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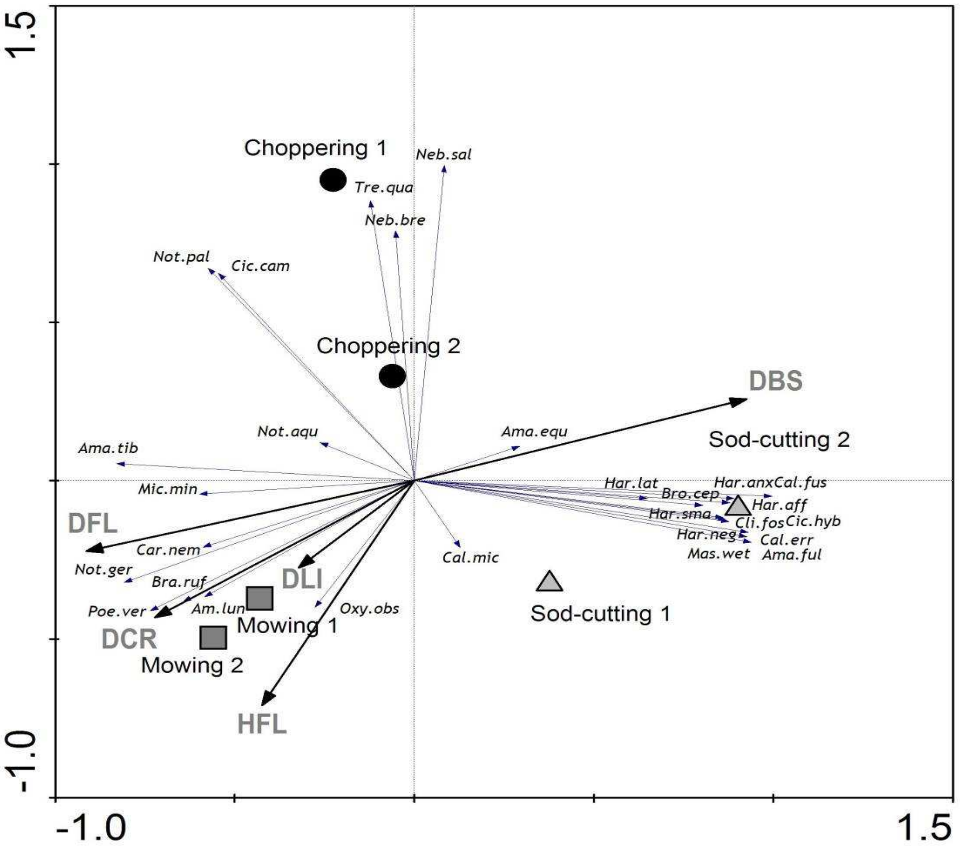

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SHORT-TERM EFFECTS OF MODERN HEATHLAND MANAGEMENT MEASURES ON CARABID BEETLES (COLEOPTERA: CARABIDAE)

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Abstract. For the maintance of dry heathland ecosystems the implementation of adequate conservation measures is required. Besides traditional land use practices (e.g. mowing) several modern management measures (e.g. sod-cutting, choppering) were developed and applied. In the present study the carabid beetle fauna of three different managed heathland sites in a coastal heathland on the Baltic isle of Hiddensee, Germany, was analysed. Pitfall trapping yielded a total of 4,018 carabid beetles belonging to 48 species. Species and individual richness was highest in the sod-cutted site followed by choppering and mowing. Diversity was highest on the mown site, due to the most even distribution of species. Species composition differed clearly among sites indicated by RDA ordination and Jaccard´s similarity index. The application of sod-cutting and mowing present two important habitats for specialised carabid beetles: Sod-cutting creates secondary, highly dynamical habitats suitable for several dune species while mowing seemed to preserve a typical (*Calluna*) heathland carabid fauna. The use of different management measures could lead to a more heterogeneous heathland, create suitable habitats for several specialised carabid beetle species and therefore might enhance diversity.
Keywords: *Choppering, coastal heathland, mowing, restoration ecology, sod-cutting*

Introduction

The main object of the European Habitats Directive is the maintenance of biodiversity by the conservation of certain habitats and of wild fauna and flora (EC Habitats Directive 92/43/EEC). For semi-natural habitats, such as dry heathlands, the implementation of adequate conservation measures is therefore required and necessary. In North-West Europe heathlands were traditionally used as grazing ground for sheep in addition with sod-cutting (also known as turf cutting or plaggen) (Webb, 1998). By such land use practices, nutrients were continuously depleted and natural succession to shrub or forest is arrested (Gimingham, 1972; Webb, 1998). In former times, this cultural landscape developed in large areas throughout the Atlantic region of Europe mainly on dry, acid, and nutrient-poor soils (Gimingham, 1972). Nowadays, heathlands are restricted to small and mainly fragmented areas (Webb, 1998). The main reasons for this decrease are changes in land-use (e.g. afforestation, agricultural intensification) and high rates of atmospheric nitrogen deposition (Heil and Diemont, 1983; Webb, 1998). Both, the abandonment of traditional land use and eutrophication, enhance successional processes including negative effects such as accumulation of soil organic matter, decreasing biodiversity and the loss of a typical heathland fauna (Marrs and Le Duc, 2000; Roem and Berendse, 2000; Irmler, 2004). Especially for invertebrates, such as carabid beetles or spiders, heathlands present an important ecosystem with higly specialised species (Usher, 1992; Buchholz, 2010).

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To preserve heathlands and counteract the negative effects of succession and eutrophication, several modern management measures were developed and applied (Barker et al., 2004; Härdtle et al., 2006). Besides mowing and burning, especially sod-cutting and choppering, two methods that are highly intensive and require the use of specialised machines, are seen as highly effective in reducing nutrient loads (Härdlte, 2006). By sod-cutting the total above ground biomass and most of the humus-rich topsoil layer (O- and A-horizon) is removed down to the mineral and sandy soil layer. Because sod-cutting is cost-intensive and results in high amounts of waste material, choppering has been applied as an alternative method (Niemeyer et al., 2007) which takes an intermediate position of intensity between sod-cutting and mowing. Thereby the above ground biomass is totally removed as well as (much of) the O-horizon, while the A-horizon remains unaffected (Maes et al., 2004). A more detailed description of both measures is given by Niemeyer et al. (2007).

All management measures aim at preserving a vital heathland landscape on a long-term basis as well as preserving a typical heathland flora and fauna. Carabid beetles are a highly usefull indicator taxon for assessing management practices or restoration effects (e.g. Buchholz et al., 2009; Malfait and Desender, 1990; Mossakowski et al., 1990), and in many studies the (short-term) response of carabid beetles to different heathland management schemes like cutting/mowing, burning or grazing has been analysed (Usher and Thompson, 1993; Usher, 1992; Gardner, 1991; Garcia et al., 2009). But especially with respect of animal conservation, not only the long-term preservation of a (homogenous) heathland vegetation but also the creation of a vegetation mosaic might be of great importance, too, as a heterogeneous heathland might enhance insect diversity (Gardner, 1991; Schirmel et al., 2010). Therefore, management measures are not only important for vegetation recovery and development on a long-term basis, but could also contribute to a high insect diversity by forming a heathland mosaic with different habitats suitable for several species.

The aim of the present study was to analyse the carabid beetle fauna of three different managed heathland sites in a coastal heathland on the Baltic isle of Hiddensee, Germany. The applied management measures were sod-cutting, choppering and mowing and an analysis of the short-term effects of these management measures (up to 3 years after realisation) on the carabid beetle fauna was done. In particular the following research questions were addressed: (i) How do species richness, diversity and abundance patterns differ among the three different managed sites? (ii) Does the carabid species composition differ? (iii) What can be concluded for nature conservation and management practices in heathlands?

Materials and methods

Study area

The study area is a coastal dune heathland on the Baltic Sea island of Hiddensee (Mecklenburg-Western Pomerania, Northeastern Germany). The island is situated west of Rügen in the National Park “Vorpommersche Boddenlandschaft” (Western Pomeranian Bodden landscape). The north-south extent of Hiddensee is about 19 km with a maximum width of about 3 km (total area of approx. 16 km^2). The island is divided geomorphologically into a Pleistocene hilly landscape in the north (up to 72.5 m a.s.l.) and an adjacent lowland in the south formed by Holocene sandy deposits (Möbus, 2000). Hiddensee has an average annual precipitation of 547 mm and an average annual

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temperature of 7.5 C (Reinhard, 1962). In the centre of Hiddensee an anthropo-zoogenically influenced coastal dune heathland is situated with a size of about 250 ha (54°32´N, 13°5´E). The heathland is dominated by dwarf-shrubs (mainly *Calluna vulgaris* (L.) Hull, but also *Empetrum nigrum* L. s. str., *Salix repens* L. and *Erica tetralix* L.). The extensive and rather homogeneous heath-stands are interrupted by sparsely vegetated grey dunes dominated by *Corynephors canescens* (L.) P. Beauv., *Carex arenaria* L. and cryptogams, grassy heath-stands (*Deschampsia flexuosa* (L.) Trin., *Molinia caerulea* (L.) Moench, *C. arenaria*), and shrub encroached stands (mainly *Betula pendula* Roth and *B. pubescens* Ehrh.).

The heathland area was traditionally used as grazing ground for domestic animals and as fuel and building material until about the second World War (Umweltministerium Mecklenburg-Vorpommern, 2003). In recent times the heathland has been kept open by several conservation measures. Manual shrub clearing has been applied sporadically since 1978 (Umweltministerium Mecklenburg-Vorpommern, 2003) and regularly since 2000 (Blindow, pers. comm.). In 2004 sheep grazing with up to 550 individuals and herd by a shepard was reintroduced. On three sites within the heathland the mechanical techniques sod-cutting, choppering and mowing were conducted.

Experimental set-up

Choppering (size: around 16,700 m²) and mowing (around 6,500 m²) were done in November 2006 and sod-cutting (20,500 m²) in November 2007 (*Table 1*). All measures were accomplished by the company Meyer-Luhdorf with specialised maschines.

Carabid beetles were sampled continuously from 09 May 2008 to 22 October 2009, i.e., 0.5 to 2 years after sod-cutting and 1.5–3 years after choppering and mowing, respectively. On each site two transects were arranged from the border to the centre, each consisting of four sampling locations at +5m, +10m, +15m, and +20m. At each sampling location one pitfall trap was installed. Pitfall traps consisting of white plastic cups (6.5 cm in diameter and 7.5 cm deep) were set flush with the soil surface. To protect the traps from precipitation a 15 × 15 cm transparent plastic roof was installed a few centimeters above each trap. Ethylenglycol and a few drops of detergent were filled up to about the half of the traps and used as a killing and preservation fluid. The traps were emptied every two (2008) or four (2009) weeks in summer and every four weeks in winter. Vegetation sampling took place at each sampling location in a 1 × 1m square once in July 2008. The densities of field layer (DFL), cryptogams (DCR) and litter (DLI) as well as the proportion of bare soil (DBS) were estimated in %. The height of field layer (HFL) was measured in cm.

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Table 1. *Charactersitics of the study sites with the different management schemes a) sod-cutting, b) choppering and c) mowing in the coastal heathland on the Baltic isle of Hiddensee, Germany*

Management	Size [m²]	Date of measure	Description
Sod-cutting	20,500	Nov 2007	Dominated by new shoots of *Calluna vulgaris*, *Rumex acetosella*, *Carex arenaria* and *Rubus fruticosus* agg. Very high proportion of bare and sandy soil.
Choppering	16,700	Nov 2006	Domination of *Carex arenaria*, *Calluna vulgaris*, *Deschampsia flexuosa* and cryptogams. High proportion of bare soil rich in humus.
Mowing	6,500	Nov 2006	Domination of *Calluna vulgaris, Carex arenaria*, *Deschampsia flexuosa* and cryptogams. High proportion of dead woody *Calluna*-sprouds and cryptogams (e.g. *Pleurozium schreberi*).

Data analyses

Müller-Motzfeld (2006) was used for species identification and nomenclature of carabid beetles. Vegetation parameters (log(x+1) transformed) among the three managed sites were compared using Kruskal-Wallis-ANOVA (SPSS 11.5). Species richness estimation of carabid beetles was done using the bias-corrected Chao1 (Chao, 1984, 2005), ACE (Chao and Lee, 1992) and the second order Jacknife (Burnham and Overton, 1978) index with the software SPADE. As diversity measures the Shannon index ($H' = \sum p_i \ln p_i$), the reciprocal Simpson index ($1 / (D = \sum p_i^2)$) and the reciprocal Berger-Parker index ($1 / (d = N_{max} / N)$) were used. Rarefied species richness (down to n = 780 individuals) and rarefaction curves were calculated and created with the software PAST. Differences between rank abundance-plots were tested using the Kolmogorov-Smirnov two-sample test where D_{max} represents the largest unsigned difference between the cumulative relative abundances of two sites. The critical value D_α was calculated as $D_\alpha = K_\alpha \sqrt{[(n_1 + n_2) / (n_1 * n_2)]}$, where $K_\alpha = \sqrt{[1/2 (-\ln (\alpha/2))]}$ (see Magurran, 2004). The Jaccard´s similarity index (C_J) where used as a measure of species overlap between managed sites. To analyse carabid assemblage response to habitat parameters of the three sites RDA ordination was performed, because preliminary conducted DCA yielded a gradient length of < 2 (Leyer and Wesche, 2006). For scaling we chose inter-species correlations and species scores were divided by deviation. For ordination analyses the four pitfall traps of one transect were treated as a unit, and number of individuals were standardised to individuals/transect/day. Only species with > 3 individuals per transect were used, and data was log transformed prior analyses. RDA ordination was done using the software package Canoco 4.5.

Results

Vegetation characteristics

Density of field layer and of cryptogams differed significantly among the three sites increasing from sod-cutting over choppering to mowing (*Table 2*). Also the proportion of bare soil differed significantly and was by far highest in the sod-cutted site followed by the choppered site. No differences could be detected in density of litter and height of field layer. However, for the latter a trend could be observed showing the highest vegetation on the mown site mainly due to the occurrence of grasses such as *D. flexuosa* and *Festuca rubra* L.

Table 2. *Comparison of vegetation parameters of different managed heathland sites a) sod-cutting, b) choppering and c) mowing. Significant differences are shown in bold (Kruskal-Wallis-ANOVA)*

	Abbreviation	Sod-cutting	Choppering	Mowing	Chi²	p
Density [%]						
Field layer	DFL	13.3 ± 4.1	39.6 ± 11.0	72.5 ± 7.4	12.611	**0.002**
Cryptogams	DCR	0	2.9 ± 1.6	21.3 ± 6.3	16.809	**< 0.001**
Litter	DLI	6.0 ± 0.5	9.0 ± 2.5	10.0 ± 1.3	3.485	0.175
Bare soil	DBS	80.3 ± 5.4	53.8 ± 11.8	18.1 ± 7.4	11.908	**0.003**
Height of field layer [cm]	HFL	17.4 ±2.9	13.1 ± 2.0	23.6 ± 4.0	5.144	0.076

Capture statistics and diversity

In total 4,018 carabid beetles belonging to 48 species were sampled. *Calathus fuscipes* Goeze (1,523 individuals, 37.9 % of total catch) and *Nebria salina* Fairmaire & Laboulbène (1,264 ind., 31.5 %) were the dominant species in all sites. Frequent species were *Calathus erratus* C.R. Sahlberg (259 ind., 6.4 %), *Poecilus versicolor* Sturm (146 ind., 3.6 %) and *Amara lunicollis* Schiödte (110 ind., 2.7 %).

Species richness (observed and estimated) and individual richness was highest in the sod-cutted site followed by choppering and mowing (*Table 3, Fig. 1*). In contrast, diversity measures indicate a higher diversity on the mown site compared to the choppered and sod-cutted sites, which both had very similar values. Rarefaction curves showed for all three management schemes no reaching of an asymptote (*Fig. 1*).

Table 3. *Species richness and diversity measures of carabid beetles in three different managed heathland sites a) sod-cutting, b) choppering and c) mowing.*

	Sod-cutting	Choppering	Mowing
Individuals	1,738	1,497	783
Species richness			
Observed	37	30	29
Chao1	40.5	32.5	31.0
ACE	44.0	32.9	32.1
2^{nd} order Jackknife	46.0	37.0	35.0
Rarefied (n=780)	29.6	26.3	29.0
Diversity			
Shannon *H´*	1.6	1.6	2.2
Simpson (1/*D*)	3.0	3.0	6.2
Berger-Parker (1/*d*)	1.9	2.0	3.6

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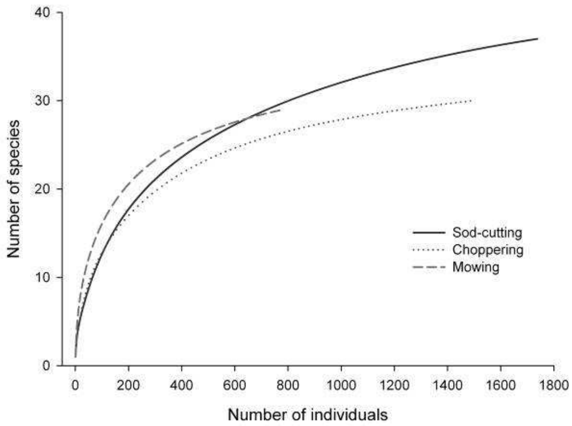

Figure 1. *Individual-based rarefaction curves based on carabid beetle data of three different managed heathland sites a) sod-cutting, b) choppering and c) mowing*

Rank-abundance plots of carabids (*Fig. 2*) did not differ significantly among sites (Komogorov-Smirnof two-sample test; sod-cutting vs. choppering: D_{max} = 0.054, D_{α} = 0.334, p > 0.05; sod-cutting vs. mowing: D_{max} = 0.241, D_{α} = 0.337, p > 0.05; choppering vs. mowing: D_{max} = 0.276, D_{α} = 0.354, p > 0.05). However, rank-abundance plot of the mown site showed a more even distribution of species while the sod-cutted and choppered sites were dominated by three or two species, respectively.

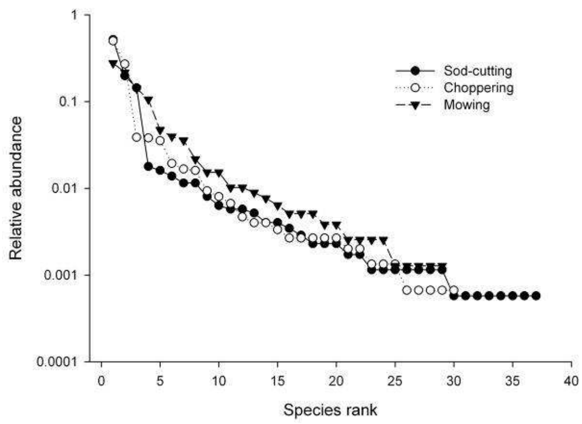

Figure 2. *Rank-abundance plots based on carabid beetle data of three different managed heathland sites a) sod-cutting, b) choppering and c) mowing.*

Species composition

The similarity index of Jaccard showed a weak similarity between carabid beetle species inventory of sod-cutting and choppering (C_J = 0.523) and sod-cutting and mowing (C_J = 0.404). The choppered site and the mown site shared 23 species of 34 and had a moderately similar species inventory (C_J = 0.676).

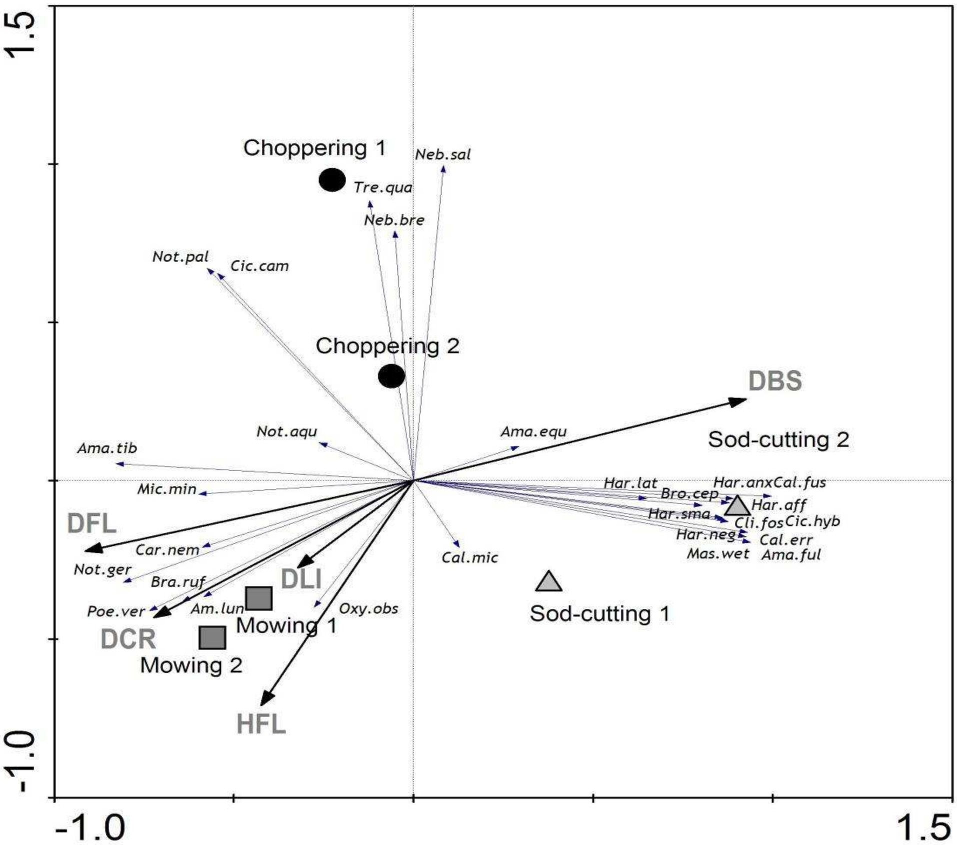

Figure 3. *RDA-ordination of carabid beetles (species > 3 ind. per transect, two transects per site) and vegetation parameters of three different managed heathland sites a) sod-cutting, b) choppering and c) mowing during the whole catching period from May 2008 until October 2009. Abbreviation of species names: Ama.equ = Amara equestris, Ama.ful = A. fulva, Ama.lun= A. lunicollis, Ama.tib = A. tibialis, Bra.ruf = Bradycellus ruficollis, Bro.cep = Broscus cephalotus, Cal.err = Calathus erratus, Cal.fus = C. fuscipes, Cal.mic = C. micropterus, Car.nem = Carabus nemoralis, Cic.cam = Cicindela campestris, Cic.hyb = C. hybrida, Cli.fos = Clivina fossor, Har.aff = Harpalus affinis, Har.anx = H. anxius, Har.lat = H. latus, Har.neg = H. neglectus, Har.sma = H. smaragdinus, Mas.wet = Masoreus wetterhallii, Mic.min = Microlestes minutulus, Neb.bre = Nebria brevicollis, Neb.sal = N. salina, Not.aqu = Notiophilus aquaticus, Not.ger = N. germinyi, Not.pal = N. palustris, Oxy.obs = Oxypselaphus obscurus, Poe.ver = Poecilus versicolor, Tre.qua = Trechus quadristriatus*

RDA ordination of carabid data showed a separation of the six transects mainly along two axis (eigenvalues of axis: 1. = 0.569, 2. = 0.375, 3. = 0.029, 4. = 0.018; *Fig. 3*). Axis 1 showed a separation along a vegetation density gradient while the second axes showed a gradient from high to low vegetation. The sod-cutting transects could be found on the right end of the ordination plot and were positively correlated with a high proportion of bare soil (DBS) and negatively with the density of total vegetation (DTV). Typical species exclusively occurring at this site were *Cicindela hybrida* Linnaeus, *Masoreus wetterhallii* Gyllenhal, *Broscus cephalotus* Linnaeus, *Amara fulva* O.F. Müller, *Clivina fossor* Linnaeus, *Harpalus neglectus* Audinet-Serville and *Harpalus smaragdinus* Duftschmid. Most frequent species were *C. fuscipes* (52%), *N. salina* (20%) and *C. erratus* (14%). An intermediate position along this gradient took the choppering transects. This site was characterised by the dominance of *N. salina* (50%),

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less abundant *C. fuscipes* (27%) and the frequent occurrence of *Nebria brevicollis* Fabricius (4%) and *Trechus quadristriatus* Schrank (4%). Other species preferring this site were *Cicindela campestris* Linnaeus and *Notiophilus palustris* Duftschmid. On the left side of the ordination plot and positively correlated with vegetation density (DTV, DCR, DLI) were the mown transects situated. Typical species in this site were *P. versicolor*, *Notiophilus germinyi* Fauvel in Grenier, *A. lunicollis*, *Amara tibialis* Paykull and *Bradycellus ruficollis* Stephens. Again *C. fuscipes* (28%) and *N. salina* (22%) were the dominant species, but also *P. versicolor* (14%), *A. lunicollis* (11%) and *B. ruficollis* (5%) were common.

Discussion

In order to preserve heathlands several different measurements were used but with different success (Power et al., 2001). As shown by (den Boer and de Vries, 1994), even by the application of the very intensive measurement sod-cutting, a typical heathland carabid beetle fauna developed on a long-term view. But besides the aim of a long-term preservation of a typical heathland vegetation and faunal composition, the use of different management measures might furthermore create a more heterogenous heathland. (Gardner, 1991) proposed, that the occurrence of different heathland successional stages could enhance carabid beetle diversity and (Schirmel et al., 2010) showed, that habitat mosaics within a heathland were of great importance for Orthoptera. So in which way do the three different managed sites differ and which value do the management measures have for carabid beetles?

Since the sites were closely related (max. distance about 250 m), were not divided by any barriers, and had a similar vegetation prior management (Blindow, pers. comm.), we assume a similar carabid beetle composition of the sites prior the the management measures. Detected differences in this study among sites, could therefore mainly be returned to the effect of each of the applied management measures. Differences were detected in species and individual richness and in species composition of carabid beetles. The site with the most intensive measure sod-cutting showed clearly both the highest species and individual richness. High individual numbers can be explained by the frequent occurrence of *Calathus fuscipes*. Individual richness was also relatively high on the choppered site while on the mown site only about the half of the individual number could be detected. Species richness between the choppered and the mown site showed similar results.

Species composition of the different managed sites differed clearly indicated by the RDA ordination and the Jaccard index. Especially composition of the sod-cutted site could be well separated. On this site several species occur exclusively. To these species belong mainly typical “dune” species such as *Harpalus smaragdinus*, *H. neglectus, Broscus cephalotus*, *Masoreus wetterhallii*, *Amara fulva* and *Cicindela hybrida* (Turin, 2000). On the other hand typical “heath” species such as the locally threatened *Bradycellus ruficollis* or *Notiophilus germinyi* (Turin, 2000) were very frequent on the mown site. Also species depending on higher vegetation (*Poecilus versicolor*, *Oxypselaphus obscurus*) found suitable habitat conditions on this site. The occurrence of these species reflects the low intensity of this management measurement and indicates a low impact on the typical heathland carabid beetle fauna. Choppering, which take an intermediate position in management intensity, also showed an intermediate position referring to species composition. However, neither typical dune nor heath species occurred frequently in this site and the assemblage mainly consist of eurytopic species (e.g. *Nebria salina*, *Notiophilus palustris*) (Turin, 2000).

From a carabid beetle conservation point of view, the application of sod-cutting and mowing therefore present two important habitats for specialised carabid beetles. Thereby sod-cutting creates secondary, highly dynamical (e.g. sand blow) habitats similar to younger and more pristine successional stages. In contrast, mowing seemed to preserve a typical (*Calluna*) heathland carabid fauna which found suitable habitat conditions shortly after application. This should mainly be caused by the fact, that the topsoil is not affected and torphobiont species (such as *B. ruficollis*) are still able to find approbiate habitat conditions which seemed not to be true for the choppered site.

By applying management for nature conservation one has always to keep in mind the costs: While sod-cutting (or topsoil removal in general) is extremely expensive (e.g. Klimkowska, in press), mowing have relatively low costs. For the conservation of heath carabid beetles species mowing seems to be an appropriate management scheme and should be preferred compared to choppering which is more expensive. But of course the future perspective and the vegetation development of these sites are of outstanding importance. If nutrient loads in heathland habitats became to high, mowing might be an unsuitable measure and e.g. choppering might be more successfull.

In conlusion, the use of different management measures have a great short-term effect on carabid beetles. While sod-cutting creates a highly dynamic habitat important for several and often threatened dune species, mowing preserve a typical heathland carabid fauna. Choppering seemd to be of low relevance on a short-term basis, because of the quasi absence of dune and heath species. The use of different managements can led to a more heterogenous heathland which might be important not only for carabid beetles but for several arthropods and might enhance biodiversity in general.

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REFERENCES

[1] Barker, C.G., Power, S.A., Bell, J.N.B, Orme, C.D.L. (2004): Effects of habitat management on heathland response to atmospheric nitrogen deposition. – Biological Conservervation 120: 41-52.

[2] Buchholz, S. (2010): Ground spider assemblages as indicators for habitat structure in inland sand ecosystems. – Biodiversity and Conservation. DOI: 10.1007/s10531-010-9860-7.

[3] Buchholz, S., Hannig, K., Schirmel, J. (2009): Ground beetle assemblages of peat bog remnants in Northwest Germany (Coleoptera: Carabidae). – Entomologia generalis 32: 127-144.

[4] Burnham, K.P., Overton, W.S. (1978): Estimation of the size of a closed population when capture probabilities vary among animals. – Biometrika 65: 625-633.

[5] Chao, A. (1984): Nonparametric estimation of the number of classes in a population. – Scandinavian Journal of Statististics 11: 265-270.

[6] Chao, A. (2005): Species estimation and applications. – In: Balakrishnan, N., Read, C.B., Vidakovic, B. (eds.) Encyclopedia of Statistical Sciences, 2nd Edition, Vol. 12:7907-7916, Wiley, New York.
[7] Chao, A., Lee, S.M. (1992): Estimating the Number of Classes via Sample Coverage. – Journal of the American Statistical Association 87(417): 210-217.
[8] Den Boer, P.J., van Dijk, Th.S. (1994): Carabid beetles in a changing environment. – Wageningen Agriculture University Papers 94(6): 1-30.
[9] Gardner, S.M. (1991): Ground beetle (Coleoptera: Carabidae) communities on upland heath and their association with heathland flora. – Journal of Biogeography 18: 281-289.
[10] García, R.R., Jáuregui, B.M., García, U., Osoro, K., Celaya, R. (2009): Responses of arthropod fauna assemblages to goat grazing management in Northern Spanish heathlands. – Environmental Entomology 38(4): 985-994.
[11] Gimingham, C.H. (1972): Ecology of heathlands. – Chapman and Hall, London.
[12] Härdtle, W., Niemeyer, M., Niemeyer, T., Assmann, T., Fottner, S. (2006): Can management compensate for atmospheric nutrient deposition in heathland ecosystems? – Journal of Applied Ecology 43: 759-769.
[13] Heil, G.W., Diemont, W.H., (1983): Raised nutrient levels change heathland into grassland. – Vegetatio 53: 113-120.
[14] Irmler, U. (2004): Die Entwicklung der Carabidengemeinschaften während der Sukzession von Heide zu Wald. – Angewandte Carabidologie Supplement III: 17-25.
[15] Klimkowska, A., Dzierża, P., Brzezińska, K., Kotowski, W., Mędrzycki, P. (in press): Can we balance the high costs of nature restoration with the method of topsoil removal? Case study from Poland. – Journal for Nature Conservation.
[16] Leyer, I., Wesche, K. (2006): Multivariate Statistik in der Ökologie. – Springer, Berlin, Heidelberg.
[17] Magurran, A.E. (2004): Measuring Biological Diversity. – Blackwell, Oxford.
[18] Marrs, R.H., Le Duc, M.G. (2000): actors controlling vegetation change in long-term experiments designed to restore heathland in Breckland, UK. – Applied Vegetation Science 3:135-146.
[19] Maelfait, J.P., Desender, K. (1990): Possibilities of short-term Carabid sampling for site assessment studies. – In: Stork, N.E. (ed.) The role of ground beetles in ecological and environmental studies pp. 217-225. Intercept, Andover.
[20] Mossakowski, D., Främbs, H., Baro, A. (1990): Carabid beetles as indicators of habitat destruction caused by military tanks. – In: Stork, N.E. (ed.) The role of ground beetles in ecological and environmental studies pp. 237-243. Intercept, Andover.
[21] Möbus, G. (2000): Geologie der Insel Hiddensee (südliche Ostsee) in Vergangenheit und Gegenwart - eine Monographie. – Greifswalder Geowissenschaftliche Beiträge 8: 1-130.
[22] Müller-Motzfeld, G. (ed.) (2006): Band 2, Adephaga 1: Carabidae (Laufkäfer). – In: Freude, H., Harde, K.W., Lohse, G.A., Klausnitzer, B. Die Käfer Mitteleuropas. 2. Auflage. – Spektrum, Heidelberg, Berlin.
[23] Niemeyer, M., Niemeyer, T., Fottner, S., Härdtle, W., Mohamed, A. (2007): Impact of sod-cutting and choppering on nutrient budgets of dry heathlands. – Biological Conservation 134: 344-353.
[24] Reinhard, H. (1962): Klimatologie. Atlas der Bezirke Rostock, Schwerin und Neubrandenburg. – VEB Topographischer Dienst, Schwerin.
[25] Roem, W.J., Berendse, F. (2000): Soil activity and nutrient supply ratio as possible factors determinig changes in plant species diversity in grassland and heathland communities. – Biological Conservation 92: 151-161.
[26] Schirmel, J., Blindow, I., Fartmann, T. (2010): The necessity of habitat mosaics for Orthoptera (Caelifera, Ensifera) in dry heathlands. – European Journal of Entomology 107: 129-132.
[27] Webb, N.R. (1998): The traditional management of European heathlands. – Journal of Applied Ecology 35: 987-990.

[28] Umweltministerium Mecklenburg-Vorpommern (ed.) (2003): Die Naturschutzgebiete in Mecklenburg-Vorpommern. – Demmler, Schwerin.
[29] Usher, M.B. (1992): Management and diversity of arthropods in *Calluna* heathland. – Biodiversity and Conservation 1: 63-79.
[30] Usher, M.B., Thompson, D.B.A. (1993): Variation in the upland heathlands of Great Britain: conservation importance. – Biological Conservation 66: 69-81.

THE AGRO-ECOLOGICAL POTENTIAL OF HUNGARY AND ITS PROSPECTIVE DEVELOPMENT DUE TO CLIMATE CHANGE

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Abstract The climate change is one of the most relevant challenges that agriculture is facing in Hungary and all over the World. It is very difficult to express the agriculture related effects of the climate change in numbers and figures, since the atmosphere-soil-plant system is very complex. A crop simulation model was used for exploring the prospective effects of climate change on the agro-ecological potential of Hungary. The model was linked to a detailed meteorological and soil database of Hungary to provide the required input data. Simulations first employing measured meteorological data then combining them with a climate change scenario were used to determine the present and the prospective agro-ecological potential of Hungary. The simulation results indicate that the Hungarian agriculture can not avoid the effects of climate change, and unfortunately the majority of these effects would be negative. The yields of the spring crops will prospectively decrease while higher yields might be expected for the autumn crops. The amount of N-based green house gases emitted from the soil will prospectively increase because of the changes in the annual distribution of precipitation. On the basis of the simulation results the role of the autumn crops is likely to become more significant in Hungary. Another alternative for the Hungarian agriculture is to find new crops maybe species of Mediterranean origin that could be profitably grown here.
Keywords: *crop model, crop production, GHGs, nitrate leaching*

Introduction

The Carpathian basin is an important area of crop production in Europe. Around 10 million tonnes yields of different crops are produced here for eight countries, not counting the exports. The majority of the agricultural land in the basin belongs to Hungary. Unfortunately the animal husbandry gradually fell into the background in the past decades, yet concerning its plant production, Hungary is among the best on the world regarding the average yields of her main crops. This result could be primarily owed to the outstanding natural endowments of the country: the majority of soils naturally have high water storing capacity and high nutrient content (Kovács et al., 2005) and the climate is favourable to many agricultural crops. Considering the performance of crops production in Hungary it is not a negligible factor that farmers are supported by such advisory systems as the RISSAC-RIA cost-effective and environmentally friendly fertilizer recommendation system (Csathó et al., 1998) that was awarded with the Hungarian Innovation Grand Prize in 2008.

One of the most important questions that the Hungarian agriculture faces is whether this performance could be maintained in the future. More and more observations prove that the Middle-European climate is changing faster than in any other period in the past. Even the most cautious climate change scenarios predict more than 1 °C temperature increase (the average of different scenarios is 3 °C) combined with a decrease of precipitation by the end of this century. Using the method of spatial analogies (Adams et al., 1998) it is quite easy to find those parts of Europe whose present climate is similar to the future climate of the Carpathian basin. The analogous locations of the Carpathian basin for 2030, 2060 and 2090 could be found in Northern Bulgaria, Northern Greece and Northern Africa (Horváth, 2008). Since agriculture is very much dependent on the climate it is no small wonder that the pattern of agricultural land use is quite different for the present Carpathian basin and for its future analogue, Northern Greece. Winter wheat, maize, sunflower and rape are the basic crops of agriculture in Hungary. Around 85% of the agricultural land is covered with these crops. Will the present way of agriculture sustainable in the future or we need new, feasible but also profitable alternatives for sustainable agriculture in Hungary? Should the present pattern of land use be modified due to climate change and economic changes? There are two main conceptions about the future of the agriculture in Hungary:

- The food production and the processing industry are based mostly on the above mentioned four crops. Despite of the changes the climatic conditions for producing the present basic crops will be given, or even if the conditions became marginal it would be too difficult to change the whole system.
- Approaching the end of this century the climate of this area will become more and more Mediterranean which may open the door for many crops which can not be grown profitably here at present, while the conditions might become marginal for some of the currently dominant crops. For example cotton is grown at 15 % of the agricultural land in Northern Greece. The increase of the fossil fuel prices has turned the attention of many to energy crops. The share of energy consumption of biomass origin has shown a continuously growing figure in the near past. These are only two simple examples that might cause bigger changes in land use in the future in Hungary as well as in the Carpathian basin.

The agriculture related effects of climate change in Hungary would prospectively be mainly negative (Erdélyi, 2009). The climate change scenarios predict several degree increase of temperature by the end of the century combined with a 10% decrease of precipitation for the crucial summer period (Pongrácz et al., 2006). Along with this the probability of extreme weather events such as frost during autumn and spring is expected to increase (Mika et al., 2008). The lack of precipitation during summer, prospectively result in an increase of yield loss and crop fluctuation. New pests, weeds, plant diseases might appear on the scene and the death rate of pests is expected to decrease due to the milder winters. The increase of winter precipitation amount and the more intense rainstorms increase the risk of soil erosion.

It is very difficult to express the agriculture related effects of the climate change in numbers and figures, since the atmosphere-soil-plant system is very complex. On one hectare agricultural land 4 TJ solar radiation and 5-6000 tonnes of precipitation reach the soil surface within a year, and from the several 10 kgs of sown seeds over 20 tonnes of biomass develops. In the meanwhile innumerable faster and slower, important and less important processes take place that more or less influence each other. The crop simulation models were created to give an approximate description of this complex system.

The primary purpose of crop simulation models is to describe the processes of the very complex atmosphere–soil–plant system, including human activities, using mathematical tools (functions, differential equations, etc.) and to simulate them with the help of computers. The ultimate aim of using these models, however, is to answer such

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production and environment related questions that otherwise could only be answered by carrying out expensive and time-consuming experiments. The main advantage of the simulation models is that they are capable of exactly describing the processes within, and interactions between complex systems. As a matter of course the precondition of using models is that they have to be calibrated and validated using quality experimental data.

Many aspects of the potential effects due to the climate change in Hungary were investigated by several researchers (Ladányi and Hufnagel, 2006; Sipkay et al., 2008; Boksai and Erdélyi, 2009; Diós et al., 2009; Ladányi and Horváth, 2010). The main objective of this study is to enlarge the scope of the previous studies (Harnos, 1996; Kovács et al., 1998; Harnos, 2000) by exploring and estimating the prospective effects of climate change on the agro-ecological potential of Hungary in more detail, more accurately using more sophisticated instruments.

Materials and methods

Meteorological, soil and plant data used in the study

A database of the Hungarian Meteorological Service for the 2002-2006 period including daily maximum temperature, daily minimum temperature and daily precipitation covering the area of Hungary with an 1/6 degree resolution grid was used in the present study (*Fig. 1*).

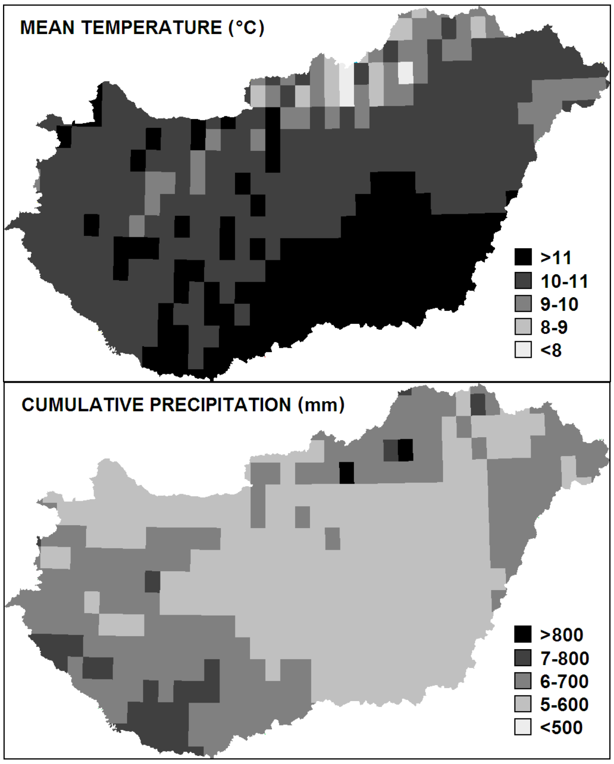

Figure 1. *The spatial distribution of average annual cumulative precipitation and mean temperature in Hungary, 2002-2006 (source: Hungarian Meteorological Service)*

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The database contains the data of 466 rectangles that are considered meteorologically homogenous. Despite of its shortness, the 2002-2006 period seems to be representative for Hungary regarding the average annual cumulative precipitation and the mean temperature as well as the distribution of the individual years within the period (*Table 1*). There is an average year, two years that are slightly under and above the average and two years that are considerably under and above the average regarding both investigated meteorological parameters. The national average of the annual precipitation totals is practically equal for the 2002-2006 period and the 1961-1990 reference period (*Table 1*). Extrapolating the linear temperature growth rate (0,76 ± 0,28 °C/100 year (α=0,05)) that was established based on the 1901-2004 period (Szalai *et al.*, 2005) the average temperature of the 2002-2006 period should be 10,25 ± 0,15 °C that is lower than the observed by a couple of tenth degrees (*Table 1*).

Table 1. *Some of the characteristic figures of the 2002-2006 period, compared to the 1961-1990 reference period for Hungary*

Year / period	(Average) annual cumulative precipitation, mm	Mean temperature, °C
2002	552	11.5
2003	469	10.6
2004	693	10.4
2005	737	10
2006	585	10.8
2002-2006	607	10.66
1961-1990	612	9.96

It could be attributed to the fact that this period was warmer than the average thus it can not be considered to be representative, or it could be ascribed to the direct manifestation of the climate change (local warming) and the linear trend of the temperature growth simply underestimate the actual growth rate at end of the 1901-2004 period. We make the latter likely since for the next 100 years even the most cautious climate change scenarios predict more than 1 °C temperature increase (Bartholy et al., 2006) that indicates that the increase of the average temperature is different from the linear, it is more like exponential. On the basis of all this we used the working hypothesis that the 2002-2006 period was representative for the present climate of Hungary.

The database of the Hungarian soils was created in the Research Institute of Soil Science and Agricultural Chemistry in the early 1990s (Várallyay et al., 1994). It is presented on *Fig. 2* in a form of a map just to demonstrate the diversity and the mosaic character of the Hungarian soils. The database categorizes the Hungarian soils into 22 soil types and characterise each of these types with a representative soil profile. The soil-physical and soil-chemical parameters as well as the water and nutrient balance characteristics of the profiles were measured in situ and were included in the database. This database was used in the study.

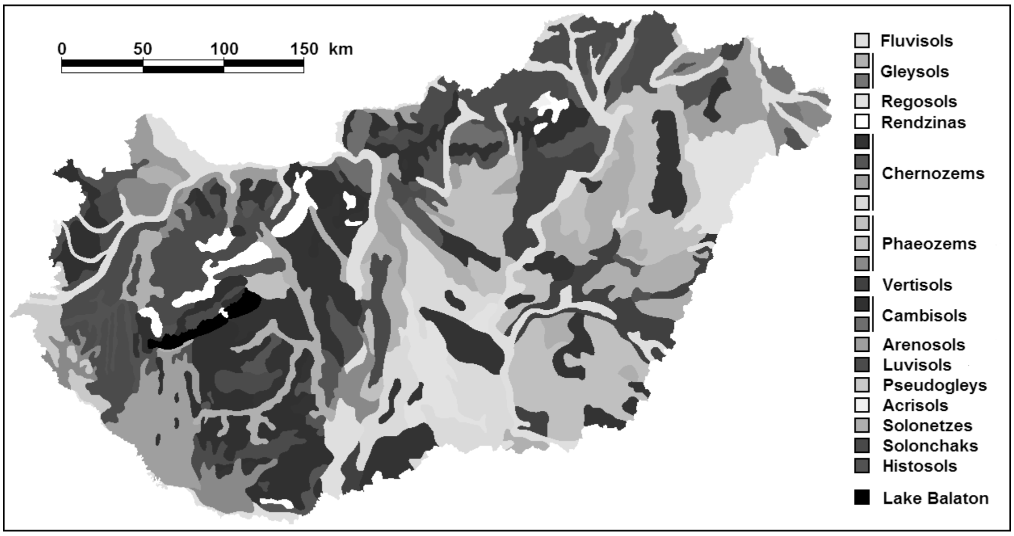

Figure 2. *FAO soil map of Hungary (source: Research Institute for Soil Science and Agricultural Chemistry, Hungary)*

The maximum yields of winter wheat, maize and sunflower in Hungary on a county level were extracted from the yearbooks of the Central Statistical Office for the 1982-2006 period (*Fig. 3*). It is obvious that even the yield of the best county in the best year can not be considered to be equal to the potential yield because none of the counties can be considered to be homogenous from pedological and meteorological point of view, to say nothing of the fact that many of the farmers can not afford to provide the ideal agricultural conditions for plant growth. Since the effect of climate change was the focus of this study it was practical to use the maxima of the yearly maximum yield levels as conditionally potential yields for each crop. Conditionally potential yield is defined as the maximum reachable yield level among the present climatic, pedological and agricultural circumstances.

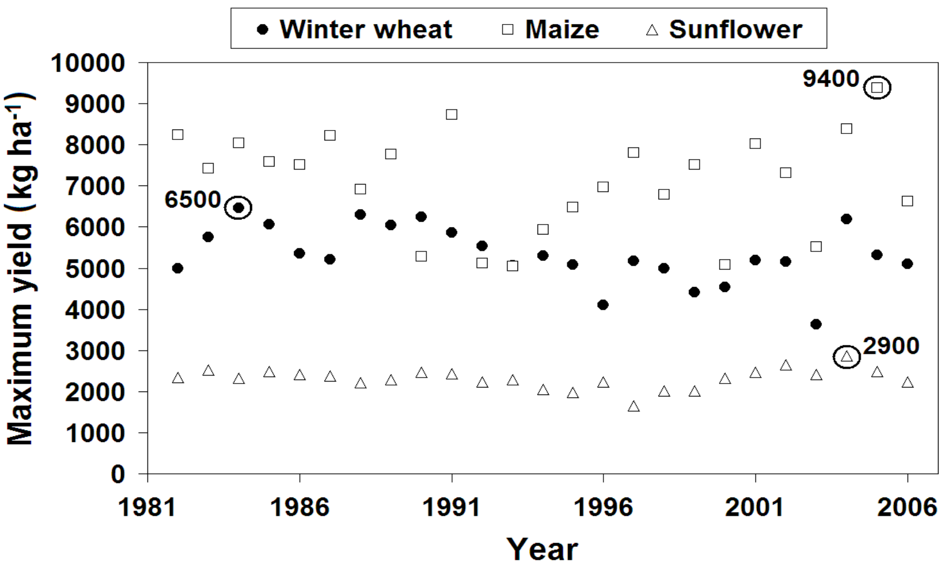

Figure 3. *Maximum yields of the three main crops of Hungary and their maxima in the period of 1982-2006, on a county level (source: Central Statistical Office, Hungary)*

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Methods and tools used in the study

The collected data were fed into the 4M crop simulation model (Fodor et al., 2002; Fodor and Kovács, 2003) so that some indices of the agro-ecological potential of Hungary and its prospective development due to climate change could be quantified. 4M is a CERES (Ritchie et al., 1998) clone. The source code of the CERES model (Ritchie et al., 1994) was used as a starting point for developing 4M. Several studies have proved CERES to be an effective crop model (Kovács et al., 1995; Jamieson et al., 1998). The entire FORTRAN code of CERES was rewritten in Delphi and a user-friendly interface was also developed for the model to ease handling input and output data. 4M inherited all the capabilities of CERES but was developed with several new subroutines and modules in the past years (Fodor, 2006; Fodor et al., 2009).

4M is a daily-step, deterministic (not stochastic) model whose functioning (computation) is determined by the numerical characteristics (parameters) of the atmosphere–soil–plants system. Besides the data that describe the physical, chemical and biological profile of the system, it is also necessary to set its initial, boundary and constraint conditions in the input file of the model. The parameters regulate the functions and equations of the model: the development and growth of plants or the heat, water and nutrient balance of the soil. The initial conditions are the measured system variables at the beginning of the simulation run such as the water or nutrient content of the soil. The boundary conditions are primarily the daily meteorological data such as the global radiation, temperature and precipitation. The constraint conditions cover the numerical expressions of the human activities such as data about planting, harvest, fertilization or irrigation.

Without going into details the outlined functioning of 4M is the following. The model calculates (simulates) the plant growth and development determined by the meteorological, soil and agro-technical conditions given by the input data. It calculates how the plant goes through the different phenological stages, the amount of the new matter produced by the plant via photosynthesis, the partition of the assimilates among the organs, the leaf growth depending on the amount of new matter that arrives into the leaves, and finally the yield. Meanwhile the model also calculates the amount of water and nutrients extracted from the soil by the plant and as a result of this how the soil dries out and becomes poorer in nutrients. If the water and/or the nutrient content of the soil decrease under a predefined limit the plant growth slows down and in serious shortage the plant even dies. In parallel with these the simulation of the processes independent of plant growth also takes place. The model calculates how the precipitation infiltrates into the soil, the amount of nitrate that percolates down under the root zone and the amount of the NO_x gases released from the soil due to denitrification: the former can contaminate the drinking water reservoirs by reaching the water table, while the latter are greenhouse gases.

Since all the required meteorological and soil data were available only the plant parameters were needed to be set. The plant parameters of the model were calibrated so that the simulated yields would be equal to the determined conditionally potential yields (*Fig. 3*) for each investigated crop switching off the effect of any kind of stress factor in the model. In other words we adjusted the crops specific input parameters till the simulated and observed conditionally potential yields were practically the same.

Only the most basic agro-technical applications were taken into account during the simulations (*Table 2*). The amount of fertilizer required by the plants was calculated by using the Cost-Saving and Environmentally Friendly Fertilizer Recommendation System of the RISSAC and RIA institutes (Csathó et al., 1998).

After feeding all the input data into the 4M model the 2002-2006 period was simulated for every meteorologically and pedologically different cell of Hungary as if winter wheat, maize or sunflower was grown on that area. This meant 1311 simulation runs for each crops, since the intersect of the maps of *Fig. 1* and *Fig. 2*, the 466 meteorologically distinguished cells and the 18 distinguished soil groups resulted in this many combinations for Hungary. It has to be noted that four of the 22 FAO soil groups that is presented in Hungary were excluded from the study since on these areas (e.g. Gleysols) there is no reason for the existence of simulating crop production not even from theoretical point of view. These soils add up to an insignificant proportion of the country area and are denoted with black colour on the result maps.

Table 2. *Characteristics of the agro-technical applications that were taken into account during the simulations.*

Crop	Planting date (day/month)	Harvest date (day/month)	Fertilization date (day/month)	N dose [kg ha^{-1}]
Winter wheat	10/10	10/07	25/09+05/03	30+60
Maize	25/04	30/09	05/04	100
Sunflower	15/04	20/09	05/04	50

The calculated 5 year average yields, denitrification and nitrate leaching were recorded in every simulation and were represented on maps. Three categories were displayed on each map: under the average, average and above the average areas. The lower and upper limits of the average category were determined by extracting and adding the standard deviation from and to the 5 year country average. Since the simulated denitrification values showed a quite skewed distribution, the median was used instead of the arithmetic mean in this case. Using the simulation results an estimation could be given for the agro-ecological potential of Hungary taking the most important limiting factors such as the climate and the soil into account.

The prospective development in the next hundred years of the above determined potentials due to the climate change was also investigated. The 4M model enables the user to systematically alter the original weather data that is representing the present climate, according to a scenario taking the effect of climate change into account during the simulations. A scenario can be visualized as a table that sets the amount and the mathematical operation for the change of the meteorological data (*Table 3*).

Table 3. *The prospective scale of climate change by 2100 in Hungary*

Parameter	Mathematical operation	Winter	Spring	Summer	Autumn
Temperature (°C)	Add	3.2	2.3	2.8	2.7
Precipitation (mm)	Multiply	1.11	1.04	0.91	0.99
CO_2 (mg kg^{-1})	Fix value	557			

The actual values in the scenario (*Table 3*) were set based upon the studies of Hungarian climate change researchers (Pongrácz et al., 2006; Haszpra, 2008) and represent the simple arithmetic mean of several existing prognoses for each meteorological variable.

The effect of the predicted increase of the possibility of frosts during spring and autumn (Mika et al., 2008) as well as the prognosticated increase of rainstorm events during summer (Bartholy et al., 2006) was ignored in the simulations. It was also postulated that there will be no significant changes in the soil conditions as well as in the plant production. It has to be noted the probable achievements of plant breeders in improving more drought resistant cultivars was not taken into account either. This was beyond the scope of this study. However the meteorological data of the 2002-2006 period was used in the study, on the maps presenting the results, for the shake of simplicity, year 2000 and 2100 were indicated denoting that the specific results related to the present or the future climatic conditions.

Results

Being a countrywide investigation, the amount of NO_x gases that is emitted from the agricultural areas of Hungary (approx. 45 000 km^2) could be estimated. An average of 1 650 000 kg of N based greenhouse gases of agricultural origin are emitted to the atmosphere in Hungary in a year and this figure is expected to slightly increase by the end of this century independently of the kind of the produced crops (*Fig. 5*).

It is fully visible on the yield maps (*Fig. 4a* and *Fig. 4b*) that both the climatic and the soil conditions are responsible for the development of the winter wheat and maize yields: the mosaic-like pattern coming from both the climate and soil database (*Fig. 1* and *Fig. 2*) is identifiable on the corresponding maps. This characteristic prospectively will not change due to the climate change. On the sunflower yield maps, however, only the pattern of the meteorological cells (*Fig. 1*) appears indicating that the yield development of this crop is determined by the climate in the first place, which can be explained with the greater water demand of the sunflower. Based on the simulation results the sunflower will be an explicit 'loser' due to the climate change: the expected yields are shifted from the 'above average' category to the 'average' while the average yields are shifted to the 'below average' category (*Fig. 4c*).

It is not so obvious to see the prospective effect of climate change for maize, though an 6-7% decrease of the average yield is expected on country level. There could be smaller areas at the central and the north-western parts of Hungary where an increase of the maize yields could be expected while an explicit decrease of yields could be expected at the eastern territories of the country (*Fig. 4b*). In case of both crops the dryer and hotter summers resulting in greater water and heat stresses could be hold responsible for the yield loss. This negative effect could not be counterbalanced by the increased CO_2 promoted photosynthesis.

The probable reaction of winter wheat is just contrary to that of the sunflower. The present 'below average' yields practically disappear due to the climate change while higher than the present average yields could be expected at the majority of the country area by the end of this century (*Fig. 4a*). It is probably due to the increased precipitation amount during the winter period (*Table 3*) which creates more advantageous conditions for the development in spring. The less summer precipitation does not really decrease the yield since the growing season of the winter wheat ends at the end of June or at the beginning of July even today and the shortening of the growing seasons is also expected (Erdélyi, 2008) due to the climate change.

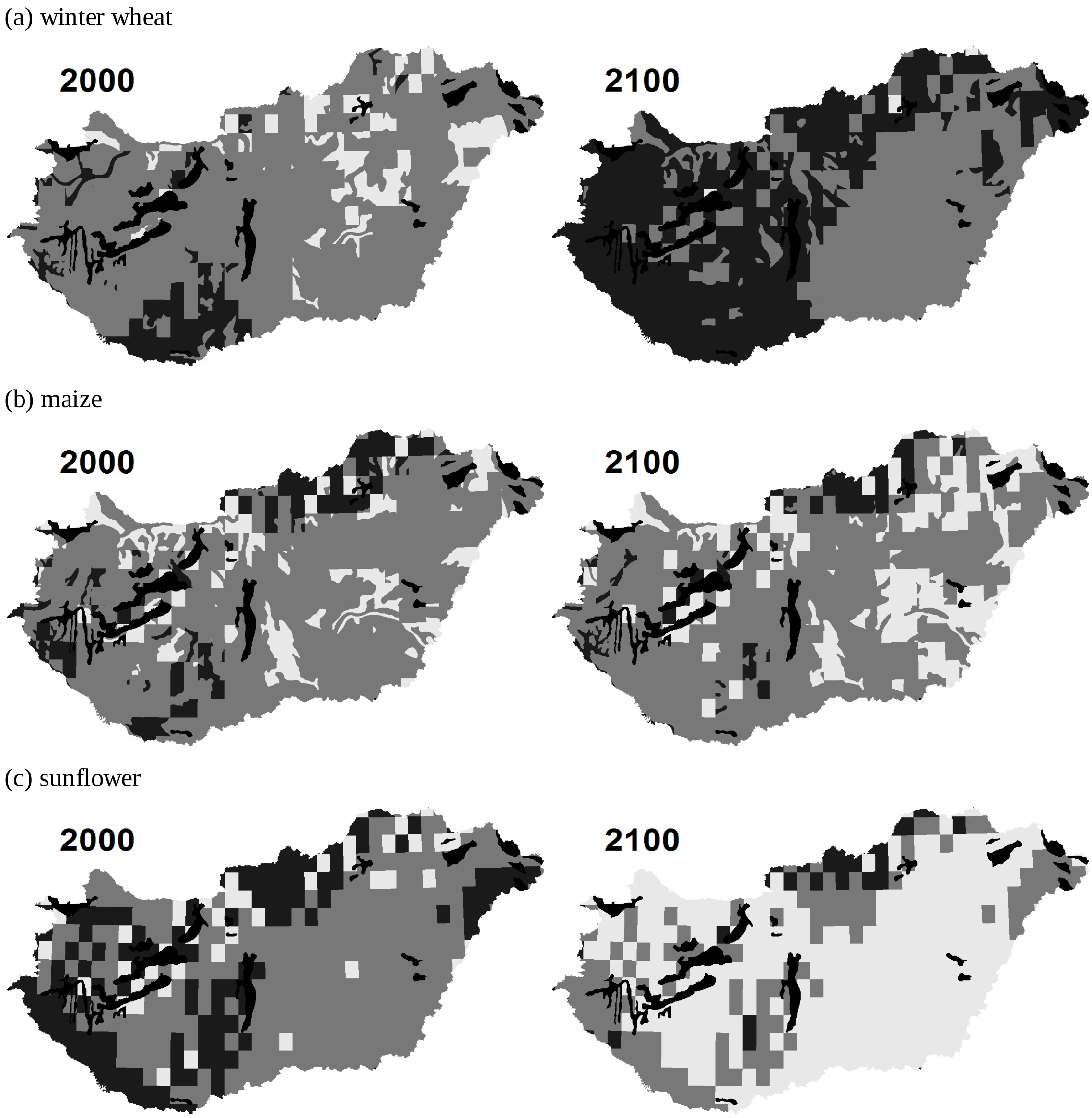

Figure 4. *The spatial distribution of the present (2000) and the expected future (2100) yields in Hungary for the three main crops. The grey colour denotes the present average yields (winter wheat: 3300-4300 kg ha^{-1}, maize: 4500-6500 kg ha^{-1}, sunflower: 2500-3000 kg ha^{-1}), while light and dark grey represent the 'below average' and the 'above average' yields respectively*

It is distinctly visible on the maps presenting the denitrification (NO_x emission) rates (*Fig. 5*) that the soils having high clay content at the eastern part of Hungary produce 'above average' NO_x emission rates in the first place. The quantity of this is expected to increase due to the climate change especially in the case of growing maize and sunflower. The increase of precipitation amounts in the winter-spring period might be responsible for this when the soil is not or scarcely covered with crops thus the plant water uptake is negligible that results in higher soil water content and an increase of denitrification rates. This phenomenon is not so stressed in case of growing winter wheat since its growing season includes the whole winter-spring period.

A similar trend can be seen on the nitrate leaching maps (*Fig. 6*) due to the climate change. The risk of nitrate leaching is expected to rise as a consequence of the

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increasing precipitation amounts during the winter and spring quarters (*Table 3*). Another factor that might contribute to the probable nitrate leaching increase is that the plants prospectively won't take up as much nitrogen as today as they develop lower yields. Since possible changes in fertilization practices were not taken into account in the simulations more and more nitrogen could be remained in the sol during the consecutive growing seasons further increasing the risk of nitrate leaching. This tendency can be observed in the case of winter wheat only to a small degree. Wheat takes up water as well as nitrogen during winter and spring that might be an explanation for this.

(a) winter wheat

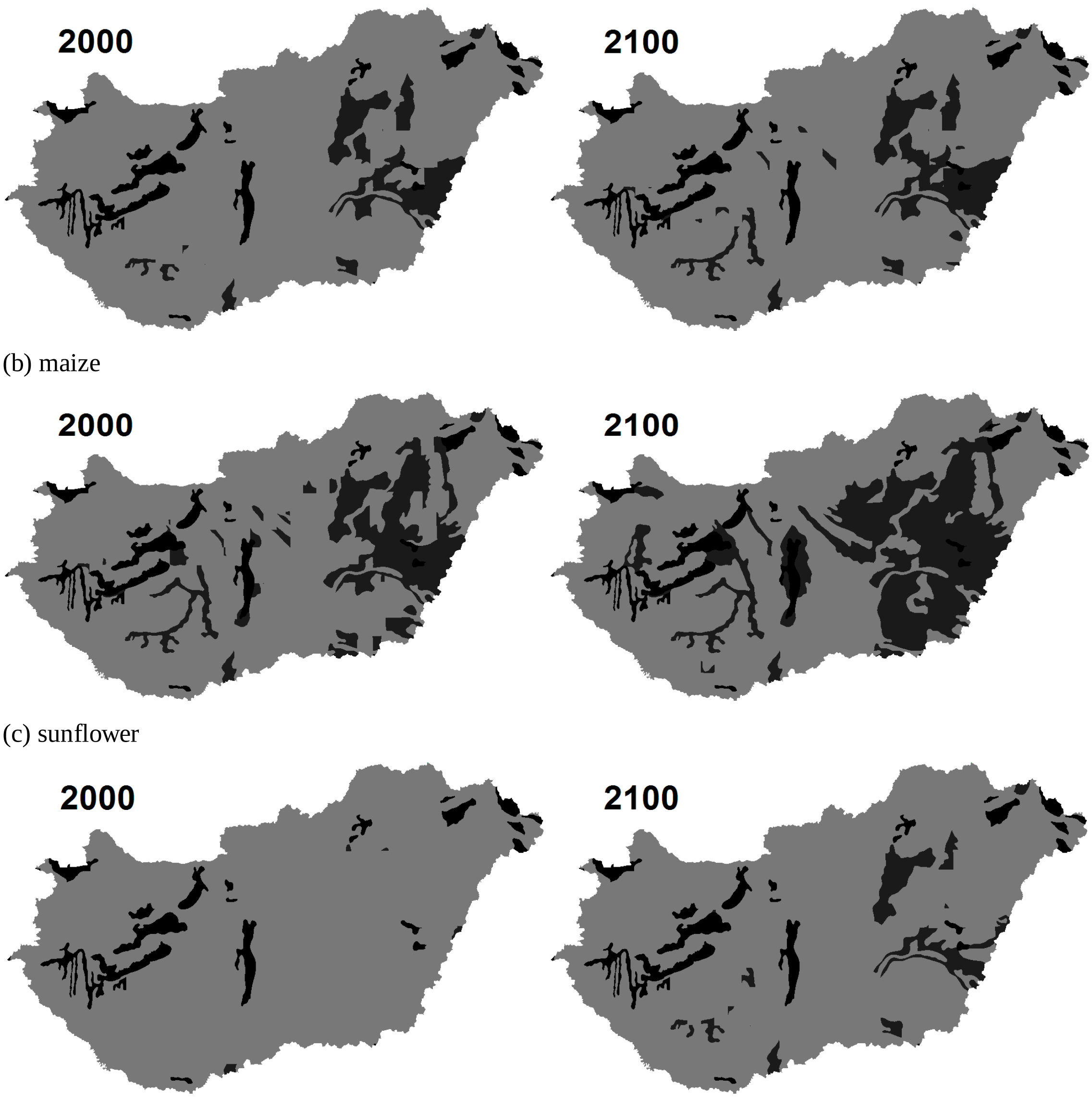

Figure 5. *The spatial distribution of the present (2000) and the expected future (2100)* NO_x *emission in Hungary growing winter wheat (a), maize (b) and sunflower (c). The grey colour denotes the present average emission rates (0-2000 kg* km^{-2} y^{-1}*), while dark grey represents the 'above average' emission rates*

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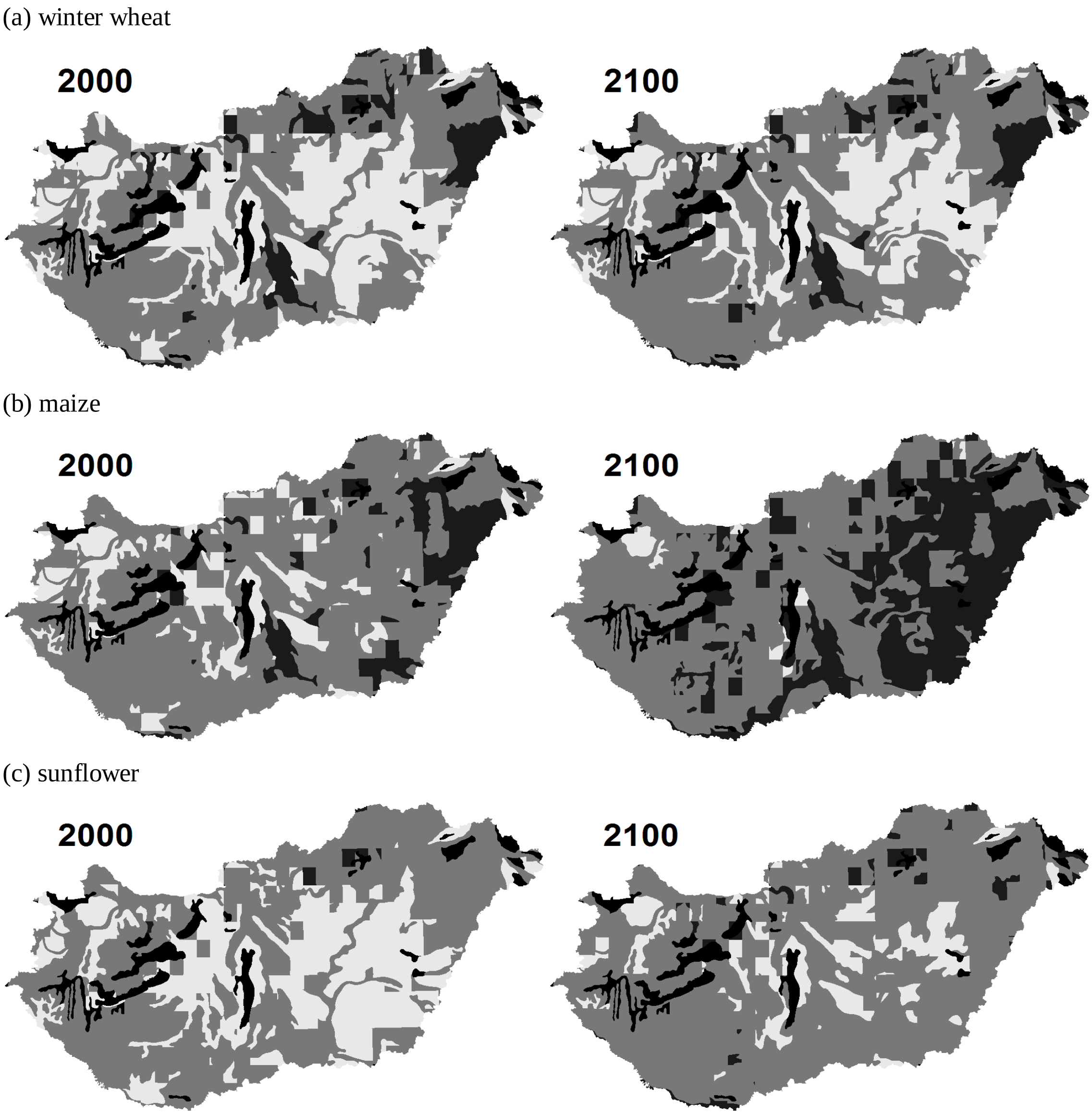

Figure 6. *The spatial distribution of the present (2000) and the expected future (2100) nitrate leaching rates in Hungary growing winter wheat (a), maize (b) and sunflower (c). The grey colour denotes the present average nitrate leaching rates (1000-3000 kg km^{-2} y^{-1}), while light and dark grey represent the 'below average' and the 'above average' leaching rates respectively*

Summary and conclusions

The 4M crop simulation model was used to quantify some indices of the agro-ecological potential of Hungary and its prospective development due to climate change. The simulation results indicate that the Hungarian agriculture can not avoid the effects of climate change, and unfortunately the majority of these effects would be negative. The yields of the spring crops (maize, sunflower, etc.) will prospectively decrease while higher yields might be expected for the autumn crops like winter wheat. The selection of the suitable variety to sow might become particularly important. The need for drought resistant varieties – especially for sunflower - might be the most important

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challenge for plant breeders hoping that this race would be won by the man and not by the nature. The feasibility of irrigation should be investigated from economic and as a matter of course from environmental protection point of view, since yield loss of spring crops could be attributed to the dryer summers in the first place. The amount of N-based green house gases emitted from the soil will prospectively increase because of the changes in the annual distribution of precipitation. The growing of spring crops might imply an additional environmental protection risk due to the prospectively increasing nitrate leaching rates. This effect might be mitigated by rational and properly timed fertilization. Considering the prospectively growing yields and the decreasing environmental protection risks the role of the autumn crops is likely to become more significant in Hungary. Another alternative for the Hungarian agriculture is to start testing and - in case of getting positive results - start growing alternative crops such as energy crops like robinia (*Robinia pseudoacacia*), poplar (*Populus*), etc. or crops native or successfully grown at Mediterranean areas such as fenugreek (*Trigonella foenum-graecum L.*), lady's thistle (*Silybum marianum (L.) Gaernt.*) or cotton (*Gossypium*).

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REFERENCES

[1] Adams, R.M., Brian, H., Lenhart, S., Leary, N. (1998): Effects of global climate change on agriculture: an interpretative review. – Climate Research 11: 19-30.

[2] Bartholy, J., Pongrácz, R. (2006): Comparing tendencies of some temperature related extreme indices on global and regional scales. – Időjárás 110: 35-48.

[3] Boksai, D., Erdélyi, É. (2009): Importance and possibilities of maize production of Hungary in the future. – In: Mihailovic, Miloradov (ed.) Environmental, Health And Humanity Issues In The Down Danubian Region: Multidisciplinary Approaches, World Scientific Publishing Company, Singapure, 297-307.

[4] Csathó, P., Árendás, T., Németh, T. (1998): New, environmentally friendly fertiliser advisory system, based on the data set of the Hungarian long-term field trials set up between 1960 and 1995. – Communic. in Soil Science and Plant Analysis 29: 2161-2174.

[5] Diós, N., Szenteleki, K., Ferenczy, A., Petrányi, G., Hufnagel, L. (2009): A climate profile indicator based comparative analysis of climate change scenarios with regard to maize (Zea Mays L.) cultures. – Applied Ecology and Environmental Research 7(3): 199-214.

[6] Erdélyi, É. (2008): The potential impacts of climate change on main field crops and their yields, case studies in Hungary. – "Klíma-21" Füzetek 55: 53-79. (English Spec. Edition)

[7] Erdélyi, É. (2009): Sensitivity to Climate Change with Respect to Agriculture Production in Hungary (2009). – Precision Agriculture '09. van Henten, E.J., Goense. D., Lokhorst, C. (ed.) Wageningen Academic Publisher, 559-567.

[8] Fodor, N., Máthéné-Gáspár, G., Pokovai, K., Kovács, G.J. (2002): 4M – software package for modelling cropping systems. – European J. of Agr. 18: 389-393.

[9] Fodor, N., Kovács, G.J. (2003): Sensitivity of 4M maize model to inaccuracy of weather and soil input data. – Applied Ecology and Environmental Research 1(1-2): 75-85.

[10] Fodor, N. (2006): 4M – software for modelling and analysing cropping systems – Journal of Universal Computer Science 12: 1196-1207.

[11] Fodor, N., Máthéné-Gáspár, G., Németh, T. (2009): Modeling the nutrient balance of the soil-plant system using the 4M simulation model. – XI. ISSPA conference, Santa Barbara, CA, USA, July 18-22, 2009.

[12] Haszpra, L. (2008): Hungarian Meteorological Service Measurement and analysis of trends of atmospheric GHGs in Hungary. – IPCC outreach event ”Climate change 2007: implications for Hungary” Key insights from the IPCC’s fourth assessment report. April 10-11, CEU, Budapest, Hungary. http://web.ceu.hu/envsci/projects/IPCCOutreach/Folders/Presentations/Haszpra_Laszlo_Budapest_10_April_2008.pdf (visited on 28/01/2009)

[13] Harnos, N. (2000): Analyzing the prospective effects of climate change on winter wheat production using simulation models. – Növénytermelés 50: 41-55. (in Hungarian)

[14] Harnos, Zs. (1996): Modelling crop response in Hungary. – In: Harrison, P.A., Butterfield, R.E., Cowning, T.E. (eds.) Climate Change, Climatic Variability and Agriculture in Europe, University of Oxford, pp: 179-189.

[15] Horváth, L. (2008): Use of spatial analogy method to analyse to possible land use change in Hungary. – “CLIMA-21" Brochures 55: 23-27.

[16] Jamieson, P.D., Porter, J.R., Goudriaan, J., Ritchie, J.T., van Keulen, H., Stol.,W. (1998): A comparison of the models AFRCWHEAT2, CERES-Wheat, Sirius, SUCROS2, and SWHEAT with measurements from wheat grown under drought. – Field Crop Research 55: 23-44.

[17] Kovács, G.J., Németh, T., Ritchie, J.T. (1995): Testing Simulation Models for the Assessment of Crop Production and Nitrate Leaching in Hungary. – Agricultural Systems 49: 385-397.

[18] Kovács, G.J., Dunkel, Z. (1998): The prospective effects of climate change in the Hungarian agriculture in the next 50 year. – In: Dunkel, Z. (ed) Climate change and its effects, HMS, Budapest, Hungary, pp. 181-193. (in Hungarian)

[19] Kovács, G.J., Csathó, P. (2005): Agricultural nutrient balances of Hungary between 1901 and 2003. – Research Institute for Soil Science and Agricultural Chemistry, Budapest. (in Hungarian)

[20] Ladányi, M., Hufnagel, L. (2006): The effect of climate change on the population of sycamore lace bug (Corythuca Ciliata, Say, Tingidae Heteroptera) based on a simulation model with phenological response. – Applied Ecology and Environmental Research 4(2): 85-112.

[21] Ladányi, M., Horváth, L. (2010): A review of the potential climate change impact on insect populations – general and agricultural aspects. – Applied Ecology and Environmental Research 8(2): 143-152.

[22] Mika, J., Lakatos, M. (2008): Extreme weather tendencies in Hungary: One empirical and two model approaches. – In: Sigro, J., Brunet, M., Aguilar, E. (ed) Regional Climatic Change and its Impacts, Tarragona, Spain, pp. 521-531.

[23] Pongrácz, R., Bartholy, J., Torma, Cs., Hunyady, A. (2006): Producing climatic scenarios using circulation models. – In: Current environmental problems – from global to local: vulnerability and adaptation, Pannon University, Georgikon Faculty of Agriculture, Keszthely, Hungary, pp. 6. (in Hungarian)

[24] Ritchie, J.T., Singh, U., Godwin, D.C., Humpries, J. (1994): CERES cereal generic model FORTRAN source code. – Michigan State University, East-Lansing, MI.

[25] Ritchie, J.T., Singh, U., Godwin, D.C., Bowen, W.T. (1998): Cereal growth, development and yield. – In: Tsuji, G.Y., Hoogenboom, G., Thornton, P.K. (ed) Understanding Options for Agricultural Production, Kluwer Academic Publishers, The Netherlands, pp 79-98.

[26] Sipkay, Cs., Horváth, L., Nosek, J., Oertel, N., Vadadi-Fülöp, Cs., Farkas, E., Drégelyi-Kiss, Á., Hufnagel, L. (2008): Analysis of climate change scenarios based on modeling of seasonal dynamics of a Danubian copepod species. – Applied Ecology and Environmental Research 6(4): 101-109.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

[27] Szalai, S., Konkolyné, B.Z., Lakatos, M., Szentimrey, T. (2005): Some climatic characteristics of Hungary from 1901 to 2004. – Hungarian Meteorological Service, Budapest. (in Hungarian)
[28] Várallyay, Gy., Szabó, J., Pásztor, L., Michéli, E. (1994): SOTER (Soil and Terrain Digital Database) 1:500000 and its application in Hungary. – Agrokémia és Talajtan 43: 87-108. (in Hungarian)

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POST-IMPACT ASSESSMENT OF OIL SPILLAGE ON WATER CHARACTERISATION

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Abstract. The coastal waters from an oil polluted area in Nigeria were examined for some physico-chemical parameters and heavy metals. The impact of seasonal changes on water qualities was also investigated. The nutrient levels particularly during the dry season are eutrophic. The trends were such that apart from electrical conductivity, total dissolved solids and Zn where averagely <10% , < 17% and none respectively exceeded the WHO guidelines for water at both seasons, other parameters exceeded the guidelines greatly. Additionally, levels obtained rendered the water unsuitable for both recreational and agricultural purposes based on USEPA guidelines. Significant correlations ($p < 0.01$) were observed between the levels obtained between the two seasons. However, only with pH was any significant difference in the mean between the two seasons recorded. The anthropogenic input of oil in the form of spill was observed to influence greatly some water qualities. Thus, not only is the water unfit for drinking but also unsuitable for livestock and agricultural activities which are the mainstay of the inhabitants.
Keywords: *Contamination, oil spill, pollution, toxicity, water quality parameter.*

Introduction

Pollution is an unwanted and detrimental change in a natural system. It is associated with the presence of toxic substances. The introduction of oil into the ocean via oil spill is one major way by which the natural coastal area of Ondo State, Nigeria, is getting polluted. Oil pollution is a serious obstacle to food chain productivity of the sea (Kavitha et al., 1999). Interestingly, a significant quantity of petroleum, estimated at 0.2million tons a years, enters the sea without any help from humans, through natural seeps and continental margin. Thus, oil is a natural constituent of the marine environment (Thomas and William, 2004). Apart from the physico-chemical damage that accompanied oil when released into the ocean, some levels of metals are equally released (Thomas and Kathleen, 1998).

Metals are usually found as ions and complex compounds in the hydrosphere and concentration of metal species in various types of water cover a wide range. The presence of toxic metal such as Pb, Cd, Ni, Cr etc. in the environment, particularly in water has been a source of worry to environmentalist, government agencies and health practitioners (Kakulu and Obibanjo, 1992; Anmar et al., 1993; Fatoki and Awofolu, 2003; Eddy and Ekop, 2007). The heavy metals have been referred to as economic pollutants, which are widely distributed in the environment (O'Neil, 1993).

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The major sources of heavy metals in water especially in the oil producing area (like the study area in this study) has been reported to include crude oil, drilling fluids or mud and production water naturally occurring in the production reservoir. These metals include V, Ni, Co, Cd, Pb, Zn, Mn, Cr and Ti (Thomas and Kathleen, 1998).

The metals in the aquatic environment are of environmental interest and importance because of their interactions with solid phase materials of geological origin and also because of their influence on biological processes. Within the aquatic system, geological weathering and dissolution of aerosol particles from the atmosphere are some of the natural sources of these metals (Gary and Stephen, 2001).

Few people are fully aware of their daily dependence upon crude oil. Awareness is growing as the fragilities of modern civilization, stemming from population growth and economic expansion become recognizable not only as world and natural problem but also a personal problem. However, as human dependence on crude-oil increases especially in a country like Nigeria, the dangers that accompanied it also increase. In spite of the growing concern by private individuals who are environmentally conscious about the associated problems with oil spillage based on global disaster records, the government especially at the state level seems adamant about this silently killing oil-containing pollutant.

The water within the present study area remains the major source of livelihood for the inhabitants. In view of the health concerns alerted by the magnitude of the problems afflicting the inhabitants within most of the communities within the studied area, especially the children, the present work is thus carried out partly to create awareness to the inhabitant and society at large and consequently recommend the urgent need for better understanding of the associated problems that oil spillage and other activities by oil companies within this zone had and still causing especially in organic, ionic and metal enrichments.

Study area

The study area for the work is the oil producing coastal region of Ondo State, Nigeria *(Fig. 1).* There have been several cases of oil spillage within these coastal communities as a result of the activities of oil companies operating within these zones. The same river leads to the Atlantic Ocean and to some other parts of the country which means that the pollution may have tremendous adverse effect on other parts of the country. The major occupation of the inhabitants are fishing and farming. Most members of these communities are not aware of the environmental impact of oil pollution and most have died due to their ignorance.

Materials and methods

Sampling and sample preservation

Sampling sites *(Table 1)* were chosen with the aim of collecting water samples at a place that truly represent the water body (Wilde et al., 1999).The global positioning system (GPS) was used for site identification to enhance future monitoring. Twenty three (23) representative sites were considered for the physico-chemical parameters so as to comply with the objectives of the study and easy access, out of which nine (9) strategic locations were studied for some heavy metals. The sampling sites were all

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located along the same river course and were averagely at a distance of 2 km from one another. Three reference locations were sampled within the same geographical location with similar ecological conditions. Sampling at each sites were in triplicate in order to ensure better precision. The sampling frequency chosen for this study was based on literature guidance (US EPA, 2004).

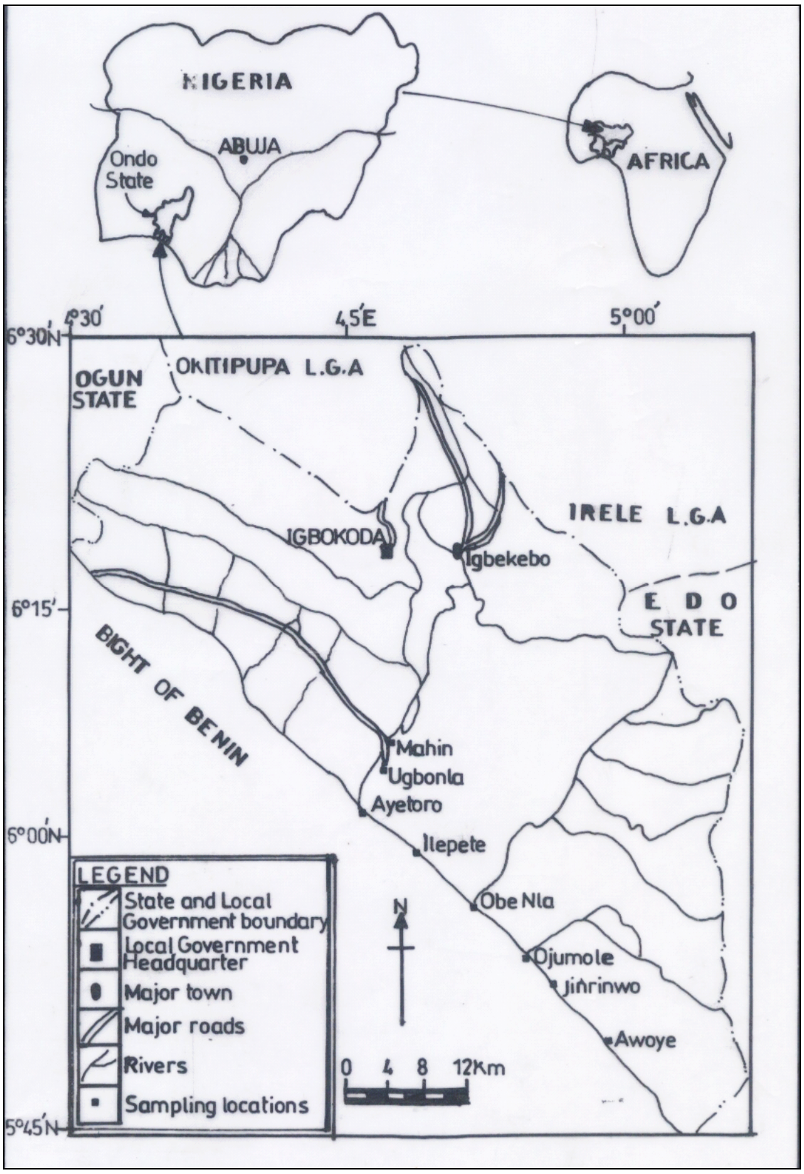


Figure 1. *Map of sampling locations*
(Inserted is the area map of Nigeria and Africa showing the geographical locations)

Table 1. *Site identification and coordinates. Igbokoda 1, 2 and 3 represent control locations*

	Abbreviation	Names of communities	Latitude (degree)	Longitude (degree)	Altitude (meters)
1	A	Ayetoro (town)	06°06’12.4”	004°46’36.0”	12
2	B	Idi ogba	06°05’56.1”	004°47’13.2”	14
3	C	Alagbin zion	06°04’48.8”	004°47’14.9”	14
4	D	Oroto	06°04’22.3”	004°48’53.7”	14
5	E	Asumaga	06°03’20.9”	004°39’58.9”	12
6	F	Ilowo	06°03’15.6”	004°50’10.1”	11
7	G	Ilepete	06°02’10.0”	004°51’23.3”	14
8	H	Obeadun	06°01’35.5”	004°51’57.5”	16
9	I	Obe Nla	06°00’51.9”	004°52’40.2”	16
10	J	Erebino	05°59’51.3”	004°53’37.1”	15
11	K	Ikorigbo	05°57’15.0”	004°53’59.6”	10
12	L	Obe iji	05°59’16.9”	004°54’09.8”	9
13	M	Obereweje	05°58’55.6”	004°54’27.2”	13
14	N	Obebowoto	05°56’52.4”	004°54’34.4”	13
15	O	Ojumole	05°56’05.4”	004°53’10.2”	15
16	P	Atlantic ocean	05°57’00.4”	004°55’34.7”	16
17	Q	Atlantic (inside)	05°56’59.8”	004°53’52.2”	16
18	R	Otumara	05°56’42.8”	004°55’55.8”	14
19	S	Odonla	05°56’24.5”	004°56’56.7”	13
20	T	Ilu abo	05°55’38.5”	004°56’44.7”	13
21	U	Jinrinwo	05°55’55.1”	004°57’28.7”	10
22	V	Odofado	05°55’18.3”	004°58’03.7”	12
23	W	Awoye	05°54’46.7”	004°57’56.2”	10
24	X	Igbokoda 1[a]	06°09’12.3”	004°44’32.4”	10
25	Z_1	Igbokoda 2[a]	06°09’12.4”	004°43’33.7”	13
26	Z_2	Igbokoda 3[a]	06°09’12.7”	004°44’32.5”	11

On collection, measurements were made of pH, temperature and conductivity using pre-calibrated pH meter (Hanna pH 211 microprocessor), thermometer and conductivity meter respectively. Samples were collected in polyethylene bottles cleansed with 50% HNO_3. Samples for anion analyses were unfiltered and unacidified. However, for cations analyses and metals, the samples were unfiltered but acidified with HNO_3 (Nickson et al., 2005). The essence of not filtering samples was to ensure accurate result. All samples were stored in the refrigerator at a temperature of about 4 °C prior to chemical analysis (Campolo et al., 2002; DWAF, 2002). As part of quality control, field blanks were introduced in the chain of custody.

Chemical analyses

Total solids (TS) and total dissolved solids (TDS) were determined using the standard method (APHA, 1989). Walkley and Black method of 1934 was adopted for the determination of total organic carbon (TOC). Chloride and sulphate were determined using the Morhs and turbidimetric standard methods respectively. The Cadmium reduction and ascorbic acid methods were respectively employed for nitrate and phosphate determination. Heavy metals in the water were analyzed after careful digestion with conc. HCl using AAS (Alpha 4AAS, Chemical Tech. Analytical, Euro). Blanks and standards were used as quality control measures. Detection limits (µg/l) were; Cd (0.002); Ni (0.05); Pb (0.004) and Zn (0.006). Statistical analysis using the SPSS for windows 13.0 versions was later employed for data presentations.

Results and discussion

Results

Table 2 and 3 present a summary statistics of some water characteristics and heavy metals during the dry and wet seasons. The results did show apparent seasonal

Table 2. *Summary statistics of some water physico-chemical properties#*

	pH	**EC (µS/cm)**	**TS (mg/l)**	**TDS (mg/l)**	**SS (mg/l)**
Range (mg/l)	4.1-6.9 (5.8-8.5)	2.2-35.4 (3.0-23.4)	209-3650 (148-2300)	150-2404 (112-1438)	59-1246 (86-612)
Mean (mg/l)	5.43 (6.89)	11.17 (12.05)	1176 (918)	760 (602)	419 (322)
S.D	0.81 (0.77)	7.3 (5.5)	691 (479)	496 (315)	314 (215)
C.V (%)	14.73 (10.00)	65.4 (46.1)	59 (52)	65 (52)	75 (67)
WHO	7.0-8.5	20	500	1000	Na
% Viol.	83(33)	8.3(12.5)	96(79)	16.7(8.3)	-
	Chl (mg/l)	**Sulp (mg/l)**	**Nit (mg/l)**	**Phosp (mg/l)**	**TOC (mg/l)**
Range (mg/l)	266-1012 (352-932)	65-605 (61-416)	40-400 (91-401)	73-703 (69-541)	8.9-41.7 (13.2-32.5)
Mean (mg/l)	664 (606)	235 (206)	164 (198)	302 (222)	20.3 (20)
S.D	216 (170)	169 (110)	101 (79)	190 (125)	9.4 (5.0)
C.V (%)	33 (28)	72 (53)	62 (40)	63 (56)	46.3 (25.0)
WHO	250	250	50	0.5	na
% Viol.	100(100)	46(46)	88(100)	100(100)	-

#: Concentrations are mean of triplicate analysis. Values in parenthesis are results of the wet season
C.V: Coefficient of variation; S.D: Standard deviation; na: Guidelines not available
Chl: Chloride; Sulp:Sulphate; Nit: Nitrate and Phosp: Phosphate;
%Viol: percent of concentration in violation of WHO standard guidelines for drinking water.
Sources: WHO (1993)

Table 3. *Descriptive basic statistics of the heavy metals contents# (mg/l) in the studied water*

Locations	Seasons	Cd	Ni	Pb	Zn
Ayetoro	D	1.01^{a} ±0.37	0.4^{a} ±0.1	14.58^{a} ±5.04	0.04^{a}±0.14
	W	$10.9^{b,c,d}$±0.14	$0.4^{a,b}$ ±0.3	$8.62^{b,c}$ ±2.64	0.04^{a}±0.03
Asumaga	D	0.19^{a}±0.14	<0.1	18.21^{b} ±189	0.09^{a}±0.31
	W	0.17^{a}±0.31	$0.6^{a,b}$ ±0.9	$6.16^{a\rightarrow d}$±1.26	0.05^{a}±0.01
Ilopete	D	$2.27^{a,b}$ ±0.14	0.5^{a} ±0.3	$12.19^{b,c}$ ±2.86	0.11^{a}±0.03
	W	$2.49^{h\rightarrow,j}$±0.23	$0.8^{a,b}$ ±1.2	4.09^{a} ±0.24	0.08^{a}±0.82
Obe nla	D	1.17^{a} ±0.04	2.3^{a} ±0.3	19.89^{a} ±1.64	0.11^{a}±0.12
	W	$1.72^{a\rightarrow d}$±0.72	2.7^{a} ±1.4	$6.19^{a\rightarrow d}$ ±0.59	0.09^{a}±1.12
Ikorigho	D	0.92^{a}±0.14	1.7^{a} ±0.9	19.96^{b} ±1.36	0.11^{a}±0.17
	W	$1.64^{,e\rightarrow d}$±1.31	$2.1^{e\rightarrow g}$ ±0.4	$7.19^{c,d,e}$ ±0.94	0.09^{a}±0.63
Ojumole	D	1.30^{a}±0.22	1.9^{a} ±0.9	20.53^{b} ±2.96	0.18^{a}±0.03
	W	$1.65^{d,e,f}$±1.24	$1.9^{e\rightarrow g}$ ±0.1	9.17^{f} ±1.31	0.11^{a}±0.24
Otumara	D	3.49^{a}±0.13	1.9^{a} ±0.7	42.35^{b} ±3.41	0.42^{a}±0.31
	W	3.26^{m}±0.27l	$1.8^{d\rightarrow g}$ ±0.4d	9.92^{f} ±2.13	0.22^{a}±0.14
Odofado	D	$1.96^{a,b}$ ±0.96	0.9^{a} ±0.2	22.39^{b} ±0.12	0.52^{a}±0.37
	W	$2.16^{f\rightarrow j}$ ±1.26	$1.8^{d\rightarrow g}$ ±1.3	$9.14^{e,f}$ ±0.89	0.31^{a}±0.16
Awoye	D	2.28^{a}±0.13a	0.4^{a} ±0.5	21.40^{a} ±1.22	0.57^{a}±0.13
	W	$2.39^{g\text{-}k}$ ±0.97	$0.9^{a\rightarrow d}$ ±0.3	$9.40^{e,f}$ ±1.36	0.22^{a}±0.21
Igbokoda*	D	$0.04^{a,b}$±0.14	$1.02^{a,b}$±0.12	$12.21^{c\rightarrow f}$±1.02	0.09^{a}±0.32
	W	$1.10^{a,b}$±0.14	$1.45^{a,b}$±1.05	$9.65^{e,f}$ ±1.12	0.04^{a}±0.53
Mean ± S.D	D	1.52 ± 0.97	1.11± 0.78	20.37 ± 8.57	0.22 ± 0.20
	W	1.76 ± 0.87	1.44 ± 0.74	7.95 ± 1.94	0.13 ± 0.09
Coeff. of Var.	D	64	70	42	91
	W	49	51	24	69
Range		0.17 – 2.49	4.09 – 42.35	< 0.1 – 2.7	0.04 – 0.57
WHO Stand.		0.003	0.02	0.01	3.0
% Viol.		100	100	100	None

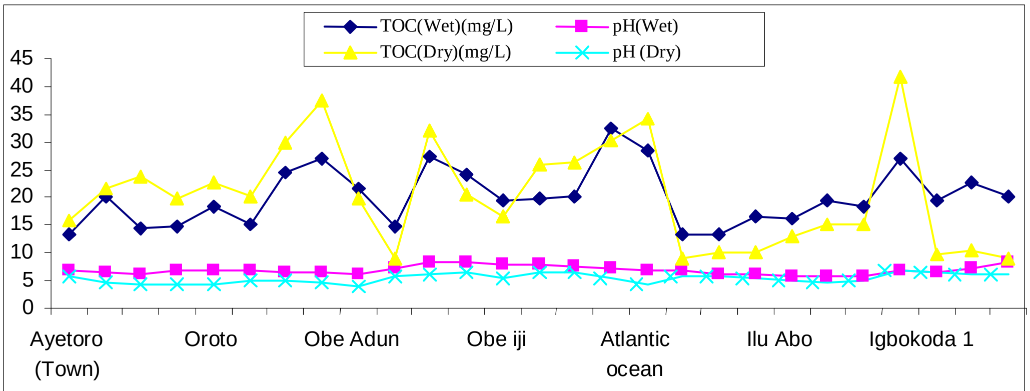
#: Concentrations are mean of triplicate analysis. Values in parenthesis are results of the wet season
*C.V: Coefficient of variation; *: Reference location; S.D: Standard deviation*
WHO Stand: World Health Organization Standards for drinking water (1993)
%Viol: percent of concentration in violation of WHO standard guidelines for drinking water.

Means in the same column followed by the same superscript are not significantly different (α = 0.05) according to Duncan's New Multiple Range Test differences. The temperature of the water samples ranged from 27.3 °C to 31.8 °C during the dry season. However, the temperature ranged from 25.9 °C to 28.7°C in the wet season. The ranges in pH and conductivity respectively were 4.1- 6.9 and 2.2-35.4μScm^{-1} for the dry season; 5.8-7.9 and 3.0-23.4μScm^{-1} for the wet season. Similarly, ranges (mg/l) during the dry and wet seasons respectively for other parameters are TS (209-3650 and 198-2300), TDS (150-2404 and 112-1438), TSS (51-1100 and 86-1140), Chloride (266-1012 and 401-769), sulphate (65-605 and 61-392), nitrate (40-400 and 91-401) phosphate (73-703 and 69-541) and total organic carbon (8.9-41.7 and 13.2-27.2). The metal displayed similar, increasing mean concentrations in the order Zn < Ni < Cd < Pb at both seasons. The highest and least variation were reported by Zn (CV: 91%) and (CV: 24%) respectively at both seasons.

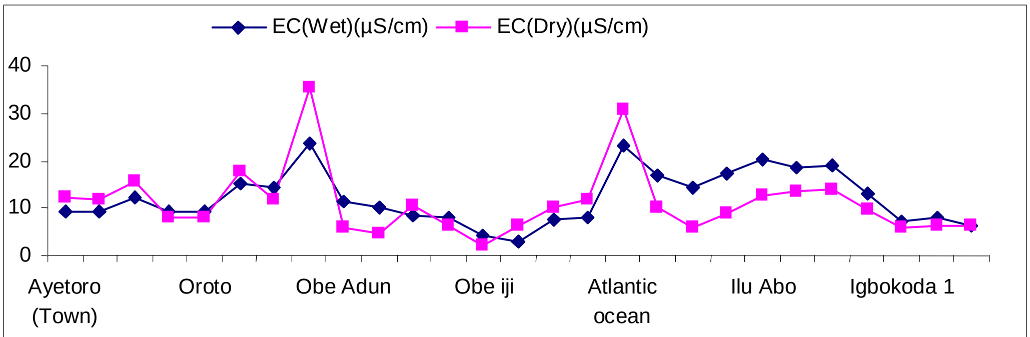
Water quality dynamics

The dynamics of some of the significant parameters, as obtained in the present study for both dry and wet seasons are shown in *Figures 2a* to *3b*. It is interesting to note the observed differences, in temporal dynamics of water quality between the different seasons. The figures indicate that water quality during the two seasons exhibits seasonal changes and distinct dry and wet periods can be identified for most parameters.

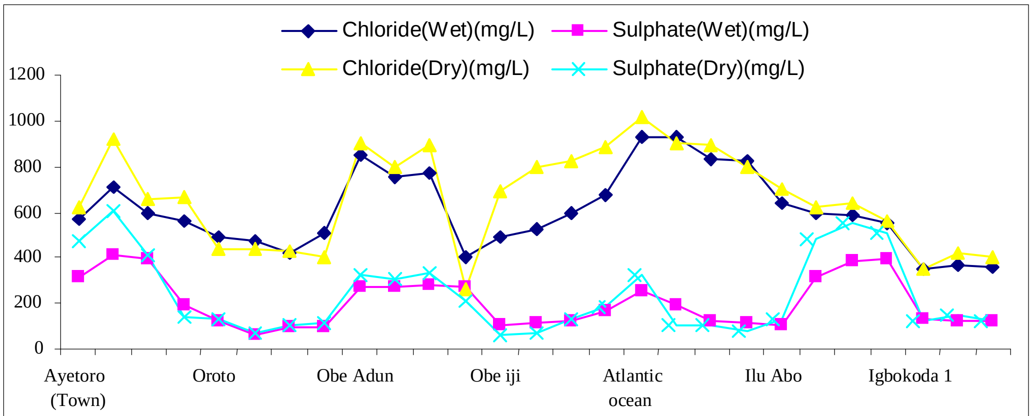
The colour of the water samples varied considerably between the two seasons and even with slight variation within some communities at the same season. In general, the colours varied from brown to light yellow. None was colourless at both seasons, a characteristic that is noted for impure water. The presence of colour at some of the locations, particularly during the dry season is indicative of greater organic and inorganic matter in suspension. This is reflective in the TOC obtained at these locations.



2a: Variation in pH and Total Organic Carbon

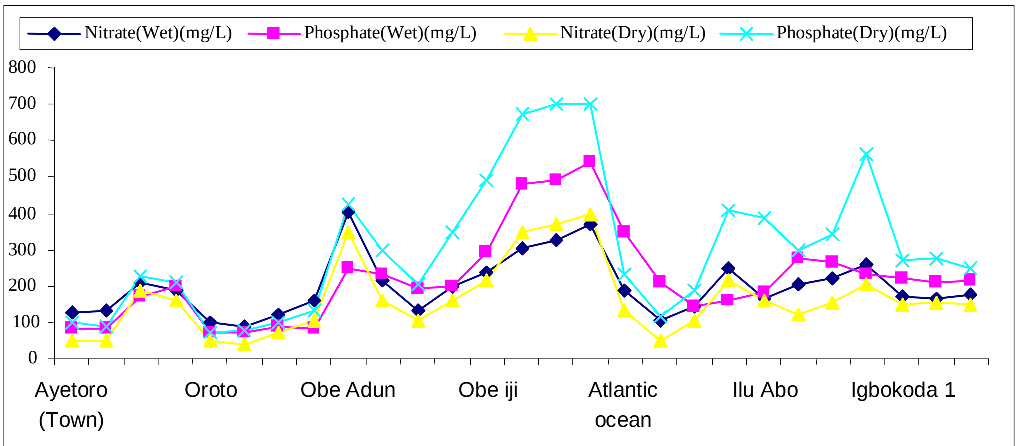


2b: Variation in Electrical Conductivity

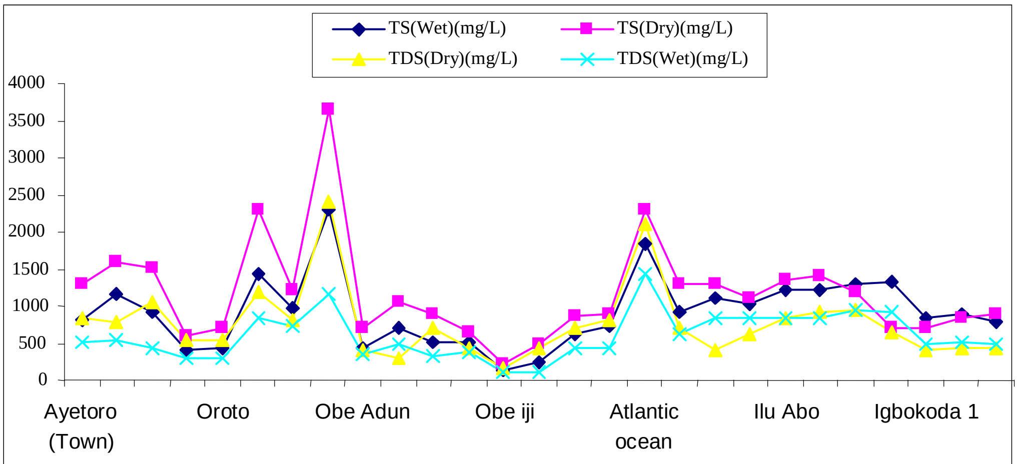


2c: Variation in chloride and sulphate

***Figure 2.** (a-c) Variations of some water physico-chemical parameters*



3a: Variation in nitrate and phosphate



3b: Variation in total solid and total dissolved solid

Figure 3. *(a-b) Variations of some water physico-chemical parameters*

The pH for most of the water samples (83.3 %) during the dry season fell within 4.13 to 5.97 while those of the wet season were not only fairly increased but fairly constant across most of the sampling locations. However, the comparatively low values of pH (i.e. greater acidity) during the dry season to those of wet season can be attributed to natural sources or some organic matters. Partial decomposition of some organic matters by bacteria and fungi has longed been recognized to produce various organic acids that are capable of lowering the pH of aqueous solution (Bowen, 1986). Most of the pH data in the dry season fall out of the 6.5-8.5 range of WHO standard for drinking water *(Table 2)* and water intended for aquatic life and recreational activities (DWAF, 2002; WHO, 2002; US EPA, 2004).The acidic nature of the water particularly during the dry season is expected to influence the solubility, availability and toxicity of metals in the aquatic ecosystems.

All the water samples did not show any measurable range of phenolphthalein alkalinity. Thus, the entire river shows no excess basic constituent. This has further supported the pH values obtained which fell entirely within the acidic-neutral range. In a similar manner, higher conductivity values in water were obtained during the dry season. Highest values were recorded at Ilepete (35.35µS/cm) and Atlantic Ocean (30.88µS/cm) during the dry season. These suggest a large amount of dissolved mineral salts within these communities. At four different locations; L, I, H and R, abnormally low values of 2.21, 4.56, 5.88 and 5.91µS/cm respectively were obtained. These low values were reflective in the amount of dissolved solids (TDS) recorded during the dry season at these locations. Except for one location, each for both season, the ranges for both season fell within the WHO (20µS/cm) for EC in water intended for domestic use (DWAF, 2002; WHO, 2002; NEMA, 2003).

A thorough assessment of the TDS which is slightly above half or almost equivalent of TS at some of the sampling locations indicate a high value for both seasons. The higher values of TDS during the dry season cannot but be associated with the nature of the medium, which is entirely acidic from the pH values recorded. Thus low value of the pH equally depicts water rich in dissolved organic matter and polluted acid drainage. Increased dissolved solid can lead to improved mineralization of receiving water or depletion of oxygen, depending on the nature (either organic or inorganic). Oxygen depletion can result from the oxidation of nitrogen and phosphorous (which are chemically combined in organic compounds) to nitrates and phosphates. The suspended solids were also at a high values even at the control sites (X,Y and Z). The consistent trading activities can be contributing factors to these values.

The organic carbon ranged from 8.9-4.17 mg/l and 13.4-32.5 mg/l during the dry and wet season respectively. The mean are very close and not significantly different ($\alpha = 0.05$, $P = 0.720$).These values are very high and far above the 0-5mg/l guidelines for domestic use (DWAF, 2002). As generally noted, organic matter influences the mobility and flux of the trace metals .The high level obtained in the study is an indication of greater metal input into the aqueous solution in the bioavailable form. This portends danger and serious threat to aquatic life.

It is of interest to note that at both seasons, none of the communities recorded normal phosphate (0.5mg/l), chloride (<250mg/l) and with not less than 88% violating nitrate WHO guideline for drinking water. The level of chloride was very high, much higher than nitrate, sulphate and phosphate especially during the dry season. Of particular interests are communities around the Atlantic ocean and the Atlantic itself (i.e. P and Q) where highest concentrations were recorded, though, this was expected. The high degree of pollution of chloride is indicative of salt-water intrusion. The chloride concentrations were found to generally decrease with distance away for the Atlantic Ocean downstream to site W *(Fig. 2c).* The normal chloride released into the sea from in-take of petroleum industry in Nigeria is <200mg/l (FEPA, 1992). Chloride values recorded at both seasons are quite higher. About 41% of the total (both seasons) exceeded the maximum allowable concentrations of Cl^- (<700mg/l) in water for agricultural purposes (e.g. irrigation) in Nigeria (FEPA, 1992).

Nitrates and Phosphates are two important nutrients that have been increasing markedly in natural waters since the mid-1960s (Hodgson, 2004). The increase in these nutrients, particularly phosphate, as exemplified in the present report over nitrate is of environmental concern and was not unexpected. This is because, in anaerobic environment, coupled with oil pollution of the sea during the dry season, nitrates are in low concentration and manganese and ferric oxides are abundant. The environmental significance of these metal oxides is that they serve a dual role. Not only are they a source of oxidants to micro organism, thus leading to 'algae blooms', they are also important for their capacity to bind toxic metals, deleterious organic compounds, phosphates and gases (Thomas and Williams, 2004). Oil spillage, is a major cause of low biological productivity which can result in appreciable amount of phosphate (Hammerton and Sherah, 1992). Since the equilibrium existing between photosynthesis (Process of cell formation) and respiration (process of cell decay) should be maintained, any disturbance as observed in the present study whereby the rate of cell decay is greater than that of cell formation, especially during the dry season (due to oil spill), will lead to accumulation of excess organic nutrients (see TOC values for dry season) which will in turn stimulate bacteria activity, thus making the water to become eutrophic.

The fairly clear appearance with low light penetration, foul smelling odour coupled with scarce varieties of fish especially during the dry season; at the time of sampling for the present study are essential features of eutrophic waters (Samir, 2003). Moreover, since primary producers in water such as cyanobacteria, phytoplankton and algae depend so much on sunlight and as such limited to the region near the surface of the ocean, where sunlight can penetrate i.e. the euphotic zone (Thomas and Williams, 2004), the presence of oil on the ocean has limited all possible microbial activities. Thus, oxygen supply of the ocean gets exhausted making the existing bacteria to shift from predominantly aerobic to anaerobic microorganisms that generate noxious products (NH_3,CH_4 and H_2S) of anaerobic metabolism. In general nitrate levels are observed to be less during the dry season while those of phosphates were observed to be higher *(Fig. 3a).* The levels of NO_3^- and PO_4^{3-} obtained in this report are exceedingly too high for both aquatic life and irrigation purposes with guideline values of <0.5mg/l for NO_3^- and <0.05mg/l for PO_4^{3-} respectively (FEPA, 1992; Campolo et al., 2002). The water is not equally suitable for livestock watering and recreational activities with guideline of <10mg/l for NO_3^- and <0.05mg/l for PO_4^{3-} respectively (FEPA, 1992; WHO,2002). The results of the sulphate obtained at both seasons are relatively comparable with almost half violating the WHO guideline values. The results of the TOC for both seasons were not with exceptions as virtually all the sampled water violated the <5mg/l quality guidelines values for domestic uses (Campolo, 2002).

In an attempt to further ascertain the pattern and trend of the water physico-chemical characteristics, a comparison was made with previous studies within the ecological zone (*Table 4)*. Most of the parameters apart from EC were observed to be recorded at higher concentrations than the previously obtained data. This can be said to be an enrichment of the aquatic ecosystem with pollutional nutrients.

Heavy metals enrichment

The range of results obtained from the river water displayed slight variation at both seasons. Cadmium and Ni were observed to be lower while Pb and Zn were at higher concentrations in the dry season. Significant mean differences occurred in Pb ($P = 0.000$) and Zn ($P = 0.030$). Based on Pearson correlation, *(Table 4),* there are 1).Significant ($\alpha \leq 0.01$) positive correlation between Cd and Zn. 2) Significant ($\alpha \leq 0.05$) positive correlations between Cd and Pb; Cd and Ni:Zn and Pb during either the wet and/or dry season(s). From the correlation result, it can be said that the heavy metals are directly and significantly related. With the limited available data, all the

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metals except Zn exceeded standard guideline values (WHO, 2002; NEMA, 2003) *(Table 3)*.

In addition, levels of Cd and Pb are higher than the maximum contaminant level (MCL) for livestock drinking (0.02 and 0.01mg/l); agricultural purposes such as aqua culture (0.01 and 0.2mg/l) and irrigation (0.2-1.8µg/l and 1.7µg/l) respectively (US EPA, 2004).This showed that water from the river is generally unsuitable for these activities with regards to the values obtained in this study, which could have chronic health effects on various users. Cadmium remains a metal without any known biological significance.

Toxicity in water is expected based on the levels obtained in this study. Chronic exposure to Pb has been linked to growth retardation in children (Schwartz et al., 1986). Lead toxicity studies conducted on female animals revealed mostly miscarriages, premature delivery and potent mortality (Tapieau et al., 2000). A concentration of Pb $\approx$ 0.1mg/l is detrimental to foetuses and children with possible development of neurological problem (Fatoki et al., 2002).

Apart from very few locations, levels of Ni obtained are higher than the guideline values. More importantly, attention has to be focused on the toxicity of Ni in low concentrations due to the fact that Ni can cause allergic reactions and that certain Ni compounds may be carcinogenic (Mokenzie and Smythe, 1998). All Ni compounds except for metallic Ni have been classified as carcinogenic to humans. Some of the health related effects of Ni are skin allergies, lung fibrosis, variable degrees of kidney and cardiovascular system poisoning and stimulation of neoplastic transformation (Awofolu and Fatoki, 2005).

Levels of Zn in the river water are quite less than the 3.0mg/l WHO guideline value. Hence, no detrimental effects from domestic water usage are expected. However, the USEPA levels for Zn in water for safe aquatic ecosystem, irrigation and livestock watering are 0.003mg/l, 0-0.1mg/l and 0-0.1mg/l respectively (US EPA, 2004). Consequently, the water is unfit for the sustenance of the aquatic ecosystem but could still be utilized for irrigation and livestock watering at some of the studied locations where concentration fell below the USEPA levels.

Generally, the elevated level of metals and physico-chemical properties examined in this study and possibly some other factors which we hope to look into in future studies complicated the economic sustenance of the inhabitants within the studied area. It equally represents what can be obtainable anywhere where oil operation (on-shore and off-shore) are being carried out It must however be emphasized that the source of these metals concentration cannot totally be associated with petroleum if one considers the percentage of metals in petroleum. Consequently, they must have been sourced, in addition, through anthropogenic and in particular natural seepage. Increased in metals could also have resulted from annual drilling and disposal, a regular operation being carried out within these communities by oil companies.

Using the available data from studies carried out within the same ecological zone and other parts of the country *(Table 5)*, the range of most of the metals are lower than most reported concentrations. The mean cadmium, Ni, and Zn levels in this study are lower than the range reported by some authors (Kakulu, 1985; Okoye, 1989; Asaolu, 1998). Lead is unique in the sense that the range reported in this study is higher than those reported by other workers identified above. This again signaled that probably, leaded gasoline has not yet been phased out of our oil operation.

Table 4. *Correlation Matrix of Physico-Chemical Properties for Dry and Wet Seasons*

	EC-1	EC-2	TS-1	TS-2	TDS-1	TDS-2	Cl^-1	Cl^-2	$SO_4^{2-}1$	$SO_4^{2-}2$	NO_3^--1	NO_3^--2	PO_4^{3-}-1	PO_4^{3-}-2	TOC-1	TOC-2	pH-1
EC-1	1																
EC-2	.734**	1															
TS-1	.840**	.841**	1														
TS-2	.741**	.908**	.866**	1													
TDS-1	.895**	.743**	.930**	.736**	1												
TDS-2	.734**	1.000**	.841**	.908**	.743**	1											
Cl^-1	.403*	.170	.144	.169	.280	.170	1										
Cl^-2	.099	.021	-.071	-.034	.033	.021	.888**	1									
$SO_4^{2-}1$	.077	.051	.086	-.032	.075	.051	.269	.249	1								
$SO_4^{2-}2$	.147	.140	.210	.072	.188	.140	.207	.212	.948**	1							
NO_3^--1	-.240	-.193	-.291	-.353	-.240	-.193	.162	.345	.092	.052	1						
NO_3^--2	-.351	-.236	-.383	-.407*	-.333	-.236	.070	.311	-.091	-.143	.956**	1					
PO_4^{3-}-1	-.283	-.127	-.291	-.364	-.209	-.127	.131	.405*	-.047	-.085	.763**	.841**	1				
PO_4^{3-}-2	-.322	-.283	-.332	-.462*	-.257	-.283	.003	.253	-.059	-.096	.866**	.925**	.841**	1			
TOC-1	.018	.333	.199	.102	.182	.333	-.082	.001	.041	.083	.332	.324	.368	.337	1		
TOC-2	.133	.532**	.280	.289	.213	.532**	.007	.058	.186	.195	.189	.186	.173	.190	.710**	1	
pH-1	-.648**	-.321	-.504**	-.402*	-.504**	-.321	-.261	-.101	-.186	-.299	.127	.257	.373	.328	.306	.122	1
pH-2	-.483*	-.449*	-.277	-.440*	-.248	-.449*	-.293	-.169	-.110	-.173	.114	.235	.337	.448*	.122	-.066	.632**

Significant / r / * ($P < 0.05$) 1 = Wet season
** ($P < 0.01$) 2 = Dry season

Table 5. *Comparison of Water Quality Characteristics of Rivers in the Present Study with Previous Studies within the Ecological Zone and FME Limits* [#]

Parameter	Present Study	Okitipupa SE Belt[a]	Ondo Coastal Water[b]	River Oluwa[c]	Niger Delta[d]
pH	4.13-8.46	5.37-7.70	6.00-7.43	6.90-7.50	
EC(µS/cm)	2.21-35.35	10.00-125.00	10.00-3658.00	-	
TSS(mg/l)	51-1246	2.15-29.40	0.20-3.75	< 20	
TDS(mg/l)	112-2404	5.19-75.45	0.0005-19.99	200	
TS (mg/l)	148-3650	10.55-85.18	0.26-21.67	-	
Cl^- (mg/l)	266-1012	6.97-29.60	10.24-1627.50	-	
NO_3^-(mg/l)	40-401	nd-42.00	nd-0.04	-	
PO_4^{3-}(mg/l)	73-703	nd-88.00	21.70-297.25	-	
SO_4^{2-}(mg/l)	61-605	2.36-11.08	nd-475	-	
Cd (mg/l)	0.17-2.49	-	0.1-9.6	-	0.67-5.07
Pb (mg/l)	4.09-42.35	-	0.2-36.0	-	0.67-5.07
Ni (mg/l)	< 0.1-2.7	-	2.0-9.3	-	nd-22.45
Zn (mg/l)	0.04-0.57	-	0.6-17.6	-	nd-42.86

Sources: [a]Ayesanmi [b]Asaolu (1998); [c]Ajayi and Osibanjo (1981); [d]Kukulu (1985)
[#] Water quality standard for aquatic life
- = Not determined
NS = Not Specified

Conclusion

Oil pollution (i.e. water pollution due to oil spills) is one problem for which no effective and final solution has been found anywhere in the World and in spite of every concerted effort to abate it, it has remained an inseparable part of the oil based operation. Some of the parameters determined were recorded at levels above the WHO standards for drinking water. It was equally observed that the levels of some of these parameters rendered the water unsuitable for recreational and agricultural purposes. The higher levels of most of the parameters over previous reports from the same ecological zone are indicative of anthropogenic enhancement and poor monitoring system. The persistent incidence of oil spillage into these coastal environments can be said to be contributory to the unusually high concentrations of some of the parameters determined in this study. The observed seasonal differences with higher concentrations of some parameters in the wet season than in the dry season may be attributed to increased land based run-off to the water body. Other possible reason may be due to increased water current and wave action, which may largely disturb the sediment, with the concomitant resurfacing of the previously leached materials into the sediment. It is therefore recommended that proper assessment and monitoring system be set up by the government so that aquatic and human lives would not be endangered.

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REFERENCES

[1] Anmar, W.S, Khalid, A.R., Thaer, I.K. (1993): Heavy metals in the water, suspended solids and sediment of the river Tigris impoundment at Samara. – Water Research 27(6): 1099-1103.

[2] APHA (1989): Standard methods for Examination of water and wastewater. – 15th edition, ByrdPras Springfield; Washington D.C.

[3] Asaolu, S.S. (1998): Chemical pollution studies of coastal waters of Ondo state. – Ph.D Thesis, Federal University of Technology, Akure, Nigeria.

[4] Awofolu, O.R., Fatoki, O.S. (2005): Levels of tracemetals in water and water from Tyume River and its effect on an irrigated farmland. – Water S.A. 31(1): 87-93.

[5] Bowen, H.J.M. (1986): Trace elements in biochemistry. – 1st ed., Academic Press, London.

[6] Campolo, M., Andrenssi, P., Soldati, A. (2002): Water quality control in the river Arno. – Journal of Science Direct 36: 2673-2680.

[7] Department of Water Affairs and Forestry, DWAF (2002): Water resources management plan in the deep river catchments: A situation assessment report No N/9210/REQ/1200 Pretoria, 150. – Affairs and Forestry, Department of Health and Water Research Commission.

[8] Eddy, N.O., Ekop, A.S. (2007): Assessment of the quality of water treated and distributed by the Akwa Ibom State water company. – E. J. Chemistry 4(2): 180-186.

[9] Fatoki, O.S., Awofolu, O.R. (2003): Levels of Cd, Hg and Zn in some surface waters from the Eastern Cape Province, South Africa. – Water SA. 29(4): 375-380.

[10] Fatoki, O.S., Lujiza, N., Ogunfowokan, O.A. (2002): Trace metal pollution in Umtata River. – Water SA. 28(2): 183-190.

[11] Federal Environmental Protection Agency, FEPA (1992): National Guidelines and standards for industrial effluents, gaseous emissions and hazardous waste Management in Nigeria. – Government notice 49.

[12] Gary, W.V., Stephen, J.D. (2001): Environmental Chemistry: a global perspective. – Oxford University Press, New York.

[13] Hammerton, C., Sherah, J.G. (1992): Analysis of raw potable and wastewater. – The Analyst 102: 82-86.

[14] Hodgson, E. (2004): A textbook of modern toxicology. – 3rd ed., Wiley Interscience, USA.

[15] Kakulu, S.E., Obibanjo, O. (1992): Trace metal analyses. – Environmental Pollution (series B)11: 315-322.

[16] Kakulu, S.E. (1985): Heavy metals in the Niger Delta: Impact of petroleum industry on the baseline levels. – Ph.D Thesis, University of Ibadan, Ibadan,Nigeria.

[17] Kavitha, N.S., Hilda, A., Gopinath, S., Latha, L. (1999): Proceedings of the International Workshop in Madras. – Biomanagement of Biogeoresources; Madras University, India. 1.

[18] Mokenzie, H.A., Smythe, L.E. (1998): Quantitative trace analysis of biological Elsevier, Amsterdam.

[19] Natural Environment Management Authority,NEMA (2003):Environmental Standards and preliminary environmental impact assessment for water quality and discharge of effluents into water/land. – Vol. 1. Prepared for the Ministry of Natural Resources, Nigeria.

[20] Nickson, R.T., McArthur, I.M., Shrestha, B., Kyaw-Myint, I.O., Lowly, D. (2005): Arsenic and other drinking water quality uses, Muzaffargarh District, Pakistan. – Applied Geochemistry 20: 55-68.

[21] O'Neil, P. (1993): Environmental chemistry. – 1st ed. Chapman and Hall, London.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

[22] Okoye, B.C.O.(1989): Levels and impact if some heavy metals in Lagos Lagoon. – Ph.D Thesis, Chemistry Department, O.A.U. Ile-Ife, Nigeria.
[23] Samir, K.B. (2003): Environmental Chemistry. – 2nd ed. Prentice-Hall, India, New Delhi.
[24] Schwartz, L., Angle, C., Pitcher, H. (1986): Relationship between childhood blood lead levels and stature. – Pediatr. **77**: 281-288.
[25] Tapieau, C., Poupon, J., None, P., Lefevie, B. (2000): Lead accumulation in the mouse ovary after treatment induced follicular atresia. – Reproductive Toxicology 15(4): 385-391.
[26] Thomas, G.S., Williams**,** M.S. (2004): Chemistry of the environment. – 2nd ed. Prentis Hall of India, New Delhi, 319-325.
[27] Thomas, L.P., Kathleen, E.S. (1998): Composition of petroleum mixture. – Total petroleum criteria working group series, Amherst Scientific Publishers, Massachusetts, 2: 2-8.
[28] United State Environmental Protection Agency (2004): EPA groundwater and drinking water current standards. – EPA office of water, Washington DC, USA.
[29] Wilde, F.D., Radtke, D.B., Gibs, J., Livasubo, R.T. (1999): Field manual for the land collection of water quality data. – US Geological survey, Book 9, Denvier.
[30] World Health Organisation (2002): Revision of WHO guidelines – 3rd ed. www.who.int/water-sanitation-health/GDWQdraftchemicals/list.htm for drinking water quality, Bilthova, The Netherlands.

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PHYTOREMEDIATION OF METAL MINE WASTE

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Abstract: Phytoremediation is a group of technologies that use plants to reduce, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim to clean-up contaminated areas. In this review paper, different types of phytoremediation processes, typical plants used and their application for clean-up of metal contaminated sites were reviewed. Plant responses to heavy metals and mechanisms of metal uptake and transport were also discussed. Phytoremediation of Pb, Zn, Cu and Fe tailings and mine spoils were carried out by grasses, herbs and shrubs, which could be categorised as As accumulator (*Paspalum*, *Eriochloa, Holcus, Pennisetum Juncus, Scirpus* and *Thymus),* Pb accumulator *(Brassica juncea, Vetiveria, Sesbania, Minuartia, Juncus, Scirpus* and *Thymus),* Cu accumulator *(Ammania baccifera, Scleranthus)* Zn and Cd accumulator *(Vetiveria, Sesbania, Viola, Sedum, Rumex).* The research work showed that, bioavailability and metal uptake by plants could be accomplished by ameliorating pH, addition of organic amendment, fertiliser and chelating agents. Further research is required to develop fast growing high biomass plants with improved metal uptake ability, increased translocation and tolerance of metals through genetic engineering for effective phytoremediation of metal mine wastes.
Keywords: *Phytoextraction, mine tailings, metal accumulator plants.*

Introduction

Phytoremediation, the use of plants for environmental restoration, is an emerging cleanup technology. Both metal and non-metal mining activities generate huge quantity of waste rocks, which damages the aesthetics of the area. Particularly, in case of metal mining, activites such as crushing, grinding, washing, smelting and all the other process used to extract, concentrate metals, generate a large amount of waste rocks and tailings which scars the landscape, disrupts ecosystems and destroys microbial communities. Waste materials or spoils that remain after the extraction of usable ores are dumped on the surrounding land, which is the sources of toxic metals, leave the land devoid of topsoil, nutrients and supportive microflora and vegetation, thus remains barren (Das and Maiti, 2008).

Most of the conventional remedial technologies like leaching of pollutant, vitrification, electrokinetical treatment, excavation and off-site treatment are expensive and technically limited to relatively small areas (Barceló and Poschenrieder, 2003). Moreover, they deteriorates the soil fertility, which subsequently causes negative impacts on the ecosystem. Establishment of vegetation cover can fulfill the objectives of stabilization, pollution control, visual improvement and removal of threats to human beings (Freitas et al., 2004). The use of plants for purifying contaminated soils and

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water has been developed much more recently. In the 1970s, reclamation initiatives of mining sites developed technologies for covering soil with vegetation for stabilization purposes and reduction of visual impact (Williamson and Johnson, 1981). It was not until the 1990s, that the concept of phytoremediation emerged as a new technology that uses plants to reduce, remove, degrade or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition useable for private or public applications. The generic term ‘phytoremediation’ consists of the Greek prefix “phyto” (plant), attached to the Latin root “remedium” (to correct or remove evil) (Cunningham and Ow, 1996). Phytoremediation is a cost effective, environmental friendly, aesthetically pleasing approach with long term applicability. Phytoremediation is well suited for use at very large field sites where other methods of remediation are not cost effective or practicable (Williamson and Johnson, 1981).

However, adverse factors such as acidity, nutrient deficiencies, toxic heavy metal ions, poor physical structure, and their interaction in most mine tailings inhibit plant establishment and growth on the tailings (Pichtel and Salt, 2003). Mine spoil or tailing dumps usually have barren surfaces, with rare plants that show signs of suffering such as stunted growth, chlorosis, necrosis and anomalous development of roots with respect to shoots (Dinelli and Lombini, 1996). Low specific diversity in the mine spoil area is the result of severe environmental conditions, but some plants can tolerate high concentration without sign of stress. Collecting plant species from contaminated soil and the evaluation of plant metal concentrations can be used to get information about specific plant behavior in this environment and to complete data about metal dispersion, with reference to their mobility to the biomass. Some plants phytostabilise heavy metals in the rhizophere through root exudates immobilization (Blaylock and Huang, 2000), whilst other species incorporate them into root tissues (Khan, 2001). Some plants also transfer metals to their above ground tissues, potentially allowing the soil to be decontaminated by harvesting the aboveground parts. Therefore, plant community established on mine spoil / tailings could be useful to minimize the impacts of mining. Moreover knowing the diversity of plant responses in contaminated sites having different metals and toxicity levels, is important to study the composition of plant community that was established on degraded soils or mine spoil, which would serve as a basic approach for mine remediation.

Phytoremediation processes

Plants have shown the capacity to withstand relatively high concentrations of organic chemicals without toxic effects, and they can uptake and convert chemicals quickly to less toxic metabolites in some cases. In addition, they stimulate the degradation of organic chemicals in the rhizosphere by the release of root exudates, enzymes and the build-up of organic carbon in the soil. For metal contaminates, plants show the potential for phytoextraction (uptake and recovery of contaminates into above-ground biomass), filtering metals from water into root systems (*rhizofiltration*), or stabilizing waste sites by erosion control and evapotranspiration of large quantities of water (*phytostabilization*) and so on (Cunningham and Ow, 1996). There are a number of different forms of phytoremediation, discussed below. All phytoremediation processes are not exclusive and may be used simultaneously. The different forms of phytoremediation may apply to specific types of contaminants or contaminated media and may require different types of plants as shown in *Table 1*.

Table 1. *Typical Plants Used in Various Phytoremediation Processes*

Process	Mechanism	Media	Contaminants	Typical Plants
1. Phyto-extraction	Hyper-accumulation	Soil, Brownfields, Sediments	Metals (Pb, Cd, Zn, Ni, Cu) with EDTA addition for Pb, Selenium.	Sunflowers, Indian mustard, Rape seed plants, Barley, Hops, Crucifers, Serpentine plants
2. Rhizo-filtration	Rhizosphere accumulation	Groundwater, Water and Wastewater in Lagoons or Created Wetlands	Metals (Pb, Cd, Zn, Ni, Cu) Radionuclides (^{137}Cs, ^{90}Sr, ^{238}U) Hydrophobic organics	Aquatic Plants: - Emergents (bullrush, cattail, pondweed, arrowroot, duckweed); - Submergents (algae, stonewort, parrot feather, *Hydrilla*)
3. Phyto - stabilization	Complexation	Soil, Sediments	Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) Hydrophobic Organics (PAHs, PCBs, dioxins, furans, pentachlorophenol, DDT, dieldrin)	Phreatophyte trees to transpire large amounts of water for hydraulic control; Grasses with fibrous roots to stabilize soil erosion; Dense root systems are needed to sorb / bind contaminants
4. Phyto-volatization	Volatization by leaves	Soil, Groundwater, Sediments	Mercury, Selenium, Tritium	Poplar, Indian mustard, Canola, Tobacco plants.
5. Phyto-degradation	Degradation in plant	Soil, Groundwater, Landfill leachate, Land application of wastewater	Herbicides (atrazine, alachlor) Aromatics (BTEX) Chlorianated aliphatics (TCE) Nutrients (NO_3^-, NH_4^+, PO_4^{3-}) Ammunition wastes (TNT, RDX)	Phreatophyte trees (poplar, willow, cottonwood); Grasses (rye, Bermuda, sorghum, fescue); Legumes (clover, alfalfa, cowpeas)
6. Rhizo-degradation	Degradation by plant rhizosphere microorganisms	Soil, Sediments, Land application of wastewater	Organic contaminants (pesticides, aromatics and polynuclear aromatic hydrocarbons [PAHs])	Phenolics releasers (mulberry, apple, orange); Grasses with fibrous roots (rye, fescue, Bermuda) for contaminants 0-3 ft deep; Phreatophyte trees for 0-10 ft; Aquatic plants for sediments

Phytoextraction

This process reduces soil metal concentrations by cultivating plants with a high capacity for metal accumulation in shoots (Barceló and Poschenrieder, 2003). The plants must extract large concentrations of heavy metals into their roots, translocate the heavy metals to above ground shoots or leaves and produce large quantity of plant biomass that can be easily harvested; when plants are harvested contaminants are removed from the soil. Recovery of high price metals from the harvested plant material may be cost effective (eg. phytomining of Ni, Tl or Au). If not, the dry matter can be burnt and the ash disposed of under controlled conditions. Phytoextraction is also known as phytoaccumulation, phytoabsorption and phytosequestration. Phytoextraction can be divided into two categories: continuous and induced (Salt et al., 1998). Continuous phytoextraction requires the use of plants that accumulate particularly high levels of the toxic contaminants throughout their lifetime (hyperaccumulators), while induced phytoextraction approaches enhance toxin accumulation at a single time point by addition of accelerants or chelators to the soil.

Rhizofiltration

This technique is used for cleaning contaminated surface waters or waste waters such as industrial discharge, agricultural runoff, or acid mine drainage by absorption or precipitation of metals onto roots or absorption by roots or other submerged organs of metal tolerant aquatic plants. For this purpose plants must not only be metal resistant but also have a high absorption surface and must tolerate hypoxia (Dushenkov et al., 1995). Contaminant should be those that sorb strongly to roots, such as hydrophobic organics, lead, chromium(III), uranium and arsenic(V). Plants like sunflower, Indian mustard, tobacco, rye, spinach and corn have been studied for their ability to remove lead from effluent, with sunflower having the greatest ability (Raskin and Ensley, 2000).

Phytostabilization

It refers to the holding of contaminated soils and sediments in place by vegetation, and to immobilizing toxic contaminants in soils. Phytostabilization is also known as in-place inactivation or phytoimmobilization. Phytostabilization can occur through the sorption, precipitation, complexation or metal valence reduction (Ghosh and Singh, 2005). Metals do not ultimately degrade, so capturing them in situ is sometimes the best alternative at sites with low contamination levels or at vast contaminated areas where a large scale removal action or other in situ remediation is not possible. Plants with high transpiration rates, such as grasses, sedges, forage plants and reeds are useful for phytostabilization by decreasing the amount of ground water migrating away from the site carrying contaminants. Combining these plants with hardy, perennial, dense rooted or deep rooting trees (popular, cottonwoods) can be an effective combination (Berti and Cunningham, 2000).

Phytovolatization

It involves the use of plants to take up contaminants from the soil transforming them into volatile form and transpiring them into the atmosphere. Selenium (Se) is a special case of a metal that is taken up by plants and volatilized. Neumann et al. (2003) found that an axenically cultured isolate of single celled freshwater microalgae (*Chlorella* sp.) metabolized toxic selenate to volatile dimethylselenide at exceptionally high rates when transferred from mineral solution to water for 24h, than those similarly measured for wetland macroalgae and higher plants. Hyper-volatilization of selenate by microalgae cells may provide a novel detoxification response. Uptake and evaporation of Hg is achieved by some bacteria. The bacterial genes responsible have already been transferred to *Nicotiana* or *Brassica* species, and these transgenic plants may become useful in cleaning Hg-contaminated soils (Meager et al., 2000).

Phytodegradation

It involves uptake, metabolisation and degradation of contaminants within the plant, or the degradation of contaminants in the soil sediments, sludges, groundwater or surface water by enzymes produced and released by the plant. Phytodegradation is not dependent on microorganisms associated with the rhizosphere. Phytodegradation is also known as phytotransformation, and is a contaminant destruction process. For instance,

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the major water and soil contaminant trichloroethylene (TCE) was found to be taken up by hybrid poplar trees (*Populas deltoids nigra*), which breaks down the contaminant into its metabolic components (Newman et al., 1997).

Rhizodegradation

Rhizodegradation is the breakdown or organics in the soil through microbial activity of the root zone (rhizosphere). Enhanced rhizosphere degradation uses plants to stimulate the rhizosphere microbial community to degrade organic contaminants (Kirk et al., 2005). Grasses with high root density, legumes and alfalfa that fix nitrogen and have high evapotranspiration rates are associated with different microbial populations. Significantly higher populations of total heterotrophs, denitrifers, were found in rhizosphere soil around hybrid poplar trees in a field pot than in non-rhizosphere soil (Jordahl et al., 1997).

Phytorestoration

It involves the complete remediation of contaminated soils to fully functioning soils (Bradshaw, 1997). In particular, this subdivision of phytoremediation uses plants that are native to the particular area, in an attempt to return the land to its natural state.

Hydraulic control

It is the use of vegetation to influence the movement of ground water and soil water, through the uptake and consumption of large volumes of water. Hydraulic control reduces or prevents infiltration and leaching and induces upward flow of water from the water table through the vadose zone. Vegetation water uptake and transpiration rates are important for hydraulic control. The application of different phytoremediation technologies for cleaning up of metal contaminated sites are given in *Table 2.*

Table 2. *Phytoremediation application*

Location	Application	Plants	Contaminants	Performance
Trenton, NJ	Phytoextraction demonstration 200 ft x 300 ft plot Brownfield location	Indian mustard *(Brassica juncea)*	Pb	Pb cleaned-up to below action level in one season.
Dearing, KS	Phytostabilization demonstration one acre test plot abandoned smelter, barren land	Poplars *(Populus* sp.)	Pb, Zn Cd Concs. > 20,000 ppm for Pb and Zn	50% survival after 3 years. Site was successfully revegetated.
Whitewood Cr., South Dakota	Phytostabilization demonstration one acre test plot mine wastes	Poplars *(Populus* sp.)	As, Cd	95% of trees died. Inclement weather, toxicity caused die-off.
Pennsylvania	Phytoextraction mine wastes	*Thlaspi caerulescens*	Zn, Cd	Uptake is rapid but difficult to decontaminate soil.
San Francisco, CA	Phytovolatization refinery wastes and agricultural soils	*Brassica* sp.	Se	Selenium is partly taken-up and volatilized, but difficult to decontaminate soil.

Plant response to heavy metals

When categorizing plants that can grow in the presence of toxic elements, the terms "metal excluder", "metal indicator", and "metal accumulator" are used. Metal excluders prevent metal from entering their aerial parts or maintain low and constant metal concentration over a broad range of metal concentration in soil; they mainly restrict metal in their roots. The plant may alter its membrane permeability, change metal binding capacity of cell walls, or exclude more chelating substances (Lasat, 2000).

A metal indicator species is one that actively accumulates metal in their aerial tissues and generally reflects metal level in soil. They tolerate the existing concentration level of metals by producing intracellular metal binding compounds (chelators), or alter metal compartmentalization pattern by storing metals in non-sensitive parts (Ghosh and Singh, 2005). Indicator species have been used for mine prospecting to find new ore bodies.

Metal accumulators can concentrate metal in their aerial parts, to levels far exceeding than soil. By definition, hyperaccumulators are herbaceous or woody plants that accumulate and tolerate without visible symptoms a hundred times or greater metal concentrations in shoots than those usually found in non-accumulators. Baker and Brooks (1989) established 0.1% as the minimum threshold tissue concentrations for plants considered Co, Cu, Cr, Pb or Ni hyperaccumulators, while for Zn or Mn the threshold is 1%. For cadmium and other rare metals, it is 0.01% by dry weight. Hyperaccumulators are found in 45 different families, with the highest occurrence among the Brassicaceae (Reeves and Baker, 2000). These plants are quite varied, from perennial shrubs and trees to small annual herbs. Some of the hyperaccumulators and their metal accumulation capabilities are listed in *Table 3.*

Table 3. *Metal concentrations (on a dry weight basis) in known hyperaccumulators*

Metal	Plant Species	Concentrations in "Harvestable" Material from Plants Grown in Contaminated Soil (dry wt basis)
Cd	*Thlaspi caerulescens*	1,800 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Cu	*Ipomoea alpine*	12,300 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Co	*Haumaniastrum robertii*	10,200 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Pb	*T. rotundifolium*	8,200 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Mn	*Macadamia neurophylla*	51,800 mg kg $^{-1}$ in shoots (Baker and Walker, 1990)
Ni	*Psychotria douarrei, Sebertia acuminate*	47,500 mg kg $^{-1}$ in shoots (Baker and Walker, 1990) 25% by wt of dried sap (Jaffre et al., 1976)
Zn	*T. caerulescens*	51,600 mg kg $^{-1}$ in shoots (Brown et al., 1994)

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How do plants take up and transport metal?

The process of metal accumulation involves several steps; one or more of which are responsible for the hyperaccumulation in plants:

Solubilization of the metal from the soil matrix

Many metals are found in soil-insoluble forms. Plants use two methods to desorb metals from the soil matrix: acidification of the rhizosphere through the action of plasma membrane proton pumps and secretion of ligands capable of chelating the metal. Plants have evolved these processes to liberate essential metals from the soil, but soils with high concentrations of toxic metals will release both essential and toxic metals to solution (Lasat, 2000).

Uptake into the root

Soluble metals can enter into the root symplast by crossing the plasma membrane of the root endodermal cells or they can enter the root apoplast through the space between cells. While it is possible for solutes to travel up through the plant by apoplastic flow, the more efficient method of moving up the plant is through the vasculature of the plant, called the xylem. To enter the xylem, solutes must cross the Casparian strip, a waxy coating, which is impermeable to solutes, unless they pass through the cells of the endodermis. Therefore, to enter the xylem, metals must cross a membrane, probably through the action of a membrane pump or channel. Most toxic metals are thought to cross these membranes through pumps and channels intended to transport essential elements. Excluder plants survive by enhancing specificity for the essential element or pumping the toxic metal back out of the plant (Hall, 2002).

Transport to the leaves

Once loaded into the xylem, the flow of the xylem sap will transport the metal to the leaves, where it must be loaded into the cells of the leaf, again crossing a membrane. The cell types where the metals are deposited vary between hyperaccumulator species.

Detoxification and/or chelation

At any point along the pathway, the metal could be converted to a less toxic form through chemical conversion or by complexation. Various oxidation states of toxic elements have very different uptake, transport, and sequestration or toxicity characteristics in plants. Chelation of toxins by endogenous plant compounds can have similar effects on all of these properties as well. As many chelators use thiol groups as ligands, the sulfur (S) biosynthetic pathways have been shown to be critical for hyperaccumulator function (Van Huysen et al., 2004) and for possible phytoremediation strategies.

Sequestration and volatilization

The final step for the accumulation of most metals is the sequestration of the metal away from any cellular processes it might disrupt. Sequestration usually occurs in the plant vacuole, where the metal/metal-ligand must be transported across the vacuolar membrane. Metals may also remain in the cell wall instead of crossing the plasma membrane into the cell, as the negative charge sites on the cell walls may interact with polyvalent cations (Wang and Evangelou, 1994). Selenium may also be volatized through the stomata.

Phytoremediation of metal mine waste

The selection of trace element tolerant species is a key factor to the success of remediation of degraded mine soils. For long-term remediation, metal tolerant species are commonly used for revegetation of mine tailings (Lan et al., 1997), and herbaceous legumes can be used as pioneer species to solve the problem of nitrogen deficiencies in mining wastelands because of their N_2 fixing ability (Lan et al., 1997).

Singh et al. (2004) describes the impact of young high-density plantations of two native leguminous (*Albizia procera* and *A. lebbeck*) and one non-leguminous timber tree (*Tectona grandis*) species on the soil redevelopment process during the early phase of mine restoration in a dry tropical environment. There was a general improvement in soil properties due to establishment of plantations. Highest soil organic C values were found in *A. lebbeck* plantations and lowest in *T. grandis* plantations. Both *A. lebbeck* and *A. procera* substantially increased levels of nitrogen in soil. However, *A. procera*, with slow decomposing litter, was not as effective in raising N levels in the soil as *A. lebbeck*, indicating that all N fixers may not be equally efficient in raising soil N levels.

Yang et al. (2003) conducted a field trial at Lechang Pb/Zn mine tailings of Guangdong Province, Southern China to compare growth performance, metal accumulation of Vetiver (*Vetiveria zizanioides*) and two legume species (*Sesbania rostrata* and *Sesbania sesban*) grown on the tailings amended with domestic refuse and/or fertilizer. It was revealed that domestic refuse alone and the combination of domestic refuse and artificial fertilizer significantly improved the survival rates and growth of *V. zizanioides* and two *Sesbania* species, especially the combination. However, artificial fertilizer alone did not improve both the survival rate and growth performance of the plants grown on tailings. Roots of these species accumulated similar levels of heavy metals, but the shoots of two *Sesbania* species accumulated higher (3-4 folds) concentrations of Pb, Zn, Cu and Cd than shoots of *V. zizanioides*. Most of the heavy metals in *V. zizanioides* were accumulated in roots, and the translocation of metals from roots to shoots was restricted.

Metal uptake capacity by Caryophyllaceae species (genera Dianthus, Minuartia, Scleranthus and Silene) were studied from metalliferous soils in northern Greece, having different concentrations of Cu, Pb, Zn, Cd, Ni, Cr, Fe, Mn, Ca, Mg (Konstantinou and Babalonas, 1996). They concluded that *Scleranthus perennis subsp. perennis* showed the highest Cu concentration (205 mg kg^{-1}), whereas *Minuartia cf. bulgarica* hyperaccumulated Pb (1175 mg kg^{-1}). Ca concentrations in plants were in most cases much higher than those in soil, whereas the contrary was true for Mg. As a result the Ca/Mg ratio, which was in almost all cases lower than 1 in the soil, was much increased in the plants.

Mine spoil dump material and plants *Silene armeriu* (Caryophyllaceae), *Salix spp*. (Salicaceae) and *Populus nigra* (Salicaceae) were sampled at 4 different growing stages from the pyrite-chalcopyrite mining area of Vigonzano (Northern Apennines, Italy). Mine spoils have high concentrations of Fe, Mg, Cu, Cr, Co and Ni, and are characterized by moderately to strongly acid environmental conditions. Water leaching tests indicate the following order of extraction: Zn ≥ Cu > Ni > Fe ≥ Cr (Dinelli and Lombini, 1996). The results indicate that metal concentrations increase with plant ageing, the highest concentrations being observed in leaves. The variations of BAC (Biological Accumulation Coefficient) for the plants growing on the Vigonzano mine spoil area indicates that Zn is the element most easily absorbed by plants. An absorption sequence Zn >Co >Cu >Ni > Fe >Cr can be generalized for plants growing on the mine spoil area indicating the importance of soil solution composition in plant absorption.

Selection of plant materials is an important factor for successful field phytoremediation. Zhuang et al. (2007) conducted a field experiment to evaluate the phytoextraction abilities of six high biomass plants – *Vertiveria zizanioides*, *Dianthus chinensis*, *Rumex K-1* (*Rumex upatientia* × *R. timschmicus*), *Rumex crispus*, and two populations of *Rumex acetosa* – in comparison to metal hyperaccumulators (*Viola baoshanensis*, *Sedum alfredii*). The paddy fields used in the experiment were contaminated with Pb, Zn, and Cd. Results indicated that *Viola baoshanensis* accumulated 28 mg Cd kg^{-1} and S. alfredii accumulated 6279 mg Zn kg^{-1} (dry weight) in shoots, with bioconcentration factors up to 4.8 and 6.3, respectively. The resulting total extractions of *V. baoshanensis* and *S. alfredii* were 0.17 kg ha^{-1} for Cd and 32.7 kg ha^{-1} for Zn, respectively, with one harvest without any treatment. The phytoextraction rates of *V. baoshanensis* and *S. alfredii* for Cd and Zn were 0.88 and 1.15 %, respectively. Among the high biomass plants, *R. crispus* extracted Zn and Cd of 26.8 and 0.16 kg ha^{-1}, respectively, with one harvest without any treatment, so it could be a candidate species for phytoextraction of Cd and Zn from soil. No plants were proved to have the ability to phytoextract Pb with such high efficiency.

Bech et al. (2002) reported the results of the screening of plant species from three different mining areas in South America: a copper mine in Peru ("Mina Turmalina"), a silver mine in Ecuador ("Mina San Bartolomé") and a copper mine in Chile ("Mina El Teniente"). The accumulation of heavy metals viz. As in shoots as a function of extractable metal concentrations in the soils was analyzed in field samples. The different plant species collected from the severely polluted soils exhibited large differences in accumulation of heavy metals and As. Among the grass species (Poaceae), the highest concentration of As was observed in the shoots of *Paspalum sp.* (> 1000 mg kg^{-1}) and *Eriochloa ramosa* (460 mg kg^{-1}) from the Cu mine in Peru, and in *Holcus lanatus* and *Pennisetum clandestinum* (> 200 mg kg^{-1}) from the silver mine in Ecuador. *Paspalum racemosum* also accumulated considerable concentrations of Cu and Zn. The species from the genus Bidens (Asteraceae) were not only able to accumulate high concentration of As in shoots (> 1000 mg kg^{-1} in *B. cynapiifolia* from Peru), but also considerable amounts of Pb (*B. humilis* from Chile). The highest concentration of Cu was found in the shoots of *Mullinum spinosum* (870 mg kg^{-1}) and in *B. cynapiifolia* (620 mg kg^{-1}). The accumulation of Zn was highest in the shoots of *Baccharis amdatensis* (> 1900 mg kg^{-1}) and in *Rumex crispus* (1300 mg kg^{-1}) from the silver mine in Ecuador.

Maiti et al. (2005) conducted a study with the aim to identify pioneering species that naturally colonize Fe tailings and accumulate heavy metals. Total, bioavailable, acid extractable and water-soluble fractions were studied. After the second year onwards, along with nine herbaceous pioneering species, four tree species (*Tectona grandis*, *Alstonia scholaris*, *Azadirachta indica* and *Peltaphorum*) were found growing naturally. The study shows that some species could accumulate relatively high metal concentrations indicating internal detoxification of metals. The study revealed that *T. grandis* accumulated a higher concentration of metals than *A. scholaris* in the Fe tailings, but all concentrations were within the normal range. Native naturally colonizing plant species may be used for the bioremediation of iron tailings as initial cover species to stabilize and reduce erosion.

A pilot scale study conducted on the Fe tailings of Noamundi, Tata- Steel by Maiti and Nandhini (2005) reported that nine plant species was able to grow naturally on the Fe tailings, out of which 4 species namely *Borhevia repens*, *Oxalis corniculata*, *Blumea lacera* and *Avera aspera* were analysed for total metal contents in the whole plant. The total metal contents in the natural vegetation varied widely between 1530-8412 mg Fe kg^{-1}, 17-102 mg Mn kg^{-1}, 28-110 mg Zn kg^{-1}, 10.8-18.8 mg Cu kg^{-1}, 5.2-35.8 mg Pb kg^{-1}, 12-32 mg Ni kg^{-1} and 5.5-31.8 mg Co kg^{-1}. Maximum accumulation of Fe was found in *Oxalis* (7442 mg kg^{-1}) whereas Mn and Zn were observed maximum in *Blumea lacera* (88 mg kg^{-1}) and *Avera aspera* (109 mg kg^{-1}) respectively. The variation of BAC (Biological Accumulation Coefficient = total metals in plants/ DTPA metals in soil) for plants growing in the Fe tailings indicated that Fe was the element most easily absorbed by the plants. An absorption sequence was in the order of Fe> Ni> Pb> Zn> Cu> Mn> Co.

Das and Maiti (2007a) conducted a field studies in an abandoned copper mine tailings (Rakha mine, Jharkhand, India), to find out accumulation of metals (Cu, Ni, Mn, Zn, Pb, Cd and Co) in the naturally colonising vegetation. They found that, out of 11 species, *Ammania baccifera* growing on copper tailings, levels of Cu accumulation in the root parts was found even more than 1000 mg $kg^{−1}$ dry weight (DW). Metals accumulated by *A. baccifera* were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them. Thus, establishment of such plant on copper tailings can be a safe method to stabilize the metals.

Das and Maiti (2007b) analyzed metal accumulation in above and underground tissues of plants belonging to 5 genera and 4 families from the abandoned Cu-tailing ponds of Rakha mines, Jharkhand, India. Tailings have high concentration of Cu, Ni and characterized by moderately acid environment and low nutrient contents. Plant communities respond differently, depending on their ability to uptake or exclude a variety of metals. Accumulated metals were mostly retained in root tissue indicating that an exclusion mechanism for metal tolerance widely exists in them. Retention of some metals more than toxic level in the above ground tissues of some plants suggests the presence of internal metal detoxification and tolerance mechanisms in them.

Freitas et al. (2004) studied the metal accumulation in the natural vegetation in the degraded copper mine of São Domingos, SE Portugal. Plants belonging to 24 species, 16 genera and 13 families were collected and samples were analyzed for total Ag, As, Cu, Ni, Pb, and Zn. The highest concentrations of metals in Cu mine soil (DW) were 11217.5 mg Pb $kg^{−1}$, 1829 mg Cu $kg^{−1}$, 1291 mg As $kg^{−1}$, 713.7 mg Zn $kg^{−1}$, 84.6 mg Cr $kg^{−1}$, 54.3 mg Co $kg^{−1}$, 52.9 mg Ni $kg^{−1}$ and 16.6 mg Ag $kg^{−1}$. With respect to plants, the higher concentrations of Pb and As were recorded in the semi-aquatic species *Juncus conglomeratus* with 84.8 and 23.5 mg $kg^{−1}$ DW respectively, *Juncus efusus* with 22.4 and 8.5 mg $kg^{−1}$ DW, and *Scirpus holoschoenus* with 51.7 and 8.0 mg $kg^{−1}$ DW, respectively. *Thymus mastichina* also showed high content of As in the aboveground parts, 13.6 mg $kg^{−1}$ DW. Overall, the results indicates accumulation of various metals by different plant species, with some of these metals being partitioned to the shoots.

In a study conducted by (Blaylock et al., 1999) at a lead-contaminated site in Trenton, New Jersey, the soil was treated for phytoremediation using successive crops of *B. juncea* combined with soil amendments. Through phytoremediation, the average surface soil Pb concentration was reduced by 13%. In addition, the target soil

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concentration of 400 mg/kg was achieved in approximately 72% of the treated area in one cropping season. It is found that the integration of specially selected metal-accumulating crop plants (*Brassica juncea* (L) Czern.) with innovative soil amendments allows plants to achieve high biomass and metal accumulation rates.

In a field study, mine wastes containing Cu, Pb and Zn were stabilized by grasses – *Agrostis tenuis* for acid lead and zinc mine wastes, *Agrostis tenuis* for copper mine wastes, and *Festuca rubra* for calcareous lead and zinc mine wastes (Smith and Bradshaw, 1979).

Shu et al. (2004) conducted a field experiment to compare the growth and metal accumulation in 4 grasses (*Vertiveria zizanioides*, *Paspalum notatum*, *Cynodon dactylon* and *Imparata cylindraca var major*) on the fields amended with 10 cm domestic refuse + complex fertilizer (NPK, Treatment A), 10 cm domestic refuse (Treatment B) and complex fertilizer (NPK, Treatment C), respectively, and without any amendment used as control (Treatment D). The results indicated that *V. zizanioides* was a typical heavy metal excluder, because the concentrations in shoots of the plants were the lowest among the four plant species tested. The most of metal accumulated in *V. zizanioides* distributed in its roots, and transportation of metal in this plant from root to shoot was restricted. Therefore, *V. zizanioides* was more suitable for phytostabilization of toxic mined lands than *P. notatum* and *C. dactylon*, which accumulated a relatively high level of metals in their shoots and roots. It was found that *I. cylindraca var. major* accumulated lower amounts of Pb, Zn Cu than *C. dactylon* and *P. notatum* and could also be considered for phytostabilization of tailings. Although the metal (Pb, Zn, and Cu) concentrations in shoots and roots of *V. zizanioides* were the lowest, the total amounts of heavy metals accumulated in shoots of V. zizanioides were the highest among the four tested plant species due to the highest dry weight yield of it. The results indicated that *V. zizanioides* was the best choice among the four species used for phytoremediation (for both phytostabilization and phytoextraction) of metal contaminated soils.

Chelant-enhanced phytoextraction of heavy metals is an emerging technological approach for a non-destructive remediation of contaminated soils. Komárek et al. (2006) studied the effect of the use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated soils. The main objectives of this study were (i) to assess the extraction efficiency of two different synthetic chelating agents (ethylenediamine-tetraacetic acid (EDTA) and ethylenediaminedisuccinic acid (EDDS) for desorbing Pb from two contaminated agricultural soils originating from a mining and smelting district and (ii) to assess the phytoextraction efficiency of maize (*Zea mays*) and poplar (*Populus sp.*) after EDTA application. EDTA was more efficient than EDDS in desorbing and complexing Pb from both soils, removing as much as 60% of Pb. Maize exhibited better results than poplar when extracting Pb from the more acidic (pH 4) and more contaminated (upto 1360 mg Pb kg^{-1}) agricultural soil originating from the smelting area. On the other hand, poplars proved to be more efficient when grown on the near-neutral (pH$\sim$6) and less contaminated (upto 200 mg Pb kg^{-1}) agricultural soil originating from the mining area. Furthermore, the addition of EDTA led to a significant increase of Pb content especially in poplar leaves, proving a strong translocation rate within the poplar plants.

Zhuang et al. (2005) conducted a field trial to evaluate the phytoextraction efficiencies of three plants and the effects of EDTA or ammonium addition [(NH4)2SO4 and NH4NO3] for assisting heavy metal (Pb, Zn, and Cd) removal from contaminated soil. The tested plants include *Viola baoshanensis*, *Vertiveria zizanioides*, and *Rumex K-1* (*Rumex patientia* × *R. timschmicus*). The application of EDTA soil was the most efficient to enhance the phytoavailability of Pb and Zn, but did not have significant effect on Cd. Lead phytoextraction rates of *V. baoshanensis*, *V. zizanioides* and *Rumex K-1* were improved by 19-, 2-, and 13-folds compared with the control treatment, respectively. The application of ammonium did not have obvious effects on phytoextraction of the three metals, except that the accumulations of Zn and Cd in shoot of *V. baoshanensis*. Among the three tested plants, *V. baoshanensis* always accumulated the highest concentrations of Pb, Zn, and Cd. The concentrations of Pb, Zn, and Cd in the shoots of *V. baoshanensis* treated with EDTA were 624, 795, and 25 mg kg^{-1}, respectively, and the phytoextraction efficiencies of this species for Pb, Zn, and Cd were also the highest among the three species. Results presented here indicated that *V. baoshanensis* had great potential in phytoremediation of soils contaminated by multiple heavy metals, although the dry weight yield was the lowest among the three plants.

Enhancement of phytoremediation by plant genetic modification

The development of commercial phytoextraction technologies requires plants that produce high biomass and that accumulate high metal concentrations in organs that can be easily harvested, i.e. in shoots. It has been suggested that phytoremediation would rapidly become commercially available if metal-removal properties of hyperaccumulator plants, such as *Thlaspi caerulescens*, could be transferred to high-biomass producing species, such as Indian mustard (*Brassica juncea*) or maize (*Zea mays*) (Brown et al., 1995). In an effort to correct for small size of hyperaccumulator plants, Brewer et al. (1997) generated somatic hybrids between *T. caerulescens* (a Zn hyperaccumulator) and *Brassica napus* (canola), followed by hybrid selection for Zn tolerance. High biomass hybrids with superior Zn tolerance were recovered.

The use of genetic engineering to modify plants for metal uptake, transport and sequestration may open up new avenues for enhancing efficiency of phytoremediation. Metal chelator, metal transporter, metallothionein (MT), and phytochelatin (PC) genes have been transferred to plants for improved metal uptake and sequestration. For example, in tobacco (*Nicotiana tabacum*) increased metal tolerance has been obtained by expressing the mammalian metallothionein, metal-binding proteins, genes (Maiti et al., 1991).

Transgenic plants, which detoxify/accumulate Cd, Pb, Hg, As and Se have been developed. The most spectacular application of biotechnology for environmental restoration has been the bioengineering of plants capable of volatilizing Hg from soil contaminated with methylmercury. Methyl-mercury, a strong neurotoxic agent, is biosynthesized in Hg-contaminated soils. To detoxify this toxin, transgenic plants (*Arabidopsis* and tobacco) were engineered to express bacterial genes merB and merA. In these modified plants, merB catalyzes the protonolysis of the carbonmercury bond with the generation of Hg^{2+}, a less mobile mercury species. Subsequently, MerA converts Hg(II) to Hg (0) a less toxic, volatile element which is released into the atmosphere (Rugh et al., 1996). Hg reductase has also been successfully transferred to *Brassica,* tobacco and yellow poplar trees (Meager et al., 2000).

Phytoremediation efficiency of plants can be substantially improved using genetic engineering technologies. Recent research results, including overexpression of genes whose protein products are involved in metal uptake, transport, and sequestration, or act as enzymes involved in the degradation of hazardous organics, have opened up new possibilities in phytoremediation. A better understanding of the mechanisms of rhizosphere interaction, uptake, transport and sequestration of metals in hyperaccumulator plants will lead to designing novel transgenic plants with improved remediation traits. As more genes related to metal metabolism are discovered, facilitated by the genome sequencing projects, new vistas will be opened up for development of efficient transgenic plants for phytoremediation. It is also expected that recent advances in biotechnology will play a promising role in the development of new hyperaccumulators by transferring metal hyperaccumulating genes from low biomass wild species to the higher biomass producing cultivated species in the times to come.

Technology development

Phytoremediation is an emerging technology, potentially effective and applicable to a number of different contaminants and site conditions. Major limitations of the present research lacks data related to the mass balance of the metals. In addition, the problem is compounded by metal leaching away from the original source. The cost associated with phytoremediation is difficult to estimate because of lack of economic data. It is likely, however, that the cost will be very much site specific. Recently, a group of scientist ranked a variety of metals with respect to phytoextraction research status, readiness for commercialization, and regulatory acceptance of the technology (Lasat, 2000). Results of this evaluation are shown in *Table 4*.

Table 4. *Current research status, readiness for commercialization, and regulatory acceptance of phytoremediation for several metal and metalloid contaminants*

Metal	Contaminant							
	Ni	Co	Se	Pb	Hg	Cd	Zn	As
Commercial readiness*	4	4	4	4	3	2	3	1
Regulatory acceptance**	Y	Y	N	Y	N	Y	Y	N

** rating: 1- basic research underway; 2- laboratory stage; 3- field deployment; 4- under commercialization.*
*** Regulatory acceptance: Y- yes, N- no.*

Conclusions

Metal mine waste generally contain anomalous concentration of metals, which inhibit the plant colonisation. Metal being non-biodegradable, phytoremediation techniques are the only viable solution to decontaminate the metal contaminated land. Even though, there are several processes of phytoremediation and different plant species have been used, role of grasses, legumes and some tree species has been well established. Out of the several grass and legume species reported, *Vetiveria* sp., *Sesbania* sp were found to be most promising for bioremediation of tailings pond. Adding organic amendment facilitates the effective establishment and colonisation of pioneer species. The research work showed that, bioavailability and metal uptake by plants could be accompalished by ameliorating pH, adding chelating agents, using appropriate fertilisers and altering soil ion composition. Further research is required to develop fast growing high biomass plants with improved metal uptake, translocation and tolerance through genetic engineering for effective phytoremediation of metal mine wastes.

REFERENCES

[1] Baker, A.J.M., Brooks, R.R. (1989): Terrestrial higher plants which hyperaccumulate metallic elements – a review of their distribution, ecology and phytochemistry. ─ Biorecovery 1: 81-126.

[2] Baker, A.J.M., Walker, P.L. (1990): Ecophysiology of metal uptake by tolerant plants. – In: Shaw, A.J. (ed.) Heavy Metal Tolerance in Plants: Evolutionary Aspects, CRC Press, Boca Raton, FL, pp 155-177.

[3] Barceló, J., Poschenrieder, C. (2003): Phytoremediation: principles and perspectives. ─ Contributions to Science 2(3): 333-334.

[4] Bech, J., Poschenrieder, C., Barcelo, J., Lansac, A. (2002): Plants from Mine Spoils in the South American Area as Potential Sources of Germplasm for Phytoremediation Technologies. ─Acta Biotechnologica 22: 5-11.

[5] Berti, W.R., Cunningham, S.D. (2000): Phytostabilizaton of metals. – In: Raskin, I., Ensley, B. (eds.) Phytoremediation of toxic metals: Using plants to clean up the environment, Wiley Interscience, New York, pp. 71-88.

[6] Blaylock, M.J., Elless, M.P., Huang Jianwei, W., Dushenkov, S.M. (1999): Phytoremediation of lead-contaminated soil at a New Jersey brownfield site. ─ Remediation 9(3): 93-101.

[7] Blaylock, M.J., Huang, J.W. (2000): Phytoextraction of metals. ─ In: Raskin, I., Ensley B.D. (eds.) Phytoremediation of toxic metals: Using plants to clean the environment, John Wiley and Sons Inc., New York, pp. 314.

[8] Bradshaw, A. (1997): Restoration of mined lands - using natural processes. ─Ecol. Eng. 8: 255-269.

[9] Brewer, E.P., Saunders, J.A., Angle, J.S., Chaney, R.L., McIntosh, M.S. (1997): Somatic hybridization between heavy metal-hyperaccumulating *Thlaspi caerulescens* and canola. ─Agron. Abstr. 1997: 154.

[10] Brown, S.L., Chaney, R.L., Angle, J.S., Baker, A.J.M (1994): Zinc and cadmium uptake by *Thlaspi caerulescens* and *Silene vulgaris* in relation to soil metals and soil pH. ─ J Environ. Qual. 23: 1151-1157.

[11] Brown, S.L., Chaney, R.L., Angle, J.S., Baker, A.M. (1995): Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. ─Soil Sci. Am. J. 59: 125-133.

[12] Cunningham, S.D., Ow, D.W. (1996): Promises and prospects of phytoremediation. ─ Plant Physiol. 110: 715-719.

[13] Das, M., Maiti, S.K. (2007): Metal accumulation in 5 native plants growing on abandoned Cu- tailings ponds. ─Applied Ecology and Env Res. 5(1): 27-35.

[14] Das, M., Maiti, S.K. (2007): Metal accumulation in A. *baccifera* growing naturally on abandoned copper tailings pond. ─Env. Monit. and Assess 127: 119-125.

[15] Das, M., Maiti, S.K. (2008): Comparison between availability of heavy metals in dry and wetland tailing of an abandoned copper-tailing pond. ─Envt. Moni & Assess Vol. 137(1-3): 343-350.

[16] Dinelli, E., Lombini, L. (1996): Metal distribution in plants growing on copper mine spoils in Northern Apennies, Italy: the evaluation of seasonal variations. — Applied Geochemistry 11: 375-385.

[17] Dushenkov, V., Nanda Kumar, P.B.A., Motto, H., Rakin, I. (1995): Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. —Environ. Sci. Technol. 29: 1239-1245.

[18] Freitas, H., Prasad, M.N.V., Pratas, J. (2004): Plant community tolerant to trace elements growing on the degraded soils of São Domingos mine in the south east of Portugal: environmental implications. — Environmental International 30(1): 65-72.

[19] Ghosh, M., Singh, S.P. (2005): A review on phytoremediation of heavy metals and utilization of it's by products. – Applied Ecology and Environmental Research 3(1): 1-18.

[20] Hall, J.L., (2002): Cellular mechanisms for heavy metal detoxification and tolerance. — J Exp. Bot. 53: 1-11.

[21] Jaffre, T., Brooks, R.R., Lee, J., Reeves, R.D. (1976): *Sebertia accuvzinata:* a nickel-accumulating plant from New Caledonia. —Science 193: 579-580.

[22] Jordahl, J.L., Foster,L., Schnoor, J.L., Alvarez, P.J.J. (1997): Effect of hybrid poplar trees on microbial populations important to hazardous waste bioremediation. — Environ. Toxicol. Chem. 16(6): 1318-1321.

[23] Khan, A.G. (2001): Relationship between chromium biomagnification ratio, accumulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. —Environ. Int. 26: 417-423.

[24] Kirk, I., Klironomos, I., Lee, H., Trevors, J.T. (2005): The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil. — Environ. Pollut. 133: 455-465.

[25] Komárek, M., Tlustoš, P., Száková, J., Chrastný, V., Ettler, V. (2006): The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. —Chemosphere 67: 640-651.

[26] Konstantinou, M., Babalonas, D. (1996): Metal uptake by Caryophyllaceae species from metalliferous soils in northern Greece. —Plant Systematics and Evolution 203: 1-10.

[27] Lan, C.Y., Shu, W.S., Wong, M.H. (1997): Revegetation of Pb/Zn mine tailings: phytotoxicity of the tailings. — In: Wise, D.L. (ed.), Global environmental biotechnology, Elsevier Science, London, pp 119-130.

[28] Lasat, M.M., (2000): Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. — J. hazardous Substances Research 2(5): 1-25.

[29] Maiti, I.B., Wagner, G.J., Hunt, A.G. (1991): Light-inducible and tissue-specific expression of achimeric mouse metallothionein cDNA gene in tobacco. — Plant Sci. 76: 99-107.

[30] Maiti, S.K., Nandhini, S. (2005): Bioremediation of Fe ore tailings and bioaccumulation of metals in the naturally ocurring vegetation: A case of Noamundi Fe-ore tailings, TISCO, Noamundi. — Int. seminar on " Mineral Processing Technology (MPT - 2005), January 6-8, 2005.

[31] Maiti, S.K., Nandhini, S., Das, M. (2005): Accumulation of metals by naturally growing herbaceous and tree species in iron ore tailings. — Int. J. of Environmental Studies 62(5): 593-603.

[32] Meager, R.B., Rugh, C.L., Kandasamy, M.K., Gragson, G., Wang, N.J. (2000): Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. — In: Terry, N., Bañuelos, G. (eds.) Phytoremediation of Contaminated Soils and Waters, CRC Press LLC, Boca Raton, FL, USA, pp 201-219.

[33] Neumann, P.M., De Souza, M.P., Pickering, I.J., Terry, N. (2003): Rapid microbial metabolism of selenate to volatile dimethylselenide. – Plant Cell Environ. 26: 897-905.

[34] Newman, L., Strand, S., Choe, N., Duffy, J., Ekuan, G., Ruszaj, M., Shurtleff, B., Wilmoth, J., Heilman, P., Gordon, M. (1997): Uptake and biotransformation of trichloroethylene by hybrid poplars. –Environ Sci.Tech. 31: 1062-1067.

[35] Pichtel, J., Salt, C.A. (2003): Vegetative growth and trace metal accumulation on metaliferous wastes. – J. Environ. Qual. 27: 618-642.

[36] Raskin, I., Ensley, B.D. (2000): Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment. – John Wiley & Sons, Inc., New York.; pp 50-70.

[37] Reeves, R.D., Baker, A.J.M. (2000): Metal accumulating plants. – In: Raskin, I., Ensley, B.D. (eds.) Phytoremediation of toxic metals: Using plant to clean up the environment, Wiley, New York, pp 193-230.

[38] Rugh, C.L., Wilde, H.D., Stack, N.M., Thompson, D.M., Summers, A.O., Meagher,R.B. (1996): Mercuric ion reduction and resistance in transgenic *Arabidopsis thaliana* plants expressing a modified bacterial *merA* gene. –Proc. Natl. Acad. Sci. USA 93: 3182-3187.

[39] Salt, D.E., Smith, R.D., Raskin, I. (1998): Phytoremediation. – Ann. Rev. Plant Mol. Biol. 49: 643-668.

[40] Shu, W., Zhao, Y., Yang, B., Xia, H., Lan, C. (2004): Accumulation of heavy metals in four grasses grown on lead and zinc mine tailings. – J. of Env. Sciences 16: 730-734.

[41] Singh, A.N., Raghubanshi, A.S., Singh, J.S. (2004): Impact of native tree plantations on mine spoil in a dry tropical environment. –Forest Ecology and Management 187: 49-60.

[42] Smith, R.A.H., Bradshaw, A.D. (1979): The use of metal tolerant plant populations for the reclamation of metalliferous wastes. –J. Appl. Ecol. 16: 595-612.

[43] Van Huysen, T., Terry, N., Pilon-Smits, E.A.H. (2004): Exploring the selenium phytoremediation potential of transgenic Indian mustard overexpressing ATP sulfurylase or cystathionine-γ-synthase. –Int. J. Phytoremediation 6: 1-8.

[44] Wang, J., Evangelou, V.P. (1994): Metal tolerance aspects of plant cell walls and vacuoles. – In: Pessaraki, M. (ed.) Handbook of Plant and Crop Phyiology, Marcel Dekker, Inc, NewYork, pp 695-717.

[45] Williamson, A., Johnson, M.S. (1981): Reclamation of metalliferous mine wastes. – In: NW Lepp (ed.) Effect of Heavy Metal Pollution on Plants. Vol. 2, Metals in the Environment. Applied Science Publishers, London, pp185-212.

[46] Yang, B., Shu, W., Ye, Z., Lan, C., Wong, M. (2003): Growth and Metal Accumulation in Vetiver and two *Sesbania* Species on Lead/Zinc Mine Tailings. – Chemosphere 52: 1593-1600.

[47] Zhuang, P., Yang, Q.W., Wang, H.B., Shu, W.S. (2007): Phytoextraction of Heavy Metals by Eight Plant Species in the Field. –Water, Air, & Soil Pollution 184: 235-242.

[48] Zhuang, P., Ye, Z.H., Lan, C.Y., Xie, Z.W., Shu, W.S. (2005): Chemically Assisted Phytoextraction of Heavy Metal Contaminated Soils using Three Plant Species. – Plant and Soil 276: 153-162.

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THE POSSIBILITIES OF BIODIVERSITY MONITORING BASED ON HUNGARIAN LIGHT TRAP NETWORKS

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Abstract Our method is presented with displaying time series, consisting of the daily amount of precipitation of 100 years, which has meant a separate challenge, as the precipitation data shows significant deviations. By nowadays, mankind has changed its environment to such an extent that it has a significant effect on other species as well. The Lepidoptera data series of the National Plant Protection and Forestry Light Trap Network can be used to justify this. This network has a national coverage, a large number of collected Lepidoptera, and an available, long data series of several years. For obtaining information from these data, the setting up of an easy to manage database is necessary. Furthermore, it is important to represent our data and our results in an easily analysable and expressive way. In this article the setting up of the database is introduced, together with the presentation of a three dimensional visualization method, which depicts the long-range and seasonal changes together.
Keywords: *biodiversity, monitoring, data mining, Lepidoptera*

Introduction

The spreading and the structure of ecological associations significantly depend on environmental factors and resources. This is called ecological niche, which can be perceived as part of an n dimensional space that is used by the given population (n dimension means the n number of different environmental effects and resources).

There is no opportunity on an examination of population dynamics to investigate all (n) environmental factors. Therefore we only looked for relationships between a few relevant environmental parameters and the data collected by monitoring the communities. In view of these correlations we attempted to draw conclusions about the future state of the population.

The size and the structure of populations are influenced by several factors: agriculture, urbanization, climate, soil, vegetation, solar radiation, etc. However, these effects are not independent from each other. Climate change has an effect on each component. Thus, environmental effects (biotic and abiotic), cannot be examined independently from each other. That is, no such ideal circumstances can be created, where it could be investigated for example how the population is effected by temperature changes, since it has an effect on other influential environmental factors as well, which can have an effect on the investigated population. The investigation is further complicated by the fact that climate is not the only thing having an effect on the population and its surroundings. Primarily human activity should be mentioned here.

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One of the big problems of these times is that the data available for us are growing at an incredible pace. Filtering out important data from the databases is getting to be an increasing problem.

The aim of our work is to create a database from the Lepidoptera data of the light trap network, which assures the availability of data for the purpose of writing this article and further researches in an easily manageable form. Besides this we have introduced a three-dimensional depicting method in this article, which presents time series figures in an expressive way.

A Visual-Basic program has been made for data processing, evaluating and visualizing the results. We chose this programming language primarily because it can easily be set up for the direct use of Excel and Access files. These programs are suitable for the graphical visualization of long time series with the help of Autocad and ArcGIS graphical programs.

Review of literature

The most widespread collection method of Lepidoptera flying at night is light trapping. This method was first employed following the experiments of Williams (1935). Light traps have been used since 1940 in Hungary. In 1952 the construction of an internationally unique trap network began (Jermy, 1961; Nowinszky, 2003a). By now, the Hungarian light trap system has been equipped uniformly with Jermy type light traps.

Those light traps that have been operating for a long time uninterrupted, in the same place are the most suitable for population dynamics investigation (Nowinszky, 2003b).

It is practical to use all the light trap data because of the effects of different abiotic factors. This way it is achievable that the effects appearing in different collection places and modifying the number of collections neutralize each other (Nowinszky, 2003b).

The longest possible time series (daily data series) is needed to define the changes in a data series and its tendencies in the most reliable way. It should cover the largest possible geographical area and data collection should be carried out with the same method all along. The data series of the National Plant Protection and Forestry Light Trap Network is the most adequate for these conditions (Hufnagel et al., 2008).

Large quantity data coming from different sources can be processed with methods of data mining. As a first step data warehouses are created from databases (Böhlen, 2003; Fan, 2009; Han and Kambel, 2004; Keim, 2004). This procedure is preprocessing (Kennedy, et al. 1998; Pyle, 1999), during which the automatically detectable defective data are removed. The rest of the defective data can be filtered out only with human assistance, in an interactive way (Han and Kambel, 2004).

With the joining of databases a data structure is created, which ensures data access according to several points of view. The most suitable structure for this is an n-dimensional data cube (Euler, 2005; Gray et al., 1997).

The moving average method can be used for filtering out extremes appearing in databases and for decreasing the fluctuations in data series (Heuvelink and Webster, 2001). It smoothes the data series at the same time(Han and Kambel, 2004).

Image visualization is closer to human thinking than large tables containing numerical data, which, though, provide exact information, but are difficult to handle and they are not suitable to present correlations (Gimesi, 2004) either. When analysing calculation results, it can be helpful if the data is presented in an easily interpretable, graphical form.

We used three-dimensional figures for the presentation of long time series, where the yearly and seasonal changes could be seen well – these are outlined by Gimesi (2009). A similar depicting method was used by Mulligan (1998) for the demonstration of the seasonal changes of vegetation. He remarked that the method is able to demonstrate both short- and long-range tendencies. For the demonstration of Lepidoptera data, three-dimensional figures were also used by Marchiori and Romanowski (2006).

Diversity indices are numerical functions defined on sets of species frequency or species occurence probability(Izsák, 2001). So the diversity of a biozoenosis – in an ecological sense – is some kind of a function of the number and abundance of species.

Biological diversity primarily means the variousness of species regarding a given area and a given period. Species, genus or genetic diversities can be studied, such as epidemiologic or population diversities (Izsák, 1994; Izsák and Juhász-Nagy, 1984).

However, diversity indices do not provide information about the spatial position of entities, which can characterize the community at least as much as the number of species or the diversity (Menhinick, 1962).

In statistical ecology numerous functions are applied as diversity indices (Dewar and Porté 2008; Izsák, 2001; Mishra et al., 2009; Sipkay et al., 2005; Tóthmérész, 1997). Different diversity indices described in the ecological literature present the diversity of a given species community from different points of view. It is general experience that the diversity of numerous fauna and flora communities measured by different indices show significant positive correlation. The main reason for this is the high sensibility of indices to the change of population with the largest number of entities. Indices depend on the size of the sample, though to different extent (Ibáňez et al.,1995).

Numerous methods have been worked out to characterize diversity, which can be assorted as follows, according to Tóthmérész (2001):

- Number of species,
- Diversity indices,
- Classical diversity statistics,
- Scale-pending characterization of diversity,
- Mosaicity, the role of patterns (β-diversity),
- Space-series analysis.

Shannon diversity index is the most commonly used in ecological literature (e.g.: Arnan et al., 2009; Balog et al., 2008; Chefaoui and Lobo, 2008; Kevan, 1999; Skalskia and Pośpiech, 2006), therefore we also investigated the distribution of collected Lepidoptera with the help of this one.

Table 1. *Trap statistics (highlighted traps worked for the longest time)*

Trap Code	Date Start	Date End	Operation Time [months]	Number of Individuals	Number of Species	Name of Trap
1	Nov. 1961.	Dec. 1975.	139	63948	572	Budakeszi
2	Jun. 1961.	July. 1991.	302	602476	798	Makkoshotyka
3	Jun. 1961.	Dec. 2006.	441	285752	776	Felsőtárkány
4	Apr. 1962.	Dec. 2006.	421	291694	677	Gerla-Gyula
5	Jan. 1962.	Apr. 1976.	147	29852	60	Kunfehértó
6	Apr. 1965.	Oct. 1990.	185	134303	588	Farkasgyepű
7	Jun. 1961.	Dec. 2006.	364	102903	662	M.Háza,M.Almás
8	Mar. 1962.	Nov. 2006.	417	298604	686	Répáshuta
9	Feb. 1962.	Oct. 2006.	393	193967	770	Sopron
10	Aug. 1967.	Jun. 1973.	45	11267	416	Szakonyfalu
11	Jan. 1961.	Dec. 2006.	354	301795	697	Szentpéterfölde
12	Mar. 1962.	Feb. 1977.	153	39242	520	Szombathely
13	Jun. 1961.	Dec. 2006.	403	325227	689	Tolna
14	Mar. 1962.	Oct. 2006.	445	715915	696	Tompa
15	Feb. 1962.	Nov. 2006.	446	476072	765	Várgesztes
16	Jun. 1969.	Aug. 1990.	149	25183	479	Gyulaj-Kocsola
17	Aug. 1969.	Dec. 2006.	334	175334	698	Erdősmecske
18	Aug. 1969.	Aug. 2003.	85	48671	552	Kömörő
19	Aug. 1969.	Aug. 1975.	48	578	33	Kőkút
20	Sept. 1969.	Jun. 1974.	28	1125	30	Alsókövesd
21	Mar. 1970.	Aug. 1975.	45	1656	30	Zalaerdőd
22	July. 1972.	Aug. 1995.	204	82770	621	Piliscsaba
23	May. 1978.	Nov. 2006.	186	200144	638	Gilvánfa-Sumony
24	Sept. 1977.	Dec. 2006.	243	557826	638	Kapuvár
25	Aug. 1976.	Sept. 1995.	152	87831	458	Karcag-Apavára
26	July. 1976.	Dec. 2006.	253	326855	656	Bugac
27	July. 1976.	Nov. 2006.	253	183090	672	Nagyrákos-Szala
28	Jun. 1976.	Oct. 2003.	147	68158	538	Szulok
29	May. 1976.	Aug. 1985.	72	41544	526	Zalaszántó-Supr
30	Sept. 1975.	Nov. 2006.	215	183190	675	Sárvár-Baj-Acsád
31	Mar. 1988.	Oct. 1990.	23	6080	62	Bejcgyertyános
32	1977. Apr.	July. 2006.	268	161841	618	Sasrét
33	Sept. 1979.	Nov. 2000.	188	190769	548	Jánkmajtis
34	May. 1990.	Nov. 2006.	154	151071	560	Diósjenő
35	Apr. 1992.	Jun. 1995.	14	1785	141	Nagylózs
36	Mar. 1992.	Oct. 1998.	38	19153	394	Telkibánya
37	Sept. 1975.	Dec. 2006.	141	108785	493	Hőgyész-Tamási
38	Mar. 1981.	July. 1981.	5	1183	159	Ivánc
40	Mar. 1977.	Sept. 2003.	128	181664	568	Ásotthalom
41	May. 1993.	May. 1993.	1	39	18	Gödöllő
42	July. 1976.	July. 1979.	26	3094	301	Nádasd
43	Jun. 1977.	Aug. 1977.	3	457	54	Albertirsa
52	Sept. 1991.	Nov. 2006.	133	204087	702	Bakonybél-Somh
53	May. 1994.	Dec. 1995.	13	9468	313	Mosonmagyaróár
54	Dec. 1993.	Oct. 1995.	15	4285	195	Ásványráró
55	Mar. 1995.	May. 2001.	49	24170	368	Barcs-Krigóc
56	Apr. 1993.	Oct. 2006.	109	117965	510	Egyházaskesző
57	Apr. 1996.	Oct. 2006.	48	53304	434	Kecskemét
58	Apr. 1996.	Nov. 2003.	57	58244	517	Pilismarót
59	Apr. 1995.	Dec. 2006.	93	136481	459	Püspökladány
60	Mar. 1999.	Nov. 2006.	71	29617	466	Kemencepatak
61	Apr. 1999.	Sept. 1999.	6	6322	195	Maroslele
62	Mar. 2005.	Aug. 2006.	12	15209	289	Csöprönd
63	Mar. 2005.	Sept. 2006.	16	21667	335	Szentendre
64	Apr. 2005.	Dec. 2006.	17	35804	365	Vámosatya

Materials and methods

In the course of our work we used the National Plant Protection and Forestry Light Trap Network, the first light traps of which were installed in 1961 (Szontagh, 1975).

These traps are operating all year round, except for those days when the temperature does not rise above 0 °C, or when the area is covered by snow (Nowinszky, 2003a).We received the data of the National Plant Protection and Forestry Light Trap Network in dBase format. Our object was to create a database out of these hardly processable data that allows access to the data easily, in a general format. This way we have created a database essential for further researches.

The data of the National Plant Protection and Forestry Light Trap Network were processed using data-mining methods. As a first step – based on a method well known in the literature(Böhlen, 2003; Fan, 2009; Han and Kambel, 2004; Keim, 2004) – we created a data warehouse out of the available databases. This process included the merge and the filtering of databases (Bogdanova and Georgieva, 2008).

The trap statistics created from the trap data can be seen in *Table 1.* In this table the beginning and the end of the operation are shown, together with the operation time in months, the total number of collected individuals, the number of collected species, and the name of the trap (its geographical position).

The merge of databases

The original (light trap collection) data can be found in separate databases for each trap. The record structure of the original databases can be seen in *Fig. 1*.

Data

SORSZ	CSAPDA	K_KOD	A_EV	A_HO	D1	D2	......	D31	FELV	FIDO	JEL	IDO
:	:	:	:	:	:	:	:	:	:	:	:	:

Figure 1. *The record structure of the original databases*

The following fields can be found in the databases:

SORSZ (sn) – ordinal number of the measurement
CSAPDA (trap) – trap code
K_KÓD (l_code) – code of the insect species(Lepidoptera)
A_EV (yoh) – year of collection
A_HO (moh) – month of collection
D1-D30 – number of individuals collected daily
FELV (rec) – name of the data recorder
FIDO (torec) – date of recording
JEL (sign) – sign
IDO (time) – date of collection

Fig. 2 presents the record structure of trap and species databases. These are linked to the structure presented in *Fig. 1*.

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Trap

CS_KOD	CS_NEV	MENT
:	:	:

Species

K_KOD	K_NEV	K_RNEV	KONYV
:	:	:	:

Figure 2. *The record structure of trap and species databases*

The following fields can be found in the databases:

CS_KOD (trap) – trap code
CS_NEV (name) – name of the trap
MENT – one boolean data
K_KOD (species) – species code
K_NEV (name) – name of the species
K_RNEV (name) – short name
KONYV (name) – name by book

Fig. 3. demonstrates the relational connections of tables (records) presented in *Fig.1*, *Fig. 2*.

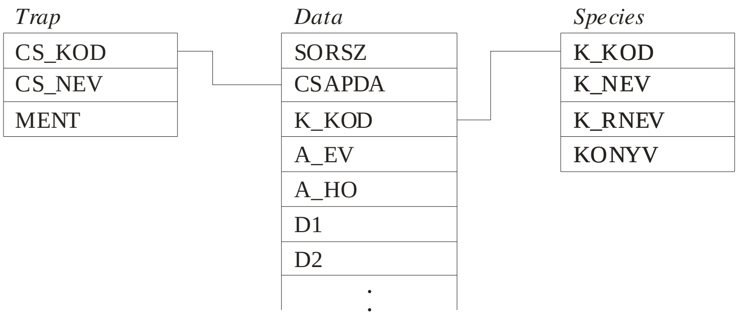

Figure 3. *The relational connections of data-tables*

With the merge of databases we created a data structure that ensures searching by trap code, Lepidoptera code, and date. The most suitable structure for this is the three-dimensional data cube that can be seen in *Fig. 4*. The dimensions of the cube are: *time*, *trap code*, *species code*. This way one elemental cube contains the number of species collected in a given trap on a definite day.

For the sake of quicker data access and the following graphical depiction we have divided the time dimension into year and day. Therefore we actually used a four-dimensional data cube.

While defining the ordinal number of the day – for the sake of uniformization – years were considered to contain 365 days, i.e. measurements made on 29th February were excluded. This did not cause any error, as during the 45-year period of investigation – considering all the traps and species – it meant leaving out altogether 109 individuals.

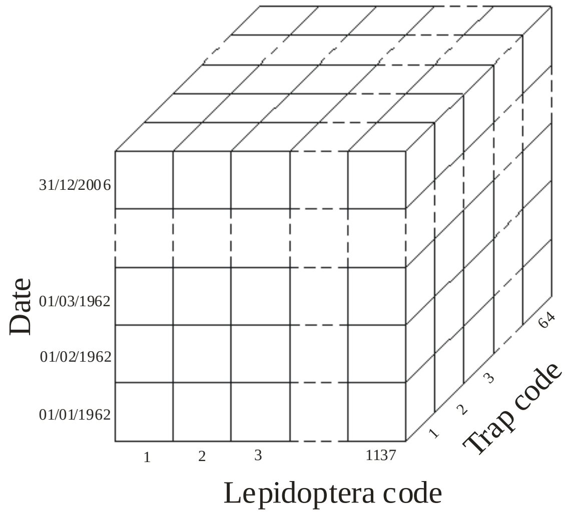

Figure 4. *The data cube*

Data filtering, data cleaning

During the course of creating the data warehouse we executed an automatically performable filtering, during which:

- incorrect dates coming from data recording mistakes were removed (only the data of the period between 1962 and 2006 were collected, the ordinal number of months had to be between 1 and 12, and the number of days had to correspond with the value belonging to the given month),
- those species codes that were not included in the species database have been filtered out,
- doubly recorded data were deleted.

The filtering out of other defective data can be executed in an automatic way only to a limited extent. In the rest of the cases an interactive (requiring human assistance) filtering can be carried out (Han and Kambel, 2004). The visualization method recited in this article is suitable for noticing flagrant (widely differing from the environment) data easily (Gimesi, 2008).

Filtering based on trap code

Those light traps that have been operating in the same place for a long time without interruption are the most suitable for the purpose of investigating population dynamics (Nowinszky, 2003b). Accordingly, we chose from the database those traps have worked for the longest time, mindful of having data of the highest possible number of days in the examined period. We have chosen those 9 traps that worked for the longest time between 1962 and 2006. The data of these are marked with highlighting in *Table 1*.

For the sake of further processing we distinguished between the cases when a trap did not operate and when it did not collect any specimen of the given species. A trap was considered not operating when no collection happened on a given day regarding all species.

The geographical position of the examined traps are demonstrated in *Fig. 5.* In this figure green (darker) rings indicate those settlements where the chosen traps can be found.

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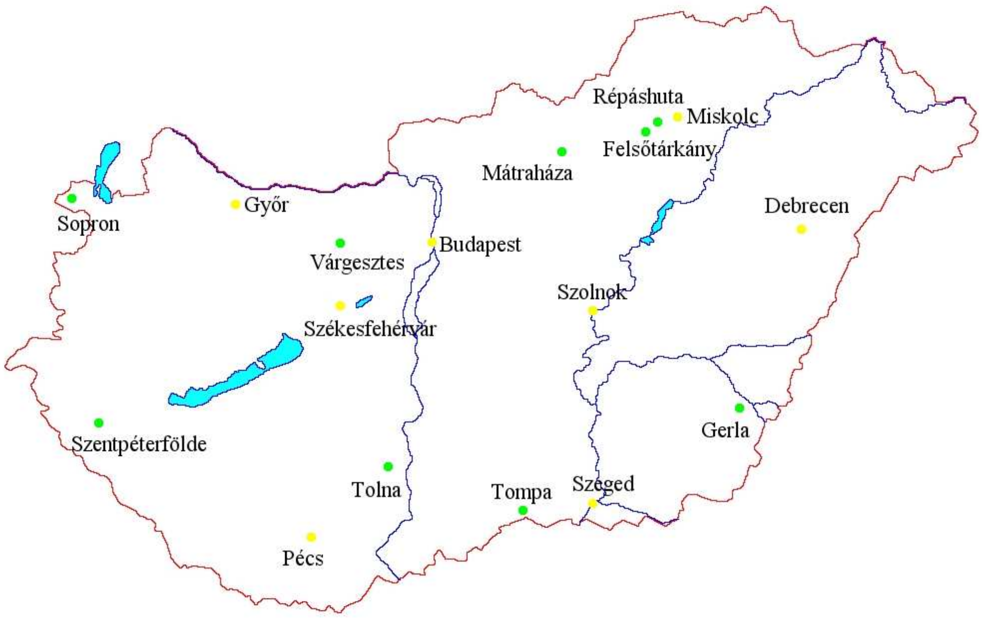

Figure 5. *The regional distribution of the examined light traps*

Filtering based on species code

From this point on only those species data were used where there was collection of at least one specimen every year in the examined period (1962-2006), considering all the traps. After filtering, altogether 281 species were left in the database.

After finishing data cleaning and data filtering the Lepidoptera database contained the data of 9 traps, 281 species, which altogether meant 4,020,614 records. The structure of the database is shown in *Fig. 6*.

Lepidoptera database

EV	NAP	CSAPDA	FAJ	DB
:	:	:	:	:

Figure 6. *The final structure of the Lepidoptera database*

The following fields could be found in the database:

EV (year) – year

NAP (day) – the ordinal number of the day within the year

CSAPDA (trap) – trap code

FAJ (species) – species code

DB – the number of individuals of the species collected on the given day by the given trap

Merging the trap data

For the sake of decreasing the different abiotic factors and the effects modifying the number of collections happening in different collection places, it is practical to use the data of the greatest possible number of light-tarps (Nowinszky, 2003b). For the creation of a national time series the data of the traps found in different places had to be merged by species. This is the so called data reducing method (Moon and Kim, 2007), that was carried out by a moving-average calculation. The method of moving-average is suitable for filtering out the extremes occurring in the daily data and to decrease the fluctuations in the data series (Heuvelink and Webster, 2001). This method is also smoothing the time series (Han and Kambel, 2004).

During the moving-average calculations we used the average of 9 days' (moving-average of 9th grade). We chose number 9, because it corresponded to the number of traps. *Fig. 7* demonstrates the window-method that we used to calculate the average, but with fictive data. The figure shows the calculation of the merged data of a given species. Days can be found in the vertical direction, and traps in the horizontal one. "-1" in a cell indicates that the trap did not operate on the given day. In such case, when calculating the average, the content of the cell is not added to the sum and the value of the divisor is not increased either.

Traps

Days

0	3	2	0	-1	-1	2	0	0
2	4	0	-1	2	1	4	3	1
-1	0	1	-1	5	1	0	5	4
1	-1	2	0	1	0	3	-1	2
0	-1	5	2	-1	5	0	4	1
0	5	1	3	0	2	-1	5	0
1	0	4	-1	5	4	0	2	3
-1	-1	2	0	5	1	5	-1	2
-1	3	0	3	-1	2	4	0	-1

Figure 7. *The window used for average calculation*

The following formula was used for the calculation of average:

$$\frac{1}{n}\sum_{i=k}^{k+8}\sum_{j=1}^{9} d_{i,j} \qquad k = 1\ldots16417$$

,where:

$d_{i,j}$ = the value of the cell (number of individuals), where i is the ordinal number of the trap, j is the ordinal number of the day (cells containing 1 are not calculated!)

n = the number of cells not containing 1

k = the first day of the window

The maximum value of k is the number of days between 1st January 1962 and 31st December 2002, minus 8.

After the calculation of average the data structure outlined in *Fig. 8* was presented, where the rows of the table show the years and the ordinal number of the day within the year (16425 rows), and the columns show the species code (281 columns).

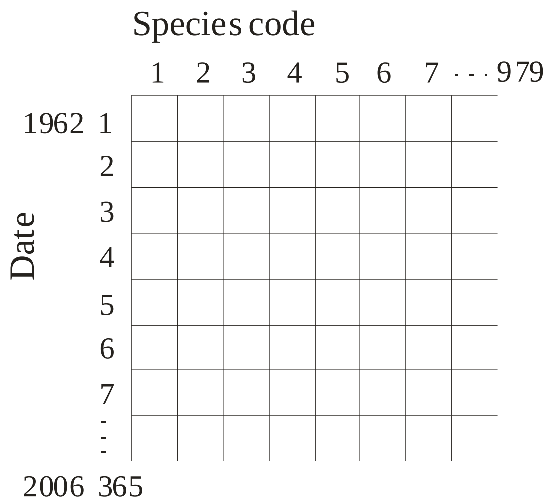

Figure 8. *The Lepidoptera data table after the average calculation*

We made a program using Visual Basic language for the creation of the Lepidoptera data warehouse, for the data filtering and for further data processing.

Results

Biological diversity means primarily the diversity of species concerning a given area and a given period of time. For the characterization of diversity we used the number of individuals, the number of species, and the Shannon diversity index.

Time series of aggregate collection

The time series of the number of Lepidoptera collected between 1962 and 2006 is demonstrated in *Fig. 9*.

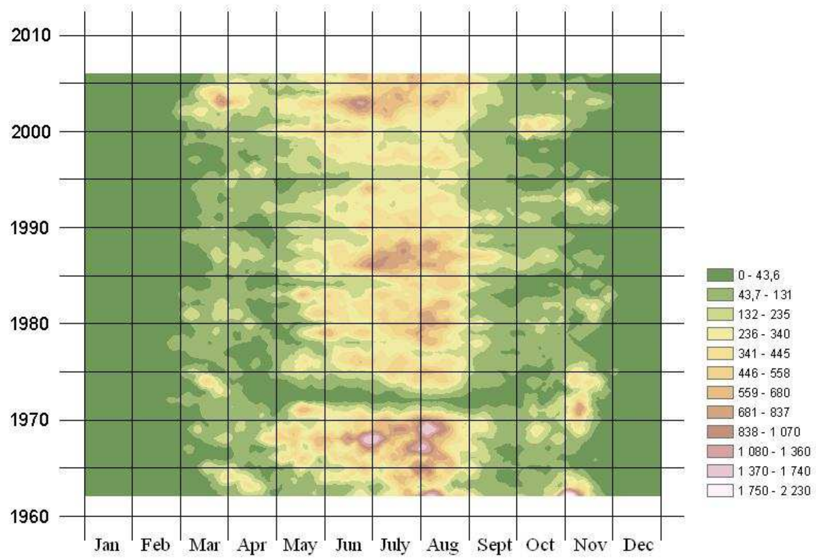

Figure 9. *The three-dimensional time series figure of the number of individuals based on the Lepidoptera data*

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It can be seen in the figure that the maximum of the number of individuals occurs in the middle of summer, but there are smaller peaks at the end of March, at the beginning of April and in November, as well.

In summer a significant increase can be observed in the number of individuals, which shows up every 15-20 years.

A remarkable anomaly can be observed in this figure and also in further time series figures in 1972 and 1973. The reason for this is that the definition of Lepidoptera was less accurate in this period.

Number of species (taxon)

One of the most important diversity indices is the number of species (Tóthmérész, 2002), the measure of which depends on the number of entities collected and the attraction zone of the traps. Its drawback is that it does not make a difference between populous species and those that are represented by one or a few entities and moreover, it is territory dependent.

Fig. 10 shows the distribution of the number of collected species. There are fractions in it as well, because the figure was made by interpolation.

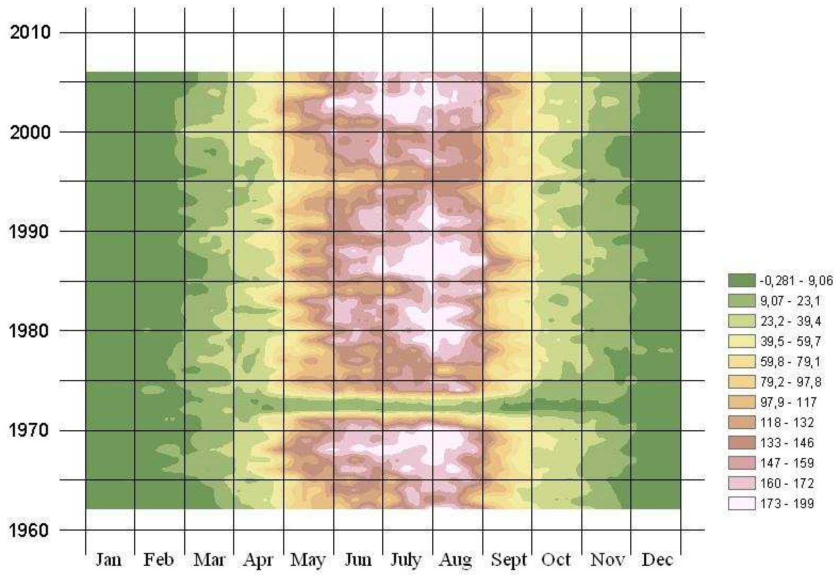

Figure 10. *The three-dimensional time series figure of the number of species based on the Lepidoptera data*

The time series of the number of species shows a smoother picture than that of the number of individuals. However, it can be observed here as well that there are periods (years) when the number of species is significantly higher compared to the neighbouring years.

In the literature Shannon index is used the most commonly for the characterization of diversity, therefore we have also used this for the analysis of our data.

This index is sensitive to the changes of rare species, that is its value decreases with the increase of the number of individuals of dominant species, but it increases with the increase of the number of species. The time series of the Shannon index can be seen in *Fig. 11*.

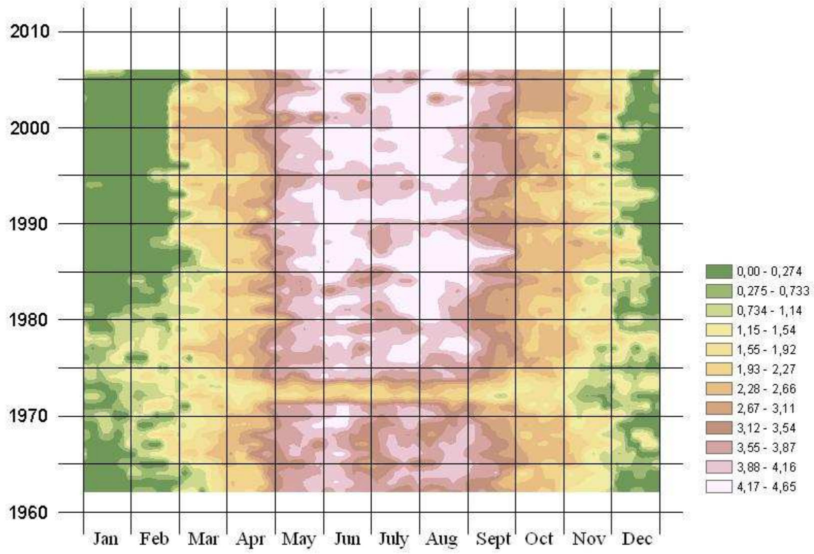

Figure 11. *The three-dimensional time series figure of the Shannon-Wiener index of Lepidoptera data*

It can be seen in the figure that the diversity index has maximums in the middle of June and of August. Accordingly, a decrease in diversity can be observed in July.

In the winter period between 1965 and 1980 relatively high diversity values can be seen, in spite of the fact that they cannot be seen either in the time series of the aggregate number of collected individuals (*Fig. 9*), or in that of the species (*Fig. 10*).

It is fully visible in all three figures that the values significantly depend on the season.

A significant anomaly can be noticed in 1972 and in 1973 in the time series figures. A likely reason for this is a personal change at that time, as a consequence of which the definition of Lepidoptera was carried out less precisely.

Fig. 12 shows the numbers of individuals collected daily during the 33- year period, *Fig. 13* show the dispersion as a function of days.

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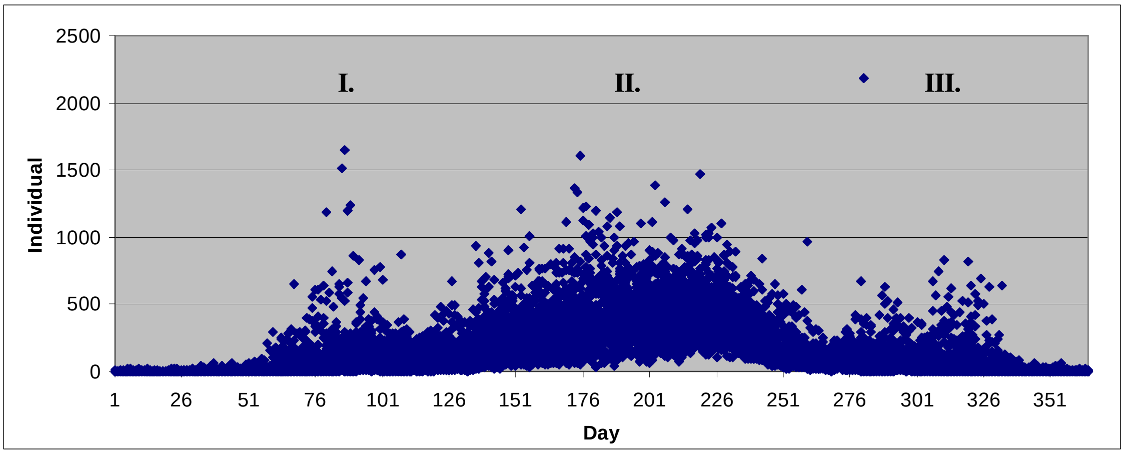

Figure 12. *The numbers of individuals collected daily based on Lepidoptera data*

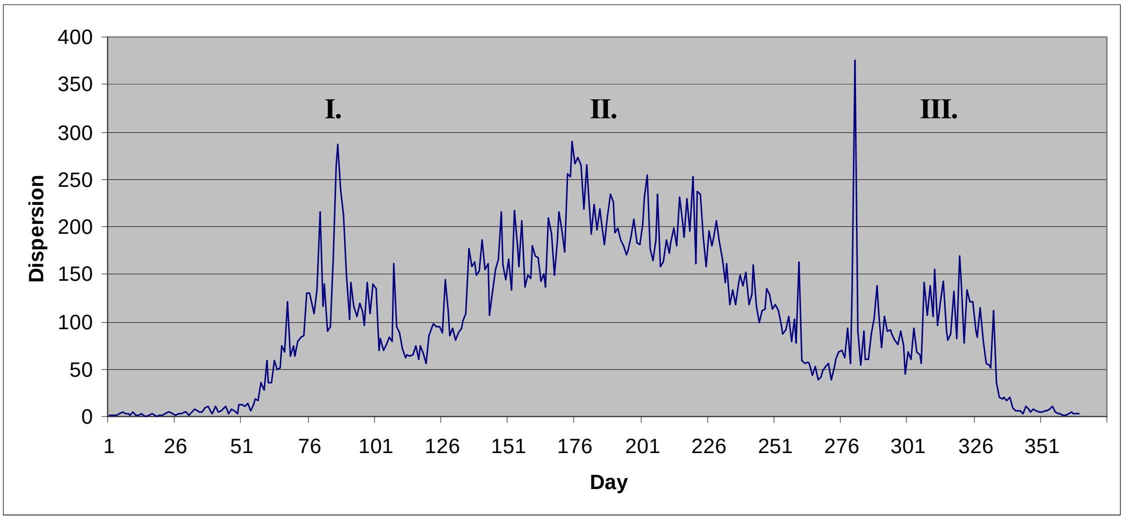

Figure 13. *Dispersion of the number of individuals collected daily*

In *Figure 13* three easily separable stages can be seen: the beginning of spring (I.), summer (II.), and late autumn (III.).

The increase in the numbers of individuals are caused by those dominant species that swarm in those periods.

We have made a list of the dominant species of the three periods, which is shown in *Table 2*. The dominant species appeare in a larger ratio during the spring and the autumn period. The reason for this is that in these periods the number of existing species is lower.

Species 42 and 43 are two-generational. 43 is also dominant in the autumn period, which can be seen in the table. Species 172 has two swarms as well, but both of them are in summer.

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Table 2. *The ratio of the dominant species in the three periods*

Period	Code	Species name	Ratio
Spring	40	Orthosia gothica (Linnaeus, 1758)	7,5 %
	41	Orthosia cruda (Denis & Schiffermüller, 1775)	15,6 %
	42	Eupsilia transversa (Hufnagel, 1766)	9,0 %
	43	Conistra vaccinii (Linnaeus, 1761)	25,2 %
	51	Alsophila aescularia ([Denis & Schiffermüller], 1775)	16,3 %
	449	Orthosia incerta (Hufnagel, 1766)	5,0 %
Summer	172	Ectropis bistortata (Goeze, 1781)	2,8 %
	240	Eilema complana (Linnaeus, 1758)	4,3 %
	398	Athetis furvula (Hübner,1808)	2,7 %
	411	Paracolax glaucinalis (Denis & Schiffermüller, 1775)	5,2 %
	515	Zanclognatha lunalis (Scopoli, 1763)	4,1 %
	519	Eilema lurideola (Zincken, 1817)	3,7 %
Autumn	43	Conistra vaccinii (Linnaeus, 1761)	3,5 %
	52	Alsophila quadripunctaria (Esper, 1800)	5,8 %
	54	Operophtera brumata (Linnaeus, 1758)	45,7 %
	63	Erannis aurantiaria (Hübner, 1799)	4,0 %
	65	Erannis defoliaria (Clerck, 1759)	16,8 %
	656	Ptilophora plumigera ([Denis & Schiffermüller], 1775)	8,7 %

Discussion

In this article the data processing of the National Plant Protection and Forestry Light Trap Network was introduced together with a possible visualization method.

The database created is based on the light trap data. It is suitable for utilization in the most important research areas of the national light trapping. These areas were summarized by Szentkirályi (2002). Among them are faunistical, zoogeographical, taxonomical, phytocenological, ethological, phenological, ecological, etc. examinations (Nowinszky, 2003c).

In case of examinations in swarming phenology the number of generations (Nowinszky, 2003b) and the seasonal changes can be determined by the daily depiction of the entity number of species. This method is widespread both in national and in international publications (Ábrahám and Tóth, 1989; Caldas, 1992; Kimura et al., 2008, Mészáros, 1993; Szentkirályi, 1984).

Investigations in population dynamics provide possibility to draw a conclusion about the tendency of change, based on the data of succeeding years (Nowinszky, 2003b). This method has also been used by several publications (Conrad et al., 2006; Leskó et al., 1997; Szentkirályi et al.,1995; Szontagh, 2001; Wolda et al., 1998). These publications depict the annual changes and those within a year separately. By merging these two methods we introduced a three-dimensional method that depicts the seasonal and long-term (annual) changes in one figure. The different time series can be depicted much more expressively with this method (Gimesi, 2008, 2009). A similar method was used by Marchiori & Romanowski (2006) to demonstrate insect collecting time series, and also by Mulligan (1998) to demonstrate the seasonal changes of foliages.

In this article we presented the sorting of those Lepidoptera data into databases which were collected by light traps and a visualization method for that. We did not

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examine the reasons considering what environmental effects led to certain changes in the time series.

In the future we are willing to perform different investigations with the help of the compiled database. For example: the behaviour of models of species abundance, the behaviour of linear quantile regressions, the regional distribution of different entities and the temporal change of that, and also the different biotic and abiotic effects on population dynamics.

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REFERENCES

[1] Ábrahám, G., Tóth, M. (1989): A káposzta-bagolylepke (Mamestra brassicae L.) rajzásdinamikai vizsgálata szexferomon-, illat- és fénycsapdával. – Növényvédelem 23(3): 119-122.
[2] Arnan, X., Gracia, M., Comas, L., Retana, J. (2009): Forest management conditioning ground ant community structure and composition in temperate conifer forests in the Pyrenees Mountains. – Forest Ecology and Management 258: 51-59.
[3] Balog, A., Marko, V., Adam, L. (2008): Rove beetles (Coleoptera: Staphylinidae) collected during the long term ecological research in a Hungarian oak forest. – Journal of Environmental Biology 29: 263-266.
[4] Bogdanova, G., Georgieva, T. (2008): Using error-correcting dependencies for collaborative filtering. – Data & Knowledge Engineering 66: 402-413.
[5] Böhlen, M. (2003): 3D visual data mining—goals and experiences. – Computational Statistics & Data Analysis 43: 445-469.
[6] Caldas, A. (1992): Mortality of Anaea ryphea (Lepidoptera: Nymphalidae) Immatures in Panama. – Journal of Research on the Lepidoptera 31(3-4): 195-204.
[7] Chefaoui, R.M., Lobo, J.M. (2008): Assessing the effects of pseudo-absences on predictive distribution model performance. – Ecological Modelling 210: 478-486.
[8] Conrad, K.F., Warren, M.S., Fox, R., Parsons, M.S., Woiwod, I.P. (2006): Rapid declines of common, widespread British moths provide evidence of an insect biodiversity crisis. – Biological Conservation 132: 279-291.
[9] Dewar, R.C., Porté, A. (2008): Statistical mechanics unifies different ecological patterns. – Journal of Theoretical Biology 251: 389-403.
[10] Fan, B. (2009): A hybrid spatial data clustering method for site selection: The data driven approach of GIS mining. – Expert Systems with Applications 36: 3923-3936.
[11] Gimesi, L. (2004). Mesterséges intelligencia alkalmazása a rekultivációban. – Acta Agraria Kaposváriensis 8(3): 1-9.
[12] Gimesi, L. (2008): The use of 3-dimensional graphic display method for presenting the changes in weather. – Applied Ecology And Environmental Research 6(1): 165-176.
[13] Gimesi, L. (2009): Development of a visualization method suitable to present the tendencies of the changes in precipitation. – Journal of Hydrology 377: 185-190.

[14] Gray, J., Chaudhuri, S., Bosworth, A., Layman, A., Reichart. D., Venkatrao, M., Pellow, F., Pirahesh, H. (1997): Data Cube: A Relational Aggregation Operator Generalizing Group-By, Cross-Tab, and Sub-Totals. – Microsoft Corporation, Data Mining and Knowledge Discovery 1: 29-54.

[15] Han, J., Kamber, M. (2004): Adatbányászat – Koncepciók és technikák. – Panem, Budapest.

[16] Heuvelink, G.B.M., Webster, R. (2001): Modelling soil variation: past, present, and future. – Geoderma 100: 269-301.

[17] Hufnagel, L., Sipkay, Cs., Drégely-Kis, Á., Farkas, E., Türei, D., Gergócs, V., Petrányi, G., Baksa, A., Gimesi, L., Eppich, B., Dede, L., Horváth, L. (2008): Klímaváltozás, biodiverzitás és közösségökológiai folyamatok kölcsönhatásai. – In: Harnos, Zs., Csete, L. (ed.): Klímaváltozás: Környezet – Kockázat – Társadalom. Szaktudás Kiadó Ház, Budapest, 229-266.

[18] Ibáňez, J.J., De-Alba, S., Bermúdez, F.F., García-Álvarez, A. (1995): Pedodiversity: concepts and measures. – Catena 24: 215-232.

[19] Izsák, J. (1994): Applying the jackknife method to significance tests of diagnostic diversity. – Methods of Information in Medicine 33: 214-219.

[20] Izsák, J., Juhász-Nagy, P. (1984): Studies of diversity indices on mortality statistics. – Annales Univ. Sci. Budapestienensis, Sectio Biologica 24-26: 11-27.

[21] Jermy, T. (1961): Kártevő rovarok rajzásának vizsgálata fénycsapdával. – A Növényvédelem Időszerű Kérdései 2: 53-61.

[22] Keim, D.A. (2004): Pixel based visual data miningof geo-spatial data. – Computers & Graphics 28: 327-344.

[23] Kennedy, R.I., Kee, Y., Van Roy, B., Reed, C.D., Lippman, R.P. (1998): Solving Data Mining Problems Through Pattern Recogntion. – Upper Saddle River, Prentice Hall.

[24] Kevan, P.G. (1999): Pollinators as bioindicators of the state of the environment: species, activity and diversity. Agriculture, Ecosystems and Environment 74: 373-393.

[25] Kimura, G., Inoue, E., Hirabayashi, K. (2008): Seasonal abundance of adult caddisfly (Trichoptera) in the middle reaches of the Shinano River in Central Japan. – 6th International Conference on Urban Pests, Budapest.

[26] Leskó, K., Szentkirályi, F., Kádár, F. (1997): A gyűrűsszövő (Malacosoma neustria L.) hosszútávú (1962-1996) populációingadozásai Magyarországon. – Erdészeti Kutatások 86-87: 207-220.

[27] Marchiori, M.O., Romanowski, H.P. (2006): Species composition and diel variation of a butterfly taxocene (Lepidoptera, Papilionoidea and Hesperioidea) in a restinga forest at Itapuã State Park, Rio Grande do Sul, Brazil. – Revista Brasileira de Zoologia 23(2): 443-454.

[28] Menhinick, E.F. (1962): Comparison of invertebrate populations of soil and litter of mowed grasslands in areas treated und untreated with pesticides. – Ecology 43: 556-561.

[29] Mészáros, Z. (1993): Bagolylepkék – Noctuidae. – In: Jermy, T., Balázs, K. (1993): A növényvédelmi állattan kézikönyve 4/B: 453-831.

[30] Mishra, A.K., Özger, M., Singh, V.P. (2009): An entropy-based investigation into the variability of precipitation. – Journal of Hydrology 370: 139-154.

[31] Moon, Y., Kim, J. (2007): Efficient moving average transform-based subsequence matching algorithms in time-series databases. – Information Sciences 177: 5415-5431.

[32] Mulligan, M. (1998): Modelling the geomorphological impact of climatic variability and extreme events in a semi-arid environment. – Geomorphology 24: 59-78.

[33] Nowinszky, L. (2003a): A fénycsapdázás kezdete és jelenlegi helyzete. – In: Nowinszky, L. (ed.) A fénycsapdázás Kézikönyve, Savaria University Press, Szombathely.

[34] Nowinszky, L. (2003b): A fénycsapdás gyűjtési adatok hasznosítása. – In: Nowinszky, L. (ed.) A fénycsapdázás Kézikönyve, Savaria University Press, Szombathely.

[35] Nowinszky, L. (2003c): A fénycsapdás gyűjtési adatok feldolgozási módszerei. – In: Nowinszky, L. (ed.) A fénycsapdázás Kézikönyve, Savaria Univ. Press, Szombathely.

http://www.ecology.uni-corvinus.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)

[36] Pyle, D. (1999): Data Preparation for Data Mining. – Morgan Kaufmann, San Francisco.
[37] Sipkay, Cs., Hufnagel, L., Gaál, M. (2005): Zoocoenological state of microhabitats and its seasonal dynamics in an aquatic macroinvertebrate assembly (hydrobiological case studes on Lake Balaton, №. 1). – Applied Ecology and Environmental Research 3(2): 107-137.
[38] Skalskia, T., Pośpiech N. (2006): Beetles community structures under different reclamation practices. – European Journal of Soil Biology 42: 316-320.
[39] Szentkirályi, F. (1984): Analysis of light trap catches of green and brown lacewings (Nouropteroidea: Planipennia, Chrysopidae, Hemerobiidae) in Hungary. – SIEEC X. Budapest, 177-180.
[40] Szentkirályi, F. (2002): Fifty-year-long insect survey in Hungary: T. Jermy's constributions to light trapping. – Acta Zoologica Academiae Scientiarum Hungaricae 48(1): 85-105.
[41] Szentkirályi, F., Szalay, K., Kádár, F. (1995): Jeleznek-e klímaváltozást a fénycsapdás rovargyűjtések? – Erdő és Klíma Konferencia, Noszvaj 1994: 171-177.
[42] Szontagh, P. (1975): A fénycsapda hálózat szerepe az erdészeti kártevők prognózisában. – Növényvédelem 11: 54-57.
[43] Szontagh, P. (2001): Rovarok okozta problémák és kihívások erdeinkben. – Erdészeti Tudományos Intézet Kiadványai 15: 35-37.
[44] Tóthmérész, B. (1997): Diverzitási rendezések. – Scientia, Budapest
[45] Tóthmérész, B. (2001): Kvantitatív ökológiai kutatások különös tekintettel a skálázási és mintázati problémákra. – In: Borhidi, A. (2001): Ökológia az ezredfordulón, Budapest, Akadémiai Kiadó, 15-25.
[46] Tóthmérész, B. (2002): A diverzitás jellemzésére szolgáló módszerek evolúciója. – In: Salamon-Albert, É. (2002): Magyar botanikai kutatások az ezredfordulón.
[47] Wolda, H., O'Brien, C.W., Stockwell, H.P. (1998): Weevil Diversity and Seasonally in Tropical Panama as Deduced from Light trap Catches (Coleoptera: Curculionoidea). – Smithsonian Institution Press, Washington, D.C.

EVALUATION OF BIODIVERSITY FOR MULTI-PURPOSE FOREST MANAGEMENT USING A NON-LINEAR OPTIMIZATION APPROACH

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Abstract. The paper applies a non-linear optimization approach to evaluate biodiversity in the multi-purpose modelling of forest management. An even-aged forest of pure Norway spruce was generated from large-scale inventory data of the Black Forest of southwest Germany. The effects of different management alternatives including five conversion schemes, two traditional age-class and single-tree-selection systems and a "Do-nothing" strategy were simulated using the forest growth model "BWINPro-S". Optimal allocation of management strategies to the forest area was found subject to the hard constraints of "area" and "protection" and the chance constraint "wood even flow". A utility function representing the value of biodiversity was estimated based on the opportunity costs of different biodiversity levels. The upper and lower boundaries of the function were determined by successive optimization runs. Subsequently, the obtained monetary value of biodiversity was directly integrated into the optimization of a particular forest to identify the optimal allocation of management schemes to entire forest enterprise. Conservation or "Do-Nothing" was the most desirable scenario combined with the partial establishment of beech regeneration. Economic and silvicultural consequences of the optimal multi-purpose forest management plan were discussed and compared to the alternative business-as-usual strategies.
Keywords: *Non-linear Optimization; Biodiversity; Multi-purpose Forest Management; Black Forest; Climate Change*

Introduction

Multi-purpose forest management and biodiversity

Sustainable management of forest ecosystems has to provide forest goods and services without jeopardizing the ecological and environmental quality. Therefore, in many countries, forest management practices have been changed and there is a trend to move away from homogeneous and non-site adapted even-aged plantation forestry to more close-to-nature ecosystems (Hanewinkel and Pretzsch, 2000; Schröder et al., 2007; Knoke et al., 2008). The conversion of pure coniferous stands into mixed-species forests, as an adaptation strategy to climate change (Böttcher, 2007) is a focus of contemporary forest management and policy in Europe (Bladt et al. 2009; Kint et al. 2009), especially in Germany (Hanewinkel and Pretzsch, 2000; Spiecker, 2003;

Schröder et al., 2007; Pretzsch et al., 2007). The scope of forest conversion is to enhance the level of biodiversity considerations in a cost-effective way based on the idea of multi-purpose forest management (Steuer and Schuler, 1978; Kangas and Kuusipalo, 1993; Briceno-Elizondo et al., 2008; Bladt et al., 2009). Nonetheless, a comprehensive analysis of forest conversion within multi-purpose forest management particularly including the valuation of biodiversity is still lacking.

One concern is that biodiversity and its related indices are very difficult to integrate into optimization models of forest management planning (Steuer and Schuler, 1978; Schulte et al., 1998; Hanewinkel and Pretzsch, 2000; Koskela et al., 2007). The reason for this is not only the lack of a unified theory of biodiversity (Schulte et al., 1998; Buongiorno and Gilles, 2003) and, consequently, of a universal indicator for biodiversity (Kangas and Kuusipalo, 1993; Zhou and Buongiorno, 2006; Yousefpour and Hanewinkel, 2009) but also the mathematical complexity (i. e. the non-linear nature) of most of the popular indices such as the Shannon index (Koskela et al., 2007).

Integration of biodiversity into forest optimization

In the present study, the management problem to be analyzed was the conversion of pure Norway spruce stands (*Picea abies* L. Karst) into mixed stands of Norway spruce and European beech (*Fagus sylvatica* L.) including economic and ecological aspects. To model forest conversion, a forest growth simulator is necessary in order to predict the effects of different silvicultural treatments on the development of the main forest characteristics (Hanewinkel, 2001; Hasenauer, 2006; Kint et al., 2009). Despite their limitations, these simulators are also practical in comparing consequences of such strategies considering a wide variety of forest characteristics such as biodiversity (Schröder, 2005). For this study, a single-tree growth simulator “BWINPro-S” was selected which included the necessary juvenile module to model the establishment of beech regeneration under the canopy of Norway spruce trees (Schröder et al., 2007; Röhle, 2009).

The integration of biodiversity in forest optimization is not new (Steuer and Schuler, 1978; Kangas and Kuusipalo, 1993; Buongiorno and Gilless, 2003; Kurttila et al., 2006; Briceno-Elizondo et al., 2008; Koskela et al., 2007). However, the studies have rarely identified the most desirable forest management plan based on a recommendable level of biodiversity, meaning a level that can be achieved under given framework conditions (e.g. constraints) and that – at same time – reaches an optimum (Kurttila et al., 2006).

In order to incorporate biodiversity into the process of forest management modeling, there is a need to monetize its value (Kangas and Kuusipalo, 1993; Kurttila et al., 2006; Koskela et al., 2007; Yousefpour and Hanewinkel, 2009). The standard approach therefore is an indirect one: biodiversity is formulated as a constraint within an optimization procedure to calculate its opportunity costs (Buongiorno and Gilless, 2003; Kurttila et al., 2006; Koskela et al., 2007). In this study, a more direct way is proposed to integrate biodiversity into a multi-objective optimization problem. A great advantage of this approach is identifying the upper and lower boundaries of the regional utility-loss function for biodiversity (regarded as a public good) and, consequently, evaluation of biodiversity for the multi-purpose modeling of a particular forest which is so far not available in the literature.

Goal of the study

The goal of the present study was to develop a new method to valuate biodiversity based on a non-linear optimization procedure. Therefore, in a first optimization step, a global utility function for a forest management problem subject to a biodiversity constraint was defined and the boundaries from which biodiversity influenced the global utility function were derived. A non-linear function related to the opportunity cost (utility-loss) of biodiversity within the derived boundaries of the feasible solution space was parameterized. The effectiveness of the developed procedure was demonstrated by applying it to the problem of finding the optimal forest management pathway for a forest conversion problem based on a recommendable level of biodiversity within a second optimization step. Furthermore, a related sensitivity analysis of the best management solution was examined in a post-optimality analysis (Buongiorno and Gilless, 2003; Kurttila et al., 2006).

The paper is organized as follows. The section the materials and methods introduces an adopted methodology to evaluate and integrate biodiversity in the modeling of forest management. This is proposed to integrate biodiversity into a multi-objective optimization problem by identifying the upper and lower boundaries of the regional utility-loss function for biodiversity and evaluation of biodiversity for the multi-purpose modeling of a particular forest. Section of results outlines the most desirable solution and analyses the trade-offs between goals. Variety of forest strategies have been compared at the end of this section too. The last section is first and foremost devoted to the discussion of the modeling approach, its limitations and advantageous. Afterwards, the main findings of provided numerical applications have been interpreted for the case of Black Forest to achieve the multiple goals of regional forest management.

Materials and methods

Forest management scenarios

Eight different forest management scenarios representing forest conversion (five scenarios), a traditional age-class management, a single-tree-selection system for the transformation of even-aged into uneven-aged stands and a nature conservation strategy were defined (*Table 1*). Five different forest conversion scenarios, from pure- to mixed-stands, were developed by applying different silvicultural systems, namely: "**G**roup **F**elling" (GF), "**Sh**elterwood" (Sh), "**S**trip **C**utting" (SC), "**A**ge-**C**lass" (AC) and "**Tr**ansformation" (Tr, by means of a "Single-tree-selection" system) all of which include regeneration (planting of 1,500 beech seedlings per ha). These scenarios were formulated in detail (*Table 1*) according to the approach of (Hanewinkel and Pretzsch, 2000). The conversion scenarios were compared to the traditional age-class system of managing pure Norway spruce in the Black Forest area of Southwest Germany to the transformation of the even-aged Norway spruce forest into an uneven-aged one and to a "Do-nothing" alternative corresponding to a nature conservation scenario. The intensity of the thinning activities of the different scenarios was expressed by the A-value according to (Johann, 1982). The baseline conversion-, age-class- and transformation-scenarios were formulated according to (Hanewinkel and Pretzsch**,** 2000) and (Hanewinkel, 2001) where details of the management prescriptions for the different alternatives can be found.

Table 1. *Management prescriptions for the different silvicultural scenarios*

Scenario	Period 1	Period 2	Period 3	Period 4
Conversion (I-V)	A-Value = 4 (= 150 Crop tree/ha)	A- Value = 6 (= 200 Crop tree/ha)	Group Felling (I) (3 * 25dm); Shelterwood (II) (25 %); Strip Cutting (III) (50 %); Age-Class (IV) (even-aged); Transformation (V) Single-tree-selection; + Plantation (1500/ha)	+ Target -diameter -harvest (50 %) D.B.H.= 45cm (scenarios I-VII)
Age-Class (VI)	A-Value = 7 (= 300 Crop tree/ha)	Age-Class even-aged	Cutting Break	
Transformation (VII)	A-Value = 6 (= 200 Crop tree/ha)	Single-tree-selection uneven-aged	Single-tree-selection uneven-aged	
Conservation (VIII)		Do-nothing		

Period I-IV: Planning periods of 10 years each
*(I) Group Felling (3*25m) = felling of three gaps with a diameter of 25 m each by "Group Felling"*
(II) Shelterwood (25 %) = removal of 25 % of the growing stock by "Shelterwood" cutting
(III) Strip Cutting (50 %) = removal of 50 % of the growing stock by "Strip Cutting"
(IV) Age-Class = a traditional even-aged management system (also in scenario VI)
(V) Transformation = Transformation of the even-aged stand into an uneven-aged one following an inverse j-shaped curve for the diameter distribution by "Single-tree-selection" system (also in scenario VII)
(VIII) Conservation = to do no interventions in the forest
Target diameter harvest (50 %)- d.b.h = 45cm = removal of 50 % of the trees having reached a target diameter of 45cm d.b.h

Simulation of decision alternatives

Simulation of forest stands and the prediction of the effects of different silvicultural treatments on these stands as management decision alternatives is a primary modeling step towards forest enterprise optimization (Hasenauer, 2006; Pretzsch et al., 2007). To accomplish this, a model of an even-age forest enterprise was generated according to the technique of strata planning (Hanewinkel and Pretzsch, 2000) deduced from large-scale inventory data (permanent plots of the national forest inventory of Germany) of the northern Black Forest. Site productivity was assumed to be constant over space and time. To test the decision alternatives for different stands, an age-class forest enterprise consisting of five model stands in five age-classes of 30, 50, 70, 90 and 110 years with 200 ha of area each was simulated over a planning period of 40 years, divided into four steps of ten years each. *Table 2* shows the main inventory data of the generated stands.

Table 2. *Description of the stands (age-classes) deduced from large-scale inventory data (permanent plots of the national forest inventory of Germany) of the northern Black Forest*

Age (years)	D100 (cm)	H100 (m)	N/ha	Dg (cm)	Hg (m)	B/ha (m2/ha)	V/ha (m3/ha)
30-50	25.6	14.4	1648	15.7	12.5	31,9	207
50-70	36.2	21.4	728	24.0	19.5	32.9	318.6
70-90	46.2	27.8	277	35.3	27.5	27	329.8
90-110	55.5	33.5	180	46.8	32.7	30.9	435.7
110-130	62.7	38.1	150	56.6	37.6	37.7	587

D100 = average diameter of 100 highest trees
H100 = average height of 100 highest trees
N/ha = number of trees (with the diameter of above 7 centimeters)
Dg = average diameter of all trees
Hg = average height of all trees
B/ha = average Basal area of all trees
V/ha = average Volume of all trees

The growth and yield simulator "BWINPro-S" which was used in this study is, aside from the model SILVA (Pretzsch, 2001), one of the two computer-based simulators currently used in Germany for a multitude of applications. Its regional relevance ranges from the Pleistocene lowland conditions to sites on Palaeozoic rocks in a chilly and humid highland climate (Schröder et al., 2007, according to Fürst et al., 2004) and can therefore be applied to site conditions of the Black Forest. In the present study, the distance-dependent version of the growth and yield simulator "BWINPro-S" was used for the simulations (Röhle, 2009). One of the unique features of "BWINPro-S" is a module to simulate juvenile growth of beech under the canopy of Norway spruce (Schröder et al., 2007), and thereby enabling the simulator to model the establishment of regeneration. *Fig. 1* illustrates how growth and yield predictions in "BWINPro-S" may be combined with thinning and harvesting operations in order to simulate different management options that can be compared by means of their financial outcomes (Röhle, 2009).

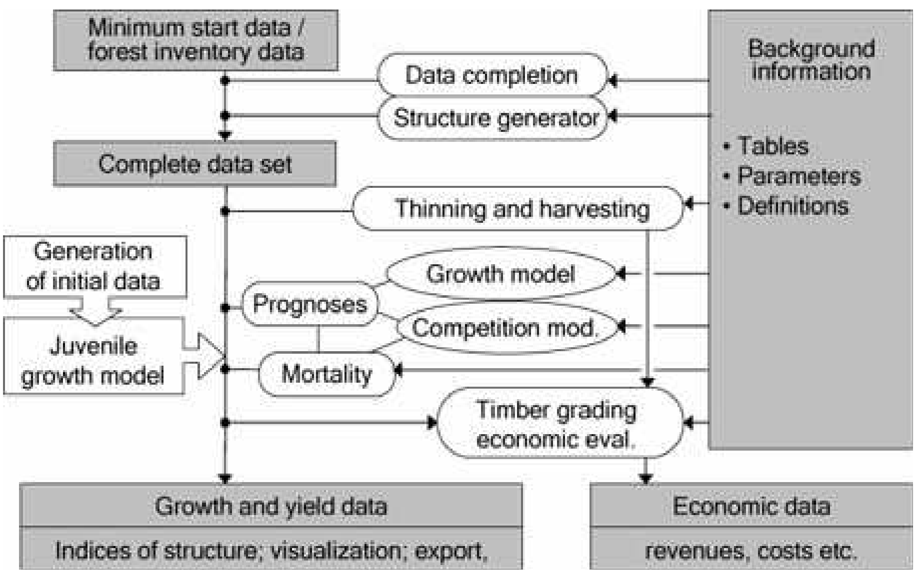

Figure 1. *Main modules of the simulator "BWINPro-S" in Röhle, 2009*

Wood prices and the costs of silvicultural and forest improvements were based on the deflated average realized prices and costs during the five-year period from 2000-2005 to calculate the net revenues of timber production. The establishment cost of beech regeneration was integrated into the harvesting cost to make the calculations straightforward. A mean price of 3 € per seedling was used to calculate regeneration costs.

The simulation and optimization procedures were run separately in different software environments. The output of the simulations runs were stored in a database and then used as an input for the following optimization procedures using the Solver Premium Platform (Buongiorno and Gilless, 2003; Dirsch and Knoke, 2007; Yousefpour and Hanewinkel, 2009).

Quantifying biodiversity

The scope of forest conversion in the present study is to enhance the level of biodiversity (structural diversity) of the managed forests. Therefore, to express this in a quantitative way, an adapted Shannon index was used. The index calculates the evenness (uniformity) of the distribution of species (i.e. Norway spruce and European beech) in the entire forest area and is therefore a good measure of conversion (*Equation 1*). A major goal of forest conversion in the Black Forest area is to improve the structural diversity in pure monocultures of Norway spruce by establishing beech (Hanewinkel and Pretzsch, 2000; Hanewinkel, 2001; Kint et al., 2009). The index reaches its maximum value when all species are represented equally.

$$Sh = -\sum_{n=1}^{N} Gn * \ln(G_n), Gn = g_n / g_N \qquad \text{(Eq. 1)}$$

Where **G**n is the proportion of the basal area in a particular species (gn), of which there are n, to the total basal area of all species in the stand (g_N, N is the number of species in a stand).

General formulation of the optimization problem

Global utility function

Multi-objective optimization procedures are often used to support the process of decision- making in forest management planning (Steuer and Schuler, 1978; Buongiorno and Gilless, 2003; Baskent and Sedat, 2005; Dirsch and Knoke, 2007; Yoshimoto and Marusak, 2007; Briceno-Elizondo et al., 2008; Yousefpour and Hanewinkel, 2009; Tahvonen, 2009). In this study, an additive utility function was defined to simultaneously consider the values of harvesting activities and standing volume. The forest optimization problem was formulated for a model age-class forest. The stand types (**s**) of this model forest standing for different age-classes were treated with different silvicultural scenarios (treatments - **t**) over several planning periods (**p**). To represent a model age-class forest optimization problem, the additive utility function **AUF** (global utility/objective function) to be maximized can be written as (*Equation 2*):

$$AUF(X) = (U_H + [U_L - U_I]) * X \qquad \text{(Eq. 2)}$$

With:

U_H = net present value (**U**tility) of all **H**arvest activities

$$U_H = \sum_{s=0}^{S} \sum_{t=0}^{T} \sum_{p=0}^{P} d_{i,p} u_H(s,t,p) \qquad \text{(Eq. 3)}$$

s = stand (age-class 1-5)
t = silvicultural treatment (1-8) (see *Table 1*)
p = planning period of 10 years (1-4)
$d_{i,p} = \frac{1}{(1+i)^{p*10}}$ is the discount factor, which depends on the period **p** and;
i = discount rate

U_L is the net present value (**U**tility) of the standing volume at the **L**ast period ***P*** which was also subject to wood stumpage prices.

$$U_L = \sum_{s=0}^{S} \sum_{t=0}^{T} d_{i,p} u_L(s,t,P) \qquad \text{(Eq. 4)}$$

U_I is the net present value (**U**tility) of the standing volume at the **I**nitial period p_0 (period zero).

$$U_I = \sum_{s=0}^{S} \sum_{t=0}^{T} d_{i,p} u_I(s,t,p_0) \qquad \text{(Eq. 5)}$$

Consequently $[U_L - U_I]$ is the change of the net present value of the standing volume during the conversion period of forty years (***P***- ***P****0*).

X is the area of the stand (s) to be optimally allocated to the treatment (t).

$$X = x(s,t) \qquad \text{(Eq. 6)}$$

The considered revenues in **AUF** (Equation 2) are U_H (*Equation 3*) and $[U_L - U_I]$ (*Equation 4 and 5*), which correspond respectively to the direct financial revenue due to harvest (as calculated by the simulator) and the value of standing volume (calculated with the subtraction of the value of the standing volume at the initial step and last step, derived from the simulator). An actual discount rate of 2% (**i** = 0.02) and stumpage prices of different stand ages were applied to obtain the present value of both coefficients. The decision variable, **X** (Equation 6), represents the total area of a stand type s devoted to treatment t. The optimization problem consists of finding the space optimal; $\{\mathbf{x}(s,t)\}_{s=1..S,t=1..T}$ set of values.

Global constraints

Along with the **AUF**, the most important forest enterprise constraints were formulated. The constraints under examination were the asset management (reality hard constraint); wood even flow (which was considered as soft/chance constraints) and the conservation of a proportion of the oldest stand area (hard constraint). The punishment function for the soft constraint (wood even flow) included the standard deviation (expression 7) which represents the absolute amount by which the constraint has gone beyond its limit and was not met. The optimization procedure was subject to these constraints which signify supplementary objectives of a forest owner.

$$100 * (\exp(deviation \ / 100) - 1) \qquad \text{(Eq. 7)}$$

The first constraint is an area (asset management) constraint ($Ca._{(max)}$) allocated to different decision alternatives. *Equation 8* expresses that for each stand type **s** symbolizing one age-class, the total area must be allocated among the entire set of available treatments.

$$\forall s, \ (s = 1,..., S); \sum_{t=1}^{T} x_{(s,t)} = A_{(s)} = Ca._{(max)} \qquad \text{(Eq. 8)}$$

In each planning period, the area of each stand type (age-class) must therefore be equal to 200 ha.

The forest enterprise should ensure that it will be able to produce a minimum volume **C**wef. of wood to be sold in each period. Equation 9 demonstrates such a volume constraint that is applied in each period, where **wef.**(s,t) represents the volume of wood to be harvested in period **p** per hectare of stand **s** where the treatment t is applied.

$$\forall p, (p = 1,..., P); \sum_{s=1}^{S} \sum_{t=1}^{T} \text{wef.(s, t, p)} * \text{x(s, t)} \geq \text{Cwef.}_{(min)} \qquad \text{(Eq. 9)}$$

The lower bound of the wood even flow constraint ($Cwef._{(min)}$) was fixed at 4.0 m^3 per year and ha (40 m^3 per 10-year-period), which on the one hand is well below the site productivity to guarantee a minimum harvest and allow for the necessary harvesting activities to install the regeneration in the conversion strategies and, on the other, prevent excessive volume accumulation in the even-aged schemes. The volume constraints of this optimization correspond to the silvicultural prescriptions that have been developed by the State Forest Service Baden-Württemberg in Southwest Germany for the management of pure spruce forests in Southwest Germany (MLR, 1999). In order for the forest types “stable mixed spruce forests” and “spruce forests to be converted into mixed beech forests” (MLR, 1999), a thinning interval of three to ten years and a maximum of 80 m^3/ha per thinning intervention was foreseen. The volume constraints of those applied to this optimization were a compromise in part between silvicultural needs, which should guarantee a successful conversion for the forest enterprise, and the other hand prescriptions to safeguard sustainability.

Finally, to represent a “protected area” policy, a conservation constraint (**C**p) was applied (*Equation 10)* to guarantee that at least a given proportion (**Pr**) of the area of the oldest stand a(**S**) is devoted to a “Do-nothing” treatment (**T**).

$$Cp. \geq \Pr * a(S),\ a(S) = x(S,T) \qquad \text{(Eq. 10)}$$

The proportion of the oldest stand (**S**) to be reserved for nature conservation purposes (treatment **T** which is Do-nothing scenario) was fixed at 10 % of its area (**Pr*a**(**S**) = 0.1*200=20 ha).

The adapted objective function and the set of constraints defined above was applied to analyze the optimization problem from the forest enterprise point of view by dedicating an optimal area of each stand to each scenario taking into account sustainable forest management goals of a virtual forest owner.

Integration of biodiversity into the optimization

A fictional additional global utility due to biodiversity (expressed as the Shannon index) was derived and integrated into the modeling of multi-purpose forest management. To develop a global utility function for biodiversity, the Shannon Index had to be computed for the entire forest enterprise due to its non-linear nature. The main reason for this choice was that it is a well-known index which is computable from the model outputs of most of the forest simulators. A two-step optimization procedure was designed (*Fig. 2*) that first defines a utility function for the Shannon index (optimization-**α**) and then – as a demonstrator of the effectiveness of the procedure – integrates the values into the optimization of a particular forest management plan (optimization- **β**).

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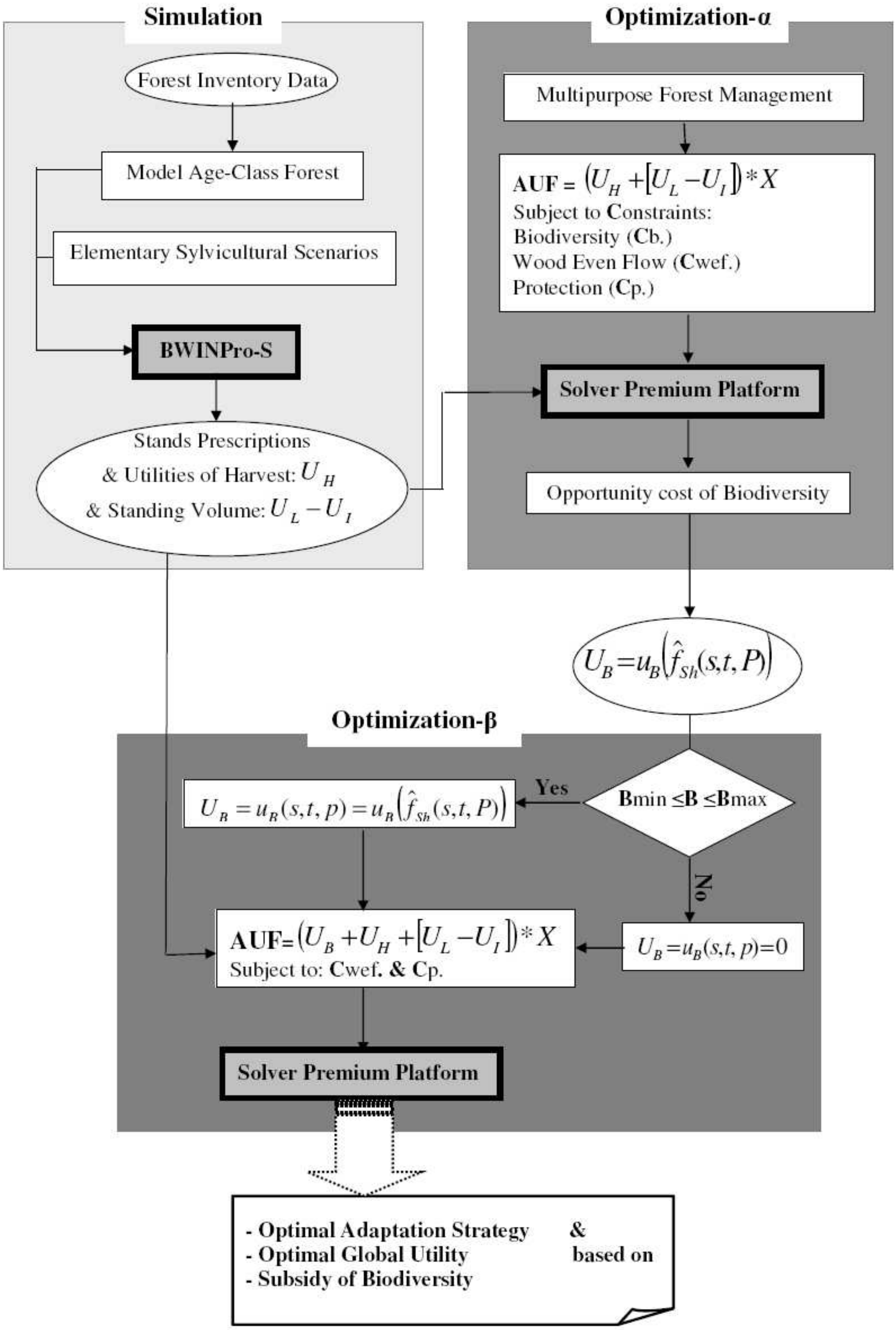

Figure 2. *Flowchart showing how biodiversity was conducted and integrated as a non-smooth objective (IF-THEN function) in the two-steps simulation-optimization procedure Parameters as described in section 2.2*

Optimization-**α** successively uses the output of the simulation runs and optimizes the global utility (*Equation 1*) subject to global constraints by iterating the variable level of the Shannon index as a global constraint (**C**b.). From a certain level on (**B**$_{min}$ – *Fig. 3*), **C**b. becomes an active constraint and consequently decreases the global utility to a level where the optimization no longer has a feasible solution (**B**$_{max}$, as described in *Fig. 3*). Afterwards, the opportunity cost of the different levels of the constraint "Biodiversity" (Shannon Index) in its active area between **B**$_{min}$ and **B**$_{max}$ was calculated based on the loss in global utility due to the constraint. The opportunity cost of different levels of Shannon index allows for the adjustment of a parametric (θ) function $\hat{f}_{Sh}\theta$ (*Fig. 3*), which can serve as a utility function of the Shannon index, $u_B\left(\hat{f}_{Sh}(s,t,P)\right)$. The obtained utility function can now be reused in any optimization of a particular forest enterprise with similar management objectives and constraints simultaneously considering the values of biodiversity and timber production.

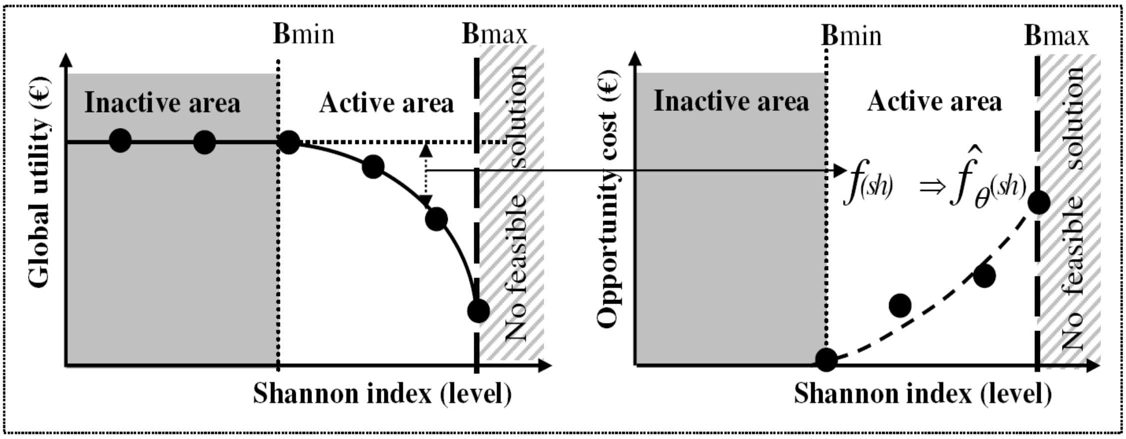

Figure 3. *Schematic depiction of how to adjust the utility function of the Shannon index by iteratively estimating the opportunity costs in the optimization runs*

The adjustment of the utility function of the Shannon index is a complex procedure. In the present study, an IF-THEN function was used to serve as the utility of biodiversity in the AUF. This type of objective function for the evaluation of the global utility leads to a non-smooth optimization procedure. This is an important issue in the integration of the utility of biodiversity in order to prevent overestimation or underestimation of its value compared to other objectives.

For demonstration purposes, the adjusted function was then integrated into the formulation of the global utility of the optimization-**β** including biodiversity as an objective (*Fig. 2*). Optimization-**β** provided the opportunity for biodiversity to compete with other objectives (e. g. timber production) and identified the optimal forest management plan with the allocation of different alternatives to the entire forest enterprise area with the most recommendable level of biodiversity as expressed by the Shannon index. Optimization-**β** therefore maximizes the optimal global utility with multiple values of biodiversity and timber production (*Equation 11*).

$$AUF(X) = \left(U_B + U_H + \left[U_L - U_I\right]\right) * X \qquad \text{(Eq. 11)}$$

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In this study, an identical dataset was used for optimization-**α** – and **β** to demonstrate the procedure of valuating biodiversity. Mathematically, this is a repetition of the optimization-**α** with **B**max as the highest, most recommendable level of biodiversity. In a practical application , the results of optimization-**α** and the developed utility function should be applied to particular forest enterprises with similar framework conditions in order to determine the financial effects of a recommendable level of biodiversity for management strategies such as forest conversion. Nevertheless, this example was demonstrated in this study to show trade-offs between timber and non-timber objectives in the process of multi-purpose forest modeling.

Results

Estimation of the utility function for the shannon index

The extracted forest characteristics from the simulator for the optimization were biomass, harvest revenues, operation costs and managed area whereas a non-linear optimization procedure was formulated based on these results and on the Premium Solver Platform of Frontline Systems© (Frontline 2009). The first step was to calculate the opportunity cost of biodiversity (optimization-**α**) by using **t**he observations of the Shannon index. A parametric function was adjusted to the calculated values for the Shannon index, $u_B\left(\hat{f}_{Sh}(s,t,P)\right)$. Subsequently, two different exponential utility functions for the Shannon index were derived and integrated into the optimization-**β** procedure with the following IF-THEN function (*Equation 12*):

$$\begin{cases} \text{If:} \quad 0.25 < Sh \leq 0.5, U_B = u_B\left(\hat{f}_{Sh}(s,t,P)\right) = 4919.1 * Sh^{3.4434} (R^2 = 0.99) \\ \text{If:} \quad 0.5 < Sh \leq 0.625, U_B = u_B\left(\hat{f}_{Sh}(s,t,P)\right) = 5729.1 * Sh^{3.8924} (R^2 = 0.95) \\ \text{Otherwise:} \quad U_B = u_B\left(\hat{f}_{Sh}(s,t,P)\right) = 0 \end{cases} \quad \text{(Eq. 12)}$$

Optimizing forest management incorporating biodiversity

Assuming of stability in the current discount rate of 2% and using the global additive utility function which include revenues from harvest as well as biodiversity (optimization-**β**) led to an overall optimum (or near optimum) utility for the entire enterprise of 7,958 €/ha. The optimal global utility consists of the utility of the harvest (1,016 €/ha), standing volume (6,134 €/ha) and biodiversity (807 €/ha) with 13, 67 and 10 percent of the total utility respectively. This optimal solution resulted in a recommendable level for the Shannon index of 0.625 (when planting beech on an area of 21 ha in stand-2 and 180 ha in stand-5) with related opportunity costs of 807 €/ha.

This optimum led to the distribution of the different silvicultural treatments to the different age-classes ("stands"). *Fig. 4* shows that the optimal solution does not consist of a unique treatment for the entire forest enterprises and all age-classes, but of a combination of different treatments in different stands (age-classes).

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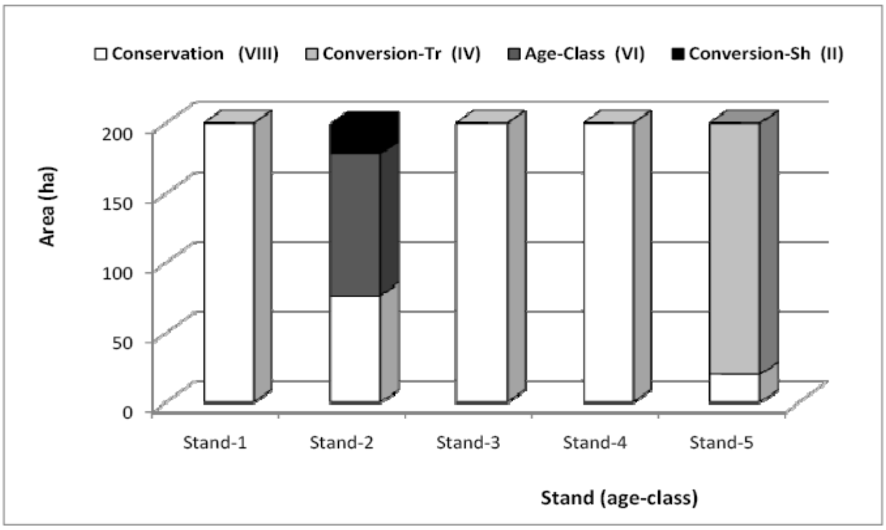

Figure 4. *Results of the optimization for the entire forest enterprise – distribution of the different silvicultural treatments to different age-classes (stands) including biodiversity Silvicultural strategies as described in Table 1:*
*(II) Conversion-Sh: "**S**helterwood" used for the gap installation in the 3rd period of Conversion*
*(IV) Conversion-Tr: **Tr**ansformation by the means of "Single-tree-selection" system used for the gap installation in the 3rd period of Conversion to transform the even-aged forest to uneven-aged one*
(VII) Age-Class: a traditional even-aged management system
(VIII) Conservation: „Do-nothing"strategy

Although the optimal pathway for stands 2 and 5 is a combination of different treatments, the conservation strategy (Do-nothing) is the dominant scenario in stands 1, 3 and 5. Despite the integration of biodiversity into the optimization, conversion which also include the introduction of beech seedlings appears as optimal only in stand-2 by shelterwood cutting and stand-5 by transformation into an uneven-aged forest). This is mainly due to the rather high costs of harvesting and planting beech and high accumulation of standing volume in the "Do-nothing" scenario that makes harvesting less attractive. Other alternative scenarios such as traditional age-class forestry are an option for more than 50 % (101 ha) of stand-2.

Comparing the results of the forest optimization with and without integration of biodiversity proved the necessity of directly integrating biodiversity as an objective into the planning process. *Fig. 5* shows the results of the optimization without taking biodiversity into account. The optimal solution is similar to that in *Fig.4* except for a replacement of the conversion scenario to non-conversion in stand-5. The absence of the notion of biodiversity in the body of the optimization causes a shift towards traditional silvicultural systems such as age-class (even-aged) forestry in the oldest stand. Conversion (with group felling) appears only in a part of stand-2 (59 ha or 27 %). Moreover, the level of Shannon index for the entire forest enterprise decreases from 0.625 to 0.183.

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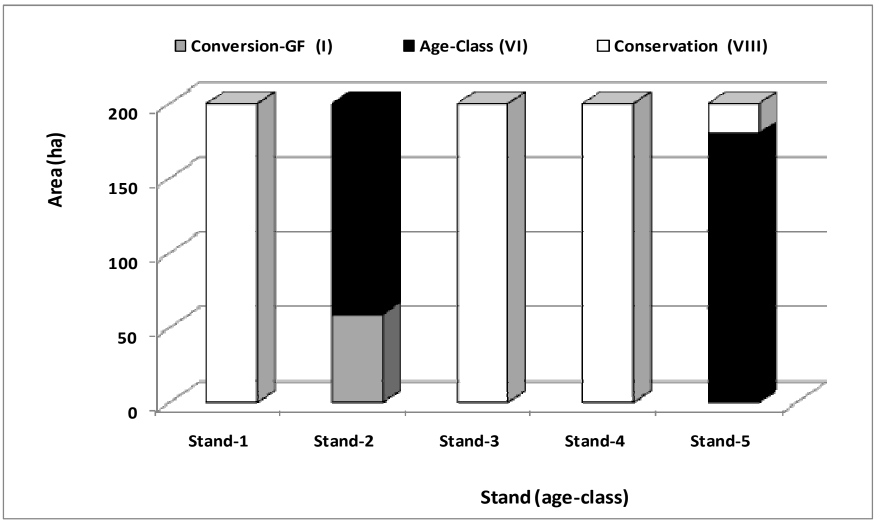

Figure 5. *Results of the optimization for the forest enterprise – distribution of the different silvicultural treatments to different age-classes (stands) without biodiversity*
Silvicultural strategies as described in Table 1:
*(I) Conversion-GF: "**G**roup **F**elling" used for the gap installation in the 3rd period of Conversion*
(VI) Age-Class: a traditional even-aged management system
(VIII) Conservation: „Do-nothing" strategy

Comparison of forest management strategies – forest enterprise level

To compare potential alternative strategies for the entire forest enterprise and their effects on the global utility, the optimization procedure was run separately for each of the main silvicultural strategies: i) "Do nothing" (Conservation), ii) Traditional "Age-Class" Forestry, iii) Transformation (even-aged to uneven-aged) and iv) Conversion (pure to mixed stands). The global utility of these main strategies was differentiated among the utility of harvest U_H, standing volume $[U_L - U_I]$ and the utility of biodiversity U_B and compared to that of the optimal forest plan (see last section) as a baseline. This allowed for the calculation of the cost of different forest strategies when applied to the entire forest enterprise and for the analysis of the effect of the different utilities (*Table 3*). In the present study, the post-optimality analysis is of specific discount in that it shows the effect of the opportunity cost of biodiversity on the whole forest enterprise level.

Table 3. *Global Utility and Utilities of different forest strategies when applied to the entire forest enterprise*

Forest Strategies	Global Utility*	Utility of Harvest *	Utility of Biodiversity*	Utility of Standing Volume*
Conservation	8344	0	0	8344
Age-Class	7220	3738 (52%)	0	3482 (48%)
Transformation	5904	4447 (75%)	0	1457 (25%)
Conversion	5769	2817 (48%)	1356 (24%)	1597 (27%)
Optimal Forest Plan	7958	1016 (13%)	807 (10%)	6134 (77%)

** = figures are all in €/ha*

Table 3 shows the utilities of different strategies when applied to the entire forest enterprise. Compared to the optimal forest plan, the cost of converting the entire forest enterprise from pure stands of Norway spruce into mixed stands of Norway spruce and European beech would amount to 7,958 – 5,769 = 2,189 € /ha (2,189,000 € overall) in 40 years. The utility of biodiversity for this scenario was 1,356 €/ha, which was noticeably (nearly two times) higher than that of the optimal forest plan. This is due to intensive forest management aiming at establishing mixed stands on the entire forest area and consequently imposes a higher opportunity cost. Transforming the entire forest enterprise from even-aged into uneven-aged stands of Norway spruce led in costs of more than 1,316 €/ha and by applying a traditional age-class system, reduced the global utility by more than 738 €/ha. This was an effect of the earlier revenues from harvesting when reducing the standing volume to install regeneration or the approach to the uneven-aged structure in the transformation and conversion strategies in the 40-year planning period. The conservation (Do-nothing) strategy comprised the maximum global utility, which is due to the fact that there is no possibility to implement the global constraint of wood even flow for this strategy.

Discussion

Limitations of the models

As in many other studies (Buongiorono and Gilles, 2003; Hasenauer, 2006; Dirsch and Knoke, 2007), the present investigation used a modern growth simulator to depict growth and yield of a model forest enterprise. Therefore, all the results that are presented here are subject to the limitations of these growth models. One of the important limitations is the lack of a natural regeneration module that applies to most of the simulators (except “BWINPro-S” with the beech regeneration module). Another limitation of the simulators is the increase of uncertainty with increasing duration of the simulation. Thus, the optimization took place in finite time and was restricted to a time span of 40 years (4 periods of 10 years each). Longer simulations lead to a distinct increase of the uncertainty linked to the prognosis of growth and yield (Pretzsch et al., 2007) and to a high risk of producing artefacts within the present goal-seeking investment problem.

In this study a procedure was developed to integrate biodiversity via an estimated utility function in the post-simulation planning of a forest enterprise in the Black Forest area of Southwest Germany. The presented utility function of biodiversity is, of course, dependent on the index for biodiversity that was used (in this case the Shannon index), the parameters of the growth model (“BWINPro-S”) and the underlying silvicultural scenarios. Therefore, the value function as well as the results should be primarily regarded as an application of the introduced methodology in the sense of a demonstration. A generalization of the findings in the model forest used in this study is therefore not possible and was not intended. However, the methodology introduced in the present investigation can be adopted to optimize forest management planning taking into account other non-monetary criteria and preferences of decision-makers.

Integration of biodiversity

Multi-criteria decision-making techniques are common tools for treating biodiversity in forest planning (Kangas and Kuusipalo, 1993; Wikström and Eriksson, 2000; Buongiorno and Gilles, 2003; Kurttila et al., 2006; Briceno-Elizondo et al., 2008). Biodiversity has been considered as an attribute for alternative management scenarios in forest planning (Wikström and Eriksson, 2000), as an objective in the analytic hierarchy process (Kangas and Kuusipalo, 1993; Briceno-Elizondo et al., 2008), as a constraint in optimization processes (Buongiorno and Gilless, 2003; Kurttila et al., 2006). Biodiversity has thus far not been directly integrated into the optimization process as an objective due to valuation problems. The present paper suggests a way of evaluating biodiversity based on the Shannon index, a commonly used and available measure of biodiversity (Önal, 1997; Buongiorno and Gilless, 2003), in forest enterprise level.

Kurttila et al. (2006) and Koskela et al. (2007) examined the first-best instrument for biodiversity maintenance on the stand level. Kurttila et al. (2006) defined the bidding price demand for the biodiversity objective and calculated the subsidy of holding the same total utility as clear cutting on a stand level. They came up with a value between 290 and 403 €/ha for a protection period of twenty year at a discount rate of 4%. Koskela et al. (2007) found that a fully synchronized combination of retention tree subsidy and harvest tax is needed to achieve the goal of biodiversity management in Boreal forests. Furthermore, it has been demonstrated that when combined with a harvest tax, the retention tree subsidy was 1,000 €/ha and 750 €/ha using a Faustmann and a Hartman model, respectively. When used with a timber subsidy or a site value tax, the retention tree subsidy was 1,700 €/ha in both models.

The results of the present study in *Table 3* show that for the optimal model, a subsidy of 807 €/ha would be needed to compensate for the opportunity costs of biodiversity. This would increase to 1,356 €/ha if a conversion scenario were applied to the total forest area (discount rate = 4%). Yoshimoto and Marusak (2007) used a similar approach for determining the price of carbon as subsidy, which can be regarded as compensation or cost for carbon loss. They found that at an discount rate of 2%, the annual cost for the amount of carbon sequestered in the remaining trees ranged between 763 and 106 Yen/Ct/year (equal to 28-206 €/ha).

Multi-purpose forest management

The results indicate that the optimal management plan may vary with and without pricing and integration of biodiversity as an objective into the optimization procedure (Kangas and Kuusipalo, 1993; Wikström and Eriksson, 2000; Briceno-Elizondo et al., 2008). The optimal solution also identifies the optimal level of all integrated objectives such as biodiversity. This is one of the main findings of this study that maybe used by forest policy-makers in order to decide which level of biodiversity is achievable and at the same time, cost-efficient when designing forest conversion strategies for different forest enterprises on a larger area. Once, the utility function is available, different forest enterprises can use it to identify optimum solutions for conversion strategies under similar conditions. The obtained optimal solution is not only efficient because of simultaneous approvals for the recommendable level of biodiversity, but also controllable due to the identified optimal management pathways. Koskela et al. (2007) came to a similar conclusion that a combination of subsidy and a corrective tax/subsidy is necessary to induce the landowner to follow the target of biodiversity maintenance in

Boreal forests, which in this case means to lengthen optimal rotation periods and to provide an incentive to leave retention trees.

In this study, including biodiversity in the optimization of the forest management plan leads to the conversion of the oldest stands of Norway spruce into mixed stands of spruce and beech. Optimal silvicultural pathways may differ not only among different stands, but also at times within a given stand when multiple values beyond timber production such as carbon sequestration or biodiversity are taken into account (Zhou and Buongiorno, 2006). Backéus et al. (2005) revealed that by assigning a monetary value to carbon storage as an objective of optimization with linear programming, the harvest levels will be influenced. Koskela et al. (2007) also found that the harvest tax rate varies within the range of 40–65% in the Faustmann model and 20–40% in the Hartman model, while timber subsidy is between 0.5–1.0% and site value tax is approximately 1.75%. The integration of criteria other than the net present values of harvesting and standing volume such as biodiversity (in the sense of the Shannon index) and the respective constraints into the forest management decision-making process usually leads to a diversification of silvicultural strategies (Schulte et al., 1998). However, the “Do-nothing scenario was the most allocated scheme in the entire forest area in this study.

Conversion as adaptation strategy to climate change

Knoke et al. (2008) compared pure and mixed forests with mixed-species stands and concluded that mixed forests are better able to compensate for disturbances than monocultures, more resistant against biotic and abiotic disturbances and by applying an extended forest economic model, mixing large blocks of native broadleaf species into pure conifer forests may lead to a significant reduction of financial risk. This is of crucial importance when taking into account an expected climate change with increasing temperatures that will deteriorate growth conditions especially for non-site-adapted secondary coniferous forests such as Norway spruce (Spiecker, 2003). One of the major adaptation strategies is the conversion of these forests into more site-adapted species such as European beech. A general problem when implementing this type of strategy is the lack of a quantitative basis to control the success of the measures that are foreseen.

The methodology presented in this study is not only able to calculate the cost of different forest management strategies such as adaptation with forest conversion, but also to propose an optimal management plan for the silvicultural interventions necessary to achieve the desired status. The results (*Fig. 3 and 4*) showed that the optimal management plan may vary with and without pricing and integration of biodiversity. Consequently, the variation imposed a shift from conservation to active, silvicultural interventions with partial introduction of beech especially in the oldest stand and this increases the share of the biodiversity in the global utility from 10 to 24 percent (*Tabl 3*). These results confirm the importance of taking the value of various ecological considerations into account in order to achieve the optimal level of ecological considerations with consequential pathways for the sustainable forest management.

Considering different adaptation strategies in the same optimization procedure can assist forest decision-makers to compare management alternatives such as adaptation with forest conservation, “Do-nothing”, traditional age-class forestry or conversion from pure to mixed stands in a quantitative way (*Table 3*). In this study, the optimization leads to a mixture of different silvicultural strategies for the entire forest enterprise with at least partial introduction of beech regeneration (*Fig. 4*). Hanewinkel (2001) and Knoke and Plusczyk (2001) evaluated different conversion strategies from pure to mixed stands. Depending on the discount rate, the conversion systems applied in these studies, proved to be financially advantageous due to the earlier revenues issued from the more intensive thinning and openings of the canopy to allow regeneration. Tahvonen (2009) also mentioned that although even- and uneven-aged systems may represent locally optimal solutions with equal economic outcomes, changes in decision parameters such as the rate of discount, timber price, or planting cost may imply that the optimal solution shifts from even- to uneven-aged management. Moreover, the optimal solution for Norway spruce represents an intermediate case between the two management systems, even – and uneven-aged forestry, and yields about 30% higher economic output compared to a solution where the even-aged forestry is predetermined. This is all an effect that is also visible in the present study, namely in the conversion strategies foreseen in the optimal solutions for stand-2 (*Fig. 4 and 5*).

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REFERENCES

[1] Backéus, S., Wikström, P., Lämas T. (2005): A model for regional analysis of carbon sequestration and timber production. – Forest Ecology and Management 216: 28-40.

[2] Baskent, E.Z., Sedat, K. (2005): Developing alternative wood harvesting strategies with linear programming in preparing forest management plans. – Turkish Journal of Agriculture and Forestry 29: 1-13.

[3] Bladt, J., Strange, N., Abildtrup, J., Svenning, J.Ch., Skov F. (2009): Conservation efficiency of geopolitical coordination in the EU. – Journal for NatureConservation 17: 72-86.

[4] Böttcher, H. (2007): Forest management for climate change mitigation: Modeling of forestry options, their Impact on the regional carbon balance and implications for a future climate protocol. – PhD Dissertation, University of Freiburg.

[5] Briceno-Elizondo, E.D., Jäger, M., Lexer, J., Garcia-Gonzalo, J., Peltola, H., Kellomäki, S. (2008): Multi-criteria evaluation of multi-purpose stand treatment programmes for Finish boreal forests under changing climate. – Ecological Indicators 8: 27-45.

[6] Buongiorno, J., Gilless, J.K. (2003): Decision methods for forest resource management. – Amsterdam, Academic Press.

[7] Dirsch, R., Knoke, Th. (2007): Zur finanziellen Analyze der Höhe des Holzvorrates, eine Anwendung der Linearen Programmierung im Rahmen der Forstbetriebsplannung. – Allgemeine Forst- und Jagdzeitung 178: 142-150.

[8] Frontline Inc. (2009): Premium Solver Platform. – User Guide copyright © 2008 by Frontline Systems, NV, USA.

[9] Hanewinkel, M., Pretzsch, H. (2000): Modeling the conversion from even-aged to uneven-aged stands of Norway spruce (*Picea abies* L. Karst) with a distance-dependent growth simulator. – Forest Ecology and Management 134: 55-70.

[10] Hanewinkel, M. (2001): Economic aspects of the transformation from even-aged pure stands of Norway spruce to uneven-aged mixed stands of Norway spruce and beech. – Forest Ecology and Management 151: 181-193.

[11] Hasenauer, H. (2006): Sustainable forest management: Growth models for Europe. – Berlin-Heidelberg, Springer Verlag.
[12] Johann, K. (1982): Der A-Wert, ein objektiver Parameter zur Bestimmung der Freistellungsstärke von Zentralbäumen. – Deutscher Verband der Forstlichen Versuchsanstalten, Sektion Ertragskunde, Weibersbrunn.
[13] Kangas, J., Kuusipalo, J. (1993): Integrating biodiversity into forest management planning and decision-making. – Forest Ecology and Management 61: 1-15.
[14] Kint, V., Lasch, P., Lindner, M. Muys, B. (2009): Multi-purpose conversion management of Scots pine towards mixed oak-birch stands - a long-term simulation approach. – Forest Ecology and Management 257: 199-214.
[15] Knoke, Th., Plusczky, N. (2001): On economic consequences of transformation of a spruce (*Picea abies* L. Karst) dominated stand from regular to irregular age structure. – Forest Ecology and Management 151: 163-159.
[16] Knoke, Th., Ammer, Ch., Stimm, B. Mosandl, R. (2008): Admixing broadleaved to coniferous tree species: a review on yield, ecological stability and economics. – European Journal of Forest Research 127: 89-101.
[17] Koskela, E., Ollikainen, M. Pukkala, T. (2007): Biodiversity policies in commercial boreal forests: Optimal design of subsidy and tax combinations. – Forest Policy and Economics 9: 982-995.
[18] Kurttila, M., Pykäläinen, J. Leskinen, P. (2006): Defining the forest landowner's utility-loss compensative subsidy level for a biodiversity object. – European Journal of Forest Research 125: 67-78.
[19] MLR (1999): Richtlinie landesweiter Waldentwicklungstypen der Landesforstverwaltung Baden-Württemberg. – MLR, Ministerium Ländlicher Raum, Stuttgart.
[20] Önal, H. (1997): Trade-off between structural diversity and economic objectives in Forest Management. – American Journal of Agricultural Economics 79: 1001-1012.
[21] Pretzsch, H., Grote, R., Reineking, B., Rötzer, Th., Seifert, St. (2007): Models for forest ecosystem management: A european perspective. – Annals of Botany 101: 1065-1087.
[22] Pretzsch, H. (2001): Modellierung des Waldwachstums. – Hamburg, Parey.
[23] Röhle, H. (2009): Waldwachstumssimulator BWINPro-S: (http://www.forst.tu-dresden.de/Waldwachstum/simulator_ww.htm), 01.05.2009.
[24] Schröder, J. (2005): Zur Modellierung von Wachstum und Konkurrenz in Kiefern-Buchen-Waldumbaubeständen Nordwestsachsens. – PhD Dissertation, Technical Unoiversity of Dresden.
[25] Schröder, J., Röhle, H., Gerold, D., Münder, K. (2007): Modelling individual-tree growth in stands under forest conversion in East Germany. – European Journal of Forest Research 127: 459-472.
[26] Schulte, B.J., Buongiorno, J., Skog, K. (1998): Optimizing uneven-aged management of Lobby pine stands. Proceedings of the Society of American Foresters. – National Convention, Michigan, pp. 306-318.
[27] Spiecker, H. (2003): Silvicultural management in maintaining biodiversity and resistance of forests in Europe-temperate zone. – Journal of Environmental Management 67: 55-65.
[28] Steuer, R.E., Schuler, A.T. (1978): An interactive multi-objective linear programming approach to a problem in forest management. – Operations Research 27: 254-279.
[29] Tahvonen, O. (2009): Optimal choice between even- and uneven-aged forestry. – Natural Resource Modeling 22: 289-321.
[30] Wikström, P., Eriksson, L.O. (2000): Solving the stand management problem under biodiversity-related considerations. – Forest Ecology and Management 127: 361-376.
[31] Yoshimoto, A., Marusak, R. (2007): Evaluation of carbon sequestration and thinning regimes within the optimization framework for forest stand management. – European Journal of Forest Research 127: 315-329.

[32] Yousefpour, R., Hanewinkel, M. (2009): Modelling of forest conversion planning with an adaptive simulation-optimization approach and simultaneous consideration of the values of timber, carbon and biodiversity. – Ecological Economics 68: 1711-1722.
[33] Zhou, M., Buongiorno, J. (2006): Forest landscape management in a stochastic environment, with an application to mixed loblolly pine-hardwood forests. – Forest Ecology and Management 223: 170-182.

THE PHEROMONE TRAP CATCH OF HARMFUL MOTHS IN CONNECTION WITH SOLAR ACTIVITY FEATURED BY Q-INDEX

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Abstract The paper deals with connections between solar flare activities and pheromone trap collection of harmful moth species. The authors have worked out the pheromone catch data of Spotted Tentiform Leafminer (*Phyllonorycter blancardella* Fabricius, 1781), Peach Twig Borer (*Anarsia lineatella* Zeller, 1839), European Vine Moth (*Lobesia botrana* Denis et Schiffermüller, 1775), Oriental Fruit Moth (*Grapholita molesta* Busck, 1916), and Plum Fruit Moth (*Grapholita funebrana* Treitschke, 1846) as were operated for the period of 1988 and 1993-2007 in Borsod-Abaúj-Zemplén County (Hungary). The results proved that the daily catches were significantly modified by the Q-index, expressing the different lengths and intensities of the solar flares. On days with high Q-index relative to the ones of the average swarming periods, the number of catches is considerably lower.
Keywords: *harmful moths, pheromone trap, Q-index, solar flares*

Introduction and review of literature

As part of global solar activity flares, eruptions can be observed in the active regions of the solar surface, that are accompanied by intensive X-ray, gamma- and corpuscular radiations, that reach also the Earth and establish an interaction with its outermost atmosphere, producing thus changes in its electromagnetic conditions (Smith and Smith, 1963). Solar flares are most powerful and explosive of all forms of solar activity and the most important in terrestrial effects. This idea led solar physicists to evaluable the daily flare index (Özgüç and Ataç, 1989).

Most daily flare activities are characterised by most authors by index Q that expresses the significance of flares also by their duration. Its calculation is made by the following formula:

$$Q = (i \times t)$$

where i = flare intensity, t = the time length of its existence

Earlier (Örményi, 1966) calculated and published the flare activity numbers based on similar theoretical principles ("Flare Activity Numbers") for the period of 1957-1965.

The solar activity also exerts influence on life phenomena. In the literature accessible to the authors, however, no publication can be found that would have dealt with the influence of flares on the collection of insects by pheromone traps. Earlier we have published our studies and demonstrated the influence of hydrogen alpha flares No. 2. and 3. on light-trap catches (Tóth and Nowinszky, 1983).

Materials and methods

The Q-index daily data for the period 1988 and 1993-2007 were provided by Dr. T. Ataç B.Ü. Kandilli Rasathanesi.

In 1988 pheromone traps were operating in Borsod-Abaúj-Zemplén County (Hungary –Europe) at 9 villages. An additional one trap operated between 1993 and 2007. These traps attracted 5 Microlepidoptera species altogether, in some of the years using 2-2 pheromone traps for each species, however, in other years not all 5 species were monitored. Catch data of the collected species is displayed in *Table 1*. We examined the trapping data of these species depending on the Q-indexes.

Table 1. *Number of examined individuals caught by pheromone trap and number of observing data*

Species	Number of individuals	Number of data
Spotted Tentiform Leafminer *Phyllonorycter blancardella* Fabricius, 1781	51 805	1766
Peach Twig Borer *Anarsia lineatella* Zeller, 1839	6 873	1913
European Vine Moth *Lobesia botrana* Denis et Schiffermüller, 1775	20 240	2320
Oriental Fruit Moth *Grapholita molesta* Busck, 1916	12 673	2299
Plum Fruit Moth *Grapholita funebrana* Treitschke, 1846	27 679	3250

From the catching data of the examined species, relative catch (RC) data were calculated for each observation posts and days. The RC is the quotient of the number of individuals caught during a sampling time unit (1 night) per the average number of individuals of the same generation falling to the same time unit. In case of the expected average individual number, the RC value is 1. The introduction of RC enables us to carry out a joint evaluation of materials collected in different years and at different traps.

At the values of Q-index showed considerable differences in course of the respective years, they were preferably expressed as percentages of the averages of swarming periods. We studied the influence of flare activities on the daily catches. To disclose the latter, the Q/Q average values were co-ordinated with the relative catch data of different observation posts for each day of the catch period. The Q/Q means values have been contracted into groups (classes), and then averaged within the classes the relative catches data pertaining to them.

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Results and discussion

The connections between Q/Q averages and daily catches of examined species are presented in *Fig. 1 – 5*.

From the results several important consequences could be drawn.

The figures 1-5 show that in those days, in which the Q /Q average value exceed the species-characteristic value, a significant decline the pheromone trap catching result. The number of Spotted Tentiform Leafminer (*Phyllonorycter blancardella* Fabr.), Peach Twig Borer (*Anarsia lineatella* Zeller), and European Vine Moth (*Lobesia botrana* Den. et Schiff.), begins to decrease when Q values are higher then averages for swarming periods. As opposed to this the number caught of the Oriental Fruit Moth (*Grapholita molesta* Busck) and Plum Fruit Moth (*Grapholita funebrana* Tr.) starts decreasing already when the Q value attains the half of average value of the swarming period.

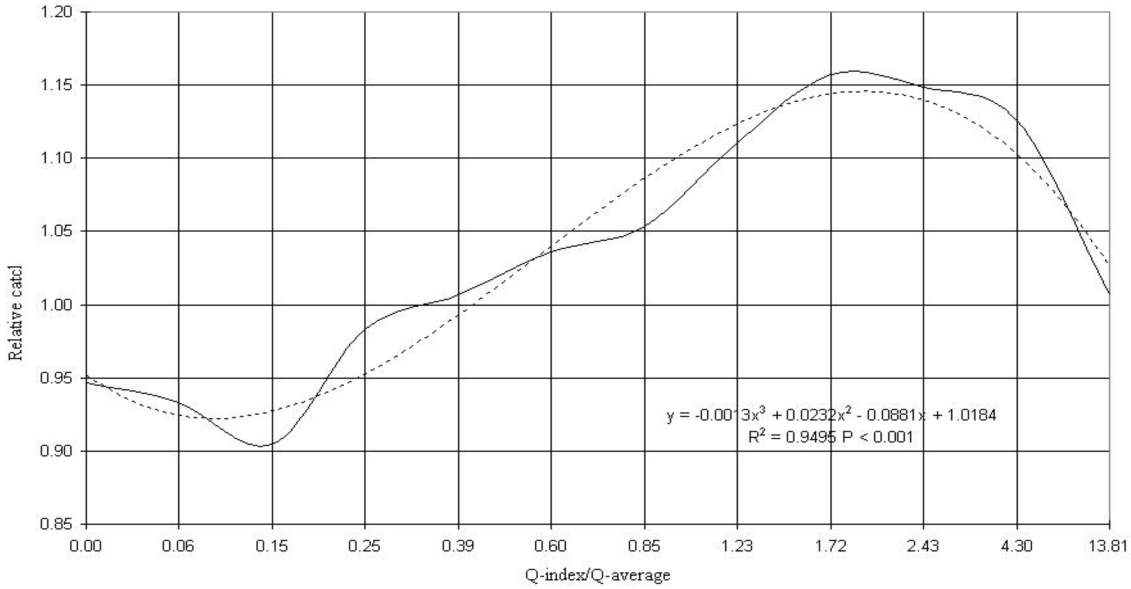

Figure 1. *The pheromone trap catch of Spotted Tentiform Leafminer (Phyllonoricter blancardella Fabr.) in connection with the Q/Q average values between 1993 and 2007*

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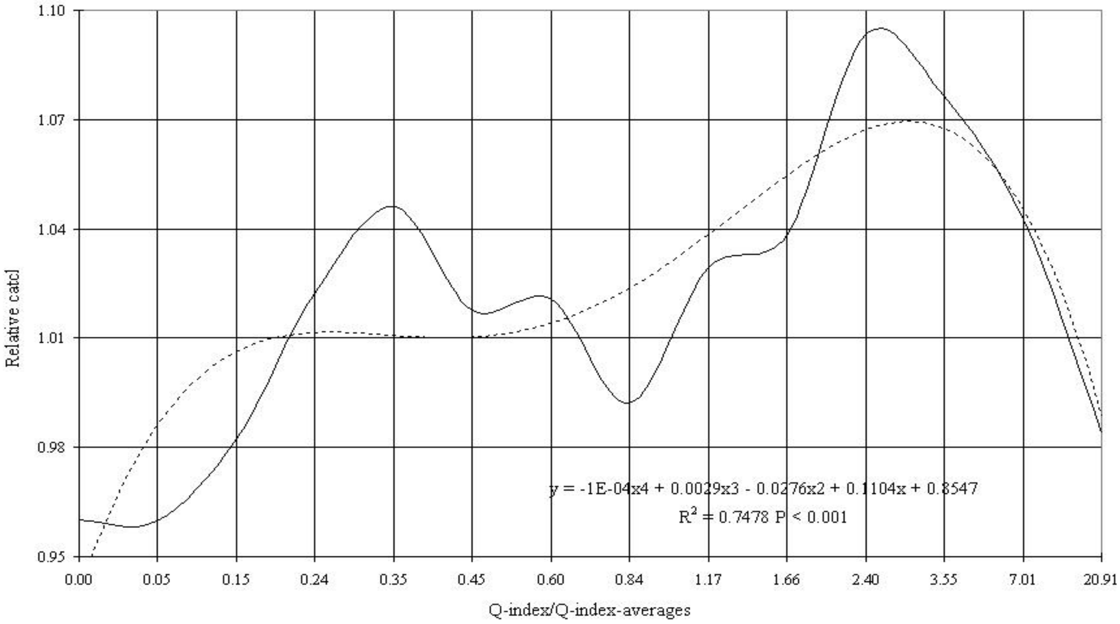

Figure 2. *The pheromone trap catch of Peach Twig Borer (Anarsia lineatella Zeller) in connection with the Q/Q average values between 1993 and 2007*

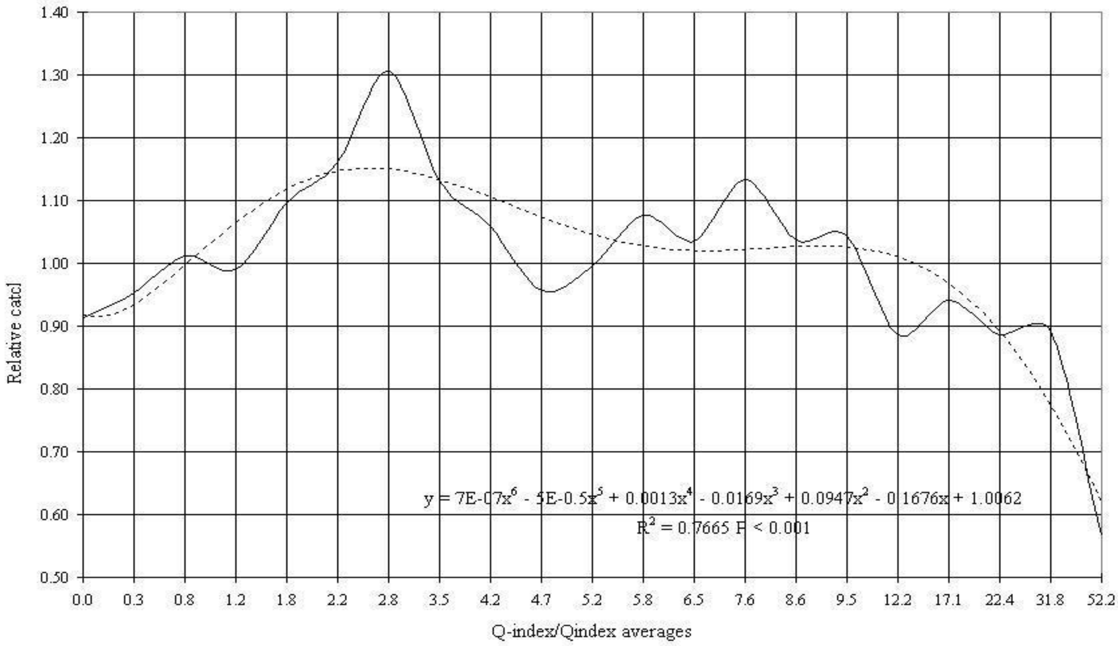

Figure 3. *The pheromone trap catch of European Vine Moth (Lobesia botrana Den. et Schiff.) in connection with the Q/Q average values between 1993 and 2007*

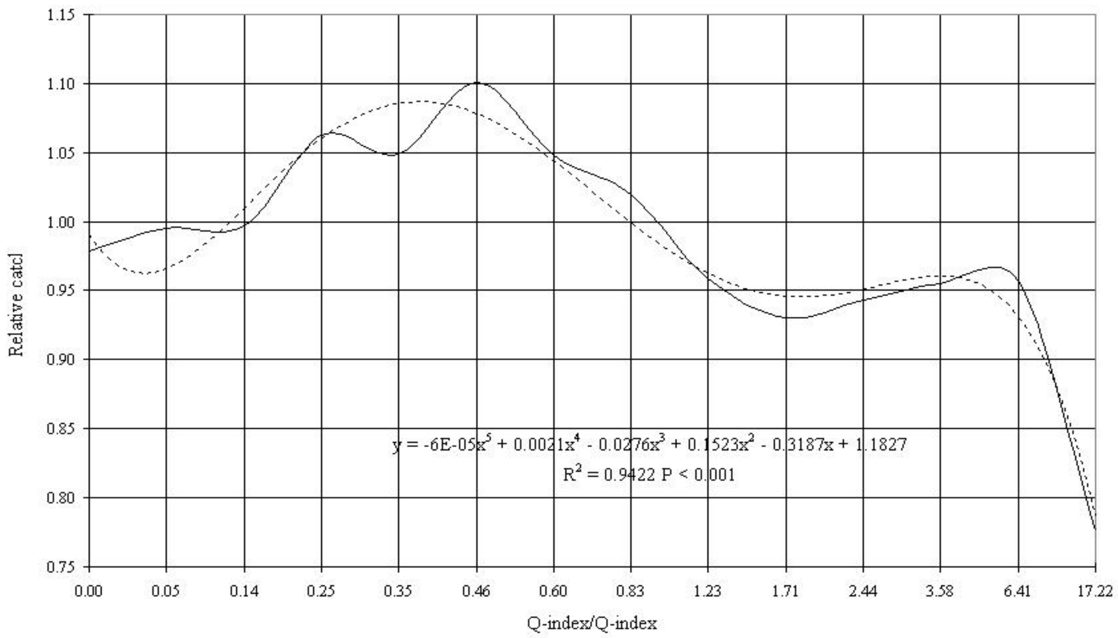

Figure 4. *The pheromone trap catch of Oriental Fruit Moth (Grapholita molesta Busck) in connection with the Q/Q average values between 1993 and 2007*

The changes of the Q-index modify the number of the harmful moths caught daily significantly according to our results. This fact is notable for the plant protection prognostic.

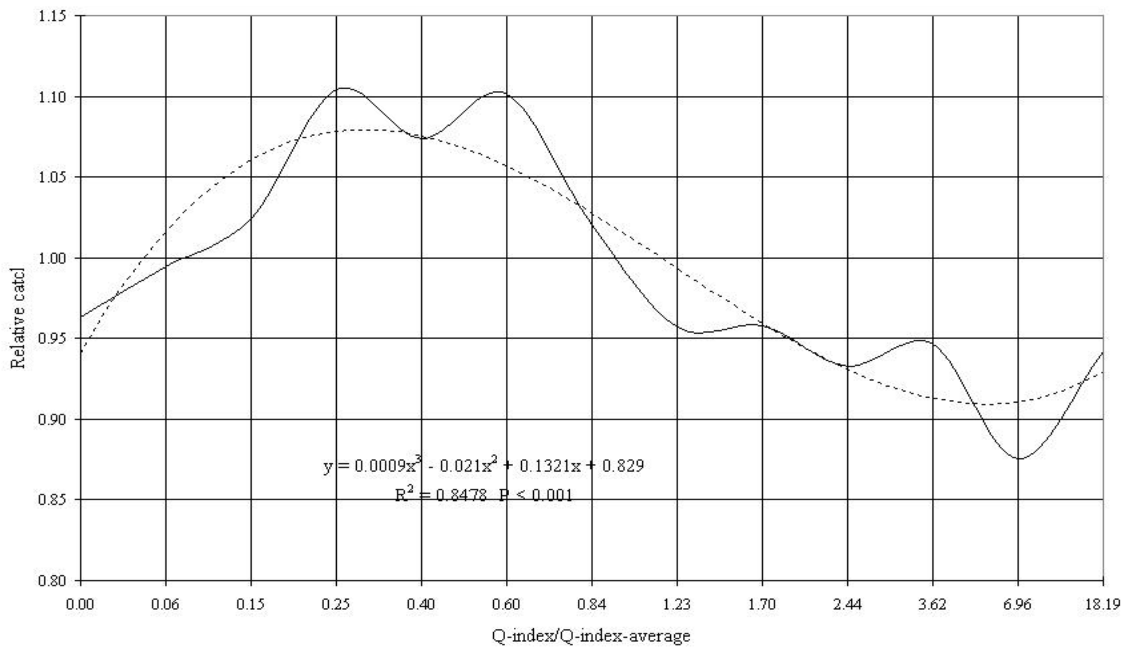

Figure 5. *The pheromone trap catch of the Plum Fruit Moth (Grapholitafunebrana Tr.) in connection with the Q/Q average values between 1993 and 2007*

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REFERENCES

[1] Örményi, I. (1966): The relationship between geomagnetic activity and chromospheric Hα-flares. – Acta Geodaet., Geophys. et Montanist, Acad. Sci. Hung. 1(1-2): 121-136.
[2] Özgüç, A., Ataç, T. (1989): Periodic behaviour of solar flare index during solar cycles 20 and 21. – Solar Physics 123: 357-365.
[3] Smith, H.J., Smith, E.V.P. (1963): Solar flares – Macmillan Co., New York, 426.
[4] Tóth, Gy., Nowinszky, L. (1983): Influence of solar activity on the outbreaks and daily light-trap catches of Scotia segetum Schiff. – Z. ang. Ent. 95: 83-92.

GENETIC POTENTIAL FOR TOXICITY: BLOOMS OF CYANOBACTERIA IN THE ITAIPU RESERVOIR, BRAZIL

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Abstract. Cyanobacteria are algae of great importance to environmental and public health because they produce toxins, such as microcystins. The same species of cyanobacteria may show microcystin-producing and non-microcystin-producing genotypes, which are impossible to differentiate based on morphological characteristics. The presence of the mcyA gene has been used as an indirect diagnosis for the identification of microcystin-producing cyanobacteria. The aim of this work was to evaluate if the blooms of cyanobacteria in the Itaipu reservoir present a microcystin-producing genotype. To that end, the phycocyanin intergenic spacer (PC-IGS) and partial mcyA gene were amplified and sequenced. The presence of these genes was observed in all the samples, indicating the toxicological potential of the blooms of cyanobacteria in the Itaipu reservoir. The phylogenetic analyses of sequences from cyanobacteria blooms isolated from the Itaipu reservoir and sequences available in GenBank demonstrated the genetic similarity between samples from Itaipu and species from the *Microcystis* genus.
Key words: *Microcystin, PC-IGS, mcyA.*

Introduction

Cyanobacteria are ubiquitous organisms that can be found in many diversified aquatic environments. Their presence is important because they are known to produce cyanotoxins (Carmichael, 1994) that are capable of affecting the aquatic biota, which results in poisonous effects even in terrestrial mammals (Sivonsen and Jones, 1999). Cyanobacteria produce several different types of toxins, including microcystins. Microcystins are a hepatotoxin produced by a cluster of 10 genes consisting of 55,000 base pairs (bp) arranged in two probable operons, mcyA-C and mcyD–J, which encode for a complex enzymatic system involved in microcystin biosynthesis (Tillett *et al.*, 2000), that acts by covalently binding to phosphatase proteins, which disrupts cellular signaling and can lead to hepatic tumors, as described by (Falconer *et al.*, 1994; Ito *et al.*, 1997). As such, the presence of microcystin-producing cyanobacteria in bodies of water has obvious relevance to public health.

In 1995, Neilan *et al*. described a pair of primers that are derived from the coding region of the α and β subunits of the phycocyanin (cpcA and cpcB) and the intergenic spacer between these two subunits (PC-IGS). These primers can be used to amplify DNA by PCR (polymerase chain reaction) and, with the use of restriction enzymes (RFLP), can aid in the identification of many cyanobacteria genera. But the primers selectively amplify both toxic and non-toxic variants of DNA of cyanobacteria that can be present in the complex microbial communities of aquatic environments.

Another way to study the taxonomy and phylogeny of cyanobacteria is to sequence the PC-IGS and compare its sequence with those of several species of cyanobacteria that are available in GenBank using BLAST (Basic Local Alignment Search Tool) (Baker *et al*., 2002). The PG-IGS of cyanobacteria is appropriate for phylogenic studies because the two phycocyanin subunits α and β that flank the intergenic spacer are extremely conserved, which allows the annealing of primers across many species. The intergenic spacer, on the other hand, is very variable and allows the distinction of species of cyanobacteria (Neilan *et al*., 1995).

The toxicity of cyanobacteria cannot be evaluated by morphological characteristics. The difficulty in the differentiation among toxic and non-toxic strains has been overcome by the development of quantitative analyses of cyanotoxins in water. Recently, amplification of a fragment of the mcyA gene by PCR allowed the identification of cyanobacteria with a microcystin-producing genotype in blooms of water reservoirs. (Tillett *et al*., 2001) synthesized a pair of specific primers to amplify the mcyA gene of *Microcystis* species; however, the primers did not effectively amplify the mcyA gene of other cyanobacteria genera. (Hisbergues *et al*., 2003) synthesized a different pair of primers that correspond to another fragment of the mcyA gene, which allowed the selective distinction of microcystin and non-microcystin-producing cyanobacteria from several genera.

These alternative approaches for the detection of toxic cyanobacteria by amplification of the mcy gene has been applied to several genera, such as: *Microcystis* (Davis *et al*., 2009; Kurmayer *et al*., 2003; Via-Ordorika *et al*., 2004; Tillett *et al*., 2001), *Planktothrix* (Kurmayer *et al*., 2004), *Leptolyngbya* and *Geitlerinema* (Richardson *et al*., 2007), *Anabaena* (Kaebernick *et al*., 2002), *Nostoc* (Hisbergues *et al*., 2003), and others.

The evaluation of toxicity by PCR techniques is consistent with results obtained by the quantitative analyses of toxins in water by methods such as HPLC (High-pressure liquid chromatography), MALDI-TOF MS (Matrix-assisted laser desorption/ionization Team-Of-Light mass spectrometry) and ELISA (Enzyme-linked immunosorbent assay). Several authors have demonstrated a correlation between the presence of mcy genes and the detection of microcystin in water (Baker *et al*., 2002; Boaru *et al*., 2006; Davis *et al*., 2009; Kurmayer *et al*., 2004; Hisbergues *et al*., 2003; Mankiewicz-Boczek, *et al*., 2006; Oberholster *et al*., 2009; Via-Ordorika *et al*., 2004).

One of the most important reservoirs of South America, the Itaipu reservoir was built in 1982 and is located in the southwest of Paraná State (24° 15’ – 25° 33’ S-latitude; 54° 00’ – 54° 37’ W-longitude), and it demarcates part of the border between Brazil and Paraguay. The reservoir presents a flooded area of 1.350 km^2 and a residence time of 40 days. In general, the annual oscillations of the water level are less than one meter (Bini, 2001). Ecological or even taxonomy work on the algae community in the Itaipu reservoir is very rare. As such, the aim of this work was evaluate the toxicity of natural blooms of cyanobacteria in the Itaipu reservoir by the investigation of the presence of the mcyA gene.

Materials and methods

Two of the eight tributaries on the Brazilian margin of the Itaipu reservoir were chosen to collect the biological samples. Specifically, the tributaries that are formed by the São Francisco Verdadeiro (SV) and São Francisco Falso (SF) rivers were selected for this study (*Fig. 1*).

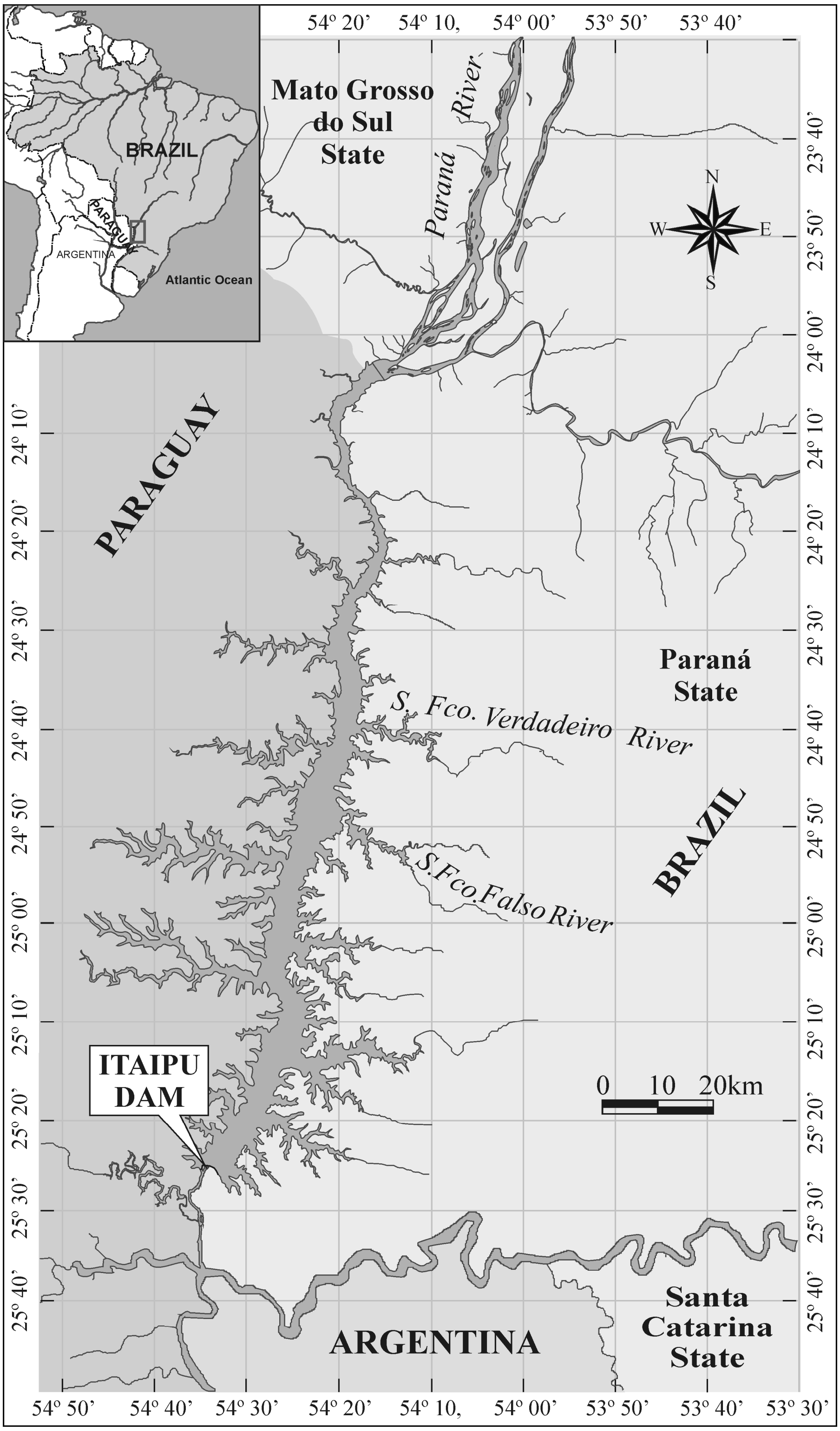

***Figure 1.** Sampling sites in the two tributaries of the Itaipu reservoir (São Francisco Verdadeiro and São Francisco Falso rivers)*

Approximately 150 ml of water from the subsurface that contains the cyanobacteria bloom was collected in October 2007. The collections were performed at four locations (SV1, SV2, SV3 and SV4) at the São Francisco Verdadeiro tributary and in one location (SF1) at the São Francisco Falso tributary. An aliquot of each sample was stored at 4°C for DNA extraction.

For DNA extraction, 2.0 ml of each sample was put into an Eppendorf tubes and centrifuged at 10,000 rpm for 10 min to pellet the cells. To each tube, 500 µl of extraction buffer was added (1 M Tris-HCl, pH 8.0, 0.5 M EDTA, pH 8.0, 140 mM β-mercaptoethanol, 5 M NaCl, 5% CTAB, and Sarcosyl 10%), in the presence of lysozyme (1 mg/ml) and incubated in a water bath at 37°C for 30 min. Proteinase K (50 µg/ml) was added and the cells were incubated for 2 h in a water bath at 60°C. Cellular debris was isolated from DNA by a phenol/chloroform extraction. The purified DNA was precipitated with a mixture of saline solution and ethanol overnight. The DNA was washed with ethanol to remove excess salt and treated with RNAse in a 37°C water bath for 2 h. Extracted DNA was quantified by agarose (1%) gel electrophoresis, using as a reference DNA from the bacteriophage λ at concentrations of 25, 50 and 100 ng/l.

The primer pair PCβF (5'-GGCTGCTTGTTTACGCGACA-3') and PCαR (5'-CCAGTACCA-CCAGCAACTAA-3') (Neilan *et al*., 1995) was used for amplification of the phycocyanin intergenic spacer (PC-IGS), which is a positive control for the presence of cyanobacteria DNA in the sample. The primer pair mcyA-Cd1R (5'-AAAAGTGTTTTATTAGCGGCTCAT-3') and mcyA-Cd1F (5'-AAAATTAAAAGCCGTATCAAA-3') (Hisbergues *et al*., 2003) was used for the partial amplification of the mcyA gene.

The PCR conditions were as described by (Prioli *et al*., 2002) and consisted of the following steps: 95°C for 10 min, 35 cycles of 95°C for 90 s, 56°C for 30 s, 72°C for 50 s and a final step of 72°C for 7 min. PCR fragments were submitted to electrophoresis in a 1,5% agarose gel with a standard DNA ladder 100 bp (Gibco BRL). The gels were stained with ethidium bromide.

Approximately 50 ng of DNA from each reaction was used in the sequencing reactions using the MegaBase automatic sequencer (Amersham), according to the manufacturer's instructions. Fifty-one sequences of the PC-IGS and 15 sequences of the partial mcyA gene from species of cyanobacteria of the orders Chroococcales, Nostocales and Oscillatoriales were selected from GenBank for the phylogeny analysis. Only sequences published in scientific journals were selected.

The sequences were aligned using the Clustal W (Thompson *et al*. 1994) computer program and edited with the Bioedit program (Hall, 1999). The choice of evolutionary model, using the Akaike Information Corrected Criterion (AICc) and Bayseian Information Criterion (BIC) procedures, was performed using the Paup 4.0b4 (Swofford, 2002) and Modeltest 3.0 (Posed and Crandall, 1998) programs. The nucleotide diversity matrix and the Neighbor-Joining dendrogram were built with the Mega 4.0.1 program (Tamura *et al*., 2007). The principal coordinate scatter plot was built using the eigenvectors after Lingoes correction criterion with the Statistica 7.1 program (StatSoft, Inc., 2005).

Results

Phycocyanin Intergenic Spacer (PC-IGS)

The results of the PCR using primers to amplify the PC-IGS produced fragments of approximately 650 bp (*Fig. 2*), which confirmed the presence of cyanobacteria DNA in the samples.

Figure 2. *A 1,5 % agarose gel including a 100 bp ladder (La) and the negative control (Br). Fragments of 650 bp correspond to the amplification product of the PC-IGS from samples of cyanobacteria blooms from São Francisco Verdadeiro (SV1, SV2, SV3 and SV4) and São Francisco Falso (SF1) tributaries, using the PCβF and PCαR primers*

The PCR products for the samples from the São Francisco Verdadeiro (SV1, SV2, SV3 and SV4) tributary were sequenced. After the alignment, a 320 bp sequence was obtained that encodes for part of the phycocyanin β subunit, the complete intergenic spacer and part of the α subunit. The Tamura-Nei evolutionary model was selected for phylogenetic analysis. The sequence indicated that there were six nucleotide substitutions points among the four samples. No substitution was found between the samples SV2 and SV3. The transition/transversion bias was 1.7 and the distribution of nucleotide bases was A=0.247, T=0.241, C=0.263 and G=0.248.

The results of the Neighbor-Joining dendrogram and the scatter plot, that were built with the Itaipu reservoir samples, in combination with sequences available from GenBank, made it was possible to characterize the three orders of cyanobacteria that were analyzed. The sequences of the cyanobacteria samples from the Itaipu reservoir aligned with species from the *Microcystis* genus in the Chroococcales cluster (*Fig. 3* and *Fig. 4*).

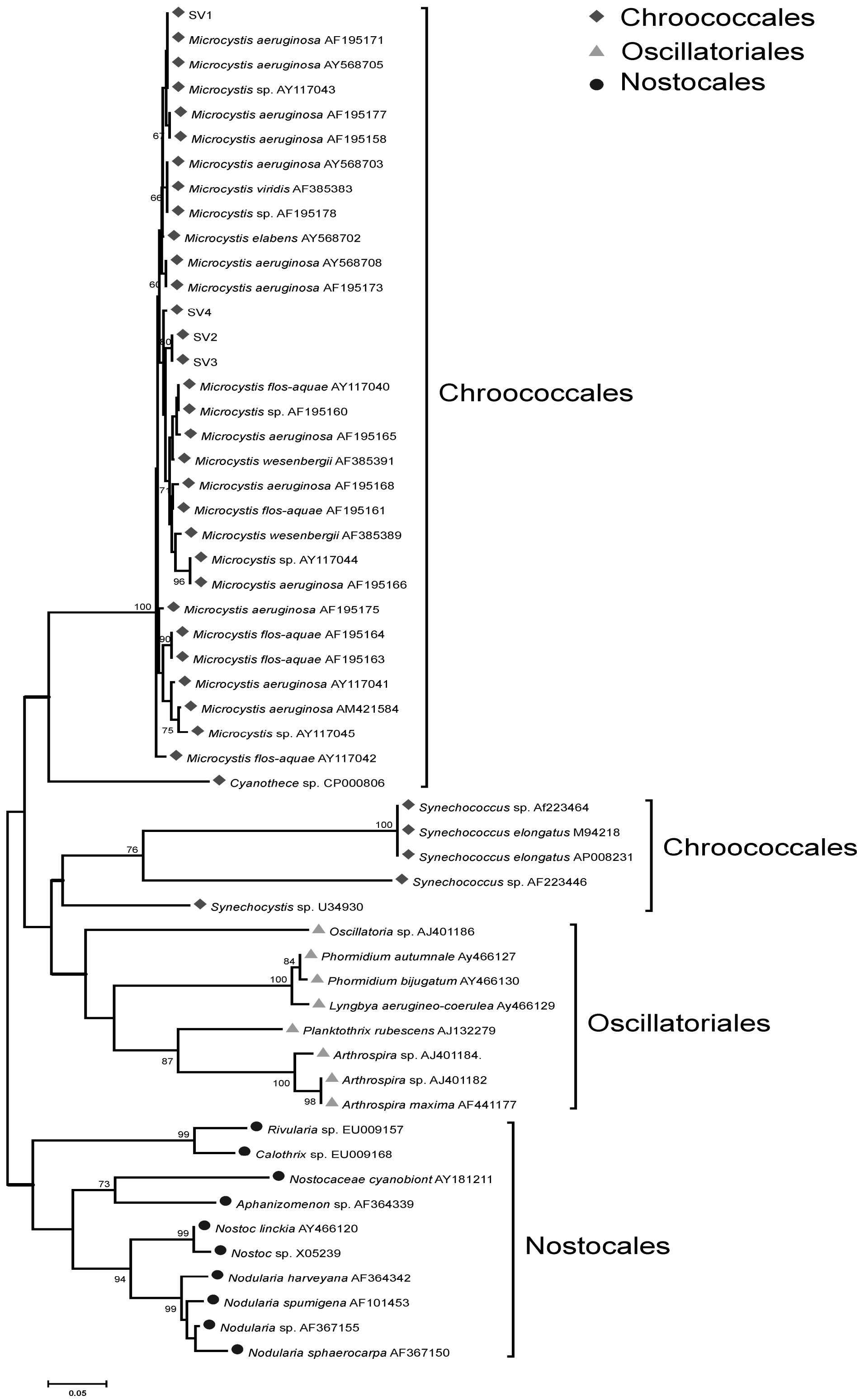

Figure 3. *Neighbor-Joining dendrogram built with the Tamura-Nei model, with 10,000 Bootstraps, from sequences of the PC-IGS from samples of cyanobacteria blooms from São Francisco Verdadeiro (SV1, SV2, SV3 and SV4) tributary and sequences available in GenBank*

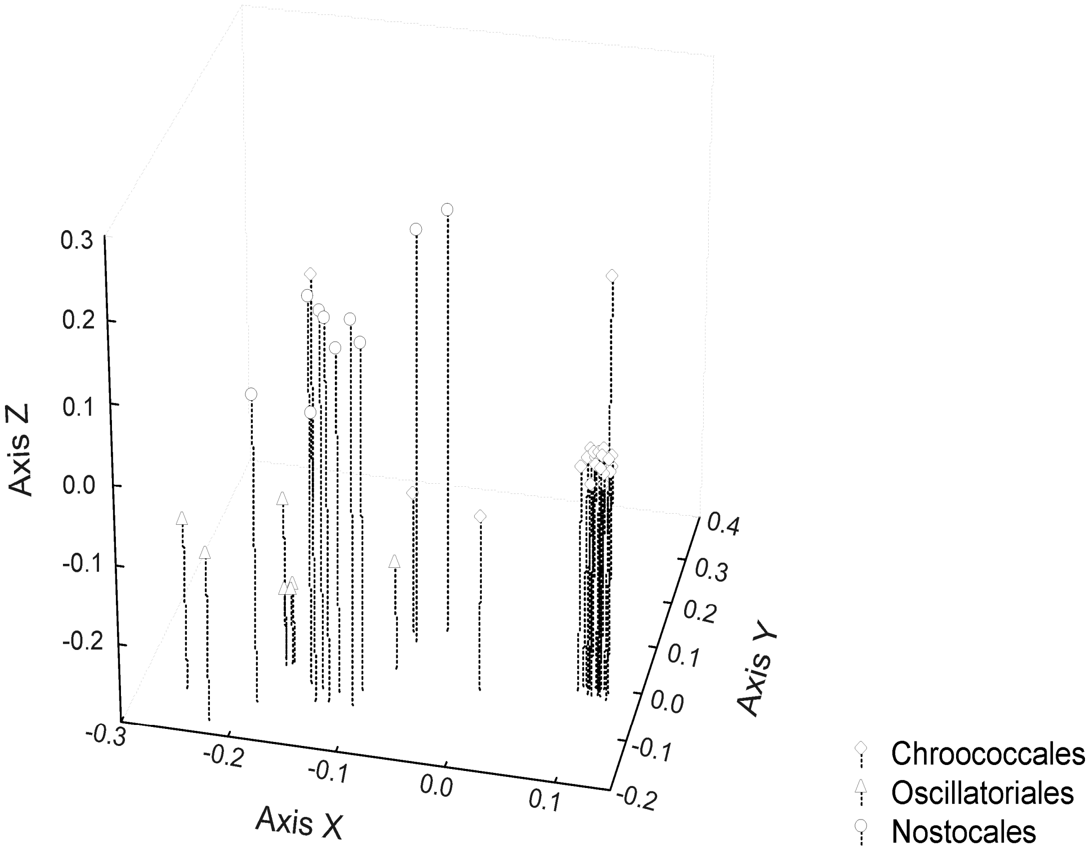

Figure 4. *Scatter plot built with the eigenvectors obtained from the Tamura-Nei genetic distance matrix from PC-IGS sequences from samples of cyanobacteria blooms from São Francisco Verdadeiro (SV1, SV2, SV3 and SV4) tributary and sequences available in the GenBank*

Toxicity

Partial amplification of the mcyA gene produced a DNA fragment of approximately 300 bp for all analyzed samples (*Fig. 5*). The presence of the mcyA gene in all samples indicates that the cyanobacteria blooms present in the Itaipu reservoir are from a microcystin-producing genotype strain.

The partial microcystin synthetase gene (mcyA) of three samples from Itaipu reservoir was sequenced. After alignment, a 236 bp sequence was obtained. Only one nucleotide substitution point was observed, which was in the sample from the São Francisco Falso (SF1) tributary. The transition/transversion bias was 1.7, with nucleotide base distribution of A=0.265, T=0.328, C=0.155 and G=0.253.

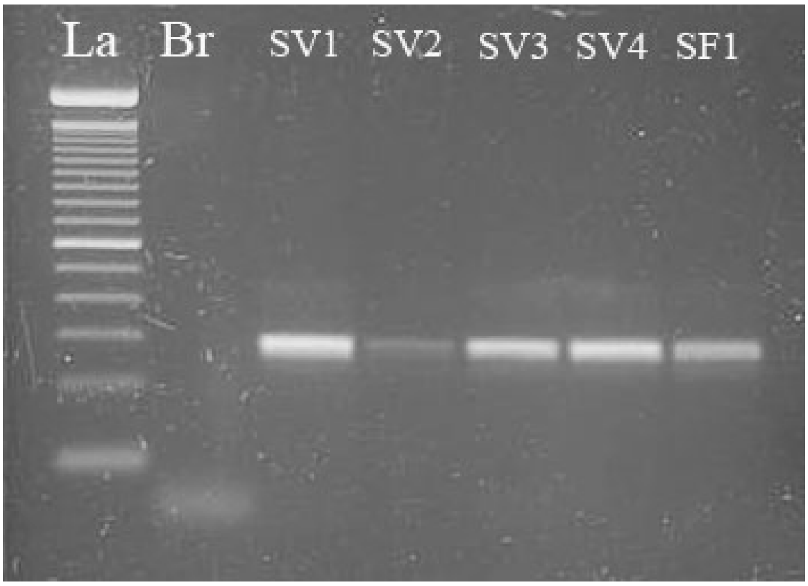

Figure 5. *A 1,5% agarose gel including a 100 bp ladder (La), and the negative control (Br) Fragments of 300 bp correspond to the amplification product of the partial mcyA gene from samples from the São Francisco Verdadeiro (SV1, SV2, SV3 and SV4) and São Francisco Falso (SF1) tributaries, using the mcyA-Cd1R and mcyA-Cd1F primers*

The Neighbor-Joining dendrogram separated the samples from the Itaipu reservoir into a single cluster, which was very close to the cluster of the species from the *Microcystis* genus (*Fig. 6*). This results were consistent with the results obtained with the PC-IGS sequences, The genetic analyses of the partial sequence of the mcyA gene were also useful in the characterization of the three orders of cyanobacteria.

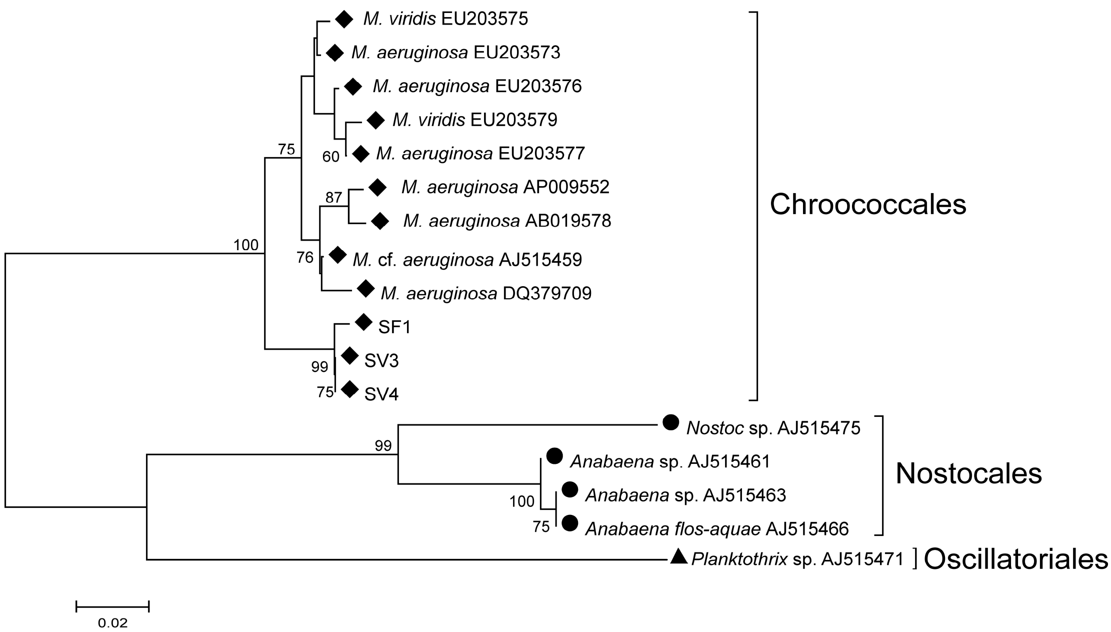

Figure 6. *Neighbor-Joining tree built with the p distance, with 10,000 bootstrap, using sequences of the partial mcyA gene from samples of cyanobacteria blooms from the São Francisco Verdadeiro (SV3 and SV4) and São Francisco Falso (SF1) tributaries and sequences available in GenBank*

Discussion

Amplification of the PC-IGS sequence is a positive control for the presence of cyanobacteria DNA in the samples. (Neilan *et al*., 1995) used primers of the PC-IGS for the selective PCR amplification of genetic material of cyanobacteria of different samples from aquatic environments. Fragments from toxic genera (*Microcystis* and *Anabaena*) had between 500 and 740 bp. All of the DNA samples studied from the Itaipu reservoir tested positive for the PC-IGS, which confirms the presence of cyanobacteria in the algae blooms that were studied.

The results of the PC-IGS sequencing demonstrate the genetic relationship among samples from the Itaipu reservoir and species from the *Microcystis* genus, such as *Microcystis aeruginosa*. The exact result, with simple nucleotide peaks in the sequencing, confirms that the extracted DNA of the samples were from one single species since the presence of more than one species (in significant abundance) would show up as multiple nucleotide peaks (Baker *et al*., 2002).

The presence of the mcyA gene in the samples collected from the Itaipu reservoir demonstrates the toxicological power of these cyanobacteria blooms. Environmental conditions, such as light intensity, nutrient concentration, temperature, age and size of the colony, appear to influence microcystin production. However, the environmental factors that control the expression of the microcystin synthetase genes are not yet completely understood (Nishizawa *et al*., 1999).

In fact, the presence of the mcyA gene alone is not enough to infer that the blooms are producing cyanotoxin. In 2009, Ostermaier and Kurmayer demonstrated that the mcyA gene could be inactivated in natural populations, due to a mutation in the gene. However, the presence of the mcyA gene has been correlated with the ability of cyanobacteria to produce microcystin (Tillet *et al*. 2000), and several studies have correlated the presence of mcy genes, included mcyA, with the presence of microcystin in water (Baker *et al*., 2002; Boaru *et al*., 2006; Kurmayer *et al*., 2004; Hisbergues *et al*., 2003; Mankiewicz-Boczek, *et al*., 2006; Via-Ordorika *et al*., 2004). Thus, the results obtained in this work indicated that the species of cyanobacteria that is present in samples from two tributaries of the Itaipu reservoir is from a microcystin-producing strain.

The present study emphasizes the importance of using molecular markers for the identification of potentially toxic cyanobacteria in environmental monitoring programs because this method allows the evaluation of algae before the toxins are released into the water. The presence of a species that contains a genotype for microcystin production is of particular concern for public health in water reservoirs, such at the Itaipu reservoir, because it is used for recreation by the riverine population and tourism

The sequencing of the PC-IGS and the partial mcyA gene is shown to be useful in the molecular analyses of cyanobacteria and must be utilized together for the identification of potentially toxin-producing cyanobacteria.

REFERENCES

[1] Baker, J.A., Entsch, B., Neilan, B.A., McKay, D.B. (2002): Monitoring Changing Toxigenicity of a Cyanobacterial Bloom by Molecular Methods. – Appl. Environ. Microbiol. 68(12): 6070-6076.

[2] Bini, L.M. (2001): Dinâmica populacional de *Egeria najas* Planchon (Hydrocharitaceae): sobrevivência de uma espécie submersa em um ambiente subtropical com elevada

turbidez (reservatório de Itaipu Binacional, Brasil Paraguai). – Tese (Doutorado em Ecologia de Ambientes Aquáticos Continentais), Universidade Estadual de Maringá, Maringá, Brasil.
[3] Boaru, D.A., Dragos, N., Welker, M., Bauer, A., Nicoara, A., Schirmer, K. (2006): Toxic potential of microcystin-containing cyanobacterial extracts from three Romanian freshwaters. – Toxicon 47: 925-932.
[4] Carmichael, W.W. (1994): The toxins of cyanobacteria. – Sci. Am. 270: 78-86.
[5] Davis, T.W., Berry, D.L., Boyer, G.L., Gobler, C.J. (2009): The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of Microcystis during cyanobacteria blooms. – Harmful Algae 8:715-725.
[6] Falconer, I.R., Burch, M.D., Steffensen, D.A., Choice, M., Coverdale, O.R. (1994): Toxicity of the blue-green alga (Cyanobacterium) Microcystis aeruginosa in drinking water to growing pigs, as an animal model for human injury and risk assessment. – Environ. Toxicol. Water Qual. 9: 131-139.
[7] Hall, T.A. (1999): Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucl. Acids. Symp. Ser. 41: 95-98.
[8] Hisbergues, M., Christiansen, G., Rouhiainen, L., Sivonen, K., Borner, T. (2003): PCR-based identification of microcystin-producing genotypes of different cyanobacterial genera. – Arch. Microbiol. 180: 402-410.
[9] Ito, E., Kondo, F., Terao, K., Harada, K. (1997): Neoplasic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin-LR. – Toxicon 35: 1453-1457.
[10] Kaebernick, M., Dittmann, E., Borner, T., Neilan, B.A. (2002): Multiple alternate transcripts direct the biosynthesis of microcystin, a cyanobacterial toxin. – Appl. Environ. Microbiol. 68: 449-455.
[11] Kurmayer, R., Christiansen, G., Chorus, I. (2003): The abundance of microcystin-producing genotypes correlates positively with colony size in *Microcystis* and determines its microcystin net production in Lake Wannsee. – Appl. Env. Microbiol. 69(2): 787-795.
[12] Kurmayer, R., Christiansen, G., Fastner, J., Börner, T. (2004): Abundance of active and inactive microcystin genotypes in populations of the toxic cyanobacterium *Planktothrix* spp. – Environ. Microbiol. 6: 831-841.
[13] Mankiewicz-Boczek, J., Izydorczyk, K., Romanowska-Duda, Z., Jurczak, T.; Stefaniak, K., Kokocinski, M. (2006): Detection and monitoring toxigenicity of cyanobacteria by application of molecular methods. – Environ. Toxicol. 21(4): 380-387.
[14] Neilan, B.A., Jacobs, D., Goodman, A.E. (1995): Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. – Appl. Environ. Microbiol. 61: 3875-3883.
[15] Nishizawa, T., Asayama, M., Fujii, K., Harada, K., Shirai, M. (1999): Genetic Analysis of the Peptide Synthetase Genes for a Cyclic Heptapeptide Microcystin in *Microcystis* spp.1. – J. Biochem. 126: 520-529.
[16] Oberholster, P.J., Myburgh, J.G., Govender, D., Bengis, R.; Botha, A.M. (2009): Identification of toxigenic Microcystis strains after incidents of wild animal mortalities in the Kruger National Park, South Africa. – Ecotoxicology and Environmental Safety. 72: 1177-1182.
[17] Ostermaier, V., Kurmayer, R. (2009): Distribution and Abundance of Nontoxic Mutants of Cyanobacteria in Lakes of the Alps. – Microb. Ecol. 58: 323-333.
[18] Posada, D., Crandall, K.A. (1998): MODELTEST: testing the model of DNA substitution. – Bioinformatics 14: 817-818.
[19] Prioli, S.M.A.P., Prioli, A.J., Júlio Jr., H.F., Pavanelli, C.S., Oliveira, A.V., Carrer, H., Carraro, D.M., Prioli, L.M. (2002): Identification of *Astyanax altiparanae* (Teleostei, Characidae) in the Iguaçu River, Brazil, based on mitochondrial DNA and RAPD markers. – Genetic and Molecular Biology. 25(4): 421-430.

[20] Richardson, L.L., Sekar, R., Myers, J.L., Gantar, M., Voss, J.D., Kaczmarsky, L., Remily, E.R., Boyer, G.L., Zimba, P.V. (2007): The presence of the cyanobacterial toxin microcystin in black band disease of corals. – FEMS Microbiol. Lett. 272(2): 182-187.
[21] Sivonsen, K., Jones, G. (1999): Cyanobacterial toxins. – In: Chorus, I., Bartram, J. (eds.) Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management, E & FN Spon London. pp. 41-111.
[22] StatSoft, Inc. (2005): STATISTICA (data analysis software system), version 7.1. www.statsoft.com.
[23] Swofford, D.L. (2002): PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer, Sunderland, Massachusetts.
[24] Tamura, K., Dudley, J., Nei, M., Kumar, S. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. – Molecular Biology and Evolution 24: 1596-1599.
[25] Thompson, J.D., Higgins, D.G., Gibson, T.J. (1994): CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequencing weighting, position-specific gap penalties and weight matrix choice. – Nucleic. Acids. Res. 22: 4673-4680.
[26] Tillett, D., Dittmann, E.; Erhard, M., VonDöhren, H., Börner, T., Neilan, B.A. (2000): Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide-polyketide synthetase system. – Chem. Biol. 7: 753-764.
[27] Tillett, D., Parker, D.L., Neilan, B.A. (2001): Detection of toxigenicity by a probe for the microcystin synthetase A gene (mcy A) of the cyanobacterial genus *Microcystis*: comparison of toxicities with 16S rRNA and phycocyanin operon (phycocyanin intergenic spacer) phylogenies. – Appl. Environ. Microbiol. 67: 2810-2818.
[28] Via-Ordorika, L., Fastner, J., Kurmayer, R., Hisbergues, M., Dittmann, E., Komárek, J., Erhard, M., Chorus, I. (2004): Distribution of Microcystin-Producing and Non-Microcystin-Producing *Microcystis* sp. in European Freshwater Bodies: Detection of Microcystins and Microcystin Genes in Individual Colonies. – System. Appl. Microbiol. 27: 592-602.

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