, diversity and evenness were significantly related to elevation, but not significantly related to other variables. Further measures for the conservation of *T. chinensis* var. *mairei* and its forests must be undertaken.

Keywords: *Forest conservation, protected plant, economic species, quantitative analysis, vegetation-environment relation, species diversity*

Introduction

Conservation of endangered species and their habitats is a priority feature of environmental policies in many countries and international organizations (Zhang et al., 2007; Oyonarte et al., 2008). Humans have inflicted so much ecological damage on the planet that a real concern is how to protect what remains (Pickett et al., 1997; Larsen and Olsen, 2007). Estimates of current and future extinction rates suggest that we will lose double-digit percentages of the existing species on the Earth in the new century (Pimm & Askins, 1995). Already, many plant species are listed as endangered (Orians, 1993; World Conservation Monitoring Center, 1992). The China red book of plants lists hundreds of endangered plant species (Fu, 1991).

Plants in genus *Taxus* produce chemicals of Taxol which is an effective medicinal compound against several cancers. Therefore, species of *Taxus* are considered as important resource plants in China. There are 5 species in the genus of *Taxus* Linn. in China. They are *T. cuspidate*, *T. wallichiana*, *T. mnnanensis*, *T. chinensis* and *T. chinensis* var . *mairei.* These species can only be found in mountainous areas in China (Wu, 1979). *T. chinensis* var. *mairei* (Lem. & H. Lev.) W. C. Cheng & L. K. Fu, a low-growing tree species, is an endemic, endangered and first-class nationally protected species in China. It is distributed in limited areas of Yunnan, Sichuan, Hubei, Shanxi provinces and Guangxi autonomous district (Wu, 1980). However, natural forests dominated by *T. chinensis* var. *mairei* can only be found in Guangxi, Sichuan and southeastern Shanxi (Ru et al., 2006).

The conservation situation of *T. chinensis* var. *mairei* and its forest is becoming worsening due to the destruction of natural vegetation and collection of its leaves, branches and even stems and roots for medicines since 1970s, the distribution of this species and its communities have been progressively reduced (Fu, 1991). Conservation of this endangered species and its habitats are becoming urgent, and basic studies are needed for its conservation. Despite widespread research on this endangered species, including its taxonomy, chemicals, genetics, reproduction and cultivation (e.g. Wu, 1979; Huo et al., 2007; Jin et al., 2007), study on community ecology of forests dominated by this species is unsatisfactory and needs to be further strengthened (Zhang et al., 2006b). This paper mainly focuses on ecological relations of forests of *T. chinensis* var. *mairei* and their environmental variables by using multivariate analysis methods. Thus, the objectives of this study were: (1) to identify the community types and analyzing their composition and structure; (2) to elucidate the relationships between forest vegetation and topographic and soil variables, and to find the most important variables to the endangered species and its forests; (3) to define the pattern of species diversity and to interpret the this pattern in relation to environmental variables in the *T. chinensis* var. *mairei* forests, Shanxi; And (4) Finally, some measures of conservation management for this species and its communities are proposed.

Methods

Study area

T. chinensis var. *mairei* and its forests are distributed in only four counties, Huguan, Lingchuan, Yangcheng and Qinshui, in southeastern Shanxi (*Fig. 1*).

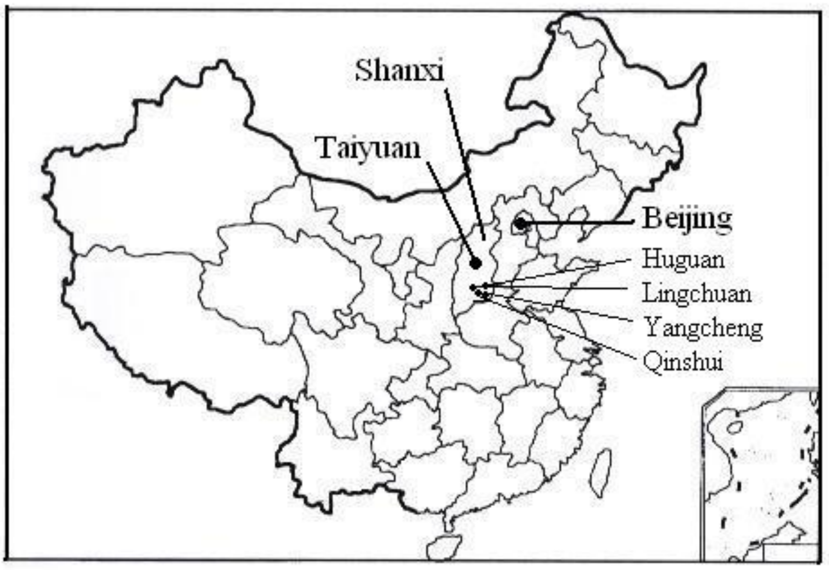

Figure 1. *Geographical position of the study sites*

This area is located at E111º33' – 113º35', N35º10' – 35º37', and including eastern part of Zhongtiao Mountains and southern part of Taihang Mountains. The elevation varies from 600 m to 1500 m. The climate of this area is warm temperate and semi humidity with continental characteristics and controlled by seasonal wind. The annual mean temperature is 5 – 13.3 °C, the monthly mean temperatures of January and July are -5 – - 0.5 °C and 23 – 26.1 °C, respectively, and the annual accumulative temperature more than 10 °C is 2700 – 3500 °C. The annual mean precipitation varies from 503 mm to 670 mm, 70% precipitation occurring in July to September within a year. Several soil types, such as cinnamon soil, mountain cinnamon soil, mountain eluviation cinnamon soil and brown forest soil can be found in this area.

Sampling

Based on a general survey of *T. chinensis* var. *mairei* species and its communities, five study sites in the four counties were determined (*Fig. 1*). Ten to thirty-five quadrats of 10 m × 10 m were established randomly at each site. The number of quadrats at each site was dependent on the area of *T. chinensis* var. *mairei* forest. The cover, height, basal area, individual number for trees, and the cover, height, abundance for shrubs and herbs were measured in each quadrat. The cover of plants was estimated by eye, and the heights were measured using height-meter for trees and using ruler for shrubs and herbs. The basal diameters of trees were measured using calipers and were used to calculate basal areas. Altogether 128 plant species were recorded in 95 quadrats. Elevation, slope and aspect for each quadrat were also measured and recorded. The elevation for each quadrat was measured by altimeter, the slope and aspect measured by compass meter. Five soil samples of 20 cm in depth in each quadrat were taken by use of soil cylindered core sampler, and were thoroughly mixed and then one quarter was collected and taken to laboratory for chemical analysis. Soil samples were air-dried and analyzed in laboratory. Soil pH, water content, organic matter, total nitrogen, total phosphorus, K, Cu, Mn, Zn were measured as soil variables. These variables were selected because some of them, such as N, P, K, organic matter, are most important nutrient elements, and some of them, such as micronutrient elements Cu, Mn, Zn, are not sufficient in the studied area (Liu, 1992; Ru and Zhang, 2000). A 1:2.5 ratio of soil to distilled water suspension was used to measure pH using a Whatman pH sensor meter. Total nitrogen was estimated using Kjeldahl extraction, and total phosphorus was measured via the $HCLO_4$-H_2SO_4 colorimetric method (molybdovanadate method). The organic matter was measured using the method of $K_2Cr_2O_7$ – capacitance. The K, Cu, Mn, Zn were measured using an Atomic Absorption Spectrophotometer. Water content was measured by oven method.

Data analysis

We used Importance Value of each species as data in community analysis and calculation of diversity indices. The importance value was calculated by the formulas (Zhang et al., 2006):

$$IV_{\text{Tree}} = (\text{Relative cover} + \text{Relative dominance} + \text{Relative frequency})/3$$
$$IV_{\text{Scrub and Herbs}} = (\text{Relative cover} + \text{Relative height})/2$$

The relative dominance refers to species basal area. The species data matrix is the importance values of 128 species in 95 quadrats.

The environmental data matrix is the values of twelve variables, nine soil factors plus elevation, slope and aspect, in 95 quadrats.

Two-way Indicator Species Analysis (TWINSPAN) (Hill, 1979) for classification, Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA) (ter Braak and Smilauer, 2001) for ordination were used to analyze the variation of communities and their relationships with environmental variables. The calculation of TWINSPAN, DCA and CCA was carried out by computer program of TWINSPAN (Hill, 1979) and CANOCO (ter Braak and Smilauer, 2001) respectively.

Three species diversity indices, one for species richness, one for species diversity, and one for species evenness were used to calculate species diversity in the forests of *T. chinensis* var. *mairei*. They are

Species number (as a richness index):

$D = S$

Shannon-Wiener diversity index:

$H' = -\sum P_i \ln P_i$

Pielou evenness index:

$E = (-\sum P_i \ln P_i)/\ln S$

Where P_i is the relative importance value of species i, $P_i = N_i / N$, N_i the importance value of species i, N the sum of importance values for all species in a quadrat, S the species number present in a quadrat (Pielou, 1975; Zhang, 2004).

The Pearson regression and correlation methods were used to analyze the relationships between species diversity indices and environmental variables.

Results

Forest communities

TWINSPAN classified 95 quadrats into 15 clusters at the last division. We chose a standard eigenvalue and got 8 groups, representing 8 associations of *T. chinensis* var. *mairei* forest. The names of the 8 associations are as follows:

I Assoc. *Taxus chinensis* var. *mairei* + *Pteroceltis tatarinowii* — *Sageretia paucicostata* + *Callicapa japonica* var. *angustata* — *Thalictrum squarrosum* + *Clerodendron trichotomum.*

II Assoc. *Taxus chinensis* var. *mairei* + *Carpinus turczaninowii* — *Vitex negundo* var. *heterophylla* — *Arthraxon lanceolatus.*

III Assoc. *Taxus chinensis* var. *mairei* + *Carpinus turczaninowii* — *Forsythia suspense* + *Zanthoxylum planispnum* — *Carex lanceolata* + *Potentilla fiagellaris.*

IV Assoc. *Taxus chinensis* var. *mairei* + *Carpinus turczaninowii* — *Clerodendron trichotomum* — *Arthraxon lanceolatus* + *Carex lanceolata.*

V Assoc. *Taxus chinensis* var. *mairei* + *Carpinus turczaninowii* — *Viburnum schensianum* + *Smilax stans*— *Carex lanceolata* + *Arthraxon lanceolatus.*

VI Assoc. *Taxus chinensis* var. *mairei* + *Carpinus turczaninowii* — *Viburnum schensianum* + *Cotoneaster multiflorus* + *Forsythia suspensa* — *Carex lanceolata* + *Epimedium grandiflorum.*

VII Assoc. *Taxus chinensis* var. *mairei* + *Carpinus turczaninowii* + *Fraxinus chinensis* — *Abelia biflora* + *Forsythia suspensa* + *Deutzia discolor* — *Carex lanceolata.*

VIII Assoc. *Taxus chinensis* var. *mairei* — *Forsythia suspense* — *Phlomis umberosa.*

The structure and environmental characteristics of the eight associations above varied with great similarity (*Table 1*).

The partitioning of the 95 quadrats in the DCA ordination space conforms closely related to their grouping by TWINSPAN (*Fig. 2*).

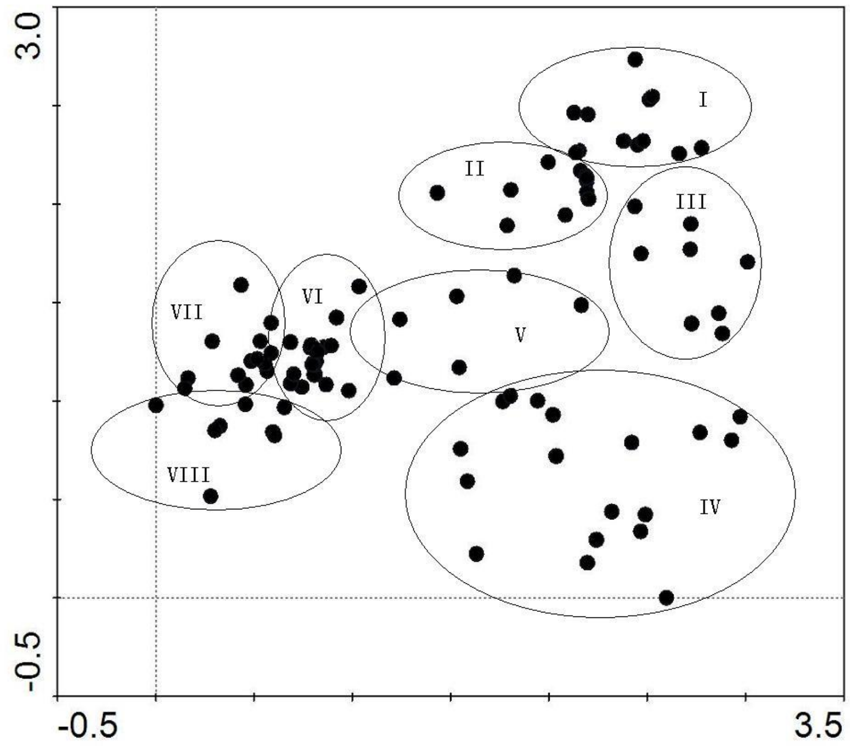

Figure 2. *Two-dimensional DCA ordination diagram of 95 quadrats and 128 species in Taxus chinensis var. mairei forests in Shanxi, China. 1, 2, ..., 95 representing quadrat number; I, II, ...,VIII representing vegetation associations*

The first two DCA axes represent the same gradients represented by the group ordering identified by TWINSPAN. These gradients are comprehensive related to topographical and soil factors.

Table 1. *Structure and environmental characteristics of the eight associations of Taxus chinensis var. mairei forests in Shanxi, China*

Community Types	**Elevation (m)**	**Slope (°)**	**Aspect**	**Total cover of community (%)**	**Cover of tree layer (%)**	**Cover of mairei (%)**	**Cover of shrub layer (%)**	**Cover of herb layer (%)**
I	900-910	5-20	W	80-85	50-60	40-55	10-15	15-30
II	760-860	15	W	70-75	40-50	40-50	35	20
III	910-935	15-30	SW	80-90	55-70	40-50	15-30	60-75
IV	730-765	10-15	W	80	50-60	25-55	15	45-60
V	910-935	20-35	N, NE	90-95	75-85	40-50	25-35	35-40
VI	890-910	10-15	W, SW	85-90	70-80	30-40	30-40	20-30
VII	940-950	35-40	SW	85-90	75-80	40-55	15-25	10-15
VIII	910-935	15-35	S, SW	65	40-50	30-40	10-25	40-50

Forests and environments

In the CCA ordination, the Monte Carlo permutation test indicated that the eigenvalues for the first canonical axis and for the all canonical axes examined were significant ($P < 0.001$) (ter Braak, 1986). The eigenvalues for the first three axes were 0.348, 0.272 and 0.201, and species-environment correlations for the first three axes were 0.906, 0.868 and 0.877 respectively (*Fig. 3* and *Fig. 4*).

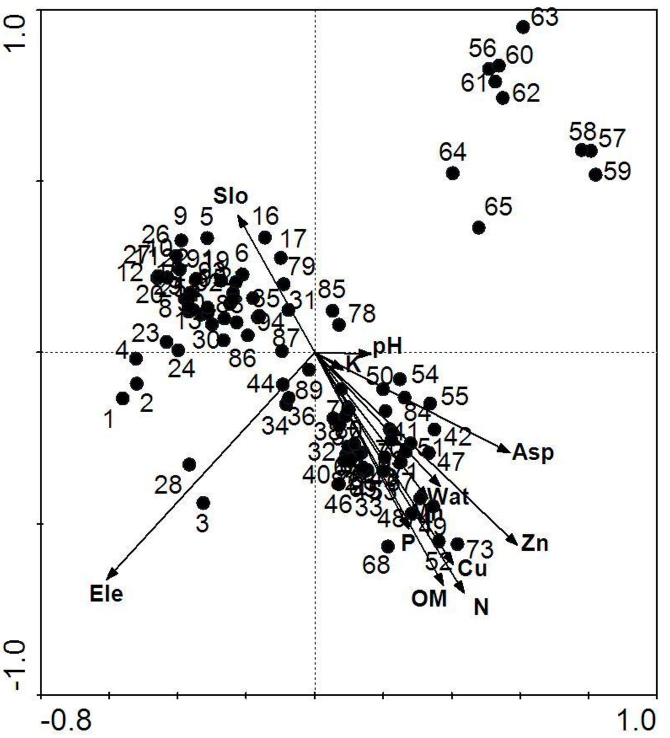

Figure 3. *Biplot of 95 quadrats and 12 environmental variables in CCA ordination of Taxus chinensis var. mairei forests in Shanxi, China. Biplot vectors shown represent the major explanatory environmental variables. Ele – Elevation, Slo – slope, Asp – aspect, OM – Soil organic matter content, Wat – soil water content, pH – soil pH value; N, P, K, Mn, Cu and Zn were all soil elements*

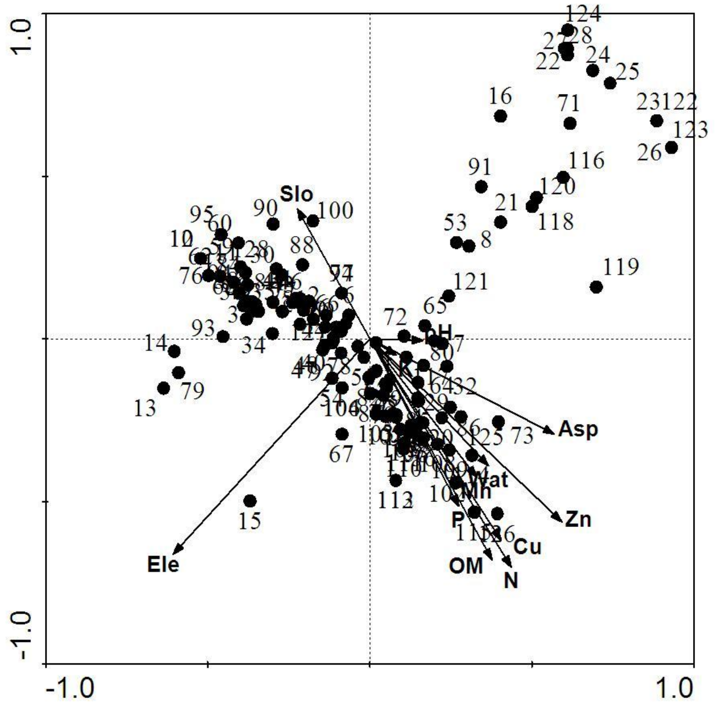

Figure 4. *Biplot of 128 species and 12 environmental variables in CCA ordination of Taxus chinensis var. mairei forests in Shanxi, China. Biplot vectors shown represent the major explanatory environmental variables.*

Ele – Elevation, Slo – slope, Asp – aspect, OM – Soil organic matter content, Wat – soil water content, pH – soil pH value; N, P, K, Mn, Cu and Zn were all soil elements. Species 1 Taxus chinensis var. mairei, 2 Carpinus turczaninowii, 3 Celtis sinensis, 4 Alangium platanifolum, 5 Diospyros lotus, 6 Toxicodendron vernicifluum, 7 Hovenia dulcis, 8 Sorbus puhuashanensis, 9 Fraxinus chinensis, 10 Rhus potaninii, 11 Crataegus kansuensis, 12 Acer grosseri var. hersii, 13 Morus cathayana, 14 Rhus typhina, 15 Cerasu stomentosa, 16 Acer truncatum, 17 Quercus variabilis, 18 Platycladus orientalis, 19 Pinus tabulaeformis, 20 Pteroceltis tatarinowii, 21 Koelrenteria paniculata, 22 Lindera glauca, 23 Idesia polycarpa, 24 Ficus heteromorpha, 25 Tilia mongolica, 26 Carpinus cordata, 27 Pistacia chinensis, 28 Quercus baronii, 29 Juglans cathayensis, 30 Ulmus lamellose, 31 Quercus liaotungensis, 32 Vitex negundo var.heterophylla, 33 Rhamnus parvifolia, 34 Leptodermis oblonge, 35 Lespedza floribumda, 36 Spiraea trilobata, 37 Spiraea pubescens, 38 Lonicera maakii, 39 Lonicera ferdinandii, 40 Forsythia suspense, 41 Cotoneaster multiflorus, 42 Viburnum schensianum, 43 Viburnum hupehense, 44 Deutzia discolor, 45 Abelia biflora, 46 Berchemia floribunda, 47 Smilax scobinicaulis, 48 Smilax stan, 49 Syringa pekinensis, 50 Zanthoxylum planispinum, 51 Grewia biloba var. parviflora, 52 Cotinus coggygris var.pubescens, 53 Clerodendrum trichotomum, 54 Deutzia parviflora, 55 Philadelphus incanus, 56 Callicarpa japonica var.angustata, 57 Sambucus williamsii, 58 Rosa davurica, 59 Euonymus alatus, 60 Schisandra chinensis, 61 Akebia trifoliate, 62 Celastrus orbiculatus, 63 Thalictrum squarrosum, 64 Aster ageratoides, 65 Arthraxon lanceolatus, 66 Carex lanceolate, 67 Elsholzia stauntoni, 68 Elsholzia patrini, 69 Viola collina, 70 Viola acuminate, 71 Viola variegtata, 72 Viola phalacrocarpa, 73 Plectranthus glaucocalyx, 74 Paris verticillata, 75 Phlomis umberosa, 76 Fragaria orientalis, 77 Epimedium brevicornum, 78 Macleaya microcarpa, 79 Mentha haplocalyx, 80 Anemone tomentosa, 81 Polygonatum odoratum, 82 Clematis fruticosa, 83 Goodyera schlechtendaliana, 84 Platanthera chlorantha, 85 Siegesbeckia pubescens, 86 Potentilla fiagellaris, 87 Aquilegia viridiflora, 88 Smilacina japonica, 89 Polygonatum sibiricum, 90 Saussurea japonica, 91 Arisaema erubescens, 92 Vicia unijuga, 93 Pedecularis artselaeri, 94 Ranunculus japonicaus, 95 Atractylodes lancea, 96 Polygonum suffultum, 97 Codonopsis pilosula, 98 Aconitum albo-violaceum, 99 Dryopteris chinensis, 100 Asplenium trichomanes, 101 Dioscorea nipponica, 102 Caryopteris tangu, 103 Asparagus cochinchinensis, 104 Begonia sinensis, 105 Allium senescens, 106 Leonurus pseudomacranthus, 107 Oenathe javanica, 108 Geum aleppicum, 109 Allium ramosum, 110 Fagopyrum tataricum, 111 Oxalis corniculata, 112 Viola chaerophylloides, 113 Carpesium cernllum, 114 Patrinia heterophylla, 115 Torilis japonica, 116 Trachelospermum jasminoides, 117 Euphorbia pekinensis, 118 Cyrtomium fortunei, 119 Girardinia cuspidate, 120 Urtica laetivirens, 121 Selaginella tamariscina, 122 Acalypha australis, 123 Eqisetum ramosisimum, 124 Anemarrhena asphodeloides, 125 Achryanthes bidentata, 126 Cimicifuga foetida, 127 Phytolacca acinosa, 128 Veratrum nigrum

The canonical eigenvalues indicated separation along the measured environmental gradients. Eleven of the twelve environmental variables were significantly correlated with species and community distribution in *Taxus chinensis* var. *mairei* forests (*Table 2*, *Fig. 3, Fig. 4*). The dominant environmental variables correlated with the first CCA axis were elevation, aspect and soil Zn. Except for these variables, the slope, soil organic matter, water content, N, P, Cu and Mn were also significantly correlated with the first CCA axis. The dominant environmental variables correlated with the second CCA axis were elevation, soil organic matter, N and Cu. Except for these variables, the slope, aspect, soil water content, P, Zn and Mn were also significantly correlated with the second CCA axis. The environmental variables correlated with the third CCA axis were elevation, slope, aspect and soil pH. Soil K was not significant on any of the first three axes. The canonical coefficients, which represent the regression coefficients between CCA axes and environmental variables produced in the CCA analysis, showed similar relationships of environmental variables with vegetation and species (*Table 2*).

Table 2. *Canonical coefficients and the correlation coefficients of environmental variables with the first three axes of CCA analysis of Taxus chinensis var. mairei forests in Shanxi, China*

Environmental variables	**Correlation coefficients**			**Canonical coefficients**		
	Axis 1	**Axis 2**	**Axis 3**	**Axis 1**	**Axis 2**	**Axis 3**
Elevation	-0.550***	-0.575***	0.301**	-0.604	-0.648	0.155
Slope	-0.202*	0.347***	0.499***	0.001	0.239	0.390
Aspect	0.516***	-0.254**	0.597***	0.444	0.086	0.772
Water content	0.331***	-0.339***	0.212*	0.086	-0.059	-0.029
pH	0.148	-0.004	-0.221*	0.200	-0.056	-0.092
Organic matter	0.341***	-0.589***	0.162	-0.596	0.381	0.402
N	0.395***	-0.609***	0.109	0.520	-0.969	-0.249
P	0.248*	-0.446***	0.114	-0.146	0.180	0.446
K	0.075	-0.044	0.152	-0.013	0.066	0.119
Cu	0.367***	-0.537***	-0.194	0.132	-0.218	-0.390
Zn	0.536***	-0.488***	0.052	0.594	-0.210	-0.340
Mn	0.299**	-0.369***	0.027	-0.149	0.238	-0.145

Note: * P<0.05, **P<0.01, ***P<0.001

Both the soil variables and the topographic variables were significant in affecting the spatial distribution of vegetation and species, because these variables interact and influence each other (Glaser et al., 2000; Zhang, 2002). Soil nutrients, such as N, P, Mn, Zn, Cu were significantly correlated with each other, and are related to soil organic matter (*Table 3*). Most soil variables with obvious effects on plant communities were correlated with aspect and slope. Soil K and pH were not correlated with other nutrients. The relationships between topographical variables were not obvious.

Table 3. *Correlation coefficients between environmental variables in Taxus chinensis var. mairei forests in Shanxi, China*

Environmental variables	**Elevation**	**Slope**	**Aspect**	**Water content**	**pH**	**Organic matter**	**N**	**P**	**K**	**Cu**	**Zn**
Slope	0.073										
Aspect	0.130	0.031									
Water content	0.064	0.088	0.384***								
pH	-0.088	-0.309**	-0.093	-0.173							
Organic matter	0.185	-0.180	0.440***	0.544***	-0.119						
N	0.127	-0.201*	0.445***	0.596***	-0.161	0.957***					
P	0.125	-0.163	0.254*	0.442***	-0.203*	0.782***	0.747***				
K	0.036	-0.046	0.175	0.084	-0.055	0.155	0.157	0.050			
Cu	0.104	-0.326**	0.292**	0.345***	-0.170	0.595***	0.638***	0.638***	0.130		
Zn	0.011	-0.229*	0.460**	0.481***	-0.116	0.873***	0.828***	0.795***	0.120	0.709***	
Mn	0.036	-0.085	0.346***	0.454***	-0.275**	0.755***	0.792***	0.616***	0.176	0.619***	0.694***

Note: * P<0.05, **P<0.01, ***P<0.001

Species diversity in forests

Species richness, diversity and evenness showed as significant linear relationships with elevation change (*Fig. 5*). This suggests that elevation is important factor to species diversity in communities of *T. chinensis* var. *mairei*. Species richness, diversity and evenness are increased with elevation increasing. The relationships between species diversities and other environmental variables were not significant except soil pH which was related to species evenness (*Table 4*).

Table 4. *Correlation coefficients between species diversity and environmental variables in Taxus chinensis var. mairei forests in Shanxi, China*

Environmental variables	**Species diversity indices**		
	Species number, *D*	**Shannon-Wiernar index, *H'***	**Evenness index, *E***
Elevation	0.205*	0.360***	0.396***
Slope	0.059	0.105	0.065
Aspect	0.067	0.036	0.042
Water content	0.039	0.048	0.032
pH	0.024	0.160	0.295**
Organic matter	0.056	0.010	0.085
N	0.061	0.010	0.059
P	0.064	0.010	0.114
K	0.152	0.142	0.033
Cu	0.109	0.066	0.039
Zn	0.132	0.109	0.010
Mn	0.092	0.045	0.067

Note: * P<0.05, **P<0.01, ***P<0.001

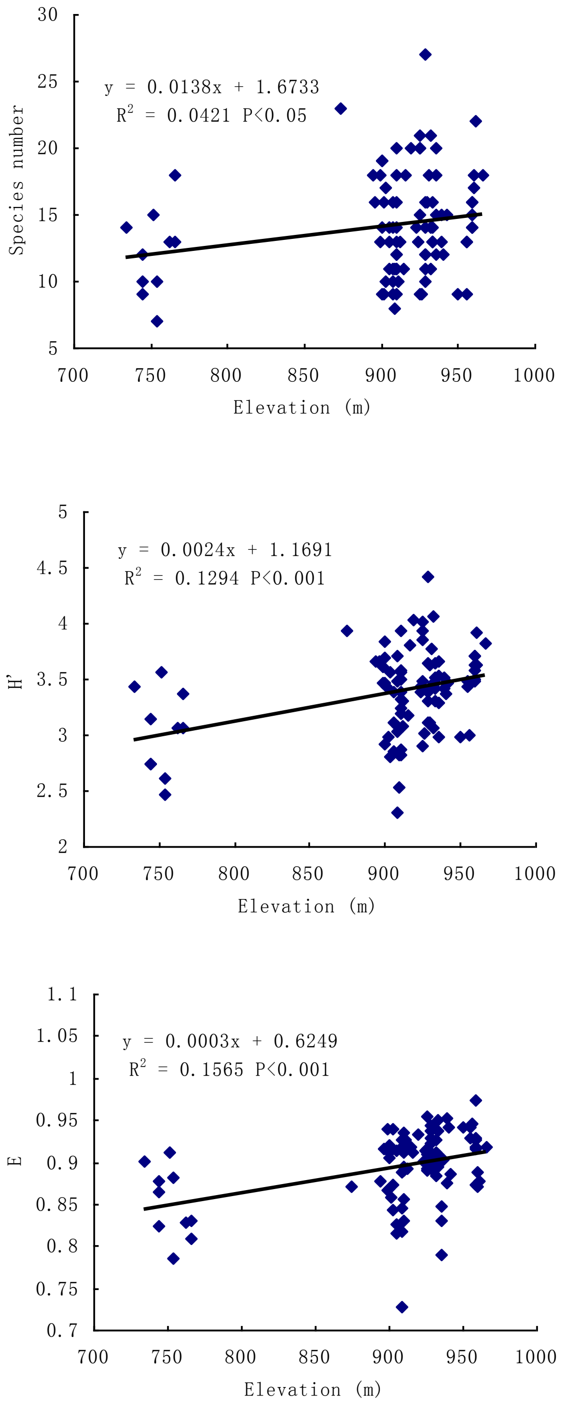

Figure 5. *The variation of species richness, diversity and evenness along the elevation gradient in Taxus chinensis var. mairei forests in Shanxi, China. The H' refers to the Shannon-Wiener index and E to evenness index*

Discussion

T. chinensis var. *mairei* forests in the study area varied in composition and structure. TWINSPAN successfully distinguished them into different forest associations. The eight associations represent the general forest types of *T. chinensis* var. *mairei* in warm-temperate area in China (Wu, 1980; Zhang et al., 2006a). They are almost all secondary natural forests. The classification scheme of forest communities is reasonable according to the Chinese vegetation classification system (Wu, 1980; Ma, 2001). The composition of tree layers of these associations is simple and very similar to each other. *Taxus chinensis* var. *mairei*, *Pteroceltis tatarinowii* and *Carpinus turczaninowii* are co-dominant species in these associations, and their difference is the proportion of these three species (Zhang et al., 2006b). The shrub layers and herb layers of associations varied greatly, and the dominant species and composition of shrubs and herbs played important roles in community differentiation (Zhang and Chen, 2007). The results of TWINSPAN were proved by DCA analysis. Each association had its own distribution area in the DCA space, and their ordination was related to environmental gradients. The DCA axes were comprehensive gradients of topographical and soil factors (Zhang, 2004).

The environmental factors are important to plant growth and vegetation development (Brunner et al., 1999; Liu, 1992; Molles, 2002). CCA analysis indicated that topographical and soil variables were significant to *T. chinensis* var. *mairei* forests in warm-temperate area (Zhang, 2005). Among topographic variables, elevation was the most important factor in relation to vegetation distribution pattern. This is mainly due to the changes of precipitation and soil moisture along altitudinal gradient, *i.e.* precipitation and soil moisture are improving with the elevation increasing. Precipitation is a limiting factor to plant growth and distribution in this area (Zhang et al., 2006a; Zhang and Chen, 2004). Beside elevation, slope and aspect were also significant to spatial variation of *T. chinensis* var. *mairei* communities in the study area. The variation of elevation, slope and aspect also affects the change of soil variables (Anderson, 1982; Molles, 2002, Paschke et al., 2003; Saeki, 2007).

The soil nutrients are key factors to plant growth and vegetation development and the importance of nutrient factors in a community or a region depends on their amount and distribution (Brunner et al., 1999; Saarsalmi et al., 2001; Oyonarte et al., 2008). Among soil variables analyzed, the organic matter, N and Cu, P, and Zn were greatly correlated with community distribution and variation. Except for these variables, soil water content and Mn were also related to the forest variation. Some of these factors were the most important nutrients and some of them were distributed unevenly in the area of *T. chinensis* var. *mairei* community distribution (Liu, 1992; Ma, 2001). The soil organic matter was significant to vegetation distribution, for many nutrients were related to organic matter in soils (Wu, 1980; Zhang and Oxley, 1994), and the accumulation and amount of organic matter were strongly related to temperature and moisture in soils (Anderson, 1982; Wu, 1980; Zhang, 2002). For micronutrients in soils, community variation was more significantly related to Mn and Zn, this is due to that the spatial distribution of these two elements are uneven, and they were not sufficient in some communities (Ertli et al., 2004; Zhang et al., 2006b). Soil water content was significant because precipitation was a limiting factor to plant growth and vegetation distribution, and it was significant to nutrient efficiency (Anderson, 1982; Zhang, 2002; Fosaa, 2004). The effects of soil K and pH on *T. chinensis* var. *mairei* communities were not apparent, because their variations among associations were small. This is identical to the results of some woodland and grassland soil studies in this area (Ma, 2001; Zhang et al., 2006b).

All the ecological factors coexist and act on plants and vegetation simultaneously in communities and ecosystems (Molles, 2002). These factors, including topographic variables, soil variables and human activities, interact with each other, and this interaction is very complicated. In our study, the most important nutrients, soil organic matter, water content, N, P, Zn and Mn were significantly correlated with each other (Paschke et al., 2003; Jin et al., 2007). The most soil variables were correlated with aspect, and some of them related to slope, *i.e.* the variation of aspect and slope affects other environmental variables that further influence plants and vegetation (Bergmeier, 2002; Fosaa, 2004). The topographical variables were not significantly correlated with each other which may be due to their small variations in *T. chinensis* var. *mairei* forests (Zhang and Zhang, 2007; Ru et al., 2006).

Species richness, diversity and evenness were all shown as a significant linear relation with elevation in the *T. chinensis* var. *mairei* forests. This is consistent with many other studies of forests (Stevens, 1992; Lomolino, 2001; Zhang et al., 2006b). They were increased with increasing elevation, which is mainly due to the improvement of water-conditions and nutrients along the altitude gradient (Zhang and Zhang, 2007). Species richness, diversity and evenness were not significantly related to other environmental variables except elevation in the studied communities, which is an exception of mountain vegetation studies (Zhang et al., 2006b; Zhang and Chen 2007). In Lishan Reserve and Taihang Mountain, species richness, diversity and evenness were significantly correlated with soil organic matter and nutrients (Zhang et al., 2006b; Zhang and Zhang, 2007). *T. chinensis* var. *mairei* forests distributed in the lower area (730 – 950 m) with comparatively poor soil, and the forest structure, composition, diversity and its soil were all under development (Ma, 2001; Ru et al., 2006). Therefore, the interaction patterns of species diversity with environmental variables were different from that in mature communities (Anderson, 1982; Zhang et al., 2006).

For conservation of *T. chinensis* var. *mairei* and its forests, one natural reserve should be established in the studied region, which must be effective in controlling cutting for medicine and other interferences (Huo et al., 2007; Sajwan and Kala, 2007; Zhang et al., 2007). In the reserve, planting *T. chinensis* var. *mairei* sapling in suitable communities can increase its cover and density, which can accelerate the development and regeneration of the forests (Sun et al., 2007; Zhang and Chen, 2007). Additionally, soil fertilization should be used for young plantations to improve the living conditions which are effective in enriching species composition and diversity (Ru et al., 2006; Jin et al., 2007).

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MODELLING THE IMPACT OF TROPOSPHERIC OZONE CONTENT ON LIGHT- AND PHEROMONE-TRAPPED INSECTS

LADÁNYI, M.[1] * – NOWINSZKY, L.[2] – KISS, O.[3] – PUSKÁS, J.[2] – SZENTKIRÁLYI, F.[4] – BARCZIKAY, G.[5]

[1]*Corvinus University of Budapest, Dept. of Mathematics and Informatics,*
H-1118 Budapest Villányi Street 29., Hungary

[2]*University of West Hungary, Savaria University Centre,*
H-9700 Szombathely Károlyi G. Square 4., Hungary

[3]*Eszterházy Károly College, Dept. of Zoology, H-3300 Eger Eszterházy Square 1., Hungary*

[4]*Plant Protection Institute of the Hungarian Academy of Sciences, Centre for Agricultural Research, Dept. of Zoology, H-1022 Budapest Herman Ottó Street 15., Hungary*

[5]*County Borsod-Abaúj-Zemplén Agricultural Office of Plant Protection and Soil Conservation Directorate, H-3917 Bodrogkisfalud Vasút Street 22., Hungary*

**Corresponding author*
e-mail: marta.ladanyi@uni-corvinus.hu

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Abstract. The study investigates the effect of the tropospheric ozone content on the relative catch of European Vine Moth (*Lobesia botrana* Den. et Schiff.), Spotted Tentiform Leafminer (*Phyllonoricter blancardella* Fabr.), Setaceous Hebrew Character (*Xestia c-nigrum* L.), Latticed Heath (*Chiasmia clathrata* L.), April Beetle (*Rhizotrogus aequinoctialis* Herbst) and *Ecnomus tenellus* Rambur trapped between 2004 and 2011 in Hungary. In order to describe the empirical connection between the ozone content of the air and the relative number of trapped insects, we introduce some nonlinear regression models of the same general model as origin. We show that elevated ozone content of air stimulates basically two different kinds of response in flying activity of insects.

Keywords: *ozone, insect, trap, Hungary, nonlinear regression models*

Introduction

According to the Fourth Assessment of the Intergovernmental Panel on Climate Change (IPCC, 2007) tropospheric ozone (O_3) is the third most important anthropogenic contributor to greenhouse radiative forcing (3-7%) with a medium level of scientific understanding. Summer daytime ozone concentration correlates strongly with temperature. Tropospheric ozone is expected to increase at 40-60% up to the end of the 21st century which is linked to air quality and climate change (Meleux et al., 2007).

Tropospheric ozone was first determined to be phytotoxic to grapes in southern California in the 1950s (Karnosky et al., 2007). Ozone is a harmful agent causing oxidative stress on plants which may vary in their tolerances. Changes in agricultural productivity can be, in one hand, the result of direct effects of ozone at the plant level, or, in the other hand the consequence of indirect effects at the system level, for instance, through shifts in nutrient cycling, crop–weed interactions, insect pest occurrence, and plant diseases (Fuhrer, 2003).

The tropospheric ozone (O_3) concentration has been monitored in Hungary at K-puszta (46°58'N 19°35') by the Hungarian Meteorological Service (HMS) since 1996, with 10 minutes averaged ozone concentration detected. Since 2004 the monitoring system was extended to 10 stations in Budapest and 37 ones in other locations throughout Hungary. The ozone content of the air is usually measured in ppm, ppb, µg or mg units (0.1 ppm O_3 by weight = 100 ppb O_3 by weight = 200 µg O_3/m^3 = 0.2 mg O_3/m^3).

The highest ozone levels occur typically in towns and cities, however, in some situations high ozone content have been measures in locations even hundreds of kilometres far away from the emission sources. Elevated ozone concentration is detected usually in summer months - from May until August – caused by bright sunshine and high temperature, or sometimes in early spring, mainly in March (Ferenczy, 2012).

Kalabokas and Bartzis (1998), Kalabokas (2002), Kalabokas et al. (2000) Papanastasiou et al. (2002, 2003) as well as Papanastasiou and Melas (2006) in Greece have studied the daily and monthly ozone content fluctuation. The ozone content is usually higher from noon to evening and decreasing from evening to dawn. It hits its lowest point in the dawn hours and begins to rise again in the early morning. However, according to Juhász et al. (2006) the ozone content of the atmosphere is occasionally still significantly high during the night.

Nevertheless, all external circumstances, including the various meteorological features (wind direction, wind speed, temperature, UV-radiation etc.) should also be considered in order to explain extreme ozone content values (Puskás et al., 2001).

In Hungary, according to the measurements of the Hungarian Meteorological Service (www.met.hu) the health protection threshold of ozone (according to the European Committee Directive, the highest 8-hour mean within one day is higher than 120 µg/m^3, http://www.eea.europa.eu/maps/ozone/legislation/eu-legislation-and-directives) are exceeded often in summer, the population information threshold (180 µg/m^3 for the mean value over one hour) are exceeded very rarely while the population warning threshold (240 µg/m^3 for the mean value over one hour) are extremely rarely.

Review of literature

Ozone in plant – insect relations

DeLucia et al. (2005) tested the hypothesis that changes in tropospheric chemistry affect the relationship between plants and insect herbivores by changing leaf quality. Their data suggest that global change in the form of elevated levels of CO and O_3 may exacerbate pest problems and, moreover, changes in tropospheric chemistry can alter the key aspects of leaf chemistry which affect the feeding and demographic performance of insects, thereby modulate the risk of crop damage by insect herbivores (Ashmore and Bell, 1991).

Through changes in metabolic processes, ozone has an impact on the quality of host plants of the phytophagous insects which indirectly can influence both the phytophagous insects and their predators and parasites (Holton et al., 2003). Agrell et al. (2005) introduced a phytophagous forest pest insect (*Malacosomadisstria*) whose food preferences changed as a result of ozone concentration change.

Holopainen et al.(1997), Peltonen et al. (2006) and Percy et al.(2002) give further examples on the phytotoxic effects of plant-mediated O_3 on the behavior and functional activity of insects.

Ozone in pest-predator relations

Pinto et al. (2007, 2008) formulated their conjecture that, during an oxidative reaction, ozone degrades herbal fragrances induced from the host plant by the herbivores. Since herbal fragrances serve as an important signal for the natural enemies (predators or parasitoids) of herbivores, elevated ozone can weaken their orientation efficiency to find their prey or host (Butler et al, 2009; Gate et al., 1995; Holton et al., 2003; Dahlsten et al., 1997). Percy et al. (2002) detected significantly lower number of parasitoids under elevated ozone circumstances.

Though several publications consider the effect of tropospheric ozone concentration on plants or on the plant-insect communities, very few papers has its object on the relationship of ozone content of the air and insect activity. In an earlier study (Puskás et al., 2001), the authors detected the increase of the number of European Corn Borer (*Ostrinia nubilalis* Hbn.; Lepidoptra: Pyraustidae) caught when the ozone content in air was high. Puskás and Nowinszky (2010) established the same in case of the Scarce Bordered Straw (*Helicoverpa armigera* Hbn.) and other harmful insects (Nowinszky and Puskás, 2011). Valli and Callahan (1968) indicated an inverse relationship between O_3 and insect activity, applying light traps.

Jones et al. (2004) have shown that elevated ozone concentration increase the susceptibility of the trees to bark beetles. Dahlstein et al. (1997) agree with Stark et al. (1968) and Grodzki et al., (2004) as they all have found that the number of *Dendroctonus brevicornis* and *Dendroctonus ponderosae* species increased while the number of their predators and parasitoids decreased on trees injured by ozone.

Ozonone as disinfectant

Extremely high concentration of ozone is harmful to insects. The study of Kells et al. (2001) evaluated the efficacy of ozone as a fumigant to disinfest stored maize. Treatment of 8.9 tonnes of maize with 50 ppm ozone for 3 days resulted in 92-100% mortality of adult Red Flour Beetle, *Tribolium castaneum* (Herbst), adult Maize Weevil, *Sitophilus zeamais* (Motsch.), and larval Indian Meal Moth, *Plodia interpunctella* (Hübner).

Biological effects of ozone have been investigated by Qassem (2006) as an alternative method for grain disinfestations. Ozone at concentration of 0.07 g/m^3 killed adults of Grain Weevil (*Sitophilus granarius* L.), Rice Weevil (*Sitophilus oryzae* L.) and Lesser Grain Borer (*Rhyzopertha dominica* Fabr.) after 5-15 hours of exposure. Adult death of Rice Flour Beetle (*Tribolium confusum* Duv.) and Saw-toothed Grain Beetle (*Oryzaephilus surinamensis* L.) was about 50% after 15-20 hours of exposure. Total adult death of all insect species was made with 1.45 g/m^3 ozone concentration after one hour of exposure. According to Bonjour et al. (2011), ozone fumigation has potential for the control of some stored grain insect pests on wheat.

Ozone effect experiments in laboratories

Direct effects of ozone on insects can be investigated mainly in laboratories while observations can supply information on complex (both direct and indirect) interactions, though exploring the relationship need high level caution (Alstad et al., 1982; Freedman, 1994; Butler et al., 2009).

Beard (1965) and Levy et al. (1972) executed long-term experiments on *Musca domestica* and *Drosophila* and *Stomoxys calcitrans.* They observed that ozone stimulated the ovipositional activity of female flies and the number of laid eggs was five times higher at elevated ozone concentration than at control circumstances.

Mondor et al. (2004) observed that in case ozone concentration was high, the intensity of escape reaction of *Chaitophorus stevensis* species has changed, the dispersity of the community increased sinificantly.

Observed ozone effect on ecosystems

Human-induced climate changes threaten the health of forest ecosystems. In particular, carbon dioxide (CO_2) and tropospheric ozone (O_3) will likely have significant but opposing impacts on forests and their associated insect communities. Hillstrom and Lindroth (2008) claim that, compared with other animal groups, insect communities are expected to be especially sensitive to changes in global climate. According to their observations between 2000 and 2003, elevated CO_2, and O_3, or both significantly affected insect community compositions in all years.

Since insects play key roles in forest ecosystems, changes in insect abundance, diversity or community composition have the potential to alter forest ecosystems. Regular monitoring and research on their response to global change is critically important to forest management and conservation.

Ozone and UV radiation

In previous studies of the authors (Puskás et al., 2001), it was proved that the ozone content of the air influences the strength of UV-B radiation which in its turn, bears an impact on the effectiveness of collecting insects by light-trap. Therefore, it seemed reasonable to find a direct empirical connection between the ozone content of the air and the number of trapped insects.

Materials and methods

The trapped species

European Vine Moth (Lobesia botrana Den. et Schiff.)

European Vine Moth is native to Southern Italy. It can be found throughout Europe in the Mediterranean, southern Russia, the Middle East, Near East, and northern and western Africa and Asia north of the Himalayas to Japan. Grape (*Vitis vinifera*) is its preferred hosts, but it has also been reported on several fruits (e.g. olive, blackberry, cherry, nectarine, persimmons and pomegranate) and a number of wild hosts (Briere and Pracros, 1998).

European grapevine moth has two generations in northern Europe, three generations in southern Europe including Hungary (Milonas et al., 2001). In May and June, first-generation larvae web and feed on the flower clusters. Second-generation larvae (July-August) feed on green berries. Third-generation larvae (August-September) cause the greatest damage by feeding inside berries. Additionally, feeding damage to berries after veraison exposes them to infection by *Botrytis* and other secondary fungi and pests

(Sáenz-de-Cabezón et al., 2005). European Vine Moth appears in all the wine-growing regions of Hungary, in very different frequency.

Spotted Tentiform Leafminer (Phyllonorycter blancardella Fabr.)

Tentiform leafminer was introduced from Europe in the 1930s. Populations in commercial orchards increased dramatically in the 1970s and 80s as the insect became resistant to organophosphate insecticides. The species is now distributed throughout Europe, the Baltic States, Byelorussia, Ukraine and Moldova as well as the European part of Russia, Transcaucasia, Urals, Asia Minor, Iran, Mongolia, and Northern America (Pfeiffer et al., 1995).

There are usually 2-5, mostly three generations of tentiform leafminer a year in Hungary. The insect overwinters as a pupa in leaves on the orchard floor. Adult moths begin to emerge when apple buds begin to break in late April and continue to emerge throughout May. The spotted tentiform leafminer infests apple. The larvae mine between layers of apple leaves, reducing photosynthetic area. Heavy infestations of leafminer affect fruit sizing, reduce vegetative growth and/or cause premature fruit drop.

Setaceous Hebrew Character (Xestia c-nigrum L.)

It is found in the Palearctic ecozone woodland. It is a common species throughout Europe, Britain and also can be found in North America, from coast to coast across Canada and the northern United States to western Alaska.

In the southern half of its range, including Hungary, there are two broods, flying in small numbers in May and June, but far more commonly in August and September. In the north there is just one generation, flying in July and August (Thompson and Nelson, 2003).

It is polyphagous, the larvae feed on a variety of herbaceous (agricultural and horticultural) plants, but especially nettle (*Urtic*a). include plants.

Latticed Heath (Chiasmia clathrata L.).

This species can be found throughout Europe from the Iberian Peninsula north to Scandinavia and east to Greece and Turkey and extends to eastern Siberia, China and Japan, North Africa, Central Asia, Siberia and the Far East.

The larvae feed on lucerne (*Medicago sativa*) and clover (*Trifolium*), however, it occurs in a range of open habitats, including moorland, grassland and waste ground.

There are usually two generations, especially in the south, flying in May and June, and August and September, and the species flies by day as well as at night.

April Beetle (Rhizotrogus aequinoctialis Herbst.).

It is distributed in Europe and in the Northern Mediterranean basin. It has a three-year development cycle. The adult does not feed; the larvae eat humus and different kinds of root. The larvae feed especially on herbaceous plants in moorland, grassland and waste ground but they can be found in orchards, vineyards and forest nurseries, too. Swarming is usually between end of March and beginning of June, mainly from noon to night (Janik et al., 2008).

Ecnomus tenellus Rambur

This caddishfly is one of most abundant insect which can be found in Hungary highland as well as in various natural and artificial lakes, backwaters, salt ponds, ditches and slow water rivers. Their presence often indicates degraded, low oxygen content water and plays an important role in aquatic ecosystems as fish food. The larvae have predatory lifestyle. The adults have cyclic pattern of flight activity from May till September with several peaks and without diapauses or parapauses (Nógrádi and Uherkovich, 2002; Kiss et al., 2006; Graf et al.,2008).

The examined sites and years

The data of investigated species and years, the number of observation stations and average value of ozone concentration in the examined period can be found in Table 1.

We worked with the ozone data measured at 23 o'clock (UT). Regardless of the number of insects caught, it was recorded whether or not the traps were successful in catching at a night (successful observation). The number of successful observations exceeds the number of the nights because not a single light-trap worked at a night.

Table 1. *Observed data of the examined species caught*

Pheromone trap catch					
			Number of		
Species	**Mean Ozone content (μg/m^3)**	**Years**	**Trap sites**	**Individuals caught**	**Successful Observations**
European Vine Moth *Lobesia botrana* Den. et Schiff. Lepidoptera Tortricidae	48.9	2004-2011	1	8 053	1 961
Spotted Tentiform Leafminer *Phyllonoricter blancardella* Fabr. Lepidoptera: Lithocolletidae	47.9	2004-2011	1	58 248	2 387

Light-trap catch					
			Number of		
Species	**Mean Ozone content (μg/m^3)**	**Years**	**Trap sites**	**Individuals caught**	**Successful Observations**
European Vine Moth *Lobesia botrana* Den. et Schiff. Lepidoptera Tortricidae	52.5	2004, 2005, 2007, 2008	1	3 820	342
	24.6	2006	1	1 628	120
Setaceous Hebrew Character *Xestia c-nigrum* L. Lepidoptera: Noctuidae	64.3	2004-2008	10	10 729	921
	47.7	2009-2011	10	3 913	694
Latticed Heath *Chiasmia clathrata L.* Lepidoptera: Geometridae	67.1	2007, 2008, 2011	10	3 564	851
	31.6	2005-2006	21	6 789	1 078
April Beetle *Rhizotrogus aequinoctialis* Herbst Coleoptera: Melolonthidae	79.3	2004-2007, 2009-2011	2	1 924	272
Ecnomus tenellus Rambur Trichoptera: Ecnomidae	25.6	2001-2005	5	21 717	848

It is clear that the sizes of the populations are very different at different sites and time intervals. Therefore we calculated the dimension-free relative catch (RC) data for each observation site and day. The RC is the quotient of the number of individuals caught by a trap during a sampling time unit (1 night) and the average number of individuals of the same generation caught in the same time unit calculated over the whole experimental area (Nowinszky, 2003).

The relationship of the ozone content of the air (μg/m^3) and the relative catch values was investigated.

The general model

We defined a general model of the form:

$$\begin{aligned}Y = \chi[X < p_0]*[p_1 + (p_2 - p_1)/(1+\exp(-p_3*(X-p_4)))]+ \\ \chi[X \geq p_0]*[p_2 + (p_5 - p_2)/(1+\exp(-p_6*(X-p_7)))]+\varepsilon\end{aligned} \qquad \text{(Eq.1)}$$

where Y denotes the relative catch (RC) while X is for the ozone content of the air [μg/m^3] and ε is a normally distributed error term with expected value of zero;

$\chi[X < p_0]$, $\chi[X \geq p_0]$ are characteristics functions which take 1 if the condition given in brackets $[X < p_0]$ or $[X \geq p_0]$ holds and zero if it is false;

p_1 is the parameter the fitting curve approaches as $X \to -\infty$;

p_2 is the parameter the fitting curve approaches as $X \to p_0$;

p_5 is the parameter the fitting curve approaches as $X \to +\infty$;

p_3 and p_6 are velocity factors of the exponential terms;

p_4 and p_7 are parameters which represent the inflexion points of the models.

The general model has the shape of *Fig. 1*. The curve can be split into four sections. The different sections can be expressed in different forms of exponential or saturation models. Section 1 and 2 or Section 3 and 4 together can be called as logistic (S-shape) models. Thus Section 1,2, 3 and 4 together can be regarded as a “bi-logistic model”.

We have chosen different special nonlinear regression models to fit the distinct type of data of different sites and time scales. All the models can be originated as a part of the general model of (*Eq.1*). We applied saturation models (Models 1, 4, 5, 8 and 9), exponential model of two different forms (Model 3 and 10), transformed saturation model (Model 11), logistic model (Model 2) and bi-logistic models (Models 6 and 7) in the forms given in the Tables 1-6. In the models Y denotes the relative catch (RC) while X is for the ozone content of the air [μg/m^3] and ε is a normally distributed error term with expected value of zero. Normality was tested by Shapiro-Wilk test (p>0.05).

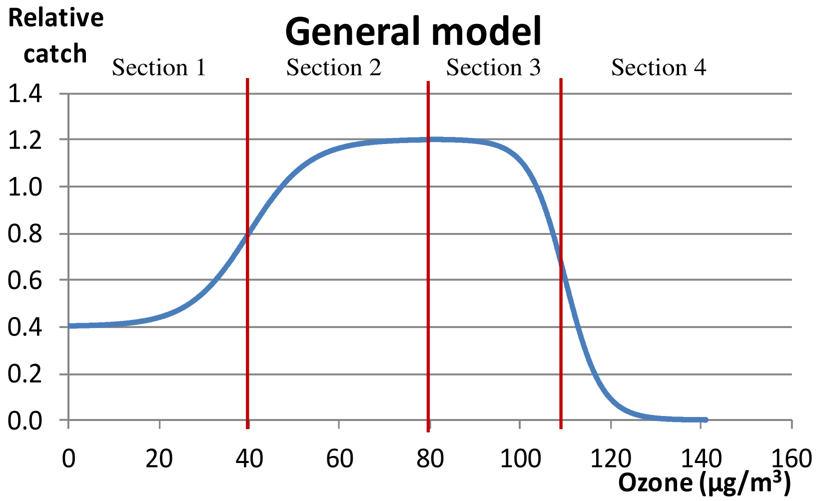

***Figure 1.** General model of relative catch depending on the ozone content of the air [$\mu g/m^3$] expressed in form (Eq. 1) with parameters $p_0 = 80$; $p_1 = 0.4$; $p_2 = 1.2$; $p_3 = 0.15$; $p_4 = 40$; $p_5 = 0$; $p_6 = 0.25$ and $p_7 = 110$.*

The parameter estimations were calculated together with their *t*-values and significance levels. The models were tested by the *F*-value and its significance level. Finally the explained variance (R^2) was evaluated.

Results

Model 1, Model 4, Model 5, Model 8 and Model 9 are saturation models where

p_1 is the parameter the fitting curve takes at point X=0;

p_2 is the parameter which represents the change of the fitting curve values on the whole range of ozone content in the experiment;

p_3 is a velocity factor of the exponential term.

In Model 2 (logistic model)

p_1 is the parameter the fitting curve approaches as $X \to -\infty$;

p_2 is the parameter the fitting curve approaches as $X \to +\infty$;

p_3 is a velocity factor of the exponential term;

p_4 is the parameter which represents the inflexion point of the model.

In Model 3 (exponential model) p_1 is a coefficient of the exponential term;

p_2 is a velocity factor of the exponential term.

In Models 6 and 7 we applied the general model in form of Eq(1).

Table 2. *Nonlinear regression models of the relative catch of Lobesia botrana Den. et Schiff.(depending on the ozone content of the air [µg/m^3] at different sites and time intervals with their parameter estimates, together with the regression diagnostics(F values of the models, t values of the parameters and the explained variance R^2)*

Species	**Site**	**Time**	**Model 1**		
Lobesia botrana Den. et Schiff.	Bodrog-kisfalud	2004-2011	$Y = p_1 + p_2 * (1 - \exp(-p_3 * X)) + \varepsilon$		
	Estimated parameters		*t*	*F*	R^2
	p_1	0.21	2.39*	2804.36***	0.96***
	p_2	0.90	11.88***		
	p_3	0.05	6.18***		
	Site	Time	Model 2		
	Cserke-szőlő	2004 2005 2007 2008	$Y = p_1 + (p_2 - p_1)/(1 + \exp(-p_3 * (X - p_4))) + \varepsilon$		
	Estimated parameters		*t*	*F*	R^2
	p_1	0.82	20.84***	1506.91***	0.92***
	p_2	1.10	40.57***		
	p_3	0.19	1.97+		
	p_4	43.51	15.28***		
	Site	Time	Model 3		
	Cserke-szőlő	2006	$Y = p_1 * \exp(p_2 X) + \varepsilon$		
	Estimated parameters		*t*	*F*	R^2
	p_1	0.46	8.33***	43.63**	0.90**
	p_2	0.30	6.60**		
	Site	Time	Joint model 4		
	Bodrog-kisfalud	2004-2011	$Y = p_1 + p_2 * (1 - \exp(-p_3 * X)) + \varepsilon$		
	Cserke-szőlő	2004 2005 2007 2008			
	Estimated parameters		*t*	*F*	R^2
	p_1	0.30	3.59**	3357.6***	0.911***
	p_2	0.84	12.82***		
	p_3	0.040	5.45***		

+significant at $p<0.1$; * significant at $p<0.05$; ** significant at $p<0.01$; *** significant at $p<0.001$

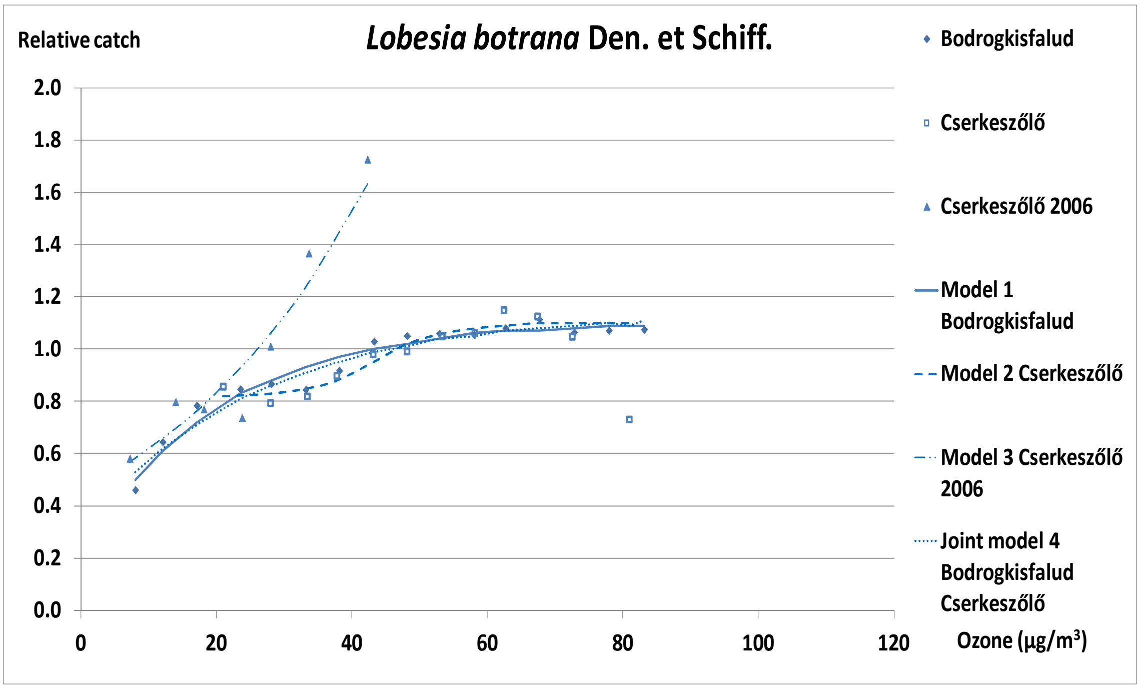

Figure 2. *Observed relative catch values and the fitted curves of nonlinear regression models relative to Lobesia botrana Den. et Schiff. depending on the ozone content of the air [µg/m^3] at different sites and time intervals*

Relative catch data of *Lobesia botrana* Den. et Schiff., Bodrogkisfalud showed saturation growth (*Model 1, Fig. 2, Table 2*). In 2006 we measured significantly lower ozone content in the air in Cserkeszőlő and detected different character of relative catch responses of *Lobesia botrana* Den. et Schiff. Therefore, we fitted a quickly growing exponential model to the relative catch data of 2006 (*Model 3, Fig. 2, Table 2*) and a slowly growing logistic model to the data in other years in Cserkeszőlő (*Model 2, Fig. 2, Table 2*). Nevertheless, considering the growing speed, Model 1 and Model 2 can be regarded similar, thus we fitted a joint (saturation) model (Model 4) which can substitute Model 1 and 2, i.e. it can represent the data a Bodrogkisfalud and also Cserkeszőlő but the year 2006 (*Fig. 1, Table 2*).

Table 3. *Nonlinear regression models of the relative catch of Lobesia botrana Den. et Schiff. depending on the ozone content of the air [µg/m^3] at different sites and time intervals with their parameter estimates, together with the regression diagnostics(F values of the models, t values of the parameters and the explained variance R^2)*

<table>
<tr><th>Species</th><th>Site</th><th>Time</th><th colspan="3">Model 5</th></tr>
<tr><td rowspan="5"><i>Phyllonoricter blancardella</i> Fabr.</td><td>Bodrog-kisfalud</td><td>2004-2011</td><td colspan="3">$Y = p_1 + p_2 * (1 - \exp(-p_3 * X)) + \varepsilon$</td></tr>
<tr><td colspan="2">Estimated parameters</td><td><i>t</i></td><td><i>F</i></td><td>R^2</td></tr>
<tr><td>p_1</td><td>0.20</td><td>2.41*</td><td rowspan="3">1979.64***</td><td rowspan="3">0.96***</td></tr>
<tr><td>p_2</td><td>1.03</td><td>16.85***</td></tr>
<tr><td>p_3</td><td>0.03</td><td>5.19***</td></tr>
</table>

* significant at p<0.05; ** significant at p<0.01; *** significant at p<0.001

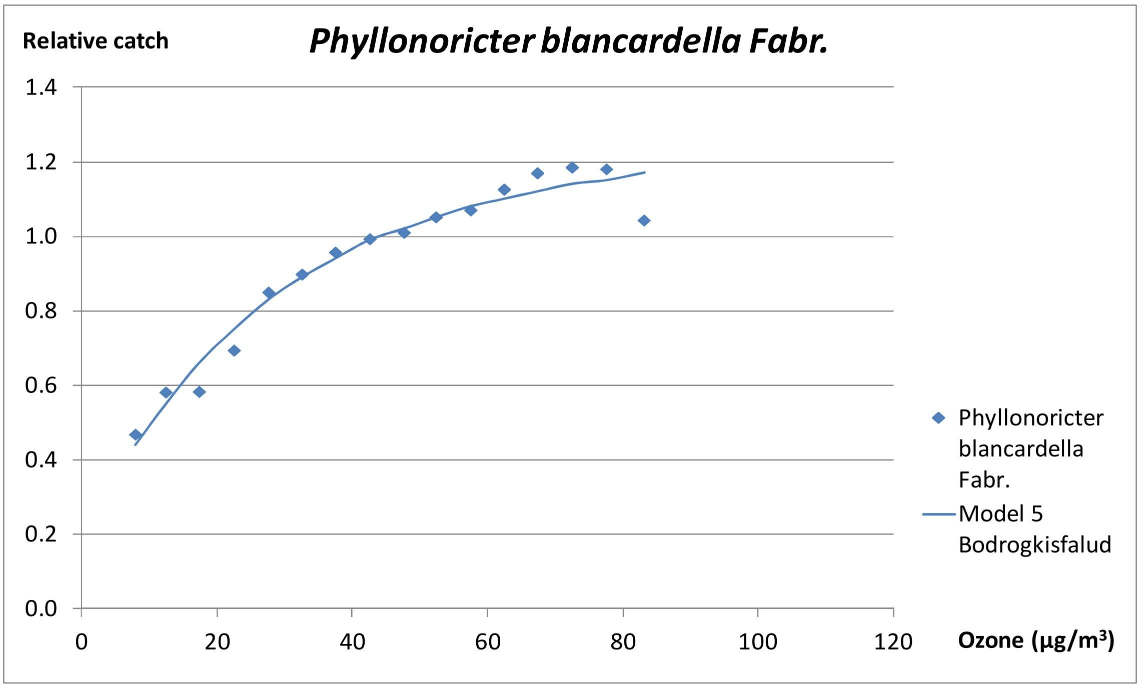

Figure 3. *Observed relative catch values and the fitted curve of nonlinear regression model relative to Phyllonoricter blancardella Fabr. depending on the ozone content of the air [µg/m^3] at Bodrogkisfalud (2004-2011)*

In case of *Phyllonoricter blancardella* Fabr., we fitted a slowly growing saturation model (*Fig. 3*) that is very similar to the Model 1 and joint Model 4 of *Lobesia botrana* Den. et Schiff. which indicates a presumably similar response strategy of the two species when the ozone content of the air is below 90 µg/m^3.

According to the calculated determination coefficients, all the Models 1-5 explain more than 90% of the variance of the observed data and have highly significant *F* values as well. By the significant (in most cases highly significant) *t* values all the parameter estimates can be judged as reliable (*Table 2* and *Table 3).*

Xestia c-nigrum L. was trapped at 10 sites between 2004 and 2011. In the years 2009-2012, during the observation period, lower mean ozone content of the air was measured than in years 2004-2008 and the relative catch data followed distinguishably different curves *(Fig. 4).* Therefore, we split the data into two (2004-2008 and 2009-2011) and fitted two different models with the same form *(Eq.1)* of the general model *(Model 6 and Model 7, Table 4, Fig. 4).*

After an increase of relative catch with increasing ozone content of air, we noticed a definite fall of the number of relative catches when the ozone content exceeded a critical value, and this was detectable in both time scales but at different critical values of ozone content (i.e. 80 µg/m^3 in earlier years with higher ozone content and 44 µg/m^3 in the later years with lower mean ozone content of the air).

Table 4. *Nonlinear regression models of the relative catch of Xestia c-nigrum L. depending on the ozone content of the air [μg/m^3] at different sites and time intervals with their parameter estimates, together with the regression diagnostics(F values of the models, t values of the parameters and the explained variance R^2)*

Species	**Number of observation sites**	**Time with higher ozone content**	**Model 6 at higher mean ozone content**		
Xestia c-nigrum L.	10	2004-2008	$Y=\chi[X<80]*[p_1+(p_2-p_1)/(1+\exp(-p_3*(X-p_4)))]+$ $\chi[X\geq 80]*[p_2+(p_5-p_2)/(1+\exp(-p_6*(X-p_7)))]+\varepsilon$		
	Estimated parameters		*t*	*F*	R^2
	p_1	0.46	6.07***	769.49***	0.96***
	p_2	1.16	37.50***		
	p_3	0.16	3.46**		
	p_4	40.36	19.32***		
	p_5	0.73	7.30***		
	p_6	0.01	0.2ns		
	p_7	80	0.11ns		
	Number of observation sites	Time with lower ozone content	Model 7 at lower mean ozone content		
	10	2009-2011	$Y=\chi[X<44]*[p_1+(p_2-p_1)/(1+\exp(-p_3*(X-p_4)))]+$ $\chi[X\geq 44]*[p_2+(p_5-p_2)/(1+\exp(-p_6*(X-p_7)))]+\varepsilon$		
	Estimated parameters		*t*	*F*	R^2
	p_1	0.62	15.08***	1383.02***	0.99***
	p_2	1.25	19.06***		
	p_3	0.22	3.57**		
	p_4	29.32	21.49***		
	p_5	0.20	0.92ns		
	p_6	0.09	3.03*		
	p_7	72.66	15.92***		

+significant at $p<0.1$; * significant at $p<0.05$; ** significant at $p<0.01$; *** significant at $p<0.00$; ns not significant

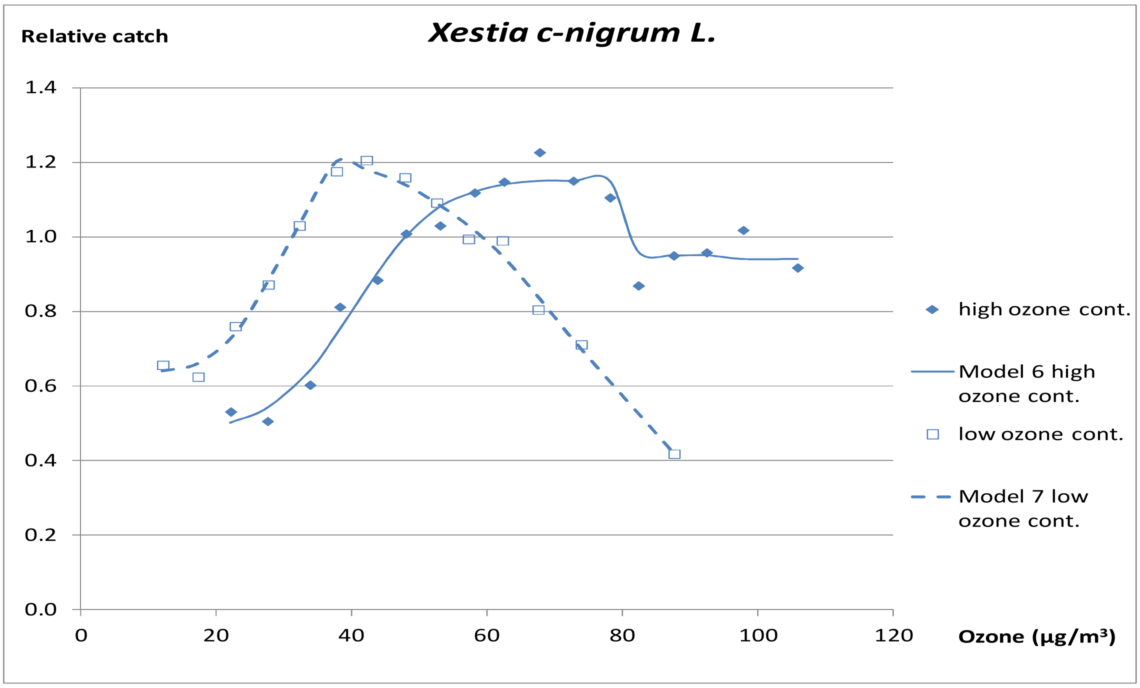

Figure 4. *Observed relative catch values and the fitted curves of nonlinear regression models relative to Xestia c-nigrum. L. depending on the ozone content of the air [µg/m^3],2004-2011*

According to the calculated determination coefficients, both Models 6 and 7 explain 96% of the variance of the observed data and have highly significant *F* values as well *(Table 4)*. Most of the estimated parameters have significant *t* values except p_6 and p_7 of Model 6 (i.e. velocity term and inflexion point of Section 3 with 4 of General model, *Fig. 1*) and p_5 of Model 7 (the limit of the model as $X \to +\infty$). In case of Model 6 the second part of the model (Section 3 and 4 of the General model) does not fit very well, indeed. For Model 7 the reason of insignificant parameter estimation is that the there is no observation in the range of ozone content of the air which corresponds to Section 4 of the General model *(Fig. 1, Fig. 4)*.

Note that also in case of *Lobesia botrana* Den. et Schiff and *Phyllonoricter blancardella* Fabr. we observed significantly lower relative catch when the ozone content exceeded 80 µg/m^3, however, we should not go far with consequences as there was not enough number of such events available to model.

Chiasmia clathrata L. was trapped altogether at 21 sites between 2005 and 2011 but in years 2009 and 2010. In the years 2005-2006, during the observation period, lower mean ozone content of the air was measured than in years 2007, 2008 and 2011, and, similarly to the case of *Xestia c-nigrum* L., the relative catch data followed distinguishably different curves (*Fig. 5*). Therefore, again we split the data into two (2004-2005 and later years) and fitted two different models with the same form (Model 8 and Model 9, *Table 5, Fig. 5*). Relative catch of *Chiasmia clathrata* L. in both time intervals followed saturation model as the ones of *Lobesia botrana* Den. et Schiff. and *Phyllonoricter blancardella* Fabr. The most important difference is that in case we detected high ozone content of the air (i.e. above 80 µg/m^3), instead of a fall we observed a slight increase of relative catch values (*Fig. 5*).

Table 5. *Nonlinear regression models of the relative catch of Chiasmia clathrata L. depending on the ozone content of the air [µg/m^3] (2005-2011) with their parameter estimates, together with the regression diagnostics(F values of the models, t values of the parameters and the explained variance R^2)*

Species	**Number of observation sites**	**Time with higher ozone content**	**Model 8 at higher mean ozone content**		
Chiasmia clathrata L.	10	2007, 2008, 2011	$Y = p_1 + p_2 * (1 - \exp(-p_3 * X)) + \varepsilon$		
	Estimated parameters		*t*	*F*	R^2
	p_1	0.48	6.18***	1670.73***	0.89***
	p_2	0.64	9.50***		
	p_3	0.04	3.81**		
	Number of observation sites	Time with lower ozone content	Model 9 at lower mean ozone content		
	21	2005, 2006	$Y = p_1 + p_2 * (1 - \exp(-p_3 * X)) + \varepsilon$		
	Estimated parameters		*t*	*F*	R^2
	p_1	0.45	3.53**	870.03***	0.85***
	p_2	0.71	6.40***		
	p_3	0.60	2.92*		

* significant at $p<0.05$; ** significant at $p<0.01$; *** significant at $p<0.001$

According to the calculated determination coefficients, both Models 8 and 9 explain more than 85% of the variance of the observed data and have highly significant *F* values as well *(Table 5)*. By the significant (in most cases highly significant) *t* values all the parameter estimates can be judged as reliable.

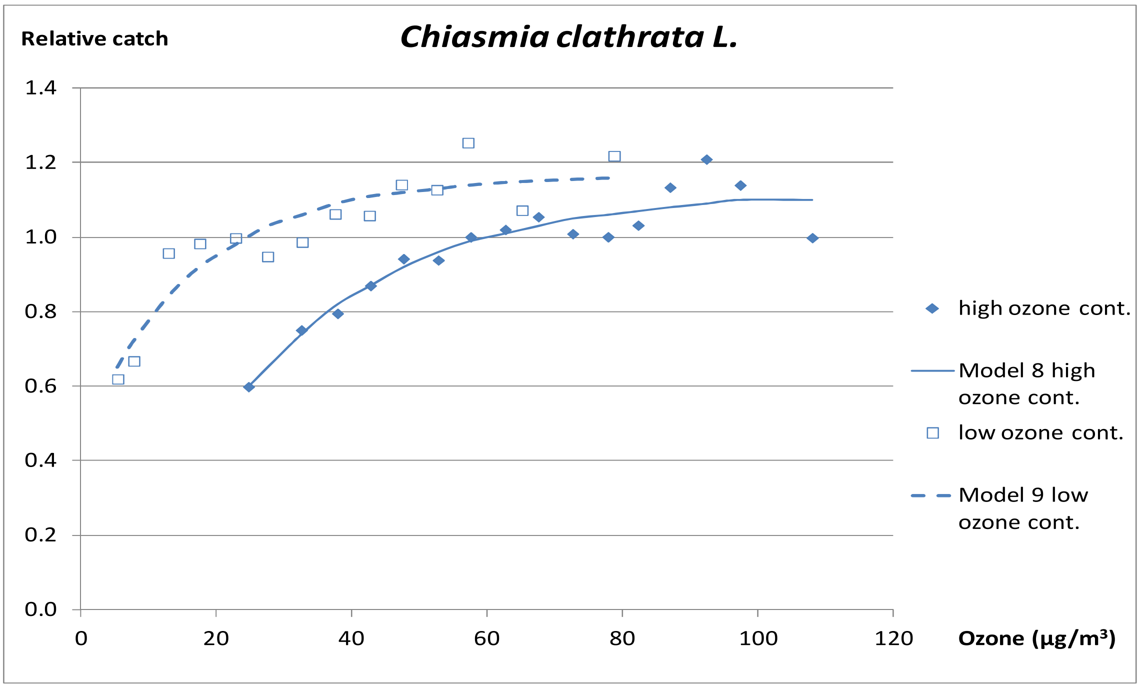

Figure 5. *Observed relative catch values and the fitted curves of nonlinear regression models relative to Chiasmia clathrata L. depending on the ozone content of the air [µg/m^3 (2005-2011)*

Rhizotrogus aequinoctialis Herbst was trapped altogether at 21 sites between 2005 and 2011 but in 2008. In contrary to the cases of *Lobesia botrana* Den. et Schiff., *Phyllonoricter blancardella* Fabr. and *Xestia c-nigrum* L., relative catch data of *Rhizotrogus aequinoctialis* Herbst show a definite increase if the ozone content of the air exceeds 80 µg/m^3 (Fig. 6). In its model (Model 10) the exponential term is multiplied by a linear term the coefficients of which are p_1 (slope) and p_2 (intersection) while p_3 is a velocity factor of the exponential term *(Table 6).*

Table 6. *Nonlinear regression models of the relative catch of Rhizotrogus aequinoctialis Herbst depending on the ozone content of the air [µg/m^3](2004-2011) with their parameter estimates, together with the regression diagnostics(F values of the models, t values of the parameters and the explained variance R^2)*

Species	**Number of observation sites**	**Time**	**Model 10**		
Rhizotrogus aequinoctialis Herbst	2	2004-2007 2009-2011	$Y = (p_1 X + p_2) * (\exp(-p_3 * X)) + \varepsilon$		
	Estimated parameters		*t*	*F*	R^2
	p_1	0.17	11.13***	44.56***	0.94***
	p_2	-0.10	-3.58**		
	p_3	-0.04	-7.31***		

* significant at p<0.05; ** significant at p<0.01; *** significant at p<0.001

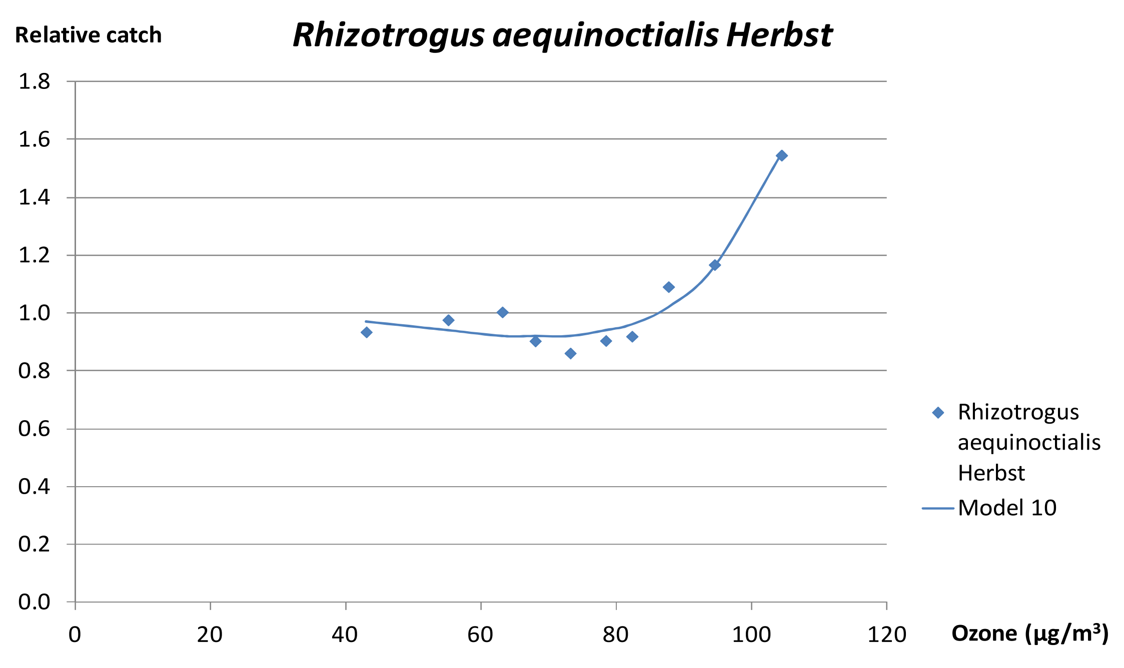

Figure 6. *Observed relative catch values and the fitted curve of nonlinear regression model relative to Rhizotrogus aequinoctialis Herbst depending on the ozone content of the air [µg/m^3] (2004-2011)*

We observed the same intensive increase of relative catch values at high ozone content circumstances for *Economus tenellus* Rambur (*Fig. 7).* In its transformed saturation model (Model 11) p_1 is the parameter the fitting curve takes at the right end of the range of the observed ozone content (X=105 µg/m^3), p_2 is the parameter which represents the change of the fitting curve values on the whole range of ozone content in the experiment while p_3 is a velocity factor of the exponential term *(Table 7).*

Table 7. *Nonlinear regression models of the relative catch of Economus tenellus Rambur depending on the ozone content of the air [µg/m^3] (2001-2005) with their parameter estimates, together with the regression diagnostics(F values of the models, t values of the parameters and the explained variance R^2)*

Species	**Number of observation sites**	**Time**	**Model 11**		
Economus tenellus Rambur	5	2001-2005	$Y = p_1 + p_2 * (1 - \exp(-p_3 * (-X + 105))) + \varepsilon$		
	Estimated parameters		t	F	R^2
	p_1	2.68	29.85***	867.15***	0.98***
	p_2	-1.74	-18.87***		
	p_3	0.13	7.10***		

* significant at p<0.05; ** significant at p<0.01; *** significant at p<0.001

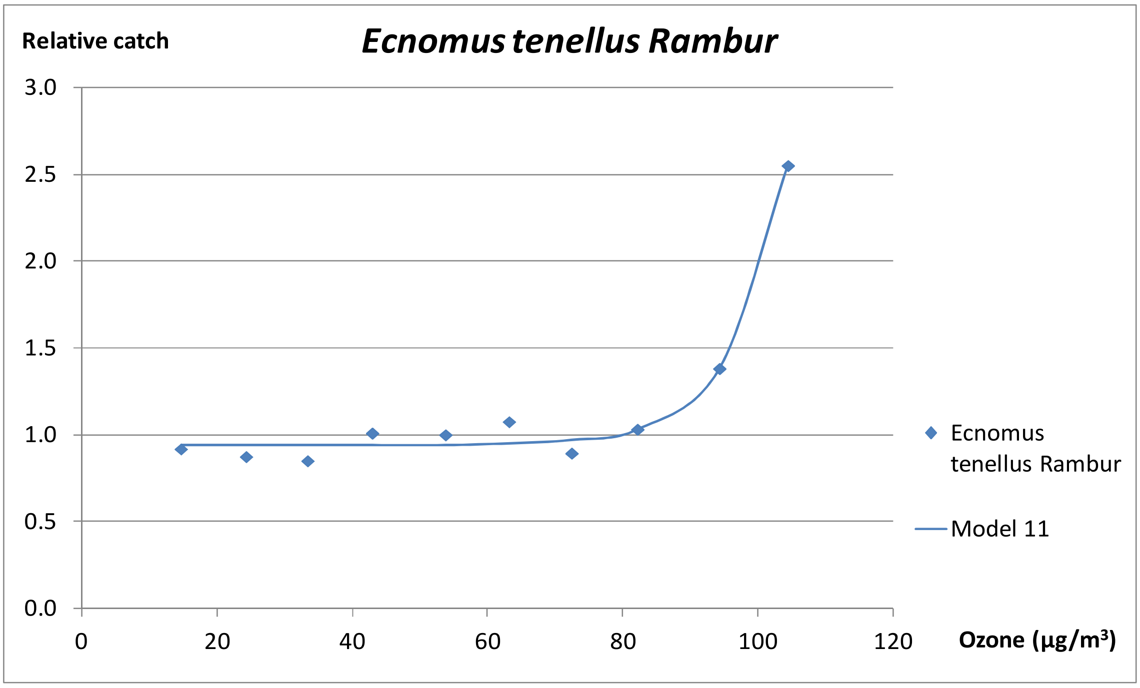

Figure 7. *Observed relative catch values and the fitted curve of nonlinear regression model relative to Economus tenellus Rambur depending on the ozone content of the air [µg/m^3] (2001-2005)*

According to the calculated determination coefficients, both Model 10 and Model 11 explain more than 90% of the variance of the observed data and have highly significant *F* values as well (*Table 6, Table 7*). By the highly significant *t* values all the parameter estimates can be judged as reliable.

Discussion

Our earlier and present results suggest that the flying activity of the European Corn Borer (*Ostrinia nubilalis* Hbn.), Scarce Bordered Straw (*Helicoverpa armigera* Hbn), European Vine Moth (*Lobesia botrana* Den. et Schiff.), Spotted Tentiform Leafminer (*Phyllonoricter blancardella* Fabr.), Latticed Heath (*Chiasmia clathrata* L.), April Beetle (*Rhizotrogus aequinoctialis* Herbst) and *Ecnomus tenellus* Rambur. increase when the ozone content is high. In case of Hebrew Character (Xestia c-nigrum L.) we detected the increasing flying activity with increasing ozone concentration up to a certain level of ozone concentration which was followed by a decreasing flying activity.

Low relative catch values always refer to environmental factors in which the flight activity of insects diminishes. However, high values are not so clear to interpret. Major environmental changes bring about physiological transformation in the insect organism. The imago is short-lived; therefore unfavourable environmental endangers the survival of not just the individual, but the species as a whole. In our hypothesis, the individual may adopt two kinds of strategies to evade the impacts hindering the normal functioning of its life phenomena. It may either display more liveliness, by increasing the intensity of its flight, copulation and egg-laying activity or take refuge in passivity to environmental factors of an unfavourable situation. By the present state of our knowledge we might say that unfavourable environmental factors might be accompanied by both high and low catch (Nowinszky, 2003; Puskás and Nowinszky, 2003).

Each case of the light trapping is unique and unrepeatable and the above results cannot be certificated with laboratory experiments. Therefore, the results of this kind of regularities are difficult to confirm. Laboratory experiments should always be supplemented by field observations because observation results throw new light upon the relation between the ozone in the air and ecosystem.

As the impact of the tropospheric ozone content on the relative catch of the insects is not widely researched field yet, our observations raise several unsolved problems. It need to be clarified whether the main reasons of the examined phenomenon should be searched in the rate of ozone sensitivity of the species, in the current phonological phase of the insects in which the high ozone content is observed, in the length of time of ozone stress or in other species-specific aspects. It is important to investigate the

Ozone content in the troposphere has increased markedly during the past century, mainly because of the release of nitric oxide, carbon monoxide and gaseous hydrocarbons from vehicles and industrial processes and from the burn of biomass in the tropics (Lelieveld et al., 1995). The elevated ozone content in troposphere can cause not only damage to crops and human health but, with interaction of other environmental effects, can also upset the balance of ecosystems. This fact indicates the importance of investigating the impact of elevated tropospheric ozone content on ecosystems, including on the flying activity of insects.

We suggest similar examinations onto other harmful insect species relevantly with other sampling methods (e.g. suction-, Malaise-, bait traps). If it were undoubtedly provable that elevated tropospheric ozone content increases the light-trap catch of certain insect species, it would be necessary to take this fact into consideration when developing the entomological researches and plant protection prognoses, too.

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ZINC AND CHROMIUM REMOVAL MECHANISMS FROM INDUSTRIAL WASTEWATER BY WATER HYACINTH, *EICHHORNIA CRASSIPES (MART.) SOLMS*

GAKWAVU, R.J.[1]* – SEKOMO, B.C.[2, 3] – NHAPI, I.[1, 2]

[1]*Department of Civil Engineering, Faculty of Applied Sciences, National University of Rwanda,*
P.O. Box 117 Huye, Southern Province, Rwanda,
(phone: + (250) (0)788 52 34 30)

[2]*UNESCO-IHE Institute for Water Education,*
P.O. Box 3015, Delft, the Netherlands

[3]*Department of Chemistry, Faculty of Sciences, National University of Rwanda,*
P.O. Box. 117 Huye, Southern Province, Rwanda

**Corresponding author*
e-mail: gakwavurjohn@yahoo.fr

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Abstract. Zinc and chromium are environmental pollutants that are toxic even at very low concentrations. Domestic and industrial wastewater discharges are probably the two most important sources for chromium and zinc in water. In Rwanda, the discharge into natural ecosystems of untreated wastewater containing heavy metals by factories and households is a growing problem. A bench-scale study was therefore conducted from May to October 2007 to investigate the major mechanisms responsible for Cr (VI) and Zn (II) removal from industrial wastewater using water hyacinth. The pH effects, plant relative growth, trace metal remaining in water samples, translocation ability, bioconcentration factor, adsorption, bioaccumulation and uptake mechanisms were investigated. The pH slightly increased from the start time pH = 6.7 (0 hr) to pH = 7.64 to 7.86 (48 hr); but after 48 hours of experiment, the pH decreased due to the saturation of bond sites, resulting in some H+ being released back into the water. The relative growth significantly decreased ($P \leq 0.05$) from 1, 3 and 6 mg/L in 1 week but it slightly decreased linearly after 1 week with increasing metal concentrations ($P \leq 0.05$). About 56.7 % of Zn (II) was accumulated in petioles, 27.0 % in leaves and 16.3 % in roots. For Cr (VI) 73.7 % was taken up in roots, 14.1 % in petioles and 12.2 % in leaves. It was observed that 17.6 %, 6.1 % and 1.1 % were adsorbed for 1, 3 and 6 mg/L of Zn (II) concentrations, respectively, by water hyacinth plants. For Cr (VI), 9.0 %, 36.4 % and 54.6 % were adsorbed for 1, 3 and 6 mg/L, respectively. The order of translocation ability for Cr (VI) was leaves<petioles<roots in water hyacinth whereas for Zn (II) it was leaves<roots<petioles.

Keywords: *Adsorption experiments, chromium removal, industrial wastewater, metal removal mechanisms, water hyacinth, zinc removal*

Introduction

Zinc and chromium are environmental pollutants that are toxic even at very low concentrations. The pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution (Nriogo, 1979). The primary sources of this pollution include the burning of fossil fuels, mining and smelting of metals, municipal wastes, fertilizers, pesticides and sewage. Heavy metals are of great concern primarily due to their known toxicity to aquatic life and human health at trace levels (Lesage, 2000). It has been reported that domestic and industrial discharges are probably the two most important anthropogenic sources of metals in aquatic systems (Stephenson *et al.,* 1980). However, the lack of a reliable method to predict metals distribution in treatment units is a key weakness in determining the fate of metals and their transportation in wastewater treatment processes, and therefore the development of effective pre-treatment guidelines (Memon, 2000). The removal of heavy metals from aqueous solutions has therefore received considerable attention in recent years. However, the practical application of physicochemical technology such as chemical precipitation, membrane filtration and ion exchange is sometimes restricted due to technical or economical constraints. For example, the ion exchange process is very effective but requires expensive adsorbent materials (Lehmann *et al*., 1999; Volesky, 2001).

The use of low-cost waste materials as adsorbents of dissolved metal ions provides economically viable solutions to this global problem and could be considered an eco-friendly solution (Volesky and Holan, 1995; Mullen *et al*., 1989). At present, emphasis is given to the utilization of biological adsorbants for the removal and recovery of heavy metal contaminants (Mullen *et al*., 1989). A research study was therefore conducted in Rwanda, where there are no appropriate systems of heavy metals removal, particularly for zinc and chromium which are some of the main contaminants released from textile and other industries. The development of a system to remove these toxic contaminants was investigated using a pilot scale bench study and conducted from May to October 2007. The aim was to investigate the major mechanisms responsible for Cr (VI) and Zn (II) removal from industrial wastewater using water hyacinth. The water hyacinth plant is rapidly infesting many aquatic ecosystems in the country. However, there are possibilities for using the hyacinth plant in heavy metal removal, considering its reported successes in general wastewater treatment (Mullen *et al*., 1989). Such a system could be inexpensive, providing Rwanda and other developing countries with an appropriate technology thus contributing to environmental sustainability.

Materials and methods

Water hyacinth collection and preparation

The pilot-scale experiments were performed using free-floating water hyacinth *(E. Crassipes)* plants that had been collected from a natural wetland area called Nyabugogo located in Kigali City. The plants were transported to the Chemistry Laboratory of the National University of Rwanda, Butare, in big plastic buckets as shown in *Fig. 1(a).* They were then placed under natural sunlight for several days to let them adapt to the new environment in the laboratory. The plants with similar size, weight and shape were selected, rinsed with distilled water to remove any epiphytes and insect larvae grown on plants and then placed in small buckets for experiments as shown *in Fig. 1(b).*

Figure 1. *Water hyacinth collection and preparation (a) plants in big container, (b) plan view of experimental set up*

All experiments were run in batch mode, using a nutrient solution containing 500 ml of tap water from the valley located at Butare near the Natural Science Centre, 500 ml of wastewater from the Nyabugogo wetland and 20 mg of $Ca(NO_3)_2 .4H_2O$ and NH_4Cl, and 40 mg of K_2HPO_4. The total fresh weight of plants in each bucket was measured before the start of each growing time, which was 1, 2 and 4 weeks.

Description of the experimental setup in the laboratory

The laboratory experimental setup for zinc and chromium removal mechanisms consisted of 12 small buckets as show in *Fig 1(b).* The three buckets without water hyacinth plants served as controls (blanks) containing 1, 3 and 6 mg/L of zinc and chromium. Nine (9) small buckets with water hyacinth plants were used with 3 buckets each containing1, 3 and 6 mg/L of zinc and chromium. The buckets were maintained for 1, 2 and 4 weeks which were the experimental periods. All experiments were performed in the laboratory at a constant temperature (25ºC). A stock solution (1,000 mg/L) of each metal was prepared in distilled water which was later used as dilution water. Individual plants were initially rinsed with distilled water to remove epiphytes, microbes, and any nutrient that might be transferred and were then placed in 2 L small plastic buckets containing 1 L of solution (0.5 L from Nyabugogo wetland + 0.5 L from Butare valley). The plants were maintained in water supplemented by the heavy metals by adding the required volume of zinc and chromium stock solutions to obtain final concentrations of 1, 3 and 6 mg/L of Cr (K_2CrO_4) and Zn ($ZnCl_2$), respectively.

Distilled water was added in order to compensate for water losses through plant transpiration, sampling and evaporation. Water samples and pH measurements were taken every 60 minutes for the first 6 hours and then one sample per day during 1, 2 and 4 weeks of exposure to the metal solution. All samples were filtered using 0.45 µm cellulose acetate filters (Whatman papers) and acidified with 5 drops of nitric acid prior to the storage of samples. The samples were then analyzed using a Perkin Elmer Atomic Absorption Spectrometer. After each test duration (1, 2 and 4 weeks), the final fresh weight for each water hyacinth plant was taken and the plants were harvested for other analyses. They were separated into petioles, roots and leaves and analyzed for relative growth, metals accumulation, translocation ability, bioconcentration factor (BCF) and adsorption on the outer surface of roots. In addition, the metals remaining in the solution were measured to assess the removal capacity of the water hyacinth plants.

Data analyses

a) Relative Growth (RG)

The relative growth of control and treated plants was calculated using *Equation 1* to assess the effects of zinc and chromium concentrations on water hyacinth plant growth.

$$RG = FFW/IFW \qquad \text{(Eq.1)}$$

Where *RG* denotes relative growth of water hyacinth plants during experimental period, dimensionless;

FFW denotes final fresh weight in grams of water hyacinth plants taken at the end of each experimental period, and

IFW denotes the initial fresh weight in grams of water hyacinth plants taken before starting experiment.

b) Bioconcentration Factor (BCF)

The BCF provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate. The BCF was calculated using *Equation 2.* A larger ratio implies better phytoaccumulation capability.

$$BCF = (P/E)_i . \qquad \text{(Eq.2)}$$

Where ***i*** denotes the heavy metal concerned,
BCF is the dimensionless bioconcentration factor,
P represents the trace element concentration in plant tissues (mgL^{-1}),
E represents the initial concentration in water (mg/L) or in the sediment (mg/kg dry wt).

c) Metal Accumulation

Metals accumulation in plant and water samples were measured. Digestion of samples in this study was performed according to the Standard Methods (APHA/AWWA/WEF, 2005). Plant biomass samples were reduced to dry matter by heating at 105°C for 24 hours in a hot air oven and the ash was digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), filtered through a Whatman® filter paper into a volumetric flask before Atomic Absorption Spectrometer analyses. The following three mechanisms were investigated to differentiate the metal adsorption, bioaccumulation, and translocation by water hyacinth during the experimental period.

Adsorption

The adsorption consists of metal attachment to the outer surface of the plant. Adsorption was quantified after the plant had been exposed to different concentrations of chromium and zinc in different periods of times (1week, 2weeks and 4 weeks). At the end of the experiment, adsorption was determined by placing roots of the water hyacinth plant in nine 100 ml of EDTA-Na_2 3, 24 mmolar respectively for 5, 10, 15, 20, 25, 30, 35, 40 and 45 minutes. This was to remove zinc and chromium trace elements on the outer surface of the roots. The EDTA-NA_2 solutions were then filtered, acidified by 5 drops of nitric acid (HNO_3), and then analyzed by Atomic Absorption Spectrometer (AAS) to determine the zinc and chromium adsorbed by the plants. The most important parameter to consider is the pH (Keith *et al.,* 2006). Generally when the pH decreases, the toxicity of metal ions increases because the proportion of the adsorbed ion on the root system decreases (Kara, 2005).

Uptake

The uptake process is a mechanism by which metal ions are transported across the cell membrane and can be used in the building of new biomass or stored in vacuoles. This mechanism was assessed at the end of the experimental period when water hyacinth plants were taken out from the small buckets and roots, petioles and leaves were separated, dried in an oven at 105°C for 24 hours. The plant samples were transformed into ash, digested and analyzed by AAS to determine the zinc and chromium concentrations in plant biomass (roots, leaves and petioles). The results from

AAS analyses show the plant parts which have higher metal accumulations. The presence of carboxyl groups at the roots system induces a significant cation exchange capacity and this could be the mechanism of moving heavy metal in the roots system where active absorption takes place.

Translocation ability (TA)

The translocation ability was calculated by dividing the concentration of a trace element accumulated in the root tissues by that accumulated in shoot tissues (Volesky and Holan, 1995). *TA* was calculated using *Equation 5*.

$$TA = (Ar/As)_i \qquad \text{(Eq.3)}$$

Where ***i*** denotes the heavy metal concerned,

TA is the translocation ability and is dimensionless,

Ar represents the amount of trace element accumulated in the roots (mgL^{-1} dw), and

As represents the amount of trace element accumulated in the shoots (mgL^{-1} dw)

Statistical analysis

In order to detect quantitative differences in the data, statistical analyses were performed.

Standard deviation

The standard deviation was obtained from the variance by extracting the square root and was expressed in the unit in which the measurements were taken:

$$S = \sqrt{\frac{\sum fd^2}{(n-1)}} \qquad \text{(Eq.4)}$$

Regression analysis

Regression is defined as the determination of statistical relationship between two or more variables. In simple regression one variable (defined as independent) is the cause of the behaviour of another one (defined as dependent variable). The correlation coefficient (r) is expressed by equation:

$$r = \frac{\sum dx.dy}{\sqrt{\sum dx^2.\sum dy^2}} \qquad \text{(Eq.5)}$$

Results and discussions

Effects of pH variations

The initial solution pH was adjusted to 6.7 in the small plastic buckets using HCl or NaOH. This is the pH tested in Nyabugogo Wetland from where the hyacinth was collected. The pH variations over time for the water hyacinth-covered containers are shown in *Figure 3.* The pH varied considerably in different buckets with water hyacinth plants over the exposure time.

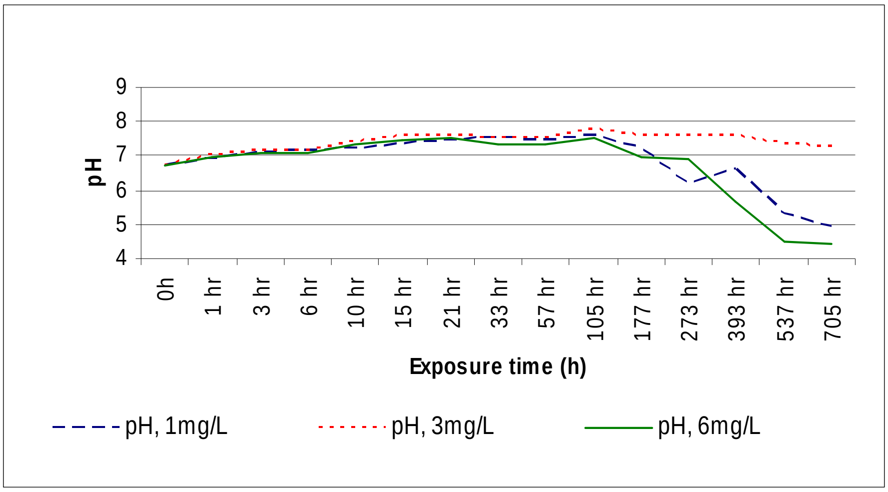


Figure 2. *Results for pH variations in water hyacinth covered containers*

Metals will precipitate as hydroxides when the pH of the wastewater is raised to pH 8 to 11 (Barron *et al.,* 1982). As a result, the extent of adsorption was rather low at low pH values. However, in the equilibrium solid phase, Zn (II) and Cr (VI) ion concentrations increased with increasing pH because of increasingly negative charges on the surfaces of the roots at high pH values. This attracted positively charged Zn (II) and Cr (VI) ions more strongly. The ANOVA with replications showed that for 1 mg/L, 3 mg/L and 6 mg/L there was no effect of exposure time but high difference between pH effects and metal remaining in water samples ($P \leq 0.05$). It was observed that the pH effect variations were due to the saturation of binding sites on root systems which affect the pH in water samples with water hyacinth plants by releasing H+ in water samples.

Plant relative growth (RG)

The relative growth of water hyacinth plants at different concentrations of zinc and chromium is shown in *Table 1.* It can be seen that the relative growth of plants decreases with the increase of zinc and chromium concentrations.

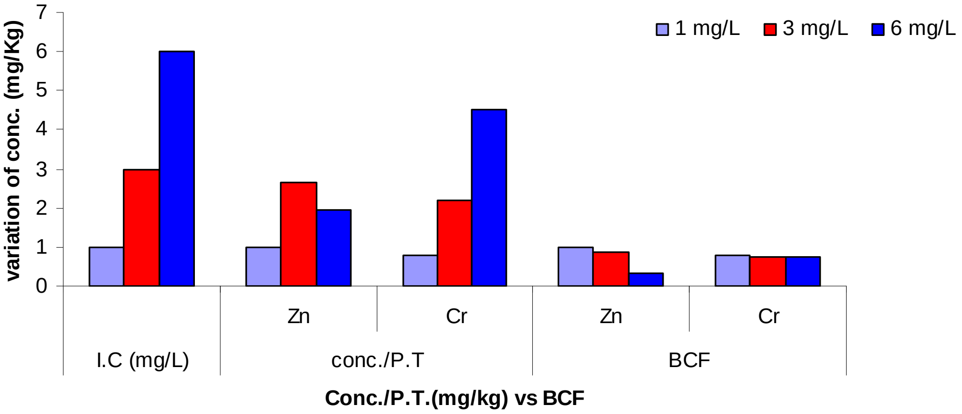
Table 1. *Relative changes in growth of plants vs. zinc and chromium concentrations*

Exposure time (week) with Zn & Cr concentrations	Initial water hyacinth fresh weight (g)	Final water hyacinth fresh weight (g)	Relative growth
1 wk, 1 mg/L	32.33	85.23	2.64
1 wk, 3 mg/L	34.50	96.91	2.81
1 wk, 6 mg/L	26.38	50.07	1.89
2 wks, 1 mg/L	29.75	55.92	1.88
2 wks, 3 mg/L	42.90	80.18	1.87
2 wks, 6 mg/L	16.24	39.96	2.27
4 wks, 1 mg/L	41.15	85.57	2.08
4 wks, 3 mg/L	39.65	98.05	2.47
4 wks, 6 mg/L	34.54	96.16	2.78

For water hyacinth plants treated with Zn and Cr, the plant relative growth significantly decreased ($P \leq 0.05$) from 1, 3 and 6 mg/L in 1 week but for 2 and 4 weeks, the relative growth decreased only slightly with increasing ($P \leq 0.05$) metal concentrations. The relative growth exhibited a decreasing trend caused by relative increases in toxicity of chromium and zinc. The analysis of variance showed that for 1 week exposure time, there is a high effect (difference is significant) of initial concentrations (1, 3 and 6 mg/L) to the growth of the plants ($P > 0.05$), but for 2 and 4 weeks related to initial concentrations, the difference is not significant ($P > 0.05$). Xiaomei et al. (2004) reported that the relative growth of water hyacinth decreases when metal concentrations increase, confirming what was observed in this investigation.

Bioconcentration Factor (BCF) of zinc and chromium

The bioconcentration factor (BCF) was calculated as the ratio of the trace element concentration in the plant tissues at harvest to the concentration of the element in the external environment (Zayed, 1998). The pattern of the bioconcentration factor of water hyacinth plants is shown in *Fig. 3*.



Note: I.C.: initial concentrations (mg/L); Conc./P.T: concentration in plant tissues (mg/Kg); BCF: Bioconcentration Factor.

Figure 3. *BCF for different initial concentrations of Zinc and Chromium*

A high competition between zinc and chromium accumulation by the plants was observed due to the kinetics of these metals. The comparison of Zn (II) and Cr (VI) showed that the BCF of zinc was higher than the chromium’s BCF for 1 and 3 mg/L, but very low for 6 mg/L for zinc. The plant accumulated more for low initial concentrations than for high ones. It was observed that there is no significant difference both for zinc and chromium when comparing initial concentrations to the concentrations in plant tissues and bioconcentration factors for zinc and chromium ($P > 0.05$).

Adsorption

Figure 4 shows the adsorption experimental results for zinc and chromium. The removal was highest for the first 15 minutes and the highest chromium concentrations observed for 6 mg/L was around 2 mg/L, for 3 mg/L was around 1.6 mg/L and for 1 mg/L was around 0.3 mg/L. The adsorption capacity of water hyacinth plants seemed to be different when zinc and chromium are compared. For zinc, 17.6 % of 1 mg/L was adsorbed by the water hyacinth plants, 6.1 % of 3 mg/L was adsorbed and the plants adsorbed 1.1 % of 6 mg/L. For chromium, 9.0 % of 1 mg/L, 36.4 % of 3 mg/L and 54.6 % of 6 mg/L were adsorbed by the water hyacinth plants. Meggo(2001) has confirmed that aquatic plants are able to accumulate metals at low concentrations.

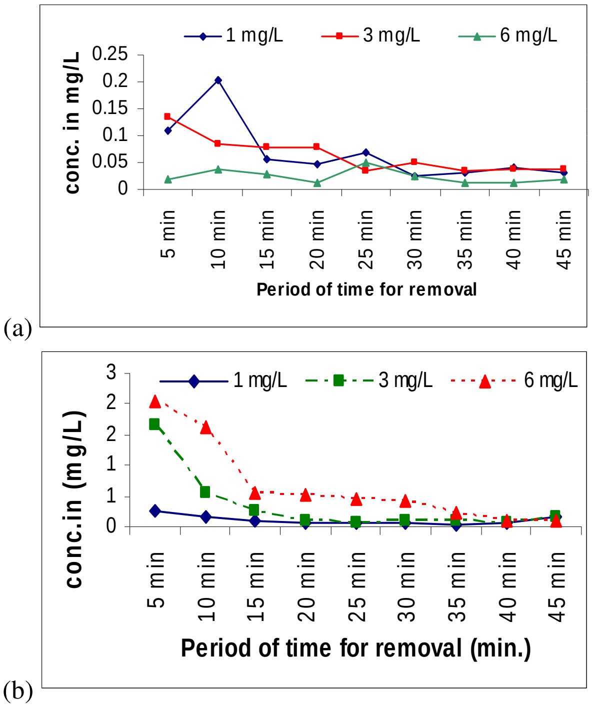


Figure 4. *Desorption over time for various initial concentrations of (a) and (b) chromium*

Uptake for zinc and chromium

It was observed that 56.7 % of zinc was accumulated in petioles, 27.0 % in leaves and 16.3 % in roots. The analysis demonstrated no significant difference for different initial concentration and exposure time ($p > 0.05$) in uptake mechanisms of zinc. There was a significant difference observed in plant parts in uptake processes ($p \leq 0.05$). For chromium, 73.7 % was taken up in roots, 14.1 % in petioles and 12.2% in leaves, demonstrating the preference of plants to store chromium in roots. The trend was the same for zinc; no significant difference existed between plant parts ($p > 0.05$) and also between initial concentrations in uptake processes ($p > 0.05$).

Translocation ability (TA)

The quantities of trace elements accumulated in the petioles exceeded those in the shoots. Roots of water hyacinth accumulated about 3 to 15 times more trace elements than did the shoots. It appears that chromium translocation is comparable to zinc as shown in *Table 2*. The ability of plants to translocate trace elements of chromium increased for roots/leaves (5.3 times for 1 mg/L, 6.5 times for 3 mg/L and 6 times for 6 mg/L). The number of times for roots/petioles decreases (4 times for 1 mg/L, 4 times for 3 mg/L and 7 times for 6 mg/L) because the order of storage was leaves<petioles<roots.

Table 2. *TA of (a) zinc and (b) chromium*

		(a) ***Initial conc. of Zn (II)***		
	Roots/ Shoots	**1 mg/L**	**3 mg/L**	**6 mg/L**
1 week	Roots/petioles	0.382^{a}	0.255^{a}	0.383^{a}
	Roots/leaves	1.114^{a}	0.732^{a}	0.164^{a}
2 weeks	Roots/petioles	0.478^{a}	0.485^{a}	0.439^{a}
	Roots/leaves	0.461^{a}	0.564^{a}	0.993^{a}
4 weeks	Roots/petioles	0.171^{a}	0.510^{a}	0.109^{a}
	Roots/leaves	0.241^{a}	1.041^{a}	0.255^{a}

		(b) ***Initial conc. of Cr (VI)***		
	Roots/ Shoots	**1 mg/L**	**3 mg/L**	**6 mg/L**
	roots/petioles	4.104a	3.663a	6.831a
1week	roots/leaves	5.288a	6.487a	5.965a
2 weeks	Roots/petioles	2.012a	2.254a	5.870a
	Roots/leaves	2.365a	3.851a	4.564a
4 weeks	Roots/petioles	1.235a	3.258a	1.239a
	Roots/leaves	2.578a	3.851a	1.008a

An ANOVA was performed on the variability in translocation ability. It was observed that the difference between metal concentrations is not significant ($p > 0.05$) and there was no significant difference between roots and shoots translocation ($p > 0.05$). Stratford *et al.* (1984) found that metal accumulations in water hyacinth increased linearly with solution metal concentration in order ***leaves<petioles<roots.*** In this research, the following order was observed: ***leaves<roots<petioles***. When the concentration is high, the water hyacinth plant can only accumulate a low concentration in plant cells. This is in agreement with the results of this study in the case of chromium concentration accumulation in water hyacinth plants, where the high concentration was accumulated in roots followed by petioles and then leaves. Water hyacinth materials were burned after all experiences to avoid the contamination.

Conclusions and recommendations

From the above results, it is concluded that water hyacinth could effectively remove zinc and chromium from industrial wastewater.. The removal mechanisms of zinc and chromium by water hyacinth plants showed that the plant concentrated a high quantity of metals. The experiments conducted under this study could be up-scaled to include treatment of actual wastewater from various industries. Essential design parameters such as hydraulic retention time needs to be determined so that the technology can be widely used in Rwanda and beyond.

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RESOURCE USE AND CROP PRODUCTIVITY IN A *COLOPHOSPERMUM MOPANE* TREE BASED AGRO-ECOSYSTEM IN INDIAN DESERT

SINGH, G.* – RATHOD, T.R.

Division of Forest Ecology, Arid Forest Research Institute, New Pali Road, Jodhpur-342005, India

**Corresponding author*
e-mail: singh_g_dr@yahoo.co.in; gsingh@icfre.org

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Abstract. A 9-year-old plantation of *Colophospermum mopane* (Kirk et Benth) kirk ex J. Leonard at a spacing of 5m x 5m was selected to study tree crop interaction with a view to find out suitability of this species for integration in agriculture land. Different treatments were (i) fixed crop of *Vigna radiata* (FC), (ii) rotation crop (*Vigna radiata* rotated by non-legume crop, RC) (iii) sole agriculture crop (AC), and (iv) sole tree (TC). *Vigna radiata* (L.) Wilczek (mungbean) was sown on 21 June 2003. Micro-plots of 1m X 1 m size were laid at 1m (near), 2.5 m (middle of two trees) and 3.5 m (centre of four trees) from the selected trees for sampling and yield recording. Tree height, dbh and crown diameter differed significantly ($P < 0.05$) between the plots. Soil water content was lowest ($P < 0.05$) in tree-integrated plots but it increased in deeper soil layers. SWC varied through non-significant in August, lowest ($P < 0.05$) at 1 m in September to significantly ($P < 0.01$) different between sampling distances in December 2003 suggesting exhaustive use of soil water by trees. Soil organic matter, available PO_4-P, NO_3-N and NH_4-N did not differ among the treatments and micro-plots though these nutrients differed in soil layers. Tree water use from 0-25 cm in August, 25-50 cm in September and 50-75 cm soil layer in December 2003 indicated a competitive nature of *C. mopane* with the associated *V. radiata* crop reducing population and yield up to a level of 97%. Reduction in grain was relatively greater than husk (pods after grain removal). Seedling population and yield were lowest at 1 m distance and increased with distance from the trees. Leaf water status of *V. radiata* was highest at 1m and it decrease with distance with a lowest value in AC plots. Relative neighbour effect values indicated a transition from strong competitive to weak facilitative nature of *C. mopane* and suggested the dominance of competitive effects during crop growth. Conclusively, integration of *C. mopane* reduces crop yield when rainfall is sufficient only for agriculture crop. Trenching around tree to reduce root overlapping between tree and agriculture crop and therefore competition for resources may be beneficial and enhance crop yield.

Keywords: *Colophospermum mopane, competition for resources, crop yield, soil nutrients, soil water, V. radiata*

Introduction

Trees growing in the agriculture land and community lands of Indian Desert minimize the risk of crop failure during drought and famines by providing fodder and fuel wood (Shankarnarayn et al., 1987; Singh et al., 2004). However, population of these are very less that requires further integration of trees to improve vegetation status and would be beneficial for the poor by providing top feed for livestock and fuel for energy generation. Trees have been found to facilitate regeneration of native and existing vegetation in forest plantation (Bone et al., 1997), sub-alpine and alpine plant communities (Callaway et al., 2002), desert communities (Vetaas, 1992) and the Middle East (Tielborger and Kadmon, 1995). However, there are also reports of neighbour interference through allelopathic suppression (Friedman et al., 1977) and competition for resources (Singh et al., 2001; Tielborger and Kadmon, 1997). In agriculture land, tree influences crop productivity (Aggarwal and Kumar, 1990; Brenan and Kessler, 1995) through soil improvement (Kater et al., 1992; Singh et al., 2000), microclimatic modification (Belsky et al., 1993) and soil water conservation (Gupta and Saxena, 1978). But limited resource availability in arid and semi-arid regions, the benefits of tree integration largely depends upon efficient and judicious management of soil and water resources. Therefore, selection of suitable tree species, an appropriate combination of tree and crop and development of suitable management practices like pruning, lopping, thinning and root clipping are important aspects to enhance productivity of an agroforestry system.

Many exotics like *Eucalyptus*, *Prosopis*, *Acacia* and *Colophospermum* etc. have been tried for their suitability to rehabilitate and to increase productivity of degraded lands because of their relatively higher growth as compared to the indigenous species (Hocking, 1993). However, before recommending into agriculture land it is necessary to study tree behaviours towards agriculture crop by studying tree crop interaction using systematically designed experiment. *Colophospermum mopane* (kirk et Benth) Kirk ex J. Leonard is an introduction to India from low altitude, high temperature regions of southern Africa and belongs to family Leguminoceae subfamily Caesalpinoideae (Bennet, 1983). It is a widespread tree of the drier parts of the Zambezian region and the river valleys of central and southern Africa (Coates, 1983). It is a preferred fuel wood and fodder species at its natural site. Because of its multipurpose uses this species has been planted to increase the productivity of degraded lands in Indian Desert. However, it is necessary to study the tree crop interaction in terms of competitive or facilitative effects on agriculture crops for its better uses in agroforestry or silvopastoral systems.

Therefore, present study was carried out to determine tree crop interactions in terms of resource use like photo-synthetically active radiations (PAR), soil water and soil nutrients and crop productivity and suggest suitable management strategy for better use of this species in enhancing productivity of dry lands.

Materials and methods

Site conditions

Study was conducted at the experimental farm of Arid Forest Research Institute, Jodhpur (Latitute 26º45`N, Longitude 72º03 E) in Rajasthan, India. The climate of the site is tropical and characterized by hot and dry summer, hot rainy season, warm autumn and cool winter (dipping down to 2 ^{0}C). Summer is the most dominant characterized by high temperature (reaching up to 49 ^{0}C in month of May) from March to mid July and experienced strong winds (usually 20-30 km h^{-1}). The period from mid July to September is the monsoon season, which receives most of the rainfall, which varied widely between the years [27]. Soil of the experimental site is aridisol “coarse loamy, mixed, hyperthermic of Typic Camborthids” as per the USDA system of classification. Soil texture is loamy sand with soil pH of 8.48, EC of 0.36 dSm^{-1} and soil organic carbon of 0.203 in 0-75 cm soil layer. The depth of the soil is about 80 cm, below which there is a hardpan of calcium carbonate aggregates called ‘Kankar’ of various sizes. Soil moisture storage in the upper 75 cm layer varies from 120 mm at -0.01 MPa to 35 mm at -1.5 MPa.

Experimental setup

A 9-year-old plantation of *Colophospermum mopane* was selected for the experiment. Different treatment plots were (i) fixed crop plot of *Vigna radiata* only (FC), (ii) rotation crop plot i.e., *Vigna radiata* rotated by non-legume crops like *Pennisetum glaucum* (RC) in second year, (iii) sole agriculture crop plot (AC), and (iv) tree only plot (TC) in three replications. Each plot was of 20 m x 15 m size with 12 experimental plants at the spacing of 5 × 5m. *Pennisetum glaucum* was harvested in RC plot in 2001 whereas no crop sowing in 2002 because of severe drought. Therefore, *Vigna radiata* was sown in both the FC and RC plots on 21 June 2003 after 64.5 mm rain between 18 to 20 June 2003. In each plot, one tree was randomly selected and micro-plots of 1 m^2 area were laid out at 1.0 m (near), 2.5 m (middle of two row of trees) and 3.5m (centre of four trees) from tree for sampling and observations recording on crop yield, soil water and soil nutrients. There were 12 plots (4 treatments X 3 replicates) in randomized block design.

Observation recording

Height, collar diameter, diameter at breast height (dbh) and crown diameter of *C. mopane* trees were recorded in June and December 2003. Percent increment in growth variables were calculated as: % increment = (x in December - x in June) × 100/ x in June. Where x is height, collar diameter, dbh or crown diameter. Photosynthetically active radiations (PAR) was measured at 1 m distance from tree trunk (under the canopy) as well as in the AC plots in September 2003 with portable CO_2 gas analyser, model *CI-301* (*CT-301 PS0*; *CID*, Vancouver, USA). Population of *Vigna radiata* seedlings was counted on 22 August 2003 from the micro-plots. Leaf samples of *V. radiata* were collected on 8th and 16th September 2003 at 14.00 hr and fresh weight was recorded immediately. Leaf dry weight was recorded after drying the samples at 65 ^{0}C and leaf water content was calculated. Crop was harvested from the micro-plots on 31 October 2003 and grain, husk (pods after removal of grain) and holm (straw) yields (air-dried) were recorded after winnowing. A relative neighbour effect (RNE) was calculated following the method of Markham and Chanway (Markham and Chanway, 1996). RNE = (X_t -X_c)/x where X was the performance variables of target species in absence (t) and presence (c) of adult neighbours (i.e., *C. mopane* trees) and x was the higher of X_t or X_c. Negative values of RNE indicated facilitation and positive values competition.

To find out the influence of *C. mopane* trees on soil nutrients and soil water, soil samples were collected from the micro-plots. Soil samples were collected in September (0-25 cm soil layer) and again in December 2003 (in 0-25, 25-50 and 50-75 cm soil layers) for soil nutrient analysis. For soil water, sampling was carried out in August (crop growing period), September (crop maturity) and December 2003 (after crop harvest). A single soil core was taken using a mechanical soil sampler and divided into 0-25, 25-50 and 50-75 cm layers to observe the soil water depletion in different soil layers. Soil water was determined gravimetrically after drying the soil at 105 °C. Gravimetric soil water content was converted to mm using the following equation to give total soil water to the soil depth of 0-75 cm. Soil water (in mm) = % soil water × soil depth in mm × bulk density of soil /100/ density of water

Soil sampling and chemical analysis

Soil samples were air dried, ground and passed to a 2 mm mesh sieve and subjected to various analyses. Organic carbon was determined by the partial oxidation method (Walkley and Black, 1934). Available nitrogen was determined after 2 M KCl extractions using UV-VIS spectrophotometer (Systronix model 117, Ahmedabad, India). Extractable phosphorous was determined by the Olson`s extraction method (Jackson, 1973).

Data processing

Data were statistically analyzed using the SPSS statistical package. Response variables were soil water, nutrients and leaf water status, seedling density (seedlings m^{-2}) and yield of *V. radiata* (g m^{-2}) per replicate. Soil nutrients in September 2003 (0-25 cm soil layer), leaf water status, seedling density, crop yield and their RNEs were analyzed using a two way ANOVA. Treatment plots and the micro-plots at different distance were the main effects and the difference was the error term. Soil water content data were square root transformed (Sokal and Rohlf, 1981). To test for variation in soil water content in FC and RC plots, three way ANOVAs were performed using soil water data as the dependent variable and treatments, micro-plot distances and soil layers as the main effects and the residual data as the error terms. Since soil sampling for soil water and nutrients in December 2003 was done at one distance only in AC and TC (1m) plots, these data were analysed by a two-way ANOVA using treatments (FC, RC, AC and TC plot) and soil depth as the main effect. RNE for soil nutrients in December and soil water was analyzing by two-way ANOVA for each soil layer independently using treatments and micro-plots distances as the main effect. Tree height, collar diameter, diameter at breast height (dbh) and crown diameter was analyzed using a one-way ANOVA. Treatment plots were the main effect. To obtain the relations between crop production and tree growth variables, soil water content and soil nutrients, a Pearson correlation was performed. To obtain homogeneous subsets in treatments, micro-plot distance and soil layers, Duncan Multiple Range Tests (DMRT) were performed for each data set.

Results

Tree growth variables

Tree height and crown diameter did not differ ($P > 0.05$) between treatments in both June and December 2003 (*Table 1*). However, collar diameter differed significantly ($P < 0.01$) at both the time mention above. Diameter at breast height was low ($P < 0.05$) in TC plot in June 2003 but it did not differ ($P > 0.05$) in December 2003. Trees were tallest in FC plots but collar diameter, crown diameter and DBH were greater in RC plots. All the growth variables were low in the TC plots. When compared with collar diameter in TC plots, trees were 1.34 and 1.30 fold thicker in FC plots and 1.47 and 1.50 fold thicker in RC plots respectively in June and December 2003. Percent increments in growth variables in December 2003 (over growth in June 2003) were highest in TC plots except of height increment which was highest in RC plots (*Table 1*).

Table 1. *Mean growth variables and percent growth increment of 114 months old C. mopane trees under agroforestry in dry area of Indian desert*

Variables	**Treatments**			**ANOVA**	
	FC	**RC**	**TC**	**F value**	**P value**
June 2003					
Height	535±10.4	503±33.8	476±4.4	2.01	NS
Collar diameter	11.9±0.45	13.1±0.68	8.9±0.24	20.12	0.002
Crown diameter	427±37.1	457±36.7	408±17.4	0.59	NS
DBH	8.6±0.30	9.6±0.71	7.4±0.23	5.51	0.044
December 2003					
Height	540±11.6	528±26.8	505±13.3	1.73	NS
Collar diameter	12.2±0.44	13.6±0.66	9.4±0.21	20.26	0.002
Crown diameter	437±37.1	482±49.4	436±18.6	0.48	NS
DBH	8.8±0.27	10.1±0.68	8.6±0.25	3.27	NS
Per cent growth increment					
Height	0.93±0.52	5.18±1.73	4.20±0.04	4.54	0.063
Collar diameter	2.26±0.32	2.24±0.60	6.06±1.46	4.44	0.066
Crown diameter	2.38±0.19	5.13±2.38	6.97±2.08	1.60	0.278
DBH	2.75±0.50	5.67±0.72	16.24±2.11	54.29	0.000

Values are mean of three replicates ±SE.

FC, fixed crop plot V. radiata only; RC, rotation crop plot in which V. radiata was rotated by non-legume crop; and TC, tree crop only.

Above ground resources

Photosynthetically active radiations (PAR) val. were 264.7, 276.0 and 308.2 µmol m^{-2} s^{-1} in the canopy zone area (1 m distance from tree trunk) of FC, RC and TC plots, respectively. Whereas PAR values in AC plots (i.e., agriculture crop without trees) was 2282.9 µmol m^{-2} s^{-1}. The reduction in PAR value under the canopy of *C. mopane* trees were 88.4 % in FC plot, 87.9 % in RC plots and 86.5 % in TC plots when compared with PAR value in the AC plots. There was 327.2 mm rain during July to September with a total number of rainfall days was 26. Main rainfall periods were 6 to 11 July (121.6 mm), 13 to18 July (83.1 mm), 22 to 25 July (19.5 mm), 28 July to 3rd August (37.1 mm), 8 August (11.7 mm) and 25 to 29 August 2003 (52.6mm) (*Fig. 1a*). There was only 1.6 mm rain in September i.e., on 26 September 2003. Total rainfall in year 2003 was 418.7 mm.

Soil water content and dynamics

Soil water content (SWC) was highest in August 2003 and lowest in December 2003 (*Figure 1b*). Two ways ANOVA (treatment X soil depth) indicated significant ($P < 0.01$) difference due to treatments and soil layers in August, September and December 2003. SWC was highest ($P < 0.01$) in the AC plots (*Table 2*). In August, AC plot had 101.49 mm soil water (0-75 cm soil layer) that reduced by 45.42 % in FC, 43.92 % in RC and 36.67 % in TC plots. In September 2003, reduction in SWC was by 38.71%, 36.85% and 24.43% in the respective plots as compared to 67.30 mm in AC plots (*Fig. 1b*). In December FC and RC plots had 31.51 % and 28.67 % less SWC as compared to 35.19 mm in AC plots whereas TC plot had 36.9 % low soil water. Average soil water depletion (average of all the four plots) from 18th August to 4th September and 4th September to 3rd December was 18.64 mm and 23.82 mm, respectively as compared to 69.12 mm on 18th August 2003.

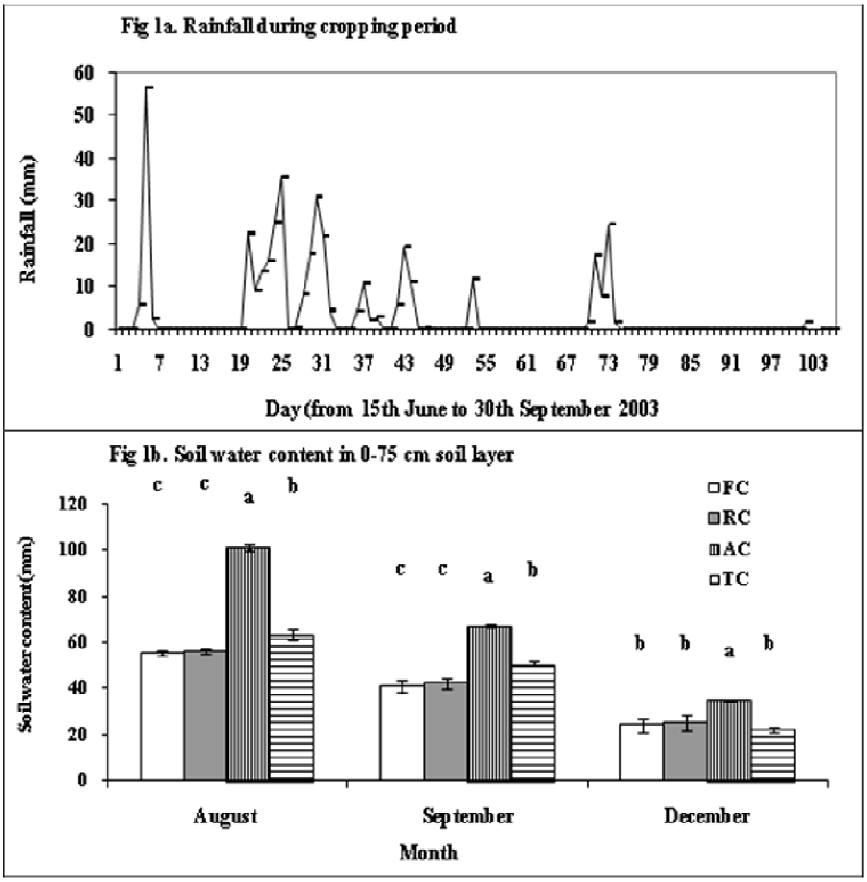
Fig 1a. Rainfall during cropping period

Fig 1b. Soil water content in 0-75 cm soil layer

Figure 1. *Rainfall distribution and soil water content in 0-75 cm soil layer. (a), rainfall during 15 June to 30 September 2003; (b), variations in soil water content during monsoon (July to September) and post monsoon period. Non-significantly ($P > 0.05$) different treatments share same letter within month*

A three way ANOVA of SWC in FC and RC plots data did not differ ($P > 0.05$) between treatments (*Table 2*). SWC differed ($P < 0.01$) between soil layers but did not vary in micro-plots at different distance in August 2003, whereas in September and December 2003, the variation due to soil layers was not significant but SWC differed ($P < 0.01$) due to distances (micro-plots). SWC was lowest near tree but 2.5 m (middle) and 3.5 m (centre) distances did not differ in SWC in September 2003 but all the distances (micro-plots) indicated significant difference in December 2003. Considering the soil depth, SWC was lowest in 0-25 cm soil layer and increased with soil depth. SWC was lowest in 0-25 cm in August, 25-50 cm in September and 50-75 cm soil layer in December 2003 (*Figure 2*). All the interaction terms indicated non-significant values.

Table 2. *Effect of Colophospermum mopane trees on soil water status (%, w/w) during crop growing (August and September 2003) and crop harvesting (December 2003) period*

Treatment	Soil depth	18 August 2003			4 September 2003			4 December 2003		
		Near	Middle	Centre	Near	Middle	Centre	Near	Middle	Centre
FC	0-25	3.54±0.17	4.06±0.32	3.83±0.27	3.04±0.13	3.65±0.43	3.89±0.40	1.25±0.08	2.22±0.09	3.00±0.10
	25-50	4.86±0.40	4.92±0.41	5.22±0.09	3.15±0.34	4.20±0.14	3.95±0.27	1.47±0.12	2.31±0.13	2.98±0.15
	50-75	5.95±0.12	6.01±0.16	6.06±0.42	2.98±0.31	4.04±0.52	4.08±0.50	1.22±0.06	1.92±0.31	2.92±0.22
RC	0-25	3.89±0.19	3.59±0.05	4.35±0.38	3.47±0.21	4.06±0.41	4.27±0.17	1.28±0.05	2.08±0.03	3.36±0.11
	25-50	5.17±0.15	4.88±0.26	5.38±0.29	2.99±0.31	3.95±0.45	3.80±0.23	1.28±0.04	2.10±0.04	3.62±0.38
	50-75	6.12±0.16	5.96±0.47	5.92±0.32	3.30±0.18	4.11±0.49	4.03±0.49	1.26±0.07	2.15±0.05	2.95±0.39
AC	0-25	7.75±0.22			4.42±0.20			1.31±0.01		
	25-50	9.26±0.10			6.73±0.08			3.32±0.13		
	50-75	10.06±0.22			6.79±0.06			4.76+0.07		
TC	0-25	4.44±0.09			3.82±0.08			1.43±0.06		
	25-50	5.44±0.32			4.45±0.17			1.82±0.07		
	50-75	7.01±0.18			5.28±0.08			2.67±0.16		
Two ways ANOVA										
F value										
Treatment		181.9**			148.7**			294.8**		
Depth		116.3**			31.78**			176.2**		
Treatment x depth		2.25NS			16.60**			90.1**		

Values are mean of three replicates ± SE.
FC, fixed crop of V. radiata only; RC, rotation crop in which V. radiata was rotated by non-legume crop; AC, sole agriculture crop; and TC, tree crop only.

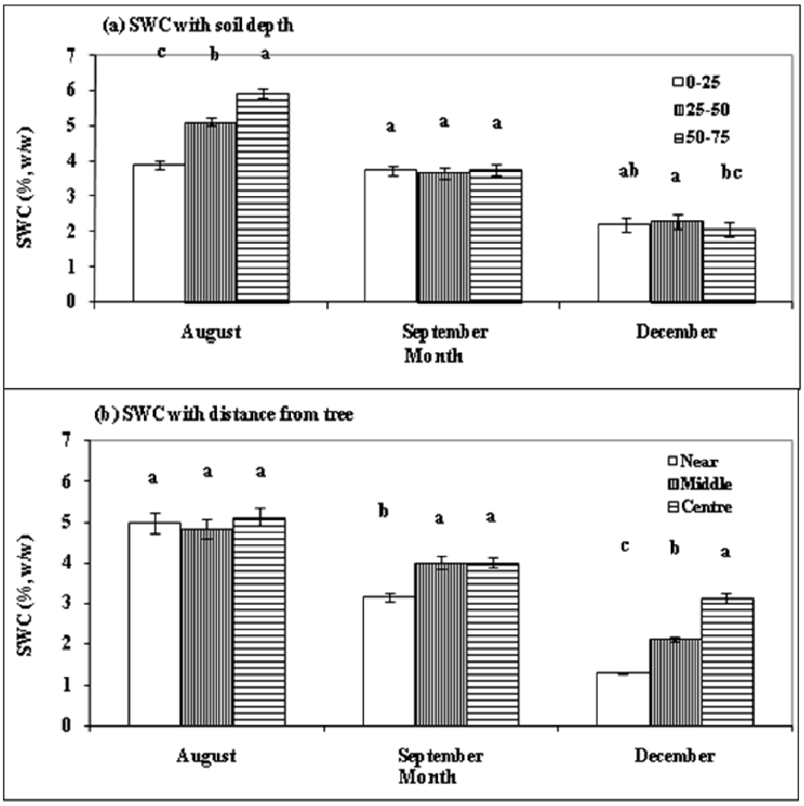

Figure 2. *Changes in soil water content with time in different soil layers and distance from C. mopane tree. (a), variations with soil depth (mean of treatments FC and RC and distance); (b) variations with soil depth (mean of treatments FC and RC and soil depth). Error bars are ±SE of three replications. Bars with similar alphabets did not differ significantly (P > 0.05)*

Soil nutrients

Soil organic matter (SOM), available PO_4-P, NH_4-N and NO_3-N of 0-25 cm soil layers in September and December 2003 did not differ between the treatments as well as the sampling position (near, middle, and center). However, available NO_3-N indicated low ($P < 0.05$) value at center whereas middle and center micro-plots did not differ in NO_3-N concentration. These nutrients decreased in December 2003 than in September 2003 in all the plots except in AC, in which there was an increase in NH_4-N and NO_3-N (*Fig. 3*). In December 2003, SOM, PO_4-P and NO_3-N did not differ due to treatments. But DMRT indicated low ($P < 0.05$) availability of PO_4-P and NO_3-N in TC and FC plots, respectively whereas NH_4-N was lowest ($P < 0.01$) in TC and highest in AC plots (*Table 3*). These soil nutrients were highest ($P < 0.01$) in availability in 0-25 cm but did not differ between 25-50 cm and 50-75 cm soil layers except for NH_4-N.

Three ways ANOVA of these nutrients for FC and RC plots indicated no variations due to treatment and distance from tree (*Table 3*). But the soil nutrients were highest ($P < 0.05$) in 0-25 cm soil layer. Duncan Multiple Range Test (DMRT) indicated greater ($P < 0.05$) availability of SOM (mean of soil depths and treatments) near tree. NH_4-N increased with distance and highest concentration was at centre. NO_3-N was highest at centre and lowest at middle while PO_4-P was highest at middle and lowest at centre of four trees. All the interaction terms were not significant ($P > 0.05$).

Table 3. *Effect of Colophospermum mopane trees on soil nutrients in December 2003 (After crop harvesting)*

Treatment	Soil depth	SOM (%)			NH_4-N			NO_3-N			PO_4-P		
		Near	Middle	Centre	Near	Middle	Centre	Near	Middle	Centre	Near	Middle	Centre
FC	0-25	0.33	0.33	0.33	4.45	4.54	4.93	3.20	3.05	3.21	12.31	12.08	11.68
		(0.02)	(0.01)	(0.01)	(0.15)	(0.12)	(0.62)	(0.07)	(0.23)	(0.26)	(1.05)	(0.66)	(0.50)
	25-50	0.23	0.18	0.17	3.17	3.04	3.13	2.11	2.22	2.28	10.17	11.03	10.79
		(0.03)	(0.03)	(0.04)	(0.31)	(0.15)	(0.01)	(0.15)	(0.09)	(0.15)	(0.46)	(0.60)	(0.83)
	50-75	0.20	0.11	0.11	2.57	2.59	2.42	2.08	2.07	2.06	10.25	10.18	9.06
		(0.12)	(0.01)	(0.01)	(0.09)	(0.52)	(0.41)	(0.21)	(0.10)	(0.04)	(0.41)	(1.91)	(1.08)
RC	0-25	0.33	0.32	0.34	4.50	4.56	4.61	3.22	3.03	3.27	12.60	12.33	12.21
		(0.01)	(0.01)	(0.01)	(0.20)	(0.11)	(0.08)	(0.29)	(0.64)	(0.29)	(1.51)	(0.97)	(0.14)
	25-50	0.22	0.20	0.20	2.97	2.85	2.77	2.28	2.23	2.35	10.72	10.83	10.74
		(0.09)	(0.05)	(0.02)	(0.27)	(0.38)	(0.10)	(0.05)	(0.12)	(0.03)	(0.11)	(0.29)	(0.48)
	50-75	0.18	0.11	0.11	2.69	2.70	2.25	2.11	2.11	1.94	9.90	10.05	9.69
		(0.02)	(0.01)	(0.01)	(0.61)	(0.63)	(0.33)	(0.14)	(0.13)	(0.02)	(0.96)	(0.83)	(0.49)
AC	0-25	0.37			4.54			4.00			13.21		
		(0.01)			(0.30)			(0.35)			(0.62)		
	25-50	0.23			3.50			2.28			11.09		
		(0.01)			(0.16)			(0.05)			(0.36)		
	50-75	0.13			2.13			1.96			9.87		
		(0.01)			(0.01)			(0.08)			(0.29)		
TC	0-25	0.34			3.54			3.33			11.29		
		0.01			(0.12)			(0.05)			(0.46)		
	25-50	0.17			3.06			2.18			10.23		
		(0.01)			(0.07)			(0.04)			(1.38)		
	50-75	0.17			2.14			2.10			9.97		
		(0.01)			(0.04)			(0.11)			(0.33)		
Two ways ANOVA													
F value													
Treatment		0.31NS			5.35**			2.53NS			1.98NS		
Depth		47.55**			140.5**			129.7**			29.24**		
T x depth		1.59NS			3.78**			3.13*			1.07NS		

Values are mean of three replicates with ± SE in parentheses.
Treatments as in Table 3.
Three way ANOVA for FC and RC plots was not significant except for soil layers.

Table 4. *Effect of over canopy tree on density of germinated seedlings and leaf water status of Vigna radiata in post dawn (14.00 hr) period*

Treatment	Distance	Seedling density (no. m^{-2})	Leaf water status at 14.00 hr (%) 8 September 2003	Leaf water status at 14.00 hr (%) 16 September 2003
FC	Near	3.67±0.88a	73.57±0.20a	71.81±0.33a
	Middle	6.67±0.88b	72.50±0.12b	70.54±0.24b
	Centre	7.00±0.58b	70.53±0.13c	69.54±0.13c
	Mean	5.78+0.66	72.20±0.45	70.63±0.35
RC	Near	3.67±0.33a	73.20±0.11a	71.18±0.12a
	Middle	4.67±0.33b	72.38±0.11b	70.58±0.26b
	Centre	6.00±0.01b	70.88±0.07c	70.25±0.22c
	Mean	4.78+0.36	72.15±0.34	70.69±0.17
AC		34.67±0.33	66.01±0.31	66.35±0.23
Two way ANOVA for FC and RC plots				
Probability of F value				
	Treatment	4.26	0.23	0.051
	Distance	12.05*	221.99**	24.85**
	T x D	1.42	4.06*	4.36*

Values are mean of three replicates ± SE. Treatments as in Table 2.
**, significant at $P < 0.05$; **, significant at $P < 0.01$.*

Density and leaf water status of V. radiata

Effect of trees on seedling population of *V. radiata* was observed since the time of germination. Density of germinated seedlings was higher ($P < 0.01$) in the AC plots (34.67 m^{-2}) as compared to the FC (5.78 m^{-2}) and RC (4.78 m^{-2}) plots (*Table 4*). Two-way ANOVA indicated no difference in seedling population between FC and RC plots (though population was relatively greater in FC plots than in RC plots) but density was less ($P < 0.01$) near tree and increased with distance being highest at centre, which did not differ with population at middle micro-plot (2.5 m, DMRT). Leaf water status (LWS) was higher on 8th September 2003 as compared to 16th September 2003 (*Table 4*). It was lowest ($P < 0.01$) in AC plots but it did not differ between FC and RC plots. In FC and RC plots LWS was highest ($P < 0.05$) at 1 m (71.5 %) and decreased with increase in distance from tree (69.9 % at centre).

Yield of V. radiata

Total yield (grain + husk + holm) of *V. radiata* was 95.61, 99.00 and 573.00 g m^{-2} in FC, RC and AC plots, respectively (*Table 5*). When compared with yield in AC plots, reduction in crop yield was by 83.3 % in FC and 82.7% in RC plots. Grain yield reduced by 97.5 % in FC and 97.3 % in RC plots, whereas the husk and holm reduced by 91.3% and 74.9% in FC plots and 90.6 % and 74.1 % in RC plots, respectively than in AC plots. Two ways ANOVA indicated no difference in treatments (FC and RC plots) and distance from tree for husk, grain and holm yield. However, these crop yield variables were relatively low at 1 m and highest at center. Per cent contribution of husk, grain and holm to the total produce was 12.6 %, 28.3 % and 59.1 %, respectively in AC plots. Average (mean of distance) contribution was 6.6 %, 4.3 % and 89.1 % in FC and 6.9 %, 4.4 % and 88.7 % in RC plots, respectively for husk, grain and holm. Reduction in grain yield was relatively greater (from 28.3 % in AC to 4.4 % in FC/RC) as compared to husk (12.6 % in AC to 6.8 % in FC/RC). Percent contribution of husk and grain to the total yield increased with distance from tree and were highest at the centre of four trees (*Table 5*).

Table 5. *Effect of over canopy of C. mopane on average yield (g m^{-2}) of V. radiata*

Treatment	Distance	Husk	Grain	Holm	Total
FC	Near	5.67±1.01a	3.67±0.93a	78.00±8.32c	87.33±10.24c
	Middle	6.00±1.15a	4.00±1.04a	83.67±6.50bc	93.67±8.67bc
	Centre	7.33±1.31a	4.67±0.93a	93.83±3.60ab	105.83±5.78ab
	Mean	6.33±0.63	4.11±0.51	85.17±3.97	95.61±5.01
RC	Near	5.83±0.88a	3.50±0.58a	79.50±7.40c	88.83±8.85c
	Middle	6.33±1.30a	4.33±1.09a	87.00±9.64bc	97.67±11.98bc
	Centre	8.33±0.93a	5.33±0.88a	96.83±5.36ab	110.50±7.08ab
	Mean	6.83±0.65	4.39±0.51	87.78±4.58	99.00±5.71
AC*		72.3±7.80	162.0±4.48	338.7±29.2	573.0±19.9
Two way ANOVA for FC and RC plots					
Probability of F value					
	Treatment	0.305	0.136	0.204	0.220
	Distance	1.974	1.190	2.780	2.695
	T x D	0.079	0.103	0.010	0.012

Values are mean of three replicates with ± SE. Treatments as in Table 2.
One-way ANOVA indicated significant ($P < 0.01$) difference among FC, RC and AC plots.
Within each column, treatments which are not-significantly different share the same letter.

Correlations in soil resources, tree growth variables and crop yield

Tree height, collar diameter and crown diameter were negatively correlated with SWC in August, September (0-25 and 25-50 cm soil layers) and December 2003 (50-75 cm soil layer), soil nutrients and crop yield i.e., husk (r = -0.917 to -0.971, $P < 0.01$), grain (r = -0.0.933 to -0.988, $P < 0.01$) and holm (r = -0.939 to -0.969, $P < 0.01$) (*Table 6*). A negative correlation was also observed between tree growth and density of *V. radiata* seedlings. However, growth variable were positively correlated SWC in 0-25 cm soil layer in December and SOM and NH_4-N in September 2003, and leaf water status of *V. radiata*. Density and yield of *V. radiata* were positively correlated with SWC in all the soil layer in August and 25-50 and 50-75 cm soil layer in September 2003. But crop yield was negatively correlated with SOM (r = -0.477 to -0.592, $P <$ 0.01) and NH_4-N (r = -0.672 to -0.713, $P < 0.01$) and positively related with PO_4-P (r = 0.904 to 0.934, $P < 0.01$) and NO_3-N (r = 0.908 to 0.955, $P < 0.01$, n = 21). Availability of SOM and NH_4-N was negatively related with SWC in 25-50 and 50-75 cm soil layer in September 2003 in but reverse trend was observed in December 2003. PO_4-P and NO_3-N were positively related with SWC in September 2003 and December 2003 (*Table 6*).

Relative neighbour effects

Relative neighbour effects (RNE) values for soil water (RNE_{SWC}) was positive except for 0-25 cm soil layer of middle and center micro-plots in December 2003 (*Figure 3a*). In August it was highest in 0-25 cm soil layer whereas RNE_{SWC} was highest in 25-50 cm and 50-75 cm soil layers, respectively in September and December 2003. $RNE_{Nutrient}$ was positive for most of the soil nutrients except for SOM and NH_4-N in September 2003. $RNE_{Nutrient}$ was also positive for SOM and PO_4-P in 50-75 cm (near tree), NH_4-N in 0-25 cm (middle and center of four trees) and 50-75 cm soil layers and NO_3-N in 50-75 cm soil layers (*Figure 3b*). RNE was also positive for density of *V. radiata* and crop yield like grain, holm and the total crop yield (Figure 3c). The values were higher near the tress as compared to middle and center of four trees. However, RNE$_{LWS}$ for *V. radiata* leaves was negative at all the three distances with lowest (more negative) value near the trees.

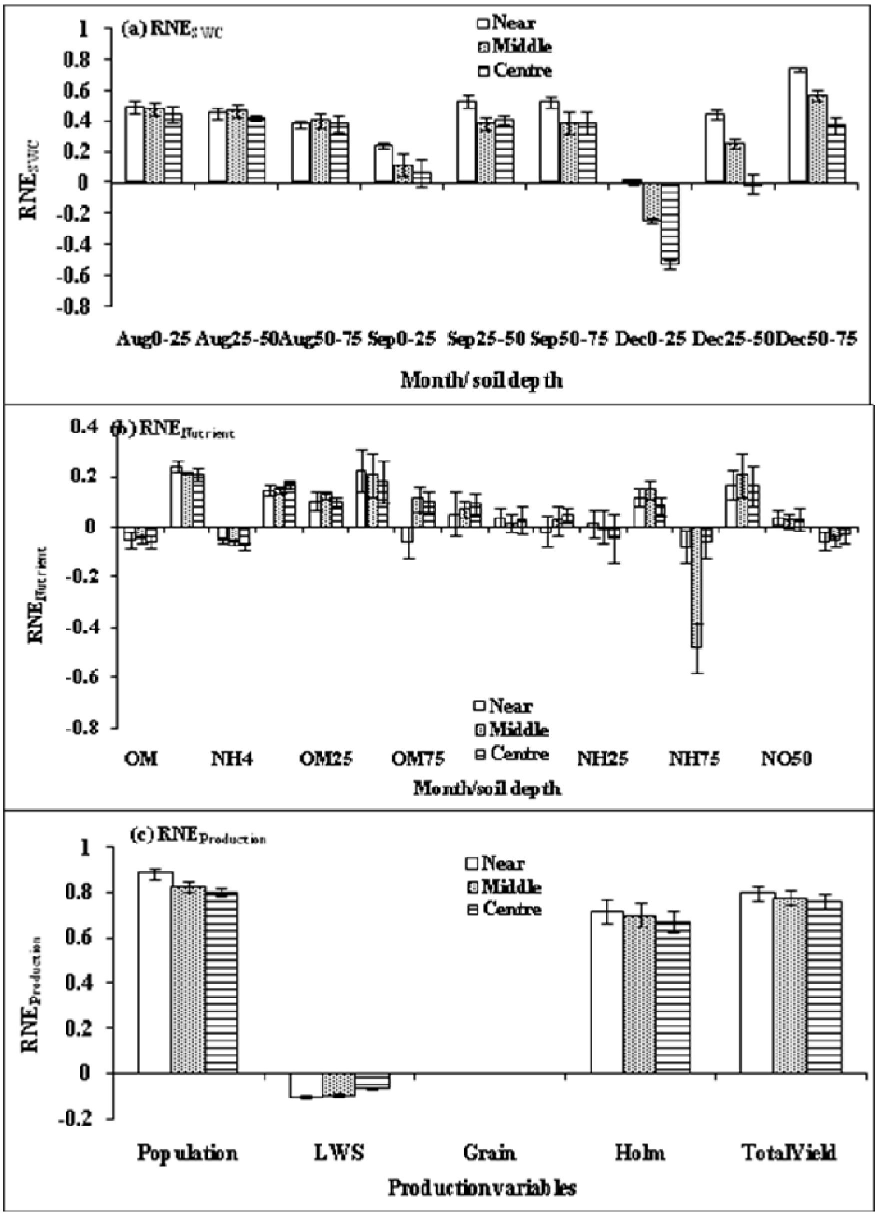

Figure 3. *Relative neighbour effects of C. mopane on (a), soil water content; (b), soil nutrients; and crop growth and yield parameters. LWS, leaf water status of V. radiata seedlings on 8 and 16 September 2003. Error bars are ±SE of three replications. Bars with similar alphabets did not differ significantly (P > 0.05) within the groups*

Table 6. *Correlation coefficient of tree growth variables of December 2003, soil resources and crop production*

Variables	Height	Crown dia.	Collar dia.	$\mathrm{AUG}_{\mathrm{SWC}}$	$\mathrm{SEP}_{\mathrm{SWC25}}$	$\mathrm{SEP}_{\mathrm{SWC50}}$	$\mathrm{SEP}_{\mathrm{SWC75}}$
$\mathrm{AUG}_{\mathrm{SWC}}$	-0.929**	-0.913**	-0.907**	-	0.446*	0.845**	0.763**
$\mathrm{SEP}_{\mathrm{SWC25}}$	-0.374	-0.461*	-0.353	0.446*	-	-	-
$\mathrm{SEP}_{\mathrm{SWC50}}$	-0.836**	-0.876**	-0.893**	0.845**	-	-	-
$\mathrm{SEP}_{\mathrm{SWC75}}$	-0.770**	-0.750**	-0.819**	0.763**	-	-	-
$\mathrm{DEC}_{\mathrm{SWC25}}$	0.352	0.316	0.406*	0.289	0.329	-0.137	-0.174
$\mathrm{DEC}_{\mathrm{SWC50}}$	-0.397	-0.399	-0.351	0.439*	0.615**	0.529**	0.406*
$\mathrm{DEC}_{\mathrm{SWC75}}$	-0.787**	-0.717**	-0.738**	0.776**	0.551**	0.814**	0.809**
$\mathrm{SEP}_{\mathrm{SOM}}$	0.564**	0.536*	0.580**	0.485*	-0.370	-0.523*	-0.638**
$\mathrm{SEP}_{\mathrm{PO4\text{-}P}}$	-0.925**	-0.906**	-0.957**	0.898**	0.476*	0.915**	0.854**
$\mathrm{SEP}_{\mathrm{NH4\text{-}N}}$	0.731**	0.621**	0.751**	-0.626**	0.154	-0.548*	-0.514*
$\mathrm{SEP}_{\mathrm{NO3\text{-}N}}$	-0.924**	-0.938**	-0.937**	0.914**	0.267	0.835**	0.754**
$\mathrm{DEC}_{\mathrm{SOM25}}$	-0.695**	-0.761**	-0.767**	-	-0.182a	0.396b	0.630**c
$\mathrm{DEC}_{\mathrm{SOM50}}$	-0.495*	-0.356	-0.450*	-	0.019a	0.392b	0.387c
$\mathrm{DEC}_{\mathrm{PO4\text{-}P25}}$	-0.405*	-0.290	-0.305	-	-0.211a	0.100b	0.167c
$\mathrm{DEC}_{\mathrm{NH4\text{-}N50}}$	-0.470*	-0.575**	-0.520**	-	-0.003a	0.473*b	0.461*c
$\mathrm{DEC}_{\mathrm{NO3\text{-}N25}}$	-0.674**	-0.519**	-0.582**	-	-0.213a	0.293b	0.551**c
Population	-0.978**	-0.924**	-0.981**	0.947**	0.432	0.918**	0.864**
LWS	0.888**	0.836**	0.880**	-0.872**	-534**	-0.881**	-0.869**
Grain yield	-0.988**	-0.933**	-0.973**	0.956**	0.390	0.875**	0.830**
Holm yield	-0.979**	-0.950**	-0.917**	0.964**	0.396	0.863**	0.828**
Total yield	-0.985**	-0.961**	-0.925**	0.955**	0.396	0.870**	0.831**

*Significant at *; $P < 0.05$ and **; $P < 0.01$. The values without * are not significant.*

SWC, soil water content . 25, 0-25 cm; 50, 25-50 cm; and 75, 50-75 cm soil layers; LWS, leaf water status of V. radiata crop.

a, b and c, correlation co-efficient in relation to SWC in December 2003 in 0-25, 25-50 and 50-75 cm soil layers, respectively.

Discussion

Interaction between tree and the associated crop for growth resources like light, water and nutrients affects the overall productivity of the system (Belsky et al. 1993). However, the types of interactions depended upon the competitive (Inchausti, 1995), and facilitative (Greenlee and Callaway, 1996) processes (i.e., shading and nutrient enrichment) of the associated trees. Taller and thicker tree in the FC and RC plot as compared to the trees in TC plots were due to soil working in the cropping plots. But greater incremental growth in trees of TC plots as compared to that in the FC and RC plots were due to greater availability of soil water discussed in next sections.

Soil water content

Differences in growth variables of *C. mopane* trees influenced the availability of soil water and nutrients. A negative correlation between tree growth variables and soil water contents (SWC) showing greater soil water use in tree-integrated plots, particularly by tree as compared to sole agriculture plot. Non-significant difference in SWC due to distance (micro-plots) in August 2003 was due to availability of sufficient amount of water received through rain in July and August. However, low soil water in 0-25 cm soil layer was due to utilization by tree and agriculture crop (*Figure 2*), though reduction in SWC in this soil layer through surface evaporation cannot be ruled out (Gibson and Bachelard, 1986). Significantly low SWC near tree zone (1m distance from tree) in September was due to soil water use by tree (*Table 2*). Relatively low input of water through rain between 18 August and 4 September (*Figure 1*) and high water use by tree in the rooting zone made a gradient in SWC, which increased with distance from tree trunk. However, relatively low SWC in 25-50 cm soil layer in September 2003 indicated that tree and probably *V. radiata* used soil water from this soil layer. Variations in SWC became more evident in December 2003 when it was highest ($P < 0.01$) at center of four trees suggesting greater amount of soil water extraction by trees from near and middle distances. A decrease in SWC in 50-75 cm soil layer in December 2003 was indicative of soil water use from this layer. This suggests that *C. mopane* utilize soil water from topsoil layer during sufficient soil water availability whereas it utilizes deeper soil water when soil water stress prevails in upper soil layers. Competition for soil water during August and September (crop growing period) might cause reduction in crop yield. Relatively greater positive RNE_{SWC} in 0-25 cm in August, in 50-75 cm in September and 50-75 cm soil layer in December 2003 as compared to the other soil layers also indicated competition for soil water use in these layers (*Figure 3a*). A facilitative effect (negative RNE_{SWC}) of *C. mopane* trees at middle and center of four trees in 0-25 cm soil layer in December 2003 was probably due to 'hydraulic lift' in which tree extracted water from deeper soil layer and redistribute it in the upper soil layer. Fuentes et al. (1984) also recorded an increase in soil water availability as a result of facilitative influence of neighbours. Competition for water and nutrients indicated overlapping of fibrous roots of *C. mopane* trees and crop as observed in other studies (Singh and Rathod, 2002; Wallace et al., 1980). Positive interactions found to increase with stress as evidenced by decreased RNE_{SWC} in 0-25 cm soil layer in December 2003 as compared to that in September 2003 (*Figure 1a*), which was comparatively wetter (Callaway et al., 2002).

Soil nutrients

The mechanism in building up of the soil nutrient level is the improvement of soil organic matter and other nutrients through addition of litter by the trees and associated vegetation and turnover of roots. Significantly higher SOM, PO_4-P, NH_4-N and NO_3-N in upper 0-25 cm as compared to 25-50 and 50-75 cm soil layers was attributed to be due to greater accumulation of organic matter on the surface of the soil through litter addition (*Table 3*). Litter addition and soil water availability regulate the organic matter decomposition and the formation of stable and labile soil organic matter pools (Vitousek and Sanford, 1986). This is evidenced by significantly ($P < 0.05$) greater availability of SOM (mean of soil depths and treatments) near tree. Though not significant but lowest availability PO_4-P, NH_4-N and NO_3-N ($P < 0.05$) near tree as compared to the middle and center of four trees distances was due to their utilization in tree/ crop growth (*Table 3*). Browald (1987) also observed higher concentration of NH_4-N and lower concentration of NO_3-N at closer distance of trees indicating an efficient uptake of nitrate and enhanced mineralization closer to trees. Low ($P < 0.05$, DMRT) availability of NO_3-N and PO_4-P in TC and FC plots was due to utilization by trees. Lowest ($P < 0.01$) availability of NH_4-N in TC and highest in AC plots was due to nitrogen addition through nitrogen fixing by *V. radiata*, a legume crop. Relatively low nutrient concentration in December 2003 as compared to that in September 2003 was resulted from their utilization in tree/ crop growth. Positive RNE values for most of the nutrients indicated competitive effect of *C. mopane* trees for utilizing soil nutrients. However a few negative RNE values near tree in 50-75 cm soil layer for SOM and PO_4-P, 0-25 and 50-75 cm soil layer for NH_4-N and 50-75 cm soil layer for NO_3-N indicated facilitative effect of *C. mopane* trees for these nutrients (*Figure 3b*), which was due to added litters or root turn over.

Light resource and leaf water status

Reduction in PAR under the canopy of *C. mopane* by 88.4 % in FC to 86.5 % in TC plots indicated a dense canopy influencing transpiration losses of the under canopy crop. That affected water status of *V. radiata* seedling growing in the vicinity of the trees. Significantly higher level of leaf water status in *V. radiata* leaves in tree integrated plots (FC and RC plots) as compared to that in AC plot suggested a reduction in transpiration loss of agriculture crop resulted from reduced PAR. Greater was the tree size higher was the leaf water status (*Table 4 & 6*). A negative RNE_{LWS} indicated facilitative effects of *C. mopane* tree by increasing water status of the associated crops (*Fig. 3c*). A decreased PAR and increased productivity under canopy of savanna trees is the most common pattern in tropical tree communities with low density, low rainfall and moderate soil fertility sites (Belsky et al., 1993).

Yield of V. radiata

Significantly low density of *V. radiata* plants on 22 August 2003 in tree-integrated plots (FC, 5.78 m^{-2} and RC, 4.78 m^{-2}) as compared to sole crop plot (AC, 34.67 m^{-2}) indicated a tree-influenced reduction in crop population. A dry spell of 15 days (from date of crop sowing on 21 June 2003) probably caused a reduction in soil water in FC and RC plots due to use of water by trees affecting germination, population and growth of *V. radiata* seedlings (personal observation). Reduced growth and population of vegetation have been recorded in other deserts (Franco and Nobel, 1989), drought affected fields (Wilson and Tilman, 1991) and in alpine vegetation (Wilson, 1993). Inouye et al. (1980) also reported that the competition among annual plants of the Sonoran Desert did not affect survival but reduced growth rate, biomass and fecundity.

Low yield of *V. radiata* in FC and RC was attributable to reduction in soil resources particularly soil water (*Table 2 & 5*) discussed earlier. Significantly high yield of *V. radiata* in AC plots suggested sufficient soil water and nutrient availability enhancing crop yield. A positive correlation between soil water content and crop yield suggested greater availability of soil water in AC plot as compared to that in FC and RC plots. Though not significant but increase in grain, husk and holm yield with increase in distance from *C. mopane* trees indicated a competitive effect of the tree on crop yield. The competition was for resource utilization like soil water and nutrients. Since there were very less difference in soil nutrient due to distance from tree, the competition was mainly for soil water utilization. A regular rain of required quantity probably caused withdrawal of soil water by trees as well as the agricultural crop from the same soil layer. Such type of water use by the integrated tree reduces soil water availability for the crop and reduce crop yield. Relatively greater reduction in grain yield as compared to husk suggested that the effect of competition for soil water was severe on grain filling and yields. Low SWC in 0-25 in August and 25-50 cm in September 2003 suggested that tree utilized soil water from the same layer from which *V. radiata* was utilizing. At less water availability in topsoil layer, tree used to utilize soil water from the deeper soil layer as evidenced by reduced soil water in 50-75 cm soil layer in December 2003.

Concluding remarks

Despite of favourable influence by reducing solar radiation and enhancing crop water status in tree-integrated plots, there was significantly high reduction in yield of *V. radiata*. Tree size, competition for resources and soil water deficit were the factors affecting crop yield. Availability of optimum amount of soil water for agriculture crop resulted in high completion between tree and agriculture crop for water use thereby reducing population and growth of associated crop. When a decline in soil water in top soil layer occur, tree use to withdraw soil water from deeper layers and improves soil water in upper soil layers thereby indicating facilitative effect under adverse condition. A transition from strong competitive to weak facilitative between August and December suggests the dominance of competitive effects over facilitation across a gradient from favourable to soil water stress conditions (Bertness and Callaway, 1994). Thus, a farmer cannot enhance crop production by integrating *C. mopane* tree when rainfall is sufficient only for agriculture crop. Silviculture practices like trenching around tree trunk to reduce overlapping of roots of trees and crop and therefore competition for resources may be beneficial for crop production when *C. mopane* is integrated in agriculture land.

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MULTI-TARGET MODELLING OF THE DIATOM DIVERSITY INDICES IN LAKE PRESPA

NAUMOSKI, A.*

University Ss. "Cyril and Methodius" - Skopje, Faculty of Electrical Engineering and Information Technologies
Karpos II bb, P.O.BOX 574, Skopje, Macedonia
(phone: + +389-2-309-9168; fax: + 389-2-306-4262)

**Corresponding author*
e-mail: andrejna@feit.ukim.edu.mk

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Abstract. In this paper we present models of relationship between the diatoms community diversity indices (DIs) and the physico-chemical parameters using machine learning techniques. By taking relative abundances into account, a diversity index depends not only on species richness but also on the evenness, or equitability, with which individuals are distributed among the different time and space. Diversity indices provide important information about rarity and commonness of species in a community. Because the physical-chemical conditions of the environmental influence on the several diversity indices of the diatoms community at once, it is more reliably to model all the diversity indices together.

For modelling of the DIs models we use the raw; as measured, values of the concentrations for the physical-chemical parameters and the diversity indices of the diatoms abundance. The well known machine learning techniques are used to express this relationship: regression trees (RTs) and multi-target regression trees (MTRT's). The MTRT are more general than the RT, which predictive target is only one variable. The diversity indices are calculated for all diatoms of one measurement for 16 months, monthly and then are placed with the given physico-chemical parameters in one table. The results from the model have captured the ecological information with correlation between 0.9 and 0.92 for unseen (test) data. Diversity indices have proved to be a reliable indicator for the influence of the environment on the diatoms community. Temperature and conductivity components together with the Zn concentration are most influenced factors on the diatoms biodiversity. This could lead to more widely research broad view in this direction of ecological modelling.

Keywords: *diatoms, Multi-target modelling, Lake Prespa, machine learning*

Introduction

Machine learning techniques into ecology have proven to be useful into obtaining knowledge for certain problems. This property is used to model the abiotic environment. Using this DIs it will be very useful to model the specific diatom communities which are known to exist only in definite environmental conditions, especially diatoms abundance which throughput last decade proved as ideal bio-indicators (Reid et al., 1995).

Taking the diatom relative abundances into account, a diversity index depends not only on species richness but also on the evenness, or equitability, with which individuals are distributed among the different species. The ability to quantify diversity in this way is an important tool for biologists trying to understand community structure (Van Dam et al., 1994). Eutrophication, metals, temperature, pH, ammonia and others are one of the few that are vital for diatom survival and life cycle. This is why we build models to see how diatoms diversity indices response on the changes of these parameters.

In order to extract this knowledge from the ecological data we will use machine learning techniques. The most researched type of machine learning is inductive machine learning, where the experience is given in the form of learning examples. Machine learning (and in particular predictive modelling) is increasingly often used to automate the construction of ecological models (Levkov et al., 2006). Most frequently, models with regression trees of diversity indices and population dynamics are constructed from measured data by using machine learning techniques. The most popular machine learning techniques used for modelling diversity indices include decision tree induction and rule induction.

From machine learning methodology we explore the two afore mentioned possibilities for habitat modelling of the diatom community in Lake Prespa (Republic of Macedonia). To learn a model for each diatom species separately we employ regression trees – STRT (Breiman et al., 1984). To build a model for the all the diversity indices, we use multi-target regression trees – MTRT (Blockeel et al., 1998; Struyf et al., 2006). The main advantages of the latter approach are: (1) the multi-target model is smaller and faster to learn than learning models for each organism separately and (2) the dependencies between the organisms are explicated and explained.

We use dataset, which has been collected from different measurement stations placed in Lake Prespa, as a part of the EU project TRABOREMA (WP3, EC FP6-INCO project no, 2005-2007). Several important parameters are measured, which reflect the physical, chemical and biological aspects of the water quality of the lake. From these measurements, several diatoms (algae) belonging to the group Bacillariophyta) will be considered for estimating a relationship between their relative abundance, and then calculated their diversity indices and the abiotic characteristics of the habitat.

The paper is organized as follows. Section 1 introduces with idea of the diversity indices modelling and the main purpose of this paper, the diversity indices model for Lake Prespa, in Section 2 we give an overview of the machine learning algorithms and introduction to machine learning for diversity indices modelling, with a briefly description of the approach to machine learning that is often used in this kind of modelling: multi-target regression trees. The measured data and experimental setup, the main diatom bio-indicators and data collection procedures are presented in Section 3, while Section 4 describes the diversity indices models which were built for several indices and single-target only. Section 5 concludes the paper and gives directions for future work in this area.

Machine learning algorithms

The output of a diversity indices model is some property of the population of the target group of organisms at the spatial unit of analysis. There are two degrees of freedom here: one stems from target property, the other from the group of organisms studied. In the simplest case, the output is just the presence/absence of a single species (or group). In this case, we simply talk about diversity indices models.

The input to a DIs model is a set of environmental variables, which in our case are two different kinds. The first kind concerns abiotic properties of the environment, e.g., physical and chemical characteristics. The second kind concerns some biological aspects of the environment, which may be considered as an external impact on the group of organism under study. In our case the biological aspects of the environment are the diversity indices of diatoms abundance calculated with mathematical equations which are known the literature (http://www.wcsmalaysia.org/stats/diversityIndexMenagerie.htm).

Machine learning for diversity indices modelling

The input to a machine learning algorithm is most commonly a single flat table comprising a number of fields (columns) and records (rows). In general, each row represents an object and each column represents a property. In machine learning terminology, rows are called examples and columns are called attributes (or sometimes features). Attributes that have numeric (real) values are called continuous attributes. Attributes that have nominal values are called discrete Attributes. The tasks of classification and regression are the two most commonly addressed tasks in machine learning. They are concerned with predicting the value of one variable from the values of other variables. The target variable is called the class (dependent variable in statistical terminology). The other variables are called attributes (independent variables in statistical terminology). If the class is continuous, the task at hand is called regression. If the class is discrete (it has a finite set of nominal values), the task at hand is called classification. In both cases, a set of data is taken as input, and a predictive model is generated. This model can then be used to predict values of the class for new data. The common term predictive modelling refers to both classification and regression. Given a set of data (a table), only a part of it is typically used to generate (induce, learn) a predictive model. This part is referred to as the training set. The remaining part is reserved for evaluating the predictive performance of the learned model and is called the testing set. The testing set is used to estimate the performance of the model on new, unseen data.

Diversity indices are important to estimate dominance of the spices in the community, evenness and richness of the community. In this way, every index is important and depends only of the questions we ask about the species that are enrolled in the experiment [11]. We are interested to model the diatoms community, which latter can be used as bio indicator of the environment. In these experiments we have calculated Chao richness, Hill's N1, Hill's N2, (Berger-Parker)$^{-1}$, (Simpson)$^{-1}$, ShannonH' - Entropy, Brillouin, Margalef, Hill's N2/N1, Brillouin Evenness, Simpson Evenness and Shannon Evenness [11].

Nevertheless, we have taken into the experiment several other indices, the results are provided later in this paper. The influences of the abiotic factors on the community structure are captured using the both single and multi regression trees.

Multi-target regression trees

Multi-objective regression trees generalize regression trees in the sense that they can predict a value of multiple numeric target attributes (Breiman et al., 1984). Therefore, as prediction, instead of storing a single numeric value, the leafs of a multi-objective regression tree store a vector. Each component of this vector is a prediction for one of the target attributes.

A multi-objective regression tree (and a regression tree (Blockeel, H., et. al., 1998)) is usually constructed with a recursive partitioning algorithm from a training set of records (known as algorithm for top-down induction of decision trees). The records include measured values of the descriptive and the target attributes. One of the most important steps during the tree induction algorithm is the test selection procedure. Each test for a given node is selected on the base of some heuristic function that is computed on the training data (Garofalakis et al., 2003). The goal of the heuristic is to guide the algorithm towards small trees with good predictive performance.

After the regression tree is constructed, it is common to prune it. With pruning some subtrees are replaced with leaves, in order to improve predictive accuracy and/or interpretability. There are two pruning approaches: pre-pruning and post-pruning. With pre-pruning approaches, the pruning is included in the tree building algorithm as a stopping criterion. Examples of pre-pruning are the stopping criteria mentioned above: the number of records in a leaf and the maximum depth of the tree. The post-pruning approaches are applied after the tree construction has ended. Example of this approach is the pruning method proposed by (Blockeel et al., 2002). Essentially, this is a dynamic programming optimization method that selects a subtree from the constructed tree with at most *maxsize* nodes and minimum training set error (mean squared error, summed over all target attributes). The restriction *maxsize* is a user defined value.

Data description

The data that we have at hand were measured during the EU project TRABOREMA. The measurements cover one and a half year period (from March 2005 till September 2006). Samples for analysis were taken from the surface water of the lake at several locations near the mout of the major tributaries. In total, 275 water samples were available, 218 from the lake measurements and 57 from the tributaries. On these water samples both physico-chemical and biological analyses were performed. The physico-chemical properties of the samples provided the environmental variables for the habitat models, while the biological samples provided information on the relative abundance of the studied diatoms.

The following physico-chemical properties of the water samples were measured: temperature, dissolved oxygen, Secchi depth, conductivity, pH, nitrogen compounds (NO2, NO3, NH4, inorganic nitrogen), SO4, and Sodium (Na), Potassium (K), Magnesium (Mg), Copper (Cu), Manganese (Mn) and Zinc (Zn) content.

The biological variables were actually the relative abundances of 116 different diatom species. Diatom cells were collected with a planktonic net or as an attached growth on submerged objects (plants, rocks or sand and mud). This is the usual approach in studies for environmental monitoring and screening of the diatom abundance [8]. The sample, afterwards, is preserved and the cell content is cleaned. The sample is examined with a microscope, and the diatom species and abundance in the sample is obtained by counting of 200 cells per sample. The specific species abundance is then given as a percent of the total diatom count per sampling site (WP3, EC FP6-INCO project no, 2005-2007). The diversity indices are calculated by the mathematical formula of the indices that are used.

We applied the methodology described in Section 2, according to the experimental setup describe in next section, to the data at hand. With the modelling procedure (with the different scenarios and the different pruning algorithms) we obtained several models. From these models we select the ones that have better predictive power, and have reasonable size (in the most cases the tree minimal records in leaf is 4). The diatom species in the models are presented with their respective abbreviations. Their complete names can be found in (Levkov et al., 2006).

With the multi-target modelling we obtain predictive model that describe all diatom species and explains the dependencies between them and the physical-chemical characteristics.

Experimental design

We perform the analysis along two different scenarios: (1) modelling with the raw, as measured data and (2) selected bio-diversity indices from the previous experiment which have best correlation coefficient and RMSE. These scenarios were applied on lake measurements data for all 18 physico-chemical parameters and 12 biodiversity indices. In the case when as target variables we had all diatoms diversity indices we learn single MORT and a regression tree or single-target regression tree (STRT) for each index separately.

We applied 3 different pruning algorithms: minimal records in a leaf, maximal depth and maximal size. The parameter setting for these algorithms was as follows: for minimal records in a leaf we set 2, 4, 8, 16 and 32; for maximal depth we set 3, 4 and 5 and for maximal size we set 7, 9, 11 and 13. For validation of the performance on unseen data we used 10-fold cross validation. To assess the predictive power of the models we compare them by their correlation coefficient and root mean squared error (RMSE).

Diversity indices models of diatoms community

Diversity indices models

The MTRT tree constructed from the raw data is given on *Fig 1.* From the tree it is obvious to see that the most influence parameters on the diatoms diversity indices is the temperature and specifically the temperature high than 5.5 °C. Secondly important physico-chemical parameters are conductivity and the Zn. If we inspect the model leafs of the tree we can note that largest values are encounter if the temperature is high than 5.5 °C and conductivity values are low than 238 µS/cm.

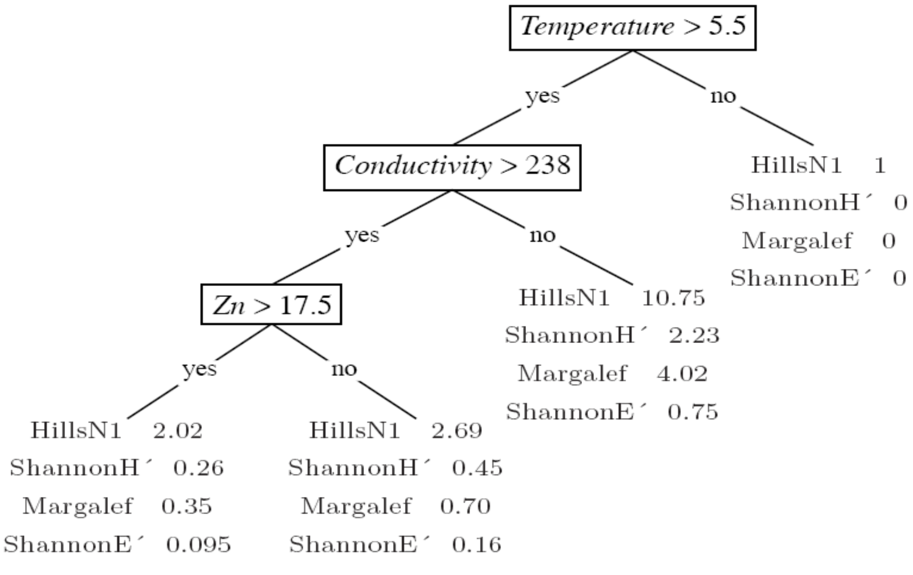

Figure 1. *MORT of the TOP 10 Diatom from lake measurements dataset*

This is expected; in fact the diatoms are known species that leaves in moderate water with low level of toxic element as the model shows. The Zn concentration negatively influences on the diversity of the diatoms, also the high temperatures. The constructed model for the measured data consist from the 4 important diversity indices, which according to the model are the most influence factors on the diatoms community. It will be very interesting to see how these indices are affected separately by the 18 physical-chemical parameters. For this purpose we build single-target regression trees – STRT.

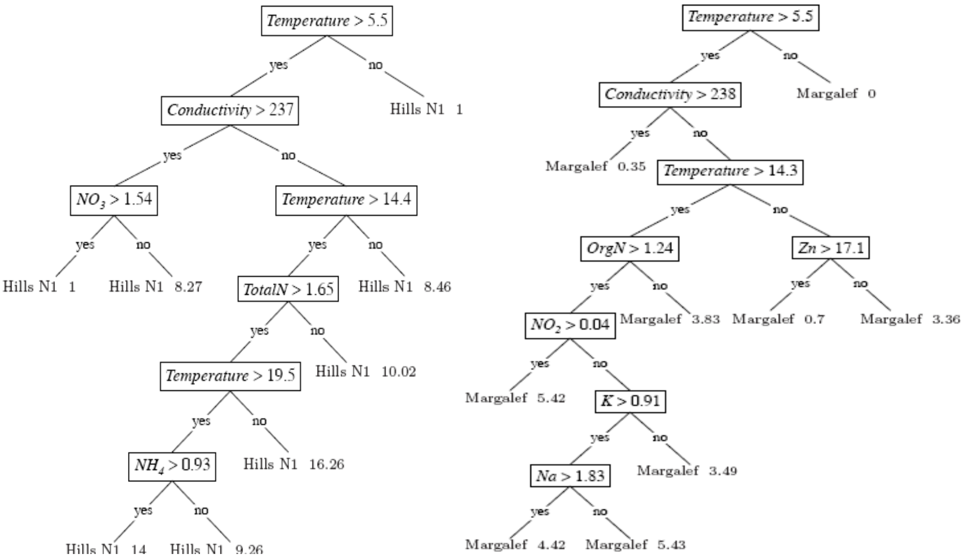

Figure 2. *Single target regression tree for the Hills N1 (left) and Margelef (right) diversity indices*

On *Fig. 2* - left, the single target regression tree for the Hills N1 diversity index is shown. It is obvious that the temperature is the most influence factor on this index, as the MTRT have shown. Beside the temperature and the conductivity, other influence factors are nitrogen components. Total Nitrogen, NO3 and NH4 play important role in the organisms' life cycle. High values of this index are expected in relative high temperatures, low conductivity and low concentrations of nitrogen components. Later in the experiment we build a model for single target model for Margelaf diversity index (see *Fig. 2* – right side). It is obvious again that the temperature is the most influence factor on this index and conductivity as second. Beside the temperature and the conductivity, other influence factors are nitrogen components. Total Nitrogen, NO3 and NH4 together with some metal parameters. Here it is interesting that metals like Na, K and Zn have influence on this index, because the Zn especially have toxic property on the environment.

High values of this index are expected in relative high temperatures, low conductivity. The nitrogen component together with the metals it is obvious that do not change much the value of this index.

Single target model was also build for the Shannon Evenness and Shannon H' - Entropy index. The single target tree for the Shannon H' tree is shown on *Fig. 3* – left side. As the rest of the model this index is mostly influence by the temperature and the conductivity parameter together with a set of nitrogen components. The Zn component capture with the multi-target regression tree has also has showed here. Low values of this index are expected with high values of the temperature and conductivity, which was confirmed by the MTRT for all indices.

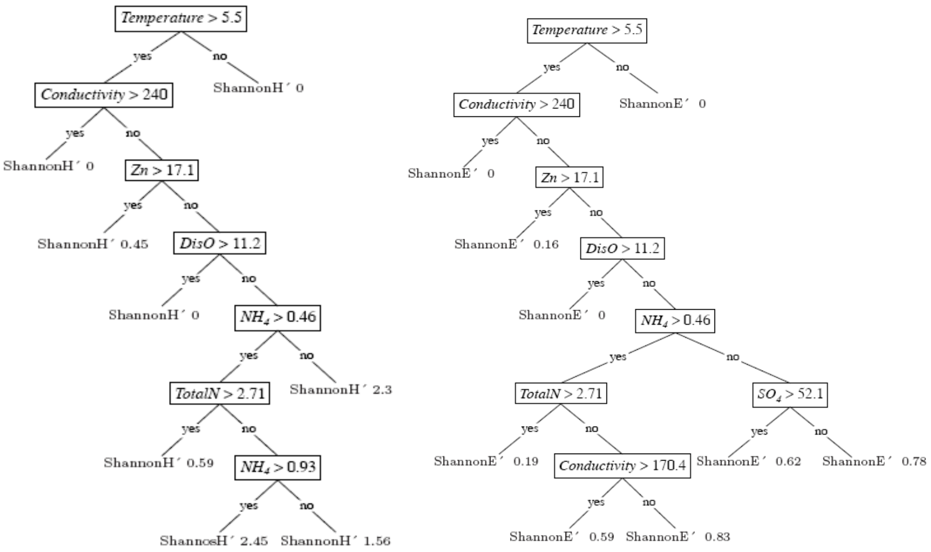

Figure 3. *Single target regression tree (STRT) for the Margelaf and Shannon H' diversity index*

Evenness property of the diatoms community is presented with Shannon E' index, which we can see from the picture the most influence parameter, is the temperature and the conductivity (*Fig. 3* – right side).

Table 1. *Correlation coefficient and RMSE coefficients for the diversity indices for both STRT and MTRT*

	MTRT					**STRT**			
	CC		**RMSE**			**CC**		**RMSE**	
	Train	**Xval**	**Train**	**Xval**		**Train**	**Xval**	**Train**	**Xval**
Chao richness	0.00	0.00	7.84	13.96		0.00	0.00	3.29	13.87
Hill's N1	0.94	0.22	1.92	6.88		0.98	0.27	1.06	6.88
Hill's N2	0.00	0.00	1.38	5.56		0.00	0.00	1.03	6.21
1 /Berger-Parker	0.00	0.00	0.66	2.55		0.00	0.00	0.66	2.56
1 / Simpson	0.00	0.00	1.77	7.16		0.00	0.00	1.74	7.65
ShannonH'	0.90	0.42	0.39	0.94		0.98	0.47	0.20	0.94
Brillouin	0.00	0.00	0.18	0.63		0.00	0.00	0.16	0.74
Margalef	0.89	0.40	0.81	1.84		0.98	0.46	0.38	1.82
Hill's N2/N1	0.00	0.00	0.05	0.17		0.00	0.00	0.03	0.19
Brillouin Evenness	0.00	0.00	0.06	0.20		0.00	0.00	0.05	0.21
Simpson Evenness	0.00	0.00	0.07	0.25		0.00	0.00	0.08	0.26
Shannon Evenness	0.87	0.46	0.14	0.28		0.98	0.63	0.06	0.25

From the model, other influence factors are the nitrogen components and the Zn parameters, and now together with the SO_4 component plays an important role in change of this index. High values of this index are expected for low temperatures, conductivity and total nitrogen component. The SO_4 component has little vary this index.

In continue of this paper, the performances of the experiments are given in *Table 1* and *Table 2*. The correlation coefficients given in table 1 are for both MTRT and the STRTs. Many of the diversity indices do not have any correlation, which later we will dismiss them from further investigation, leading to the second experimental setup. The performance data of the experiments conducted on the 4 left diversity indices in the second experiments are given in *Table 2*.

It is obvious from the table that the experiments on the unseen data have correlation coefficient 0.6 for multi-target trees and 0.63 for the single target trees. But this do not mean that the multi-target tree approach is better, but overall power prediction of the multi-target tree are better with mean value of the four parameters of 0.55 while STRT have only 0.41. Also the multi-target tree has produced a decision tree which is smaller in size and takes all the diversity indices at once.

Table 2. *Correlation coefficient and RMSE coefficients for the diversity indices for both STRT and MTRT only for best four parameters*

	MTRT				STRT			
	CC		RMSE		CC		RMSE	
	Train	**Xval**	**Train**	**Xval**	**Train**	**Xval**	**Train**	**Xval**
Hill's N1	0.91	0.44	2.37	5.87	0.98	0.27	1.06	6.88
ShannonH'	0.92	0.58	0.36	0.82	0.98	0.47	0.20	0.94
Margalef	0.91	0.57	0.74	1.60	0.98	0.46	0.38	1.82
Shannon Evenness	0.90	0.60	0.12	0.25	0.98	0.63	0.06	0.25

Conclusion

In this paper, we applied machine learning methodology, in particular single regression trees and multi-target regression trees, to model the abiotic influence of the environment on the diversity indices of the diatom community abundance in Lake Prespa. The models of the lake diatom communities have different structure and different environmental preferences and they interact with those different diversity indices according the models.

The learned diversity indices models show that the most important factors influencing the diatoms diversity indices are the temperature and conductivity, while the Zn and the nitrogen components are second important (from the Single-target RT). All the models given in this paper are first attempt to model the diversity indices of the diatoms in Lake Prespa.

Important to note here, that variable of the temperature and conductivity concentration, together with the Zn component highly depends from outside factors. Zn concentration loading from the human activates – industry, while the temperature from the human activity – CO_2. According the climate models the temperature in the next 50 years will increase, the models indicate and puts in danger existents of the diatoms that depends from the temperature factor.

The experiments showed that machine learning tools can extract some valuable knowledge in a relatively comprehensible form, even when the application area is so extremely complex also for humans and the data are far from being perfect. The predictive power (testing procedure) of the models is weak, but the knowledge representation (training procedure) has reach values of 0.9 in some cases. We have in mind that any ecosystem cannot be fully described with all its inside process, because the model will be complex for any analysis to be performed.

We do strongly believe that these models will help explaining the very complex environmental patterns of influence within the Prespa Lake ecosystem and emphasize the most important variables to be monitored or put in the focus of the decision makers regarding the mitigation of the detected forced eutrophication processes and their consequences. In this direction, an expert system that will automatically generate decisions rules will point out the relationship between the eutrophication parameters (like Secchi Disk, Total Phosphorus, Total Nitrogen and etc.) and the diatom community. For further work, we plan to model the diatom communities using multi-label classification methods.

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MICROBIAL DIVERSITY AND DENSITY IN THE CORAL REEF ENVIRONMENT OF THE GULF OF MANNAR

BALASUBRAMANIAN, R.[1]* – KANNAN, L.[2]

[1]People's Action for Development (PAD),
2/29 Sebastian Koil Street, Vembar – 628 906, Thoothukudi – Dist., India
(phone: + 91 9443426469)

[2]Centre of Advance study in Marine Biology
Annamalai University, Parangipettai –608 502.

**Corresponding author*
e-mail: coralbalu@gmail.com

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Abstract. Microbes are important in the coral reef environment in the recycling of nutrients and as a source of food to a variety of animal species. They also have an important trophic role to play in the reef environment, goverened by different physico-chemical and biotic factors. The present study has revealed the presence of more THB (total heterotropic bacteria) population density in water (5.2 to 22.5 × 10^5 CFUml^{-1}) and in the sediments (7 to 24.2 × 10^7 CFUg^{-1}) than in the surrounding coastal environments. A total of 24 species of planktonic cyanobacteria and 11 species of benthic cyanobacteria were recorded. Among them, *Phormidium tenue* was the most dominant species and the families Oscillatoriaceae and Chroococcaceae were predominant in the present study. A total of 35 species of fungi belonging to 20 genera representing the families Labyrinthulomycetes, Oomycetes, Zygomycetes and Deuteromycetes were isolated. Among them, the deuteromycetous fungi formed the bulk of the mycoflora of all the samples at both stations.

Keywords: *Coral reef microbes, Gulf of Mannar, Bacteria, Cyanobacteria , Fungi THB*

Introduction

Under normal conditions, corals co-exist with the microorganisms that are abundant in the marine environment. However, when corals are stressed by high temperatures, increased nutrient concentrations or sedimentation, these microbes may cause disease to them (Baird, 2000). Rising water temperatures or reduction in water quality will reduce the resistance of corals and increase the virulence of the microbes.

Much is known about the role of microbes in terrestrial and fresh water ecosystems when compared to the marine environment. Marine microbes play a major role in the biogeochemical cycles, regulating the distribution of nutrients throughout the ocean (Hoppe and Gocke, 1993), in addition they are important in the maintenance of the community (Hoppe *et al.,* 1988). In fact, the microbial population in the reef system is responsible for the decomposition of organic material reaching the coral reef environment, thus supplying the needed nutrients. They also serve as a direct source of food for a variety of reef organisms. At the same time it should be noted that the microbes viz. bacteria, cyanobacteria and fungi can affect the corals by causing diseases which may lead even to devastation.

Heterotrophic bacteria have long been recognized as important agents of carbon and nitrogen cycling in the coral reefs (Ducklow, 1990) and bacterial respiration is an important component of coral community metabolism (Sargeant and Austin, 1949).

Of the different microbes, cyanobacteria, which are the oxygen evolving photosynthetic prokaryotes, inhabit various types of marine environs. They play a vital role in the carbon cycle of oligotrophic waters (Hagstrom *et al.,* 1988).

Marine fungi are the potential source for a variety of extra cellular enzymes (Rohrmann and Molitoris, 1992) and calcium carbonate substrata such as shells of dead molluscs, coral skeletons, as well as coralline algae are the common habitat of these fungi. They decompose the organic matter into nutrients thus playing an important role in the cycling of nutrients.

Though there have been some attempts to study the bacteria and fungi of the coral reef environment of India, a holistic approach to assess the microbial (bacteria, cyanobacteria and fungi) diversity and density in the water and sediments of the coral reef environment of the Gulf of Mannar is lacking. Further, this assessment is essential because these microbes present in the ambient environment may turn into pathogens, when coral species are stressed. Having this in mind, the present study was carried out at two stations viz. Manoli island (*Station 1*) and Krusadai island (*Station 2*) in the Gulf of Mannar.

Materials and methods

The Gulf of Mannar is situated on the southeast coast of India. In the Gulf area, there is an arc of 21 islands starting from the northern most Rameswaram island to Tuticorin (8^0 46’ and 9^0 14’ N Lat; 78^0 9’ and 79^0 14’ E Long.) forming the ‘archipelago’, called ‘Mannar Barriers’. Among these islands only two were selected for the present study, *station 1* Manoli island and *station 2* Kurusadai island.(*Fig.1*)

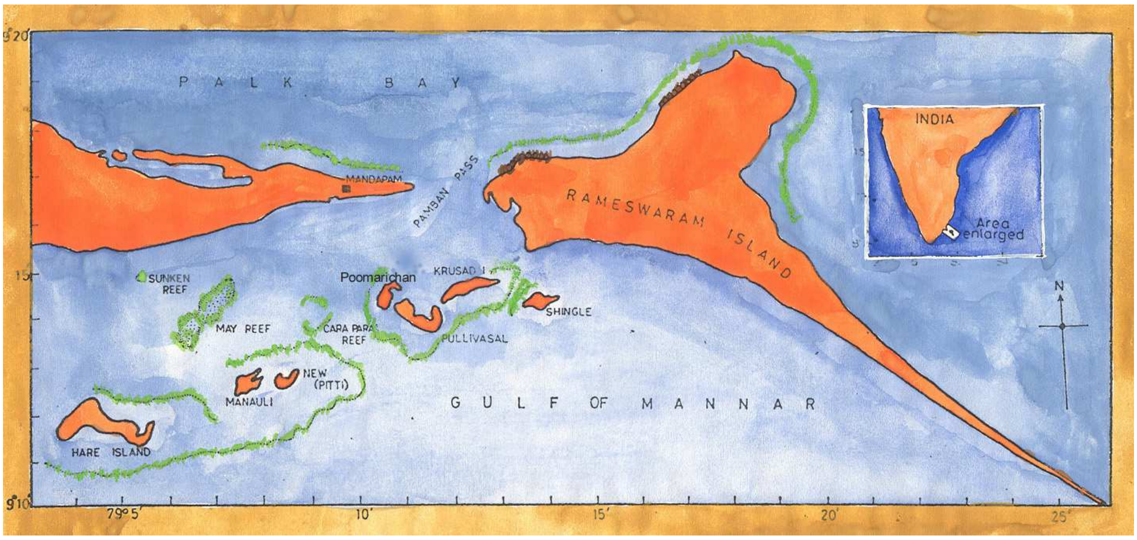

Figure 1. *Study area*

For microbiological assessment, water and sediment samples were collected from both the stations.

Bacteria

Isolation of total heterotropic bacteria (THB)

Total heterotrophic bacteria were isolated from water and sediments, adopting the serial dilution plating technique using Zobell Marine Agar 2216 medium (Himedia Chemicals Pvt. Ltd., Mumbai). Identification of the pure cultures up to generic level was done with the schemes of Buchanan and Gibbons (1975), Krieg (1984) and Sneath (1986).

Cyanobacteria

Planktonic cyanobacteria

The planktonic cyanobacteria were collected using a plankton net made of bolting silk cloth of no. 25 with pore size of approximately 64 µm. The net was towed for about 10 minutes in the surface water and the samples were collected. The filtered plankton samples were transferred to wide mouthed polythene bottles and preserved in 4 % formaldehyde and then analyzed under a light microscope.

Benthic cyanobacteria

Benthic cyanobacteria were collected from the intertidal cyanobacterial mats. The mats were removed from the substratum and suspended in 50 ml of filtered water taken from the collection site and shaken gently to free the algae from the sand particles. The particles were allowed to settle for few minutes and the supernatant consisting of the benthic cyanobacteria were retained in the suspension. The samples were then analyzed after adding a few drops of 4% formaldehyde and Lugol's solution. Identification of species was carried out using the taxonomic publications of Desikachary (1959), Hum and Wicks (1980) and Anand (1989).

Fungi

For mycological analysis, the method of Prabhu *et al.,* (1991) was followed. Standard pour plate technique was used to isolate and enumerate fungi, by making suitable dilutions of water and sediment samples. All the isolated colonies were identified up to species level referring to the works of Booth (1971), Ainsworth *et al*., (1973a,b), Ellis (1976) and Kohlmeyer and Kohlmeyer (1979).

Multiple regression analysis was done in order to determine the influence of the physico-chemical variables on the populations of THB, cyanobacteria and fungi. Summary of the multiple regression analysis is given station-wise in Tables 4 and 5 with regression equation. THB, cyanobacterial and fungal populations were considered as dependent variables and other parameters namely wind speed, rainfall, air temperature, surface water temperature, pH, salinity, dissolved oxygen, particulate organic carbon, total phosphorus, inorganic phosphate, total nitrogen, nitrate, nitrite, silicate, calcium and magnesium were considered as independent variables.

Results

Total Heterotrophic Bacteria (THB)

Among the bacterial strains isolated from the water and sediments at both the stations, a total of 245 strains were randomly sub-cultured and identified to generic level. Totally nine genera viz. *Pseudomonas, Vibrio, Aeromonas, Flavobacterium, Enterobacter, Bacillus, Micrococcus, Arthrobacter* and *Corynebacterium* were identified.

In general, gram negative bacteria showed a higher proportion (59.13 %) than the gram positive bacteria (40.87 %). When compared to station 1, station 2 recorded more gram negative genera.

THB population density in water varied from 5.2 to 22.5 × 10^5 CFU ml^{-1} at both the stations (*Fig. 2*). At both the stations, the minimum was recorded during the premonsoon and the maximum was recorded during the monsoon seasons.

Population density of THB in the sediments varied from 7 to 24.2 × 10^7 CFU g^{-1} at both the stations (*Fig. 3*). The same trend was recorded as in the case of water.

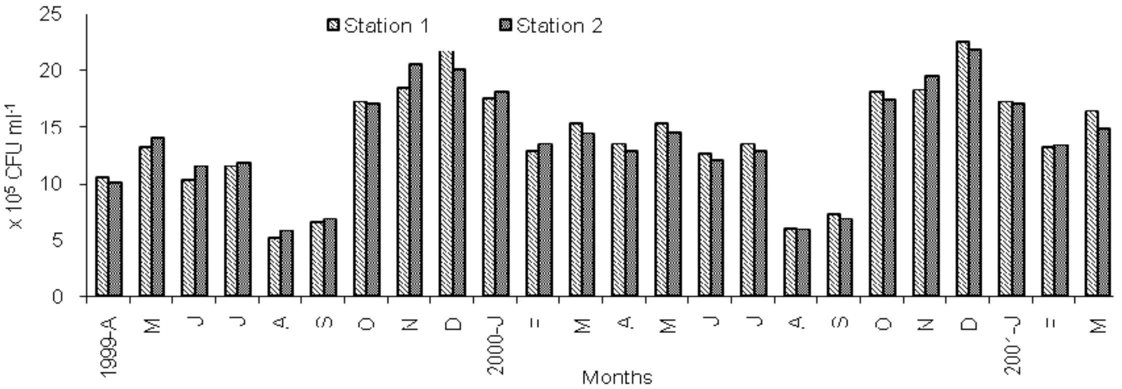

Figure 2. *THB population density recorded in water at stations 1 and 2*

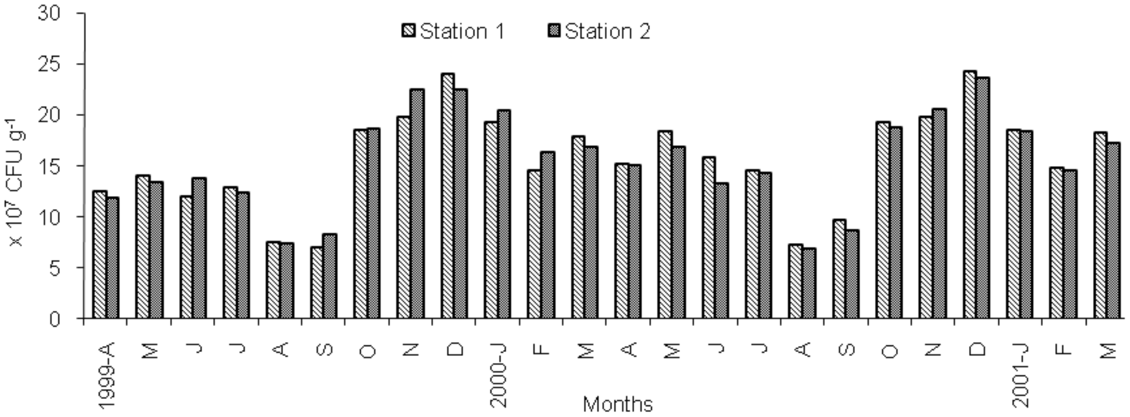

Figure 3. *THB population denstity recorded in water at stations 1 and 2*

Cyanobacteria

Among the planktonic cyanobacteria, station1 recorded a total of 24 species belonging to 15 genera and 5 families while station 2 recorded a total of 22 species belonging to 15 genera and 5 families (*Table 1*).

Table 1. *List of planktonic cyanobacteria recorded from stations 1 and 2*

S. No.	Species	Station 1	Station 2
	Chroococcaceae		
1.	*Microcystis* sp.	+	+
2.	*Chroococcus turgidus*	+	+
3.	*Gloeocapsa* sp.	+	+
4.	*Aphanocapsa montana*	+	+
5.	*Aphanothece clathrata*	+	+
6.	*Synechocystis salina*	+	+
7.	*S. pevalekii*	+	-
8.	*S. aquatilis*	+	-
	Oscillatoriaceae		
9.	*Spirulina subsalsa*	+	+
10.	*Oscillatoria nigroviridis*	+	+
11.	*O. salina*	+	+
12.	*O. tenuis*	+	+
13.	*Oscillatoria* sp.		
14.	*Trichodesmium erythraeum*	+	+
15.	*Phormidium fragile*	+	+
16.	*P. tenue*	+	+
17.	*P. ambiguum*	+	+
18	*Phormidium* sp.	+	+
19.	*Lyngbya* sp.	+	+
	Nostocaceae		
20.	*Nostoc* sp.	+	+
21.	*Anabaena* sp.	+	+
	Scytonemataceae		
22.	*Plectonema terebrans*	+	+
23.	*Plectonema* sp.	+	+
	Rivulariaceae		
24.	*Calothrix* sp.	+	+

+ denotes presence - denotes absence

In the case of benthic forms, a total of 11 species belonging to 8 genera and 4 families were recorded at both the stations (*Table 2*). *Oscillatoria* and *Phormidium* were found to be the dominant genera.

Table 2. *List of benthic cyanobacteria recorded from stations 1 and station 2.*

S. No.	Species	Station1	Station 2
	Chroococcaceae		
1.	*Chroococcus minutus*	+	+
2.	*C. minor*	+	+
3.	*Gloeocapsa* sp.	+	+
4.	*Aphanocapsa montana*	+	+
	Oscillatoriaceae		
5.	*Oscillatoria* sp.	+	+
6.	*Phormidium* sp.	+	+
7.	*P. fragile*	+	+
8.	*P. tenue*	+	+
9.	*Lyngbya* sp.	+	+
	Nostocaceae		
10.	*Anabaena* sp.	+	+
	Rivulariaceae		
11.	*Calothrix* sp.	+	+

+ denotes Presence - denotes absence

Planktonic cyanobacteria

Population density of cyanobacteria in water varied from 22 to 190 individuals/l at both stations (Fig. 4). At *Station 1*, the minimum and the maximum was recorded during the premonsoon and summer season respectively. At *Station 2*, the minimum was recorded during the premonsoon season and the maximum was recorded during the postmonsoon season.

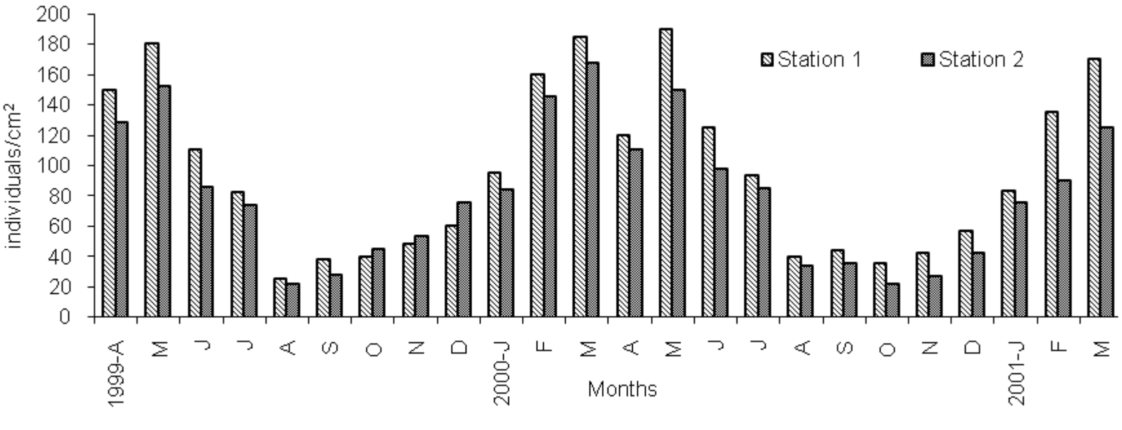

Figure 4. *Population density of planktonic cyanobacteria recorded at stations 1 and 2*

Benthic cyanobacteria

Population density of cyanobacteria in sediments varied from 12 to 40 individuals/cm^2 (*Fig. 5*). At station 1, the minimum was observed during the premonsoon season and the maximum was observed during the summer season. At station 2, the minimum population density was observed during the monsoon season and the maximum was observed during the postmonsoon season.

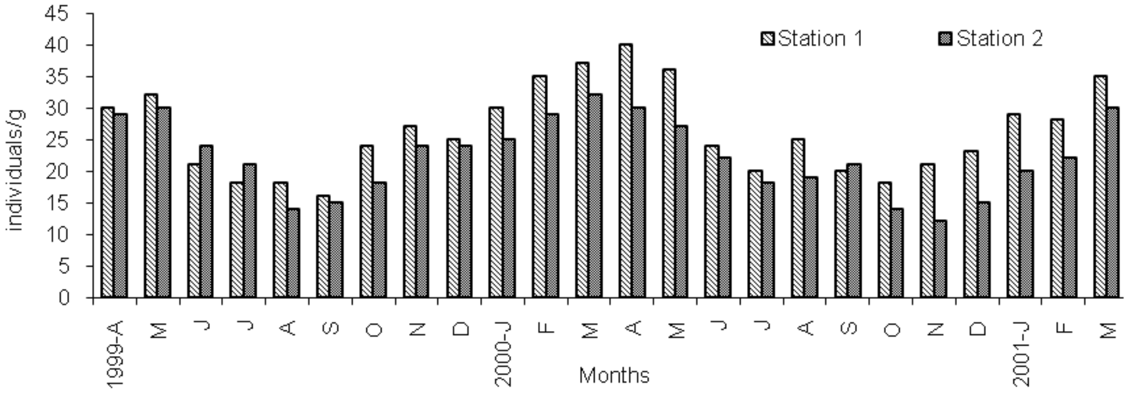

Figure 5. *Population density of benthic cyanobacteria recorded at stations 1 and 2*

Fungi

In the present study, a total of 35 species of fungi belonging to 20 genera (*Table 3*) were recorded from the water samples and 32 species of fungi belonging to 20 genera were recorded from the sediment samples collected from both stations.

Of the 35 species of fungi, 1 species belongs to 1 genus of Labyrinthulomycetes, 2 species belong to 1 genus of Oomycetes, 1 species belongs to 1 genus of Zygomycetes and 31 species belong to 17 genera of Deuteromycetes.

A total of 35 species belonging to 20 genera were identified from the water samples. Among the fungal colonies in water, *Aspergillus niger* contributed the maximum at *station 1* (15.7 %) than at *station 2* (12.52 %). *A. terreus* ranked second in its contribution at station 1 (11.12 %). *Penicillium chrysogenum* was the third dominant species (8.43 %) while non-sporulating black and white colonies contributed 6.50 % at station 1 and 5. 68 % at *station 2*.

Table 3. *List of fungi recorded from the water and sediment samples of stations 1 and 2.*

S. No.	Title	Water	Sediments
	LABYRINTHULOMYCETES		
1.	*Labyrinthuloides minuta*	+	+
	OOMYCETES		
2.	*Thraustochytrium aureum*	+	-
3.	*T. benthicola*	+	+
	ZYGOMYCETES		
4.	*Mucor hiemalis*	+	+
	Deuteromycetes		
5.	*Alternaria alternata*	+	+
6.	*A. longipes*	+	+
7	*Aspergillus candidus*	+	+
8	*A. flavus*	+	-
9.	*A. fumigatus*	+	+
10	*A. glaucus*	+	+
11	*A. niger*	+	+
12	*A. sydowi*	+	+
13	**A. nidulans**	+	+
14	*A. terreus*	+	+
15	*Cephalosporium humicola*	+	+
16	*Cladosporium cladosporioides*	+	+
17	*C. macrocarpum*	+	+
18	*Fusarium equiseti*	+	+
19	*F. oxysporum*	+	+
20	*Penicillium chrysogenum*	+	+
21	*P. citrinum*	+	+
22	**P. funiculosum**	+	+
23	*Scolecobasidium humicola*	+	+
24	*Trichoderma koningii*	+	+
25	Lulworthia grandispora	+	+
26	L. yorkensis	+	-
27	*Halosphaeria salina*	+	+
28	*Halosarpheia marina*	+	+
29	*Nimbospora octonae*	+	+
30	*Ulkenia visurgensis*	+	+
31	*Curvularia lunata*	+	+
32	**Cladosporium herbarum**	+	+
33	**Dendryphiella saline**	+	+
34	**Trichoderma viride**	+	+
35	**Phomamedi caginis**	+	+
36	Non sporulating black mycelium	+	+
37	Non sporulating white mycelium	+	+

+ denotes presence - denotes absence

Fungi in water (Species composition and population density)

The fungal population density in water ranged from 3 to 20 × 10^1 CFU ml^{-1} at both the stations (*Fig. 6*). At *station 1*, the minimum was recorded during the summer season and the maximum in monsoon season. At station 2, the same trend was noticed.

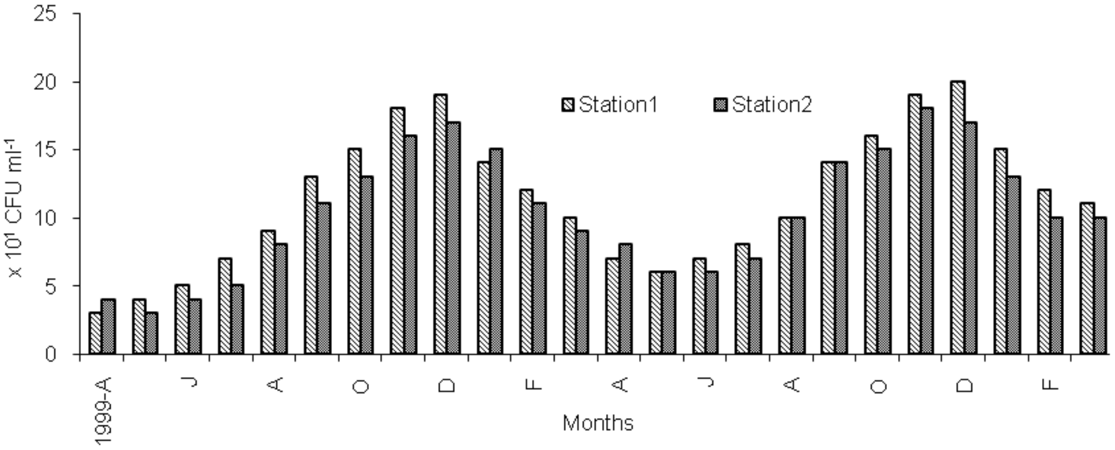

Figure 6. *Population density of fungi recorded in water at stations 1 and 2*

Fungi in sediments (Species composition and population density)

A total of 32 species belonging to 20 genera were identified from the sediment samples. Among the fungal colonies in sediments, *A. niger* contributed the maximum at station 1 (18.26 %) than at station 2 (19.12 %), followed by *A. terreus* i.e. 14.32 % at station 1 and 16.18 % at station 2. *P. chrysogenum* contributed 8.92 % at station 1 and 9.14 % at station 2 and non-sporulating black and white colonies contributed together 5.72 % at *station 1* and 4.78 % at *station 2*.

Aspergillus was the dominant genus recorded from both the stations. *Thraustochytrium aureum*, *Aspergillus flavus* and *Lulworthia yorkensis* were present only in the water samples at both the stations.

The fungal population density varied from 5 to 24 × 10^2 CFU g^{-1} at both the stations (*Fig. 7*). At both the stations, the minimum was recorded during the postmonsoon and the maximum was recorded during the monsoon season.

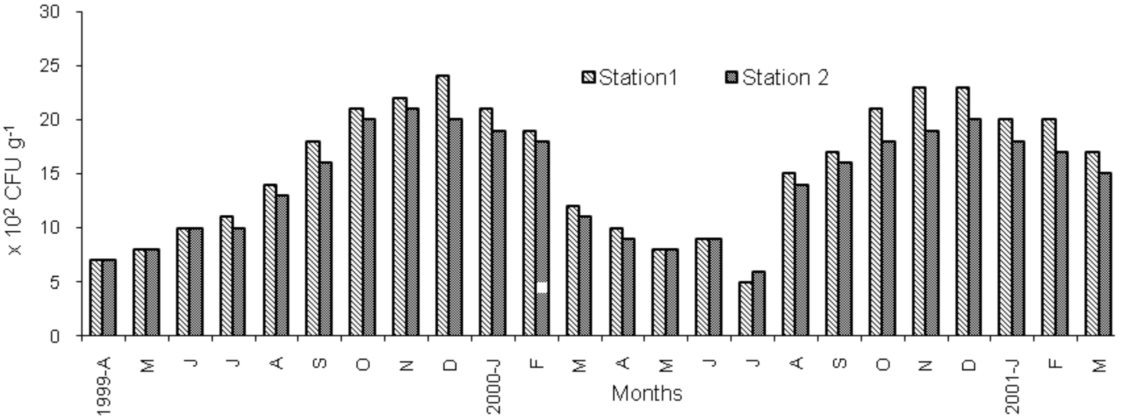

Figure 7. *Population density in fungi recorded in sediments at stations 1 and 2*

Discussion

Bacteria are important in the coral reef environment in the recycling of nutrients and as a source of food to a variety of animal species (Disalvo, 1974; Sorokin, 1974). They also have an important trophic role to play in the reef environment, goverened by different physico-chemical and biotic factors. The coral reef environment supports the growth of bacteria and even the coral mucus which consists mainly of polysaccharides and proteins (Meikle *et al.,* 1988) acts as a good growth substrate for bacteria (Fukami Kimio, 1994). In fact, some of the studies (Paul *et al.,* 1986; Kannapiran, 1997) have revealed the presence of more bacterial population in coral mucus than in water. Hence, monitoring the THB population in the coral reef waters is considered to be one of the coral reef management practices.

It is in this line, the present study has revealed the presence of more THB population density (5.2 to 22.5 × 10^5 CFU ml^{-1}) in the coral reef waters and in the sediments (7 to 24.2 × 10^7 CFU g^{-1}) than in the surrounding coastal waters and sediments. Velankar (1955) has also recorded higher values of THB density in the sediments from the inshore waters of Mandapam. The THB population density recorded in the coral reef environment of the Gulf of Mannar during the present study is comparable to that of the other similar environment of Coconut Island (Sorokin, 1973) and Kaneole Bay (Disalvo, 1969).

Among the two stations studied, Manoli island recorded higher THB population density. This could be ascribed to the fact that seaweeds, seagrasses and mangroves are abundant here which produce a lot of detrital matter that gets drifted into the adjacent reef environment and favours the growth of THB.

Table 4. *Multiple regression equation obtained for THB (water and sediments) vs. physico-chemical parameters; cyanobacteria (planktonic and benthic)*

Type	Sample	Multiple regression equation
THB	Water	Y= -85.836-.161var1+.00708var2- .658var3+1.189var4+1.145var5 -.411var6+ .467var7+ .203var8 +.424var9 -7.249var10+.208var11 +2.393var12 -.445var13+ 2.428var14-.0206var15+ .00455var16
	Sediments	Y= -79.995-.300var1+.00282var2-.442var3+.923var4+10.076var5-.135var6+.322var7+.153var8+1.242var9-7.929var10+.240var11+1.882var12-.333var13+2.285var14-.0149var15+.00229var16
Cyanobacteria	Planktonic	Y= -1029.565-8.282var1-.06067var2-8.143var3+19.015var4+80.620var5+11.310var6-10.844var7+2.699var8 +16.785var9-156.361var10+11.042var11+83.212var12-21.982var13+15.028var14-.337var15-.009878var16
	Benthic	Y= +11.913-1.485var1+.01692var2-1.086var3+1.360var4-6.714var5+2.007var6+1.174var7-.02623var8 +.487var9-12.461var10+.359var11+2.095var12-.470var13+.989var14-.02071var15+.01171var16
Fungi	Water	Y= +49.342-.06179var1-.001489var2-.732var3+.274var4-3.738var5-.08693var6+1.523var7-.07417var8-.633var9+6.814var10-.02442var11-2.081var12-.01885var13-.03422var14-.006156var15-.0003981var16
	Sediments	Y= +115.390-.196var1-.003354var2-.636var3-.678var4-15.853var5+1.226var6+2.522var7+.06166var8-1.359var9+11.331var10+.03012var11-2.934var12+.199var13-1.252var14+001687var15+.007805var16

In the present study, higher THB population densities were noticed during the monsoon and postmonsoon seasons at both the stations in water and sediments. This could be attributed to the land runoff, which is expected to bring more amount of organic and inorganic nutrients to the reef environment, a situation favourable for the growth of THB.

With regard to the environmental parameters, changes in salinity and temperature could influence the distribution of bacteria (Morita, 1974; Mohan Kumar *et al.,* 1979). This could be observed in the present study also. Higher THB populations were recorded during the monsoon season when the salinity was lower due to the freshwater inflow. The lower salinity and the excess nutrients would have favoured the growth of the THB populations. Carlucci (1974) observed that the availability of nutrients particularly phosphate and total nitrogen as yet another important factor governing the distribution of marine bacteria. In the present study, during the monsoon season, higher values of phosphate (4.6 µM at stations 1 and 2) and total nitrogen (44.2 µM at station 1 and 39.0 µM at station 2) were noticed, coinciding with higher bacterial population density.

Table 5. *Multiple regression equation obtained for THB (water and sediments) vs. physico-chemical parameters; cyanobacteria (planktonic and benthic) vs. physico-chemical parameters and fungi (water and sediments) vs*

Type	Sample	Multiple regression equation
THB	Water	Y= -95.321-.03513var1+.009261var2-.461var3+1.011var4+11.889var5-.386var6+.228var7 +.187var8+.358var9-7.115var10+.317var11+2.758var12-.392var13+2.110var14-.01759var15+.004033var16
	Sediments	Y= -91.407-.04594var1+.01053var2-.279var3+.824var4+12.205var5-.509var6+.667var7+.118var8+ .294var9-7.300var10+.08174var11-.905var12+.109var13+2.447var14-.01343var15+.003159var16
Cyanobacteria	Planktonic	Y= -786.883-7.025var1-.01251var2-7.522var3+15.060var4+88.720var5+3.416var6-7.774var7+2.565var8+13.096var9-144.812var10+9.187var11+88.717var12-21.092var13+14.528var14-.350var15+.003440var16
	Benthic	Y= +18.590-.830var1+.02354var2-.447var3+1.010var4+4.729var5-.310var6-1.604var7+.114var8-1.201var9-12.078var10+.188var11+2.692var12-1.444var13+2.426var14-.04572var15-.001074var16
Fungi	Water	Y= +69.056+.115var1+.008787var2-.648var3-.103var4-4.370var5-.219var6+1.239var7-.08866var8-2.466var9+9.678var10-.636var11-10.308var12+1.282var13+.491var14+.003607var15+.0007551var16
	Sediments	Y= +92.960-.172var1+.002077var2-.604var3-.305var4-12.018var5+.774var6+1.949var7+.06151var8-.796var9+7.837var10+.07119var11-2.000var12+.148var13-1.074var14-.0004744var15+.005478var16

The above facts are substantiated by the regression analysis performed among the different variables such as rainfall, surface water temperature, pH, dissolved oxygen, particulate organic carbon, total phosphorus, total nitrogen, nitrite, reactive silicate and magnesium which showed influence on the THB populations in water and sediments of both the stations (*Tables 4* and *5*).

In the present study, *Bacillus, Micrococcus, Arthrobacteium, Corynebacterium, Pseudomonas, Vibrio, Aeromonas, Flavobacterium* and *Enterobacter* were recorded from both the stations. Among them, *Bacillus* was found to be the dominant genus in the gram positive group and in the gram negative group, *Pseudomonas* was the dominant genus. *Vibrio* was the second dominant genus in the gram negative group. In general, the gram negative bacteria recorded a higher proportion than the gram positive bacteria. This lends support to the observations of Kannapiran (1997) and Velankar (1955).

In the present study, a total of 24 species of planktonic cyanobacteria belonging to 15 genera and 5 families were recorded. The detailed study revealed that 6 genera comprising 8 species are assignable to the family Chroococcaceae, 5 genera comprising 11 species to Oscillatoriaceae, 2 genera comprising 2 species to Nostocaceae, 1 genus with 2 species to Scytonemataceae and 1 genus with 1 species to Rivulariaceae. Among these, 4 species (*Aphanocapsa montana, Aphanothece clathrata, Synechocystis pevalekii* and *S. aquatilis*) belonging to 3 genera of the family Chroococcaceae have been reported as fresh water forms by Desikachary (1959).

A total of 11 species of benthic cyanobacteria belonging to 8 genera were also recorded in the present study. Among these, 4 species of 3 genera belong to the family Chroococcaceae, 5 species of 3 genera belong to Oscillatoriaceae, 1 species of 1 genus belongs to Nostocaceae and 1 species of one genus belongs to Rivulariaceae. Out of these, 3 species (*Chroococcus minutus, C. minor* and *Aphanocapsa montana*) belonging to 2 genera have been reported as freshwater forms by Desikachary (1959). This could be due to the remarkable adaptability of cyanobacteria to any environment (Desikachary, 1959; Fogg, 1973; Carr and Whitton, 1982; Hof and Fremy, 1993).

Among the different cyanobacteria recorded in the present study, *Phormidium tenue* was the most dominant species and the families Oscillatoriaceae and Chroococcaceae were predominant in the present study, thus conforming to the observations of Thajuddin and Subramaniyan (1992) in the Bay of Bengal, adjoining the south east coast of India.

During the present study, higher nutrient contents (especially nitrogen) were noticed, which could be correlated with the maximal diversity and density of the cyanobacteria. Regression analysis (*Tables 4* and *5)* has also indicated that surface water temperature, pH, salinity, particulate organic carbon, total phosphorus, total nitrogen, nitrate and reactive silicate have influence on the planktonic cyanobacterial populations at station 1, in addition to magnesium which has influence on these organisms at station 2. In the case of benthic cyanobacteria, rainfall, surface water temperature, salinity, dissolved oxygen, total phosphorus, total nitrogen, nitrite, reactive silicate and magnesium have influence on these organisms.

In the present study, a total of 35 species of fungi belonging to 20 genera representing the families Labyrinthulomycetes, Oomycetes, Zygomycetes and Deuteromycetes were isolated. Among them, the deuteromycetous fungi formed the bulk of the mycoflora of water and sediment samples at both the stations. Predominance of deuteromycetes observed in the present study is in conformity with the earlier observations of Upadhyay *et al.* (1978), Venkatesan (1981), Dasarathy (1983) and Nadimuthu (1998) in different parts of the southeast coast of India.

Aspergillus with 8 species (*Aspergillus candidus, A. flavus, A. fumigatus, A. glaucus, A. niger, A. sydowi,* A. *nidulans* and *A. terreus*) was found to be the most dominant genus in the water and sediment samples at both the stations. Rai and Chowdhery

(1978) have also noted the *Aspergillus* dominance as the characteristic feature of soils of warmer regions.

During the present investigation, higher fungal population density in water at station 1 (20 × 10^1 CFU ml^{-1}) and at station 2 (18 × 10^1 CFU ml^{-1}) and in sediments at station 1 (24 × 10^2 CFU g^{-1}) and at station 2 (21 × 10^2 CFU g^{-1}) was recorded during the monsoon season. In water, lower density (3 × 10^1 CFU ml^{-1}) was recorded during the summer season at both the stations. In sediments, lower density was recorded at station 1 (5 × 10^2 CFU g^-1) and station 2 (6 × 10^2 CFU g^{-1}) during the postmonsoon season. Copious amount of rainfall during the monsoon season might have provided the necessary nutrients and substratum for fungi to thrive well and multiply thereby leading to their higher counts during this season (Untawale and Parulekar, 1976; Matondker *et al.,* 1980; Muniyandi, 1985). The lower population density of fungi recorded during the summer season could be ascribed to the toxicity caused by higher concentration of chloride ions (Swart, 1958).

From the present study, it is understood that the THB, cyanobacteria and fungi have distinct spatio-temporal variations in their qualitative and quantitative distributions at both the stations. Under the normal conditions, these microbes can co-exist with other organisms including corals in the marine environment without harming them (Andrew Baird, 2000). But, the environmental stresses can make them virulent and cause disease to their associated flora and fauna. The influence of different environmental parameters on the microflora (THB, cyanobacteria and fungi) could be understood in the present study through the multiple regression analysis (*Tables 4* and *5*). This would suggest that a continuous monitoring of the microflora and the physico-chemical parameters in the coral reef environment of the Gulf of Mannar is therefore essential, as a measure of coral reef management practice, since there are abundant populations of microflora in the reef environment as well as coral microbial diseases.

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INFLUENCE OF PLANT ASSOCIATIONS ON THE SILICON CYCLE IN THE SOIL-PLANT ECOSYSTEM

BOCHARNIKOVA, E.A.[1] – MATICHENKOV, V.V.[2]*

[1]Institute of Physical-Chemical and Biological Problems in Soil Science Russian Academy of Sciences, Pushchino, Moscow region, 142292, Russia, E-mail: mswk@rambler.ru

[2]Institute Basic Biological Problems Russian Academy of Sciences, Pushchino, Moscow region, 142292, Russia

**Corresponding author*
e-mail: Vvmatichenkov@rambler.ru

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Abstract. Soluble Si compounds such as monosilicic and polysilicic acids affect chemicals and physical properties of the soil. The main aim of this study is to evaluate Si cycle in the various soil-plant systems via the determination of mobile Si forms in the soil and of the total content of Si in the plant associations. The concentrations of monosilicic acid, polysilicic acids and acid-extractable Si in unmowed meadow, mowed meadow, birch-aspen forest, spruce wood and agricultural land soil-plant systems, were tested at the soil depths of 0-10, 20-30 and 50-60 cm in a Russian region, south of Moscow. The annual content of Si within the investigated soil-plant systems was calculated. Forty to 80 kg ha^{-1} of Si is annually removed from Grey Forest Soil (Luvisol). The concentration of monosilicic acid in the upper soil horizon depended on the type of plant association and on the total content of adsorbed Si. The removal of plant remains from the ecosystems resulted in decreased monosilicilic acid concentration in the upper soil horizon. The ecosystems which utilize annual plant remains increased the content of monosilicilic acid of the surface soil horizon. The concentration of monosilicic acid in the upper soil layer can be used as indicator of the stability of plant association. The unmowed meadow and the birch-aspen forest were characterized as ecosystems with accumulative type of Si cycle. The agricultural land, the mowed meadow and spruce wood all had alluvial type of Si cycle.

Keywords: *monosilicilic acid, plant association, polysilicic acid, silicon cycle, soil-plant ecosystem*

Introduction

Silicon (Si) is the second most abundant element of the earth surface. Numerous chemical, physical and biological soil processes are realized and controlled by this element (Matichenkov and Bocharnikova, 2001; Snyder et al., 2006). The content of Si usually ranges from 20-35% for clay or silt soils to 40-44% for sandy soils (Essington, 2003). Mainly, Si is present as quartz, alkaline earth and aluminum silicates, which forms a soil skeleton and these forms of Si are chemically or biochemically inert, but have influence on the soil physical properties (Conley 2002; Sommer et al., 2006). In the element mobility classification, Si is located in two positions as inert and as mobile element (Matichenkov et al., 2000). Mobile Si substances correspond to monosilicic acid, polysilicic acid, organo-silicon compounds and complex compounds with organic and inorganic substances (Matichenkov and Bocharnikova, 2001). These substances play a primary role in the global biogeochemical cycle of Si (Matichenkov and Bocharnikova, 1994; Wollast and McKenzie, 1983). Plant adsorbs Si in monosilicic acid or their anion forms (Epstein, 1999; Ma, 2003). The removal Si from the soil by plants can range from 20-40 kg/ha for terrestrial ecosystems characterized by low level of biomass increasing up to 3000 kg/ha, which typifies the tropical and subtropical zones (Anderson, 1991; Wattean and Villemin, 2001). About 27.5 million tons of Si are adsorbed by plants and removed from cultivated areas annually (in comparison to P-absoption, whose annual amount is of 18 million tons) (Bocharnikova and Matichenkov, 1994). Our calculations demonstrate that about 460 million tons of Si is annually involved in the global biological cycle, of which about 341 million tons of Si are in the continental ecosystems (Matichenkov and Bocharnikova, 1994). The intensification and volume of Si cycle in the terrestrial ecosystems can be compared with the cycle of such elements as phosphorus, potassium and calcium (Alexander et al., 1996; Exley, 1998; Lucas et al., 1993). However, the information about the terrestrial Si cycle and the effect of plant association relative to the forms and concentration of Si substances in the soil, are scanty and inadequate in order to better define the role and function of this element in the nature. Besides, to evaluate the biogeochemical Si cycle, only the total content of mobile Si is usually analyzed (Alexander et al., 1996; Bartoli, 1985; Basile-Doelsch et al., 2005; Lucas et al., 1993). Whereas there are at least three forms of mobile Si which are present in the soil (Dietzel 2002; Iler, 1979; Matichenkov and Bocharnikova, 2001). These are: monosilicilic acid, polysilicic acid and organo-silicon compounds.

Monosilicilic acid is highly biochemical and geochemical active (Iler, 1979; Ma, 2003; Matichenkov et al., 2000). Monosilicilic acid can control the mobility of phosphates and can transform plant-unavailable P into plant-available by the replacement of P from Ca, Al and Mg phosphates (Matichenkov and Ammosova, 1996). Al, Mg and heavy metal toxicity may be also suppressed by monosilicic acid (Birchall, 1992; Bocharnikova et al., 1999). Monosilicilic acid has an effect on soil pH level (Iler, 1979). It controls the formation of secondary minerals and also the intensity of mineral weathering processes in the soil (Orlov, 1985; Olier, 1990; Horigushi, 1988). Monosilicic acid may be absorbed by plants and microorganisms (Epstein, 1999; Ma, 2003).

Monosilicilic acid is a product of Si containing minerals dissolution (Essington 2003; Iler, 1979). The solubility of minerals depends on the chemical structure and size of its particles. The highest solubility is inherent in amorphous silica with high surface area (Iler, 1979). In the surface soil horizons, amorphous silica is represented by biogenic silica forms (plant and microorganism phytoliths) and abiogenic substances formed as a result of precipitation and dehydration of soluble Si compounds (Clarke 2003; Bobrova, 1995; Summer et al., 2006). In plants and soil microorganisms, about 90% of adsorbed monosilicilic acid transforms into polymers or biogenic amorphous silica (Alexandre et al., 1996). Biogenic amorphous silica returns to the soil with plants or microbial remains and becomes a new source for monosilicilic acid (Sommer et al., 2006). By this means Si cycle is realized via monosilicic acid migration and transformation (Matichenkov and Bocharnikova, 1994). We suggested that monosilicilic acid plays a primary role in the terrestrial biogeochemical cycle of Si and provides for the movement of this element in the cycle itself (Matichenkov et al., 2000).

Polysilicic acid has two or more atoms of Si (Iler, 1979; Dietzel, 2002). Some molecules of polysilicic acid have thousands of Si atoms and can represent chains and branch of spherical forms of molecules (Chadwick et al., 1987 a; Chadwick et al., 1987 b). The term of “polysilicic acid” is under discussion now (Iler, 1979; Knight and Kinrade, 2001). It is recognized the following forms of polysilicic acid, as soluble silicic acid, which has more then 2 atoms of Si: oligomers (low molecular weight polysilicic acid), polysilicic acid (high molecular polysilicic acid, but without solid nucleus) and colloids, which according chemical classification must have solid nucleus and size from 10^{-7} to 10^{-5} cm. Oligomers of silicic acids are characterized by high chemical activity (Dietzel, 2002; Knight and Kinrade, 2001). Our and literature data showed that this substances can be measured together with monosilicic acid, because during 10 minutes of interaction with molybdenum ammonium all monosilicic acid and most part of oligomers are formed colored compels (Dietzel, 2002; Matichenkov, 1990). The colloidal particles of polysilicic acid stir up solution, therefore we suggested to use centrifuge or fine filter for removing of these particles form other form of high molecular weight polysilicic acid (Matichenkov, 1990). It is necessary to recognize truth polysilicic acid and colloidal polysilicic acid because there are has different physical and chemical properties (Yazinin 1989, Matichenkov, 1990). Polysilicic acid has definite effect on soil adsorption capacity and soil structure (Yazinin, 1989; Matichenko et al., 1996). Polysilicic acid is chemically inert and is formed as the result of monosilicic acid polymerization (Iler, 1979; Matichenkov et al., 1996). Other ways of polysilicic acid formation were hypothesized but were not experimentally confirmed, for example, the direct formation from weathered minerals (Nazarov, 1976).

Plant associations and soil interplay and has great influence on each other (Alexandre et al., 1997; Bartoli, 1983; Basile-Doelsch et al., 2005). However the processes of interaction between single plant and plant association on the content and behaviors of Si are investigated poor. The understanding of the biogeochemical cycle of Si processes require more information about behavior of various forms of mobile Si in he soil-plant syetm.

We hypothesized that various plant associations absorb monosilicilic acid differentially and may control the concentration of monosilicilic acid and polysilicic acid in the upper soil horizons. As a result, a natural or anthropogenic change in plant association can modify Si cycle and influence soil mineral formation, weathering processes and some chemical and physical soil parameters.

The main aim of this study was to determine soil mobile Si substances in the five soil-plant systems, the total content of Si in plant association for boreal climatic zone and to evaluate the biogeochemical cycle of Si.

Materials and methods

The selected ecosystems were located on the third terrace of the right side of river-bank of Oka River, south of Moscow region and 5 km west of Pushchino. This area was described as having identical quaternary period sediments, exposition and type of soil (Bazilevich,1993; Yermolaev and Shirshova,1994). The scheme of tested area is presented on *Fig.1*.

The unmowed and mowed meadows were used for long-term experiment, conducted by the Institute of Physical-Chemical and Biological Problems in Soil Science (Yermolaev and Shirshova, 1994). Sixteen years ago these areas were separated from plough land, which was used for agriculture over hundred of years. During this experiment, natural evolution of plant associations was examined by supporting meadows under unmowed and mowed technologies. Both plots were located on a slightly (3-5°) northern slope.

Plant from each plant association was described. The soil profiles for each plant association were prepared in third replication and type of each soil was determined.

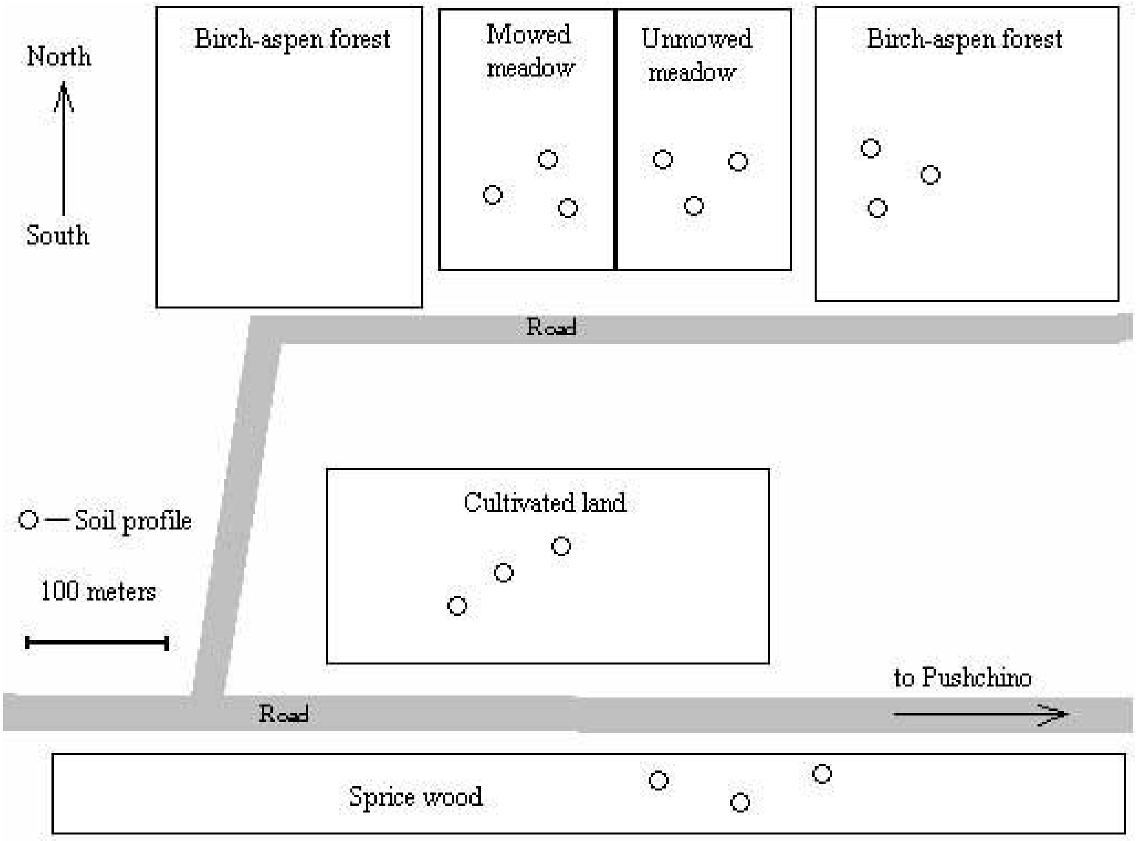

Figure 1. *The scheme of the ecosystems and location of soil profiles*

Grass and grass-rich herb phytocenosis were predominant in the meadow vegetation. The main components of the herbage were the following types: loose-tusock, and quitches grasses: *Elytrigia repens L., Nevski*, *Bromopsis inermis Leyss. Holub., Dactylis glomerata L., Festuca pratensis Huds., Pod compressa L., Phleum pratense L., Anthoxanthum odoratum L.,* and *Trifolium hybridum L., Trifolium pratense L.*

Various cereals (*Festuca pratensis Huds., Phleum pratense L.,* and *Pod compressa L.*) were characteristic of the unmowed meadow. *Trifolium pratense L., Lathyrus pratensis L.* and other non-cereals were typical of the mowed meadow.

Soil profiles of both meadows were identical. The soil was determined as Typical Grey Forest Soil (Russian Soil Classification) or Luvisol (FAO, 1991). Soil profile has the following description:

Horizon A (0-30 cm) was dark gray, with many average- and small-sized roots, damp clay-sand texture, courses of earthworms, clear boundary, and gradual transition to deeper horizon.

Horizon AB (30-70 cm) was brown with gray stains, small-sized roots, courses of earthworms, and damp clay-sand texture.

The ploughed land with barley (*Hordeum vulgare*) was located near the investigated meadows (200 m to the north-west). The soil was identical to the meadow soil, except the surface horizon (0-30 cm), which was formed by annual tillage. It didn’t have roots and its color was more homogeneous. This field has been used in agriculture for over one hundred years (Yermolaev and Shirshova, 1994).

The next plant association was described in the forest. The secondary birch-aspen forest was located beside the mowed and unmowed meadows, on a slight northern slope

(*Figure 1*). This area had the following plant composition and structure: the lime-bircth-aspen wood – hazel, rich in grasses (*Tilia cordata Mill. + Betula pendula Roth + Populus tremula L. – Corulus avellana L. – Lomicera Xylosteum L. Dum - Pulmoniaria obscura + Conval baria majlis L - Fragaria vesca L. + Veronica chamaedris L.).* Plant samples in these points were collected separately for grasses, trees leafs and trees branches. The type of soil was classified as Light Grey Forest. The soil profile had the following structure:

Horizon A (0-12 cm) was gray, moist sand-clay with many large-, average-, and small-sized roots, and gradual transition to deeper horizon.

Horizon A2B (12-30 cm) was light gray with light brown stains, damp sand-clay texture, average and large roots, and gradual transition to deeper horizon.

Horizon AB (30-70 cm) was characterized by heterogeneous mixture of grays, browns and whites and damp sand-clay texture.

The final selected plant association was located in coniferous forest. The spruce wood was located on a hill with a few equal horizontal river platforms (10-15 m) alternated with southern slopes (5-10 m and 20-35°) (*Figure 1*). It had the following plant composition and structure: *Picea abies L. Karst* + sin*gle Populus tremula L.* without grass cover. Plant samples in these ecosystems were collected separately for needles and for branches of spruce. The type of soil was classified as Grey Forest with the following profile structure:

Organic litter (0-3 cm) represented damp and friable old needles and branches.

Horizon A1 (3-12 cm) was dark gray, with few large roots, damp clay-sand texture, unclear boundary and gradual transition to deeper horizon.

Horizon A2B (12-40 cm) was characterized by heterogeneous light gray color with brown stains, damp clay-sand texture, unclear boundary and gradual transition to deeper horizon.

Horizon AB (40-80 cm) was similar to horizon A2B, brown without light gray material.

Soil and plant samples were pick out from each described ecosystems. Double soil samples were collected form each soil profile at the depth of 0 to 10 cm, 20 to 30 cm and 50-60 cm. The mixed plant samples (total herb cutting from 25 × 25 cm area) were collected from each point of soil sample, with three replications. Plant samples were measured and dried at 65°C. Then plant samples were grounded and then passed through a 0.5-mm sieve. Total Si was tested by Eliot and Snyder (1991) method with four replications for each sample. Samples of plant tissue weighing 100 mg were wetted with 2 mL of 50% H_2O_2 in 100-mL polyethylene tubes. To each tube was added 4.5 g of 50% NaOH at ambient temperature, and each tube was gently vortexes. The tubes were individually covered with lose-fitting plastic caps. The rack of tubes was placed in an autoclave at 138 kPa for 1 h. After atmospheric pressure was reached, the tubes were removed and the contents brought to 50 mL with distilled water (Eliot and Snyder, 1991). Monosilicilic acid in final solution form tubes was determined colorimetrically (Mallen and Raily method cited by Iler, 1979). The average content of total Si was calculated for each point.

Each fresh soil sample was divided into 2 sub-samples. One-half of the sub-samples was air-dried and grounded to pass a 1-mm sieve. These dried soil samples were tested for biogeochemically active amorphous silica (Matichenkov et al., 1997). The remaining sub-samples were maintained under fresh condition after removing roots and passing through a 2-mm sieve. Monosilicilic acid was determined in water extracted from fresh soil samples (Mallen and Raily method cited by Iler, 1979). The extraction procedure was conducted in the following manner: 6 g of fresh soil were placed into beakers. Thirty mL of distilled water were added to each beaker. After 1-hour shaking, the sample was filtered through filter paper No.40, then the extract was analyzed for monosilicilic acid. Polysilicic acid was analyzed in the same extract after depolymerization. For depolymerization, an aliquot (20-25 mL) was subjected to ultrasound for 1 min. During this time, all of polysilicic acid was transformed into monosilicic acid (Matichenkov et al., 1997). It should be noted from our earlier work that a change in soil moisture from 50 to 500 g kg^{-1} has no effect on the sensitivity of this method (Matichenkov et al. 1997; Matichenkov and Snyder, 1996).

Biochemically active amorphous Si was tested in dry soil samples after a 1day acid extraction procedure (20 mL 0.1 M HCl from air-dried, 2-g sample) (Barsykova and Rochev, 1979). The extract was filtered through filter paper No.40 and analyzed for Si content using the colorimetric method (Mallen and Raily method cited by Iler, 1979). The concentration of Si was determined photo metrically at 660 nm. pH level and moisture of tested soils was determined by standard methods (Thomas,1996).

All obtained data was subjected to a statistical analysis based on comparative methods using Duncan’s multiple range tests for mean separation at the 5% level of significance (Duncan, 1955).

The literary data on biomass productivity of tested ecosystems was used for the calculation of Si including the biological cycle (Yermolaev and Shirshova, 1979; Bazilevich, 1993). Only above-ground biomass was used for these calculations.

Results

The maximum amount of monosilicilic acid in the surface horizons was observed for unmowed meadow (17.7 mg Si kg^{-1}) and birch-aspen forest (16.2 mg Si kg^{-1}) (*Table 1*). Lower concentrations of monosilicic acid in the surface horizons were tested for cultivated soil (6.5 mg Si kg^{-1}), the mowed meadow soil (8.6 mg Si kg^{-1}) and the spruce wood soil (9.9 mg Si kg^{-1}). It is necessary to note that concentrations of monosilicilic acid in the soil surface horizons of all investigated ecosystems were significantly different. With depth, the concentration of monosilicilic acid tended to increase, ranging from 10.2 to 15.6 mg Si kg^{-1}.

The cultivated soil was characterized by the highest concentration of polysilicic acid: 25 mg Si kg^{-1}. The soils of both meadows at the depth of 20-30 cm were also characterized by high concentration of polysilicic acid (26.2-30.3 mg Si kg^{-1}) (*Table 1*). Both forest soils had the lowest concentration of polysilicic acid, except the subsurface horizon under birch-aspen forest. In the rest soil horizons of the forest ecosystems, the polysilicic acid concentration ranged from 1.8 to 9.6 mg Si kg^{-1}.

The difference in amorphous silica concentration in the investigated soils wasn't as high as that in silicic acids (*Table 1*). The maximum concentration of amorphous silica was determined in the deeper horizons. Its value in the lower horizons for forest ecosystem and moved meadow were not significantly different (*Table 1*). The unmowed meadow soil and cultivated soil exhibited the maximum concentration of amorphous silica in the surface soil horizons (527 and 500 mg Si kg^{-1}, respectively) (*Table 1*).

Table 1. *Concentration of soil Si compounds (mg Si kg^{-1})*

Samples	**pH**	**W%**	**Monosilicic acid**	**Polysilicic acid**	**Acid-extractable Si**
			----------------mg Si kg^{-1}--------------------		
Mowed meadow					
0-10 cm	5.87	19.4	8.6**i**	18.9**d**	415**cd**
20-30 cm	5.66	14.6	10.8**g**	26.2**b**	455**c**
50-60 cm	6.01	13.2	12.1**e**	7.8**h**	537**b**
UNMOWED MEADOW					
0-10 cm	6.24	20.8	17.7**a**	15.8**e**	527**b**
20-30 cm	6.05	14.5	10.2**gh**	30.3**a**	468**c**
50-60 cm	6.01	13.2	13.1**e**	7.5**h**	548**b**
AGRICULTURAL LAND					
0-10 cm	6.28	13.0	6.5**j**	25.0**c**	500**bc**
20-30 cm	6.14	13.0	6.5**j**	25.0**c**	510**b**
50-60 cm	6.06	17.6	13.9**d**	12.7**f**	592**a**
Birch-aspen forest					
0-10 cm	6.22	13.4	16.2**b**	4.1**i**	382**d**
20-30 cm	5.12	12.3	11.5**f**	18.6**d**	428**c**
50-60 cm	5.47	12.9	15.3**c**	1.8**j**	532**b**
Spruce wood					
0-10 cm	4.76	25.6	9.9**h**	8.1**h**	432**c**
20-30 cm	5.87	13.7	14.4**d**	1.8**j**	539**b**
50-60 cm	6.16	10.8	15.6**c**	9.6**g**	534**b**

†Value within a column followed by the same letter are not different using Duncan's Multiple Range test ($P<0.05$).

The total content of Si in plant association is presented in *Table 2*. The highest concentration of Si (1.75 % from dry mass) was observed in the spruce needles. This data correspond with Bazilevic's content of Si in the needles of *Picea abies,* which was determined in same region (1993). It is necessary to note that content of Si in the spruce needles from mountain region area are lover (Carnelli et al., 2001), which can be explain by difference in the mobile Si content in the soils.

The plant association in the unmowed meadow and barley collected from cultivated area was also characterized by high content of Si in green mass of plants (*Table 2*). This content of Si is typical for cereals (Ma, 2003). Then ext level of total Si in plant association was 1.15% and this level was determined in grass cover of birch-aspen forest. The content of total Si which was determined for plants from mowed meadow and leafs from birch and aspen trees fluctuate around 1.03 - 1.06 % of Si (*Table 2*). The lowest concentrations of Si in plants were determined in the trees branches. It is important to note that the content of Si in the spruce wood was three times higher then in birch or aspen trees (*Table 2*).

The obtained and literature data allowed us to calculate the balance of Si in the tested plant associations (*Table 3*). The calculated data of Si pool in the ecosystem biomass has demonstrated that the maximum Si (87.6 kg Si ha^{-1}) was present in the spruce wood biomass (*Table 3*). About half of the plant adsorbed Si was concentrated in the wood. Trees were accumulating this Si during 30-40 years. The annual Si absorption by spruce was only 40.2 kg Si ha^{-1} plus about 2–4 kg Si ha^{-1} accumulated in the wood. Consequently, we can suggest that the most active annual Si uptake by plants occurs in the unmowed meadow and agricultural ecosystem (79.1 and 81.5 kg Si ha^{-1}, respectively) (Table 3). The lowest Si absorption was calculated for the birch-aspen forest.

Table 2. *The total content of Si in the tested plant associations*

Area	**Total Si, % from dry mass**
Unmowed meadow	1.49**b**
Mowed meadow	1.03**d**
Cultivated land	1.34**b**
Birch-aspen forest	
grass	1.15**c**
trees leafs	1.06**d**
trees branches	0.36**e**
Spruce wood	
needles	1.75**a**
branches	0.97**d**

†Value within a column followed by the same letter are not different using Duncan's Multiple Range test ($P<0.05$).

The amount of annually returned biomass into the soil is important in order to calculate Si balance in an ecosystem (Bazilevich,1993). The removal of plant biomass as crops from cultivated land and meadow cutting, is approximated 95% of above-ground vegetable mass. In wood ecosystems, some Si absorbed is found in the wood mass (50% in birch-aspen forest and 54% in spruce woods) (*Table 3*). Probably all this Si will not return into the soil because local forest district moves out wood material. Only in the unmowed meadow soil-plant system, all of the absorbed Si returns into the soil (*Table 3*).

Table 3. *The biological Si cycle in various soil-plant systems*

Area	**Annual increase in biomass t/ha/year**	**Annual returning plant debris into soil, %**	**Annual removal of Si from soil, kg Si ha^{-1}**	**Annual return of Si into soil, kg Si ha^{-1}**	**Balance of Si**
Unmowed meadow	5.21	100	78.09	78.09	0
Mowed meadow	5.98	5	61.14	3.07	-58.07
Cultivated land	6.10	5	81.50	4.07	-77.43
Birch-aspen forest					
green mass	2.5	50	27.75	27.75	-16.99
wood	4.72	0	16.99 sum - 44.74		
Spruce wood					
green mass	2.3	46	40.25	40.35*	-47.40*
wood	4.99	0	47.40 sum - 87.65		sum -87.65*

* Needles fallen don't mix with soil forming surface layer (0-3 cm).

Discussion

Primary and secondary soil minerals are the principal sources of soluble Si forms. Soil Si-rich minerals differs in solubility and are influenced by numerous factors such as pH, particle size, chemical composition, climate, plants, soil microorganisms activity et al. (Lucas et al., 1993). It is possible to distinguish 3 main directions of Si migration and transformation in the soil: leaching of soluble Si-rich compounds without transformation, adsorption of monosilicilic acid by plant roots with consequent transformation of monosilicic acid into amorphous silica, and transformation of soluble forms of Si into the soil without movement from the soil profile. The pool of Si leached depends on the amount of atmospheric precipitates and velocity of weathering (Matichenkov et al., 2000). Polysilicilic acids are more leacheable, then monosilicic acid, and this is due to the peculiar chemical structure of these substances. (Matichenkov et al., 1996). Monosilicic acid has a positive charged anion which is absorbed very well by the soil particles, while the non-compensated electrical charge of polysilicic acid can be self- neutralized by the high flexibility of polysilicic acid molecule (Iler, 1979). Plant roots and soil microorganisms produce organic acids that accelerate mineral weathering (Alexandre, et al., 1996; Drever, 1993).

Higher plants are responsible for the absorption of Si via monosilicic acid through the soil profile. In plant tissues, Si precipitates as phytoliths in cell walls or filling of the cell lumen and in the intercellular spaces (Alexandre et al., 1996). Soil microorganisms adsorb monosilicic acid and then transform it into polysilicic acid and amorphous Si. When plant or soil microorganism die, the adsorbed and transformed Si returns to the soil. The distribution of phytoliths through the soil profile is conditioned by two different mechanisms: translocation into lower horizons and accumulation in upper soil horizon (Bobrova, 1995).

This means that soil minerals, climate conditions, and living organisms are the prime factors that determine Si movement and transformation also known as Si cycle of the soil-plant ecosystem. There are two main Si cycles in nature: cumulative and eluvial (Matichenkov et al. 2000). The accumulative Si cycle is characterized by soils with accumulation of Si substances in the upper soil layer. Ecosystems with accumulative Si cycles, phytolith formation dominates over leaching Si-rich substances. The steppe ecosystems are an example of this type of Si cycle. The content of phytoliths in such soil may range from 6% to 12% of mass in surface horizon (Bobrova, 1995).

The eluvial Si cycle is characteristic of soils having high levels of physical and chemical weathering besides intensive Si leaching, caused by climatic conditions. Plant activity, in these kinds of ecosystems characterized by this type of Si cycle, may be very high but absorbed Si doesn’t accumulate in the soil. For example, tropical rain forests often have the eluvial Si cycle where about 90% of biologically active Si of this ecosystem is found in the biomass, not in the soil (Matichenkov and Bocharnikova 1994). Eighty to 90% of total SiO_2 can be removed from the soil profile in humid tropic zones (Kovda, 1985).

In our study, all examined soil-plant systems were located on similar parent material and formed under equal climatic conditions. Only plant associations were different. As a result the role of plant association in these tested ecosystems had critical significance due to the distinction of Si cycles.

It is obvious that monosilicic acid concentration in the soil depends on the type of plants and the amount of vegetative biomass removed from the ecosystem. Returning biomass in unmowed meadow and birch-spruce forest was responsible for increasing monosilicic acid in the surface horizon of these ecosystems (*Table 1*). It is interesting that a negative Si balance in birch-spruce forest emerged at the depth of 20-30 cm (*Table 1*). This layer was supplied with tree roots.

The increased concentration of monosilicic acid in unmowed meadow soil is the result of the transformation of Si containing minerals into the following mechanism. A parent material was the primary source of monosilicic acid. First plants absorbed Si and formed phytholites (*Table 2*). Than plant remains, with formed phytoliths, returns into soil. Phytoliths represent fine amorphous silica whose solubility is usually higher than that of most soil minerals (Iler, 1979). This process repeated annually increased, over time, the content of phytholites in soil and consequently the concentration of monosilicic acid also increased (*Table 1*).

In spruce wood, absorbed silica is accumulated mainly in the needles (*Table 2*). Fallen needles form a compact surface layer and this layer does not mix with the soil. The decomposition of spruce needles is a long-term process (Bazilevich, 1993). In the upper soil horizon of spruce wood, only small amount of monosilicic acids was observed (*Table 1*).

The cultivated soil and mowed meadow soil were characterized by smaller concentration of monosilicic acids in the upper horizons because in both ecosystems absorbed Si is harvested with the crop and doesn't return to the soil (*Tables 1, 2 and 3*). It is important to notice that this situation has a negative effect on plant growth. A deficiency in Si nutrition leads to decreasing cereals viability and resistance to diseases and insect attacks (Datnoff et al., 1997; Snyder et al., 2006). Cereal growth decreases when Si nutrition is very small (Bocharnikova, 1996; Matichenkov, 1990). Probably, this explains why Si deficiency was responsible for a change in plant association on the mowed meadow where wild Si-rich cereals were displaced by other plants (*Lathyrus pratensis L., Trifolium pratence L.*) (Yermolaev and Shirshova, 1994).

The increases content of polysilicic acid in plough soil was probably due to mechanical compaction of agricultural equipment. These results correlate very well with our previous data regarding Chermozem soil (Matichenkov et al., 1996). Machine pressure may increase polysilicic acid in the upper soil horizons, however the chemistry of these processes has been poorly investigated.

The soil at the depth of 20-30 cm in both meadow ecosystems contained high amount of polysilicic acid as well (*Table 1*). This is probably caused by the same reason as in arable areas, where the land was used as tillage 16 years ago and polysilicic acid might have been retained (Yermolaev and Shirchova,1994).

The concentrations of acid-extractable Si were similar in the upper horizons of all tested soils besides the unmowed meadow and plough land (*Table 1*). This increasing of acid-extractable Si can be ascribed to the acceleration of the weathering process in the cultivated soil and to a significant amount of plant phytholites in the unmowed meadow soil (Bobrova, 1995; Kovda, 1985). Both processes have identical data on chemical testing (Barsukova and Rochin, 1979; Matichenkov et al., 1997; Matichenkov et al., 2000). The monosilicic acid concentration in both ecosystems may serve as an indicator for the direction of mineral transformation processes that are: the accumulation of phytoliths for the unmowed meadow (high level of monosilicic acid) and the acceleration of weathering for the cultivated soil (low level of monosilicic acid).

The correlating coefficients between different Si forms in the soil (*Table 1*) and the balance of elements in the soil-plant systems (*Table 3*) were calculated. A linear dependence of the type $Y = AX + B$ was used.

Calculations have demonstrated that a high correlation exists between the concentration of monosilicic acid in the soil and the balance of Si in the ecosystem: $r=0.98$, $p=0.001$, $sd=0.759$. The relationship between other examined forms of Si in soil and the balance of Si in the ecosystem had no significant level:

$r = 0.0313$, $p = 0.96$, $sd = 10.82$ for sum of soluble Si forms,
$r = -0.75$, $p = 0.26$, $sd = 7.29$ for polysilicic acid, and
$r = 0.100$, $p = 0.87$, $sd = 73$ for acid-extractable Si.

These calculations showed that monosilicic acid concentration in the upper soil horizon strongly depends on the type of plant association when compared with ecosystems having similar type of soil. Monosilicic acid represents the main component in the biogeochemical Si cycle of the soil-plant ecosystem (Matichenkov, Bocharnikova,1994; Nazarov,1976). Monosilicic acid also controls many soil properties and has a direct effect on plants and microorganism growth. In turn, plants (type of plant cover) can control the monosilicic acid concentration in the upper soil horizons as well. As a result, we are dealing with a complicated and self-regulating Si biogeochemical cycle in the soil-plant ecosystem.

The unmowed meadow and birch-aspen forest plant associations are responsible for increasing the concentration of monosilicic acid in the upper soil layer which optimizes plant Si nutrition. Silicon-accumulative plants (grasses) have competition priority. Both ecosystems are characterized by accumulative type of Si cycle. The removal of Si as a result of agricultural activity, grazing or accumulation of Si in unavailable forms (spruce wood) can change Si cycle and cause conversion changes for the soil-plant system: degradation of soil minerals, replacement of plant population where non Si accumulative plants have competitive priority. By this means that agriculture land, mowed meadow and spruce wood have typical eluvial Si cycle.

Conclusion

This investigation clearly shows that the biological cycle of Si is characterized by 40 to 80 kg Si ha^{-1} annually removed from Grey Forest Soil. The monosilicic acid concentration in the upper soil horizons strongly depends on the type of plant association. The removal of plant biomass from the ecosystem results in decreasing monosilicic acid concentration in the upper soil horizons. Si movement from lower soil horizons to surface layer can occur via the adsorption of monosilicic acid by plants and by returning phytoliths into the soil. The concentration of monosilicic acid in the upper soil layer may serve as an indicator of straining in the Si biological cycle, stability of existing plant association and direction of the ecosystem evolution. The unmowed meadow and the birch-aspen forest were characterized as ecosystems with accumulative type of Si cycle. The agricultural land, the mowed meadow and spruce wood are all eluvial type Si cycle.

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OCCURRENCE OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI IN TROPICAL FOREST COMMUNITIES OF INDIA

TRIPATHI, P.* – KHARE, P.K.

Department of Botany, Dr. H.S. Gour Central University,
Sagar (M.P.) 470003, India

**Corresponding author*
e-mail: priyanganaeco@gmail.com

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Abstract. The paper deals with the quantitative and qualitative assessment of vesicular arbuscular mycorrhizal fungi (AMF) associated with the rhizosphere of dominant plants of three vegetation viz., Tropical Evergreen Forest, Gibbon Wild-life sanctuary, Jorhat, Assam, Tropical Moist Forest, Kanha National Park, Mandla, M.P., and Tropical Dry Deciduous Forest, Sagar, M.P. Root and rhizosphere soil samples, collected near different trees species were analyzed for root colonization, spore count, extramatrical hyphae, vesicles, Paris coil and intraradical spores. VAM spores were isolated by wet sieving and decanting method and estimation of spores was carried out. In evergreen forest vegetation (Site-I), maximum root colonization (33 ± 0.35 %) was observed in *Terminalia myrocarpa Henrck & Mull.* and minimum in *Walsura robusta Roxb.* (19 ± 0.61 %).VAM spore count per 25g of soil was also highest (133 ± 0.41) in *Terminalia myrocarpa Henrck &Mull.* while it was low in *Mesua ferrea Linn.* (48 ± 0.21). In tropical moist forest (Site-II), *Bridelia retusa Spreng.* was observed to support least root colonization (33 ± 0.4 per cent) and spore density (43 ± 0.25/25 g soil). Maximum root colonization (64 ± 0.49%) and spores density (167 ± 0.46) were found in *Lagerastroemia parviflora Roxb.* In tropical dry deciduous forest (Site-III), root colonization (62 ± 0.62 %) and spores density (121 ± 0.31) were maximum in *Albizzia lebbek Benth.*-highest among all the three sites. Interestingly, these attributes were also lowest at this site in *Wrightia tinctoria Linn.* In all, 25 VAM species were identified from all the three forest sites. Out of these, 5 VAM species were common to three forest sites. More number of exclusive VAM species (6) were recorded at site-II. *Glomus* spp. was found as dominant VAM at all the three forest sites. Besides, the other morphological details of different VAM fungi, the results of present investigation envisage that diverse conditions of the habitat i.e. Tropical Moist Forest, support more VAM species not only in terms of numbers but also as the root association and potential propagules in the form of spores.

Keywords: *VAM, Root colonization, Qualitative and Quantitative characters*

Introduction

The Vesicular Arbuscular Mycorrhizal (VAM) association with most of the terrestrial plants is perhaps as old as the evolution of terrestrial flora (Taylor et al., 1995). They are ubiquitous in distribution from tropical (Chaurasia and Khare, 2005), temperate (Vestberg, 1995) and arctic (Dalpe and Aiken, 1998) regions. They are responsible for enhancing the growth and improving the health of plants and soils (Hodge et al., 2001; Rausch et al., 2001) and play a crucial role in mineral nutrition of forest trees thus becoming important nutrient acquiring mechanisms (Pate, 1994).

Although the occurrence of VAM species is non specific, they exhibit variation in both qualitative and quantitative attributes. Variation may be found due to climatic, edaphic and composition of vegetation types.

The present paper deals with the occurrence and population variations of arbuscular mycorrhizal fungi in three different tropical forests of India namely tropical evergreen forest, tropical moist deciduous forest and tropical dry deciduous forest. Root colonization, spore population and qualitative characteristics like external hyphae, vesicle, Paris coil, intraradical spores were determined and discussed.

Study area

Site-I The site belongs to a tropical evergreen forest within Gibbon National Park near Jorhat, Assam, about 30 km West of Jorhat, situated at 26°46'N latitude and 96°16'E longitude Climate is a seasonal with average monthly minimum and maximum temperature of 19ºC and 30ºC in January and May respectively. Annual rainfall is more than 300 cm received throughout the year. The soil is clay to sandy clay with pH ranging from 4.3 to 4.9. The forest types as classified by (Champion and Seth, 1968) is semievergreen to evergreen. Dominant forest tree species are *Dipterocarpus myrocarpa Vesque, Walsura robusta Roxb., Dillenia indica Linn.*

Site-II The site, tropical moist forest, is a part of Kanha National Park, a protected area dedicated to Project Tiger in the state of Madhya Pradesh in Central India. It is located in between 22°1' and 22°27'N, and 80°26' and 81°4'E longitude at an altitude of 800-900 msl. The climate is distinctly seasonal with three well marked reason viz., rainy, winter and summer. Maximum temperature of 40ºC reaches in summer and minimum as 0.0ºC during the month of December in winter season. The total annual rainfall is 140 cm principally received during the months of July to September. Edaphically it constitutes the Central Indian highlands that forms main peninsula of India. The terrain is undulating with clayey laterite rich in Bauxite on tops and plains, and valley with granite gneisses. The soil is near neutral or alkaline type. Forests are tropical moist deciduous types dominated chiefly by *Shorea robusta Gaertner F.*

Site-III This site is a part of tropical dry deciduous forests in Central India, located in between 23°5' – 24°25' N and 78°10' – 79°15' E longitude at an elevation of 583 msl near the town of Sagar in the state of Madhya Pradesh. The climate is distinctly seasonal with summer, rainy and winter seasons. Total annual rainfall is 120 cm, nearly 90% of which is received in rainy season. Summer is hot with maximum temperature touching 47ºC and winter is pleasant with minimum temperature of 8-9ºC. A long dry season of about 7-8 months is responsible for the occurrence of tropical dry deciduous forests principally dominated by *Tectona grandis* with associates like *Terminalia tomentosa W.& A., Diospyros melanoxylon Roxb.* and *Butea monosperma Lamk.Taub.* Soil is thin, rich in calcium with pH ranging from 6.5 to 8.5.

Materials and methods

Sample collection

Soil and root samples were collected during the month June (Late Summer) from selected dominant and codominant forest tree species of all the three sites. Individual non-adhering rhizosphere soil samples were taken in triplicate from the depth of 10-15 cm, after removing upper detritus. Fine rooflets (feedar roots) were collected from the dominant species of each sites. Samples were air dried, sieved and stored at 4ºC until further processing.

Processing of root samples

Root samples were rinsed with tap water and cut into 1 cm pieces, and the staining was done with trypan blue (Phillips and Haymann, 1970; Kormainik et al., 1980). One hundred root pieces were randomly picked and mounted on glass slides in lactophenol and examined under compound microscope. The colonization was calculated using the following formula

$$\%\ \text{colonization} = \frac{\text{no. of root segments colonized}}{\text{Total no. of root segments studied}} \times 100 \qquad \text{(Eq.1)}$$

Qualitative characteristics of mycorrhizal fungi i.e. external hyphae, presence of vesicles, Paris coil and occurrence of intraradical spores, were observed in stained root samples.

Extraction of spores and their identification

Spores from the soil samples (25g) were extracted by wet sieving and decanting method (Gerdemann and Nicolson, 1963). For the estimation of VAM species, a modified method (Gour and Adholeya, 1994) was followed and identification was done on current descriptions and identification manuals (Schenk and Perez, 1990; Mehrotra and Baizal, 1994; www.invam.caf.wvu.edu/my-info/Taxonomy/species.htm). Species name code of VAM fungi were followed (Perez and Schenck, 1990).

Results

Data of rhizospheric soil analysis of three study sites is presented in the table 1 to 4. Twelve tree species were analyzed for quantitative and qualitative characters of VAM fungi from site I. The lowest root colonization was observed in *Walsura robusta* Roxburgh (19%) and highest in *Terminalia myrocarpa* Henrck & Mull. (33%) followed by *Terminalia nodiflora* (R. Ar.) (32%) and *Dillenia indica* Linn. (32%). VAM fungal spore population per 25 g of rhizospheric soil of 12 tree species from site I is presented in the *Table 1*. It is evident that the lowest spore population (48/25 gm) was observed in rhizosphere soil of *Mesua ferrea* Linn.and highest spore population (133/25 g) in *Terminalia myrocarpa* Henrck & Mull. Qualitative characters of VAM fungi viz., external hyphae, vesicle, Paris coil, intraradical spores are given in *Tables 1 to 3*. The presence of extrametrical hyphae were recorded in *Terminalia myrocarpa* Henrck & Mull., *Chukrasia tabularis (*A. Juss.*),* *Tetrameles nodiflora (* R. Ar.), *Meusa ferrea* Linn.*,* *Artocarpus lakoocha* Roxb.and *Walsura robusta* Roxb.. Vesicles were less common although observed in *Dillenia indica* Linn.*,* *Mesua ferrea* Linn.*,* *Artocarpus lakoocha* Roxb. and *Walsura robusta* Roxb. Paris type coils were observed in root pieces of *Dipterocarpus macrocarpus* Vesque, *Castanopsis spp* Blume.*,* *Chakrasia tabularis(*A. Juss.), *Terminalia myrocarpa* Henrck & Mull. and *Artocarpus chama* Buch.Ham. Intraradical spores were abundant with the exception of *Dipterocarpus macrocarpus* Vesque, *Dillenia indica* Linn.*,* *Artocarpus lakoocha* Roxb. and *Artocarpus chama* Buch.Ham. Total 15 VAM species were recovered from this site, out of which 5 VAM species were exclusive, i.e. restricted to this site only.

Table 1. *Quantitative and Qualitative characters of VAM fungi associated with some important forest tree species at Site-I (Jorhat)*

S. No.	Plant	Quantitative Characters				Qualitative Characters	Vesicle	Paciscoil	Intraradical	VAM Species
		% Root colonigation		No.of spores in 25g soil		Exhamatrica Hyphae				
1	*Dipterocarpus macrocarpus Vesque*	29	SD±0.30 SE±0.15	85	SD±0.45 SE±0.26	-	-	+	-	ARHM, LFSC, SPKS, LABS, LMSS
2	*Castanopsis* spp.Blume	23	SD±0.45 SE±0.23	66	SD±0.25 SE±0.14	-	-	+	+	AUDL LFSC, LAGR, LABS LMSS
3	*Dillenia indica Linn.*	32	SD±0.38 SE±0.19	92	SD±0.42 SE±0.24	-	++	-	-	ASPN, LFSC, LAGR, LABS, LHTS
4	*Lagerostromia speriosa (L.)*	22	SD±0.31 SE±0.15	105	SD±0.51	-	-	-	+	CPLC, LFSC, LAGR, LOCT LMSS, LHTS
5	*Tetrameles nodiflora(R.Ar.)*	32	SD±0.64 SE±0.32	94	SD±0.45 SE±0.26	+	-	-	+	ASPN, ARHM, LPKS, LAGR, LHTS
6	*Chukrasia tabularis (A.Juss.)*	20	SD±0.42 SE±0.21	102	SD±0.83 SE±0.48	+	-	+	+	AUDL, LFSC, LAGR, LMSS, LABS
7	*Amoora wallichii Benth.*	28	SD±0.56 SE±0.28	99	SD±0.58 SE±0.33	-	-	-	+	LMSS, GRSA, LPKS, SSNS, LMSS
8	*Terminalia myrocarpa Henrck&Mull.*	33	SD±0.35 SE±0.18	133	SD±0.41 SE±0.21	+	-	+++	+	ASPN, LFSC, SPKS, LOCT, LMSS, LHTS
9	*Mesua ferrea* Linnaeus	27	SD±0.95 SE±0.48	48	SD±1.21 SE±0.70	+	+	-	+	GRSA, ARHM, LAGR, LABS
10	*Artocarpus lakoocha Roxb.*	27	SD±0.78 SE±0.39	79	SD±0.39 SE±0.22	+	+	-	-	GRSA, LFSC, LAGR, LOCT, LHTS
11	*Artocarpus chama Buch._Ham*	30	SD±0.59 SE±0.31	122	SD±0.47 SE±0.27	-	-	+	-	ATBC, LFSC, SPKS, LOCT, LHTS
12	*Walsura robusta* Roxburgh	19	SD±0.61 SE±0.31	57	SD±0.53 SE±0.30	+	+	-	+	ATBC, ARHM, LAGR

Table 2. *Quantitative and Qualitative characters of VAM fungi associated with some important forest tree species at Site-II (Mandla)*

S. No.	Plant	Quantitative Characters				Qualitative Characters	Vesicle	Paciscoil	Intraradical	VAM Species
		% Root colonigation		No.of spores in 25g soil		Exhamatrica Hyphae				
1	*Bridelia retusa* Spreng	33	SD±0.47 SE±0.24	43	SD±0.25 SE±0.15	+	-	+	++	LGSP, LETC
2	*Syzygium cuminii* (L.) skeels	48	SD±1.28 SE±0.64	96	SD±0.25 SE±0.14	+	+	+	+	LAGR, LFSC, LCVL, LDST
3	*Diospyros melanoxylon* (Roxb.)	43	SD±0.45 SE±0.23	84	SD±0.36 SE±0.41	+	+	-	+	LMSS, LFSC, LDST
4	*Cassia fistula* Linn.	41	SD±0.58 SE±0.29	103	SD±0.08 SE±0.	+	-	+	-	LAST, LETC, LDST, LHTS
5	*Careya arboreya* (Roxb.)	52	SD±0.69 SE±0.35	91	SD±0.94 SE±0.55	+	+	+	-	LABS, LFSC, LCVL
6	*Terminalia tomentosa* W. & A.	55	SD±0.39 SE±0.21	127	SD±0.08 SE±0.05	+	+	+	-	CFLG, LMSS, LETC, LHTS
7	*Shorea robusta Gaertner F.*	60	SD±0.31 SE±0.15	157	SD±0.52 SE±0.31	-	+	-	+	LGSP, LFSC, LDST, LHTS
8	*Schliechera oleosa* (Lour) Oken	46	SD±0.42 SE±0.21	83	SD±0.43 SE±0.25	+	-	+	+	LHTS, LETC, LDST
9	*Terminalia chebula Retz.*	57	SD±0.77 SE±0.38	133	SD±0.42 SE±0.25	-	+	+	-	LGSP, LFSC, LETC
10	*Adina cordifolia* Hook.F.	39	SD±0.50 SE±0.25	139	SD±0.08 SE±0.05	-	-	+	+	LHTS, LFSC, LCVL
11	*Bombox ceiba* Auct.	40	SD±0.50 SE±0.25	123	SD±0.53 SE±0.30	-	+	+	-	LGSP, LHTS, LETC. LFLV
12	*Lagerashtroemia parviflora* Roxb.	64	SD±0.49 SE±0.24	167	SD±0.45 SE±0.26	+++	+	+	-	ATBC, LAST, CPLC, LTNB, LMCC
13	*Embellica officinalis* Gaerth.	53	SD±0.19 SE±0.11	91	SD±046. SE±0.26	+	+	+	+	LGSP, LFSC, LDST, LHTS
14	*Mitragyna parviflora* Korth.	54	SD±0.66 SE±0.33	100	SD±0.65 SE±0.37	-	+	-	-	LGSP, LHTS, LDST

Table 3. *Quantitative and Qualitative characters of VAM fungi associated with some important forest tree species at Site-III (Sagar)*

S. No.	**Plant**	**Quantitative Characters**				**Qualitative Characters**	**Vesicle**	**Paciscoil**	**Intraradical**	**VAM Species**
		% Root colonigation		**No.of spores in 25g soil**		**Exhamatrica Hyphae**				
1	*Holoptelia integiefolia* Planch	16	SD±0.89 SE±0.44	47	SD±0.43 SE±0.25	+	+	+	+	LAGR, LFSC, ASCB, LMSS
2	*Flacourtia indica* (Burm F) Merr.	29	SD±1.06 SE±0.27	64	SD±0.90 SE±0.52	-	+	+	+	CFLG, ASCB, LAGR, LMSS
3	*Wrightia tinctoria* Linn.	14	SD±0.85 SE±0.42	29	SD±0.67 SE±0.39	-	+	-	-	LFSC, ASCB, LAGR, LMSS
4	*Acacia catechu* Willd.	21	SD±0.60 SE±0.30	33	SD±1.20 SE±0.71	-	+	-	-	SPKS, SSNS, LFSC, LAGR
5	*Butea monosperma* (Lamk. Taub)	38	SD±0.49 SE±0.25	99	SD±0.29 SE±0.17	-	++	+	+	LAGR, LTNB, ARHM, SPKS, SSNS, ASCB
6	*Anogeissus latifolia* Wall	54	SD±1.08 SE±0.54	103	SD±0.74 SE±0.43	+	+	+	+	LHTS, LABS, SPKS, SSNS, LMTC
7	*Buchanania lanzan* Spreng	35	SD±0.71 SE±0.36	67	SD±0.17 SE±0.10	-	+	-	+	LFSC, LAGR, ASCB, LMSS
8	*Lannea coromandelica* (Houtt.) Merr.	58	SD±0.76 SE±0.38	111	SD±1.13 SE±0.57	-	+	++	+	ASCB, LAGR, SSNS, LMSS, LOCT
9	*Aegle mormelos* Correa.	33	SD±0.25 SE±0.15	86	SD±1.09 SE±0.55	+	+	-	-	LFST, LAGR, ASCB, LABS
10	*Albizzia lebbek* Benth.	62	SD±0.62 SE±0.36	121	SD±0.31 SE±0.18	+	+	-	-	ASCB, LAGR, SSNS, LMSS, LOCT
11	*Miliusa tomentosa* (Roxb.) J. sinclair	41	SD±0.72 SE±0.36	52	SD±0.27 SE±0.15	++	-	-	-	LETC, LFSC, LAGR, LMSS
12	*Bauhinia retusa Ham.*	53	SD±1.29 SE±0.64	97	SD±0.55 SE±0.32	-	-	-	+	LFSC, LAGR, ASCB

Table 4. *Occurence of different VAM Species at three different forest vegetation*

Jorhat (site-I)	*Mandla (Site-II)*	*Sagar (Site-III)*
LFSC*, LABS*, LMSS*, LHTS*	LFSC*, LCVL, LGSP, LAGR*	LAGR*, LFSC*, LMSS*, LABS*
LAGR*, LOCT, LMST, ARHM	LETC, LMSS*, LDST, LAST	LTNB, LHTS*, LMTS, LOCT
AUDL, ASPN, ATBC, SPKS	LHTS*, LABS*, LTNB, LMCC	LETC, ASCB, ARHM, CFLG
SSNS, CPLC, GRSC	CFLG, CPLC, ATBC, LFLV	SPKS, SSNS

*= common species, # =exclusive species
- species code of VAM fungi were followed after Perez and Schenk (1990)

LABS= G. ambisoporum smith and schenck.
LTNB= G. tenebrosum(Thaxter) Berch.
LHTS= G. heterosporum Smith and shenck.
LMST= G. multisubtensum mukerji, Bhattacharjee and Tiwari.
LOCT= G. occultum Walker.
LETC= G. etunicatum Becker and Gerdemann.
LCVL= G. convolutum Gerdemann and Trappe.
LGSP= G. geosporum (Nical and Gerd.) walber.
LDST= G. deserticola Trappe, Bloss and Menga.
LAST= G. australe (Berk) Berch.
LMCC= G. macrocarpum Tulasne and Tulasne.
LPST= G. postulatum Koske, friese. walker and Dalpe.
LMTC = G. multicaule Gerdemann and Bakshi.
ASCB= Acaulospora Scrobiculata Trappe.
ARHM= A. rehmii sieverding and Toro.
LAGR= Glomus aggregatum schenck and smith emend. koske.
LFSC= Glomus fasciculatum (Thaxter) Gerdemann and Trappe emend. walker and koshe.
LMSS= G. moseae(Nicolson and Gerdemann) Gerdemann and Trappe.
ATBC= A. tuberculata Janos and Trappe.
AUDL= A. undulata Sieverding.
CFLG = scutellespora Fulgida Koske and Walker.
CPLC = Scutellospora pellucida (Nicol & Schenck) walker and Sander.
SSNS = Sclerocystis sinosa Gerdemann and Bakshi.
GRSA = Gisaspora rosea Nicolson and Schenk
SPKS = Sclerocystis pakistanica Iqbal and Bushra

At site II, 14 tree species were selected for analysis of different attributes. Lowest root colonization was observed in *Bridelia retusa* Spreng. (33%) and highest (64%) in *Lagerastroemia parviflora* Roxb. followed by *Terminalia chebula* Retz. (57%). VAM fungal spore population per 25 g of soil collected from the rhizosphere of 14 tree species (*Table* 2) showed wide variations from 43 in *Bridelia retusa* Spreng to 167 in *Lagerastroemia parviflora* Roxb.

Data indicate that extramatrical hyphae were present in all the tree species except in *Terminalia chebula* Retz., *Adina cordifolia* Hook.F., *Bombox ceiba* Auct. and *Mitragyna parviflora* Korth.. Occurrence of vesicles was common however, not observed in *Bridelia retusa* Spreng., *Cassia fistula* Linn., *Schliechera oleosa* (Lour) Oken. and *Adina cordifolia* Hook. F.. Paris coil type association was a common feature in all the tree species barring *Diospyros melanoxylon (*Roxb.), *Shorea robusta* Gaertner F. and *Mitragyna parviflora* Korth.. Intraradical spores were found in *Bridelia retusa* Spreng, *Syzygium cuminii (*L.)Skeels, *Disopyros melanoxylon (Roxb.) Shorea robusta* Gaertner F., *Schliechera oleosa*(Lour) Oken, *Adina cordifolia* Hook. F. and *Embellica officinalis* Gaerth.

Total 16 VAM species were found at the site II, 6 species were exclusive to this site.

From site III, twelve tree species were considered and root colonization was minimum in *Wrightia tinctoria* Linn. (14%) and maximum (62%) in *Albizzia lebbek* Benth. followed by *Lannea coromandelica* (Houtt.) Merr. (58%). VAM fungal spore population per 25 g of rhizospheric soil for 12 selected tree species (Table 3) & showed highest spore population (121/25 g) in *Albizzia lebbek* Benth.and lowest (29/25 g) in *Wrightia tinctoria* Linn.

External Hyphae were present in *Holoptelia integrifolia* Planch and *Anogeissus latifolia* Wall.. Vesicles were found in 10 tree species except in *Miliusa tomentosa* and *Bauhinia retusa*. Paris type coils were recorded in the *Holoptelia integrifolia* Planch, *Flacoutia indica* (Burm F) Merr., *Butea monosperma* (Lamk.Taub.), *Anogeissus latifolia* Wall, *Lannea coromandelica (*Houtt.) Merr.. Intraradical spores were found within most of the tree species except in *Wrightia tinctonia* Linn., *Acacia catechu* Willd, *Aegle marmelos* Correa, *Albizzia lebbek* Benth., *Miliusa tomentosa* (Roxb.) J. Sinclair.

Total 14 VAM species were recorded from site III in which 2 VAM species were exclusive.

Total 25 VAM species were observed from all the three study sites. Out of which 15 were from site I, 16 from site II and 14 VAM species from site III (*Table 4*). 5 VAM species were common to all the three sites.

Discussion

Arbuscular mycorrhizal fungi are most widely distributed symbiotic organisms in nature. The soil borne mycorrhizal fungi colonize the root cortex biotropically followed by the development of external mycelium which is a bridge connecting the root with the surrounding soil microhabitats. The VAM fungi have been reported from different plant communities of varied geographical regions such as – Tropical region (Chaurasia and Khare, 2000); Temperate region (Vestberg, 1995) and arctic region (Dalpe and Aiken, 1998).

Results of the present study indicate that all the tree species from tropical forests had VAM association with variation in the degree of infection. Such variability of colonization has been observed by number of workers in tropical forests and attributed to a number of local edaphic and climate factors (Safir and Dunisway, 1982; Thapar and Dunisway, 1992; Byra Reddy et al., 1994; Rahangdale and Gupta, 1998; Kalita et al., 2004).

Highest spore population was observed in rhizsophere soils of site II than at site I and site III. These differences can be attributed to the type of VAM species associated with their plants (Pringle and Bever, 2002). Seasonal variation in spores population in tropical forests depend upon the climatic factors, soil type, nutrients status growth and metabolic activities of plants, together with preferences of the fungi to host species and spatial distribution of host species (Louis and Lim, 1997; Harinikumar and Bagyaraj, 1988; Johnson and Wedin, 1997; Allen et al., 1998; Lovelock et al., 2003). Moreover, VAM spore densities vary greatly according to the physiological state of the host plant and relationship with the plant species. It is interesting that Arbuscules (Arum type) in VAM fungi were absent from all the three sites, although as a defining characteristic, arbuscules may be difficult to find under natural conditions due to their short life span (Smith and Read, 1997).

Paris type (Hyphal coil) association were more frequently observed. They were particularly prolific at the site II than in the other two sites. The dominance of Paris type association imply that coiled hyphae live longer and are more tolerent to stress conditions such as drought, suggesting that external factors determine the dominance of the type of endomycorrhizae (paris Vs arum) over another.

The frequent occurrence of vesicles in most of the tree species from all the three study sites showed the presence of VAM fungi belonging to the Glomineae. The highest frequency of oval to elongate vesicles over the irregular and lobbed vesicles showed the dominance of species of the genus *Glomus* among other genera. Predominance of Glomineae has already been reported from tropical sites (Sieverding, 1991; Onguene and Kuyper, 2001). Results also showed the dominance of Glomineae over Gigasporineae. In the present study, the rhizosphere of different forest tree species from three forest sites showed common as well as variant VAM flora. Such variations are common features of VAM (Klironomos et al., 1993; Vishalakshi, 1997; Rawat and Mukerjii, 1998; Muthukumar and Udaiyan, 2000; Rama Bhat and Kaveriappa, 2001; Tamuli and Boruah, 2002).

A large number of VAM speices belong to the genus *Glomus* in the present study. The genus *Glomus* has been reported to be the dominant VAM fungus in a number of forest communities (Sharma et al., 1986; Byra Reddy et al., 1994; Uniyal and Uniyal, 2000; Tamuli and Boruah, 2002; Mohan Kumar and Sivaswamy, 1992). This may be explained due to the wider adaptability of *Glomus* spp. as compared to other species (Sparling and Tinker, 1978; Sharma et al., 1986; Byra Reddy et al., 1994; Schenk and Kinloch, 1980).

Comparative assessment of occurrence of VAM fungi from three sites envisage that tropical moist forests (site II) harbour more VAM population in terms of richness and diversity than tropical evergreen and deciduous forest communities.

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