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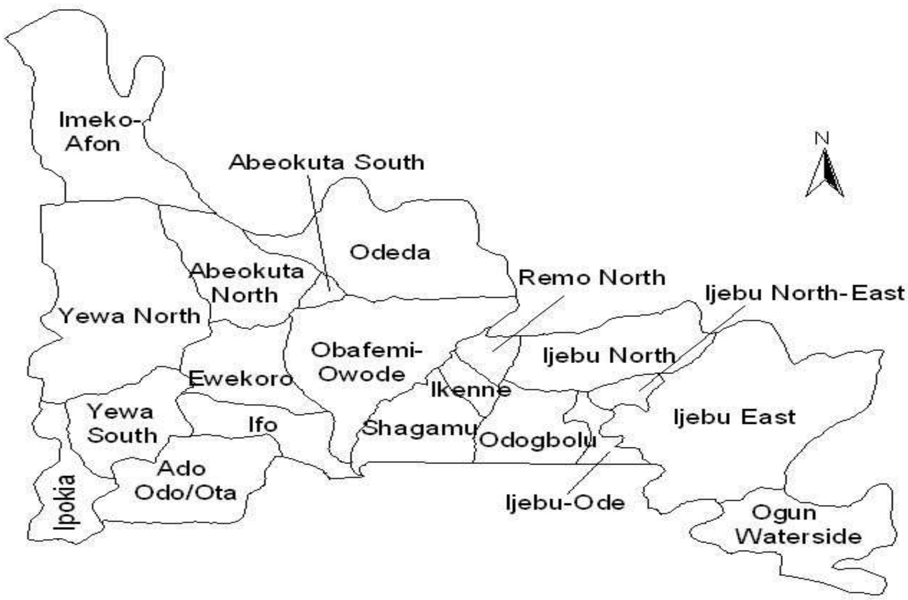

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ECOLOGICAL CONSEQUENCES OF CHANGING FUELWOOD CONSUMPTION PATTERNS IN REMOTE VILLAGES OF NORTHWESTERN CHINA

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Abstract. Consumption of ecosystem goods and services is the foundation of coupled human-natural systems. This paper reported the change in fuelwood consumption in remote northwestern Chinese villages and the ecological consequences that have occurred as a result of the Sloping Land Conversion Program (SLCP), one of China's biggest ecological restoration programs. We conducted this study using structured questionnaires that collected data on fuelwood consumption in 1999 and 2008. For these villages, fuelwood was the most important fuel source (84% of total fuel consumption in 1999). The SLCP restricted cutting of forests, so fuelwood consumption decreased to 39% of the 1999 total by 2008. In response to this decrease and increased planting of trees, the forest area increased. However, a spatial imbalance in fuelwood consumption persisted; the remaining demand for fuelwood meant that forests near villages were under high pressure, with harvesting often exceeding the natural productivity. To meet the demand for fuel and replace the fuelwood, coal consumption increased by 562%. The switch from fuelwood to coal increased CO_2 emissions by 339% from 1999 to 2008. These results have clear consequences for the region's ecology and suggest the need to take measures to account for the consequences of the SLCP.
Keywords: *Ecological consequences; Fuelwood; Fuel consumption; Sloping Land Conversion Program; Northwestern China*

Introduction

Consumption of ecosystem goods and services is a key factor in coupled human–natural systems (J. Liu et al., 2007; Zhen et al., 2010b). The steady increase in human activities and consumption of ecosystem services has led to degradation of around 60% of the world's ecosystems (MEA, 2005), accompanied by a 30% increase in atmospheric CO_2 level (Vitousek et al., 1997). The consumption of ecosystem services has exceeded the productive capacity of world's ecosystems by as much as 50% (WWF, 2010). However, the share of the biosphere's resources and ecosystem services consumed by humanity will continue to increase.

Differences and changes in consumption patterns will have different consequences for human-environment systems. For example, many case studies have shown obvious differences in food consumption patterns between China and other countries, leading to different ecological consequences (Gerbens-Leenes and Nonhebel, 2005; Gerbens-Leenes et al., 2010; Zhen et al., 2010a, 2010b). The increasing consumption of beverages and foods of animal origin is likely to create additional pressure on natural resources, especially in Asia (Gerbens-Leenes et al., 2010), as well as the loss and fragmentation of native forests (McAlpine et al., 2009) and additional emissions of greenhouse gases (Fearnside, 2005; McAlpine et al., 2009).

Fuelwood consumption is a particularly important problem, since approximately half of the world's population uses fuelwood in their daily lives (He et al., 2009). Fuelwood collection has been recognized as an important factor in habitat fragmentation and biodiversity loss (Bearer et al., 2008; He et al., 2009; J. Liu et al., 1999). For instance, the increased population and fuelwood collection intensities in China's Wolong National Nature Reserve have dramatically decreased the forest habitat that sustains populations of the giant panda (Ailuropoda melanoleuca) (J. Liu et al., 1999). As fuelwood becomes more difficult to find and collection sites move higher in elevation and become more remote, the high-quality panda habitats face an increasing risk of fragmentation and degradation (He et al., 2009). However, fuelwood consumption that does not exceed the natural growth rate of the forest could potentially decrease net CO_2 emissions if fuelwood replaces the consumption of fossil fuels (Eriksson and Gustavsson, 2010). A potential reduction of from 2 to 30% has been estimated for various European countries (Schwaiger and Schlamadinger, 1998). This suggests that *sustainable* use of fuelwood may represent a good option to reduce the environmental impacts of energy production.

National policies affect human activities and the resulting consumption of ecosystem services, with related ecological consequences. The "greening" of global consumption patterns by deliberate development or modification of policies is an important goal (Spaargaren and Mol, 2008). In this context, many scientists have studied the impact of such policies on ecosystem services in coupled human–natural systems. For example, to prevent further habitat degradation and restore panda habitat, the Chinese government implement several conservation policies (J. Liu et al., 2007). These policies prohibited the development of cropland in forested areas, limited the sites where fuelwood can be harvested and the amount of fuelwood that can be harvested from these sites, but the effects appear to have been imperfect (Chen et al., 2009). Adjusting the conservation policies to provide payments to compensate people for conservation costs, increasing job availability in cities, and encouraging the migration of young people to cities appear to have been more helpful (Chen et al., 2009; J. Liu et al., 1999). A recent study on the Mongolian Plateau concluded that different land-use policies, and particularly changes in land ownership, caused major differences in food and fuel consumption by herders in Mongolia and China's Inner Mongolia autonomous region (Zhen et al., 2010b). And traditional nomadic grazing pattern under public land ownership in Mongolia leaded to less degradation in rangeland quality, comparing to the concentrated and continuous herding pressure on the small patches of grassland managed by individual households, under the grassland household contracting policy (Zhen et al., 2010b).

In rural areas of northwestern China, fuelwood is a major fuel for heating and cooking. In recent years, fuelwood consumption has varied greatly as a result of socioeconomic development and policy changes. One major policy that has affected lives in this region is the Sloping Land Conversion Program (SLCP, also known as the

"Grain for Green Program"), which is one of the largest ecological restoration programs in the world. Under this program, farmers who convert cropland on steep slopes to forest and grassland are rewarded with grain and cash subsidies from the government (J. Liu et al., 2008). The SLCP was originally planned to continue from 2001 to 2010, with a goal of converting 14.63×10^6 ha of sloping farmlands, including 4.4×10^6 ha on slopes steeper than 25° and in desertified fields. In addition, the program's goal was to afforest or revegetate another 17.33×10^6 ha of sparsely vegetated mountainous, hilly, and eroded lands (Yin and Yin, 2010).

The primary trade-offs for ecosystem services that the policy-makers considered were the need to preserve the food supply of local peoples, while improving soil and water conservation. Research has shown that the SLCP achieved great successes in terms of reducing surface runoff and soil erosion, maintaining soil fertility, and lowering river sediment loads, while conserving water resources and reducing desertification (J. Liu et al., 2008). In addition, the program has promoted socioeconomic development that has helped numerous farmers change their income structure, thereby increasing their income (J. Liu et al., 2008; Uchida et al., 2005; Xie et al., 2006). The subsidy program focused on grain and cash because the primary consumption targeted by the SLCP was food consumption. However, the SLCP also affected the ecosystem service of fuelwood supply due to the increased forest area, and affected fuelwood consumption due to the change in forest management (i.e., increased restrictions on use of the forests).

Unfortunately, there have been no studies of the changes in fuelwood consumption in remote northwestern villages and the associated ecological consequences. The goal of the present study was to provide this information in the context of the SLCP and to discuss the ecological effects of these changes to guide the adjustment of government policies in this remote part of China.

Study area

This study focused on four villages (Wuzhuang, Xiakou, Sanshilipu, and Shimo) located in Guyuan District of the Ningxia Hui Autonomous Region, a remote mountainous region in northwestern China (*Fig. 1*). Elevations range from 1248 to 2942 m above mean sea level, and the region has a continental monsoon climate, characterized by a mean annual rainfall of 472 mm and an annual average temperature of 9°C ranged between -15 to 30°C (Zhen et al., 2010a). The long winter (4 months) and low temperatures (mean monthly temperatures range from -2 to -15°C) create a high demand for heating fuel.

As a part of Loess Plateau, this area is sensitive to water and wind erosions due to the special soil characteristics (e.g., loess soil), climatic conditions, and topography. As a result of unsustainable human practices such as excessive cultivation and forest harvesting on slopes, soil erosions has been severe, leading to seriously degraded ecological conditions in many areas (Shi and Shao, 2000). The decades of these activities has intensified and accelerated the ecological degradation, thereby jeopardizing the ecological security of downstream regions of China's Yellow River. The SLCP started in 2000 in this region, with the goal of converting arable land on steep slopes (>15°) into forests and afforesting sparsely vegetated areas of hilly and bare land. The native forests are a mix of broadleaf trees and shrubs, which the shrubs were usually used as fuelwood by local residents.

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In addition to its ecological fragility, this area is home to some of the poorest people in China, with an average total income of 10273.0 RMB (1580.5$ with currency of 6.5 RMB equal to 1.0 $) and net income of 2613.9 RMB (402.1$) in Guyuan District in 2008, which is much lower than the national average for rural areas. The population density is 140 people per km^2, with 86.1% of the population living in rural areas. The total population increased by 18% from 1999 to 2008. However, due to high levels of migration to cities, the rural population only increased by 7% during this period. There are many villages scattered throughout mountainous areas that lack a transportation infrastructure – some areas lack even basic roads.

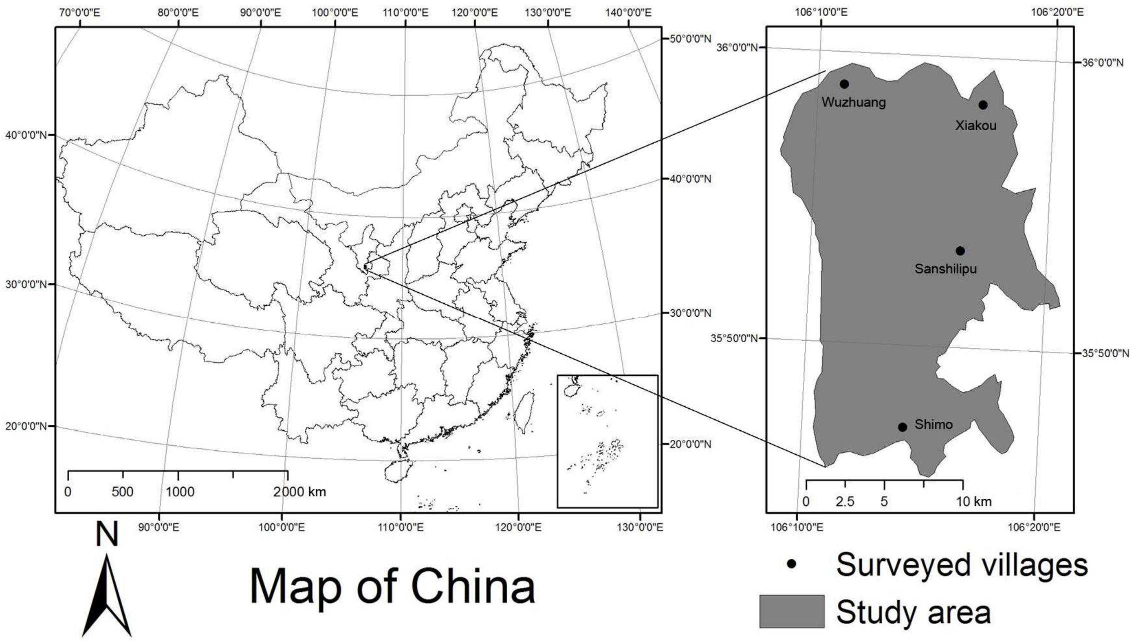

Figure 1. *Location of the study area*

Research methods and data sources

Household surveys and data collection

We conducted a household survey from July to August 2009 using structured questionnaires to obtain our primary data. We randomly selected 208 households in the four villages (n = 34 to 77 per village) and interviewed their residents. We asked about the following socioeconomic characteristics of each household: family composition, levels of education, income, and income sources. Our goal was to obtain basic background information on the household. The fuel consumption information we collected included the types, amounts, and distances from home of the market or collection locations. The information on income and fuel consumption was obtained for 1999 and 2008 to provide an indication of changes during this 10-year period. As most of the households had recorded detailed family accounts of their income, fuel consumption, and costs, we believe that despite the potential inaccuracy of this approach, the information we collected provided a good overview of changes in fuel consumption and other characteristics of the families during this period.

The fuel consumption values were recorded as weights. The distance and associated time cost to reach each fuel market used by the household were recorded. For fuelwood collection, the distance to the typical collection locations and the most distant location were determined for 1999 and 2008, as was the time required to reach these locations and return with fuelwood. The locations of the four villages were also recorded using GPS and input into a GIS program (version 9.3 of ArcGIS, ESRI, Redlands, CA).

Data derived from statistical yearbooks and land-use maps were also used in this paper. The statistical yearbooks include county-level yearbooks produced by the government of Guyuan District and national rural statistics books from 1999 to 2008. Land-use maps of this region were created by interpretation of Landsat TM images by the Data Center for Resources and Environmental Sciences of the Chinese Academy of Sciences (http://www.resdc.cn/english/default.asp). The land-use maps were categorized into six types: arable land, forest, grassland, construction land, water, and bare land.

Data analysis

The analysis of fuelwood consumption was divided into two parts. First, we analyzed the changes in fuelwood consumption. The consumption data obtained from the interviews were analyzed using Microsoft Excel 2003 and version 10.0 of the SPSS software (SPSS Inc., Chicago, IL). The consumption values for all fuel types were normalized to standard coal equivalents (kg sce) using the original recorded weight and the conversion factors in the Chinese federal standard (Standardization Administration 2008).

Second, we analyzed the fuelwood collection patterns using our GIS software. The fuelwood collection patterns were demonstrated with an indicator of fuelwood collection intensity. The formula of calculating fuelwood collection intensity is as following:

$$I_p = \sum_{i=1}^{4} \frac{n_{pi}}{n_{total\ i}} / 4 \quad \text{(Eq.1)}$$

I_p means the intensity of fuelwood collection from the specific forest of location p;

n_{pi} means the amount of households, which the distances from the their village i to the usual fuelwood collection locations are larger than the distance from location p to the village i;

$n_{total\ i}$ means the total amount of households in village i;

$0 < I_p < 1$, where $I_p = 1$ means all the households from all four villages were usually collecting fuelwood in the forest of location p, while $I_p=0$ means none of the households did so. To facilitate interpretation of this information, we divided the intensity of fuelwood collection into five classes: very high intensity ($0.4 < I_p < 1$) means that 40% or more of the households usually collected fuelwood in this area, versus 30% for high intensity ($0.3 < I_p < 0.4$), 20% for moderate intensity ($0.2 < I_p < 0.3$), 10% for low intensity ($0.1 < I_p < 0.2$) and very low intensity ($0 < I_p < 0.1$) means many of the households may have reached that area at some time, but did not usually collect fuelwood there. The distance from each settlement to the most distant fuelwood collection location was assumed to represent the scope of human activity of fuelwood collection for the village. We used the buffer analysis feature provided by ArcInfo to determine the boundaries for human activity and for fuelwood collection intensity in each class. In addition to analyzing fuelwood consumption, we examined the possible driving forces responsible for changes in fuelwood consumption based on the consumption and socioeconomic data obtained from our survey.

The analysis of ecological consequences was also divided into two parts. First, we analyzed the forest area and associated net fuelwood productivity changes. The change in forest area was calculated based on the land-use maps from 1999 and 2008. The formula of calculating of net fuelwood productivity was as following:

$$P_n = P_t - \mathrm{C}_t = A_f \times P_{average} - n_t \times C_{average} \qquad \text{(Eq.2)}$$

P_n means the net fuelwood productivity;
P_t means the total fuelwood productivity;
C_t means the total fuelwood consumption;
A_f means the forest area;
$P_{average}$ means average natural productivity per hectare per year for the four major shrubs tree species of different age growing in the Guyuan area, which are *Amygdalus davidiana*, *Hippophae rhamnoides, Caragana korshinskii* and *Caragana microphylla* (X.L. Liu, 2009);
n_t means the total amount of households;
$C_{average}$ means the average fuelwood consumption per household;

We assumed that 40, 30, 20, and 10% of the fuelwood consumption was supplied by the forests with very high, high, moderate, and low fuelwood consumption intensities, respectively. The net fuelwood productivity (P_n) was calculated respectively in the forest with each estimated fuelwood collection intensity level. Net productivity was assumed to represent the threshold for potential ecological degradation of the forest, which would occur when fuelwood was harvested at a rate exceeding the forest's natural growth rate (Ghilardi et al., 2009).

We also calculated the estimated emissions of greenhouse gases resulting from the consumption of different types of fuel using the associated emission factors. The fuelwood use was considered to be carbon-neutral, yet would still release gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), particularly if the combustion is incomplete for the latter two gases (Bhattacharya and Abdul Salam, 2002; Schwaiger and Schlamadinger, 1998). We considered the emissions of CH_4 and N_2O due to fuelwood consumption using the associated emission factors for cooking and heating provided by Bhattacharya and Abdul Salam (2002). The emission factors for CO_2, CH_4, and N_2O from coal and liquefied petroleum gas (LPG) were obtained from IPCC (2006). The CH_4 and N_2O emissions were converted into CO_2 equivalents based on their global warming potential (IPCC, 2006). All total CO_2 emissons reported in this paper represent the sum of actual CO_2 emissions and the equivalent CO_2 emissions from CH_4 and N_2O.

Results

Patterns of and changes in fuelwood consumption

We calculated the average consumption per household of different types of fuel for the four villages based on the survey data. Our survey revealed that the fuel types

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consumed in the villages included fuelwood, dry grass, straw, animal dung, coal, LPG, and electricity. As the consumption of animal dung in the four villages was limited (less than 1%) and wasn't recorded in national statistics, we excluded the animal dung from the rest of our analysis. Similarly, electricity did not become a significant source of power to the four villages before 2008, so we have not discussed the impact of electricity. We also combined the data for dry grass and straw, since they are recorded as a single category in national statistics and because our focus was on fuelwood rather then on fine distinctions within fuel categories.

Fuelwood was the most important type of fuel in 1999, accounting for 84% of the total fuel consumption *(Fig. 2*). By 2008, the total fuel consumption per household had decreased by 13.6 %. The structure of the fuel consumption also changed. Biomass fuel (wood, grass, and straw) decreased from 91.3 % of the total in 1999 to 41.9 % in 2008, and fossil fuels (coal, LPG and electricity from coal-fired power plants) increased from 8.7 % of the total to 58.1% during the same period. This change mainly resulted from a decrease in fuelwood consumption per household, from 1518.1 kg sce in 1999 to 593.4 kg sce (i.e., to 39% of the 1999 level), whereas coal consumption per household increased from 144.7 kg sce to 813.4 kg sce (i.e., to 562% of the 1999 level).

Northwestern China is less developed than other regions of China. Compared with the national averages, the fuel consumption and patterns of change were therefore different. First, residents of the remote northwestern villages consumed more fuels than the national rural average, by around 18% in 1999 and 7% in 2008, mainly due to the long and cold winter. Second, residents of the remote northwestern villages preferred biomass fuels (fuelwood, dry grass and straw), which accounted for more than 90% of the total fuel consumption in 1999, versus a national rural average of only about 50%. The national rural average for fuelwood consumption accounted for around 20% of the total fuel consumption, and remained stable from 1999 to 2008, but coal consumption decreased by two-thirds, accounting for only 11% of the total in 2008. In contrast, with decreasing fuelwood consumption, coal consumption in the remote northwestern villages has increased greatly, and accounted for more than half of the total fuel consumption in 2008.

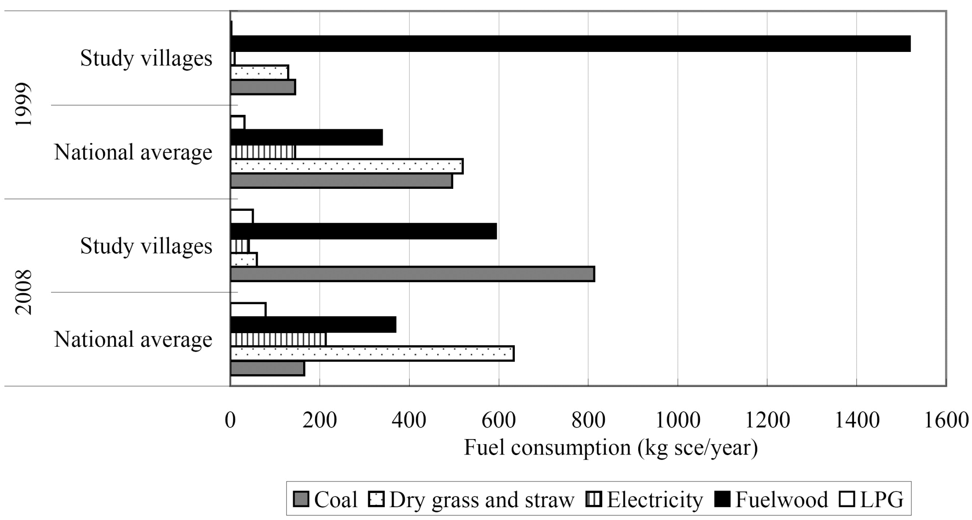

Figure 2. *Structure of and changes in fuel consumption in four remote villages in northwestern China and national rural averages. (sce, standard coal equivalent)*

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Changes in fuelwood collection patterns

Villagers in the study villages collected fuelwood in a traditional way. They traveled by foot to collect the fuelwood and transported the fuelwood to their homes on their back, which is hard work. Normally, most villagers indicated that they preferred to go to nearby forests to collect fuelwood, as long as wood was available. However, due to the SLCP policy, 46.7 % of the forest in this area was closed to human uses in 2000, and people were forbidden to harvest wood. The villagers were allowed to enter other parts of the forest, but were forbidden to cut the branches of live trees; they were only allowed to collect dead wood from the ground. Thus, some villagers chose to harvest branches illegally or sneak into the close forest, others chose to go farther to collect.

For the four villages as a whole, the average distance to the fuelwood collection locations was 2.92 km in 1999. In 2008, this distance had decreased to 2.58 km. This decreased average distance coincides with the decrease in average fuelwood consumption from 1999 to 2008 (*Fig. 2*). The fuelwood collection intensity from distant forests decreased, but the forests near the villages sustained a very high fuelwood collection intensity because fuelwood demand remained high.

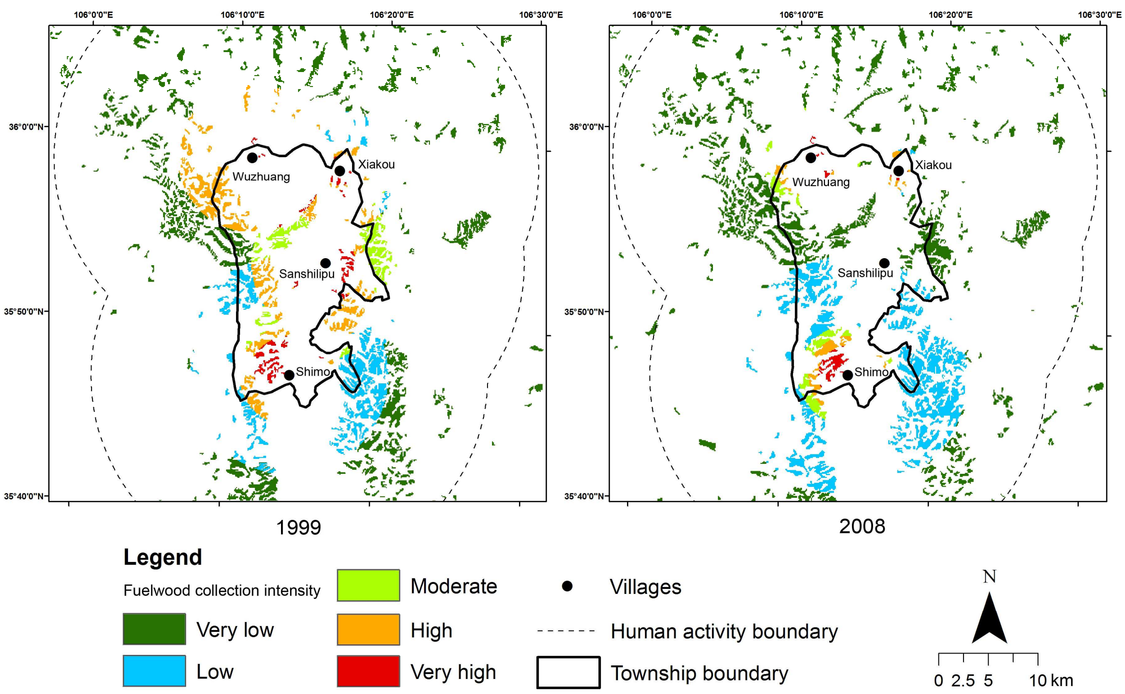

Figure 3. *Patterns and changes in fuelwood collection intensity from 1999 to 2008*

The average distances to the fuelwood collection locations varied among the villages, with different patterns of change from 1999 to 2008. For the villages of Wuzhuang, Xiakou, and Sanshilipu, the average distance decreased from 3.0 to 2.6 km, 1.9 to 1.2 km, and 2.9 to 2.4 km, respectively. The areas with very high, high, and moderate fuelwood collection intensity around these villages also decreased to different degrees (*Fig. 3*). The fuelwood collection intensity decreased from very high to very low in 144 ha of forest, from high to very low in 1581 ha of forest, from high to low in 924 ha of forest, and from moderate to very low in 1226 ha of forest around these villages. The decreasing fuelwood collection intensity coincided with the decrease in average fuelwood consumption per household, which decreased to 20.6, 9, and 66.6 % of the

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total for Wuzhuang, Xiakou, and Sanshilipu, respectively. For Shimo village, the fuelwood consumption also decreased (to 64.5 % of the total), but the average distance to the fuelwood collection locations increased from 3.2 to 3.4 km. The area with low fuelwood collection intensity increased from 3615 ha to 6079 ha. This change means that about 10% of the households in Shimo village had to travel farther to collect enough fuelwood, but that they still consumed less fuelwood than in 1999.

Driving factors for changes in fuelwood consumption

Income was one of the main factors that affected fuelwood consumption. We divided the households into six groups according to their total income in 2008. The average total income (10 273 RMB) was lower than in other rural areas of China (*Table 1*); at the national level, the average total rural income was 23 164 RMB per household in 2007. In 1999, total fuel consumption increased with increasing average total household income, from 967 kg sce at the lowest average total income (2215 RMB) to 4079 kg sce at the highest average total income (7686 RMB). In 2008, the same relationship existed (*Table 1*). From 1999 to 2008, the average total income of most households increased, except for the poorest households, whose total income remained less than 3000 RMB in 2008. The income gained from full-time or part-time off-farm employment in towns or cities contributed most to the income increase, as this income accounted for an average of 68% of the total income in 2008. However, for most households, total fuel consumption did not increase along with the increase in total income; the total fuel consumption decreased for nearly 50% of the households with total incomes greater than 12 000 RMB in 2008.

Table 1. *Fuel consumption patterns of the households as a function of annual household income levels. (sce, standard coal equivalent)*

		Total household income in 2008 (RMB)					
		>20 000	12 001 to 20 000	9001 to 12 000	6001 to 9000	3000 to 6000	<3000
Proportion of the 208 households (%)		7.7	16.3	19.2	21.6	19.2	15.9
Average total household income (RMB)	1999	7686	5726	3040	3345	2588	2215
	2008	34675	16629	10183	7676	5009	1998
Average total fuel consumption (kg sce)	1999	4079	3132	1553	1279	1299	967
	2008	2419	1643	1535	1589	1272	1387
Proportion of total fuel consumption as fuelwood (%)	1999	85.7	88.3	83.7	84.9	77.9	76.7
	2008	31.0	34.7	35.5	43.1	38.4	43.5
Proportion of total fuel consumption as coal (%)	1999	3.3	8.2	8.9	10.1	9.5	9.1
	2008	57.7	56.5	56.0	48.1	51.2	44.8
Proportion of total fuel consumption as LPG(%)	1999	0	0	0.4	0	0.3	0
	2008	7.7	3.9	2.1	4.4	1.2	0.7

Government policy was an important factor that affected the consumption behavior. In addition to converting arable land on slopes into forest, the SLCP forest management policy forbids the villagers to cut live branches for fuelwood. Thus, for the villagers who still need fuelwood after 2000 legally, they had to travel around more to visit more place collecting dead wood. The time consumed by this travel increased by 127, 191, 122, and 112% from 1999 to 2008 in Wuzhuang, Xiakou, Sanshilipu, and Shimo

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villages, respectively (*Table 2*). The increased difficulty of collecting fuelwood led to decreases of fuelwood consumption by 20.6, 9.0, 66.6, and 64.5% in Wuzhuang, Xiakou, Sanshilipu, and Shimo villages, respectively. However, based on the results in Table 1, as the mean income increased by 280%, the mean fuel consumption also increased (although the increase was not consistent for all income groups). To fill the gap created by decreased fuelwood consumption and the overall increase in total fuel consumption, all villagers were forced to start purchasing fossil fuel. The mean decrease in fuelwood consumption amounted to 924.7 kg sce per household from 1999 to 2008; simultaneously, average fossil fuel consumption increased by 717.2 kg sce. Coal consumption accounted for most of the increased fossil fuel consumption, because the coal was easy to obtain and there were many coal markets near each village. In contrast, there was only one LPG market in the township, and it was far from all four villages. Time consumed in traveling to the coal markets amounted to only 56, 60, 78, and 29% of the time required to reach the LPG market for villagers in Wuzhuang, Xiakou, Sanshilipu, and Shimo villages, respectively. The price of coal (1260 RMB/kg sce) was also only 38% of the LPG price (3290 RMB/kg sce) in 2008. The advance of the coal price was another factor that led the villagers to choose coal to fill the fuel consumption gap caused by their decreased fuelwood consumption.

Table 2. *Driving forces responsible for the differences in fuelwood consumption for the individual villages. (sce, standard coal equivalent)*

	Wuzhuang	Xiakou	Sanshilipu	Shimo
Proportion of total fuelwood consumption (%)				
Fuelwood	22.3	9.1	56.1	58.8
Coal	60.6	69.9	38.3	30.6
LPG	0.2	15.7	0.4	0.0
Time consumed (min.) for travel to				
Fuelwood collection location in 1999	52.2	48.5	62.3	54.2
Fuelwood collection location in 2008	66.3	92.5	75.8	60.7
Coal market in 2008	18.1	19.7	25.6	24.8
LPG market in 2008	32.5	32.9	32.7	86.5

Ecological consequences of the changes in forest area and net fuelwood productivity

The change in fuelwood consumption had direct impacts on the region's forests. We analyzed the changes in forest area and net fuelwood productivity as a function of the scope of human activities (*Fig. 3*) using land-use maps and statistical data. As a result of the SLCP, the area of forests increased by 2100 ha from 1999 to 2008 (*Table 3*). Of this total, 83% of the forest resulted from conversion of arable land and 17% resulted from planting trees on bare land. Only 2% of the newly planted trees were fuelwood forests based on government statistics collected from 2000 to 2008. The forest area under different fuelwood collection intensities also changed greatly during this period. From 1999 to 2008, the areas of forest under very high, high, and moderate fuelwood collection intensity decreased by 57, 20, and 64%, respectively. The areas of forest with low and very low fuelwood collection intensity increased by 184 and 118%, respectively (*Table 3*).

The total net fuelwood productivity in the study area increased by 25.8% from 1999 to 2008 due to increased forest productivity and decreased fuelwood consumption.

Excluding forest with a very low fuelwood collection intensity, the remaining forest had a total fuelwood productivity of 43.9 and 41.4 kt in 1999 and 2008, respectively, versus only 21.7 and 9 kt of fuelwood consumption. Thus, in both 1999 and 2008, the forest could supply more fuelwood than required by the consumption demand. However, due to the spatial imbalance between fuelwood availability and proximity to a village, there was 5.4 kt of overextraction of fuelwood in 700 ha of forest surrounding the villages in 1999. In 2008, this overextraction decreased, but there was still overextraction of 1.9 kt of fuelwood in 400 ha of forest surrounding the villages (*Table 3*). The forests that were under very high fuelwood collection intensity therefore face a serious risk of degradation.

Table 3. *Changes in forest area and productivity from 1999 to 2008 in forests with different fuelwood collection intensities*

	Area of forest (thousand ha)		Fuelwood productivity (kt/ year)		Consumption demand on the forest (kt)		Net productivity (kt/year)	
	1999	2008	1999	2008	1999	2008	1999	2008
Fuelwood collection intensity								
Very low	13.8	16.3	63.7	75.5	0	0	63.7	75.5
Low	3.8	7.0	17.7	32.6	2.2	0.9	15.5	31.7
Moderate	1.4	0.9	6.6	4.0	4.3	1.8	2.3	2.2
High	3.5	0.7	16.3	3.1	6.5	2.7	9.7	0.4
Very high	0.7	0.4	3.3	1.7	8.7	3.6	-5.4	-1.9
Total	23.2	25.3	107.6	116.9	21.7	9.0	85.8	107.9

Ecological consequence of changes in CO2 emissions

The change in fuelwood consumption directly affected the CO_2 emissions from fuelwood. The average fuelwood consumption decreased from 1518.1 to 593.4 kg sce from 1999 to 2008, leading to a decrease of CO_2 emission by 290.1 kg per household (*Table 4*). However, the decreased fuelwood consumption was compensated for by increased consumption of other fuel types, particularly coal, indirectly changing CO_2 emissions. Coal consumption increased from 144.7 kg sce to 813.4 kg sce from 1999 to 2008, leading to an increase of CO_2 emissions from 628.4 kg to 3531.8 kg per household. The CO_2 emissions from increased coal consumption were greater than the reduction in CO_2 emissions caused by decreased fuelwood consumption. For an average household, 668.7 kg sce of fuelwood consumption was replaced by coal consumption, causing a net increase of CO_2 emissions by 2693.6 kg from 1999 to 2008; this is equivalent to the release of 4 more kg of CO_2 per 1 kg sce when fuelwood consumption was replaced by coal consumption.

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Table 4. *Changes in emission of greenhouse gases due to the changes in fuelwood consumption. (sce, standard coal equivalent)*

	Emission factors (g/kg)			Global warming potential (GWP)			Consumption per household (kg sce)		CO_2 emissions per household (kg)	
	CH_4	N_2O	CO_2	CH_4	N_2O	CO_2	1999	2008	1999	2008
Fuelwood	6.76	0.08	0	23	296	1	1518.1	593.4	476.3	186.2
Coal	0.031	0.047	3086.7	23	296	1	144.7	813.4	628.4	3531.8
LPG	0.015	0.0015	964.1	23	296	1	2.0	50.5	1.1	28.4
Overall							1664.8	1457.3	1105.8	3746.4

Some households used LPG to replace fuelwood consumption. Despite the higher CO_2 emission from LPG than from fuelwood, the replacement of fuelwood by LPG only increased CO_2 emission by 0.2 kg per kg sce, which was much lower than for coal. However, based on our analysis of *Table 2*, the cheaper prices (by 38%) and reduced time required to reach the markets (by 56%) for coal compared with LPG led most households to choose coal rather than LPG to replace fuelwood. On average, only 48.5 kg sce per household of fuelwood consumption was replaced by LPG, which amounts to around 7% of the fuelwood consumption replaced by coal (*Table 4*).

From 1999 to 2008, despite a decrease of average total fuel consumption per household to 87.5 % of the 1999 value, the CO_2 emissions per household increased greatly (to 339% of the 1999 value). This tremendous increase in CO_2 emission mainly resulted from decreased fuelwood consumption and increased coal consumption.

Discussion

The residents of the four remote villages in northwestern China depended greatly on the ecosystem service of fuelwood supply from the forest in their daily lives due to their low income levels and the region's long, cold winter. Villagers chose their fuels mainly based on cost, although the travel time to obtain the fuel was also a factor. Because fuelwood was free, it was the preferred choice. However, after the SLCP began, villagers were forbidden to cut live branches or enter closed forest areas, making fuelwood more difficult to obtain. Some villagers continued to illegally harvest branches because they required the free fuelwood for survival. To prevent implementation of the SLCP from harming local people, the government encouraged workers to change from agriculture to various industries. By 2008, 8 years after the implementation of the SLCP, many villages had successfully shifted from traditional farming to other activities, mainly construction, transportation, and the restaurant business (Uchida et al., 2007; J. Liu et al., 2008). As the difficulty of and time consumed by fuelwood collection increased, most villagers started to purchase fossil fuels to save time that they could use for part-time work. Because of its much lower price and greater availability than LPG, coal was the preferred choice to replace fuelwood. However, as *Table 4* shows, decreased fuelwood consumption and its replacement by coal consumption had potentially severe ecological consequences.

As the human population and the demands it places on natural resources increase, the increasing consumption of ecosystem services often leads to degradation of the natural systems (WWF, 2010). Conversely, if the consumption of these services can be decreased, this should have positive ecological consequences. For example, reduced fuelwood consumption would alleviate habitat fragmentation and the degradation of natural forests (Bearer et al., 2008; Naughton-Treves et al., 2007). However, if we consider consumption of ecosystem services in the context of coupled human–natural systems, direct and indirect ecological consequences are revealed. Most previous studies have reported large and positive ecological consequences from the SLCP, the synergies of many ecosystem services such as soil and water conservation, biodiversity, ecosystem productivity and carbon sequestration (J. Liu et al., 2008; Yin et al., 2010). In our study area, a remote part of northwestern China that is governed by the SLCP policies, the decreased fuelwood consumption was accompanied by increased forest area. However, the fuelwood consumption showed a high spatial imbalance with respect to the location of the new sources of fuelwood. The forests that surround the villages face a risk of degradation because fuelwood consumption exceeded the natural productivity of these forests. In addition, the decreased fuelwood consumption required villagers to purchase substitute fuel (mainly coal). The resulting increase in coal consumption caused by decreased fuelwood consumption led to a large increase in CO_2 emissions. Increase of soil and water conservation and other forest ecosystem services might have to tradeoffs with the increase in fuelwood consumption reducing atmospheric CO_2 concentration.

Because of the increasing fuel consumption demand and the still strict forest management guidelines, coal consumption is likely to continue increasing based on the results of our study. For the whole area covered by the SLCP, 32.5 million households participated in 20 provinces (Yin and Yin, 2010), with five of the provinces in the northwest. This means that millions of households in northwestern China are experiencing situations similar to those of the households in our study. The potential additional CO_2 emissions that will result from the observed changes in fuelwood consumption should not to be ignored by policy-makers. We have two key suggestions that could mitigate these problems. On the one hand, the construction of more infrastructures for LPG markets should be considered and prices of LPG should be subsidized to encourage villagers to choose LPG instead of coal. On the other hand, reforestation should consider introducing and planting more fuelwood forests composed of fast-growing species near the villages to benefit the households that still need fuelwood. However, because such trees tend to have low water-use efficiency, managers should carefully ensure that the trees will not extract more water from the soil than the environment can provide, since this can exacerbate ecosystem degradation (Cao, 2008). It may also be possible to provide support for villagers so they can establish small-scale businesses to transport fuelwood from more distant forests to each village, thereby both improving employment stability and reducing the spatial imbalance in fuelwood availability.

Conclusions

In this study, we analyzed the changes in fuelwood consumption in four remote villages in northwestern China and the ecological impacts on the forest area, net productivity, and CO_2 emissions. We found that:

- The decreased fuelwood consumption between 1999 and 2008 was mainly associated with the SLCP, which started in 2000 in the study area. This program forbids people from cutting live branches for fuelwood, and closed

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nearly half of the forest areas to villagers. These policies made it difficult for the villagers to collect enough fuelwood.

- The consumption of fuelwood decreased greatly from 1999 to 2008. Simultaneously, the fuelwood collection intensity decreased in most of the forests and the scope of the fuelwood collection by most villagers (i.e., the distance they traveled to harvest the fuelwood) decreased from 1999 to 2008. To compensate for the decrease in fuelwood consumption, coal consumption increased greatly.
- The decreased fuelwood consumption was accompanied by an increased area of forest and increased total net fuelwood productivity. However, the demand for fuelwood remained high, so the forests nearest to the villages sustained a very high fuelwood collection intensity that exceeded the natural productivity of the forests, leading to a risk of degradation. The decreased fuelwood consumption also led to increased coal consumption, thereby increasing net CO_2 emissions.

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CUT MOWING AND GRAZING EFFECTS WITH GREY CATTLE ON PLANT SPECIES COMPOSITION IN CASE OF PANNON WET GRASSLANDS

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Abstract. Examined area can be found at Balaton Uplands National Park (Hungary). 5 sample areas were examined in Badacsonytördemic: 1: 32 hectare under-grazed pasture, 2: 38 hectare overgrazed pasture, 3: 34 hectare hayfield, 4: trampled area, 5: beaten track. Livestock population was 118 in the monitored pastures. Sampling was executed along five 52m long circular transects, within 5cm × 5cm interlocking quadrates. Based on the data we can state that the curve of the drinking area was the highest of species-area examinations however weed appeared because of degradation which provided more species. According to species-area examinations overgrazed areas were richer in species then other examined areas. Based on diversity data drinking area considered degraded, while meadow and overgrazed areas was considered as proper state. Diversity of meadow was larger, but dominance of economically useful species was smaller. The amount of less valuable species – *Carex hirta* – increased.
Keywords: *grazing, pasture, hayfield, species composition, nature conservation*

Introduction

In Pannon region certain animal species bred because they can adapt to specific climatic conditions. Domestic grey cattle kept on wet grasslands, but it almost entirely disappeared from Hungarian steppes. Similar to the EU countries, heavy agro-intensification was typical to Hungary between 1960 and 1980 and it ended in a similar way as other countries (Gregory et al., 2005). After the changing of regime productivity significantly fall back (Báldi and Faragó, 2007), which resulted of extensive areas and biodiversity (Tilman et al., 2002). This intensification process meant the use of more herbicides and chemical fertilizers and also the homogenization of landscape (Poschlod and WallisDeVries, 2002; Robinson and Sutherland, 2002; Benton et al., 2003; Tscharntke et al., 2005). The decreasing diversity is mainly depending on farm size because of management intensity at field scale. Positive effect were find in case of slope grassland topography while negative influence in case of farm size on species richness, orthopterans and butterflies (Marini et al., 2009). In EU countries agro-environmental programs were launched in order to stop or even reverse the decline of biodiversity (Kleijn and Sutherland, 2003). Payments to farmers were linked to ecological goods representing differently ranked quality-levels of vascular plant diversity (Kohyani et al., 2008; Klimek et al., 2008). In Hungary this effort was supported by the National Agro-environmental Program (NAKP) and the 2253/1999 (X.7.) government decree (Haraszthy et al., 2004).

In Hungary grey cattle took into focus thank to the incentive of sustainable use of grasslands, it became decisive factor in grassland preservation under nature protection. Though grey cattle are kept extensively, livestock management methods are the same as in case of beef cattle. Contrary to the traditional grazing methods grey cattle can be kept on the pasture for a longer period (from April until November, 200-240 days). Demand of manpower is low and rotational grazing system proved to be the most effective. Besides the usability of grey cattle in nature conservation works, does not change the area compared other livestock area. Beef productivity per area depends not only on the performance of the animal but on the number of animal per area and utilization of pasture as well. There is negative correlation between the effectiveness of pasture usage and the production per animal. Positive correlation was examined between amount of ingestion and grass supply, if animals have the chance to select (Penning et al., 1986).

Grasslands are not only important from nature conservation aspects but they have economic importance as well. Therefore it is vital to know the species composition of the pastures. The aim of this survey is to find answers in this matter. In the sample area we examined the species combinations of the grassland, which is important for the survival of the grassland. Our question is how certain nature conservation methods, like mowing or grazing, change the inner structure of vegetation? Which method is the best for sustaining biodiversity, preserving the pasture considering nature conservation and economic aspects? It is important to know detailed composition of the area since the local interactions and the limited expansion determine dynamics of vegetation and set the rules of symbiosis for species (Durett and Levin, 1994; Tilman and Kareiva, 1997; Chesson, 2000). Environmental structures and management methods are determining factors in symbiotic processes. They can either strengthen or weaken symbiosis, depending on their conditions (Law et al., 2003; Bolker et al., 2003; Snyder and Chesson, 2004). Quality and diversity of the species combination around individual – so called neighbourhood diversity - is important from functional aspect as well (Oksanen, 1997). With the use of Juhász-Nagy model, micro-phytosociological landscape can be modelled. Fine-scale structure examinations can reveal diversity, structure of micro-habitats (species combinations) and also the impacts of certain events on the vegetation which make nature conservation a more controllable task.

Materials and methods

Sample areas

Our sample area can be found near Balaton which is one of the richest regarding natural values in Hungary (Balaton Uplands National Park). The basin is surrounded by basalt peaks. There have always been intensive agricultural activities for centuries but

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the support programs after the changing of the regime changed and strengthened them. As result, grey cattle appeared in the region and now we can examine their effects.

Sample areas are in Badacsonytördemic:

1: 32 ha grassland space with low intensity grazing (under-grazed pasture)

2: 38 ha overgrazed pasture (space)

3: 34 ha meadow (hayfield)

4: trampled space near to the watering point

5: trodden path where animals go to drink etc., trampled space along the road

During examination 118 animals were found on pasture. Method of continuous grazing was applied. *Agostio-Deschampsietum caespitosae* Újvárosi 1947 associations were dominant on utilized areas, except for trodden parts next to the roads where *Lolio-Cynodontetum dactylidi* Jarolímek et al. 1997 were typical. The examined area applied as pasture and meadow in the past.

Phytosociological analysis

The main purpose of this article to explore the condition of the grasslands regarding naturalism and degradation using fine-scale patterns based on micro-phytosociological examinations with the use of characteristic functions describing spatial structures (Juhász-Nagy and Podani, 1983). Samplings executed along five 52m long circular transects, within 5cm x 5cm interlocking quadrates, where the presence of rooted species recorded. This sampling method provides detailed data about the condition of vegetation. It is relatively fast method and it causes insignificant disturbance (Bartha, 2001; Bartha et al., 2004). Complete sampling at each spatial-series step were executed, samples collected from all the possible positions in basic transects, allowing overlaps (Juhász-Nagy and Podani, 1983; Bartha and Kertész, 1998).

In order to avoid the artefacts – caused by stochastic behavior of rare species - whose frequency did not reached 3% (Tóthmérész and Erdei, 1992). Theoretical and methodological basis for examination of crop micro-structure and the degeneration – caused by disturbance – were provided by the models of Juhász-Nagy Pál (1993), Juhász-Nagy and Podani (1983) and their application methods (Bartha, 2001; Bartha and Kertész, 1998; Bartha et al., 1998, 2004; Campetella et al., 2004; Czárán and Bartha, 1989, 1992; Podani, 1993; Tóthmérész and Erdei, 1992).

Examined phytosociological status parameters were the followings: number of species, variance of species, number of species combinations, floral diversity, association and relative association. Variance of species refers to spatial heterogeneity. Number of species combinations shows the diversity of symbiosis and structural complexity of crop.

Detailed description of models and functions can be found in the works of Juhász-Nagy (1980, 1984), Juhász-Nagy and Podani (1983), Podani et al. (1993) and Bartha (2001).

Results

Couples of dominant species were found in the areas under disturbance or stress (drinking area 4th stand, path 5th stand). Their frequency was significant compared to the other species. Number of species which have 10% or higher frequency was low. In other stands, where disturbance was lower, there were more species with frequency higher then 10%.

Examining the number of species whose frequency is over 3% in each spatial unit, we can see that the curves made around the trampled stand near to the watering point (stand 4), on the overgrazed area (stand 2), on the meadow, hayfield (stand 3) and on the under-grazed area (stand 1) reach the minimum area at 30m, 40m, 25m, and 45 m respectively. This means they contain all the species of at least 3% frequency (*Fig.1*). It means 16 species at the drinking area, 15 on the overgrazed area, 12 on the meadow and the under-grazed area and 2 along the road. The numbers of matrix species were: 5, 6, 6, 5, 1. Along the road the only matrix species was *Polygonum aviculare* which explains the *Fig. 1*.

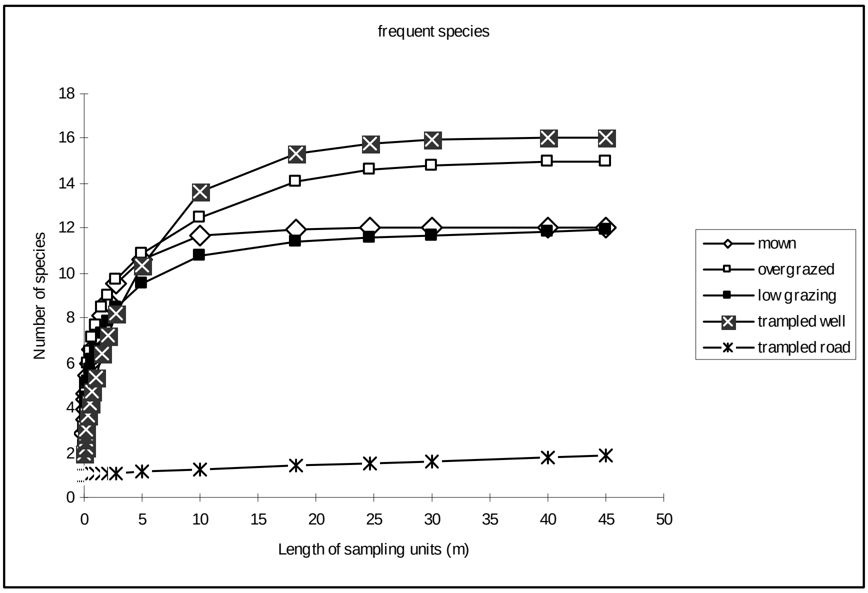

Figure 1. *Variation in number of species at different spatial units in Badacsonytördemice sampling areas (species with frequency over 3%) (Filled square: mown grassland; cross: overgrazed stand; star: grassland stand with low intensity grazing; x: trampled stand near to the watering point; filled triangulum: trampled stand along the road.)*

Variance in number of species refers to the amount of heterogeneity. Based on it and counted with finer spatial units significant differences were not found (*Fig. 2*). Low maximum were found of trampled stand curve along the road (5) and the high variance maximum of species number in the vegetation around the trampled stand near to the watering point (4). Maximums appeared along with big spatial units in both cases. Meadow showed the highest variance regarding species. These belongs to the useful grass-management category. Usage of small units, - 80cm-5m units - the under-grazed area showed the highest heterogeneity.

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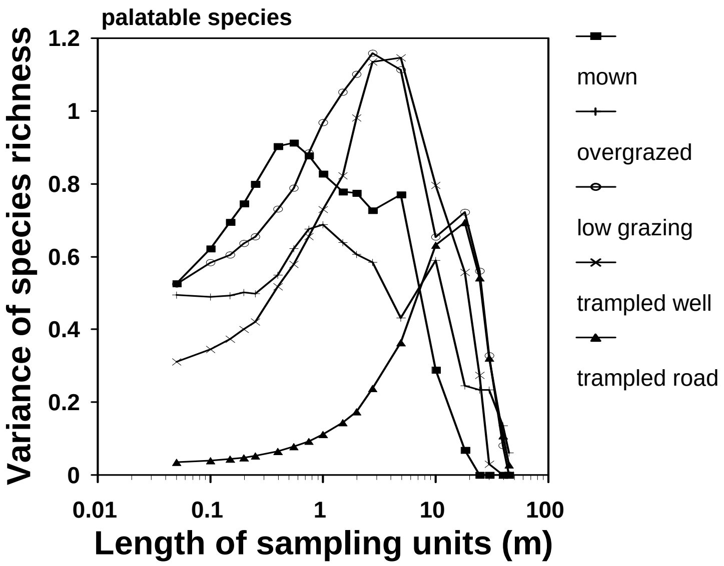

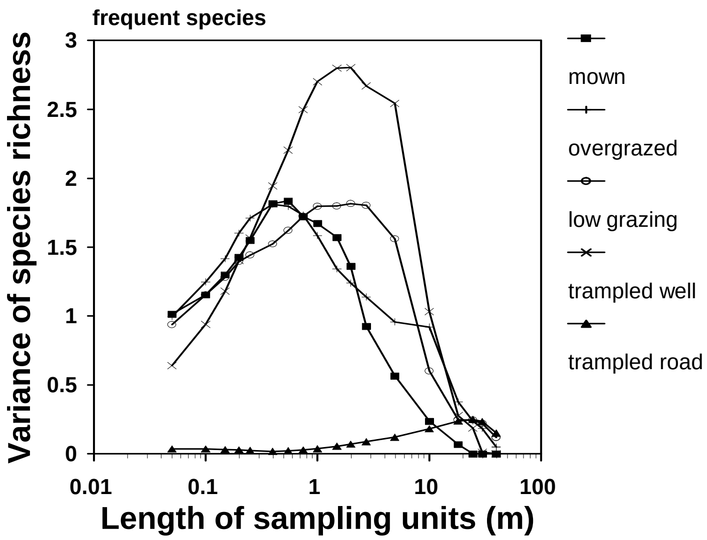

Figure 2. (2a and 2b diagrams). *changing of species number variance in sample areas of Badacsonytördemice regarding palatable and frequent (useful) species (Filled square: mown grassland; cross: overgrazed stand; star: grassland stand with low intensity grazing; x: trampled stand near to the watering point; filled triangulum: trampled stand along the road.)*

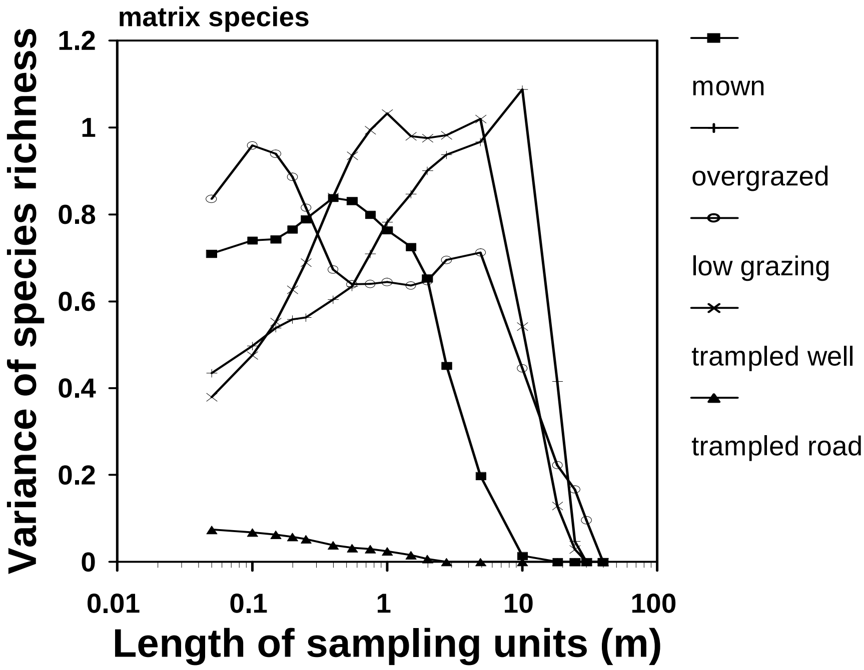

Figure 3. *changing of species numbervariance in sample areas of Badacsonytördemice regarding matrix species (Filled square: mown grassland; cross: overgrazed stand; star: grassland stand with low intensity grazing; x: trampled stand near to the watering point; filled triangulum: trampled stand along the road.)*

Number of species combinations was the highest in the overgrazed area while application of 10cm and 40cm units in case of meadow was the best. This means that these unit sizes contain the most species combinations. Their curves later steeply decrease and it reached 0 at 30m. Matrix species showed the largest segregation in meadow (3) when 10 cm units were applied (*Fig. 3*). It is followed by the under-grazed area using the same quadrate size. Number of species combinations was the highest in case of meadows and overgrazed pastures at 10cm and 40cm scales respectively. It means that these scales represent the largest species combinations amongst examined associations. Afterwards their curves steeply decrease and they reached zero at 30m. The greatest separation was shown by the examination with matrix species (3). In case of meadow it appeared at 10cm spatial unit which was followed by the under grazed area at the same quadrate unit.

Discussion

In the examined lines on the degraded areas, the number of species – with 10% frequency – was low compared to other areas. These species were mainly dominant *gramineae* and sedge species of the grasslands, which compose the basic texture of the vegetation. Other species fit into their matrix and the stability of the association depends on them. According to Kun et al. (2007) the steadiness of the dominance conditions show the stability of grassland (e.g.: in meadow and overgrazed areas), since in this case there are numerous species which can prevent the degradation of the structure if a dominant species disappear.

If we consider the primary and secondary *gramineae* and pulses important of grassland management aspects only, than the meadow curve (3rd sample area) reaches its maximum only in 30m. Number of species of this line was six. Most of the taxa useful of grassland management aspects (8 pcs) are in the overgrazed grassland.

Maximums of species variance also appeared on the mostly used, degraded areas. It refers to the homogeneity of vegetation (5), and to strong spatial heterogeneity. Besides matrix species- with small spatial units – grazed areas shows the highest species variance, namely spatial heterogeneity. It is also bad for the stability of species composition of grasslands. If we consider that the species belongs to the useful grassland management categories only, than meadow showed the highest variance, while – In 80cm-5m spatial unit - overgrazed area proved to be the most heterogeneous.

Number of species combinations indicates the diversity of symbioses methods between species and structural complexity of the association. Counting with all the species over 3% appearance frequency, 1040 theoretical maximum did not occur in any cases. It would only be possible if there were different species combinations in all micro-quadrates. The most species combinations were found in meadow at small 10cm spatial unit, this was followed by the under grazed area at the same quadrate unit.

Drinking area had the highest species-area examinations but weeds appeared to provide more species due to their degradation According to species-area examinations overgrazed areas were rich in species. Drinking area is considered degraded in case of compositional diversity, while meadow and overgrazed areas considered useful. Diversity of meadow was bigger, but the dominance of economically useful species was smaller and the amount of less valuable *Carex hirta* increased. It proves that mowing is a good method from nature protection aspects though it can ruin the feed value of the grass in long term. Regarding the matrix species, meadow has the best values thanks to the regular annual mowing. Second autumn mowing could be omitted. Due to the results of overgrazed areas summer and autumn increments should be grazed in order to maintain the species diversity of grassland and draw them into grassland cultivation method. .In case of meadow and overgrazed sample areas, mutual cultivation method used, since meadow was grazed for a shorter period at late summer or at fall in the previous years.

Production of sample area was significant so it could carry more species than the estimated animal carrying capacity. Due to the continuous burden grass can not reached significant height on the overgrazed area. Greater amount of feed produced because of continuous grazing results in greater production and continuous fertilization Contrary to the nature protection regulations, this specifies only 1 grey cattle per hectare while the area was capable to carry 3 animals. With this method utilization and sustainability regulations can be specified for a given area. It helps the directory of the national park to plan better nature protection actions . If they lead the animals to the under grazed area during dry period in August, it helps the regeneration of the grassland. Compositional diversity showed that the overgrazed area had a greater animal carrying capacity and its species composition was valuable from nature protection aspects.

In under grazed area, due to the lack of proper treatment, *Festuca arundinacea* – which is a bog composing species which well adapts to extreme conditions – became dominant from species with feeding importance. Other species were present in low number. As a result of the lack of May-June mowing, number of species increased and grassland shifted toward "one-species" grassland. Its performance was high though animals did not like it. The species number and diversity was low. Prospect of becoming one-species grassland was a threat. Species composition on the overgrazed are seemed to be stable, number of grazing animals maintains the diversity of the grassland if their number was determined according to the distribution of the yield. Grazing compacted grass in overgrazed areas. Combination of useful species was examined in this study. It was good for the animal from health, fitness, etc. aspects if it grazed from different kind of species.

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ISOLATION, CHARACTERIZATION AND GROWTH CONTROL OF A SULFATE REDUCING BACTERIA FROM SEWAGE WATER

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Abstract: Sulfate Reducing Bacteria (SRB) are a diverse heterogeneous group of anaerobic microorganisms inhabiting in various environmental conditions. SRB are one of the most important groups of microorganisms that participate in various nutritional cycles of the environment and cause degradation of various organic matters through the process of dissimilatory sulfate reduction. They are known to be implicated in cases of microbially influenced corrosion arising in a wide range of natural and industrial circumstances. SRB are known to utilize the indigenous sulfate source and hence produce the deadly poisonous hydrogen sulfide (H_2S) gas. In the present study one strain of SRB designated as WS-1 was collected from sewage water. The sample was enriched in a basal mineral media in an anaerobic condition using lactic acid as carbon source. The isolated strain (WS-1) was Gram negative cocci shaped and occured in chains and pairs. Biochemical characterization of the strains revealed that, the strain WS-1 was closely related to *Veillonella*. Sulfate reduction and sulfide formation were evident both in case of lactic acid and acetic acid as carbon source. It was observed that sodium nitrate and potassium nitrate were effective in controlling sulfide formation.
Keywords: *Sulfate Reducing Bacteria, nitrate, sulfide*

Introduction

The Sulfate Reducing Bacteria (SRB) constitutes a morphologically and physiologically diverse anaerobic group of microorganisms. They have the capacity to reduce sulfate present indigenously in the environment and reduce it to deadly poisonous hydrogen sulfide gas.

$$\underset{\text{(Sulfate)}}{SO_4^{2-}} + \underset{\text{(Organic Substrate)}}{COD} \rightarrow \underset{\text{(Sulfide)}}{H_2S} + \underset{\text{(Carbon Dioxide)}}{CO_2} \qquad \text{(Eq.1)}$$

Typically, SRB are found mainly in marine and freshwater sediments where sulfate is present in abundance (Nielsen et al., 1999). But they are also found in agricultural and industrial waste water systems, in oil fields and also in cooling towers (Dan et al., 1996; Postgate, 1984; Rao et al., 2000). These microbial consortia can utilize sulfate, thiosulfate, sulfite and elemental sulfur as electron acceptors but cannot utilize nitrate, nitrite or formate (Azabou et al., 2007). They can also utilize environmental substances such as benzene, toluene, ethylbenzene, xylenes, naphthalene, phenanthrene and alkanes and halogenated compounds (Ensley et al., 1995; Zhang and Young 1997). The bacterial strains are also able to utilize peptone, asparagine, glycine, alanine, aspartic acid, ethanol, propanol, butanol, glycerol, glucose, lactate, succinate and malate .The main disadvantages of these SRB's are that they cause the souring of oil and corrosion of pipelines thereby making huge economical loss to oil industries. Hence it lowers the economic value of the produced oil and imposes safety hazards due to H_2S toxicity. Therefore, it is important to study the growth and control the activity of SRB.

Previously it was reported that biocides targeting SRB were widely used (Jayaraman et al., 1999) in controlling the growth of SRB in surface facilities but mostly the biocides cannot penetrate bio-films readily within reservoirs or on the metal surfaces of industrial equipment (Gardner and Stewart, 2002). It is also reported (Gardner and Stewart, 2002) that biocide-resistant SRB can often emerge in due course of time due to frequent use of the same.

Several methods for sulfide removals are in common use today. Some of these are physio-chemical process that involves direct air stripping, oxidation and chemical precipitation. The major disadvantages of these conventional systems are the relatively high-energy requirement or the high chemical and disposal costs (Busiman et al., 1989). Therefore the need arises to make an extensive study on biological control of SRB. Nitrate is applied to control the growth of SRB and result in both transient and long-term inhibition of sulfide production (Allen, 1949). Naphthalene is also proved to be an effective controlling agent of SRB. As reported earlier the addition of high concentrations of nitrate can generate nitrous oxide, which increases the redox potential, leading to long-term inhibition of sulfide production (Allen, 1949; Jenneman, 1986). Naphthalene also proved to be toxic for the microorganisms using polycyclic aromatic hydrocarbons as carbon source (Gracia et al., 1998).

Keeping in view the above aspects, the current study deals with purification and characterization of SRB isolated from sewage water sample collected from the campus of Institute of Minerals and Materials Technology, Bhubaneswar during December, 2006. The present investigation deals with hydrogen sulfide production by utilization of various substrates like lactic acid and acetic acid. Another set of experiment deals with whether various nitrate sources as well as naphthalene could be useful for controlling growth of SRB and reducing the production of hydrogen sulfide.

Materials and Methods

Site description and sampling procedure

The experiments were carried with sewage water sample. The water sample designated as WS-1 was collected from the sewage canal of Institute of Minerals and Materials Technology, Bhubaneswar, Orissa. Sample collections were performed in December, 2006.

The water sample (20mL) was collected in sterile screw capped glass bottle entirely filled and sealed with cap to maintain anoxic condition.

Isolation and cultivation of pure cultures

Samples were inoculated in enrichment media within 24h of sampling. The inoculated sample was incubated at 35 °C for a period of at least 2 weeks. Growth of the microorganism was analyzed by microscopic study in every 7 days. Pure cultures of the microorganism were obtained by repeated application of streaking in Basal mineral media (3% agar) supplemented with K_2HPO_4 0.3g/L, KH_2PO_4 0.3g/L, $(NH_4)_2SO_4$ 0.3g/L. The inoculated Petri plates were incubated at 35°C in anaerobic chambers in an atmosphere of CO_2 which contains less than 1% oxygen. Anaerogen sachets were also provided in the anaerobic chamber. After an incubation period of 1-2 weeks well isolated colonies were observed in the plates. Purity of isolated strains was checked by direct microscopic observation in leica phase microscope.

The basal mineral media used for cultivation and isolation of SRB contained in $g.L^{-1}$: $(NH_4)_2SO_4$– 3, $MgSO_4$- 1.5, NH_4Cl-1, K_2HPO_4-0.65, $CaCl_2.2H_2O$-0.08, $FeNH_4SO_4$,-0.01 $CH_3CHOHCOOH/CH_3COOH$-5mL, Yeast Extract-0.2, Trace Elements-1.5mL, Riboflavin-2mL, Resazurin-0.002g. Resazurin was added as a redox indicator. The pH of the medium was adjusted to 7.5-7.8 with 1N NaOH. The constituents of trace element solution used in mineral media contained in g/L: $MnCl_2$- 1, $CoCl_2$-1, $NiCl_2$-0.2, $CuSO_4$-0.1, $ZnSO_4$-0.1 and H_3BO_3-0.1. Isolation was done in specially designed gas passing bottles. The bottles were supplemented with 1mL water sample respectively and fluxed with nitrogen and CO_2 gases to maintain anaerobic condition.

Morphological, physiological and biochemical characterization

A Leica phase microscope was used to observe the Gram staining as well as cell shape and motility. The physiological characterization of the isolated SRB strain (WS-1) was studied according to the ability to grow in basal mineral media for a period of two weeks. To determine the ability of the SRB to utilize various carbon sources, isolated bacteria were grown in basal mineral media replacing lactic acid with acetic acid. The media turned black which indicates the positive result. For biochemical characterization, media were used supplied by HI media. The isolated strains were inoculated in the freshly prepared sterilized media and incubated at 35 $^{\circ}C$ inside the anaerobic chambers which was vacuumed and subsequently fluxed with CO_2 gas.

Analytical techniques

Amount of sulfide was analyzed by titrometric method using 0.1N sodium thiosulfate as titrant and starch as indicator with 10ml of sample solution [Vogel, 1961].

Table 1. *Colony Morphology of the strain WS-1*

Agar medium used for isolation	Colour	Form	Elevation	Margin	Size(mm)	Surface	Structure
Basal Mineral Media fortified with agar	Pinkish	Small, circular	Irregular	Entire	0.5-2	Glistening	Opaque

Results and Discussion

Isolation

It was observed that the colour of the fresh media first turned blue then to colourless and lastly colour changed to pink after 2-3 days of incubation. Then finally the media turned to deep black colour with black precipitate at the bottom of the culture vessel after two weeks of incubation at 35^0C. This indicates the formation of hydrogen sulfide due to the degradation of the carbon source. The sample (WS-1) was taken in duplicates. In one set of sample Nitrogen gas was fluxed and in the other set Nitrogen gas was not supplied instead the bottles were stoppered after filling the culture vessels fully with media. Sulfate accumulated concomittantly with hydrogen sulfide. Cultures having the highest dilution with growth (10^5) cells/mL were used for further isolation studies. It was interesting to note that microscopic examination revealed one major bacterial morphotype that occurred predominantly having colony morphology given in (*Table 1*). The type was coccoid and non-motile. Purification of the culture was done by single colony isolation method on basal mineral media fortified with agar and a single colony was taken for biochemical characterization study.

Table 2. *Biochemical Tests*

NAME OF BIOCHEMICAL TESTS	WS-1
INDOLE	–
MR	–
VP	–
CITRATE UTILIZATION	–
LYSINE DECARBOXYLASE	+
ORNITHINE DECARBOXYLASE	+
UREASE	–
CATALASE	+
PHENYLALANINE DEAMINATION	–
GLUCOSE	+
ADONITOL	+
ARABINOSE	+
LACTOSE	+
SORBITOL	+
MANNITOL	+
RHAMNOSE	+
SUCROSE	+
OXIDASE	+
Identified as (Maximum resemblance with)	Veillonella

Characterization

Cells of strain WS-1 was found to be Gram negative cocci shaped occurring in short chains as seen under higher magnification. These strains were found to be positive for lysine decarboxylase, ornithine decarboxylase, oxidase, catalase and also for all the carbohydrate utilisation as shown in (*Table 2*). The colony morphology is shown in (*Table 1*). From the characterization study the isolated strain was observed to be having close similarity with *Veionella* species (Holt, 1989).

From the protein assay it was observed that the result was in match with the direct microscopic count. In WS-1 sample the growth and activity of the bacteria increased up to 14 days that indicates the log phase of growth of SRB in the particular sample. Subsequently the stationary growth phase of the organism was achieved which was noted with the decline in growth and activity of the bacterial cells. After 28 days of incubation, the growth was completely retarded, indicating the initiation of death phase of the microorganisms.

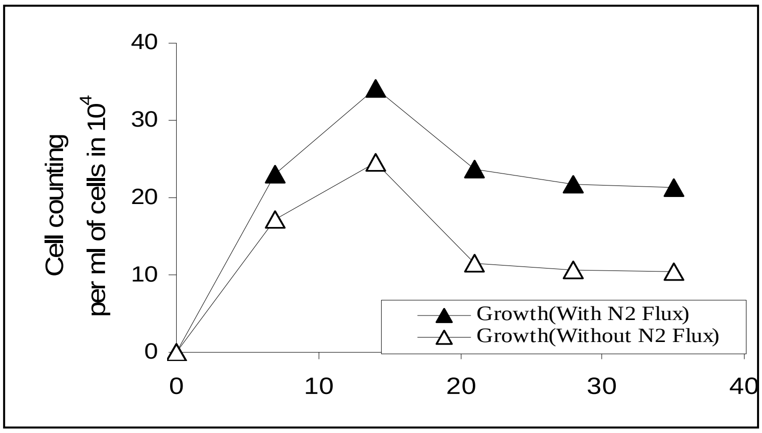

Figure 1. *Growth of isolated WS-1 strain of SRB in anaerobic basal mineral media with different conditions of nitrogen flux with lactic acid as substrate*

From the above observations it was believed that the bacterial log phase continued till 14 days of incubation. The observations were concordant with the increase in bacterial cell count which increased up to 10^5cells/mL during this period. This fact was further justified when the results of the protein assay increased up to 14 days and then a decline was observed in the amount (*Fig.7*). Same result was also observed in case of carbohydrate assay, which further supported the fact. The amount of protein obtained was in the range of 0.05 to 0.2 mg/mL of bacterial culture as shown in (*Fig.7*) and the amount of carbohydrate was in the range of 0.01 to 0.1 mg/mL of bacterial culture as shown in (*Fig.7*).

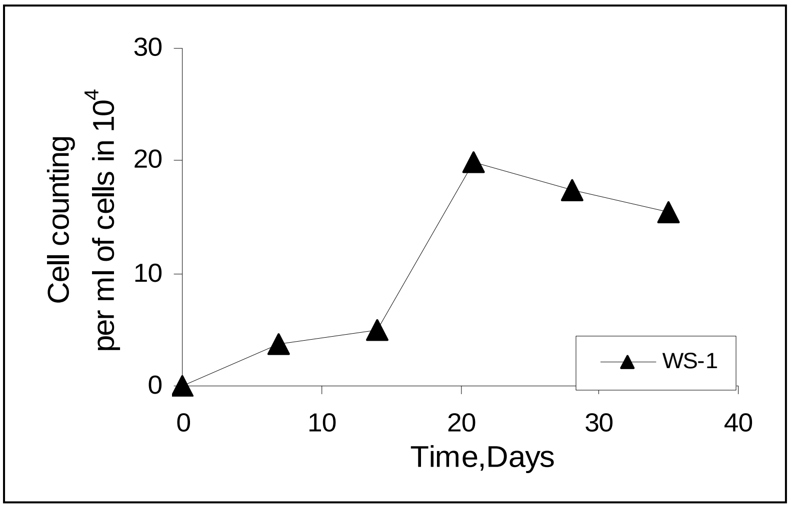

Figure 2. *Growth of isolated WS-1 strain of SRB in anaerobic basal mineral media with different conditions of nitrogen flux with acetic acid as substrate*

Growth studies

From the growth studies of WS-1 it was observed that there was an increase in the bacterial cell count up to 14 days which was in the range of 10^4 to 10^5cells/ml indicating the log phase of growth of SRB and then stationary phase was achieved. A decline in growth was observed after 28 days indicating the initiation of death phase of cells. The growth results were slightly higher in nitrogen flux samples indicating that by nitrogen flux better anaerobic condition was maintained as shown in (*Fig.1*). It was observed that growth was better in case of lactic acid as substrate than with acetic acid as substrate (*Fig.2*), which implies that lactic acid is a more preferable carbon source for the growth of SRB.

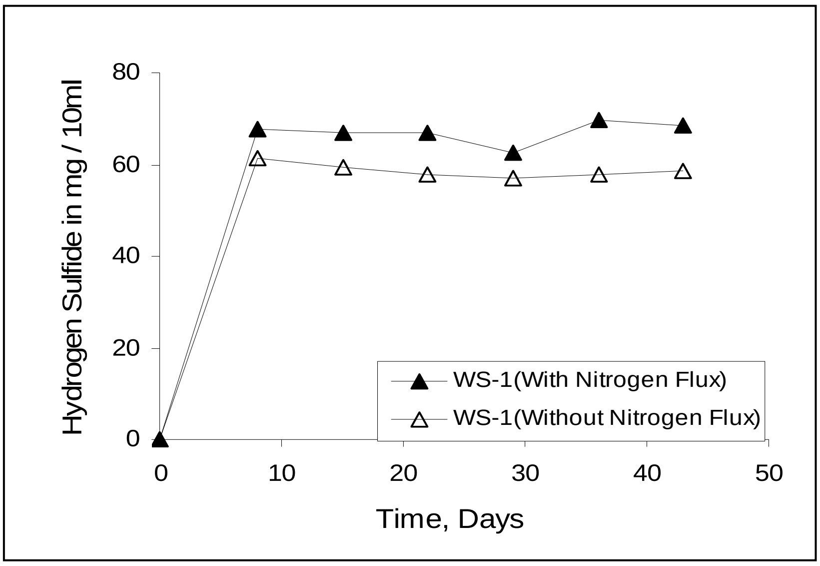

Figure 3. *H_2S production by the WS-1 strain in anaerobic basal mineral media with different conditions of nitrogen flux with lactic acid as substrate*

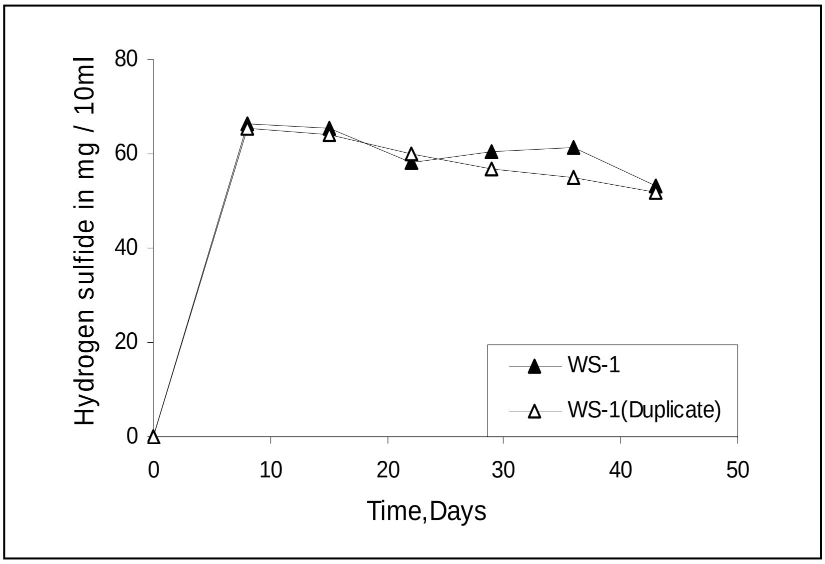

Figure 4. *H_2S production by the WS-1 strain in anaerobic basal mineral media with different conditions of nitrogen flux with acetic acid as substrate*

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Analysis of H_2S production with lactic acid as substrate

The lactate that was used as a carbon source was degraded in the culture medium and the same was associated with sulfate reduction and sulfide production. The H_2S production was increased up to 15 days of incubation at 35 °C and from 22 days to 43 days the H_2S production remained the same. From the experiments conducted it was observed out that slightly lower H_2S production was seen in the culture medium not fluxed with N_2 as compared with N_2 flux condition as shown in (*Fig.3*) supporting the fact that under N_2 flux conditions the anaerobic condition is better maintained which helps maintaining the sound growth and activity of the microorganisms.

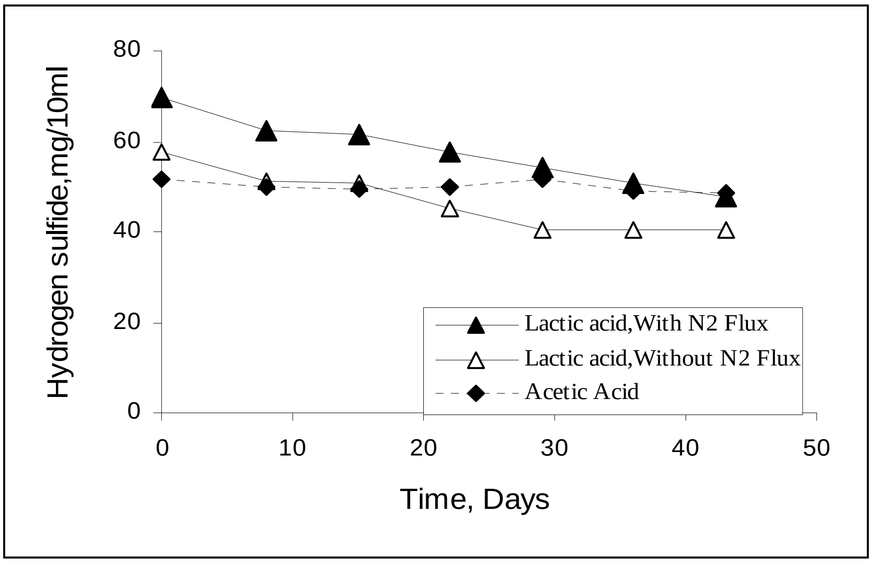

Figure 5. *Effect of sodium nitrate on H_2S production by WS-1 strain in anaerobic basal mineral media with different conditions of nitrogen flux with lactic acid as substrate and with acetic acid as substrate*

Analysis of H_2S production with acetic acid as substrate

Acetate was degraded slowly as a carbon source, so there was lower H_2S production in comparison to the lactic acid as carbon source as shown in (*Fig.4*). In this experimental set up N_2 was fluxed as better results were obtained in case of N_2 flux from the previous experiments. Therefore it can be concluded that the isolated strains of SRB utilized lactic acid as a more preferable carbon source than acetic acid.

Effect of sodium nitrate salts on H_2S production

Effect of addition of sodium nitrate to the growth media was studied in order to determine the effect of the same on the H2S production. 80mM of sodium nitrate was added to two bottles. In one bottle Nitrogen gas was fluxed and the other bottle was stoppered after filling the bottle with freshly prepared media. The addition of nitrate consistently diminished sulfate reduction and H2S production in both lactic acid and acetic acid as substrate (*Fig.5*). In the experimental set up with acetic acid as substrate N2 was fluxed as better results were obtained in case of N2 flux than without N2 flux condition. It was observed that the addition of higher concentration (80Mm) of nitrate

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prevented sulfidogenesis (*Fig.5*). Nitrate salts decrease the activity of utilization of sulfate source by SRB.

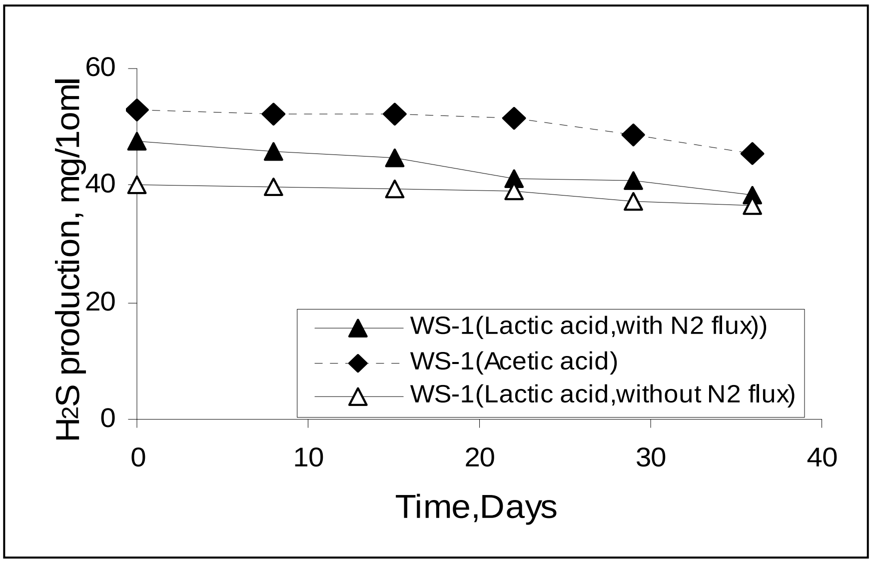

Figure 6. *Effect of potassium nitrate on H_2S production by WS-1 strain in anaerobic basal mineral media with different conditions of nitrogen flux with lactic acid as substrate and with acetic acid as substrate*

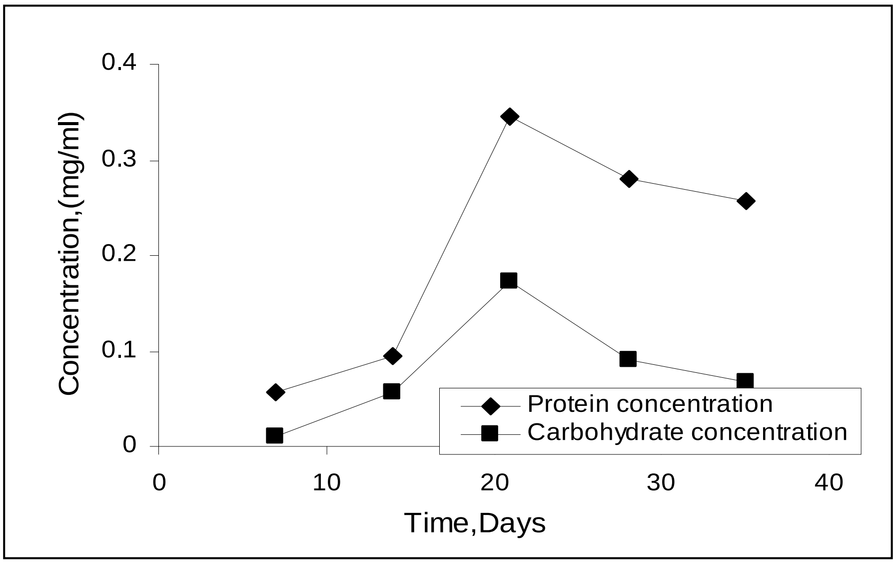

Figure 7. *Protein and carbohydrate estimation of WS-1 strain by the digestion of biomass with 2N NaOH and direct heating and the absorbance was taken at 750nm and 620nm respectively*

Effect of potassium nitrate salts on H_2S production

Effect of potassium nitrate on the H_2S production was studied in comparison to sodium nitrate. 80mM of potassium nitrate was added with varying carbon sources. Potassium nitrate was added to two bottles with varying carbon sources (lactic acid and

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acetic acid) and also in both the bottles N_2 gas was fluxed to maintain proper anaerobic condition but the control effect was less in comparison to sodium nitrate as shown in (*Fig.6*). From the study it was observed that the reduction in H_2S production was almost same for acetic acid and lactic acid substrate. Therefore it can be concluded from these results that carbon source has no effect on control conditions.

Conclusion

From the present study it was confirmed that SRB were indigenous to the sewage water. When the sample was incubated under anaerobic conditions, the microorganisms oxidized the supplied substrate and elemental sulfur present in the media that was disproportionate to hydrogen sulfide.

From the growth studies it can be concluded that lactic acid was preferred as a substrate in compared to that of acetate. It was observed that under sulfate reducing conditions, acetate was degraded more slowly than lactate, with no appreciable decrease after 2weeks of incubation. This indicates the metabolic capacity of SRB to couple the degradation of a wide variety of substrates to poisonous hydrogen sulfide gas.

It was clear from the observations that the cultures that were fluxed with nitrogen show better growth and better H_2S production. The H_2S production was also substrate dependent and with lactic acid as a substrate better H_2S production was observed as compared with acetic acid as substrate.

The present study reveals that nitrate limited sulfate reduction and sulfide formation in cultures, regardless the variety of substrate used. It was observed that sodium nitrate was very effective in diminishing sulfate reduction whereas potassium nitrate has little controlling effect.

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LONG-TERM CHANGES IN PRECIPITATION AND TEMPERATURE PATTERNS AND THEIR POSSIBLE IMPACTS ON VEGETATION (TOLFA-CERITE AREA, CENTRAL ITALY)

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Abstract. Climate change is a major global issue that impacts vegetation, agriculture, biodiversity and human safety. These impacts are predicted to be intense in the Mediterranean region. The aim of this paper is to define how local climatic trends are affecting plant communities in the Tolfa-Cerite area (Northern Latium), which is a semi-coastal area with Mediterranean to broad-leaf vegetation. Climate data analysis covered a long time period (1951-2007), considering 18 gauging stations. Data were analyzed using geostatistical methods and descriptive statistics. Climate trends and drought indicators, in relation to different vegetation associations, were analyzed using the zonal statistic tool (ArcGIS). During the investigated period, rainfall showed a uniform decreasing trend, while temperature increased, with an irregular trend. The specialization of climatic data showed a shift towards a thermo-Mediterranean bioclimate. Local climatic trends showed to have more severe impacts on specific plant communities (mesophilous forests, endangered shrubland-pastures, relict associations of meadows, etc). The observed trends towards aridity occurred in many areas covered by vulnerable plant communities. Considering the predicted changes in climate conditions for the Mediterranean area, these communities will face a further aridity increase. A permanent monitoring of these communities may increase the effectiveness of conservation policies and sustainable regional planning.
Keywords*: Bioclimate analysis, climate change, Mediterranean plant communities, Italy*

Introduction

Climate change is currently a major global concern since it could have heavy impacts on living beings, including humans (e.g., Thomas et al., 2004; Hoffman et al., 2009; Fiorillo and McCarthy, 2010). Projected impacts encompass a broad range of effects: the evolution of new plant associations (Jackson and Overpeck, 2000), shifts in the spatial distribution of tree species (e.g., Iverson and Prasad, 1998; Tchebakova et al., 2005; Téllez-Valdéz et al., 2006), animal and insect population decline or shifts (Parmesan et al., 1999; Gibbons et al., 2000; Bombi et al., 2009), reduced food availability and loss in agricultural yield (Ciais et al., 2003; Mendelsohn and Dinar, 2003; Eiji Maeda et al., 2010; Tirado et al., 2010). Many studies (Melillo et al., 1995; Bachelet et al., 2001; Hansen et al., 2001; Shafer et al., 2001; Neilson et al., 2005) agree in predicting widespread disruption of native ecosystems caused by the change in climate (see IPCC, 2000) being portrayed by General Circulation Models (GCM) for the near future (Crookston et al., 2010).

The Mediterranean regions are transitional climate regions where it has been hypothesized that climate changes may have pronounced effects (Lavorel et al., 1998; De Luis et al., 2001; Giorgi, 2006; Giorgi and Lionello, 2008). In general, the Mediterranean climate is characterized by cool, wet Winter and hot, dry Summer (Giacobbe, 1964; Henderson-Sellers and Robinson, 1991). The study of several GCM simulations shows a robust picture of climate change over the Mediterranean basin, consisting of a long-term downward trend in rainfall amount (Maheras, 1988; Kutiel et al., 1996; Palutikof et al., 1996; Esteban-Parra et al., 1998; Osborne et al., 2000; IPCC, 2007; Giorgi and Lionello, 2008) and temperature warming (Kutiel and Maheras, 1998; IPCC, 2007) especially in the hot season. Finally, it has been well documented that climate change “will lead to effects such as changes in frequencies of extreme weather events” (IPCC, 2007). An increase in the seasonal variability of climate has also been predicted. However, the Mediterranean basin is characterized by a great variability of climate types (Lionello et al., 2006) and mesoscale features. These features determine climatic gradients within a region, driven by the effects of mountains, valleys and local winds (Somot et al., 2008).

Moreover, climate change in synergy with desertification processes (Puigdefabregas and Mendizabal, 1998; De Luis et al., 2001; Sivakumar, 2007; Salvati et al., 2008), soil erosion (Favis-Mortlock and Savabi, 1996; Williams et al., 1996; Favis-Mortlock and Guerra, 1999; Nearing, 2001; Pruski and Nearing, 2002; Nearing et al., 2005) and land degradation (Attorre et al., 2007) may affect, to different extents, vegetation (e.g., Sabatè et al., 2002; Walther, 2003; Piovesan et al., 2008, Jump and Penuelas, 2005; Jiao et al., 2009).

Bioclimatology is an ecological science dealing with the relations between climate and the distribution of living species, which define specific bioclimatic regions (Rivas Martinez, 1993; Rivas Martinez, 1996). Bioclimatic indicators are based on formulas that measure climatic factors and conditions that may positively/negatively affect vegetation and may correlate to the main type of vegetation of an area. The Tolfa-Cerite area is a coastal and semi-coastal area in Central Italy, where it has previously been hypothesized a transitional shift from a defined Mediterranean to a Temperate bioclimate (Blasi et al., 1999). Thus, vegetation of this area is likely to be sensitive in relation to a shift in climate patterns, since it is already on the boundary between two bioclimatic areas.

Hence, the objective of this paper is to analyze and integrate geostatistical methods and a bioclimatic approach to quantify the effects of recent climate variations on actual vegetation. The aims of this paper are to (i) describe the bioclimate and define the climate trends within a sensitive study area through geostatistical analyses, and (ii) relate these trends to vegetation defining which are the plant communities that will experience the greatest change in climate conditions. This analysis may be useful to define which are the most vulnerable plant communities in the area in prediction of the projected climate changes for the Mediterranean basin.

Methods

Methodology for the analysis of climate and vegetation data is detailed in separate paragraphs.

Study area

The investigated area is located in the Rome prefecture (Latium, central Italy) and covers a total surface area of 556 Km^2 (*Fig. 1*). The area is delimited between longitude 11° 44' – 12° 11'and latitude 41° 55' – 42° 14' and it is bounded by the Tyrrhenian Sea on the West, by the Monti Sabatini on the East, and the Monti Cimini and the Mignone river on the North. The area is characterized by lowlands, hills and low mountains (the highest peak of Tolfa Mountains is the Monte delle Grazie, 616 m), which constitute a single morphological element, but are defined by different geological features. Some mountains are of volcanic origin, others are formed by older sedimentary deposits of flyschoid origin (Devoto and Lombardi, 1977; Contoli et al., 1980; Angelelli and Faramondi, 1995; Lombardi, 2000). The Southern sector, along the coastline, is typified by more recent geological formations with marine and fluvial sediments.

The typical landscape is characterized by dispersed towns and villages, a mosaic of pastures, cultivated land and woodlands. This landscape has been modified by a millenarian human activity: the area is inhabited since the end of the Bronze Age (9^{th}-8^{th} centuries B.C.) (Mandolesi, 1999) and there are several archeological settlements and historical monuments.

The 60% of the total surface of the study area is covered by woodland and semi-natural vegetation (Blasi, 2010). In the lower belt, vegetation is mainly Mediterranean with forests dominated by *Quercus ilex* L. and plant communities of the *Quercetea ilicis.* However, the 47% of woodlands is composed by broad-leaf species (especially on the hilly belt): *Fagus sylvatica* L., *Quercus cerris* L., *Carpinus betulus* L., *Ostrya carpinifolia* Scop. and *Castanea sativa* Miller (Anzalone, 1961; Spada, 1977; Di Pietro, 2010). Atypical plant communities consist of prairies of Sulfur springs, low belt beech forests and uncommon pastures communities (e.g., *Cynaro-Cichorietum pumili*) (Fanelli et al., 2007). The area includes many sites of the Natura 2000 network [the Tolfa hilly area is a SPA (Special Protection Area) (Council Directive 79/409/CEE)].

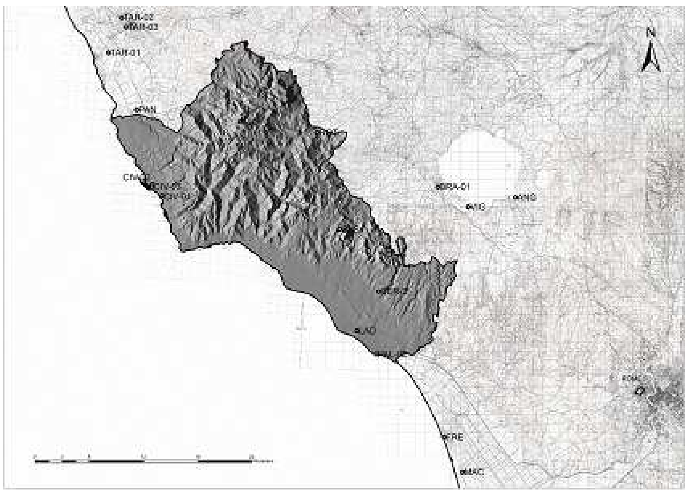

Figure 1. *Geographical position of the considered gauging stations within and nearby the study area*

Climate data

Climate stations within 30km of the research area, encompassing the broadest possible elevation range, were selected to capture the variation of the region (altitude and distance from the sea).

Climate data were obtained from the CRA-CMA (Consiglio per la Ricerca e la Sperimentazione in Agricoltura – Unità di Ricerca per la Climatologia e la Meteorologia applicate all'Agricoltura) for the period 1951-2007. Stations with limited years of registration were not considered. As regards the selected stations, years with lacking periods of registration were eliminated from the pool of data.

At the end of this initial screening, we analyzed data registered at six thermo-pluviometric gauging stations (Allumiere, Bracciano 1, Cerveteri 2, Civitavecchia 1, Maccarese, Tarquinia 1), seven pluviometric gauging stations (Anguillara Sabazia, Fregene, Ladispoli, Pantano Tarquinia, Rota, Sasso Furbara, Tarquinia 2) and five gauging stations with only temperature data (Civitavecchia 2, Civitavecchia 3, Palo Laziale 2, Tarquinia Portaccia, Vigna di Valle). Gauging stations with rainfall data are 13, with temperature data are 11. During the period 1951-1980 some of the gauging stations were inactive (three for rainfall and four for temperature). However, we statistically interpolated the missing values for temperature (see below). Nine stations are within the study area while nine are nearby (*Fig. 1*).

Climate indicators

For each station, the average was calculated of the monthly and annual values of maximum, minimum and medium temperature of monthly and annual amounts of rainfall. Higher and lower values of both temperature and rainfall were calculated. Trends in temperature and in rainfall were also calculated on a seasonal basis.

Bioclimatic indexes allow synthesizing complex relations among different sets of climatic data (Blasi, 1996). The following bioclimatic indexes were calculated: Bagnouls-Gaussen Aridity Index (BGI) (Bagnouls and Gaussen, 1953); Mitrakos indexes, for cold and drought stresses (YCS, WCS, YDS, SDS) (Mitrakos, 1980); Emberger rainfall index (Q) (Emberger, 1955); De Martonne aridity index (De Martonne, 1926); Rivas Martinez indexes (It, Ic, IO, Ios2, Ios3, Ios4) for defining the bioclimatic areas (Rivas Martinez, 1993; Rivas Martinez, 1996; Rivas Martinez and Loidi Arregui, 1999). The moving average of temperature and rainfall was also calculated, considering a window range of ten years for the whole study period (1951-2007). The outputs of these analyses were used to create graphs and spotlight trends in variations of rainfall and temperature regimes and to define the most likely period of shifting of climate conditions.

Temperature and rainfall data were then analyzed parting data in two periods (1951-1980 and 1978-2007), considering the 1980s as a break point, to highlight the variability pattern and trends in their distribution (Giavante et al., 2009). For each station, the averages of monthly and yearly rainfall and temperature values were calculated considering the two new periods and then compared, reporting any monthly, seasonal and annual variation. Bioclimatic indexes were elaborated for these new periods as well. Three gauging stations (Cerveteri 2, Tarquinia 1, and Fregene) were not used in this comparison as regards the rainfall data since data were missing for one of the two periods. Temperature data were lacking at Cerveteri 2, Palo Laziale 2, Tarquinia 1, and Tarquinia Portaccia for the period (1951-1980). These missing data were interpolated using a simple linear regression method (Goovaerts, 2000). Rainfall is a parameter with a nonlinear distribution, with random variations that could seldom be inferred. Temperature, on the other hand, has more regular trends in a small area and is mainly affected by elevation.

Geostatistical methods were used to interpolate the spatial correlation between neighboring observations to predict attribute values at unsampled locations in relation to the distance and similarity of close stations (Ordinary Kriging - Tabios and Salas, 1985; Phillips et al., 1992; Goovaerts, 2000; Attorre et al., 2007). Rainfall and temperature data were regionalized using a standardized Ordinary Kriging interpolation method (Benavides et al., 2007) using the Spatial Analyst tool in ArcGis 10.0 (ESRI, Redlands, CA, 2006).

Climatic maps for the two periods were produced in relation of several climate parameters (Annual mean of medium temperature, annual mean precipitation, mean of minimum temperature of January and February; mean of maximum temperature of July and August; mean precipitation of October, November, December and July). A standard error prediction map was produced to estimate the precision of the spatialization process for each map (Hartkamp et al., 1999). Afterward, the raster files contained in the ordinary kriging maps of the annual mean of medium temperature, the maps of annual mean precipitation and the maps of the BGI index were used to obtain three new derived maps. Values of the datasets of the period 1978-2007 were subtracted from the values of the period 1951-1978 using the ArcGIS tool “raster calculator” (in numerical values), obtaining the following maps:

(i) map of the variation of the annual mean precipitation (difference between the total amounts);

(ii) map of the variation of the annual mean of medium temperature (difference between the two values);

(iii) map of the difference of BGI values.

Vegetation and climate analysis

The vegetation map of the Rome Province (Fanelli et al., 2007) was uploaded in ArcGIS. This map has a scale of 1: 25.000 and it depicts the real vegetation areas detailing the plant community syntaxa. Each polygon is characterized by a vegetation type. However, since some vegetation categories were describing very similar plant communities, these polygons were re-categorized in more comprehensive categories. Then, the raster files (and related layers) of the three above mentioned (i-iii) maps where overlaid to the vegetation map using ArcGIS.

The “zonal statistic” tool provided by ArcGIS was used to define the variation of precipitation, temperature and BGI index values for each vegetation category and each polygon, considering minimum, maximum and the average values. Polygons defined by the same vegetation category have a diverse distribution in relation to climate patterns.

Results

Results on climate analysis are showed in summary paragraphs. The analysis of climate change in relation to vegetation data are reported subsequently.

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Rainfall distribution

The Ordinary Kriging map of annual mean precipitation is reported in *Fig. 2* along with the related standard error prediction map. It is possible to define an increasing gradient of rainfall amount from the coast to the uplands.

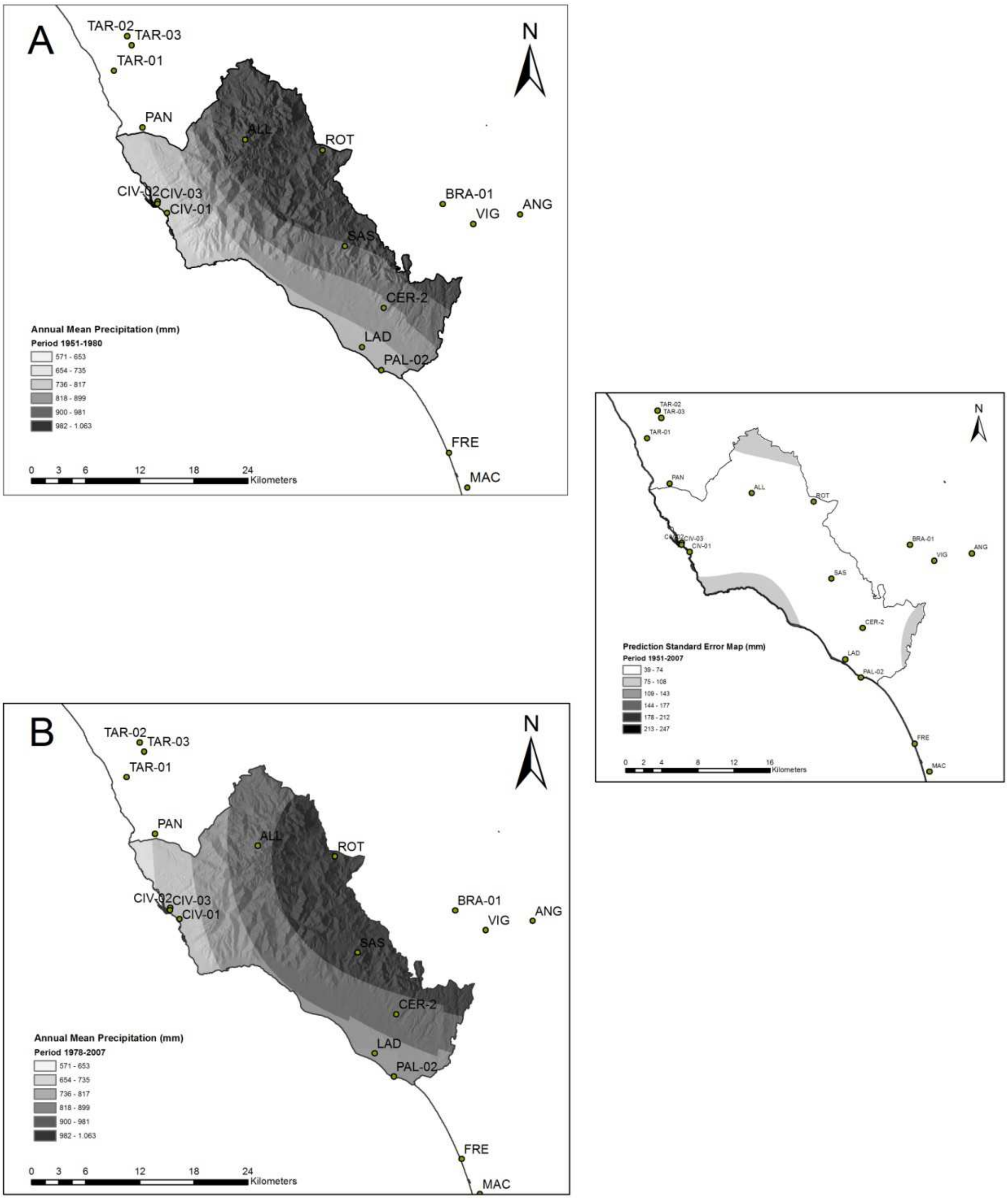

Figure 2. *Ordinary Kriging spatialization of annual mean precipitation (in mm) considering the periods 1951-1980 (a) and 1978-2007 (b) and standard error map related to the whole period*

During 1951-1980 (*Table 1*) the rainiest month was November, while the driest month was July. The highest value of annual rainfall (1117.8 mm) was registered at Rota (191 m above sea level); only at four gauging stations the average annual rainfall is higher than 1000 mm. The highest value (166.5 mm) of monthly rainfall was registered at Bracciano 1 (228 m above sea level). The lowest value of annual rainfall (670.4 mm) was registered at Tarquinia 2 (14 m above sea level, a.s.l.). The lowest value of monthly rainfall (7.8 mm) was registered at Ladispoli (5 m a.s.l.) in July.

During 1978-2007 (*Table 1*) the rainiest month was October while the driest month was July. The highest value of annual rainfall (998.3 mm) was registered at Bracciano 1; at all the gauging stations the average annual rainfall was less than 1000 mm. The highest value (155.4 mm) of monthly rainfall was registered at Anguillara Sabazia (160 m a.s.l.) in October. The lowest value of annual rainfall (569.0 mm) was registered at Civitavecchia 1 (5 m a.s.l.). The lowest value of monthly rainfall (10.0 mm) was registered at Ladispoli (5 m a.s.l.) in July.

In *Fig. 2* it is showed the Ordinary Kriging map of Annual mean precipitation (in mm) of the two considered periods (1951-1980; *Fig. 2a* – 1978-2007; *Fig. 2b*). The decrease of rainfall is evident for the whole area, and especially for the hilly areas. In the study area, a decrease of annual rainfall in nine out of 10 stations was observed ranging from 18.5 % (81.5 mm) at Maccarese to 4.4 % (29.3 mm) at Tarquinia 2. A slight (1.1 mm, 0.1 %) increase in annual rainfall amount was observed only in Sasso Furbara, an upland and wooded area. A reduction in rainfall was also highlighted on a seasonal basis. The decrease, considering the rainfall amount, was maximum during Autumn, a typical wet season in the Mediterranean region. The highest decrease in annual rainfall, considering the absolute value in mm, was calculated for the Rota station, with an estimated reduction of 203.8 mm (18.2 % of the total).

The moving average of the annual rainfall, calculated for pluviometric stations, also highlighted a decreasing trend (an example is provided in *Fig. 3*).

Table 1. *Average monthly and annual values of precipitation (in mm) for the two periods (1951-1980 and 1978-2007). The variation in rainfall was calculated subtracting the more recent period to the first one. The rainiest months are in bold, the driest months in italic*

Code	Station	Jan (mm)	Feb (mm)	Mar (mm)	Apr (mm)	May (mm)	Jun (mm)	Jul (mm)	Aug (mm)	Sep (mm)	Oct (mm)	Nov (mm)	Dec (mm)	Annual (mm)	Variation* (mm) (%)
ALL	Allumiere (1951-1980)	135.0	118.9	98.1	82.9	67.8	34.4	*12.8*	39.2	71.9	119.2	**148.5**	132.8	1061.5	-127.2 (-12.0%)
	Allumiere (1978-2007)	97.1	87.7	87.5	85.3	56.1	30.5	*21.0*	29.3	71.3	**128.2**	119.8	120.6	934.3	
ANG	Anguillara Sabazia (1951-1980)	**124.9**	109.8	82.2	85.3	68.0	41.8	*19.0*	74.8	77.2	105.5	118.5	107.9	1014.8	-71.0 (-7.0%)
	Anguillara Sabazia (1978-2007)	78.9	67.2	61.6	102.2	53.1	45.8	*15.4*	34.0	84.1	**155.4**	128.5	117.5	943.7	
BRA-01	Bracciano 1 (1951-1980)	116.9	127.7	93.5	84.3	71.1	39.3	*18.6*	43.3	101.6	110.9	**166.5**	137.1	1110.8	-112.5 (-10.1%)
	Bracciano 1 (1978-2007)	95.6	91.2	82.6	98.8	63.6	33.5	*21.6*	41.6	78.7	**136.7**	123.7	130.7	998.3	
CER-02	Cerveteri 2 (1951-1980)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Cerveteri 2 (1978-2007)	59.8	60.7	62.2	68.7	49.1	39.6	*18.6*	37.3	98.6	**126.5**	101.7	95.5	818.3	
CIV-01	Civitavecchia 1 (1951-1980)	84.5	81.6	65.7	50.9	38.6	21.7	*11.1*	22.6	54.1	77.8	**104.5**	79.6	692.7	-123.7 (-17.9%)
	Civitavecchia 1 (1978-2007)	55.3	47.1	45.5	54.6	30.1	19.1	*15.3*	20.3	52.9	**99.4**	70.4	67.0	569.0	
FRE	Fregene (1951-1980)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Fregene (1978-2007)	50.2	41.6	41.2	54.3	37.1	16.0	*10.9*	20.0	62.5	**86.1**	67.6	74.1	561.6	
LAD	Ladispoli (1951-1980)	95.4	78.8	68.3	54.2	44.9	23.4	*7.8*	27.5	83.6	83.2	**129.2**	91.5	787.8	-47.9 (-6.1%)
	Ladispoli (1978-2007)	77.2	62.8	60.3	72.7	38.9	27.9	*10.0*	27.2	64.0	**119.0**	89.9	90.0	739.9	
MAC	Maccarese (1951-1980)	93.5	83.7	74.6	51.9	41.6	23.6	*10.1*	28.2	79.4	89.6	**138.3**	92.5	807.1	-149.5 (-18.5%)
	Maccarese (1978-2007)	67.0	63.5	51.4	53.6	27.1	*17.3*	19.7	21.8	62.5	**106.4**	82.4	90.4	657.6	
PAN	Pantano Tarquinia (1951-1980)	80.3	82.2	75.1	51.4	37.1	26.1	*10.1*	26.1	54.4	82.9	**113.6**	88.7	727.9	-100.4 (-13.8%)
	Pantano Tarquinia (1978-2007)	66.0	49.8	50.8	55.4	41.0	21.2	*12.2*	18.8	49.2	**108.6**	100.7	64.4	627.5	
ROT	Rota (1951-1980)	129.7	127.7	91.6	83.4	67.8	45.4	*18.4*	41.1	87.3	132.1	**157.2**	136.0	1117.8	-203.8 (-18.2%)
	Rota (1978-2007)	92.6	76.8	76.5	89.3	56.9	32.6	*16.3*	26.7	76.0	**130.5**	129.3	110.7	914.0	
SAS	Sasso Furbara (1951-1980)	128.9	105.8	91.4	75.6	56.2	35.9	*17.3*	31.8	74.2	94.7	**138.9**	121.8	972.6	+1.1 (+0.1%)
	Sasso Furbara (1978-2007)	87.7	91.4	83.8	91.8	62.0	38.5	*14.2*	32.0	78.8	136.3	**137.1**	118.2	973.7	
TAR-01	Tarquinia 1 (1951-1980)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Tarquinia 1 (1978-2007)	50.3	45.6	47.3	40.7	35.9	19.9	*7.3*	31.4	54.5	**105.1**	85.2	71.4	594.5	
TAR-02	Tarquinia 2 (1951-1980)	70.1	81.5	56.4	45.9	40.4	27.4	*10.7*	28.7	59.9	77.5	**94.2**	77.6	670.4	-29.3 (-4.4%)
	Tarquinia 2 (1978-2007)	58.8	53.0	53.4	54.7	40.9	22.8	*15.0*	28.9	58.6	**94.9**	85.5	80.4	641.1	

*Variation in Annual Rainfall was obtained subtracting the Average Annual Rainfall of the period 1978-2007 to the Average Annual Rainfall of the period 1951-1980.

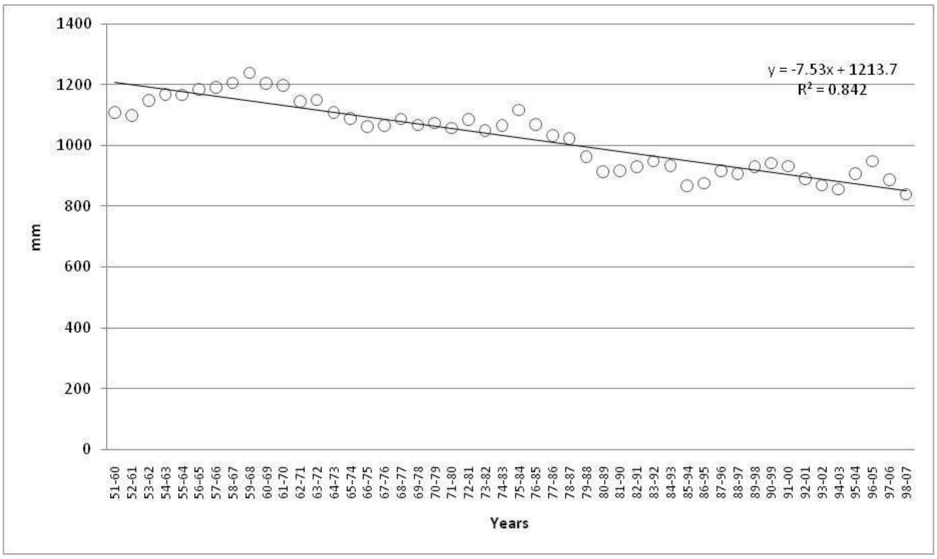

Figure 3. *Moving average of annual rainfall measured at the Rota station with the related trend line and R-squared value*

Temperature regimes

The Ordinary Kriging map of annual medium temperature is showed in *Fig. 4*. The coastal area of Civitavecchia showed to be the hottest area, while the Tolfa hilly area the coldest one.

During the period 1951-1980 (*Table 2*) the hottest months were August and July, with average medium temperatures between 21.6 °C (Allumiere) and 23.9 °C (Civitavecchia 2). Gauging stations that are located along the coast (stations of Civitavecchia, Fregene, Ladispoli, Maccarese, Palo Laziale 2, Pantano Tarquinia, Tarquinia 1 and Tarquinia 2) registered an average annual temperature of 15.9 °C. In inland areas (Allumiere, Anguillara Sabazia, Bracciano 1, Cerveteri 2, Rota, Sasso Furbara and Vigna di Valle) the average annual temperature was lower (14.8 °C). The highest value of the monthly average of maximum temperature (27.5 °C) was registered at the gauging station of Maccarese. The coldest month was January with the lowest values for Allumiere (6.5 °C medium temperature and 3.3 °C minimum temperature). The average minimum temperatures never fell below 0 °C, except in one monthly registration at Vigna di Valle (-1.4 °C in February 1956).

During the period 1978-2007 (*Table 2*) the hottest months were July and August, with average temperatures between 22.2 °C (Allumiere) and 25.1 °C (Civitavecchia 1). At sea level (same stations as above), the average annual temperature was 16.6 °C, while in the inland area was lower (15.2 °C). The highest values of maximum temperature were registered in August (mainly) with values ranging from 27.8 °C (Civitavecchia 1) to 26.3 °C in July at the station of Allumiere. The coldest months were January and February, with the lowest value for Allumiere (6.1 °C medium temperature and 3.4 °C minimum temperature). The average minimum temperature never fell below 0 °C, except in one monthly registration of Allumiere (-0.5 °C, January of 1981).

In all stations, the comparison of the two temperature datasets indicated that the annual average temperature increased from 0.1 °C to 1.0 °C. The increase in

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temperature was higher for minimum temperature than for maximum temperature, generally for all the considered stations. In all stations, the maximum temperature increased during the Summer, with a variable trend for the other seasons. The moving averages of mean annual temperature (calculated for the thermo-gauging stations) generally showed an increasing trend. *Fig. 5* shows the moving average of the minimum annual temperature of the station of Vigna di Valle.

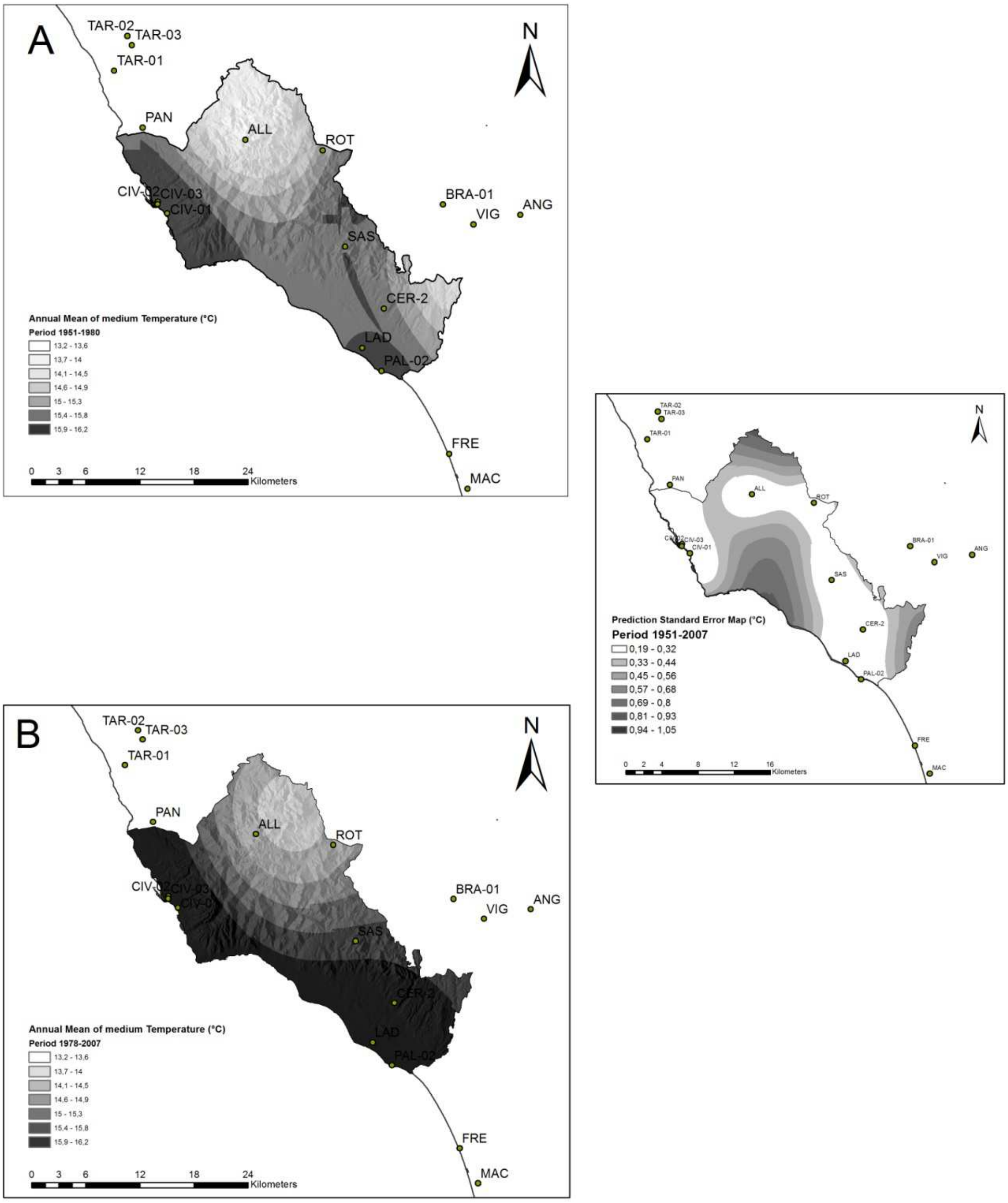

Figure 4. *Ordinary Kriging spatialization of annual mean medium temperature (in °C) considering the periods 1951-1980 (a) and 1978-2007 (b) and standard error map related to the whole period*

Table 2. *Average monthly and annual values of medium temperature (in °C) for the two periods (1951-1980 and 1978-2007). The variation in temperature was calculated subtracting the more recent period to the first one. The coldest months are in bold, the hottest months in italic*

Code	Station	Jan (°C)	Feb (°C)	Mar (°C)	Apr (°C)	May (°C)	Jun (°C)	Jul (°C)	Aug (°C)	Sep (°C)	Oct (°C)	Nov (°C)	Dec (°C)	Annual (°C)	Variation ** °C)
ALL	Allumiere (1951-1980)	**6.5**	7.0	9.0	11.6	15.7	19.2	*21.6*	21.4	18.9	14.8	10.6	7.3	13.6	0.4
	Allumiere (1978-2007)	**6.1**	6.6	8.9	11.4	16.0	19.7	*22.2*	22.1	19.0	15.3	10.2	7.2	13.7	
ANG	*Anguillara Sabazia (1951-1980)	**8.4**	9.0	10.6	12.9	16.9	20.4	22.7	*22.9*	20.8	16.9	12.7	9.3	15.3	0.5
	*Anguillara Sabazia (1978-2007)	**8.6**	9.1	11.0	13.2	17.5	21.0	23.2	*23.7*	21.3	17.8	12.9	9.8	15.8	
BRA-01	Bracciano 1 (1951-1980)	**7.7**	8.6	10.5	12.4	16.8	20.2	21.7	*22.8*	20.4	16.7	12.1	8.7	14.9	0.4
	Bracciano 1 (1978-2007)	**7.9**	8.6	10.7	13.0	17.6	21.1	22.0	*23.1*	20.8	17.0	12.2	9.1	15.3	
CER-02	*Cerveteri 2 (1951-1980)	**8.8**	9.4	10.9	13.2	17.1	20.6	22.9	*23.2*	21.1	17.3	13.2	9.8	15.6	0.6
	*Cerveteri 2 (1978-2007)	**9.1**	9.6	11.4	13.5	17.7	21.3	23.5	*24.0*	21.7	18.3	13.5	10.4	16.2	
CIV-02	Civitavecchia 1 (1951-1980)	**9.2**	9.8	11.4	13.7	17.5	21.2	23.6	*23.8*	21.5	17.8	13.9	10.4	16.2	1.0
	Civitavecchia 1 (1978-2007)	**10.2**	10.7	12.4	14.6	18.8	22.2	24.5	*25.1*	22.6	19.3	14.6	11.5	17.2	
CIV-02	Civitavecchia 2 (1951-1980)	**9.6**	10.2	11.3	13.7	17.6	21.0	23.7	*23.9*	21.8	17.8	13.8	10.6	16.2	0.4
	Civitavecchia 2 (1978-2007)	**10.1**	10.2	11.9	13.7	17.7	21.2	23.6	*24.4*	22.0	18.9	14.5	11.3	16.6	
CIV-03	Civitavecchia 3 (1951-1980)	**10.0**	10.6	11.7	13.6	17.0	20.5	23.1	*23.5*	21.4	17.9	14.1	11.0	16.2	0.5
	Civitavecchia 3 (1978-2007)	**9.8**	10.2	11.9	13.9	17.8	21.4	24.2	*24.5*	22.6	18.9	14.1	11.1	16.7	
FRE	*Fregene (1951-1980)	**9.2**	9.8	11.2	13.5	17.3	20.8	23.2	*23.5*	21.5	17.7	13.6	10.2	16.0	0.6
	*Fregene (1978-2007)	**9.7**	10.1	11.8	13.9	18.0	21.5	23.7	*24.3*	22.1	18.8	14.0	10.9	16.6	
LAD	*Ladispoli (1951-1980)	**9.2**	9.8	11.2	13.5	17.3	20.8	23.2	*23.5*	21.4	17.7	13.6	10.2	15.9	0.6
	*Ladispoli (1978-2007)	**9.6**	10.1	11.8	13.8	18.0	21.5	23.7	*24.3*	22.1	18.7	14.0	10.9	16.5	
MAC	Maccarese (1951-1980)	**8.3**	9.1	10.6	13.1	17.1	20.5	22.5	*22.7*	20.8	17.0	13.1	9.2	15.3	0.7
	Maccarese (1978-2007)	**9.1**	9.6	11.3	13.4	17.8	21.2	23.1	*23.7*	21.4	18.4	13.4	10.2	16.0	
PAL-02	*Palo Laziale 2 (1951-1980)	**9.2**	9.8	11.2	13.5	17.3	20.8	23.2	*23.5*	21.4	17.7	13.6	10.2	15.9	0.7
	*Palo Laziale 2 (1978-2007)	**9.7**	10.1	11.8	13.8	18.0	21.5	23.7	*24.3*	22.1	18.8	14.0	10.9	16.6	
PAN	*Pantano Tarquinia (1951-1980)	**9.0**	9.6	11.1	13.3	17.2	20.7	23.1	*23.3*	21.3	17.5	13.4	10.0	15.8	0.6
	*Pantano Tarquinia (1978-2007)	**9.4**	9.9	11.6	13.7	17.9	21.4	23.6	*24.2*	21.9	18.5	13.8	10.6	16.4	
ROT	*Rota (1951-1980)	**8.2**	8.8	10.5	12.8	16.8	20.3	22.6	*22.8*	20.6	16.7	12.5	9.1	15.1	0.5
	*Rota (1978-2007)	**8.4**	8.9	10.9	13.0	17.4	20.9	23.1	*23.6*	21.1	17.6	12.7	9.6	15.6	
SAS	*Sasso Furbara (1951-1980)	**7.6**	8.2	10.0	12.3	16.4	20.0	22.2	*22.4*	20.1	16.1	11.8	8.5	14.6	0.4
	*Sasso Furbara (1978-2007)	**7.6**	8.1	10.2	12.5	17.0	20.6	22.7	*23.1*	20.4	16.8	11.9	8.8	15.0	
TAR-01	*Tarquinia 1 (1951-1980)	**9.2**	9.8	11.2	13.5	17.3	20.8	23.2	*23.5*	21.5	17.7	13.6	10.2	15.9	0.7
	*Tarquinia 1 (1978-2007)	**9.8**	10.1	11.8	13.8	18.0	21.5	23.7	*24.3*	22.1	18.8	14.0	10.9	16.6	
TAR-02	*Tarquinia 2 (1951-1980)	**9.1**	9.7	11.2	13.4	17.3	20.8	23.1	*23.4*	21.4	17.6	13.5	10.1	15.9	0.6
	*Tarquinia 2 (1978-2007)	**9.6**	10.0	11.8	13.8	18.0	21.5	23.7	*24.3*	22.1	18.7	13.9	10.8	16.5	
TAR-03	*Tarquinia Portaccia (1951-1980)	**9.1**	9.7	11.2	13.4	17.3	20.8	23.1	*23.4*	21.4	17.6	13.5	10.1	15.9	0.6
	*Tarquinia Portaccia (1978-2007)	**9.6**	10.0	11.7	13.8	18.0	21.5	23.7	*24.3*	22.0	18.7	13.9	10.8	16.5	
VIG	Vigna di Valle (1951-1980)	**6.9**	7.7	9.4	11.9	16.2	20.0	*22.3*	22.1	20.0	15.6	11.1	7.8	14.3	0.4
	*Vigna di Valle (1978-2007)	**7.2**	7.8	9.9	12.1	16.7	20.4	22.8	*22.9*	20.4	16.5	11.4	8.3	14.7	

* Values interpolated using the linear regression method.

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**Variation in Annual Mean Medium Temperature was obtained subtracting the Annual Mean Medium Temperature of the period 1978-2007 to the Annual Mean Medium Temperature of the period 1951-1980

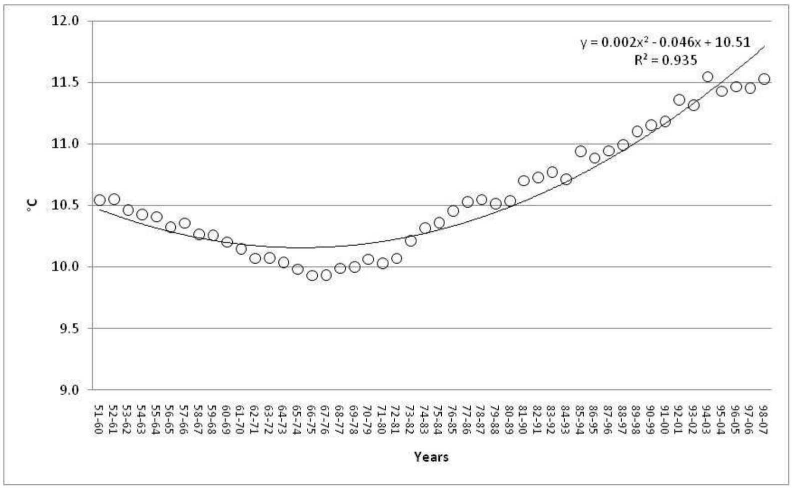

Figure 5. *Moving average of the annual minimum temperature at the station of Vigna di Valle with the related trend line and R-squared value*

Bioclimatic Indexes

During the period 1951-1980 (*Table 3*), the calculation of Mitrakos indexes showed that the station with the higher drought stress (both annual and Summer) was Civitavecchia 1. The station with the highest cold stress (both annual and Winter) was Allumiere. According to the bioclimatic classification of Emberger (Q), all stations with available data were classified as humid. According to the elaboration of Rivas Martinez indexes, all the gauging stations belonged to the Mediterranean area, excluding Bracciano, Anguillara Sabazia and Rota, which classified within the Temperate area. The values of the BGI indicated a humid climate for all the stations.

During the period 1978-2007 (*Table 3*), the calculation of Mitrakos indexes showed that the station with the highest drought stress (both annual and Summer) was Fregene. The station with the highest cold stress (both annual and Winter) was Allumiere. According to the bioclimatic classification of Emberger (Q), Allumiere, Anguillara Sabazia, Bracciano 1, Cerveteri 2, Ladispoli, Rota, Sasso Furbara and Tarquinia 2 were humid, while Civitavecchia 1, Fregene, Maccarese, Pantano Tarquinia and Tarquinia 1 were sub-humid. According to the elaboration of Rivas Martinez indexes all the gauging stations belonged to the Mediterranean area.

The comparison of Mitrakos indexes shows that, for all the stations, the annual and Winter cold stresses have markedly decreased in a percentage ranging from 1.3% to 31.7%. Summer drought stress has increased for the majority of stations (not for Ladispoli); the annual drought stress has increased too (but not for Ladispoli and Tarquinia 2). The Emberger biclimatic classification of Civitavecchia 1, Fregene, Maccarese, Pantano Tarquinia and Tarquinia 1 shifted from humid to sub humid. According to the Rivas Martinez classification all the stations that were classified as Temperate shifted into the Mediterranean belt. According to the Rivas Martinez classification three stations (Allumiere, Bracciano 1 and Rota) change their ombrotype (Rivas Martinez 1993) from humid to sub-humid; other three other stations (Civitavecchia 1 and Maccarese and Pantano Tarquinia) changed from sub-humid to dry. The variations of the BGI values over the two periods are showed in *Fig. 6*.

Table 3. *Bioclimatic Indexes values for the two periods (1951-1980 and 1978-2007). YCS= Year Cold Stress; WCS= Winter Cold Stress; YDS= Year Drought Stress; SDS= Summer Drought Stress; Q= Emberger rainfall index; AI= De Martonne Aridity Index; IT= Thermicity Index; Ic= Continentality Index; IO= Ombrothermic Index; IOS2= Ombrothermic Index of the warmest two months of Summer; IOS3= Ombrothermic Index of Summer; IOS4= Ombrothermic Index of Summer plus the previous month); BGI= Bagnouls-Gaussen Aridity Index*

Code	Station	YCS	WCS	YDS	SDS	Q	m	IA	IT	Ic	IO	IOS2	IOS3	IOS4	BGI
ALL	Allumiere (1951-1980)	231.1	149.5	127.3	127.3	158.0	3.3	45.0	265.3	15.2	6.5	1.2	1.4	2.0	9.6
	Allumiere (1978-2007)	219.8	145.8	138.4	138.4	142.7	3.4	39.4	258.4	16.1	5.7	1.1	1.3	1.7	11.8
ANG	Anguillara Sabazia (1951-1980)	149.7	107.8	78.5	78.5	147.3	5.0	40.1	319.1	14.5	5.5	2.1	2.1	2.5	2.2
	Anguillara Sabazia (1978-2007)	123.6	96.1	109.7	109.7	135.5	5.5	36.7	329.9	15.1	5.0	1.1	1.4	1.7	7.4
BRA-01	Bracciano 1 (1951-1980)	189.7	128.8	97.7	97.7	157.9	4.0	44.6	302.4	15.1	6.2	1.4	1.6	2.1	7.0
	Bracciano 1 (1978-2007)	166.6	118.6	106.4	106.4	147.3	4.5	39.5	309.7	15.2	5.5	1.4	1.5	1.9	9.0
CER-02	Cerveteri 2 (1951-1980)	123.4	94.7	N.A	N.A.	N.A.	5.6	N.A.	333.5	14.4	N.A.	N.A.	N.A.	N.A.	N.A.
	Cerveteri 2 (1978-2007)	97.8	81.3	110.9	109.1	115.9	6.1	31.3	348.6	14.9	4.2	1.2	1.4	1.7	10.5
CIV-01	Civitavecchia 1 (1951-1980)	91.6	76.3	211.9	189.1	100.4	6.3	26.4	345.4	14.6	3.6	0.7	0.8	1.1	20.4
	Civitavecchia 1 (1978-2007)	57.9	52.1	245.2	190.6	79.2	7.3	31.8	282.8	14.9	2.8	0.7	0.8	0.9	24.1
CIV-02	Civitavecchia 2 (1951-1980)	81.4	66.1	N.A.	N.A.	N.A.	6.9	N.A.	354.1	14.4	N.A.	N.A.	N.A.	N.A.	N.A.
	Civitavecchia 2 (1978-2007)	66.2	57.8	N.A.	N.A.	N.A.	7.0	N.A.	362.9	14.3	N.A.	N.A.	N.A.	N.A.	N.A.
CIV-03	Civitavecchia 3 (1951-1980)	66.1	55.6	N.A.	N.A.	N.A.	7.2	N.A.	362.1	13.5	N.A.	N.A.	N.A.	N.A.	N.A.
	Civitavecchia 3 (1978-2007)	65.1	56.3	N.A.	N.A.	N.A.	7.2	N.A.	368.9	14.7	N.A.	N.A.	N.A.	N.A.	N.A.
FRE	Fregene (1951-1980)	112.3	88.8	N.A.	N.A.	N.A.	5.8	N.A.	342.6	14.3	N.A.	N.A.	N.A.	N.A.	N.A
	Fregene (1978-2007)	89.2	74.8	266.5	206.2	79.1	6.4	21.1	359.5	14.6	2.6	0.6	0.7	1.0	23.0
LAD	Ladispoli (1951-1980)	113.0	89.3	192.9	182.7	112.1	5.8	30.4	341.2	14.3	4.1	0.8	0.9	1.2	19.1
	Ladispoli (1978-2007)	89.9	75.3	191.9	169.6	104.3	6.4	27.9	358.0	14.7	3.7	0.8	0.9	1.2	24.6
MAC	Maccarese (1951-1980)	210.2	141.3	193.0	176.2	108.6	3.7	31.9	320.2	14.4	4.4	0.9	0.9	1.2	17.4
	Maccarese (1978-2007)	165.9	117.6	228.2	182.5	88.7	4.6	25.3	342.8	14.6	3.4	0.9	0.9	1.0	28.5
PAL-02	Palo Laziale 2 (1951-1980)	112.6	89.1	N.A.	N.A.	N.A.	5.8	N.A.	341.4	14.3	N.A.	N.A.	N.A.	N.A.	N.A.
	Palo Laziale 2 (1978-2007)	89.5	75.0	N.A.	N.A.	N.A.	6.4	N.A.	358.8	14.6	N.A.	N.A.	N.A.	N.A.	N.A.
PAN	Pantano Tarquinia (1951-1980)	120.6	93.2	201.4	175.6	104.0	5.6	28.2	336.8	14.3	3.8	0.8	0.9	1.2	18.0
	Pantano Tarquinia (1978-2007)	95.7	79.7	215.2	195.6	88.8	6.2	23.8	352.3	14.8	3.2	0.6	0.8	1.1	21.5
ROT	Rota (1951-1980)	157.0	111.5	90.0	90.0	162.9	4.6	44.5	313.9	14.6	6.2	1.3	1.6	2.1	5.2
	Rota (1978-2007)	131.9	100.3	148.7	148.7	131.7	5.3	35.7	324.2	15.2	4.9	0.9	1.1	1.6	14.9
SAS	Sasso Furbara (1951-1980)	185.7	125.8	130.0	130.0	144.2	4.3	39.5	296.4	14.8	5.5	1.1	1.3	1.7	11.0
	Sasso Furbara (1978-2007)	163.9	116.4	130.5	130.5	142.6	4.6	39.0	302.1	15.5	5.4	1.0	1.3	1.8	12.0
TAR-01	Tarquinia 1 (1951-1980)	112.5	88.9	N.A.	N.A.	N.A.	5.8	N.A.	341.5	14.3	N.A.	N.A.	N.A.	N.A.	N.A.
	Tarquinia 1 (1978-2007)	89.4	74.9	225.2	182.8	83.8	6.4	22.4	359.1	15.5	3.0	0.8	0.8	1.1	26.9
TAR-02	Tarquinia 2 (1951-1980)	114.9	90.4	193.7	166.3	95.5	5.8	25.9	340.3	14.3	3.5	0.9	1.0	1.3	17.0
	Tarquinia 2 (1978-2007)	91.5	76.5	184.8	166.6	90.4	6.3	24.2	356.8	14.7	3.2	0.9	1.0	1.2	18.0
TAR-03	Tarquinia Portaccia (1951-1980)	115.1	90.5	N.A.	N.A.	N.A.	5.8	N.A.	340.2	14.3	N.A.	N.A.	N.A.	N.A.	N.A.
	Tarquinia Portaccia (1978-2007)	91.7	76.6	N.A.	N.A.	N.A.	6.3	N.A.	356.6	14.7	N.A.	N.A.	N.A.	N.A.	N.A.
VIG	Vigna di Valle (1951-1980)	213.1	136.6	N.A.	N.A.	N.A.	3.9	N.A.	280.6	15.2	N.A.	N.A.	N.A.	N.A.	N.A
	Vigna di Valle (1978-2007)	185.8	124.7	N.A	N.A.	N.A.	4.3	N.A.	290.0	15.7	N.A.	N.A.	N.A.	N.A.	N.A

N.A.=Not Available

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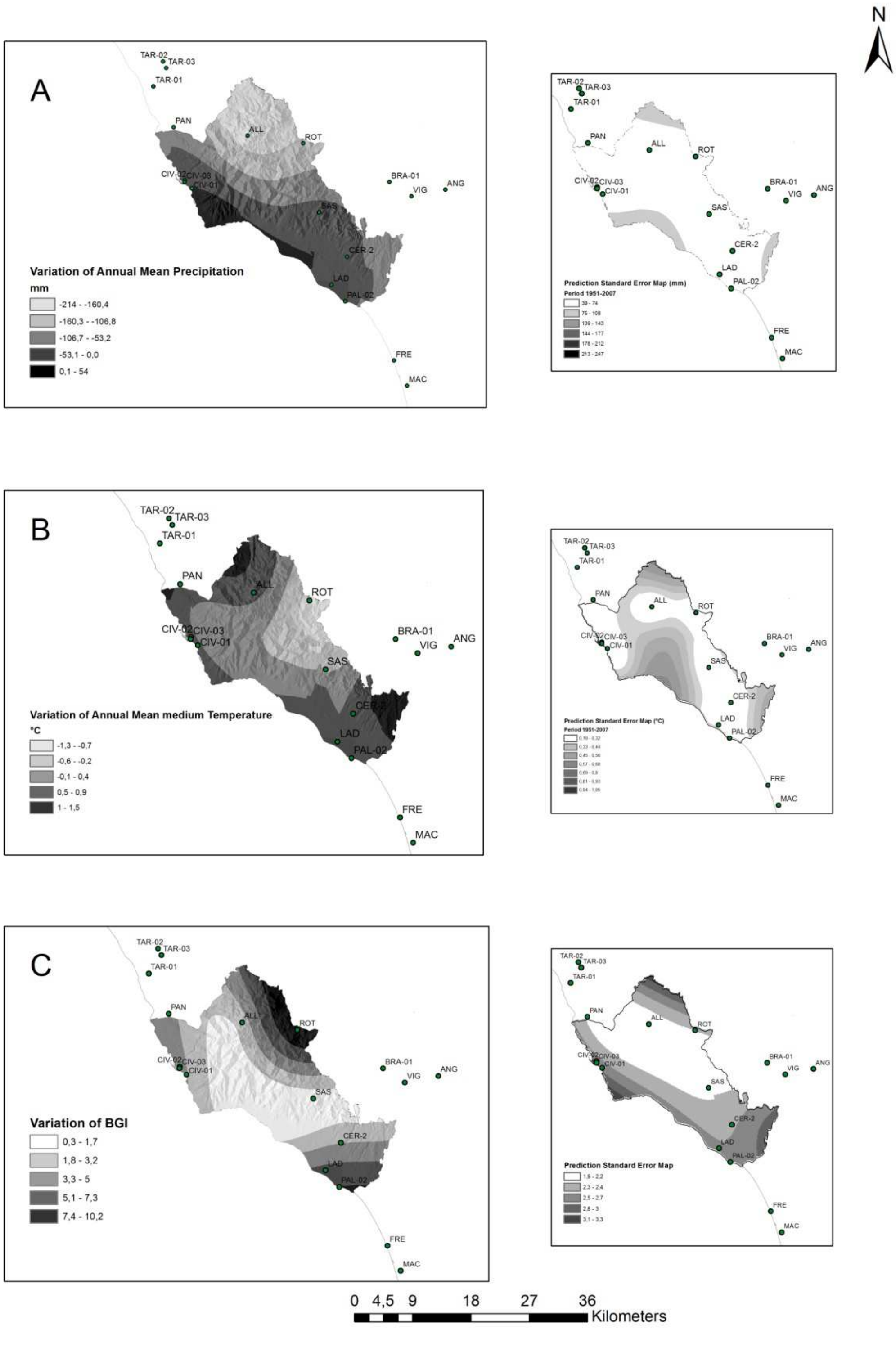

Figure 6. *Ordinary Kriging spatialization of the variation of the annual mean precipitation (a), annual mean of medium temperature (b) and of the BGI value (c). Related standard error maps are on the right*

Climate change and vegetation

According to our analyses, the broadleaf forests are the most affected by decrease in annual rainfall amount (*Table 4*) in the study area. Woodlands belonging to the vegetation association *Carpino-betuli-Coryletum avellanae*, which are located in narrow valleys on tuff substrata (Fanelli et al., 2007), were subjected to an average decrease of rainfall of 196.4 mm over thirty years (the whole area, on average, had a annual rainfall of 896.3 mm for the period 1951-1980 and of 767.2 mm for the second period). Temperate woodlands dominated by *Quercus robur* L. (*Hieracio racemosi-Quercetum petraeae* Pedrotti, Ballelli, Biondi 1982) experienced a decrease in rainfall that ranges from a minimum of 46.0 mm to a maximum of 214.3 mm (-188.7 mm on average). Chestnut forests of the *Carpinion* alliance, located on acid vulcanite soils, experienced an average decrease of 187.0 mm while the forest communities dominated by *Fagus sylvatica* L. and *Ilex aquifolium* L. [*Anemono apenninae-Fagetum sylvaticae* (Gentile 1969) Brullo 1984] in Allumiere were subjected to a decrease of 180.0 mm of rainfall (*Fig. 7*).

An average decrease of 161.2 mm of rainfall was calculated for the thermophilous sub-acidophilus woodland dominated by *Quercus cerris* L. [*Rubio-Quercetum cerridis* (Pignatti E. e S., 1968) Bas Petroli et al. (1988)], which is the most widespread woodland. *Quercus ilex* L. forests [*Viburno-Quercetum ilicis* (Br.-Bl. 1936) Rivas-Martinez, 1975] were not really affected by a decrease in rainfall (only 27 mm). The acidophilus community *Rusco aculeati-Quercetum ilicis* Biondi, Gigante, Pignateli, Venanzoni 2002 var. *Erica arborea* L. experienced an average decrease of 144.0 mm of rainfall. Some pastures also faced an important reduction of rainfall: e.g., meadows with *Gaudinia fragilis* (L.) P. Beauv. and *Cynosurus cristatus* L. (-169.7 mm) (*Gaudinio-Cynosurietum cristati* Fanelli 1997), prairies dominated by *Dasypyrum villosum* (L.) Borbàs (*Vulpio-Dasypiretum* Fanelli 1998) (-92.0 mm).

The plant communities more heavily affected by temperature increases are the igrophilous broadleaf woodlands of the flood plains. Specifically, the alluvial forests dominated by *Alnus glutinosa* (L.) Gaertn. (*Aro italici-Alnetum glutinosae* Pedrotti and Gafta 1996) and the riparian poplar woodlands (*Populetum albae* Tchou Yen-Cheng 1949) faced a temperature increase of 0.8 °C (average of the annual medium temperature). The alophilous and sub-alophilous communities of dunes experienced an increase of 0.7 °C. A moderate increase of temperature was also calculated for *Castanea sativa* forests of the *Carpinion* alliance (0.5 °C).

Aridity index calculations display that shrub communities faced the highest aridity increase, including evolving stadia of the Mediterranean *Quercus cerris* woodland (8.54) and communities of the *Rusco aculeati-Quercetum ilicis* Biondi, Gigante, Pignateli, Venanzoni 2002 var. *Erica arborea* L. (7.02). Woodlands of *Carpinus betulus* L. and *Corylus avellana* L. (*Carpino betuli-Coryletum avellanae* 1982 Ballelli, Biondi & Pedrotti 1980) (5.81) also faced an increase of aridity. Among the forests, an increase of aridity was also calculated for the re-colonizing woodland dominated by *Quercus pubescens* (*Roso sempervirentis-Quercetum* pubescentis Biondi 1986) (5.05) (BGI shifting from 0.58 to 9.98 in different localities).

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Table 4. *Zonal statistic analysis of the annual mean precipitation, annual mean of medium temperature and of the BGI value [with the related surface area (Area), Minimum (min), Maximum (max), Mean (Mean) and standard deviation (STD)] in relation to vegetation syntaxa (from Fanelli et al., 2007, mod)*

Vegetation Syntaxa	Temperature					Precipitation					BGI				
	Area (ha)	Min	Max	Mean	STD	Area (ha)	Min	Max	Mean	STD	Area (ha)	Min	Max	Mean	STD
Alophilous and sub-alophilous vegetation of dunes	43.3	0.56	1.21	0.72	0.18	43.3	-70.06	10.58	-23.87	36.22	432.5	1.30	4.70	2.90	1.56
Anemono apenninae-Fagetum sylvaticae (Gentile 1969) Brullo 1984	43.8	0.42	0.51	0.46	0.02	44.6	-184.41	-173.48	-180.02	3.06	437.5	1.94	2.79	2.33	0.21
Aro italici-Alnetum glutinosae Pedrotti and Gafta 1996	2.8	0.78	0.81	0.80	0.01	2.0	-45.65	-41.44	-43.21	1.41	27.5	2.01	2.24	2.15	0.08
Aro italici-Ulmetum minoris Rivas-Martýnez ex Lopez 1976	2.0	0.38	0.41	0.40	0.01	2.5	1.29	5.04	3.58	1.23	20.0	0.53	0.61	0.57	0.03
Arundinetum pliniane Biondi, Brugiapaglia, Allegrezza et Ballelli 1992	0.8	0.38	0.39	0.38	0.01	0.8	1.45	2.25	1.85	0.32	7.5	0.53	0.56	0.55	0.01
Arundini donax-Convolvoletum sepium R. Tx. et Oberd. Ex O. Bol_s 1962	49.8	0.23	0.73	0.54	0.15	49.6	-32.73	35.52	-7.17	18.11	497.5	0.33	6.48	3.39	1.53
Asparago acutifolii-Ostryetum carpinifoliae Biondi 1982	539.3	-0.79	0.89	0.02	0.54	539.0	-214.02	-34.45	-120.32	72.89	5392.5	0.44	6.68	3.13	1.60
Associations of the *Cistion ladaniferi* Br.-Bl. 1940	0.3	0.74	0.74	0.74	0.00	0.3	-193.46	-193.46	-193.46	0.00	2.5	2.50	2.50	2.50	0.00
Association with *Phragmites australis* (Cav.) Trin. Ex Steud and *Arundo donax* L.	0.8	0.78	0.79	0.79	0.00	1.5	-61.02	-59.70	-60.38	0.45	7.5	4.66	4.67	4.67	0.01
Association with *Rubus ulmifolius* Schott and *Rubus caesius* L.	143.0	-0.60	1.43	0.51	0.47	143.0	-200.84	34.31	-46.04	55.91	1430.0	0.56	7.67	2.95	1.62
Association with *Spartium junceum* L.	76.8	-0.10	0.97	0.50	0.38	75.4	-211.67	37.31	-83.76	95.82	767.5	0.31	4.66	2.05	1.28
Association with *Spartium junceum* L. and *Rubus ulmifolius* Schott	3.0	0.47	0.50	0.49	0.01	4.0	-45.63	-42.90	-44.38	0.84	30.0	1.31	1.34	1.32	0.01
Associations of the *Pruno-Rubion*	260.0	-0.57	1.45	0.55	0.36	258.0	-177.19	27.46	-74.17	46.78	2600.0	0.88	7.88	2.84	1.86
Carici remotae-Fraxinetum oxycarpae (Koch ex Faber 1936) Pedrotti (1970) 1992	0.8	0.23	0.23	0.23	0.00	0.5	24.83	25.09	24.96	0.13	7.5	0.69	0.72	0.70	0.01
Carpino betuli-Coryletum avellanae 1982 Ballelli, Biondi & Pedrotti 1980	1.0	0.22	0.24	0.23	0.01	0.5	-196.49	-196.34	-196.42	0.07	10.0	5.77	5.85	5.81	0.03
Cercidi-Aceretum monspessulani Lucchese e Pignatti 1998	103.0	-0.99	0.59	0.04	0.26	104.4	-212.01	-50.30	-142.27	39.31	1030.0	1.02	8.73	3.00	2.56
Chestnut forests of the *Carpinion* alliance	174.5	-0.22	0.95	0.50	0.27	170.3	-205.52	-112.63	-186.97	13.97	1745.0	1.91	4.96	2.99	0.63
Community with *Rubus ulmifolius* Schott sensu Fanelli 2002	111.0	-0.27	1.42	0.25	0.44	111.9	-205.24	4.30	-93.58	55.48	1110.0	0.31	7.67	2.16	1.82
Cynaro-Cichorietum pumili Lucchese et Pignatti 1990	955.5	-0.92	1.31	-0.01	0.43	953.2	-212.57	44.98	-72.62	46.58	9555.0	0.30	9.78	2.46	2.33
Cytiso villosi-Quercetum suberis Testi, Lucattini et Pignatti 1994	217.5	-0.05	0.72	0.25	0.15	216.1	-178.55	-15.93	-66.58	42.65	2175.0	0.60	3.64	1.47	0.61
Evolving stadia of *Aceri-Quercetum ilicis* Brullo e Marcenò 1984	1.5	0.23	0.23	0.23	0.00	1.5	2.55	3.57	3.13	0.32	15.0	0.44	0.46	0.45	0.01
Evolving stadia of *Quercus cerris* L. forests	7.8	-0.46	-0.40	-0.43	0.02	7.5	-177.97	-175.82	-176.97	0.57	77.5	8.30	8.75	8.54	0.11
Fraxino orni-Quercetum ilicis Horvatic	365.8	-0.64	1.49	0.43	0.44	360.6	-214.05	-11.01	-94.18	73.74	3657.5	0.51	7.48	2.59	1.79

(1956) 1958															
Gaudinio-Cynosurietum cristati Fanelli 1997	6.8	0.03	0.21	0.08	0.06	7.3	-170.99	-168.27	-169.73	0.64	67.5	2.82	3.87	3.57	0.34
Hieracio racemosi-Quercetum petraeae Pedrotti, Ballelli, Biondi 1982	1037.3	-0.72	0.88	0.36	0.26	1034.1	-214.28	-46.01	-188.72	21.01	10372.5	1.08	8.77	4.31	1.85
Laguro ovati-Dasypiretum villosi Fanelli 1998	559.0	-0.01	1.24	0.31	0.20	561.7	-67.43	47.67	-5.06	18.36	5590.0	0.31	4.99	1.45	0.94
Lonicero etruscae-Rosetum sempervirentis Cutini, Fabozzi, Fortini, Armanini, Blasi 1996	375.8	-0.94	1.01	0.13	0.34	375.4	-208.43	45.55	-74.07	67.06	3757.5	0.49	9.46	2.07	2.03
Mespilo germanicae-Quercetum frainetto Biondi, Gigante, Pignatelli, Venanzoni 2001	6449.5	-1.17	1.49	-0.06	0.47	6424.8	-165.70	-13.88	-60.58	25.32	64495.0	0.34	8.92	1.81	1.32
Mosaic of alophilous vegetation	42.3	0.67	0.77	0.71	0.03	42.3	-20.89	-12.73	-16.34	2.49	422.5	4.21	5.52	4.97	0.37
Mosaic with *Rubus* sp. pl. and *Prunus* sp. pl.	2.3	0.17	0.21	0.19	0.01	2.5	-33.83	-32.68	-33.34	0.34	22.5	1.07	1.08	1.08	0.00
Pastures of the *Echio-Galaction* with *Pyrus spinosa* Forssk. Trees	3534.0	-1.23	1.10	-0.19	0.54	3542.0	-214.38	26.52	-100.83	56.37	35340.0	0.34	10.13	3.74	2.70
Phragmitetum australis (Allorge 1921) Pignatti 1953	1.8	0.45	0.45	0.45	0.00	1.8	-6.58	-6.39	-6.45	0.06	17.5	3.18	3.38	3.28	0.07
Populetum albae Tchou Yen-Cheng 1949	38.5	0.32	1.44	0.75	0.24	36.3	-191.44	-4.44	-32.41	28.92	385.0	2.03	6.31	4.63	1.19
Potentillo-Polygonetalia	19.0	-0.24	0.62	0.07	0.31	19.5	-101.17	-0.67	-53.88	41.51	190.0	0.43	4.27	2.05	1.19
Prunetalia spinosae R. Tuxen 1952	1939.0	-1.22	1.47	0.09	0.39	1916.4	-213.49	42.80	-110.34	62.95	575.0	1.23	5.29	3.34	1.19
Pruno-Crataegetum Hueck 1931	4.3	0.18	0.22	0.20	0.01	3.8	13.94	17.16	15.78	0.98	42.5	1.17	1.34	1.25	0.06
Querco-Ulmetum Issler 1924	82.0	-0.68	1.47	0.37	0.38	85.2	-176.70	24.46	-35.51	52.89	820.0	0.47	10.04	2.13	1.74
Roso sempervirentis-Quercetum pubescentis Biondi 1986	521.8	-1.06	1.06	-0.05	0.69	522.9	-214.27	29.80	-143.34	60.48	5217.5	0.58	9.98	5.05	2.66
Rubio-Quercetum cerridis (Pignatti E. e S. , 1968) Bas Petroli *et al.* (1988)	6062.8	-1.26	1.02	0.08	0.56	6067.9	-214.39	-30.73	-161.20	38.20	60627.5	0.67	10.15	4.50	2.55
Rusco aculeati-Quercetum ilicis Biondi, Gigante, Pignateli, Venanzoni 2002 var. *Erica arborea* L.	37.5	-1.04	0.75	-0.49	0.79	35.8	-198.90	-32.60	-144.05	38.50	375.0	2.23	8.89	7.02	2.37
Salicetum albae Issler 1926	2.8	-0.31	0.47	0.32	0.29	3.8	-153.45	-19.14	-69.49	47.40	27.5	3.62	4.92	3.89	0.48
Silybo-Urticetum Br.-Bl. 1936	420.5	-0.54	0.87	-0.09	0.20	420.5	-212.14	-34.58	-108.26	39.44	4205.0	0.63	9.96	2.26	2.35
Thero-Brachypodion	1.8	0.41	0.43	0.42	0.00	2.0	-17.45	-15.48	-16.36	0.67	17.5	3.45	3.51	3.48	0.02
Urban areas and cultivated surfaces	25152.2	-1.25	1.50	0.48	0.41	25054.6	-214.29	54.40	-48.32	58.60	251522.0	0.30	10.06	3.13	2.01
Viburno-Quercetum ilicis (Br.-Bl. 1936) Rivas-Martinez 1975	117.8	-0.10	0.74	0.11	0.17	118.2	-77.89	2.16	-27.00	13.95	1177.5	0.30	3.46	0.61	0.67
Viburno-Quercetum ilicis (Br.-Bl. 1936) Rivas-Martinez 1975 with *Phillyrea latifolia* L.	3238.5	-0.09	1.06	0.37	0.32	3233.2	-210.80	46.70	-53.90	67.06	32385.0	0.30	6.67	1.49	0.88
Vulpio-Dasypiretum Fanelli 1998	2550.3	-1.06	1.05	-0.01	0.49	2559.5	-214.39	13.38	-92.06	60.17	25502.5	0.37	9.88	2.55	2.23

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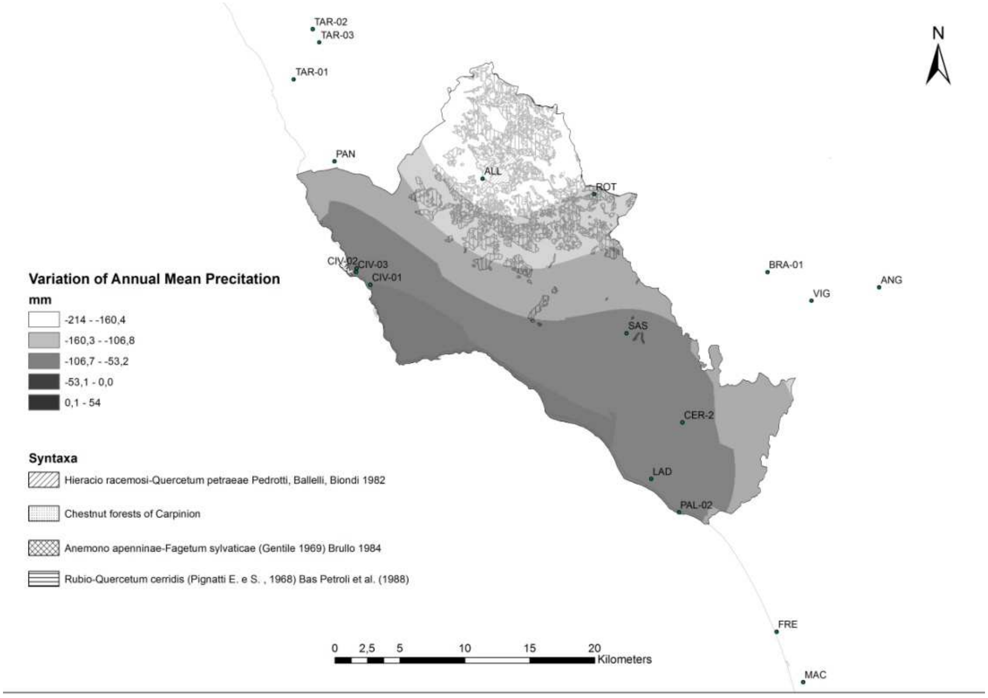

Figure 7. *Forest communities (from Fanelli et al., 2007, mod.) in the area experiencing the highest decrease in annual rainfall*

Discussion

Climate change is a cutting edge topic of research and it has been widely analyzed, predicted and forecasted at regional, national and supranational scale. However, how climate could change is still controversial (especially at local scale), as well as how it could impact plants and other living beings. In the Mediterranean basin, which has strong mesoscale features (Somot et al., 2008), local climatic studies can contribute to provide data in supporting or contrasting provisional models and theories at larger scale. Our research, developed in a Mediterranean-Temperate boundary area (Blasi et al., 1999) could provide useful data in this sense and create a positive background for further studies. Our approach tested different methods, bioclimatic and geostatical, to depict various aspects of the climate change in the Tolfa-Cerite area in relation to vegetation.

Our analysis seems to support the general negative trend in precipitation amount that has been observed in other Mediterranean areas (Giorgi and Lionello, 2008). Projected changes for the future (2071-2100) in rainfall consist in an average reduction of 8-36% (minimum and maximum) in Central Italy (Giorgi and Lionello, 2008). According to our results, precipitations are currently decreasing at comparable rates. Moreover, an increase in temperature has been observed, even if this trend is less evident in the analyzed period. Projected changes in temperature (Giorgi and Lionello, 2008), for the same period, consist in an average increase of 2.7-4.2 °C (minimum and maximum). Analyses suggest that climate change in the study region is trending to greater aridity, and projections of climate change point to a further aridity increase. Current trends in climate conditions and future scenarios have implications for regional scale vegetation as it has been tested for some Mediterranean plant communities or species (Martinez-Vilalta et al., 2002; Thuiller et al., 2004; De Dato et al., 2008; Ogaya et al., 2011).

It was observed a reduction in Autumn rainfall and this phenomenon may reduce the groundwater recharge. This reduction is partially balanced by a certain increase of rainfall during Summer, but this increase, which is expressed in percentage, correspond to a low increase in millimeters of rainfall. This phenomenon is in countertendency at projected increase of Summer drought. Geostatistical methods for the regionalization of rainfall were useful in defining its spatial distribution; the comparison of the two periods provided a visual and immediate output of changes in precipitation patterns. This analysis spotlights a clear decrease in rainfall amounts, especially in the hilly belt, while this decrease is less marked along the coast. This result may be useful in planning *ad hoc* management policies, especially as regards agriculture, irrigation, management of groundwater resources and natural vegetation conservation. Two out of the four forest communities that experienced the highest decrease in rainfall amount are listed in the Habitat Directive (92/43/CEE): the *Castanea* (9260) and *Fagus* forests (9210).

Changes in temperature are less evident, but these results might have been flattened by average values. However, it was possible to highlight a certain increasing trend in temperature, and this increase was higher for minimum than for maximum temperatures. In the study area it seems also that hilly areas were more affected by this increase in temperature values. This trend could be particularly worrying for the Tolfa area since there grow extra-zonal forests of *Fagus sylvatica* and other broad leaf woodlands. An increase of temperature seems to positively affect the growth of this species (Sabatè et al., 2002), but not if associated with a decrease in rainfall (Piovesan et al., 2008).

The area showed transitional bioclimatic features (Blasi et al., 1999) in the first analyzed period (1951-1980), but during the second period it is possible to highlight a reclassification of the whole area in the Mediterranean bioclimatic belt. This may indicate an ongoing process of vegetation shift, and probably also a change in plant distribution patterns but more analyses are required to better understand this ongoing processes.

In general, it seems that vegetation is experiencing a reduction of rainfall on the hilly belt and an increase of temperature on the coastal areas. However, it is not possible to quantify the possible effects of climate change on vegetation since long term studies are required to better understand this dynamic and complex interaction. A reduction of rainfall and an increase of temperature, together, lead to an increase in climate aridity which, however, is more marked on the hilly belt. Also, it is noteworthy that many forest associations are facing this problem with a greater extent than other plant communities. Among them, the less vulnerable, and the most affected by aridity, are the riparian forest and the broadleaf tree communities.

Conclusions

This study highlighted a variation in climatic conditions using different analyses and approaches. It also highlighted that these variations occurred in many areas covered by vulnerable plant communities. These variations may be particularly dangerous for extra-zonal forests that usually grow at higher elevations and represent a very distinctive element of the landscape of the area. Moreover, a reduction in rainfall and a shift of precipitation patterns could reduce the recharge of groundwater, thus affecting both agriculture and water availability for the rapidly growing urban settlements in the area. Conservation policies and sustainable regional planning are needed to effectively protect these vulnerable plant communities. They may benefit from a permanent and integrated monitoring system of climate conditions and plant distribution.

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UPTAKE OF ZN (II) BY AN INVASIVE WEED SPECIES *PARTHENIUM HYSTEROPHORUS* L.

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Abstract. The ability of the invasive weed species *Parthenium hysterophorus* L. for the accumulation of the heavy metal Zn was studied in a greenhouse experiment. This study was aimed at identifying a metal tolerant species from natural vegetation and assess the phytoextraction potential of the plant. To compare metal concentrations in the aboveground biomass to those in roots and in soils along with nutrients. To study their effect on growth and comparison with metal amended soils treated with EDTA. The Zn accumulated by the test species was increased significantly after 0.1g/kg of EDTA was added to the medium. An increase in metal uptake with increase in test concentrations was observed. The metal uptake could effect the leaf pigments as it reduced with increase in metal exposures and time especially in EDTA treatment. This could be due to the accumulation of Zn. The phosphorus levels were found to be low in higher uptakes of the plant. The BCFs of shoots and roots and TFs being greater than 1 shows the validity of the weed species for hyperaccumulation of the metal and can be a promising species for phytoextraction of heavy metals and remediation of metal contaminated soils which is economical and ecofriendly.
Keywords: *Accumulation, EDTA, bioconcentration factor, translocation factor.*

Introduction

Contamination of heavy metals represents one of the most pressing threat to water and soil resources as well as human health. Plants have been frequently used as indicators in the search of metal pollution or as accumulators for soil remediation called phytoremediation (Baker et al., 1994). Heavy metals may be bound or accumulated by particular plants, which may increase or decrease the mobility and prevent the leaching of heavy metals into groundwater. Growing plants can help to reduce heavy metals pollution. Plants can be easily monitored to ensure proper growth; and valuable metals can be reclaimed and reused through phytoremediation. One of the strategies of phytoremediation is phytoextraction i.e. uptake and accumulation of metals into plant shoots, which can then be harvested and removed from the site. It is necessary to use plants that can tolerate high levels of heavy metals. Certain heavy metals are also trace elements for e.g., Zn, Mn, Mg, Fe, Cu, Mo and Ni which are essential in plant nutrition (micronutrient) but plants growing in a polluted environment can accumulate trace elements at high concentrations (Alloway, 1990; Hovmand et al., 1983; Huchabee et al., 1983; Kabata-Pendias and Pendias, 1992). Elevated levels of these metals in soil may lead to increased uptake by plants, because it depends on many different factors, such as soil metal bioavailability, plant growth and metal distribution to plant parts (John, 1973; John et al., 1972; Kuboi et al., 1986; Xian, 1989).

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Plants called hyperaccumulators are preferred for metal decontamination of soils because they take up 100 times the concentration of metals over other plants (Cunningham et al., 1995). They accumulate toxic metals through their roots and transport them to the stems or leaves. Researchers hope that these metal-scavenging plants, called hyperaccumulators, could be grown in contaminated soil and harvested like hay (Bennet et al., 2003; Lombi et al., 2001 O'Connor et al., 2003). The metal could then be recovered and recycled when burned and the ash collected (Comis, 1996).

Metals uptake by roots depends on both soil and plant factors (e.g. source and chemical form of elements in soil, pH, organic matter, plant species, plant age etc.). Consequently, metal mobility and plant availability are very important when assessing the effect of soil contamination on plant metal uptake and related phytotoxic effects. The mobility and availability of heavy metals in the soil are generally low, especially when the soil is high in pH, clay and organic matter (Jung and Thornton, 1996; Rosselli et al., 2003). Interactions between metals occurring at the root surface and within the plant can affect uptake, as well as translocation and toxicity (Luo and Rimmer, 1995).

More than 400 plants are known as hyperaccumulators of metals, which can accumulate high concentration of metals into their aboveground biomass. The plants include trees, vegetables crops, grasses, and weeds. Weed species appear to be a good choice for metal accumulation studies since these hardy tolerant species can grow in most harsh conditions over large areas and give a good amount of biomass as a secondary product. It is important to use native plants to study metal accumulation because these plants are often better in terms of survival, growth and reproduction under environment. There has been a continuing interest in searching for native plants that are tolerant to heavy metals. Heavy metals can cause severe phytotoxicity, and may act as powerful force for the evolution of tolerant plant populations. Therefore, it is possible to identify metal-tolerant plant species from natural vegetation in field sites that are contaminated with various heavy metals.

Recently, chelating agents have been used as decontaminants in metal-polluted soils facilitating phytoextraction. Early studies showed that application of synthetic metal chelates such as Ethylenediamine tetraacetic acid (EDTA) to soils enhances Pb accumulation by plants (Jorgensen, 1993). EDTA was particularly effective in facilitating the phytoextraction by plants of Cd, Cu, Ni, Pb and Zn. EDTA acts by complexing soluble metals present in soil solution. In view of such diverse reports, an attempt was made to understand the plant-nutrient-chelate interaction in order to examine the heavy metal uptake.

The present study was conducted using phytoextraction to explore the accumulation and removal potential of the heavy metal Zn by an invasive weed species *P.hysterophorus* L. belonging to the family Asteraceae wildly grown over large areas in Visakhapatnam city. Zn though a micronutrient, is known to be toxic at higher concentrations (Agarwal et al., 1977; Bradshaw, 1952; Chapman et al., 1939; Goldbold et al., 1983; Hagemeyer et al., 1993). The test species is one of the most popular and dominant indigenous weed species which is well adopted to the polluted environment as affected by the various activities which can accumulate significant amounts of heavy metals. Initially a preliminary study was carried out to screen the uptake efficiency and accumulation potential of Zn by this species. The uptake was found to be in the range 36.1 – 414 µg/g in shoots and 17 – 417.2 µg/g in roots. Basing on these results pot studies were carried out for this species with amendments of different concentrations of Zn.

The experiment described in this paper was designed in part to assess measurements of total andavailable metal content of Zn in soil as predictor of metal uptake by an experimental plant. Soil pH, conductivity, available N,P,K in soils, N, P, K, Na and Ca in shoots and roots were measured to investigate their possible modifying influences on metal uptake. As a part of this study the effect of a chelating agent EDTA was also examined which was added to the amended soils in the pots after successful growth of the plants. The overall objectives of this work were:

- To identify a metal tolerant species from natural vegetation and assess the phytoextraction potential of the plant.
- to determine concentrations of Zn in plant biomass and soils to examine the translocation capacity of the plant.
- to investigate the effect of Zn on plant growth and the plants ability to accumulate and tolerate the metal.
- to analyse N, P, K, Na, Ca and leaf pigments (chl a, chl b and carotenoids) and study their influences on metal uptake.
- comparison of metal and other parameters of soils and plant biomass grown in amended soils treated with EDTA and without EDTA.
- to assess the feasibility to use green plants for phytoextraction of heavy metals.

Information obtained from this study should provide insight for using native plants to remediate metal contaminated sites.

Materials and methods

Soil samples and pot experiment

A green house study was carried out in an artificially amended sandy soil (Typic Ustochrepts) with different concentrations of 250(TC1), 500(TC2) and 1000(TC3) mg/kg Zn $SO_4.7H_20$ with control. Some selected soil characteristics are as follows: pH(1:2) 8.02 in deionised water; EC 0.116ms; organic carbon 0.41 %; calcium carbonate 1.50 % and 10.9 mg/kg of extractable Zn.

Pot culture experiments were conducted in botany farmhouse using soil treated (spiked) with zinc sulphate salt and for comparison an unamended (control). The salt was uniformly mixed with air-dried soil filled in pots (6kg soil) and left for stabilization for 2 days. Soil in farmhouse was used for the experiments. One week old seedlings of the weed species *P.hysterophorus* wildly grown in the farmhouse were procured and 5 plants were transplanted in each pot; out of which only 3 were allowed to grow at a uniform distance after their survival was confirmed. Another two sets of pots of each treatment were maintained for EDTA application. EDTA (0.1g/kg soil as disodium salt) in solution form was added at pre-flowering stage (20 days from the day of survival) to examine the EDTA effect on metal uptake. Three replicas of each treatment were considered and care was taken to protect from rainwater leaching. Plants were grown under natural light and ambient temperature in order to keep all plants under conditions as similar as possible. Plants were watered with limited amount of well water in the farm at regular intervals. Artificial fertilizers or soil amendments were not added to enhance growth or metal uptake during the course of experiment.

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Plant growth and harvesting

For growth studies individual plants were grown under similar conditions and 9 plants for each concentration were harvested at a time interval of pre-flowering (20 days), flowering (40 days) and post flowering (60 days) from 3 replicate pots, without damaging the roots. EDTA treated pots were harvested at an interval of one week (I-scoring) and two weeks (II-scoring) from the day of addition.

Maximum recoverable portion of roots were procured and plants were rinsed in distilled water to remove dust and soil mineral particles. The plants were taken to the laboratory, washed with deionised distilled water, separated into root and shoot (including stem, leaves, fruits and inflorescence) parts, dried in oven at 65 ºC for 72 hours (Xian and Shokohifard, 1989). Shoot and root length (cm) and dry biomass (g) of the plant parts (shoot and root) were taken for each treatment and interval. All calculations of Zn and extraction were done on dry weight basis excepting for leaf pigments where fresh weight was taken into considered.

Analysis of plant mass

Metal uptake is also depicted as Bioconcentration Factor (BFC). It provides an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate (Zayed et al., 1998). It was determined as the ration of $[Metal]_{plant}$ tissue/$[Metal]_{soil}$. It has limited applications if one wishes to compare uptake of a plant species under different treatments. Since change in BCF is related to the individual plant biomass and soil elemental concentrations, the efficiency of BCF is a better understood when compared between different harvests, plant species or elements.

Translocation Factor (TF) gives the shoot to root Zn concentration and depicts the ability of the plant to translocate the metal species from roots to shoot at different concentrations . It was determined as the ratio of $[Metal]_{shoot}/[Metal]_{root}$. The translocation property describes that the contents of heavy metals accumulated in shoots of a plant should be higher than those in its roots, i.e. TF>1 to be considered typical of an accumulator plant (Baker, 1981). The shoot to root ratio of contaminant concentrations in all confirmed hyperaccumulators are > 1, whereas the ratios are invariably < 1 in non-accumulators (Raskin and Ensley, 2000).

Chemical analysis of plant biomass

From the oven-dried ground plant materials 0.5g was accurately weighed and transferred into Kjeldahl flask and digested slowly using Mixed Acid Digestion procedure (Stewart et al., 1974) with a mixture of 1ml of 60 % $HClO_4$, 5ml of Con.HNO_3 and 0.5ml of H_2SO_4 on moderate heat and increased later. The digestion was continued for 10-15 minutes after the appearance of white fumes. It was then cooled, diluted with deionised distilled water and filtered using Whatman filter paper No.44 and then made upto 50ml with deionised distilled water. A blank was prepared in similar manner. This filtrate was analysed for the following:

The nitrate nitrogen in plant material was determined by phenol disulphonic acid method by UV-VS Spectrophotometer (Stewart et al., 1974).The phosphorus was determined by molybdenum blue method by UV-VS Spectrophotometer (Stewart et al., 1974). Potassium was determined by the Flame photometric method (APHA, 1989). Micronutrients Na and Ca in plant materials were determined by the Flame photometric method (Stewart et al., 1974). Zn metal in sample solutions was quantified using

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Atomic Absorption Spectrophotometer (Perkin Elmer Precisely AAnalyst 200) (Stewart et al., 1974).

Leaf pigments chlorophyll a, chlorophyll b and carotenoids of the leaves in the test species was measured on all the harvest days. For this purpose the second leaf from the top (expanding leaf) was taken into consideration. 0.5g of the fresh material was weighed accurately, ground to a fine pulp using a mortar and pestle with the addition of 80% acetone, centrifuged and the clear supernatant was transferred to a 50ml volumetric flask. This process was repeated until the residue turns to colourless and the entire solution was made upto the mark with 80% acetone to read the values in UV-VS Spectrophotometer (Sharma and Rao, 1985).

Chemical analysis of soils

The soils were air dried for 72 hours, powdered to pass through a 2 mm sieve and subjected to physico-chemical analysis. Soil pH was measured in 1:2 of soil to water ratio, organic matter was determined by Walkley Black Method (Sims and Heckendorn, 1991), nitrate nitrogen in soils was extracted with deionised distilled water in the ratio1:1 (w/v) and determined by phenol disulphonic acid method and phosphorus was extracted with 2.5 % acetic acid in the ratio 1:1 (w/v) and determined by molybdenum blue method in U.V – V.S. Spectrophotometer (Stewart et al., 1974). Potassium was extracted with 1M neutral ammonium acetate (pH 7) in the ratio 1:1 (w/v) and determined by the Flame photometric method (APHA, 1989).

Total metal concentration in the soil samples was determined by Mixed Acid Digestion procedure (as followed for plant biomass analysis) and the available metal i.e acetic acid (2.5 % in 1:1 w/v) extractable metal were determined by Atomic Absorption Spectrophotometer (Stewart et al., 1974).

Metals in sample solutions were quantified using atomic absorption spectrophotometer (AAnalyst 200 Perkin Elmer Precisely Version 5.0).

Statistical analysis

Mean values and SE of the element content were calculated, and analysis of variance (ANOVA) and Student's t-test were performed to observe differences between samples. The differences were considered to be significant at $p \leq 0.05$ and highly significant at $p \leq 0.005$ level of significance.

Results and discussions

Soil properties, nutrients and metal concentrations

The pH including EDTA treatments ranged from 8.6 to 9.16. Conductivity was found to be significantly high in metal exposure TC1, when compared to control, TC2 and TC3 in all the harvest days excepting on day 60 including EDTA treatments. Nitrates showed no significant difference within the test concentrations ($p>0.05$) and a significant difference was observed between harvest days ($p<0.05$) of without EDTA treatment as determined by two-way anova (*Table 1*). A significant increase was found from 20 to 60 days ($p<0.05$) of without EDTA treatment and a significant increase could be reported with EDTA treatment when compared to without its treatment ($p<0.05$) by student's t-test. This shows the effect of EDTA as a chelate. No significant difference was observed between the scorings of EDTA treatment by student's t-test

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($p>0.05$). No particular trend of increase or decrease in values was observed with metal exposure (*Table 7, 8*).

Table 1. *Two-way Anova for nitrates in soil (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	0.0053842	0.0017947	1.46	0.316
Harvest days	2	0.0249687	0.0124843	10.17	0.012
Error	6	0.0073648	0.0012275		
Total	11	0.0377177			

S = 0.03504   R-Sq = 80.47 %   R-Sq (adj) = 64.20 %

Phosphorus results showed no significant difference within the test concentrations and harvest days including EDTA treatments by two-way anova ($p>0.05$). No significant difference could be reported between the scorings of EDTA treatment or in between EDTA treatment and without its treatment by student's t-test ($p>0.05$). Hence, the phosphates seemed to be unaffected in all soil amendments (*Table 7* and *8*).

Potassium showed a significant difference within test concentrations ($p<0.05$) and a highly significant difference in between the harvest days ($p<0.005$) of without EDTA treatment by two-way anova (*Table 2*). There was a significant increase in results from 20 to 40 days by student's t-test ($p<0.05$). A highly significant decrease from 20 to 60 days was observed i.e when there was complete maturity of the plants ($p<0.005$) as determined by student's t-test. Potassium concentrations have reduced with EDTA treatment when compared to those without its treatment showing a highly significant difference ($p<0.005$) as determined by student's t-test (*Table 2*). No difference was found between the harvests of EDTA by student's t-test ($p>0.05$).

Table 2. *Two-way Anova for potassium in soils (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	0.0000147	0.0000049	11.27	0.007
Harvest days	2	0.0000465	0.0000232	53.23	0.000
Error	6	0.0000026	0.0000004		
Total	11	0.0000638			

S = 0.0006606   R-Sq = 95.90 %   R-Sq (adj) = 92.48 %

The available metal concentrations in soils have shown a highly significant increase with increase in test concentrations on all harvest days ($p<0.005$) of without EDTA treatment and the increase was significant in EDTA treatments ($p<0.05$) by two-way anova (*Table 3, 4*). A highly significant increase was observed in between the test concentrations and control as well as within the test concentrations on all harvest days ($p<0.005$) excepting between TC2 and TC3 on 40^{th} day of without EDTA treatment and II-scoring of EDTA treatment as determined by student's t-test ($p>0.05$) as shown in *Figure 1. to 5*. These results are in agreement with literature (Samiullah Khan and Nazar Khan, 1983).

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Table 3. *Two-way Anova for available Zn in soils(without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	11446.7	3815.58	19.44	0.002
Harvest days	2	396.4	198.20	1.01	0.419
Error	6	1177.4	196.24		
Total	11	13020.5			

S = 14.01 R-Sq = 90.96 % R-Sq (adj) = 83.42 %

Table 4. *Two-way Anova for available Zn in soil (with EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	9429.99	3143.33	29.67	0.010
Harvest days	1	103.68	103.68	0.98	0.395
Error	3	317.85	105.95		
Total	7	9851.52			

S = 10.29 R-Sq = 96.77 % R-Sq (adj) = 92.47 %

The total metal concentrations in soils also have shown highly significant increase with increase in test concentrations including EDTA treatments ($p<0.005$) and no significant difference was found between harvest days including EDTA treatments ($p>0.05$) by two-way anova (*Table 5, 6*). As determined by student's t-test a highly significant difference was reported inbetween the control and test concentrations and within the test concentrations including EDTA treatments on all harvests ($p<0.005$).

Table 5. *Two-way Anova for total Zn in soils (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	48142.7	16047.6	52.54	0.000
Harvest days	2	67.9	34.0	0.11	0.897
Error	6	1832.8	305.5		
Total	11	50043.4			

S = 17.48 R-Sq = 96.34 % R-Sq (adj) = 93.29 %

Table 6. *Two-way Anova for total Zn in soil (with EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	24222.3	8074.11	126.45	0.001
Harvest days	1	44.6	44.56	0.70	0.465
Error	3	191.6	63.85		
Total	7	24458.4			

S = 7.991 R-Sq = 99.22 % R-Sq (adj) = 98.17 %

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Table 7. *Physicochemical parameters of soils in different test concentration on different harvest day.*

Physical & Chemical Parameters	Harvest Days											
	20 days				40 days				60 days			
	C	TC1	TC2	TC3	C	TC1	TC2	TC3	C	TC1	TC2	TC3
pH	8.74 ± 0.024	8.60 ± 0.032	8.76 ± 0.03	8.67 ± 0.03	9.14 ± 0.028	9.1± 0.028	9.16 ± 0.02	9.26 ± 0.02	8.838± 0.036	8.85± 0.024	8.91 ± 0.024	8.86 ± 0.026
Conductivity (mS/cm)	0.30 ± 0.002	0.33 ± 0.002	0.27± 0.112	0.295 ± 0.002	0.40 ± 0.002	0.43± 0.007	0.42 ± 0.003	0.411± 0.002	0.192± 0.002	0.140± 0.007	0.214± 0.002	0.207± 0.002
Nitrate Nitrogen (mg/100gm)	0.51 ± 0.003	0.071± 0.001	0.06± 0.002	0.03± 0.001	0.07± 0.001	0.08± 0.003	0.08 ± 0.001	0.05 ± 0.001	0.02 ± 0.001	0.12 ± 0.005	0.16 ± 0.001	0.11 ± 0.003
Phosphorus (mg/100gm)	0.69 ± 0.28	0.75 ± 0.001	0.76± 0.028	0.66 ± 0.028	0.75± 0.001	0.70± 0.001	0.75 ± 0.001	0.70 ± 0.001	0.70 ± 0.001	0.70 ± 0.001	0.70 ± 0.001	0.70 ± 0.001
Potassium (%)	0.004 ± 0.002	0.04 ± 0.02	0.04± 0.031	0.40 ± 0.025	0.037 ± 0.022	0.04± 0.04	0.036± 0.03	0.037± 0.05	0.036± 0.03	0.038± 0.04	0.035± 0.025	0.035± 0.025

Results are means ± SE (n = 5)

***Table 8**. Physicochemical parameters of soils in different test concentration on different harves harvest day*

With EDTA Treatment								
Physical & Chemical Parameters	**EDTA – I Scoring**				**EDTA – II Scoring**			
	C	**TC1**	**TC2**	**TC3**	**C**	**TC1**	**TC2**	**TC3**
pH	9.01 ± 0.024	8.84 ± 0.026	8.79 ± 0.024	8.79 ± 0.026	8.92 ± 0.048	8.86 ± 0.020	8.86 ± 0.024	8.79 ± 0.032
Conductivity (mS/cm)	0.19 ± 0.004	0.25 ± 0.002	0.19 ± 0.014	0.24 ± 0.004	0.17 ± 0.03	0.39 ± 0.002	0.25 ± 0.002	0.26 ± 0.017
Nitrate Nitrogen (mg/100gm)	0.33 ± 0.003	0.25 ± 0.001	0.24 ± 0.001	0.21 ± 0.001	0.06 ± 0.001	0.21 ± 0.001	0.23 ± 0.001	0.24 ± 0.001
Phosphorus (mg/100gm)	0.75 ± 0.001	0.68 ± 0.034	0.75 ± 0.001	0.63 ± 0.034	0.75 ± 0.001	0.60 ± 0.001	0.70 ± 0.001	0.70 ± 0.001
Potassium (%)	0.033± 0.042	0.037 ± 0.033	0.034 ± 0.041	0.033 ± 0.02	0.032 ± 0.01	0.034 ± 0.015	0.03 ± 0.02	0.035 ± 0.044

Results are means ± SE (n = 5)

Zinc uptake , root length, shoot length and plant biomass

All the analysis and calculations were done on dry weight basis excepting leaf pigments, because dry weight of a tissue can be expected to correlate closely to the nutritional status of the plant, and it depends on the relative rates of photosynthesis and respiration. The fresh weight of a tissue reflects tissue water content and is influenced considerably by the climatic conditions prevailing when the samples are removed.

The shoot and root uptakes have decreased with increase in harvest days and increased with increase in test concentrations in both EDTA and without EDTA treatments ($p<0.05$, 0.005) by two-way anova (*Table 9, 10)* which indicates that the efficiency of the plant uptake has reduced with maturity (Ghosh and Singh, 2005). The shoot and root uptakes have shown no significant difference in metal uptake between harvest days of EDTA treatments and also between the EDTA treatment and without its treatment as determined by student’s t-test ($p>0.05$) as shown in *Figure 1.* to *5.*

The shoot and root uptakes have shown a highly significant difference in between the control and test concentrations as well as within the test concentrations including EDTA treatments by student’s t-test ($p<0.005$) excepting between TC1 and TC2 60th day of without EDTA treatment as shown in Figure 1.1 to 1.5. An increase in uptake with increase in test concentrations was observed and the results were in agreement with literature (Yu-Hong and Youn-Guan 2005). This was an expected trend in accumulation studies. These results indicate that the test species could be a hyperaccumulator. The concentrations of metal uptake by the shoot were high when compared to root uptakes in all the metal exposures on all the harvest days. The results indicate that the test species could be a hyperaccumulator.

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Table 9. *Two-way Anova for metal uptake by shoot (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	18089.1	6029.70	8.77	0.013
Harvest days	2	10139.7	5069.83	7.37	0.024
Error	6	4124.9	687.48		
Total	11	32353.6			

S = 26.22   R-Sq = 87.25 %   R-Sq (adj) = 76.63 %

Table 10. *Two-way Anova for metal uptake by root (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	6125.0	2041.67	20.67	0.001
Harvest days	2	8465.8	4232.89	42.86	0.000
Error	6	592.6	98.76		
Total	11	15183.4			

S = 9.938   R-Sq = 96.10 %   R-Sq (adj) = 92.84 %

The shoot lengths showed a significant difference in between 20 and 40 as well as 20 and 60 harvest days of without EDTA treatment as determined by student's t-test ($p<0.05$). No significant difference was reported within the test concentrations and also between test concentrations and control on day 40 and 60 as determined by student's t-test ($p>0.05$). No significant difference within test concentrations and scorings of EDTA treatment was observed as determined by student's t-test ($p>0.05$) indicating that there was no growth. No significant difference could be reported between EDTA treatment and without treatment as determined by student's t-test ($p>0.05$) as shown in *Figure 6* to *10*. Maximum growth was attained in TC3 on day 60 (20.06 cm).

The root lengths showed a significant difference between 20 and 40 as well as between 20 and 60 days by student's t-test ($p<0.05$). A significant difference was observed in EDTA treated samples when compared to those without its treatment by student's t-test ($p<0.05$). No significant difference was reported between 40 to 60 days and the harvests of EDTA treatment ($p>0.05$) as determined by student's t-test. No significant difference between the control and test concentrations and within the test concentrations on day 20 could be reported by student's t-test showing no growth ($p>0.05$). There was no significant difference in between the scorings of EDTA treatment and also between EDTA treatment and without EDTA treatment by student's t-test ($p>0.05$). A significant increase was found between control and the test concentrations ($p<0.05$) and no significant difference within the test concentrations ($p>0.05$) was observed by student's t-test on day 40 as shown in *Figure 6.* to *10.* The overall results of the shoot length and root length indicate that *P. hysterophorus* is tolerant to Zn.

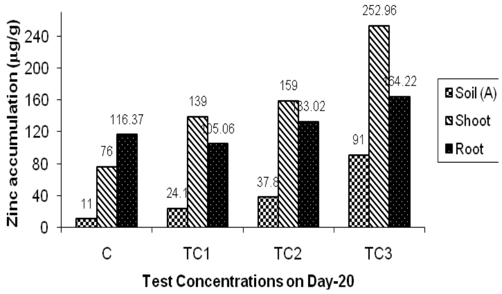

Figure 1. *Zinc accumulations of soil, shoot and root in different concentrations on day - 20*

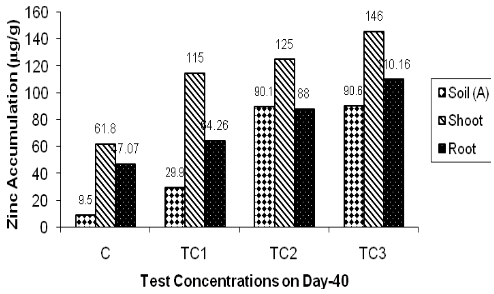

Figure 2. *Zinc accumulations of soil, shoot and root in different concentrations on day – 40*

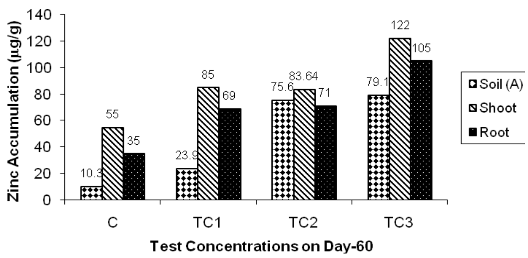

Figure 3. *Zinc accumulations of soil, shoot and root in different concentrations on day - 60*

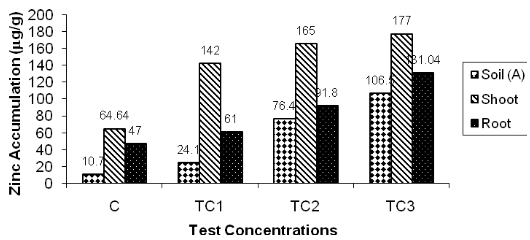

Figure 4. *Zinc accumulations of soil, shoot and root in different concentrations on I-scoring of EDTA treatment*

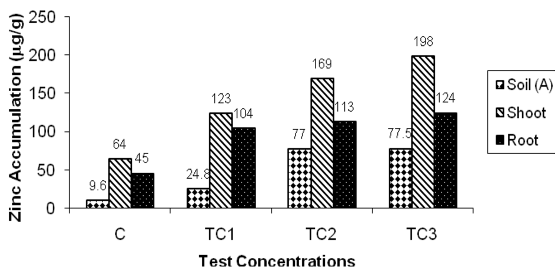

Figure 5. *Zinc accumulations of soil, shoot and root in different concentrations on II-scoring of EDTA treatment.*

A =Available metal concentrations in soil

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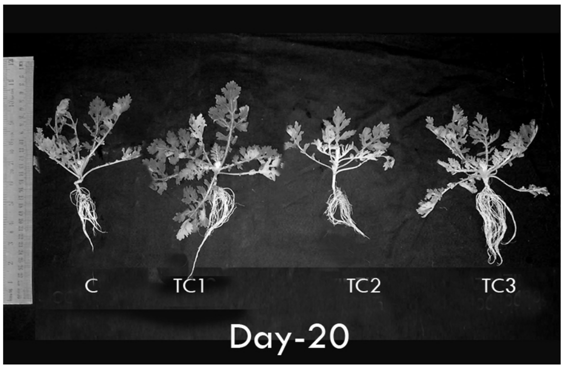

Figure 6. *Test species showing variation in different Concentrations on day-20*

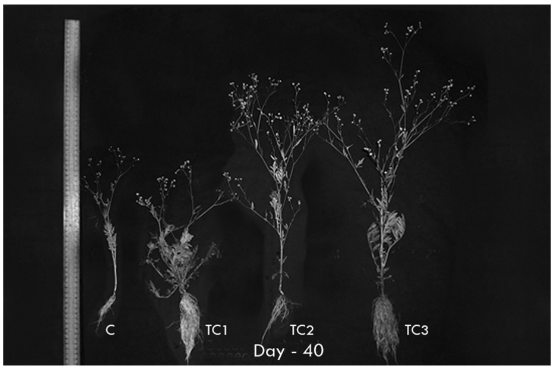

Figure 7. *Test species showing variation in different Concentrations on day-40*

Figure 8. *Test species showing variations in different concentrations on day - 60*

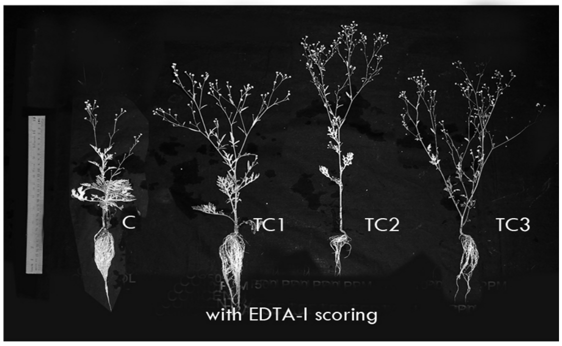

Figure 9. *Test species showing variations in different concentrations on I-scoring of EDTA treatment*

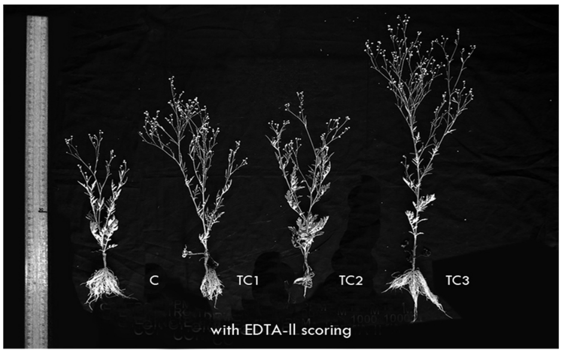

Figure 10. *Test species showing variations in different concentrations on II-scoring of EDTA treatment*

The pattern of addition of biomass is important for phytoextraction studies to estimate the best time to harvest the biomass, in this case harvesting after 60 days was best, as no mass was added beyond this period.

The shoot biomass (dry matter) showed no significant difference with time including EDTA treatment and between EDTA and without EDTA treatments showing no growth ($p>0.05$) excepting from 20 to 60 days where the biomass increased showing enough growth as determined by student's t-test ($p<0.05$). A significant increase was observed in TC1 when compared to control and decreased in TC2 when compared to TC1 on day

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20 ($p<0.05$). On day 40 also there was increase in shoot biomass of TC1, TC2 and TC3, and on day-60 TC1 and TC2 have increased when compared to control ($p<0.05$) by student's t-test. No significant difference was observed within test concentrations on day 60 and EDTA scorings and between test concentrations and control in EDTA scorings by student's t-test ($p>0.05$) as shown in *Figure 11* to *15*. The maximum amount of shoot biomass was obtained in TC2 on 60th day of without EDTA treatment.

A significant increase was reported in the root biomass (dry matter) from 20 to 60 days of without EDTA treatment ($p<0.05$) indicating enough growth and no significant difference was found to be reported in EDTA scorings as well as between EDTA and without EDTA treatments ($p>0.05$) by student's t-test as shown in *Figure 11.* to *15*. The increase in root biomass was significantly high ($p<0.005$) in all the test concentrations when compared to control on day 40 and on day 60 and a significant increase could be reported only in TC1 and TC2 when compared to control.

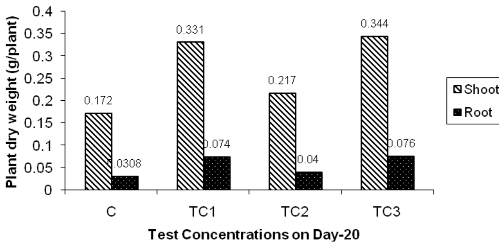

Figure 11. *Plant dry wt in different concentrations (g/plant) on day – 20*

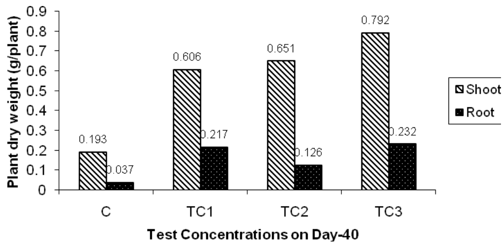

Figure 12. *Plant dry wt in different concentrations (g/plant) on day - 40*

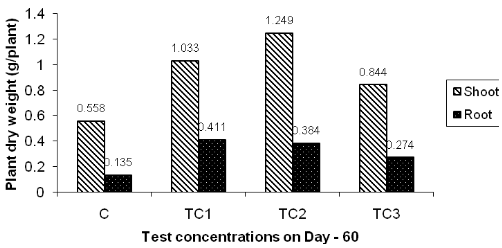

Figure 13. *Plant dry wt in different concentrations (g/plant) on day - 60*

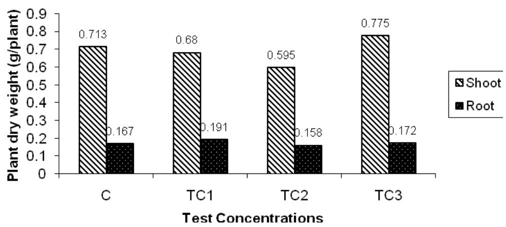

Figure 14. *Plant dry wt in different concentrations (g/plant) on I-scoring of EDTA treatment*

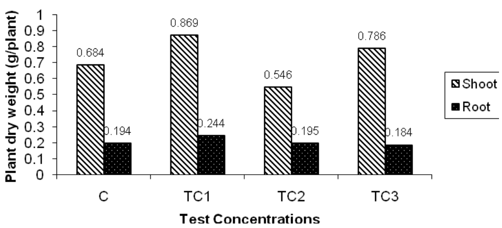

Figure 15. *Plant dry wt in different concentrations (g/plant) on II-scoring of EDTA treatment*

Maximum amount of root biomass was obtained in TC1 on day-60.Usually, when the concentration of a heavy metal in soils is not higher than the critical value that affects the growth of a plant, the aboveground biomass of a plant will not decrease significantly even though the concentration of the heavy metal in soils is very high. Once when it exceeds the critical value, would the growth of a plant be adversely inhibited, followed by occurrence of abnormal leaf colour, reduction in plant height and decrease in aboveground biomass (Sun et al., 2001; Wei et al., 2004; Zhou et al., 2004).

The results of the plant height and biomass as shown in Figure 6. to 15 suggest that P. hysterophorus L. is tolerant to Zn typical of a hyperaccumulator (Chaney et al., 1997).

Leaf pigments nutrients and in plants

Chl ‘a’ has shown a highly significant decrease in values with time from 20 to 40 days and 20 to 60 days of without EDTA treatment as determined by student’s t-test ($p<0.005$) and no difference was reported between 40 and 60 days as determined by student’s t-test ($p>0.05$). The chl ‘a’ content in EDTA treatments showed a highly significant reduction when compared to those without its treatment indicating its effect as determined by student’s t-test ($p<0.05$). A highly significant difference was observed between controls and the test concentrations, within test concentrations on 20, 40, 60 days of without EDTA treatment and the I-scoring of EDTA treatment as determined by student’s t-test ($p<0.005$). In the II-scoring of EDTA treatment highly significant difference was reported between controls and test concentrations ($p<0.005$) whereas no difference was observed in between within test concentrations ($p>0.05$) as shown in *Figure 16* and *17.* The lowest content of chl ‘a’ was obtained in TC2 on the II-scoring of EDTA treatment.The overall results have shown that metal uptake could effect the chl ‘a’ content as it reduced with increase in metal exposures and time.

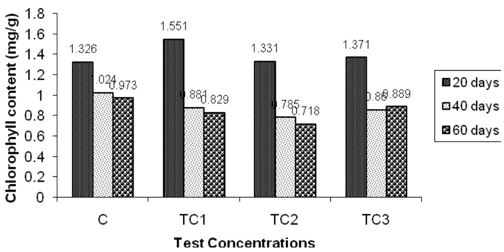

Figure 16. *Chlorophyll a content in different concentrations at different harvests (mg/g)*

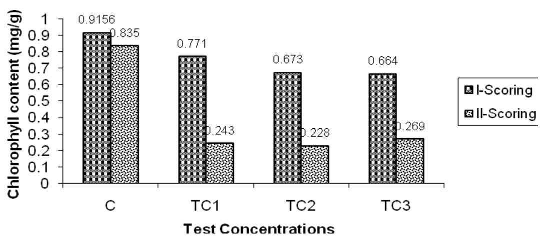

Figure 17. *Chlorophyll a content in different concentrations at different harvests (mg/g) with EDTA treatment*

Chl ‘b’ has shown highly significantly decrease with increase in time i.e from 20 to 40 and 20 to 60 days ($p<0.005$) and no significant difference between 40 and 60 days and the scorings of EDTA treatments ($p>0.05$) was observed by student’s t-test. A significant difference was observed between EDTA and without EDTA treatments as determined by student’s t-test ($p<0.05$). There was a highly significant difference between control and test concentrations on 40, 60 days and between the test concentrations of EDTA treatment as determined by student’s t-test ($p<0.005$) which is indicative of the metal effect as shown in *Figure 18* and *19.* The lowest content of chl ’b’ was obtained in TC2 on the II-scoring of EDTA treatment.

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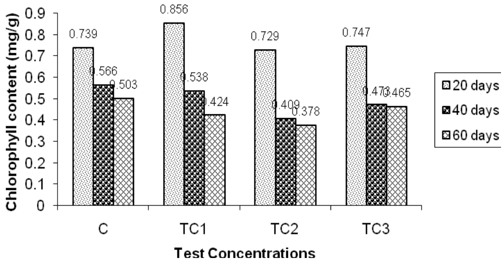

***Figure 18.** Chlorophyll b content in different concentrations at different harvests (mg/g)*

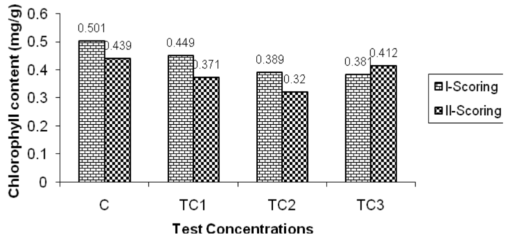

***Figure 19.** Chlorophyll b content in different concentrations at different harvests (mg/g) with EDTA treatment*

Carotenoids have shown a significant reduction in its values from 20 to 40 days and 20 to 60 days as determined by student's t-test ($p<0.05$). A significant difference in EDTA treatment was observed when compared to without its treatment as determined by student's t-test ($p<0.05$). There was no difference between 40 and 60 days as well as between EDTA scorings by student's t-test ($p>0.05$) as shown in *Figure 20* and *21*. A significant difference was observed between control and test concentrations as well as within the test concentrations on day 40 and 60 days including EDTA ($p<0.005$) as determined by student's t-test. The lowest content of carotenoids was obtained in TC2 on II-scoring of EDTA treatment.

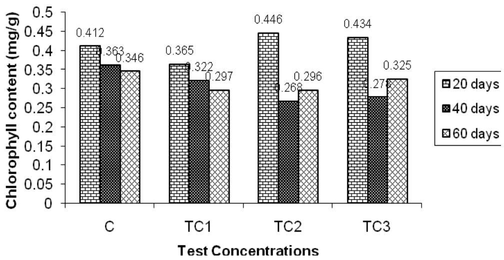

***Figure 20.** Carotenoids content in different concentrations at different harvests (mg/g)*

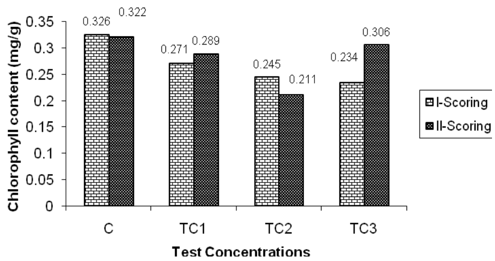

***Figure 21.** Carotenoids content in different concentrations at different harvests (mg/g) with EDTA treatment*

The yellowish colour of the leaves and the reduction in leaf pigments as shown in *Figure 16* to *21* are indication of Zn accumulation which has caused chlorophyll degradation (Prasad and Strzalka, 1999).

The shoot and root nitrates showed no significant difference within the metal exposures and the harvest days including EDTA treatment as determined by two way anova ($p>0.05$). A student's t-test was also conducted and found a highly significant increase in EDTA treatment of shoot when compared to without its treatment ($p<0.005$) of shoot. This could be due to the effect of EDTA as a chelating agent with respect to shoot.

The results of shoot and root phosphorus showed no significant difference within exposures and harvest days including EDTA treatment by two way anova ($p>0.05$). The shoot and root phosphorus showed no significant difference between individual harvest days ($p>0.05$) including EDTA treatment excepting for a significant decrease from 20 to 40 days in root phosphorus ($p<0.05$) as determined by student's t-test. Whereas a highly significant increase was observed in shoots and roots of EDTA treatment when compared to those without its treatment ($p<0.005$) showing the effect of the chelate (*Table 18, 19*).

Shoot and root potassium showed no significant difference within test concentrations ($p>0.05$) including EDTA treatment but a highly significant difference ($p<0.005$) was reported between harvest days of without EDTA treatment where no siginificant difference with EDTA treatment ($p>0.05$) was observed by two way anova (*Table 11, 12*). No significant difference between EDTA and without EDTA treatments and within the scorings of EDTA treatment of both shoot and root ($p>0.05$) was observed by student's t-test. A significant difference could be reported between all the harvest days in shoots ($p<0.05$) and a highly significant reduction from 40 to 60 days and 20 to 60 harvests ($p<0.005$) in roots by student's t-test (*Table 18, 19*). No particular trend of either shoot or root potassium was followed for metal effect.

Table 11. *Two-way Anova for potassium in shoot (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	0.50117	0.16706		
Harvest days	2	4.99338	2.49669	1.81	0.245
Error	6	0.55282	0.09214	27.10	0.001
Total	11	6.04737			

S = 0.3035 R-Sq = 90.86 % R-Sq (adj) = 83.24 %

Table 12. *Two-way Anova for potassium in root (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	0.04776	0.015921	0.72	0.576
Harvest days	2	0.81989	0.409943	18.51	0.003
Error	6	0.13286	0.022144		
Total	11	1.00051			

S = 0.1488 R-Sq = 86.72 % R-Sq (adj) = 75.65 %

The shoot and root sodium results have shown no significant difference within exposures ($p>0.05$) and a significant difference was observed between harvest days ($p<0.005$ for shoot & $p<0.05$ for root) of without EDTA treatment as determined by two-way anova (*Table 13, 14*). EDTA harvests showed significant difference within test concentrations in shoots and between harvests as well as within test concentrations in roots ($p<0.05$) by two way anova (*Table 15, 16*). Using student's t-test it was found that sodium in shoots have decreased from 40 to 60 days and 20 to 60 days ($p<0.05$ and $p<0.005$) and in roots from 20 to 60 days ($p<0.05$). There was no significant difference between EDTA and without EDTA treatment in shoots ($p>0.05$) whereas, an increase in values of EDTA treatment when compared to without its treatment were observed in roots ($p<0.05$) by student's t-test. No significant difference could be reported between the scorings of EDTA in either the shoot or root sodium levels ($p>0.05$) by student's t-test (*Table 18, 19*).

Table 13. *Two-way Anova for sodium in shoot (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	0.021150	0.0070500	3.48	0.090
Harvest days	2	0.080825	0.0404123	19.96	0.002
Error	6	0.012148	0.0020246		
Total	11	0.114122			

S = 0.04500 R-Sq = 89.36 % R-Sq (adj) = 80.49 %

Table 14. *Two-way Anova for sodium in root (without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	0.0049753	0.0016584	1.83	0.242
Harvest days	2	0.0169965	0.0084983	9.38	0.014
Error	6	0.0054385	0.0009064		
Total	11	0.0274103			

S = 0.03011 R-Sq = 80.16 % R-Sq (adj) = 63.62 %

Table 15. *Two-way Anova for sodium in shoot with EDTA treatment*

Source	DF	SS	MS	F	P
Test concs	3	0.028576	0.0095253	9.36	0.049
Harvest days	1	0.001800	0.0018000	1.77	0.276
Error	3	0.003052	0.0010173		
Total	7	0.033428			

S = 0.3190 R-Sq = 90.87 % R-Sq (adj) = 75.65 %

Table 16. *Two-way Anova for sodium in root with EDTA treatment*

Source	DF	SS	MS	F	P
Test concs	3	0.0158954	0.0152985	22.72	0.015
Harvest days	1	0.0128801	0.0128801	19.13	0.022
Error	3	0.0020204	0.0006735		
Total	71	0.0607959			

S = 0.2595 R-Sq = 96.68 % R-Sq (adj) = 92.25 %

Shoot and root calcium showed no significant difference within exposures and between harvests including EDTA treatments by two way anova ($p>0.05$) excepting between harvests of shoots of without EDTA treatment (*Table 17*). A highly significant increase in shoot of EDTA treatment when compared to without its treatment was observed by student's t-test ($p<0.005$) showing its effect as a chelate. From student's t-test both shoot and root showed no significant difference ($p>0.05$) between harvests of EDTA and without EDTA could be reported (*Table 18, 19*).

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Table 17. *Two-way Anova for calcium in shoo t(without EDTA treatment)*

Source	DF	SS	MS	F	P
Test concs	3	0.0280477	0.0093492	4.54	0.055
Harvest days	2	0.0283954	0.0141977	6.89	0.028
Error	6	0.0123585	0.0020597		
Total	11	0.0688016			

S = 0.4538 R-Sq = 82.04 % R-Sq (adj) = 67.07 %

Bioconcentration and translocation factors

The BCF of shoot has decreased with time (harvests) ,this means the power of Zn accumulation reduced with maturity of the plant. It is bound to get reduced in soils and the same trend was observed in shoot and root and also in EDTA treatment of shoot. Whereas the BCF of root has increased with time in EDTA treatment showing that the plant is extracting more metal and was in agreement with earlier studies (Ghosh and Singh, 2005). The BCF being greater >1 indicates that *P.hysterophorus* is efficient in bioconcentrating zinc into shoots and roots though the roots showed lower efficiency.

Plants must have the ability to translocate Zn from root to shoot or compartmentalize it in order to continue absorption of Zn from the substrate. Better translocation is advantageous for uptake (Ghosh and Singh, 2005). Translocation to shoot is one of the mechanisms of resistence to high Zn levels. The TF of without EDTA treatment has increased from the first (20 days) to the second (40 days) harvest and reduced on the third (60 days) harvest i.e decreased on complete maturity. The TF has decreased with increase in metal exposures in the I-scoring of EDTA and in the II-scoring of EDTA there was a slight decrease from TC1 to TC2 and then a slight increase from TC2 to TC3. The overall results showed that the TF>1 which is an indication of the test species efficiency in uptake and translocation of Zn and can be considered as a hyperaccumulator (Baker, 1981). The shoot to root ratio of contaminant concentrations in all confirmed hyperaccumulators are >1, whereas the ratios are invariably <1 in non-accumulators (Raskin and Ensley, 2000).

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Table 18. *Nutrients of Shoot and Root in different test concentration on different harvest days*

Parameter	Harvest Days											
	20 days				40 days				60 days			
	C	TC1	TC2	TC3	C	TC1	TC2	TC3	C	TC1	TC2	TC3
Nitrate Nitrogen (mg/100g) Shoot	0.17 ± .001	0.27 ± 0.001	0.27 ± 0.18	0.35 ± 0.18	0.26 ± 0.009	0.32 ± 0.006	0.32 ± 0.001	0.22 ± 0.001	0.23 ± 0.001	0.26 ± 0.006	0.19 ± 0.023	0.28 ± 0.006
Root	0.86 ± 0.001	0.42 ± 0.020	0.56 ± 0.001	0.27 ± 0.001	0.31 ± 0.008	0.43 ± 0.001	0.19 ± 0.270	0.34 ± 0.020	0.32 ± 0.02	0.29 ± 0.002	0.19 ± 0.024	0.23 ± 0.002
Phosphorus (mg/100g) Shoot	11.2 ± 0.8	10.82 ± 0.37	9.12 ± 0.12	9.56 ± 0.56	8.24 ± 0.001	9.64 ± 0.64	8.1 ± 0.18	8.16 ± 0.16	8 ± 0.001	9.2 ± 0.001	8.16 ± 0.001	10.32 ± 0.34
Root	8.62 ± 0.001	8.1 ± 0.40	6.42 ± 0.001	6.08 ± 0.176	4.82 ± 0.024	5.24 ± 0.024	5.48 ± 0.05	5.34 ± 0.48	5.4 ± 0.001	5.16 ± 0.76	6.64 ± 0.64	6.48 ± 0.48
Potassium (%) Shoot	3.93 ± 0.32	3.88 ± 0.33	3.01 ± 0.32	3.05 ± 0.42	2.76 ± 0.45	2.51 ± 0.5	2.41 ± 0.58	2.12 ± 0.50	1.89 ± 0.30	1.90 ± 0.30	1.72 ± 0.33	2.14 ± 0.40
Root	2.35 ± 0.30	2.22 ± 0.28	2.34 ± 0.25	2.42 ± 0.35	2.38 ± 0.44	2.12 ± 0.7	1.95 ± 0.58	1.92 ± 0.62	1.70 ± 0.72	1.69 ± 0.75	1.82 ± 0.68	1.59 ± 0.70
Sodium (%) Shoot	0.43 ± 0.20	0.40 ± 0.40	0.38 ± 0.40	0.45 ± 0.26	0.52 ± 0.20	0.46 ± 0.23	0.35 ± 0.27	0.34 ± 0.22	0.30 ± 0.36	0.25 ± 0.22	0.18 ± 0.19	0.23 ± 0.23
Root	0.24 ± 0.5	0.23 ± 0.52	0.28 ± 0.48	0.17 ± 0.54	0.21 ± 0.44	0.16 ± 0.44	0.14 ± 0.48	0.14 ± 0.55	0.14 ± 0.59	0.12 ± 0.60	0.16 ± 0.62	0.13 ± 0.61
Calcium (%) Shoot	0.79 ± 0.70	0.82 ± 0.62	0.73 ± 0.65	0.76 ± 0.73	0.92 ± 0.62	0.98 ± 0.55	0.9 ± 0.62	0.76 ± 0.58	0.88 ± 0.72	0.83 ± 0.75	0.77 ± 0.68	0.75 ± 0.65
Root	0.48 ± 0.40	0.19 ± 0.35	0.34 ± 0.25	0.26 ± 0.32	0.39 ± 0.28	0.22 ± 0.32	0.24 ± 0.31	0.28 ± 0.25	0.29 ± 0.51	0.24 ± 0.60	0.28 ± 0.45	0.30 ± 0.40

Results are means ± SE (n = 5)

Table 19. *Nutrients of Shoot and Root in different test concentration on different harvest days*

Parameter		With EDTA Treatment							
		EDTA – I Scoring				EDTA – II Scoring			
	C	TC1	TC2	TC3	C	TC1	TC2	TC3
Nitrate Nitrogen (mg/100g) Shoot Root	0.40 ± 0.006 0.26 ± 0.006	0.42 ± 0.012 0.39 ± 0.002	0.27 ± 0.14 0.30 ± 0.013	0.31 ± 0.001 0.30 ± 0.006	0.30 ± 0.006 0.70 ± 0.14	0.46 ± 0.02 0.32 ± 0.006	0.5 ± 0.001 0.37 ± 0.001	0.5 ± 0.001 0.37 ± 0.012
Phosphorus (mg/100g) Shoot Root	11.24 ± 0.24 5.9 ± 0.4	13.16 ± 0.16 8 ± 0.001	16.74 ± 0.24 9.79 ± 0.001	14.74 ± 0.24 7.9 ± 0.001	11.48 ± 0.48 7.16 ± 0.76	13.8 ± 0.001 9.16 ± 0.16	18.16 ± 0.16 9.64 ± 0.64	20 ± 0.001 11.2 ± 0.001
Potassium (%) Shoot Root	2.30 ± 0.4 1.91 ± 0.22	2.44 ± 0.48 2.03 ± 0.27	2.53 ± 0.6 2.08 ± 0.3	2.58 ± 0.52 1.97 ± 0.3	1.20 ± 0.54 2.02 ± 0.38	2.15 ± 0.61 2.04 ± 0.3	2.44 ± 0.45 2.4 ± 0.4	2.7 ± 0.53 2.16 ± 0.43
Sodium (%) Shoot Root	0.34 ± 0.18 0.12 ± 0.60	0.46 ± 0.2 0.26 ± 0.55	0.46 ± 0.22 0.28 ± 0.49	0.36 ± 0.28 0.25 ± 0.51	0.26 ± 0.32 0.15 ± 0.47	0.43 ± 0.38 0.37 ± 0.45	0.44 ± 0.4 0.36 ± 0.43	0.38 ± 0.35 0.36 ± 0.39
Calcium (%) Shoot Root	1.04 ± 0.69 0.24 ± 0.51	1.00 ± 0.60 0.22 ± 0.35	0.96 ± 0.52 0.22 ± 0.40	0.82 ± 0.48 0.19 ± 0.45	0.98 ± 0.42 0.23 ± 0.5	0.86 ± 0.55 0.25 ± 0.58	0.96 ± 0.48 0.31 ± 0.70	1.03 ± 0.55 0.31 ± 0.75

Results are means ± SE (n = 5)

Conclusions

A significant increase in shoot and root concentrations of the heavy metal Zn with increase in test concentrations in the medium shows a positive response of the *P.hyterophorus* for its accumulation (Wei, 2004). The plant tolerance for Zn was evident in form of increase in plant height and total biomass of the plant. The plants in all the concentrations grew till maturity. An increase in plant height and plant biomass with respect to time was positive. There was an increase in uptake with increase in test concentrations which is in agreement with earlier studies (Yu-Hong and Youn-Guan, 2005). The highest uptake was observed in TC3 on day 20 in both shoot and root (252.96µg/g & 164.22µg/g). In EDTA treatment also the highest uptake was observed in TC3 for both shoot and root on both the scorings (177µg/g, 131.04µg/g on I-scoring & 198µg/g & 124µg/g on II-scoring).

The BCF of shoot has decreased with time indicating that Zn accumulation reduced with maturity of the plant. Whereas the BCF of root has increased with time in EDTA treatment showing that the plant is extracting more metal and metal enhancement due to EDTA treatment. The increase in BCF with time was in agreement with literature (Ghosh and Singh, 2005). The translocation property described that the content of heavy metals accumulated in shoot (including stems and leaves) of a plant should be higher than those in its roots, i.e TF>1. On the basis of our work, endurance property and enrichment factor property should be considered as judging standards of hyperaccumulators too (Ma et al., 2001; Wei and Zhou, 2004; Zhou and Song, 2004).

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The enduring characteristic means that hyperaccumulators should have strong endurance to heavy metals contamination. For plants tested under experimental conditions, the aboveground biomass (the sum of dry stems, leaves and inflorescence) of hyperaccumulators should not significantly be decreased compared with the control when they are growing in soils contaminated by heavy metals seriously. At least the aboveground biomasses of plants should not be significantly reduced when the levels in soils are high enough to make the contents of heavy metals absorbed by plants reaching the critical concentration standards what hyperaccumulators should accumulate. Enrichment factor characteristic should be > 1, and at least the EF should be higher than 1 when the content of a heavy metal in soil is roughly equal to the minimum of a hyperaccumulator. According to these standards, *P. hysterophorus* can be validated as a hyperaccumulator, because the plant displayed strong tolerance to and translocation ability of Zn and EF in shoots was > 1. The concentration of Zn accumulated by the test species was increased significantly after 0.1g/kg of EDTA was added to the medium. This was in agreement with the findings in literature (Turgut et al., 2004). Phosphorus is one of the chemical constituent of the soil which interferes with zinc uptake by plants (Lindsay, 1972). The uptake will be high in low phosphorus soils. A similar trend was observed in the present study. Shoot and root potassium have decreased with decrease in metal uptake and increase in time including EDTA treatment. The sodium levels in shoot and root of without EDTA treatment decreased with drecrease in metal uptake and increase in time, root sodium of EDTA treatment decreased with increase in metal uptake and increase time. The plant potassium and sodium (without EDTA treatment) were directly proportional to the metal uptake. Shoot nitrates and calcium, root sodium , shoot and root phosphorus have shown significant increase with EDTA treatment showing the effect of EDTA in enhancement of nutrient uptake. Shoot calcium without EDTA treatment increased with growth and slightly reduced at complete maturity. The overall results have shown that the metal uptake could effect the chl a, b and carotenoids content as it reduced with increase in metal exposures and time and especially in edta treatment. This could possibly be due to the accumulation of the Zn. It has been reported that heavy metals are accumulated in the plants maximally and cause chlorophyll degradation due to heavy metal stress (Prasad and Strzalka, 1999).The accumulator species such as *Brassica species* and other accumulator crop species required special care in field applications. Whereas weeds such as *P.hysterophorus* in the present study required no care to grow, this is a practical problem that will require care in filed applications. The BCFs of shoots and roots and TFs being > 1 showing its validity for hyperaccumualtion of the metal can be a promising species for phytoextraction of heavy metals and remediation of metal contaminated soils.

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COMPOSITION OF ZOOPLANKTON ASSEMBLAGES ALONG THE ZAGYVA RIVER

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Abstract. The species composition, longitudinal distribution and seasonal dynamics of zooplankton were studied in the Zagyva River, Hungary. A total of 108 taxa was recorded from which 61 were new for the river. Rotatoria was the most abundant group, microcrustaceans were less important, only nauplii and copepodites were represented in similar individual numbers. Frequent species included *Anuraeopsis fissa*, *Pompholyx* spp., *Keratella cochlearis, Brachionus angularis, Bdelloida* sp., *Bosmina longirostris*. Dominance of cosmopolitan species was observed both in the river and its reservoir, and species characteristic of eutrophic waters were of major importance in the latter. There was a downstream decrease in zooplankton densities, which was explained by modified conditions. The relatively large number of individuals in autumn months, and the characteristic large number of individuals in the upper section contrasted general findings of potamoplankton dynamics. On the basis of the species abundance matrix, three river sections can be distinguished (upper, middle, lower section). Due to waste water discharges - received from the Tarján Stream - we found extremely high number of individuals and the lowest diversity at the sampling site Nagybátony (148 rkm).
Keywords: *Rotatoria, Copepoda, Cladocera, reservoir, spatial distribution.*

Introduction

Despite rapidly growing insights into zooplankton spatio-temporal dynamics, investigations on riverine zooplankton have not yet been given much effort in comparison with lentic systems, mainly due to historical constraints and the flowing character. This is particularly true for low order streams and rivers. With this end in view, however, the distribution of zooplankton along rivers has received relatively considerable attention for a long time. The downstream increase in zooplankton density is well-documented in some rivers (Saunders and Lewis, 1989; Gulyás, 1995a, b; Kim and Joo, 2000; Maria-Heleni *et al*., 2000; Zimmermann-Timm *et al*., 2007), however, some authors have found just the opposite (Basu and Pick, 1996; Burger *et al*., 2002). Generally microcrustaceans play a secondary role in rivers as compared to rotifers, which is explained by the shorter generation time of Rotatoria (Akopian *et al*., 2002; Lair, 2006) or rotifers are supposed to benefit indirectly from river turbidity because their crustacean competitors and predators are relatively more susceptible to suspended sediments (Thorp and Mantovani, 2005). Microcrustacean communities are often dominated by small-bodied cladocerans (e.g. bosmininds) and juvenile forms (copepodite, nauplii) of copepods (Pourriot *et al.*, 1997; Kobayashi *et al.*, 1998; Reckendorfer *et al.*, 1999; Kim and Joo, 2000).

The Zagyva has been poorly investigated regarding plankton communities. Zooplankton studies (Megyeri, 1955, 1971; Gulyás *et al.*, 1995) have not covered the whole river stretch, nor have they analysed spatio-temporal dynamics, moreover those investigations have not focused on the Zagyva alone.

To fill this gap, we aimed to determine the species composition, longitudinal distribution and seasonal dynamics of zooplankton in the Zagyva River within a two-year study. The Environmental Authority does not measure chemical and hydrological features of the river regularly, therefore the authors cannot address the question how the flow regime governs plankton dynamics in the Zagyva. It follows that we had to confine our discussion to much more descriptive levels.

Materials and methods

Study site

The Zagyva is the most remarkable tributary of the River Tisza on its middle section. Its catchment area by the estuary covers 5,677 km^2 and it flows into the River Tisza at rkm 334. The fall of water ranges from 16.7 m km^{-1} at the upper, mountainous section, to 1.7-0.64 m km^{-1} at the middle section and 0.12 m km^{-1} at the lower section. The water discharge is primarily determined by rainfall, reservoirs and mines play secondary roles. The 700 m long Maconka reservoir lies along the upper section of Zagyva and has an average depth of 2-4 m. It is characterized with a prominently rich and diverse fish stock, the shoreline vegetation is comprised of reed, sedge and seaweed.

When selecting the sampling sites we considered (1) preference towards sites downstream of the inflows of the most important tributaries; (2) representative sampling procedure, thus samples were taken from the streamline and (3) conformity to the sampling program of the Environmental Authority. Sampling sites are marked on *Fig. 1*: Nemti (Ni); Nagybátony (Nb); Petőfibánya (Pb); Gesztenyefasor (Gf); Jászfényszaru (Jf); Szentlőrinckáta (Szl); Jászberény (Jb); Jásztelek (Jt); Újszász (Új). In addition, we collected samples in the Maconka reservoir as well.

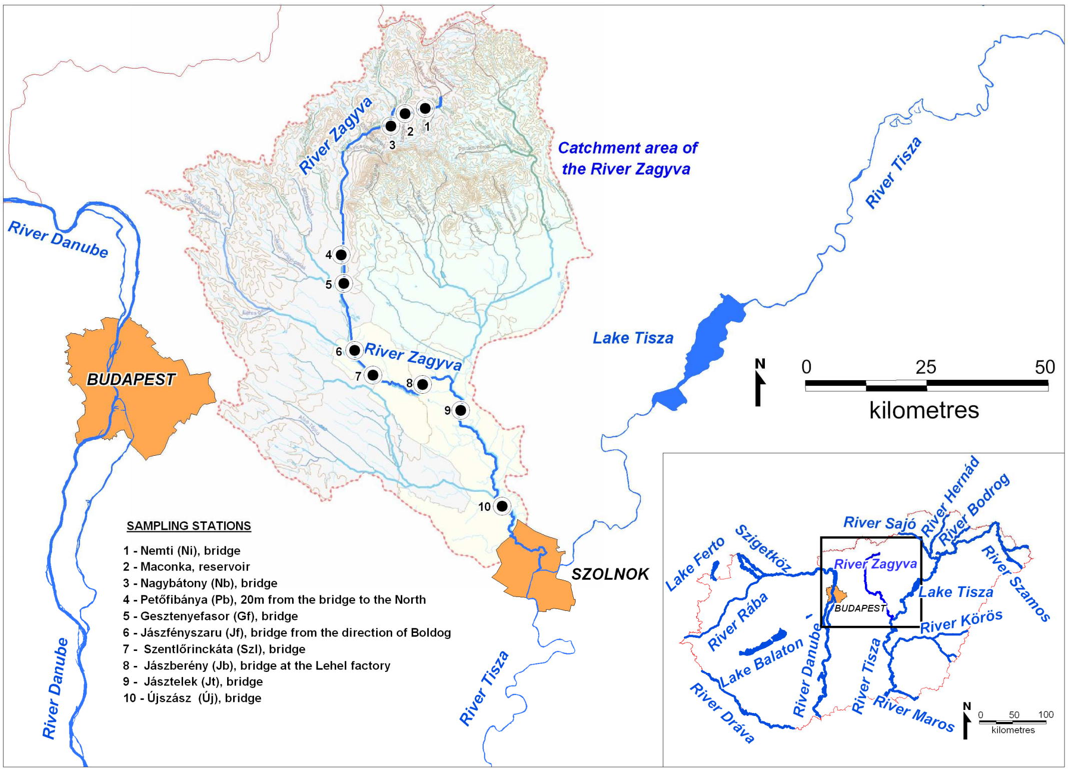

***Figure 1.** The study area with the sampling sites*

Sampling and data processing

Samples were collected at nine sampling sites along the river and in the Maconka reservoir at biweekly to monthly intervals between March and October 2006, and between June and October 2007, respectively. Samples were taken from the streamline, mostly from bridges. For the purpose of study 50 litres of water (in case of the Maconka reservoir 10 litres) was filtered through a plankton net of 50µm mesh size. The collected material was preserved *in situ* in 4% formaldehyde solution. Rotifers and microcrustaceans were counted in 5 ml subsamples in special counting chambers (70 × 52 × 5 mm) after homogenization. For the taxonomic determination of the animals identification keys by Bancsi (1986, 1988) and Gulyás and Forró (1999, 2001) were used.

In order to explore the spatial patterns of zooplankton assemblages, cluster analysis and non-metric multidimensional scaling (NMDS) using the Euclidean distance were performed (with standardized data). All data analyses were performed using the PAST software (Hammer *et al*., 2001).

Results

During the study period in the whole section of the Zagyva River – including the Maconka reservoir as well – 108 taxa were identified from which 61 were new for the river (*Table 1*). *Brachionus* spp., *Cephalodella* spp., and *Lecane* spp. were of particular importance. Taxa of *Bdelloida*, *Colurella* and *Keratella* groups almost always occured.

Crustacean plankton was dominated by nauplii and copepodites, while the cladoceran *Bosmina longirostris* was the only abundant microcrustacean.

Table 1. *The list of taxa identified during the investigation. The ones marked with "n" are new species for the river. Species found in the Maconka reservoir are marked with "Mac" and those found in the inflow of the reservoir are designated with "Inlet"*

Rotatoria	*F. terminalis*[n]	*P. sulcata*
Anuraeopsis fissa[n]	*Itura aurita*[n]	*Proales sp.*[n]
Asplanchna priodonta	*Keratella cochlearis*	*Synchaeta oblonga*
Bdelloida sp.[n]	*K. quadrata*	*S. pectinata*
Brachionus angularis	*K. testudo*[n]	*Synchaeta sp.*
B. bennini[Mac]	*K. valga*	*Testudinella mucronata*[nInlet]
B. budapestinensis[n]	*Lecane arcuata*[n]	*T. patina*
B. calyciflorus	*L. bulla*	*T. truncata*[n]
B. diversicornis[n]	*L. clara*[n]	*Trichocerca agnata*[n]
B. falcatus[n]	*L.closterocerca*	*T. rattus*[Mac]
B. leydigi	*L. cornuta*[nMac]	*T. taurocephala*[n]
B. nilsoni[nMac]	*L. elsa*[n]	*T. tenuior*[n]
B. quadridentatus	*L. flexilis*[n]	
B. urceolaris	*L. hamata*[n]	*Trichotria pocillum*
Cephalodella catellina	*L. inermis*[n]	*T. tetractis*[nInlet]
		T. truncata[n]
C. gibba	*L. luna*[n]	*Trichotria sp.*
C. megalocephala[n]	*L. lunaris*	**Cladocera**
C. misgurnus	*L. quadridentata*	*Alona rectangula*
C. obvia[n]	*L. scutata*[n]	*Alonella sp.*[nInlet]
		Bosmina longirostris
C. stenroosi[n]	*L. subtilis*[n]	*Ceriodaphnia laticaudata*[n]
C. sterea[Mac]	*L. tenuiseta*[n]	*C. recticulata*
	Lecane sp.	
C. ventripes	*Lepadella ovalis*	*Chydorus sphaericus*
Cephalodella sp.		
Colurella adriatica	*L. patella*	*Daphnia cucullata*[n]
C. colurus	*Mytilina mucronata*[n]	*Daphnia longispina*[nMac]
C. obtusa[nMac]	*Notholca acuminata*	*Disparalona rostrata*[n]
Conochilus dossuarius[n]	*N. squamula*	*Leptodora kindtii*[Mac]
Dicranophorus forcipatus[Mac]	*Notommata diasema*[n]	*Leydigia leydigi*[n]
D. grandis[nMac]	*N. dentata*[n]	*Macrothrix laticornis*
D. uncinatus[n]	*N. pachyura*[n]	*Moina micrura*[n]

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Dipleuchlanis propatula^{n}	*Paradicranophorus hudsoni*^{n}	*Pleuroxus aduncus*^{n}
Encentrum incisum^{n}	*Pleurotrocha petromyzon*^{n}	*Scapholeberis mucronata*^{nMac}
E. saudersiae	*Polyarthra dolichoptera*	*Simocephalus vetulus*^{n}
Euchlanis dilatata		**Copepoda**
Filinia cornuta^{n}	*P. euryptera*^{n}	*Acanthocyclops robustus*^{nMac}
F. longiseta	*P. major*^{n}	*Macrocyclops fuscus*^{nMac}
F. opoliensis^{n}	*Polyarthra sp.* *Pompholyx complanata*^{n}	*Thermocyclops crassus*^{nMac}

In the first year, by taking samples in spring between 15 March and 14 May, 38 Rotatoria, 9 Cladocera and 2 Copepoda species were found. According to the samples taken along the whole river section, a significant difference in the quantitative distribution of the zooplankton assemblage was observed. Our results suggested, that in the samples taken in spring the cladoceran stock was considerable only in the area of Jásztelek and Újszász. Species number and the densities of cladocerans increased during the fall after flood. At the examined sampling sites we found several species of Rotatoria (*Notholca acuminata*, *N. squamula*, *Keratella quadrata* and *Synchaeta oblonga*), that were the characteristics of colder, spring periods and either disappeared completely from the samples taken in summer or their individual number decreased considerably.

Between 28 May and 27 October 2006, 38 Rotatoria, 6 Cladocera and 1 Copepoda species were identified. Owing to the different ecological conditions in summer – in accordance with the seasonal changes – in the changed composition of zooplankton species exclusively characteristic of the summer survey were found e.g. *Anuraeopsis fissa, Encentum saundersiae, Lecane bulla, Pompholyx complanata, P. sulcata, Trichocerca agnata*. While examining the samples taken in this period, we observed that number of species of cladocerans decreased in downstream direction. *Moina micrura* and *Daphnia cucullata* were characteristic for the upper section, while *Ceriodaphnia laticaudata, Ceriodaphnia reticulata* and *Chydorus sphaericus* appeared in the lower section. Nauplii and copepodites of Copepoda were found at each sampling sites in each season. Among adult individuals only *Acanthocyclops robustus* at Petőfibánya and *Thermocyclops crassus* at Nemti were found.

By studying the samples taken between 9 June and 28 October 2007, we identified 61 Rotatoria and 9 Cladocera species. In comparison with the previous year some new species appeared in 2007 such as *Brachionus falcatus, Cephalodella strenroosi, Lecane quadridentata, Notommata pachyura, Pleurotrocha petromyzon, Trichocerca taurocephala, T. tenuior* and *Macrothrix laticornis*. There was no considerable difference in the common species between the summer and autumn samples but we found three more individuals of cladoceran species compared with the previous year. The number of the existing cladoceran species seemed to be doubled in the studied months. The dominance of Rotatoria was typical except the outflow and the inflow of the reservoir. So, in accordance with the observed quantitative changes we found a more remarkable Copepoda and Cladocera fauna only at the outflow and inflow of the reservoir. Only at these sites – influenced by the reservoir – egg-carrying female individuals in considerable number were found. As regards the registered number of all

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species, the upper section of the river was more various than the lower one, but even so it did not come to the observed number of species in 2006.

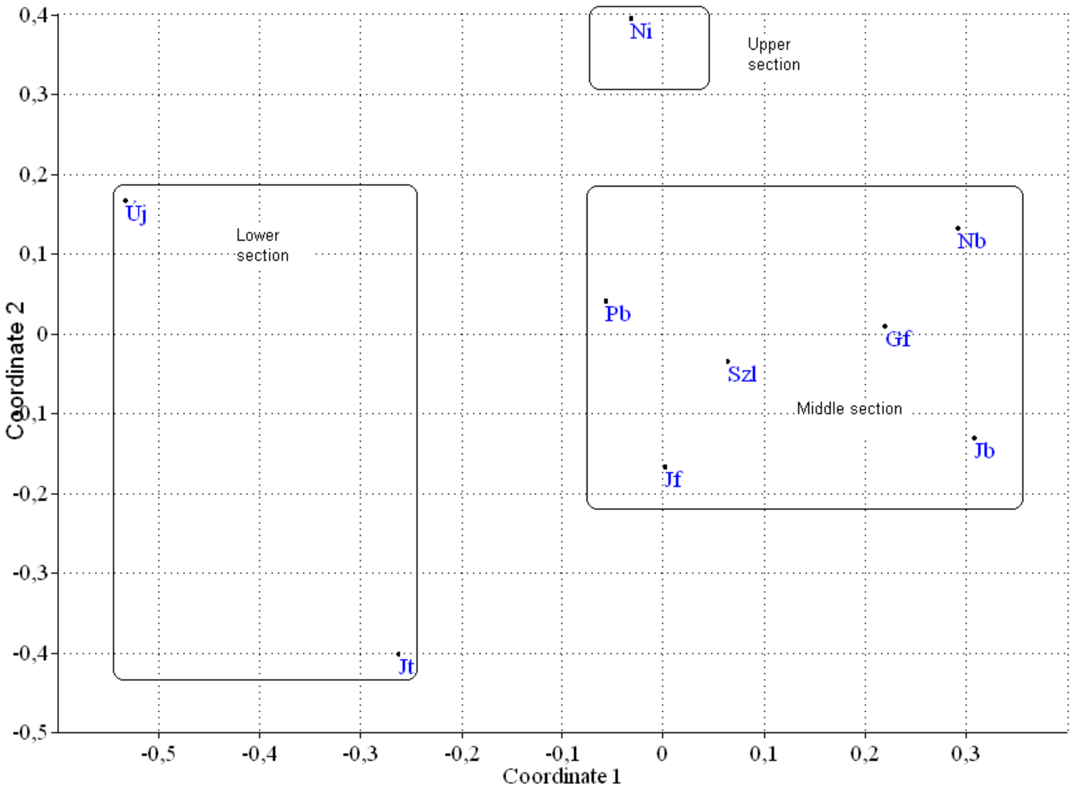

Figure 2. *The NMDS plot of the sampling sites. For the abbreviations see Materials and Methods*

The NMDS ordination pointed out that the sampling stations form three groups, which can be the equivalents of the upper, middle and lower sections roughly (*Fig. 2*). The results of the cluster analysis supported these findings (figures not given in the text). In the upper section the Maconka reservoir determines the water quality. The reservoir breaks the ”river continuum” with its lentic conditions. So the second section – between the reservoir and Jászberény – is mainly influenced by the reservoir. Jászberény is one of the most important towns in the area with significant industry. At this section the Zagyva is affected by both the waste water loading from the town and by the Tarna that flows into the Zagyva at Jászberény. Therefore we suggest that the third section (between Jászberény and the mouth of the Zagyva) is mostly affected by the above mentioned features.

Beside the NMDS analysis the different sampling sites can be also grouped depending on what kind of species had been taken from the given sampling site. Although the representatives of the most various plankton assemblages can be found on the whole river section there are clear differences in the consistences of the samples collected from the different sections (lower, middle, upper) of the river. Taking samples in 2006 *Cephalodella megalocephala*, (Ni) *Cephalodella obvia, Lepadella ovalis* (Pb), *Cephalodella misgurnus* (Ni, Pb) and *Lecane inermis* (Nb) were observed on the upper section of the river. Interestingly both *Filinia cornuta* and *Itura aurita* could be found

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only in Jászfényszaru (Jf). While *F. opoliensis* was collected from Jf and Pb in 2006, next year this species could be also identified in other sampling sites at the lower section of the river, such as Jt and Új. However *Encentrum incisum* could be found only in the sample of Jásztelek. Species *Brachionus bennini, Dicranophorus forcipatus, Dicranophorus grandis, Trichocerca rattus, Simocephalus vetulus, Macrocyclops fuscus* were collected only from the Maconka reservoir. These results might give us a hint about the special fauna of the reservoir.

The following two figures (*Fig. 3, 4*) show the diversity conditions of two sampling years.

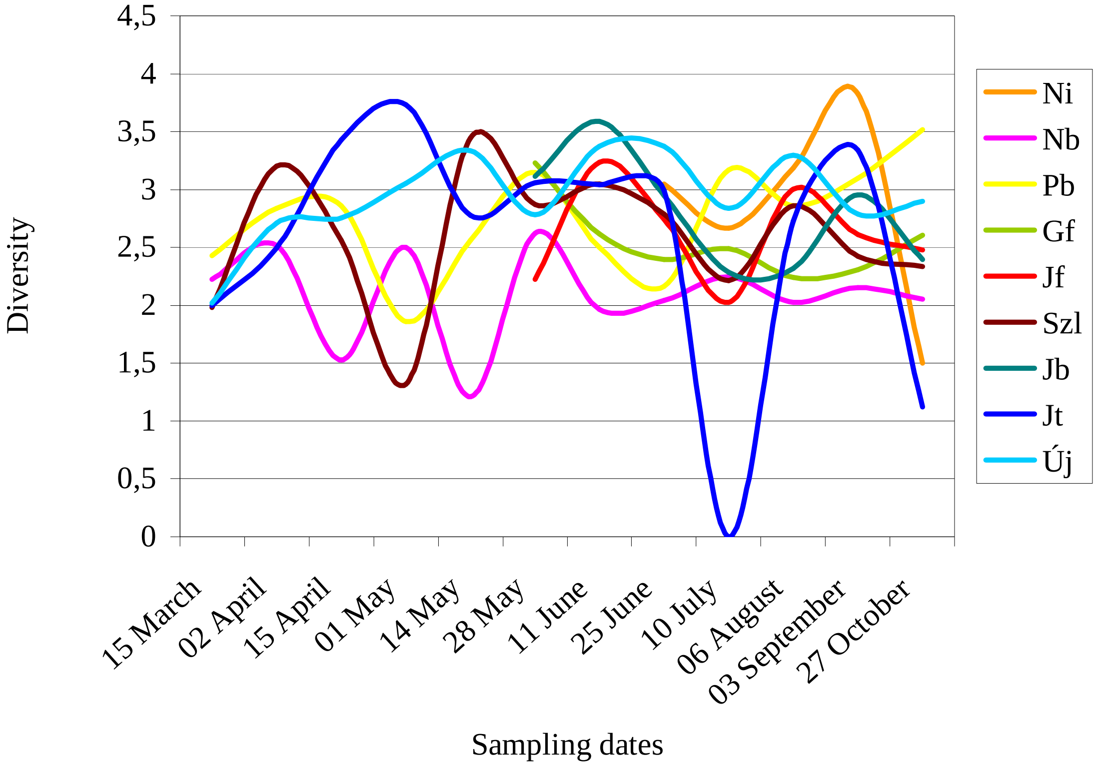

***Figure 3**. Diversity conditions (2006)*

In 2006, the average diversity reached 2,62. *Fig. 3* shows that the diversity varied significantly from March to June. In April the diversity increased at Nagybátony (Nb) but at the same time it decreased at the very next sampling site, Petőfibánya (Pb). This fact can be explained by local conditions. The result of the samples taken on 10 of July can be regarded to be the most interesting point of *Fig. 3*. This point shows that diversities decreased at all sampling sites with the exception of Nagybátony, Gesztenyefasor and Petőfibánya. Interestingly the most significant decrease in both diversity and the number of individuals was recorded by the sample taken at Jásztelek. This fact may have been caused by the waste water of Jászberény – it is 1,656,077 m^3 $year^{-1}$ according to the data kindly provided by the Environmental Authority – flowing into the Zagyva above Jásztelek resulting in a drastical decrease of the species number. This observation can be also explained by the poor quality of the water carried by Tarna

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Stream. Although this above mentioned decline in diversity is the most remarkable it is worth mentioning that similar declines can also be observed at some other sampling sites.

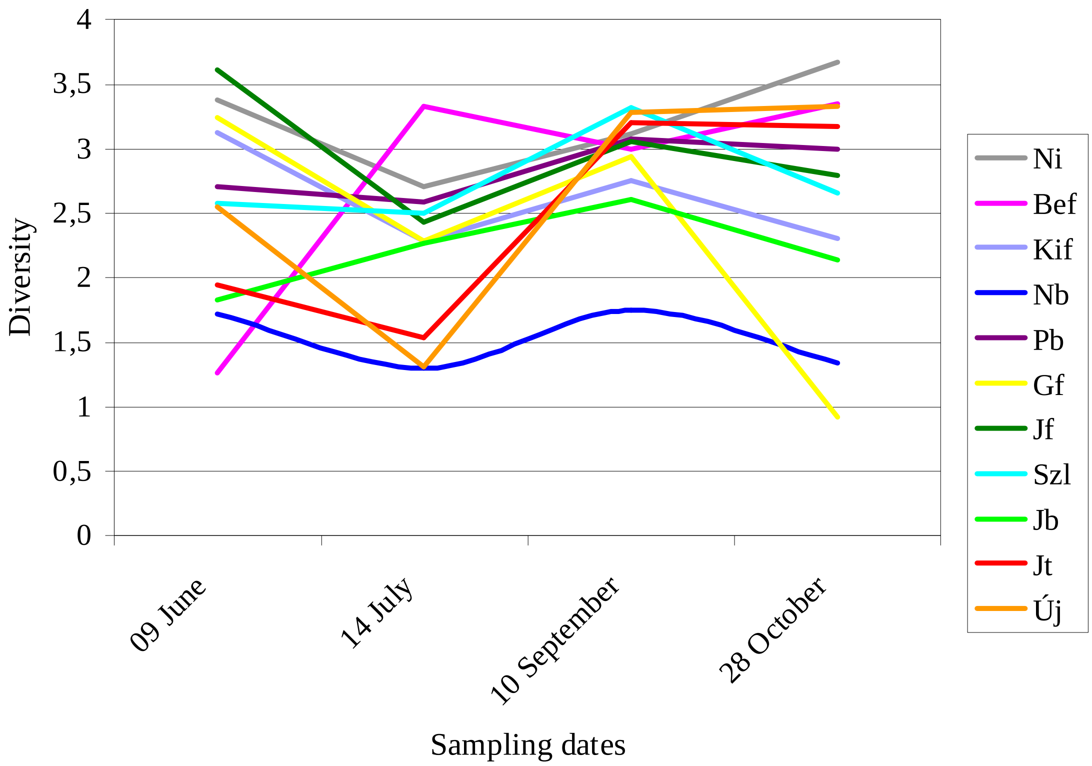

Figure 4. *Diversity conditions (2007)*

In the year of 2007, the diversity proved to be more balanced as the average diversity was 2,53 compared with the 2,62 average of the previous year observed in the same period (*Fig. 4*). The lowest diversity was measured in July but it increased gradually till September. Although the diversity of sampling site marked with Gf seems relatively high and constant till September, it shows a huge drop at the end of October.

In the year of 2006, densities recorded prior to May were extremely low (*Fig. 5*). We observed remarkably high densities in May at Nagybátony (Nb), but at the other sampling sites densities also increased significantly compared with the previous rate. However, we observed a decrease from May till August. From this time densities rose again considerably and at Nagybátony (Nb) and Gesztenyefasor (Gf) extremely high values were recorded in August.

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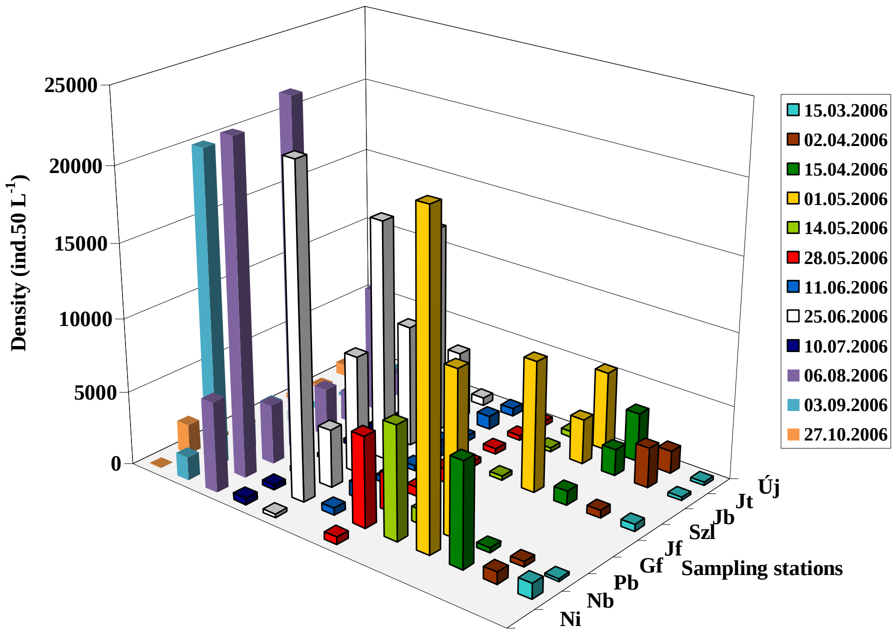

Figure 5. *Spatio-temporal dynamics of zooplankton assemblages in the Zagyva River (2006)*

In the year of 2007, densities were much more balanced contrasted with the year of 2006 (*Fig. 6*). In the year of 2007, the sampling date was less determining than the sampling site when densities are concerned, i.e. the spatial dimension explained much of the variability in densities compared to that of the temporal dimension.

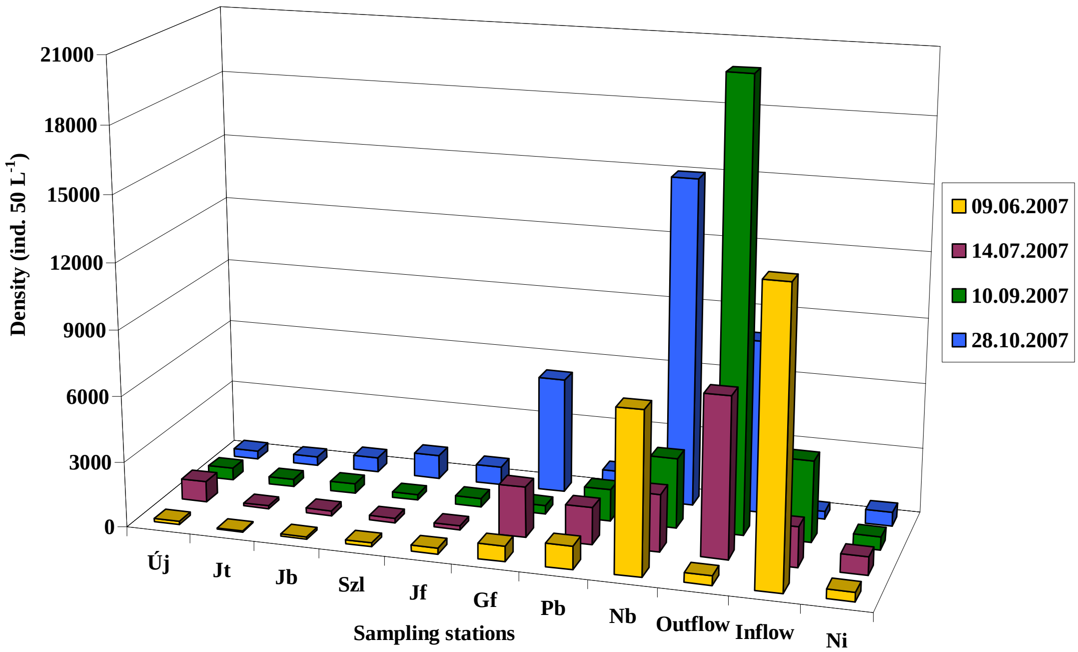

Figure 6. *Spatio-temporal dynamics of zooplankton assemblages in the Zagyva River (2007)- The terms "Inflow" and "Outflow" refer to the Maconka reservoir*

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In *Fig. 6* we can observe that densities in 2007 were higher at the upper river sections while appeared to be rather low at the lower river sections.

Discussion

Generally, findings of potamoplankton studies suggest a downstream increase in densities (Saunders and Lewis, 1989; Gulyás, 1995a,b; Kim and Joo, 2000; Maria-Heleni *et al*., 2000; Zimmermann-Timm *et al*., 2007) and in summer months a larger number of individuals is typical. But the Zagyva indicated distinct features in several aspects because in the majority of samples, we found that the number of species showed a decreasing tendency from the second sampling site. The above mentioned feature applies to the density as well *(Fig. 5, Fig. 6)*. At Nagybátony, the number of individuals has always been very high except 11 June and 10 July (data not shown). One would argue that the Maconka reservoir, which is situated upstream of Nagybátony, may account for the high number of individuals found at Nagybátony. The abundant nutrient content of waste water carried by Tarján Stream can be a more likely explanation. It carries 11,292,000 m^3 of waste water to the Zagyva every year. The river receives 4,200,000 m^3 waste water of municipal and 7,100,000 m^3 waste water of industrial origin. The total waste water loading exceeds the average water supply of the water body by more than 50% (data provided by the Environmental Authority). We suggest that the high number of individuals and the dominant species – that are typical of eutrophic waters – such as *Keratella, Polyarthra, Filinia* are the consequences of waste water loading, because loading the waste water from Győr into the Danube resulted in similar findings (Gulyás, 1995a).

While at Nagybátony the number of individuals was relatively high it was quite low at Újszász and Jásztelek except flood periods. These facts refer to a modified state. In the two-thirds of cases the samples taken at Jászberény showed a larger number of individuals than those taken at Jásztelek or Újszász. Either the waste water from Jászberény loading into the Zagyva just downstream of Jásztelek – it is 1,656,077 m^3 $year^{-1}$ according to the data given by the Environmental Authority – or the water of poor quality flowing from the Tarna into the Zagyva River may have caused the decreased number of individuals at Jásztelek and Újszász. These ideas can be a good explanation as Gulyás *et al*. (1995) also reported a relatively poor fauna of the Zagyva which was attributed to the effects of industrial and communal waste waters. Megyeri (1971) recorded 25 species of Rotatoria, 11 of Cladocera and 2 of Copepoda in the 50s – 60s.

The year of 2007 brought a change because samples were collected at the inflow and outflow of the reservoir regularly. The results we got at the outflow helped us to decide whether the low number of species, and the low value of diversity originate from the previously presented reasons or the difference is due to the effect of the Maconka reservoir. In quantitative respects, in the upper section of the river the number of the individuals was much higher than in the lower section. Generally we suggest the same fact as for the year of 2006, but there was no significant difference in those figures. So based on the quantitative changes of zooplankton the modified state that we experienced in 2006 also existed in 2007. It is important to emphasize that downstream of Nagybátony the mean of the total number of all individuals decreased dramatically. First it fell back up to the one fifth, and one third then from Jászfényszaru up to less than the one-fourteenth. In 2007, we recorded 70 species (61 Rotatoria and 9 Cladocera). It is much less than those recorded in 2006 but it can be explained by the

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less frequent sampling. The dominant species of the river (*Bdelloidea, Brachionus quadridentatus, Brachionus urceolaris, Euchlanis dilatata, Keratella cochlearis, Keratella quadrata, Pompholyx sulcata*) were typically cosmopolitans. The cosmopolitan *Bosmina longirostris* of Cladocera occurred at all sampling sites.

Species number and densities of microcrustaceans varied in a similar way along the river. In spite of the significant water supply the number of individuals taken in the lower section was less than those recorded in the upper or middle sections. In this respect we found relatively high number of individuals in the reservoir and at the sampling sites of Petőfibánya (Pb), and Nagybátony (Nb), all situated in the upper section of the river. It can be attributed to the lentic character of the reservoir and the altered environmental conditions downstream, respectively.

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SIMULATION, VALIDATION AND APPLICATION OF CERES-MAIZE MODEL FOR YIELD MAXIMIZATION OF MAIZE IN NORTH WESTERN HIMALAYAS

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Abstract. Maize (*Zea mays* L.) is one of the most important cereal crops of the world. Investigations were carried out for determination of genotypic coefficients of important varieties of maize by using CERES-Maize model in the Decision Support System for Agrotechnology Transfer (DSSAT v 3.5). The CERES-Maize model was evaluated with experimental data collected during two field experiments conducted in Palampur, India. Field experiments comprising of four dates of sowing (June 1, June 10, June 20 and June 30) and four varieties (KH 9451, KH 5991, early composite and local) of maize were conducted during *Summer* 2003 and 2004 in split plot design. Observations on development stages, dry matter accumulation at 15 days interval, yield attributes, yield (grains, stover and biological), nitrogen content and uptake were recorded. Genotypic coefficients of important varieties of maize were worked out. CERES-Maize model successfully simulated phenological stages, yield attributes (except single grain weight), yield and also N uptake with coefficient of variation (CV) nearly equal to 10 %. CERES-Maize model was validated with fair degree of accuracy. Simulation guided management practices were worked out under potential production and resource limiting situations. Best time of sowing of both hybrids (KH 9451, KH 5991) was worked out to be last week of April. While for early composite (EC), first week of May proved advantageous and for local variety second fortnight of April was the best time of sowing. The best schedule of N application was 60 kg ha^{-1} at sowing time and 30 kg ha^{-1} at knee high stage for all varieties except for local where it was 60 kg ha^{-1} at sowing and 30 kg ha^{-1} each at knee high and silking stages.
Keywords: *Crop simulation, CERES-Maize, Yield, Decision Support, Validation*

Introduction

Increased food grain production depends upon judicious use of resources. The soil, climate, genotype and management factors determine the response of crops to irrigation, fertilizer and other inputs. Working out appropriate crop management strategies under uncertainties of weather and other resources have major economic and environmental implications. Computer simulation models of the soil-crop-atmosphere system can make a valuable contribution to both advancing our understanding of the processes determining crop responses and predicting crop performance in different regions and decision making system. It facilitates the task of optimizing crop and nutrient management and also investigates environmental and sustainability issues of agro-ecosystem. A scientific model can be defined as an abstraction of some real system that can be used for purposes of prediction and control. Crop simulation models are

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mathematical representations of plant growth processes as influenced by interactions among genotype, environment, and crop management. They have become an indispensable tool for supporting scientific research, crop management, and policy analysis (Fischer et al., 2000; Hammer et al., 2002; Hansen 2002).

Crop simulation models have been used for many different applications in various countries around the world. The Decision Support System for Agrotechnology Transfer (DSSAT v 3.5) is a comprehensive decision support system (Hoogenboom et al., 2004; Tsuji et al., 1994) that includes the CERES-Maize model (Jones et al., 2003; Ritchie et al., 1998). Crop growth and development are simulated by the CERES-Maize model with a daily time step from planting to maturity and are based on physiological processes that describe the response of maize to soil and aerial environmental conditions. Potential growth is dependent on photosynthetically active radiation and its interception, whereas actual biomass production on any day is constrained by suboptimal temperatures, soil water deficits, and nitrogen deficiencies.

Crop simulation models can predict crop yields well before harvest by using expected or historical weather data. Crop performance can also be predicted for climates where the crop has not ever been grown before or not grown under normal conditions. The models can be used to address various management options like scheduling of irrigation (Boggess and Ritchie 1988; Bosch and Ross 1990), scheduling of N fertilization, time of sowing (Anapalli et al., 2005), risk analysis in rainfed cropping, selection of suitable varieties under varying agro-climatic situations, etc. Besides, they can also be used for rational planning of field experiments and as a teaching aid. A major advantage of models is their ability to simulate the temporal components of crop production. This capability allows models to evaluate different crop management scenarios with less cost and in short time (Reddy and Umamaheshwari 2004).

To simulate, yield, crop growth parameters, soil water and nutrient balances, the model requires a minimum set of data on certain parameters *vi*z. (i) weather parameters (solar radiation, maximum and minimum temperature and rainfall on daily basis), (ii) soil characteristics (soil texture, bulk density, volumetric water content at field capacity and permanent wilting point), (iii) genotypic coefficients which define the varietal characteristics of each variety to be used in the model and (iv) crop management consisting of time of different field operations. Therefore, generation of minimum data set for validation of the model is a prerequisite.

In Himachal Pradesh, models capable of forecasting maize as a decision support system are yet to be tested. Also, input data sets on ‘genotypic coefficients’ for the varieties cultivated in Himachal Pradesh are lacking. The present study proposes to generate and compile the minimum data set required to test the validity of CERES-Maize model.

Materials and methods

Site description

Field experiments on maize crop were conducted at the experimental farm of Department of Agronomy, Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur (32° 6' N latitude, 76° 3' E longitude, and 1290.8 m elevation above mean sea level). Before conducting experiments, soil samples from 0-15, 15-30, 30-45 and 45-60 cm depth were collected. Results of various physicochemical properties of soil have been summarized in *Table 1*.

The soil at the experimental field was silty clay loam in texture, acidic in reaction. The 0-60 cm soil layer had on an average bulk density of 1.35 g cm^{-3}. Moisture content at 0.3 atm and at 15 atm suction was about 0.27 and 0.19 cm cm^{-3}. Soil was rich in organic matter in the upper layer that decreased with increase in soil depth. The soil was rated as high in total N, and medium in available P_2O_5 and K_2O in upper 0-15 cm layer in both seasons. These values decreased with soil depth.
Agroclimatically the experimental area falls in sub-temperate humid zone. On an average, annual rainfall of the place is 2500 mm, of which about 80 % is received during June to September. Weather data recorded at the Meteorological Observatory of Department of Agronomy, during the crop season 2003 and 2004 has been illustrated in *Figs. 1* and *2*, respectively.

Table 1. *Physicochemical properties of experimental soil*

Properties	Layer (cm)				Method employed
	0-15	15-30	30-45	45-60	
A. Physical properties					
Sand (%)	19.1	17.7	18.5	29.1	International Pipette method (Piper, 1966)
Silt (%)	43.2	37.7	35.0	23.2	
Clay (%)	37.7	44.6	46.5	47.7	
Bulk density (g cm^{-3})	1.35	1.43	1.33	1.33	Core sampler technique
Moisture content at 0.3 atm suction(cm^3 cm^{-3})	0.267	0.269	0.270	0.270	Pressure plate apparatus (Richards, 1965)
Moisture content at 15 atm suction (cm^3 cm^{-3})	0.166	0.195	0.195	0.190	Pressure plate apparatus (Richards, 1965)
B. Chemical properties					
pH	5.6	5.5	5.6	5.5	1:2.5 soil water suspension glass electrode pH meter (Jackson, 1967)
Organic Carbon (%)	1.06	0.87	0.73	0.71	Walkley and Black’s Rapid titration method (Piper, 1966)
Total nitrogen (kg ha^{-1})	3592	3143	2470	449	Modified Kjeldahl’s method (Jackson, 1967)
NO^-_3N (kg ha^{-1})	13.47	12.35	6.74	2.25	Steam Distillation method (Jackson, 1967)
NH^+_4N (kg ha^{-1})	168.38	121.23	78.58	2.25	
Available phosphorous (kg P_2O_5 ha^{-1})	22.1	22.0	19.3	20.0	Olsen’s Method (Olsen et al., 1954)
Available potassium (kg K_2O ha^{-1})	269	292	269	269	Neutral normal ammonium acetate method (Black, 1965)

During *Summer* 2003, weekly minimum temperature ranged between 22.6 °C in 23rd standard week (4th -10th June) to 15.1 °C in 43rd standard week (22nd-28th October) in 2003 and 21.0 °C in 27th standard week (2nd-8th July) to 15.6 °C in 40th standard week (1-7th October) during second year of experimentation. Mean weekly maximum temperature during the year 2003 ranged between 35.6 °C in 23rd standard week (4th - 10th June) to 25.3 °C in 43rd standard week (22nd -28th October) and during *summer* 2004, between 31.1 °C in 22nd standard week (28 May-3rd June) and 23.5 °C in 40th standard week (1-7th October).

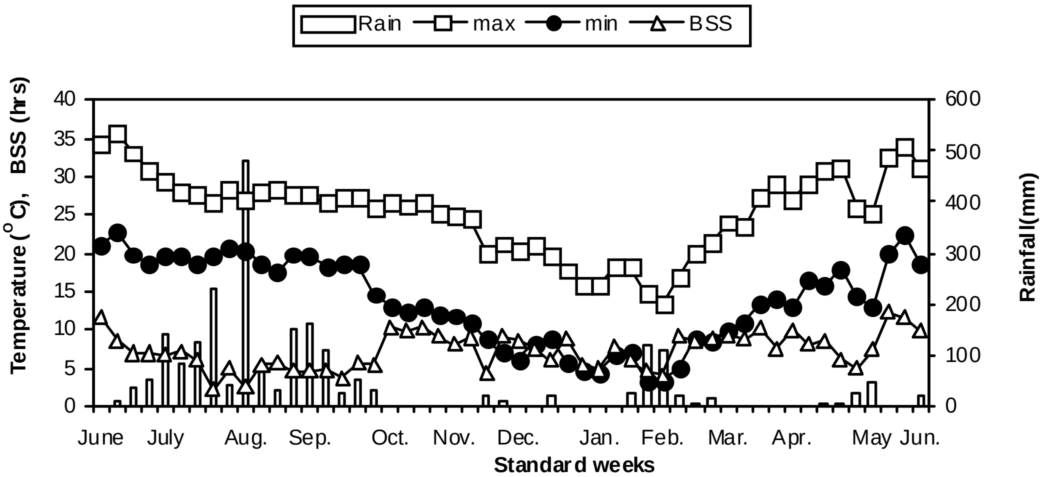

Figure 1. *Week-wise meteorological observations from 28th May 2003 to 28th May 2004 of Palampur*

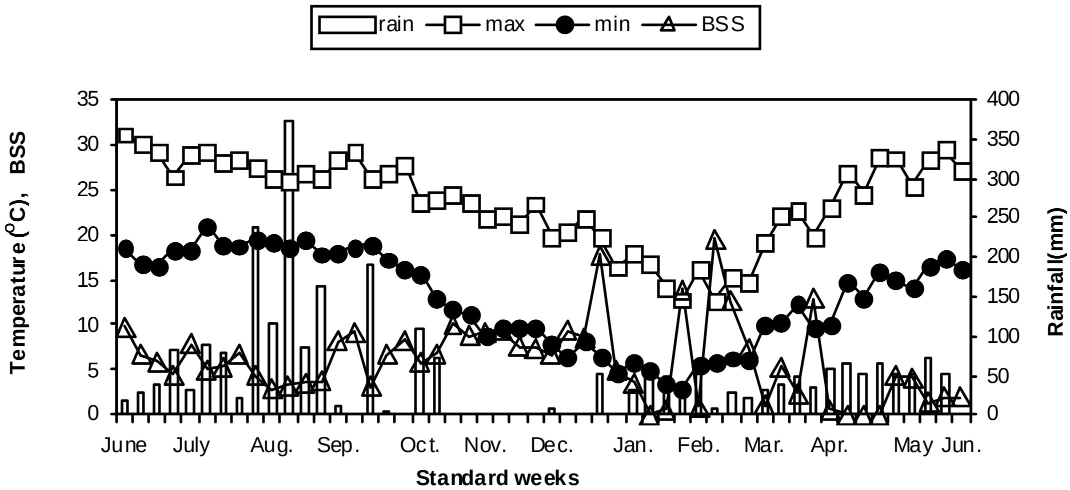

Figure 2. *Week-wise meteorological observations from 28th May 2004 to 28th May 2005 of Palampur*

Field experiments

Two field experiments on maize crop were conducted in *Summer* 2003 and 2004 in split plot design with a combination of four dates of sowing (June 1- D1; June 10- D2; June 20- D3; June 30- D4) and four varieties [KH 9451 (Hybrid) – V1; KH 5991 (Hybrid) - V2; Early Composite- V3; Local -V4]. The plot size was 21.6 m^2 (7.2 m X 3.0 m). Each treatment was replicated four times. Farm yard manure (FYM) @ 10 t ha^{-1} on dry weight basis was incorporated uniformly in all the plots and mixed well at the time of field preparation. Whole of P and K were applied @ 60 kg ha^{-1} and 40 kg ha^{-1}, respectively at the time of sowing through single super phosphate (16% P_2O_5) and muriate of potash (60% K_2O), respectively. 1/3rd N was applied at sowing and remaining 2/3rd N was top dressed in two equal splits *i.e.,* at knee-high and silking stage. Weeds were managed by spraying atrazine @ 1.75 kg ha^{-1} within 48 hours of sowing.

All the test varieties were sown on respective dates as per treatment schedule. Line sowing with a row to row spacing of 60 cm and plant to plant spacing of 20 cm to accommodate 8 plants/m^2. Data used for model evaluation were the means of four replications.

Plant measurements

The observations on dry matter accumulation were recorded at fortnightly interval. For these observations 1 m row length was marked with sticks at three observation units in the net plot area. Total number of plants m^{-1} row length were counted at emergence and at harvesting of maize crop and data reported as plants m^{-2}. For the vegetative phase, phenology was recorded by counting the leaves' collar appearance on daily basis for all experiments. Number of plants bearing silk was counted on every alternate day when silk appearance started. The day when 50% of plants in observational row (1 m) borne the silk, the stage was considered to have reached and data was reported as days after sowing.

To establish physiological maturity stage, 2 cobs plot^{-1} were randomly taken from sampling row on alternate days and dried in oven. Grains were threshed and 100 grains were counted and their weight was recorded. This procedure was continued till the two consecutive readings of 100-grain weight became constant. The earlier date was recorded as date of physiological maturity and data reported as days after sowing.

All the cobs borne by the plants growing in net plot area were counted at maturity stage and mean value was converted as number m^{-2}. Five maize cobs were randomly selected from net plot area and total number of grains was counted with the help of digital grain counter after threshing and average was reported as number of grains cob^{-1}. 100 grains were counted and weighed after drying to determine mean single grain weight.

The crop from the net plot was harvested with the help of sickles and was left for sun drying for 3-4 days, and then total produce was weighed and recorded as biological yield. The cobs were removed from the plants in the net plot area and weighed immediately after sheath removal. Five cobs were randomly selected, weighed and transferred to polythene bags. The grains of these five cobs were removed manually and weighed. The moisture content of grains was also measured immediately. The shelling percentage was calculated as per following formula:

$$\text{Shelling percentage} = \frac{\text{Grain yield of 5 cobs}}{\text{Total weight of 5 cobs}} *100 \qquad \text{(Eq. 1)}$$

To get the grain yield, the shelling percentage was multiplied by weight of cobs from net plot and after adjusting at 15% moisture content yield was then expressed as kg ha^{-1}.

Crop model

The crop model CERES-Maize (Jones and Kiniry, 1986) was used in this study. The model simulates daily growth, development and production of maize crop for any climatic and agronomic inputs. This model was chosen because of its ability to simulate both the stressed and potential yield and possibility to introduce multiple soil layer subroutines.

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Model accuracy

The data recorded on various parameters were subjected to statistical analysis, following Analysis of Variance techniques for Split Plot Design as described by Gomez and Gomez (1984) and were tested at 5% level of significance to interpret the significant findings.

The accuracy of model prediction was evaluated by testing the significance of linear regression coefficients (‘a’ and ‘b’) and degree of goodness of fit (R^2) between simulated and observed values. The root mean squared error (RMSE) between simulated and observed data were also used. RMSE is a frequently used measure of the differences between values predicted by a model or an estimator and the values actually observed from the thing being modeled or estimated. A smaller RMSE indicates less deviation of the simulated values from the observed values (McMaster et al., 1992). RMSE was further used to work out the coefficient of variation (CV) between the observed and simulated values. CV was worked out with the following formula:

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{n}(Y_i - X_i)^2} \qquad \text{(Eq. 2)}$$

$$CV(\%) = RMSE * 100 / \mu \qquad \text{(Eq. 3)}$$

Where N is the number of observations and μ is the mean observed value, Yi and Xi are the simulated and observed values of i^{th} observation.

Genotypic coefficient

The genotype file contains the genotypic coefficients, which describe the varietal characteristics, were worked out by using Gencalculator (GENCAL). Crop genotypic input data, which explains how the life cycle of a cultivar responds to its environment, are not usually available and therefore these are derived iteratively using Hunt’s method (Hunt et al., 1993). Minimum crop data set required for these calculations include date of emergence, anthesis, maturity, grain yield, above ground biomass, grain density (grains cob^{-1}) and individual grain weight. The coefficients calculated by the software were fine tuned to simulate the development stages and yield parameters. The procedure for determining coefficients involved running the model using range of values of each coefficient in the order indicated above, until the desired level of agreement between simulated and observed values were reached. CERES-Maize model requires 5 cultivar specific genotypic coefficients. The ‘P’ coefficients (P1, P2, and P5) predict flowering and maturity, while the genotypic coefficient (G2 and G3) represent the grain dry weight under non-limiting conditions (*Table 2*).

Table 2. *Genotypic coefficients for maize varieties used in the study*

S. No.	Genotypic Coefficients	KH-9451	KH-5991	Early Composite	Local
1	**P1-** Juvenile phase (expressed in degree days)	200	200	200	218
2	**P2**- Photoperiod sensitivity	1.91	1.86	1.16	1.99
3	**P5**-Thermal time from silking to physiological maturity (in degree days).	660.4	643.8	642.0	611.4
4	**G2**-Maximum possible number of kernels per plant.	933.0	829.0	756.0	740.0
5	**G3**-Kernel filling rate during the linear grain filling stage and under optimum conditions (mg/day).	8.5	8.5	8.5	8.5
6	**PHINT**-Phylochron interval; the interval in thermal time (degree days) between successive leaf tip appearance.	38.9	38.9	38.9	38.9

Model validation

CERES-Maize was validated for grain productivity (of different maize varieties of which the genotypic coefficients were worked out in this study) only for which reasonably good number of experimental data were available. Validation of the CERES-Maize for grain yield was attempted by using reported/published data of several field experiments on maize conducted during and preceding to the year of present investigation. Input data *viz.* crop management practices (sowing date, fertilizer application, irrigation management, organic manure, etc.) weather data and soil characteristics were modified in accordance with the reported year and place of experimentation. Simulation runs were made and model predicted data was generated. Actually reported and simulated data were compared. CV and regression between observed and simulated data were worked out and tested for their statistical significance.

Simulation guided management

After the model being validated satisfactorily for prediction of grain yield, simulation guided management practices were worked out for yield maximization of different varieties. This was achieved by systematically altering the various management practices (time of sowing, time and methods of fertilizer N application) as input by using sensitivity analysis option in model and recording the output (grain yield) after each run. Following this procedure simulation guided management practices for yield maximization of maize under potential production and resource limiting (no N application) situations were worked out by running model over a period of 5 years.

Irrigation levels and time of application was also considered to be an important resource that limit yield under field conditions. But the model did not simulate the effect of irrigation levels in maize. Therefore, simulated data could not be generated due to different irrigation schedules.

Results

Phenology

The genotypic coefficient P2 varied from 1.16 for early composite to 1.99 for local (*Table 2*). P1, G3 and PHINT coefficients were same for all the four varieties except P1 for local. There was considerable variation amongst varieties in P2, P5 and G2 coefficients, indicating their differential behavior to attain various development stages and sink capacity. The difference between simulated and observed days to flowering and physiological maturity of different varieties sown on different dates over the 2 years ranged from 0 to 7 days only. Therefore, *Fig. 3* shows a close correspondence between observed and simulated number of days to flowering and physiological maturity with small CV of 3.46 and 2.30 %, respectively. Similarly, goodness of fit (R^2) as well as regression coefficients between observed and simulated data was significant.

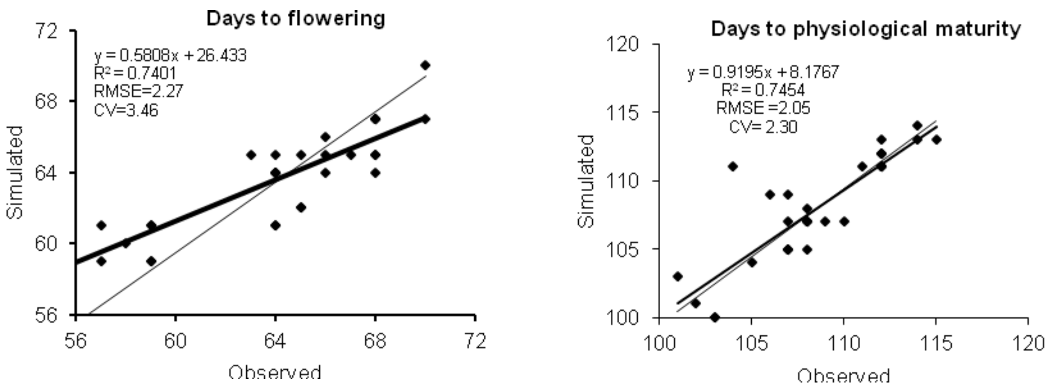

Figure 3. *Observed and simulated phenology of maize crop (Straight line indicates the 1:1 line)*

Yield attributes

Fig. 4 reveals that number of grains cob^{-1} simulated by the model matched closely with observed values. Consequently, the goodness of fit ($R^2 = 0.545$) between observed and simulated values was significant. CV was also within the acceptable level of 8.93%. Similarly significant association between observed and simulated values of grain numbers m^{-2} was supported by significant R^2 (*Fig. 4*) as worked out by comparison of calculated F- ratio with tabulated F-ratio value. Lower values of CV (9 and 10%) also supports the fact that model estimated the parameter with a reasonable degree of reliability. Unlike number of grains, single grain weight was not correctly simulated by the model and the association between simulated and observed values was not significant (*Fig. 4*). Consequently, the goodness of fit between observed and simulated data was very poor ($R^2 = 0.002$) and higher CV (13.78 %).

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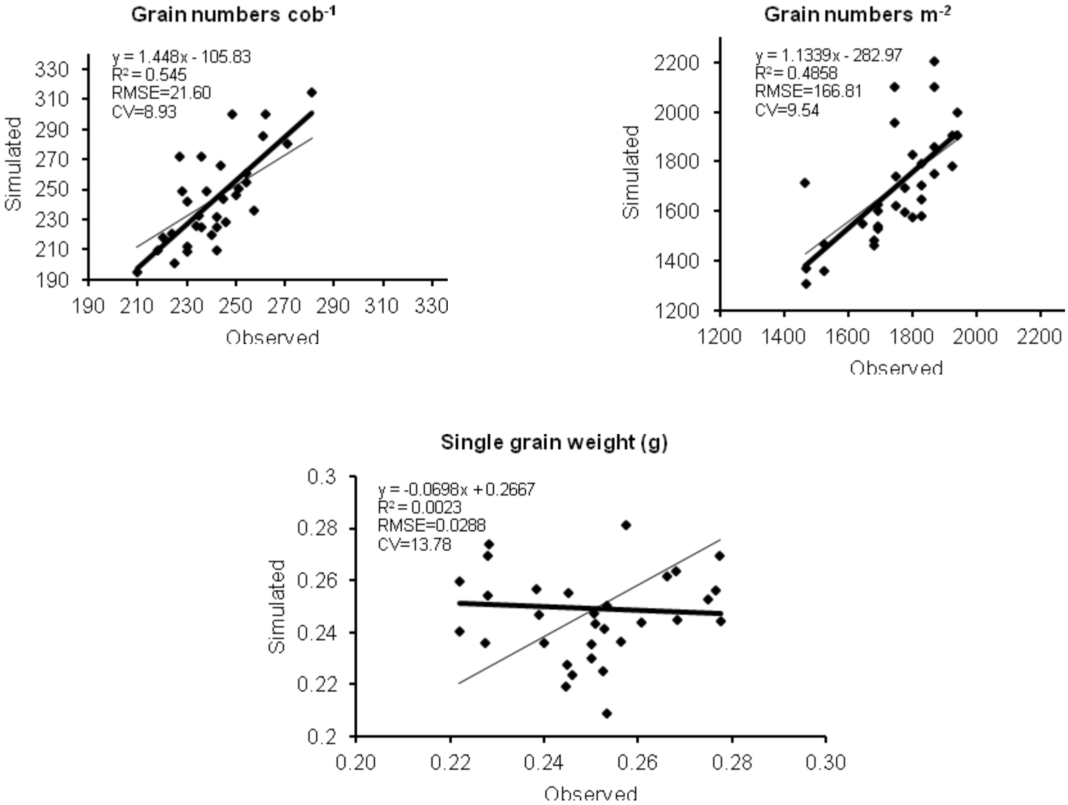

Figure 4. *Observed and simulated yield attributes of maize crop (Straight line indicates the 1:1 line)*

Yield

Like the development stages, grain yield was very well simulated by the model. Significant association between observed and simulated grain yield values were indicated by significant coefficients of regression and slope of regression line (0.875) (*Fig. 5*). The goodness of fit (R^2 = 0.843) between simulated and observed field data was also significant. Satisfactory performance of the model in predicting the grain yield is further suggested by very low CV (6.95 %) which is very well within the acceptable limits. The simulated yields ranged from 3336 kg ha^{-1} for the local to 5341 kg ha^{-1} for the hybrid KH- 9451 and the observed yields ranged from 3046 kg ha^{-1} for local to 5137 kg ha^{-1} for the hybrid KH- 9451 (*Table 3*).

Table 3. *Observed and simulated grain yield of maize (kg ha^{-1}) [Pooled over two years]*

Dates of sowing	Varieties									
	KH- 9451		KH- 5991		EC		Local		Mean	
	Obs.	Sim.	Obs.	Sim.	Obs.	Sim.	Obs.	Sim.	Obs.	Sim.
D1	**5124**	5341	**4369**	4686	**4019**	3917	**3690**	3948	**4300**	4473
D2	**5137**	5077	**4369**	4480	**4001**	4286	**3757**	3796	**4316**	4410
D3	**4492**	4740	**3874**	4134	**3463**	3739	**3410**	3498	**3809**	4028
D4	**4051**	4444	**3564**	3894	**3280**	3686	**3046**	3336	**3485**	3840
Mean	**4701**	4900	**4044**	4298	**3691**	3907	**3476**	3644		
RMSE										**276.98**
CV										**5.57**

Like grain yield, stover yield was also simulated fairly well. Although the value of R^2 = 0.4961 as well as the regression coefficients (a = 2401 and b = 0.5575) between observed and simulated data were lower than those for grain yield, yet these were statistically significantly at 5% level of significance (*Fig. 5*). Thus the model predictions are considered to be reliable and acceptable.

Mean biological yield predicted by the model agreed reasonably well with mean measured biological yield. Simulated values of biological yield of maize generated by the model were consistently lower than the measured values of all the treatments. However, association between simulated and observed data were significant with R^2=0.7747. The intercept as well as the slope of regression line were also found to be statistically significant (*Fig. 5*). CV was also within acceptable levels and lower values of this coefficient (10.73 %) further support the biological yield prediction by model was reasonability good. *Fig. 5* revealed that the association between simulated and observed harvest index values were significant (R^2 = 0.4622). Significant values of intercept and slope of regression line also authenticate the significant association.

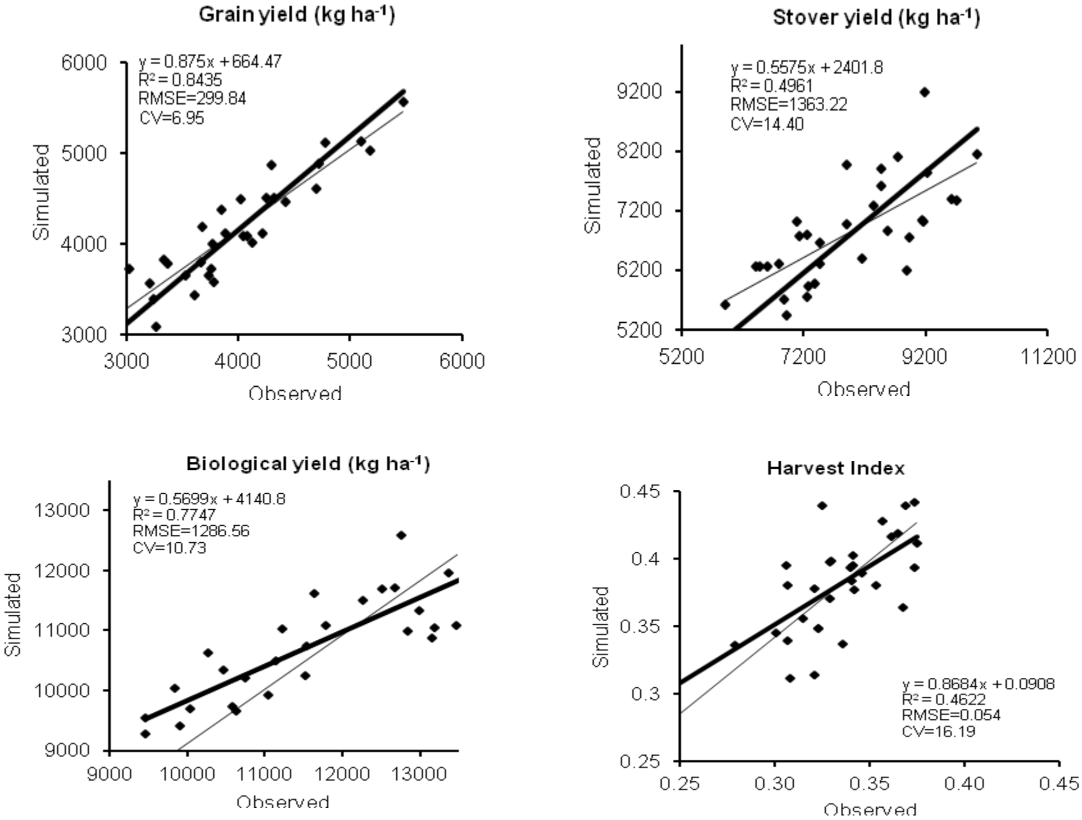

Figure 5. *Observed and simulated yield and harvest index of maize crop (Straight line indicates the 1:1 line)*

Nitrogen content and uptake

Simulated N content in maize grains were consistently, higher than the observed values. The regression line between observed vs. simulated data presented in *Fig. 6* showed that the R^2 (0.315) was not significant. The simulated and observed values for uptake of N in grains were significantly associated as supported by the tests of significance for intercept and slope of regression line. Similarly goodness of fit (R^2=0.8328) between simulated and observed data was significant. RMSE value was 6.70 kg ha^{-1} and CV 9.89%. The association between simulated and observed values for uptake of N in stover was also significant (*Fig. 6*). Goodness of fit (R^2=0.462) between the two values was also significant. RMSE value was 10.56 kg ha^{-1}. For total N uptake in maize, association between simulated and observed values was significant (*Fig. 6*). Goodness of fit (R^2=0.8065) was significant and RMSE was 23.17 kg ha^{-1}. CV was also on lower side 16.57%. In general, the model underestimated the N uptake in all the treatments.

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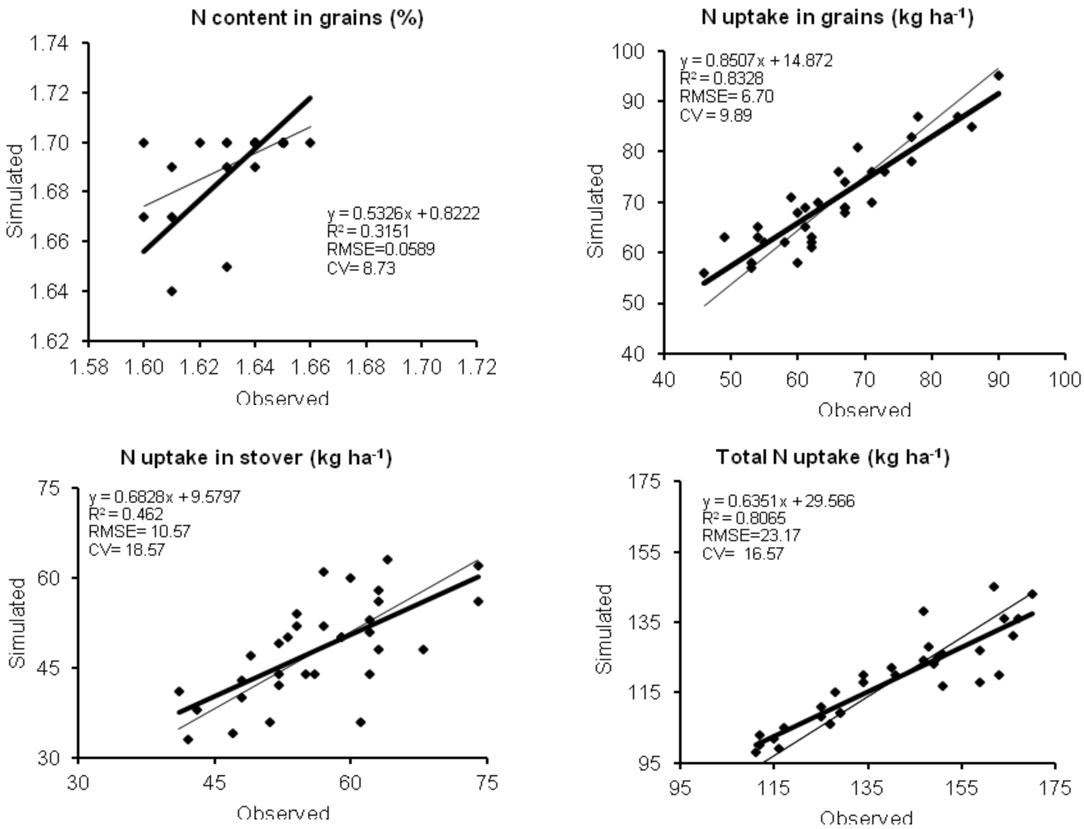

Figure 6. *Observed and simulated nitrogen content and uptake of maize crop (Straight line indicates the 1:1 line)*

Validation of CERES-Maize

Validation of the CERES-Maize for grain yield was attempted by using reported/published data of several field experiments on maize conducted during and preceding to the year of present investigation and the data is presented in *Table 4*. A perusal of *Table 4* revealed that deviation of grain yield simulated by the model varied from (-)1090 to 10 kg ha^{-1} obtained from different experiments.

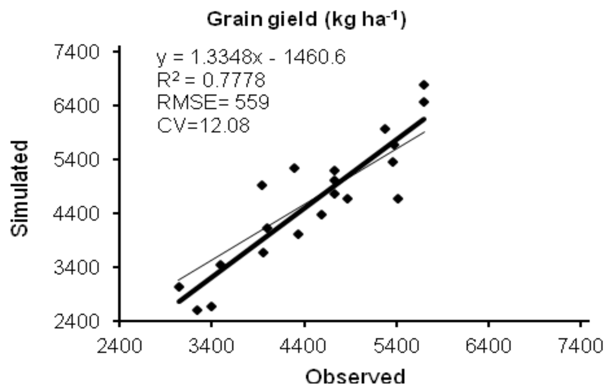

Figure 7. *Validation of CERES-Maize model for grain yield (Straight line indicates the 1:1 line)*

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Fig. 7 indicates that the simulated and observed values of grain yield for all the four varieties were close in all the experiments. Simulated and observed values were significantly associated as supported by high value of goodness of fit (R^2 = 0.7778). This was further supported by tests of significance of intercept and slope of regression line. The RMSE value was 559 kg ha^{-1} which further reveals the level of precision of model in yield prediction. CV was also within an acceptable limit of 12%. All these statistical tools indicate that the CERES-Maize model was validated with a fair degree of accuracy and hence can be used as a decision support system and work out simulation guided management practices for yield maximization of maize under different resource base situation.

Table 4. *Grain yield (kg ha^{-1}) data used for validation of CERES-Maize model*

Experiment No.	**Grain yield (kg ha^{-1})**		
	Observed	**Simulated**	**Deviation from observed**
1	4920	3944	-976
2	4670	4863	193
3	2670	3399	729
4	5010	4724	-286
5	3670	3952	282
6	5180	4724	-456
7	5240	4299	-941
8	4011	4339	328
9	2593	3235	642
10	5350	5364	14
11	5958	5272	-686
12	5671	5370	-301
13	4670	5415	745
14	4120	4001	-119
15	6469	5695	-774
16	6788	5698	-1090
17	4380	4586	206
18	4760	4724	-36
19	3450	3496	46
20	3030	3040	10

Simulation guided management practices

The validated model was used to design agronomic practices for yield maximization of maize. From simulated results (*Table 5*) it was concluded that best time for sowing of KH 9451 and KH 5991 hybrids was last week of April to first week of May. Grain yield of both the hybrids increased with increase in level of N and up to 90 kg N ha^{-1}. The best schedule for application of N was 60:30 kg ha^{-1} i.e. 60 kg N at the time of sowing and 30 kg N at knee high stage. Application of N at silking did not show any appreciable increase in yield. With these management practices hybrid 9451 registered 51-5.5 t ha^{-1} yield over the years. Under potential production situation (no N stress and

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no water stress) this variety has a yield potential of 6.0-6.4 t ha^{-1}. However under resource constraints situation i.e. under no nitrogen and rainfed conditions the yield declined to 27-29 q ha^{-1}. Grain yield of second hybrid, KH 5991 with 60:30 kg N ha^{-1} was 4.5-4.8 t ha^{-1} over the years. Under potential production situation this variety has a yield potential of 48-55 q ha^{-1}. However under resource constraints situation i.e. under no N and rainfed conditions the yield declined to 2.3-2.6 t ha^{-1}. Early composite being an early maturing variety, the appropriate time of sowing for this variety was worked out to be first week of May. This variety also responded upto 90 kg N ha^{-1}. Grain yield leveled off at 90 kg N ha^{-1}. The best schedule for application of N was same as for the hybrids. With these management practices this variety registered 3.8-4.4 t ha^{-1} yield over the years. Under potential production situation this variety has a yield potential of 4.2-4.8 t ha^{-1}. However, under resource constraints situation i.e. under no nitrogen and rainfed conditions the yield declined to 2.2-2.6 t ha^{-1}.

'Local' being a long duration variety, earlier sowing during second fortnight of May was better. Local was also found to be fertilizer responsive and yield increased with increase in N upto 120 kg N ha^{-1}. The fertilizer schedule for this variety worked out to be 60:30:30 kg N ha^{-1} i.e. 60 kg at the time of sowing, 30 kg at the time at knee high stage and 30 kg N at silking stage. This variety has a yield potential of 4.2-4.5 t ha^{-1} under potential production situation and 1.8-2.2 t ha^{-1} under resource constraints situation i.e. under no nitrogen and rainfed conditions.

Table 5. *Simulation guided management practices for yield maximization of maize*

Variety	Optimum Time of Sowing	Grain Yield (t ha^{-1})		
		Without N application	Optimum N application	Potential yield
KH 9451	Last week of April to first week of May	2.7-2.9	51-55 (60+30 kg N ha^{-1})*	6.0-6.4
KH 5991	Last week of April to first week of May	2.3-2.6	45-48 (60+30 kg N ha^{-1})	4.8-5.5
Early Composite	First week of May	2.2-2.6	38-44 (60+30 kg N ha^{-1})	4.2-4.8
Local	Second fortnight of May	1.8-2.2	35-43 (60+30+30 kg N ha^{-1})#	4.2-4.5

* 60 kg N ha^{-1} at the time of sowing and 30 kg N ha^{-1} at knee high stage
# 60 kg N ha^{-1} at sowing time, 30 kg N ha^{-1} at knee high stage and 30 kg N ha^{-1} at silking stage

Discussion

The CERES-Maize model was able to simulate phenology of 4 maize varieties grown in North West Himalayas in India. Smaller value of CV indicates that the model's performance was satisfactory in this parameter. Roman-Paoli et al. (2000), Gungula et al. (2003) and Tojo Soler et al. (2007) have also reported close prediction of days to flowering in maize by using CERES-Maize in different environments. Model

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closely simulated number of grains per cob (R^2=0.545), number of grains m^{-2} (R^2=0.485) but could not simulate single grain weight (R^2=0.002).

Grain yield was very well simulated by the model. In general, simulated grain yield data were slightly higher than the observed data. Mastrorilli et al. (2003) reported less than 13% variation in grain yield of simulated and observed grain yield under Mediterranean conditions by using CERES-Maize model. Like grain yield, stover yield was also simulated fairly well and the association between observed and simulated values was significant. In general, the model under predicted stover and total biological yields and over-estimated the harvest index. The model failed to simulate N content in grains of all varieties at all sowing dates. But N uptake in grains, stover as well as total uptake in grain+stover was closely simulated by model.

CERES-Maize model was validated with a fair degree of accuracy as supported by high value of goodness of fit between the observed and simulated data, low RMSE (559 kg ha^{-1}), CV (12.08 %) and hence can be used as a decision support system for prediction of grain yield. Best time for sowing of both hybrids was worked out to be last week of April. While for early composite, first week of May proved advantageous and for local, second fortnight of April was the appropriate time. The best schedule of N application was 60+30 kg N ha^{-1}, 60 kg N ha^{-1} at sowing time and 30 kg N at knee high for all varieties except for local where it was 60 kg N ha^{-1} at sowing and 30 kg each at knee high and silking stages.

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QUANTITATIVE DIVISION OF VEGETATION ECOTONES IN NORTHEAST CHINA

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Abstract. The present study aims to quantitatively divide the forest vegetation transition zones, the transition zone between humid forest and semi-humid meadow steppe and that between semi-humid meadow steppe and semi-arid grassland in Northeast China, using the rate of eco-climatic guarantee of the warmth and humidity index. The results indicated that there are two categories of vegetation ecotones in the Northeast China. 1) The first one has the major determinant of temperature factor. There are two sub classifications under this category. One lies in the place between cold temperate coniferous forest and temperate broad-leaved coniferous mixed forest, distributing mainly in the Northern Da Hinggan Mountains. The warmth index of it is the range of 37.8-52.2°C month. The other one locates at the place between warm temperate deciduous broad-leaved forest and temperate broad-leaved coniferous mixed forest, the warmth index value of which is 77.8-92.2°C month. It mostly distributes in the zone between Changbai Mountains and the mountains of Eastern Liaoning Province and, 2) the second category of vegetation ecotones is mainly decided by water factor. One is located between humid forest and semi-humid meadow steppe, the humidity index of which is the range of 6.63-8.37 mm/(°C month), mainly distributing in the Heilongjiang-Songhua-Wusuli Rivers Plain (the Sanjiang Plain) and in the transition region between the Northeast China mountains area and the Liaohe-Songhua-Nenjiang Rivers Plain (the Songliao Plain). The other one is the transition zone between semi-humid meadow steppe and semi-arid grassland, whose humidity index value is 4.47-6.53 mm/(°C month), mostly locating in the Hulun Buir plateau and the narrow and long region in the Songliao Plain.
Keywords: *Vegetation ecotone; rate of eco-climatic guarantee; vegetation-climate classifications; Kriging interpolation*

Introduction

Physical regional boundary lies the most significant change section of natural complex characteristics. It is the zone where qualitative changes between two adjacent and different natural complexes occur (Huang et al., 1999). There are obvious differences between the both sides of the boundary, not only in objective reality, climatic characteristics, vegetation and soil types of surface nature, but also in agricultural productivity, among others. The studies of dividing them were based on vegetation-climate classification. The early quantitative studies of vegetation-climate classification adopting single climate factors as classification indices began at the period from the 19th century turning to the 20th century. Then, since the 1940s, vegetation-climate classification had progressively researched based on synthetic climate indices, such as annual biotemperature (Holdridge, 1947), potential evapotranspiration (Thornthwaite, 1948), potential evapotranspiration and dry tolerance (Penman, 1956; McCulloch, 1965), net radiation and radiative dryness (Budyko, 1974), warmth, coldness and aridity/humidity (Kira, 1991) and humidity (Xu, 1985) and so on.

Therefore, there are many bifurcations and disputes when dividing them (e.g. Fang and Yoda, 1991; Yang and Li, 1999).

There are mainly five viewpoints regarding the boundary between the cold temperate coniferous forest (CTF) and temperate broad-leaved coniferous mixed forest (TF) in Northeast China, which were reported by scholars (Xu, 1980; Xu et al., 2008), including 1. the boundary from southern slope of the Yilehuli mountain to Aihui; 2. the southwest of Huma; 3. moved the first boundary to southeast slightly; 4. between 50°N and 50°20'N; and 5. the boundary of xin’an mountains vegetation, which was nearby the area of Aihui-Guosongou-south of Nenjiang River. Also, there were different opinions on the boundary between TF and warm temperate deciduous broad-leaved forest (WTF). Three of them were representative (Wu, 2002), including 1. the traditional ‘Shenyang-Dandong’ boundary based on the flora of Changbai Mountains and North China’s flora; 2. the east of ‘Shenyang-Dandong’ boundary, which was from the Yalu River, through Kuandian-Fengchengdabao-Caohekou-Benxi-Nanzamu to Kaiyuan; and 3. the west of ‘Shenyang-Dandong’ boundary along the Liaoyang Laoheishan to Fengcheng Maokuishan based on warmth index. What’s more, there are different opinions on the boundaries between humid forest (HF) and semi-humid meadow steppe (SMS) as well as between SMS and semi-arid grassland (SG), which the formation are mainly caused by water difference (Xu et al., 2008; Wu et al., 2003). These boundaries have been, in fact, obtained by forebears through the theoretical analysis and actual examination with practical values. However there were differences existed in those previous studies. The possible reasons are as follows. Firstly, the previous studies used different zoning indices (Ni, 1998), which inevitably led to difference. Secondly, climate controls the distribution of vegetation (Fang et al., 2002). Temperature and precipitation are the essential factors determining vegetation distributions (Fang and Yoda, 1990a; Woodward, 1987). Their long-term changes and short-term fluctuations are the major causes of the formation and distribution of eco-geographical boundary. The difference of research time would lead to different results and lastly, the systems of materials, energy, structure and function are different in the both sides of a boundary where it is the interlaced zone of two eco-systems. Therefore, it is difficult to distinguish the boundary in some spatial context, which should be the basic reason of difficulty in distinguishing the boundary. Namely, the ‘boundary’ should be, exactly, the ‘zone’. To solve these problems, we believe that it is necessary to comprehend and analyze these ‘boundaries’ using the theory of vegetation ecological transition zone (vegetation ecotone).

Vegetation ecotone (VE), the transition zone between two adjacent eco-systems, has a set of unique characteristics determined by spatio-temporal scales and the strength of the interactions between the two adjacent eco-systems (Gosz, 1993). Its unique characteristics involve that 1) VE is rich in natural resources, 2) VE controls the adjacent eco-systems, 3) part of VE is generally the eco-vulnerable zone, and 4) eco-risks occur mainly within VE zone comparing with the adjacent eco-systems. According to all these aspects, VE becomes the key area of researching on the effects of climate change (Gao and Zhang, 1997; Goldblum and Rigg, 2005; Kharuk et al., 2010; Sjögersten and Wookey, 2009). Guo (2001) studied the scientific significance of the forest VE between Da Hinggan Mountains and Xiao Xinggan Mountains to global climate change. Ma et al. (2006) studied the ecological niche of constructive species of a VE in Heng Mts. Shanxi. Huang and Wang (2010) studied the vegetation change of ecotone in west of Northeast China plain using time-series remote sensing data.

Dividing VE, especially quantitatively dividing, has significant scientific meaning. For these reasons mentioned above, the authors focus, thus, on quantitatively dividing VE using the theory of rate of eco-climatic guarantee. The cultivation and deforestation have led to serious damage of natural vegetation and environment, especially in VE in most part of Northeast China in the past 200 years. Therefore, the authors perform, specially, the analysis in Northeast China. It is expected to be valuable for the restoration and reconstruction of eco-system in transition zones of Northeast China.

Materials

The original data used in this study consist of daily precipitation and temperature obtained from the National Weather Service. There are totally 131 meteorological stations with data available from 1951 onwards in Northeast China. For many stations there are incomplete or inadequate data in the early operating period 1951-1960. The analysis period in this study is, therefore, confined to 1961-2007. We selected data from 93 stations and from those data we performed the analysis. The distribution of the 93 stations in Northeast China is presented in *Fig. 1*. We can see that the sites are evenly spaced in the study area. As for the study area, Northeast China is located between 115°52′-135°09′E and 38°72′-53°55′N, which covers Heilongjiang Province, Jilin Province, Liaoning Province, as well as Chifeng, Tongliao, Hulun Buir City and Hinggan League in Eastern Inner Mongolia (*Fig. 1*). Da Hinggan Mountains is located at the northwest, Xiao Hinggan Mountains at the northeast, and Changbai Mountains, composed of several series of parallel mountains, is locate at the southeast in the internal region of Northeast China. The Japan Sea is in the east while the Yellow Sea Bohai Seas are located in the south. The Mongolian plateau is situated in the north. The study area is a physically geographical region.

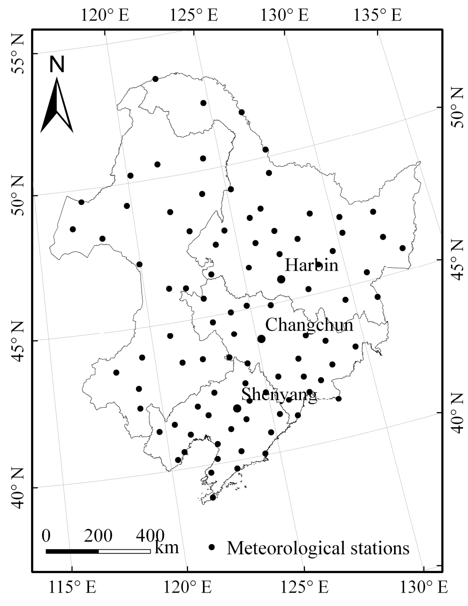

Figure 1. *Distribution of meteorological stations in Northeast China*

Methods

There are mainly four vegetation-climate classifications for dividing eco-geographical boundaries. They are Penman (1956), Thornthwaite (1948), Holdridge (1947) and Kira (1991) models, respectively. The Penman model can calculate the index precisely and has physical significance. However, this model is limited in actual applications because that the calculation of it is intricate, and that it needs too many parameters and some of them are not recorded in general meteorological stations, such as the defective value of vapour pressure (Ni, 1998). For Thornthwaite model, there are three major reasons that show this model's deficiency as follows. First, temperature, which is as the major variable of the model, is not the most perfect indicator of evapotranspiration rate. Second, this model was put forward by Thornthwaite using the data of Eastern United States, which couldn't be suitable for all places and, the last, if the mean monthly temperature is below 0°C, the corresponding monthly PE value will be set as 0 cm, which is suspected by many scholars. Especially in Northeast China, the stations generally have 3-5 months which the mean month temperature is below 0°C, and mostly stations have 5 months. The vegetation divisions of Northeast China based on this model may be not the optimal model, such as the study by Wu (2002). Holdridge method was deemed to be good vegetation-climate classification (Ni, 1998). Whereas we adopt the Kira model developed from the researches on the relationship between vegetation and climate in East Asia for calculation of the warmth index *WI* of the vegetation. Because sufficient evidence confirms that this model excellently describes the relationships between thermal climate and the macro-distribution of plant formations (Fang and Yoda, 1989). Kira (Fang and Yoda, 1990b), and Yim and Kira (1975) and Yim (1977) effectively divided the distribution of forest zones in Japan and Korea Peninsula using the *WI*, respectively. The index, which was obtained in the area where the relationship between temperature and precipitation is simple, is suitable for regions where temperature exhibits a good relationship with precipitation; this is especially true for Northeast China (Ren and Yang, 1961) where the rains mainly occur in summer. Therefore, we quantitatively divided the VEs by the methods of rate of eco-climatic guarantee based on Kira's climate classification in the Northeast China.

Calculations for the WI and HI

These indices were calculated using the following equations:

$$WI = \sum(t_n - 5) \quad \text{(Eq.1)}$$

$$HI = P/WI \quad \text{(Eq.2)}$$

where t_n is the > 5°C monthly mean temperature and P (cm) is the amount of annual precipitation.

In accordance with the actual conditions in Northeast China, *WI* = 45°C month was used as the boundary between CTF and TF; *WI* = 85°C month was used as the boundary between WTF and TF; *HI* = 7.5 mm/(°C month) was adopted as the boundary between HF and SMS; *HI* = 5.5 mm/(°C month) was used as the boundary between SMS and SG (Ni, 1998; Xu et al., 2008).

VE divisions

We calculate the possible and stable limits of vegetation using rate of eco-climatic guarantee based on the eco-geographical boundary, afterwards we obtain the VE. We assume that there are two adjacent eco-systems A and B growing perennials or crops, and the demand of A for the *WI* (or *HI*) is more than that of B. First, the dominant species of the two eco-systems compete, and both species have equal chances of survival nearby the eco-geographical boundary between A and B. The guarantee rate of them is 50% (WI_{50} or HI_{50}). With increasing *WI* (or *HI*) on one side of WI_{50} (or HI_{50}), the competitiveness of the dominant species of B declines and their chances of survival are reduced. By contrast, the survival chances of the species of A increase. A species dominate, whereas B species can grow only in certain habitats when the *WI* (or *HI*) increases to WI_{90} (or HI_{90}), i.e., if the rate of guarantee is 90% (Agricultural Meteorology, Shenyang Agricultural University, 1982). Thus, the lower limit of the stable distribution of A is WI_{90}, which is also a limit of the transition zone between A and B. By contrary, the decrease in the *WI* or *HI* on the other side of WI_{50} (or HI_{50}) results in the decline of the competitiveness of A species. Conversely, the competitiveness of B species is enhanced. When the *WI* (or *HI*) drops to WI_{10} (or HI_{10}), that is, the rate of guarantee is 10%. A species can occur only in certain habitats whereas B species dominate. This dominance constitutes the upper limit of the stable distribution of B species. It is also the other limit of the transition zone between A and B. For the annual plant or crop, they are similar to the perennials, while their growth generally requires 80% guarantee rate. According to *Fig. 2*, we can intuitively understand the transition zone as follow:

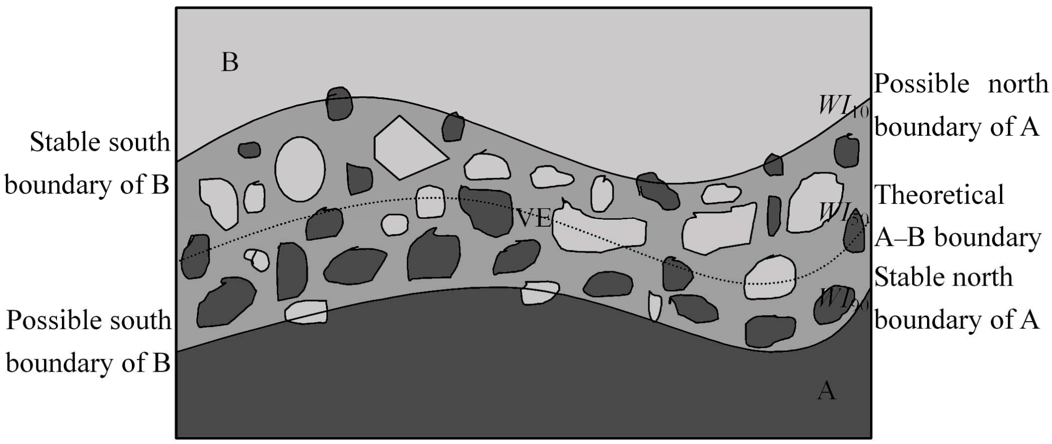

Figure 2. *The schematic diagram of vegetation ecotone (VE)*

We present the correlation and regression analysis between *WI* and ≥10°C accumulated temperature ($T_{\geq 10°C}$) with the 93 meteorological stations in Northeast China, then obtain the equation:

$$WI = 0.027 \times T_{\geq 10°C} - 7.552 \qquad \text{(Eq.3)}$$

Table 1. *Correlation and regression analyses between WI and $T_{\geq 10°C}$*

Correlation coefficient (*R*)	R^2	Sig.*	Regression coefficient (*a*)	Sig.**	Constant
0.997	0.994	0.000	0.027	0.000	-7.552

* Correlation coefficient is significant at the 0.05 level (1 – tailed).
** Regression coefficient is significant at the 0.05 level (2 – tailed).

According to *Table 1*, we learn that *WI* is highly relevant to $T_{\geq 10°C}$. The value of coefficient of determination R^2 is 0.994 (Sig. is 0), which shows that Eq. (3) is available. Additionally, we calculate the change range of $T_{\geq 10°C}$ of Northeast China in 1961-2007. The mean variation range is -388°C to 451°C, which also indicates that Northeast China belong to unstable climate type, obviously. When a guarantee rate of 90% was calculated using the guarantee rate formula, $\Delta T_{\geq 10°C}$ = 266°C (Agricultural Meteorology, Shenyang Agricultural University 1982). Then $\Delta WI = 0.027 \times \Delta T_{\geq 10°C}$ = 7.2°C month was obtained using Eq. 3. Thus, WI_{90} = 52.2°C month is the upper limit of the VE between CTF and TF. Similarly, the values of the limits of the other transition zones are calculated. Subsequently, the guarantee rate of the perennials or crops in each meteorological station is calculated based on the standard deviation formula of guarantee rate (Agricultural Meteorology, Shenyang Agricultural University, 1982).

$$WI_{90} = WI_{50} + 1.28\sigma_{WI} \text{ or } HI_{90} = HI_{50} + 1.28\sigma_{HI} \quad \text{(Eq.4)}$$

$$WI_{10} = WI_{50} - 1.28\sigma_{WI} \text{ or } HI_{10} = HI_{50} - 1.28\sigma_{HI} \quad \text{(Eq.5)}$$

For annual plant or crop:

$$WI_{80} = WI_{50} + 0.84\sigma_{WI} \text{ or } HI_{80} = HI_{50} + 0.84\sigma_{HI} \quad \text{(Eq.6)}$$

$$WI_{20} = WI_{50} - 0.84\sigma_{WI} \text{ or } HI_{20} = HI_{50} - 0.84\sigma_{HI} \quad \text{(Eq.7)}$$

where WI_{50} and HI_{50} are the boundaries of the temperature and water between different vegetation zones, respectively; 1.28 and 0.84 are the coefficients of dispersion of the normal distribution (when bias coefficient is 0); σ_{WI} and σ_{HI} are standard deviations of *WI* and *HI*, respectively.

Interpolation of the WI and HI

According to the raw data and methods mentioned above, we have obtained the *WI* and *HI* values of the 93 sites in the study area. However, this study needs the data of the entire surface. Therefore, the values at unknown locations have to be estimated or interpolated by some special methods. By placing an evenly spaced site over the area for which we have known values we can obtain an estimated surface. Kriging interpolation (Oliver et al., 1990; Mason et al., 1994), developed by Matheron and Krige (Cressie, 1992), is based on the theory of regionalized variables. The basic premise of Kriging interpolation is that each unknown point can be estimated by the weighted sum of the known points, but unlike inverse distance weighted interpolation, the weights depend on the spatial correlation and the function values of the data set (i.e., on the variogram and anisotropism), which is the biggest advantage of the Kriging technique over all the other classical statistical procedures. Kriging method also quantifies the estimation variance, which will lead to define the precision of the resulting estimates. For more information, we refer the readers to the Refs. (Cekli and Gunes, 2006; Davis, 1986; Largueche, 2006). In this study, the spatial interpolation of the *WI* and *HI* values is, therefore, presented using the Kriging technique.

Results and Discussions

CTF-TF VE division

Based on the *WI*, the boundary between cold temperate zone and temperate zone is along the ‘Southern Aershan-Southern Yakeshi-central Oroqen Autonomous Banner-central Huma-Western Tahe-Mohe’. On the basis of the guarantee rate formula, WI_{90} = 52.2°C month is as the contour line from Southern Eerguna southward through Eastern Chen Barag Banner and central Evenk Autonomous Banner to Southern Xin Barag Left Banner, then along Southern Aershan northward through Southern Yakeshi, Western Arun Banner, central Oroqen Autonomous Banner, central Huma to Eastern Tahe. The contour line represents the stable north limit of TF and the possible south limit of CTF, which is the upper limit of this VE (*Fig. 3*). WI_{10} = 37.8 °C month is the contour line from Southern Eerguna, through Eastern Chen Barag Banner and central Yakeshi, along central Oroqen Autonomous Banner, through Western Huma and Tahe to Mohe, represents the possible north limit of TF and the stable south limit of CTF. That is, this line is the upper limit of this VE (*Fig. 3*). The annual average temperature is about -2.6-0°C, $T_{\geq 10°C}$ about 1500-2000°C, annual precipitation about 340-480 mm in this VE. And the north boundary of this VE is basically consistent with the north boundary of *Quercus mongolica*.

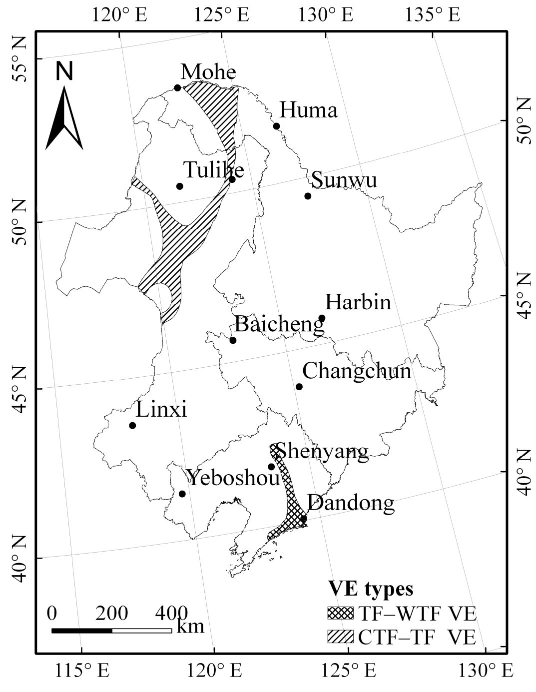

Figure 3. *Latitudinal (vertical in Mountains) VEs in Northeast China. Abbreviations: vegetation ecotone (VE), cold temperate coniferous forest (CTF), temperate broad-leaved coniferous mixed forest (TF), warm temperate deciduous broad-leaved forest (WTF)*

In addition, there are broad-leaved coniferous mixed forest zone, coniferous forest zone, erman's birch forest zone and alpine tundra zone from the basal zone to the summit in Changbai Mountains existed, respectively. Therefore there is a transition zone between broad-leaved coniferous mixed forest zone and coniferous forest zone nearby 1100 m.

TF-WTF VE division

The *WI* value of the boundary between TF and WTF is WI_{50} = 85°C month. On the basis of the guarantee rate formula, WI_{90} = 92.2 °C month is as the contour line from central Zhuanghe, along the border of Youyan and Fengcheng northward to Benxi, then through Shenyang to Tieling, which constitutes the stable upper boundary of WTF and the possible lower boundary of TF. This contour line is also the upper limit of this VE (*Fig. 3*). And the contour line WI_{10} = 77.8 °C month is obtained from Northeastern Dandong northward along the Eastern Fengcheng, through Benxi, Eastern Fushun, then northward to central Tieling, which forms the possible upper boundary of WTF and the stable lower boundary of TF. Namely, this line is the lower limit of this VE (*Fig. 3*). We name it as 'Shenyang-Dandong transition zone'. Not only the terrain and soil, but also flora shows obvious transition features in this transition zone. Terrain is gradually from Jilin Hada Ling and Longgang Mountains in Changbai Mountains transition to the Qianshan Mountains of Eastern Liaoning; soil gradually changes from dark brown forest soil to brown forest soil; the two major floras, North China's flora and flora of Changbai Mountains, interlace distribution in this transition zone. And the annual average temperature is about 5.6-9.4 °C, $T_{\geq 10°C}$ about 3000-3400°C, annual precipitation about 570-920 mm in this transition zone.

HF-SMS VE division

The forest and the meadow steppe distribute widely in the humid mountains (e.g., Changbai Mountains, Zhangguangcai Mountains, Xiao Hinggan Mountains and Da Hinggan Mountains etc.) and in the central region in the Northeast China, respectively. Hence, a forest-meadow steppe transition zone must exist between them. The terrain is composed mostly of 200-300 m altitude hills and mounds. And the forests interlace distribution with meadow in the transition zone between the mountains and plains, which form the transitional vegetation type-forest-meadow steppe between HF and SMS. The *HI* value of the boundary between them is 7.5 mm/(°C month), namely, HI_{50} = 7.5 mm/(°C month). On the basis of the guarantee rate formula, we divide the contour line HI_{90} = 8.37 mm/(°C month) into four parts (*Fig. 4*). The first part originates in Southern Eerguna, along Southwestern Yakeshi southward to Northern Aershan. The second part derives from Northern Horqin Right Wing Front Banner, northward to Zhalantun, Arong Banner, then turns northeastward through Oroqen, Huma to Tahe. The third part originates in Hehe, southward along Sunwu, Beian, Suiling and Tieli, then turns to Tangyuan until the east of Luobei. The last part derives from the foothills of Western Changbai Mountains, northward to Shangzhi, then turns southward along the southwest border of Mudan River, the northeast border of Dunhua and the northeast border of Antu to Longjing. Those parts constitute the stable limit of the forest and the possible limit of the meadow steppe, which is also the upper limit of the transition zone. The contour line, HI_{10} = 6.63 mm/(°C month), which constitutes the possible limit of the forest and the stable limit of the

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meadow steppe, is divided into two parts (*Fig. 4*). The first part derives from Southern Eerguna southward across Eastern Chen Barag Banner and Evenk Autonomous Banner until Eastern Xin Barag Left Banner. The second part originates in the Horqin Right Wing Front Banner, northward to Zhalantun, Arong Banner and Nenjiang River, then turns southward along Keshan, Mingshui, Bayan, Acheng, Yushu, Changchun, Tieling, Shenyang, and Liaoyang to Western Zhuanghe. All above constitute the lower limit of this VE.

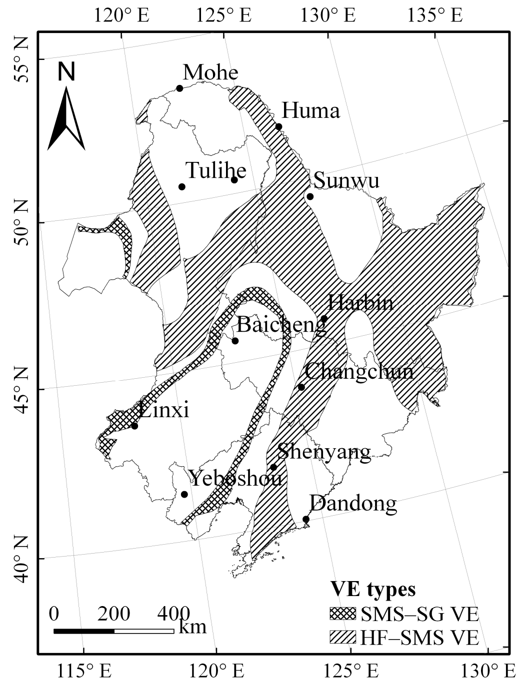

Figure 4. *Longitudinal VEs in Northeast China. Abbreviations: vegetation ecotone (VE), humid forest (HF), semi-humid meadow steppe (SMS), semi-arid grassland (SG)*

The annual average temperature is about 2-5.7 °C, $T_{\geq 10°C}$ about 2400-3200°C, annual precipitation about 450-700mm in this VE. The spatial distribution of this transition zone can be divided into three parts. One shows half ring around the Songliao Plain. Another is in the junction area between Hulun Buir plateau and Western Da Hinggan Mountains and, the last one is in Sanjiang Plain. The black soil zone is almost coincident with this transition zone, in which vegetation type is forest-meadow steppe vegetation.

SMS-SG VE division

According to eco-climatic law, a guarantee rate of 80% should be able to satisfy the eco-climatic demands of the annual vegetation growing in the Songliao Plain and Hulun Buir Plateau (Zhao and Zhang, 1999). On the basis of the guarantee rate formula, we conclude that HI_{80} = 6.53 mm/(°C month) and HI_{20} = 4.47 mm/(°C month) constitute the upper and lower limits of SMS-SG VE, respectively (*Fig. 4*). This VE is quite narrow, which originates in Western Chen Barag Banner, along Western Evenk Autonomous Banner to central Xin Barag Left Banner nearby Hulun Buir plateau. It shows tongue-shaped distribution around the Songliao Plain, and derives from Linxi, along Bairin Left Banner, central Jarud Banner, Horqin Right Wing Rear Banner, Jalaid Banner, Southern Lindian, Southern Anda, Western Zhaodong, Western Fuyu, Western

Nongan, Changling, Horqin Left Wing Rear Banner, Zhangwu, and Western Heishan to Jianchang nearby the Sonliao Plain. The annual average temperature is about 4-7°C, $T_{\geq 10°C}$ about 2 500-3 200°C, annual precipitation about 350-500 mm. The soil is mainly chestnut soil in the transition zone of Western Da Hinggan Mountains, mostly meadow chernozem and carbonate meadow soil in the transition zone of Eastern Northeast Plain. The vegetation types are both forbs meadow and dry grassland. The representative plants species include *Aneurolepidium chinense*, *Stipa Baicalensis*, *Stipa grandis*, *Artemisia frigida*, and *Agropyron cristatum* etc. This VE is quite narrow, which changes complexity, is more sensitive to climate change, and also more vulnerable damage than other transition zone. Therefore protection of such a transition zone should be taken seriously.

Discussions

To date, there are five kinds of boundaries between cold temperate zone and temperate zone in Northeast China divided in the previous studies (Xu et al., 2008). The northernmost boundary is the southwest of Huma (Liu et al., 1959). The southernmost boundary is along Aihui-Guosonggou to the south of Nenjiang River (Xu et al., 2008). The CTF-TF VE divided in this study locates between the two boundaries (*Fig. 3*). Meanwhile, intensive works have been carried out to divide the boundary between TF and WTF (e.g. Hou, 1963; Zhou, 1981; Xu et al., 2003). However, there are obvious differences among them. The result of the division of TF-WTF VE in this study is similar as that obtained by Xu et al. (2003), although they just divided the boundary between TF and WTF. That is, the 'Shenyang-Dandong transition zone' basically conforms to the actual situation. The area growing zonal vegetation, such as *Carpinus cordata*, *Abies holophylla* and broad-leaved Korean pine forest, is the north region of the transition zone, which distributes in southern region of TF. Natural Korean Pine disappears nearly in this transition zone. The secondary forests distribute widely in Eastern Liaoning Province Mountains, such as *Fraxinus mandshurica*, *Juglans mandshurica*, *Phellodendron amurense*, *Tilia amurensis*, *Fraxinus rhynchophylla*, *Corylus mundshurica* and *Sambueus williamsli*.

Validation on the VE divisions

In this section, we validate the VE divisions using the Vegetation Map of People's Republic of China (1 : 1 000 000) compiled chiefly by Institute of Botany, Chinese Academy of Sciences in 1979-2007 (Editorial Committee of Vegetation Map of China, Chinese Academy of Sciences 2007). We extract the vegetation map of Northeast China, and divide the vegetation into 10 vegetation types. *Fig. 5* shows the distributions of the vegetation types in Northeast China. Subsequently, we calculate the area of the four VEs divided in this study, as well as the area of the major vegetation types discussed in this study. The results are presented in *Table 2*.

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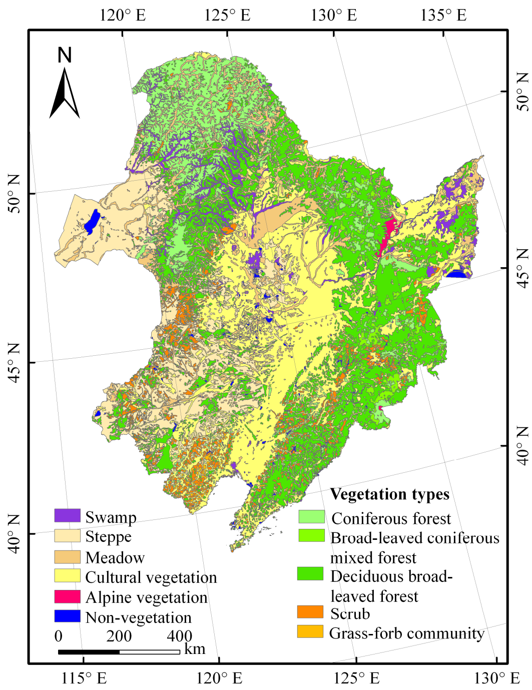

Figure 5. *Vegetation types of Northeast China*

Table 2. *The validation data of the VE divisions*

VEs / Items	CTF-TF		TF-WTF		HF-SMS		SMS-SG	
Total area (km^2)	63683.7		11313.3		481257		59166.3	
Vegetation types	CTF	TF	TF	WTF	HF	SMS	SMS	SG
Area (km^2)	23709.8	14749.1	1411.6	5353.4	212524	90891.5	9521.8	20348.2
Area ratio	CTF : TF = 1.61 : 1		TF : WTF = 0.26 : 1		HF : SMS = 2.34 : 1		SMS : SG = 0.47 : 1	

Abbreviations note: vegetation ecotone (VE), cold temperate coniferous forest (CTF), temperate broad-leaved coniferous mixed forest (TF), warm temperate deciduous broad-leaved forest (WTF), humid forest (HF), semi-humid meadow steppe (SMS), semi-arid grassland (SG).

The validation data presented in *Table 2* is obtained from the vegetation map of Northeast China using ARCMAP 9.3. We calculate the total area of the CTF-TF, TF-WTF, HF-SMS, and SMS-SG VEs, and analyze the area ratio of the discussed vegetation types in the four VEs, respectively. According to *Table 2*, it can be easily found that each VE covers mostly the vegetation types of the both sides of the VE. The variation range of the area ratio of the discussed vegetation types in each VE is 0.26 to 2.34. The most perfect VE division is CTF-TF VE with the area ratio of CTF : TF = 1.61 : 1. The last one is, unfortunately, TF-WTF VE with the area ratio of TF : WTF = 0.26 : 1. In this VE, the area of WTF is bigger than that of TF, increasing by about 4 times. The major reason may be that the marine climate influences the vegetation growth, e.g. the warm and humid airflow is beneficial to the growth of WTF in Eastern Liaoning Province Mountains. We can say, in summary, that the VEs divided in this

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study fit in with the actual vegetation distribution of Northeast China, which is, therefore, strong evidence that the method of dividing VE in this study is believable and valuable. Nevertheless, there are more works to do. For example, the division indexes need to be improved. The VEs may vary with time due to the climate changes, human activities and other disturbances. It is important to study of the relationships between the changes of VE and the variations mentioned above or the interactions of them.

Conclusions

The eco-climatic factors consisting of temperature and water are the energy and material foundation of determining VE. It is also an important basis for researching on vegetation distribution. In this paper, we employed the existing models to quantitatively divide the VEs of Northeast China based on the rate of eco-climatic guarantee.

There are two categories of VEs in Northeast China. 1) The latitudinal VEs mostly determined by temperature factor. One is CTF-TF VE, whose *WI* value is 37.8-52.2 °C month, mainly distributing in the Northern Da Hinggan Mountains. The other one is WTF-TF VE, the *WI* value of which is 77.8-92.2 °C month, mostly distributing in the zone between Changbai Mountains and the mountains of Eastern Liaoning Province. 2) The longitudinal VEs mainly decided by water factor. One is HF-SMS VE, the *HI* value of which is 6.63-8.37 mm/(°C month), mainly distributing in the Sanjiang Plain and in the transition region between Northeast China mountains area and the Songliao Plain. The other one is SMS-SG VE, whose *HI* value is 4.47-6.53 mm/(°C month), mostly locating in the Hulun Buir plateau and the narrow and long region in the Songliao Plain.

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CONSEQUENCES OF SPECIES GROUPING FOR FOOD WEB STRUCTURE

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Abstract. Effects of trophic species aggregation on the structural properties of food webs in six Mediterranean streams were assessed here. In each stream, we created three different variations of the original food web, according to the trophic species aggregation applied. The aggregations used here were based on predator-prey relationships, i.e., species were lumped at different levels of trophic habits in the food web into top (T), intermediate (I) and basal (B) species. In our studied food webs, these species corresponded to fishes, macroinvertebrates and different types of detritus, respectively. No significant differences were found in structural properties when we analyzed the stream effect, but these were found in the analysis of aggregation variation effect. Species grouping, and the level within the food web at which it occurs, influenced the structural properties of the food web. However, these properties were comparable between all the studied streams when the same resolution was considered. The relationship between omnivory and connectance was not affected by trophic species grouping. These properties were positively and significantly related in all aggregation variations of the food webs, suggesting that the stabilizing role of omnivory is maintained regardless of the particular grouping method utilized.
Keywords: *structural properties, Mediterranean streams, omnivory, connectance*

Introduction

Published food web studies vary in terms of methodology, criteria for defining links and in the level and standardization of taxonomic resolution. Some of these factors have now been shown to affect estimates of food web properties (Closs, 1991; Cohen et al., 1993; Tavares-Cromar and Williams, 1996; Thompson and Townsend, 2000; Hildrew, 2009).

Identification of the elements that comprise an ecosystem, and the connections between these elements, involves critical assumptions that have proven influential for the results of further analysis (Goldwasser and Roughgarden, 1997; Martinez et al., 1999; Abarca-Arenas and Ulanowicz, 2002; Allesina et al., 2005). In many studies, even when a certain resolution is desirable, adhering to a criterion of resolution to the level of species may not be possible because detailed information is inaccessible and/or the hypothetical sampling effort is unfeasible (Gaedke, 1995; Martinez et al., 1999). Thus, the aggregation of food web components by researchers is a complex task in which decisions taken regarding grouping method may introduce bias to the created food web.

Researchers have been criticised for their failure to resolve webs to the highest possible level of taxonomic resolution (Cohen et al., 1993). For this reason, the effect of taxonomic resolution is a critical issue in various works (Martinez, 1991; Thompson and Townsend, 2000). The effects of lumping upon food web analysis have been

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explored, (e.g. Yodzis, 1984; Lawton and Warren, 1988; Martinez, 1993; Martinez, 1994; Martinez and Lawton, 1995; Polis and Hurd, 1995; Winemiller, 1996; Pimm, 2002) but this issue requires further examination. In order to study, control and possibly reduce the effects of this variability in ecological network, some authors have evaluated the effects of aggregation on study results and conclusions (Hall and Raffaeli, 1991; Martinez, 1991; Goldwasser and Roughgarden, 1997: Sugihara et al., 1997; Abarca-Arena and Ulanowicz, 2002; Allesina et al., 2005).

Aggregation can range from the simplest of schemes involving primary producers, consumers and decomposers/detritus (Fath et al., 2007), to the most disaggregated scheme based on available data, where the concept of a food web compartment may refer to a species or even to an age- or size-class of a species (Johnson et al., 2005). In a review of available food web data, we observed that the level within the food web at which species lumping normally occurs is dependent upon the type of ecosystem in question. For example, the most common task in marine ecosystems is the aggregation of primary producers into phytoplankton, microphytobenthos and macrophytes, according to size and habitat. Since identifying organisms to species is a laborious task, species in the consumer groups such as bacteria, zooplankton and meiofauna usually remain undifferentiated, while information about higher trophic levels is more available and aggregation of data is not required to the same extent. In smaller ecosystems such as lagoons or streams however, lower or intermediate trophic levels (invertebrates, zooplankton or phytoplankton) are defined with more resolution while higher trophic levels appear grouped in single taxa (e.g. fish, birds) (Motta and Uieda, 2005; Liu et al., 2006).

This study focused on the common exercise of grouping species in order to highlight the part of the food web of most interest to researchers. This practice is carried out, according to the criteria and convenience of each researcher, because it is impossible to include the whole food web even if its boundaries are relatively limited. In this study, the food webs of six previously analysed Mediterranean streams were used to assess the effects of species lumping at different trophic habit levels through the construction of aggregated variations of food webs, thereby focusing attention upon a specific level of the trophic web. Thus, the purpose here is not to analyse these food webs exhaustively or to comment on the patterns they display. The existence of these six comparable food webs allowed us to investigate the consequences of grouping the components of one part of the food web upon the structure of that web.

Methods

Study areas

The Mediterranean streams analyzed as part of this study are located within three hydrologic demarcations located in the Southern Iberian Peninsula: South basin, Guadalquivir basin and Guadiana basin.

The South basin extends along the eastern edge of Andalusia, covering a strip some 50 km wide and 350 km long, extending over an area of 18425 km^2. This basin includes the Hozgarganta (HZ) and Guadiaro (GDR) streams. The former (HZ) is located in the Alcornocales Natural Park (Cádiz, Andalusia) and is one of the few unregulated streams throughout the basin in the Iberian Peninsula, and the only one in Andalusia (Garrido and Hidalgo, 2000). Its basin has a length of 56.4km and an area of 357.68 km^2 and is characterized by notable ecological richness and an excellent state of conservation. The

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Guadiaro stream is 101 km in length and covers an area of 1489 km^2. The banks of these streams feature communities with an abundance of deciduous plant species, including poplar (*Populus alba*), alder (*Alnus glutinosa)* and willow (Salix alba).

The Guadalquivir basin has an area of 57527 km^2. This basin includes the Rivera de Cala (CL) and Guadiamar (GDM) streams. The former (CL) marks the boundary between two protected natural areas: the Aracena Mountain Range and Aroche Peaks Natural Park and the North Mountain Range Natural Park. The Guadiamar has its source in the western foothills of the Sierra Morena and flows more than 80 km to enter the Guadalquivir river, running through the marshes of the Guadalquivir in the Doñana National Park. The Sierra Morena area is physically very homogeneous and features a mountainous environment with gentle slopes. It provides a habitat for bushes of rockrose (*Cistus ladanifer*), holm oak (*Quercus rotundifolia*) and cork oak (*Quercus suber*).

The Guadiana basin is located in the south-western quadrant of the Iberian Peninsula, forming the border between Spain and Portugal along its last stretch. This is a territory typified by both wet and arid areas, with 67147 km^2 of river network. This basin includes the Odiel (OD) and the Rivera de Chanza (CH) streams. The Odiel rises in the Aracena Mountain Range at an altitude of 660 m. The Rivera de Chanza rises in the western foothills of the Sierra Morena and, in its final stretch, forms the border with Portugal until its confluence with the Guadiana River. In terms of vegetation, the higher area is characterized by pasture with Holm oak (*Quercus ilex*) and scrub with areas of pine repopulation.

These streams are subject to a characteristic climatic regime of seasonal droughts (summer to the beginning of autumn) and inflows (from the end of autumn through the winter), which are predictable but highly variable between years (Gasith and Resh, 1999). They are characterized by a low diversity and species richness of fish. The native freshwater fish fauna of the Iberian Peninsula is characterized by a low number of families, with most species belonging to the family Cyprinidae, a high degree of diversification at species level, and the highest percentage of endemism in Europe (Doadrio, 2001). However, the macroinvertebrate fauna has higher values of diversity and species richness.

Food web construction

Binary food webs were constructed for each of the six streams. Sampling of macroinvertebrate communities and fish assemblages was conducted during the spring of 2004 to avoid both winter flooding and the arid conditions of summer, which cause high habitat loss, decrease in habitat connectivity and a large decline in the fish populations.

Samples of benthic macroinvertebrates were taken from twenty-four Suber Samplers (area 0.05 m^2, mesh size 250 µm), located at regular intervals along each selected reach, and preserved in 70% ethanol. Macroinvertebrates were sorted and identified to the highest taxonomic resolution possible using Tachet et al. (2003). 63% taxa were identified to genus or species and 37% to family or subfamily. Fish were sampled using electrofishing, operated at 300 W with a voltage of 220 V and a direct current of 0.6-0.7 A. General identification of fish was conducted following Kottelat and Freyhof (2007). Depending on the catch, a maximum of fifteen individuals were selected from each species and age per stream to represent the full size range; these were used for gut analysis while the remainder were returned to the water. Gut analysis was carried out

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under a binocular magnifying glass, and involved identification of each different component of the gut contents. The fresh weight of each item identified in the gut was expressed as a percentage of the total diet, assuming the fresh weight of the total stomach contents to represent 100% (Pinkas et al., 1971).

In the construction of the binary food webs for each stream, data from gut contents were used to describe the trophic structure of the fish assemblages. Macroinvertebrate trophic structure was described using data sourced from relevant literature (Cummins and Klug, 1979; Tachet et al., 2003). Each macroinvertebrate was assigned a Functional Feeding Group (FFG). This technique groups species together based on similarity of feeding characteristics. The groups used in this analysis were based on those of Merrit and Cummins (1996). Species were classified as scrapers (feed on algae), shredders (feed on coarse organic material, e.g., leaves, wood), collectors (feed on fine detritus deposited on the substrate), filterers (feed on fine detritus suspended in the water column) and finally, predators (feed on other macroinvertebrates).

Lower trophic taxa were aggregated into the following larger taxa groups: periphyton algae, macrophytes, allochthonous vegetal debris, allochthonous animal debris, coarse particulate organic matter (CPOM), suspended and deposited fine particulate organic matter (FPOM), and benthic detritus.

The term *taxa w*as used to refer to groups of organisms identified by the investigators as the core units of analysis in the food webs. These range from species (e.g. barbels), to species grouped by trophic habit, taxonomy, or other criteria (e.g. benthic filter feeders, zooplankton), to mixed pools (e.g. detritus – a combination of live organism, organic matter, and inorganic matter) (Dunne et al., 2004). *Trophic species* are groups of taxa whose members share the same set of predators and prey (*sensu* Paine, 1980). This study focused on the food webs of trophic species.

Construction of aggregations variations of food webs

The expanded original food web (of maximum resolution in fish and macroinvertebrates and considering different forms of detritus) was considered to represent the control, from which a series of aggregation variations of the food web were produced.

In common with the goals of Abarca-Arenas and Ulanowicz (2002) in their study regarding the effects of taxonomic aggregation on network analysis, our objective in this study was to assume the role of a researcher who would normally lump species, or groups of species, in an intuitive way. For this reason, no one numerical classification method of species aggregation was used. The aggregations used in this study were based on predator-prey relationships, i.e. species were lumped at different levels of trophic habits in the food web: Top species (T: species with prey but no predators), intermediate species (I: species with both prey and predator) and basal species (B: species with predator but no prey). In our studied food webs, these types of species corresponded to the fishes, macroinvertebrates and different types of detritus, respectively. The variations on the original extended food web are presented in *Table 1.* Throughout this study, we used the key of variations shown in this table. The cluster column identifies those species that were aggregated, while maintaining the other components as they are in the original food web.

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Table 1. *Key of food web aggregation variations to the original extended food web*

Aggregation variations of food webs	***Key***	***Clusters***
Original	OFW	*None
Fish	FFW	*Aggregation of Top species: Fish species cluster
Macroinvertebrates	MFW	*Aggregation of Intermediate species: Macroinvertebrate cluster in Functional Feeding Groups
Detritus	DFW	*Aggregation of Basal species: Detritus taxa cluster

In FFW, fish species were aggregated into single taxa. In another aggregation, macroinvertebrate taxa were lumped according to their Functional Feeding Group: scrapers, shredders, filterers, collectors and predators (MFW), while different types of detritus (CPOM, suspended FPOM, deposited FPOM and benthonic detritus) were aggregated into single taxa (DFW). Proceeding in this way, 24 different food webs were created, comprising three variations on the original food web in each of the six studied streams. Each food web consisted of a different combination of lumped species in each stream.

Structural properties of food webs

We calculated a suite of 18 properties for each food web (Williams and Martinez, 2000; Bersier et al., 2002; Dunne et al., 2004; Romanuk et al., 2006) using software developed for previous food web studies (Williams and Martinez, 2000; Williams et al., 2002; Yoon, 2004). These food web properties were: *Species richness (S)* (number of all species compartments in the web), *Fish species richness* (*F*) (number of fish species in the web) and *Macroinvertebrate species richness* (*Inv*) (number of all invertebrate taxa in the web). Six properties give percentages related to the types of species found within the web: *top species (T)* (percentage of species with prey but no predators or parasites), *intermediate species (I)* (percentage of species with both prey and predators), *basal species (B)* (percentage of species with predator but no prey), *omnivory (Omn)* (percentage of species with food chains of different lengths, where a food chain is a linked path from a non-basal to a basal species, i.e., the percentage of species that feed directly on more than one trophic level), percentage of herbivores plus detritivores (*Herb*), and the percentage of species involved in looping (*Loop*) by appearing in a food chain twice.

Two standard measures of trophic interaction richness in the food webs are reported: *linkage density* (*L/S*), links per species, the number of all trophic links in the web (L) divided by S, and *Connectance* (*C*), where $C = L/S^2$, the proportion of all possible trophic links (S^2) that are actually realized (L), ranges from 0 (no taxa preys on any other taxa) to 1 (every taxa preys on every other species including itself).

As carried out by Dunne et al., (2004), we calculated the measure of trophic level known as the *Short-weighted trophic level* (*TL*), this property gives the most accurate estimate of trophic level based on binary link information (Williams and Martinez 2004), and also its maximum value, the *Short-weighted trophic level maximum* (*MaxTL*).

We report measures related to predator and prey species: standard deviation of mean generality (*GenSD*), the number of prey items a species has, the standard deviation of vulnerability (*VulSD*), and how many predators a species has. These two measures quantify the variability of the normalized predator and prey counts for a given species. *Trophic similarity* (*Sim*) is the number of predators and prey shared in common by a pair of species divided by the total number of predators and prey of that pair. The maximum index of similarity was calculated for each species in order to calculate the average maximum trophic similarity (*MaxSim*). A simple measure of prey:predator ratio (*P:P*) ([percentage of basal + intermediate species]/[percentage of top + intermediate species]; Cohen, 1977) was used to describe the shape of the food web (high values, more triangular; low values, a "square" food web) (Thompson and Townsend, 2003). All of these are commonly calculated properties that have been reviewed in previous studies (Williams and Martinez, 2000; Dunne et al*.,* 2004; Romanuk et al*.,* 2006).

Data analysis

A two way nested ANOSIM was used to examine the properties of food webs (Clarke and Warwick, 2001), testing for similarities between streams, with aggregation variations nested within site. The same analysis was carried out to determine the effect of applying aggregation. In addition, a single factor analysis of similarities was performed in this case to test for significant similarities between pairs of food webs with different groupings. Two-dimensional MDS (Multidimensional Scaling) ordination was carried out for each aggregation variation within the original extended food web. All (24) food webs were clustered using hierarchical agglomerative clustering (Bray-Curtis similarities). In these analyses, we removed those properties related to food web size (S, F and Inv). Spearman R correlations were carried out using the statistical software SPSS 14.0 to examine the relationships between food web properties in each aggregation variation. Although the relationships between all the studied structural properties in the different food web aggregation variations were determined, only those of most interest are presented here.

Results

As expected, a decline of the resolution in trophic species led to a reduction in the number of elements within the food web *(Fig. 1.a.).* Macroinvertebrate food webs (MFW) exhibited the greatest decrease in *S,* since this food web aggregation variation grouped the highest percentage of species; specifically, between 45 and 60% of the total number of species in all streams. This decrease in the number of components of the food web affected the top (T), intermediate (I) and basal species (B) proportions differently, depending on the level at which the lumping of elements occurred. The proportion of intermediate species was reduced by the grouping of macroinvertebrate taxa, producing an increase in *T* and *B* species (*Fig. 1.b***.**, *1.c***.** and *1.d.*). Most intermediate species were herbivores or detritivores, and thus this variation (MFW) produced a similar effect in *Herb* property (*Fig. 1.e.)*

The property with the lowest variation between aggregation variations of food webs was *Omn* (*Fig. 1.f***.**)*,* i.e. values of omnivory in different variations food webs were closer to the original than those of any other properties. The largest deviation from the original food web was found in the Chanza stream, where FFW and MFW underestimated and overestimated control values, respectively. On the one hand, the

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Chanza had the highest number of omnivorous fish, thus grouping the fish into a single taxa (in FFW) and decreasing the proportion of omnivorous species, while on the other, aggregation of the macroinvertebrate taxa into five functional groups caused a reduction of intermediate species and the establishment of simple trophic links between the top and basal species. This caused the proportion of species that feed over different trophic levels to increase relative to the original extended food web.

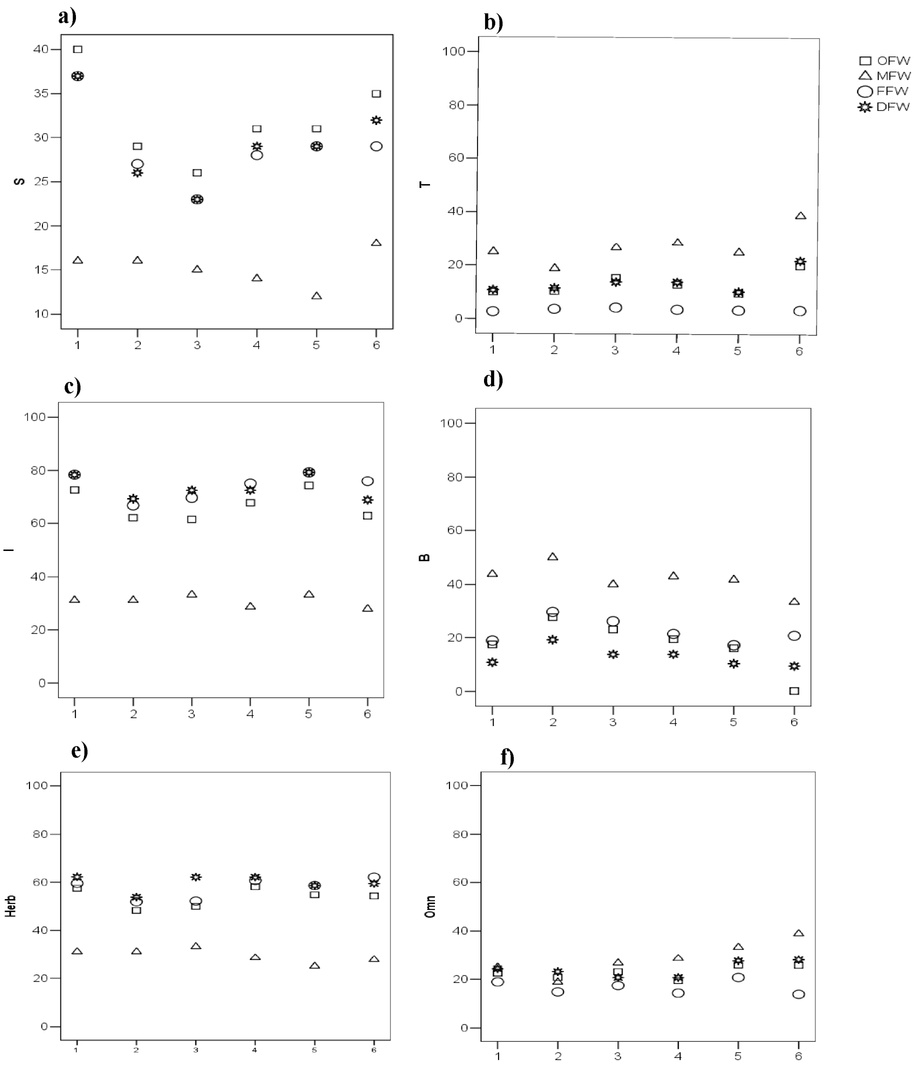

Figure 1. *Richness and proportion of taxa (S, T, I, B, Omn and Herb) for each food web aggregation variation (OFW: , MFW: Δ, FFW: ○ and DFW: ☼) in each studied stream. (1 = Hozgarganta, 2 = Guadiaro, 3 = Guadiamar, 4 = Rivera de Cala, 5 = Odiel and 6 = Rivera de Chanza)*

Regarding the properties of food webs related to link richness and linkage complexity, connectance (C) and linkage density (L/S) were affected in different way by species clustering (*Fig. 2.a* and *2.b*). FFW underestimated these properties, since a reduction in the number of fish species produced a greater decrease in link richness than in species richness, leading to lower L/S and C values. The opposite effect was observed when different forms of detritus were joined as single taxa (DFW), i.e. both properties

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were overestimated due to the decrease in species richness, while link richness remained constant.

TL tended to decrease with increased aggregation of food web components; MFW was therefore the variation which caused the most underestimation of this property, followed by FFW. DFW produced greater values than those obtained by the original extended food web (OFW) (*Fig.2.c*). When the number of intermediate or top species declined (MFW and FFW respectively), those elements of the food web with TL > 1 were deleted, producing an estimated mean trophic level of the food web below that of the original. However, when basal elements of the food web were lumped, those species with lower TL (TL = 1) were deleted, resulting in an overestimation of the mean trophic level for the whole food web.

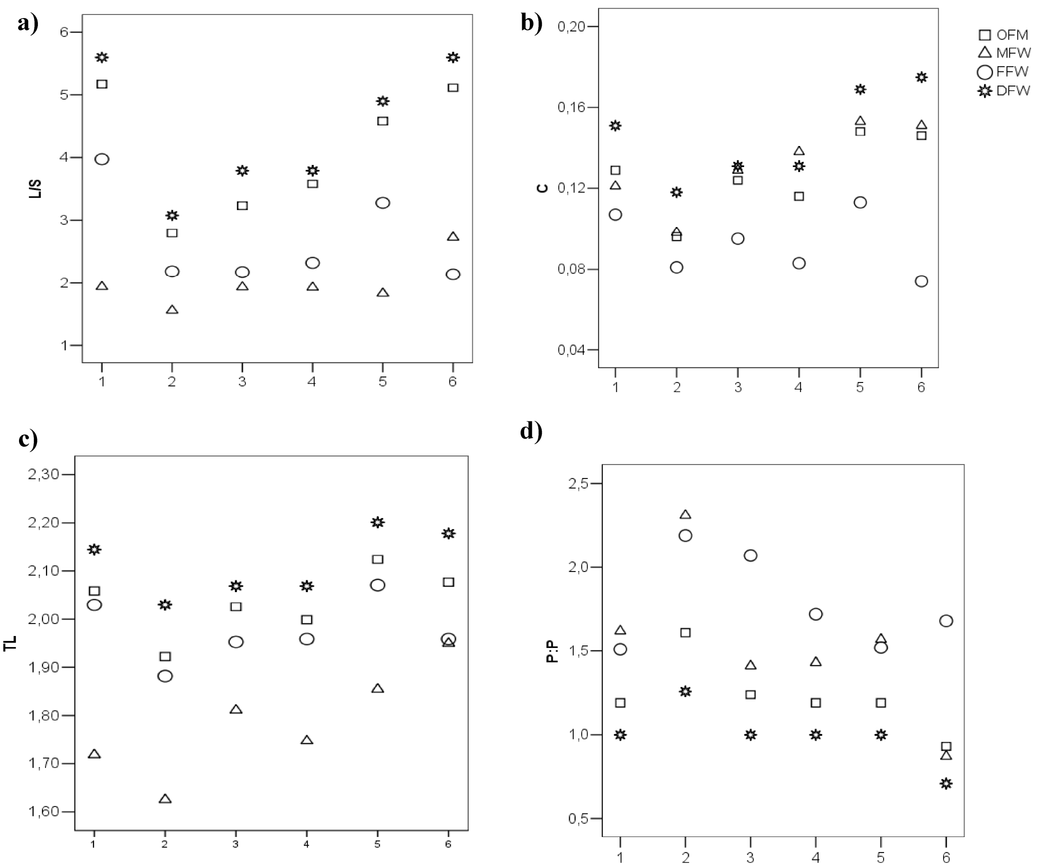

Figure 2. *Properties of trophic interaction richness, trophic level and prey: predator ratio (L/S, C, TL, PP) for each food web aggregation variation (OFW: , MFW:∆, FFW:○ and DFW: ☼) in each studied stream. (1 = Hozgarganta, 2 = Guadiaro, 3 = Guadiamar, 4 = Rivera de Cala, 5 = Odiel and 6 = Rivera de Chanza)*

Prey: predator ratio (P:P) was greatly affected by aggregation at different habit trophic levels (*Fig.2.d.*). When only basal species were aggregated (DFW), the result was a reduction in prey items, producing a P:P ratio lower than that of the original food web. Aggregation of the top and intermediate species (FFW and MFW, respectively) produced a reduction in the number of predators in the food web, increasing the P:P ratio relative to the original values of the control food web (OFW).

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The 24 constructed food webs were plotted together on an ordination graph according to the analyzed structural properties (*Fig. 3*). They are represented in clusters related to aggregation variations, but with no relation to stream effects: We found that the stream effect was insignificant (ANOSIM: Global Rho = 0.016, p = 0.367) while the effect of aggregation variations was found to be significant (ANOSIM: Global Rho = 0.625, p = 0.001). DFW showed the closest values to OFW. Thus, the results for the pairwise test in this analysis showed that no significant differences existed between the original extended food web (OFW) and the food web where different forms of detritus were aggregated into a single group (DFW) (*Table 2*).

Table 2. *Results of a pairwise test in Anosim analysis between aggregation variations. OFW: Original Food Web, FFW: Fish Food Web, MFW: Macroinvertebrate Food Web and DFW: Detritus Food Webs*

	Global Rho	***p***
OFW-MFB	0.741	*0.002*
OFW-FFW	0.331	*0.011*
OFW-DFW	0.035	0.264
MFW-FFW	1.000	*0.002*
MFW-DFW	0.896	*0.002*
FFW-DFW	0.552	*0.002*

In terms of empirical structural properties, percentages of similarity were high within and between different aggregation variations of food webs: values were found to be greater than 85% in all cases (*Table 3*). As had been expected, OFW and DFW comprised the pair of aggregation variations with the greatest similarity values. The main properties which explained dissimilarity between different food web variations were those related to the trophic habit of species: *T*, *I*, *B*, *Loop*, *Herb* and *Omn.*

The effects of aggregation on the estimation of empirical structural properties caused a variation in the relationship between food webs, within a variation type (*Table 4*). Therefore, the relationships between structural properties differed according to a given aggregation variation of the food web. One exception to this was the relationship between connectance and omnivory, which was significant and positive in all the food webs derived from the different aggregation variations.

Table 3. *Similarities in structural properties within and between different food web aggregation variations. Results are expressed as percentage similarity. OFW: Original food web, FFW: Fish food web, MFW: Macroinvertebrate food web and DFW: Detritus food web.*

	OFW	**FFW**	**MFW**	**DFW**
OFW	92.25			
FFW	90.91	94.59		
MFW	87.81	86.02	96.58	
DFW	92.67	90.05	87.50	93.33

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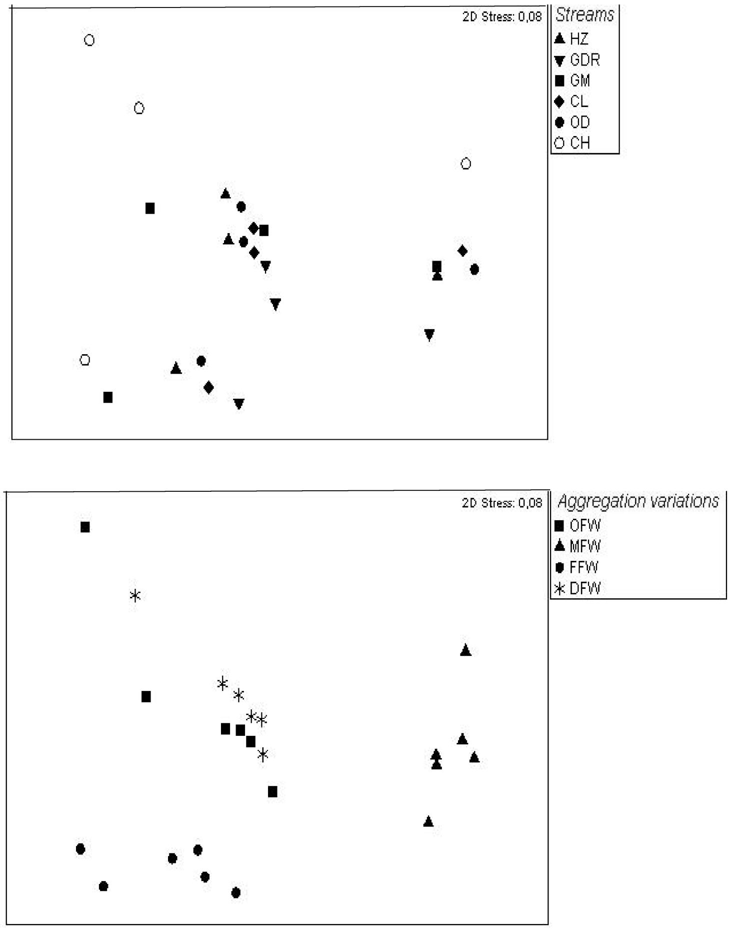

Figure 3. *Two-dimensional MDS ordination of 24 constructed food webs by their structural properties. Hierarchical agglomerative clustering was used (Bray-Curtis similarities) (up: labels according to streams; down: labels according to aggregation variations)*

Table 4. *Spearman rank correlation coefficient between selected structural properties in each food web aggregation variation. (Numbers shown in italics represent significant correlations). OFW: Original food web, FFW: Fish food web, MFW: Macroinvertebrate food web and DFW: Detritus food web.*p < 0,05 y **p < 0,01*

	OFW	FFW	MFW	DFW
Inv-L/S	_0.886*_	0.657	0.414	_0.841*_
Inv-C	0.657	0.429	-0.621	0.667
C-Omn	_0.886*_	_0.943**_	_0.943**_	_0.824*_
_C-L/S_	0.714	0.771	0.200	_0.824*_
_C-TL_	_0.999**_	0.667	_0.886*_	_0.941**_

Discussion

In general, it is neither possible to identify all the species, nor measure all the flows present within a food web. For this reason, it is broadly accepted that food webs can normally be aggregated into a manageable size for interpretation and analysis, and many studies have focused on the effects of taxonomic aggregation on food web properties (Yodzis and Winemiller, 1999; Jordan, 2003; Ludczkovich et al., 2003; Krause et al., 2003).

Omnivory and weak interactions between detritivorous and detritus play stabilizing roles in the food web of Mediterranean streams. In this work, we review food web structure in such streams and demonstrate that the lumping of their components and the level at which this occurs has a great impact upon the properties of the empirical structure of the food web. Utilization of the same resolution for analysis in different streams allowed these properties to be comparable within these ecosystems. Thus, the intuitive belief that better information is gained from looking more closely (increasing the level of resolution) would only be valid if standard levels of resolution can be maintained across different studies (Thompson and Townsend, 2000).

As expected, the original (control) food web maintained the highest *S* values. A decrease in resolution produced a concomitant reduction in the number of food web elements and it may be that the change in connectance reflects smaller web size rather than a change in resolution *per se.* (Thompson and Townsend, 2000). In our study, the food web aggregation variation that joined macroinvertebrates in five functional feeding groups (MFW) had values of connectance cosest to those of the extended original food web (OFW). In spite of this, these types of food webs differed mostly in terms of the number of total elements. We suggest that here, food web size did not dramatically affect connectance when most of grouped species were detritivorous with weak interactions with the detritus.

Our study achieved an appropriate resolution at the macroinvertebrate and fish level; however, we were not able to include meiofauna or bacteria, and the periphyton algae were left as a single group. Some studies have demonstrated the importance of meiofauna in food webs (Schmid-Araya and Schmid, 2000; Schmid-Araya et al., 2002). In these studies, the inclusion of meiofauna in the food web analysis produced increased complexity and pattern modification. However, although it is important to increase the accuracy of analysis, it is not always feasible to describe all the trophic components in exhaustive detail. Taxa clustering effects prey: predator ratios such that failure to resolve lower trophic levels adequately is likely to produce the false impression of food webs being very "square" in structure (featuring low prey: predator ratios) and this can obscure any dietary specialisation occurring among primary consumers (Thompson and Townsend, 2000).

As Yodzis and Winemiller (1999) pointed out, the main issue is to clarify the constraints of the community and be rigorous in food web depiction, which is a reliable method for producing comparable food webs. In this study, we considered clustering the periphyton algae into a single group in all the food web aggregation variations. However, we observed that lumping another basal species instead (the different forms of detritus) had the same effect as expected by the grouping of periphyton algae, i.e. lower prey: predator ratio values.

The type of grouping carried out and the level (i.e. with species of superior, intermediate or basal level) at which it is done, proved to be important and therefore must be taken into account. We suggest that, in order to study food web structure in Mediterranean streams, it is advisable to utilize a resolution which goes beyond considering superior and intermediate taxa as one or more groups. Authors such as Haven et al. (1996) point out that conducting studies that consider all trophic levels is necessary for correct analysis of food webs. However, most studies performed in streams have been designed with a special emphasis upon macroinvertebrates only (Closs and Lake, 1994; Tavares-Cromar and Williams, 1996; Corigliano and Malpassi 1998; Thompson and Townsend, 1999). Hence, we propose that fish should be included

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in the food web at a resolution level of species instead of simply being considered as a single group. The use of this aggregation is common practice in this type of ecosystems that produce values of structural properties that differ from the original values.

In general, the properties of those food web aggregation variations which lumped different forms of detritus into a single group were those most similar to original values. This outcome suggests that considering different forms of detritus as a single compartment could produce a reliable estimate for assessing food web topology. However, Allesina et al. (2002) suggest that considering detritus as one compartment would result in the neglect of important information regarding ecosystem functioning. We need to focus further on this issue to obtain more conclusive results; however, according to our findings in Mediterranean streams, the consideration of detritus as a single group did not significantly affect the general food web structure.

An interesting observation arising from this study was that aggregation did not affect one of the clearest and most relevant relationships in Mediterranean stream food webs- that which exists between omnivory and connectance. Omnivory, defined broadly as feeding on more than one trophic level, occupies a prominent position in discussions concerning the structure and dynamics of food webs. Classical conceptual syntheses suggest that omnivory should be a strongly stabilizing factor in food webs (McCann et al., 1998), and the stabilizing role of omnivory has been the topic of more recent works (Melián and Bascompte, 2002; Bascompte and Melián, 2005). The properties of connectance and omnivory were both positive and significantly related in all the food web aggregation variations, suggesting that the stabilizing role of omnivory is maintained regardless of which part of the ecosystem is under investigation.

Our study contributes to understanding the consequences of lumping species into groups of convenience in the assessment of food webs. The grouping of some food web components affects structural properties and the relationships that exist between them. The only connection unaffected by this practice is that which exists between connectance and omnivory. We recognize that more comprehensive data is required to draw fully conclusive results; however, we suggest that aggregating fish or macroinvertebrates into large groups could produce the greatest modification to the overall structure of the food webs in these rivers. Thus, when drawing conclusions about general trophic structure, investigators should consider how the components of the ecosystem have been grouped.

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THE EFFECT OF CLIMATE CHANGE ON THE PHENOLOGY OF MOTH ABUNDANCE AND DIVERSITY

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Abstract. When examining the ecological effects of climate change those databases which contain monitoring results of long-term time series have a key role. We used the daily Lepidoptera data of the Hungarian Plant Protection and Forestry Light Trap Network between 1962 and 2006 to analyse the influence of the change of temperature on the ecological dynamics of Lepidoptera. Using the quantile regression analyses we concluded that in the examined period, for the total number of individuals, seasonal phenomena shifted forward, however the days with higher diversity occurred over a shorter period. The analysis of the heat sums shows that the spring warming started earlier, although, at the same time the low quantiles of the captured Lepidoptera occurred at lower and lower heat sums over the years, while the heat sums at which the high quantiles of the number of Lepidoptera occurred showed a steady increase, meaning that the active period of the Lepidoptera community has expanded. A strong correlation was observed between diversity and temperature.
Keywords: *biodiversity, quantile regression, climate change, Lepidoptera, light trap*

Introduction

Since the last decades the effects of climate change on the dynamical behaviour and the size of Lepidoptera populations have become more and more obvious. Seasonal community dynamics (ecological processes) are, out of the meteorological parameters, primarily influenced by temperature.

Our objective was to examine the effects of temperature change on community dynamics. We chose the light-attracted species assembly of Lepidoptera, which is an adequate indicator of the above ecological phenomena (Hufnagel et al., 2008).

The data was collected from the database of the Hungarian Plant Protection and Forestry Light Trap Network. The description of the database is presented by Gimesi and Hufnagel (2010). This choice had been motivated by the national coverage of the light trap network, the large number of captured Lepidoptera individuals, and also by the availability of a long-term data series (between 1962 and 2006).

Various methods exist that demonstrate changes in a community: different diversity indices, gradations, species abundance models, quantile regression. We chose quantile regression for our study, which is ideal for examining population dynamics and demonstrating changes (Cade et al., 2005; Helmus et al., 2007). Linear regression demonstrates the ratio of occuring changes in a simple way (Chamaillé-Jammes et al., 2007). With the help of this method we observed that both heat sum and the appearance of species shifted earlier and earlier over the years.

Many authors agree on the seasonal dependence of community dynamics, regardless of the climate (temperate or tropical) of the area where the research was carried out. Szabó et al. (2007) used the number of species and the number of individuals for the demonstration of seasonal changes, while Fisher at al. (1943) used a diversity index. Species abundance models are also suitable for the examination of seasonal dynamics. With the help of the above methods different periods can be well separated.

We used the traditional two-dimensional time series figures for the presentation of seasonal changes, in which the seasonal dependence of population dynamics can be seen clearly.

Review of literature

The most common method of collecting night-flying insects is light trapping. This method has been applied since Williams' experiments (1935). In Hungary light traps have been used since 1940; in 1952 the construction of a worldwide unique trap network began (Jermy, 1961; Nowinszky, 2003a). Today, the Hungarian light trap network is equipped uniformly with Jermy-type light traps.

In order to discover the occurring changes and their tendencies in a data series, a long time series (daily data series) is needed, with great geographical coverage, and data collection needs to be done with the same method along the way, in every location. The data series of the Hungarian Plant Protection and Forestry Light Trap Network meets these requirements (Hufnagel et al., 2008).

For the investigation of population dynamics those light traps are best suited which have been operating for the longest time without interruption, in the same place (Nowinszky, 2003b). Also because of the effects of different abiotic factors, the largest possible number of light trap data coming from different locations should be used. This ensures that the effects occurring at different capturing sites, and modifying the number of captures, compensate each other (Nowinszky, 2003b). A large quantity of data, coming from various sources, can be processed with data mining methods. In our case the detailed description of the database has been reviewed by Gimesi and Hufnagel (2010).

The number of Lepidoptera captured significantly depends on several biotic and abiotic environmental factors. Naturally, the different environmental effects do not appear independently from each other, but mostly in interaction (Nowinsky, 2003b). A major part of the literature (Nowinszky, 1977; Persson, 1976; Rácz and Bernáth, 1993; Williams, 1962) primarily studies the elements of weather and their combined effects.

In Hungary the correlations between the number of captured individuals and weather elements have been investigated since the end of 1950s. Nowinsky et al. (2003) came to the conclusion, based on the works of several authors, that temperature had a fundamental role in flying activity, which derives from the physiological features of Lepidoptera. This assumption is supported by Kádár and Erdélyi (1991), as well as by Schmera (2002), among others.

Nowadays several diversity indices are used for the characterization of diversity. Among these Shannon-entropy is used the most frequently (Juhász-Nagy, 1993). A major part of the literature (e.g. Acara et al., 2007; Arnan et al., 2009; Balog et al., 2008; Chefaoui and Lobo, 2008; Kevan, 1999; Skalskia and Pośpiech, 2006; Suzuki et al., 2002) applies primarily this index.

Besides species diversity investigations, species abundance models have also been worked out (Izsák and Szeidl, 2009). They examine the number of individuals (abundance) within different species that are part of the studied community (Magurran, 1988; Bartha et al., 2007).

Besides these two approaches, quantile regression is also used, which is an effective tool for the demonstration of changes (Cade at al., 2005; Helmus, et al., 2007). Linear regression demonstrates the ratio of occuring changes in a simple way (Chamaillé-Jammes et al., 2007). With the help of quantile regression such correlations can be discovered, that cannot be with traditional statistical methods.

In case of quantile regression the changes of a given proportion of the measurement data are demonstrated. Similar analyses were carried out by Austin (2007), Anderson (2008) and Cade and Noon (2003), as well as Koenker and Hallock (2001). Kovács et al. (2009) used linear quantile regression for the demonstration of population dynamics.

Williams and Liebhold (2002) drew attention to the fact that insects were affected by even a relatively small climate change. Williams (1940) investigated the long-range effects of weather on population changes, and its short-range effects on the activity.

With the increase of temperature due to climate change, the living areas of species get shifted north (Virtanen and Neuvonen, 1999). Continental species are shifted towards the poles by an average of 6.1 kilometers every 10 years, or every 6 meters higher in the mountains (Parmesan and Yohe, 2003).

Environmental effects do not only influence the given year, but also affect the future development of populations, as it is referred to by Hufnagel et al. (2008). Therefore, besides seasonal change, numerous publications deal with long-term tendencies and the effects of climate change. According to Tobin et al. (2008) the number of pest insects increases with the rise of temperature, which can have significant ecological and economic consequences.

Wolda et al. (1998) described the yearly seasonal changes of Lepidoptera captured with light traps at different geographical locations, and studied the connection between these and different meteorological parameters. They found that most species were characterized by seasonality, even in tropical environment, which showed similar patterns in consecutive years. This phenomena is also supported by Caldas (1992), who, based on a 1-year data series, explained seasonal dependence with the change in precipitation, although he has not investigated temperature.

In Japan, Kimura et al. (2008), examining Trichoptera species, found seasonal dependence as well as a significant correlation between abundance and temperature in spite of the fact that the abundance maximum of the insects they observed occured in September, while the temperature maximum were in August.

Schmera (2002) examined Trichoptera captured with light traps from May to October. To present seasonal dependence he used the species gradation figure, the Rényi-type diversity index and the right tail sum, and found that no significant difference could be seen in the data figures between May and September, however October was significantly different from the other months.

Researches by Szabó et al. (2007) on the fauna change of Macroheterocera captured over an 8-year period showed the seasonal dependence of the number of species and individuals. According to it, a smaller peak was reached at the end of March and in the beginning of September, while in late May and early July there were higher ones. At the end of June there was a decline.

In England, Fisher et al. (1943) studied a 4- year time series of Macrolepidoptera captured with light traps, from April to October, and depicted seasonal changes on a graph indicating the number of species and individuals as well as Fisher’s alpha value.

Material and method

In the course of our work the database of the Hungarian Plant Protection and Forestry Light Trap Network was used, whose first light traps 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 with snow (Nowinszky, 2003a).

The collection data of the Hungarian Plant Protection and Forestry Light Trap Network were analysed with data-mining methods.

Due to the effects of various abiotic factors it seemed reasonable to work with the maximum number of data, so that the effects occurring at different capturing sites, modifying the number of captures, could compensate each other (Nowinszky, 2003b). To produce a national time series the trap data found at different locations needed to be merged for each species. After the work of Moon and Kim (2007) this process is called data reduction.

To filter out extremes occurring in the databases and in order to reduce fluctuations in the data series the moving average method was used. When calculating the moving-average we considered the 4-4 adjacent daily data as well, that is the average of 9 days (9^{th} grade moving average). Number 9 was chosen, because it equals to the number of traps. After calculating the average, a 16425-line database was created containing the daily data series of 281 Lepidoptera species captured between 1962 and 2006.

To create the insect data warehouse, for data filtering, and for further data processing a Visual Basic software was created. Using this programming language Excel and Access files could be managed directly.

As meteorological data source the “kutdiak” database (Ferenczy, 2008) and the “KKT” database (Szenteleki, 2007; Szenteleki et al., 2007) were used. The second one, in addition to meteorological data, contains the geographical coordinates of Hungarian cities. For the filtering and checking of the databases, data published on the website of the National Meteorological Service (2008) were used.

In Hungary, the connections between captured individuals and the elements of weather have been investigated since the late 1950s. In the literature it is a generally accepted fact that flying activity, and so the number of captures is primarily influenced by temperature. Precipitation, wind and other environmental effects influence it only to a small extent and only locally.

To study community changes, the method of linear quantile regression was used and for our analyses the GRETL (GNU Regression, Econometric and Time-series Library) (Cottrell and Lucchetti, 2009) and Microsoft Excel programs were used.

Results

One of the methods to investigate community changes is the calculation of linear quantile regression, where the changes of a given ratio (10, 20, 30, 40, 50, 60, 70, 80, 90%) of the measurement data are characterized by a linear quantile regression.

Examination of the number of captured individuals

The days of the year, on which the number of captured individuals reached 10, 20…90% of the annual sum were investigated.

Due to the fact that in the years 1972 and 1973 trapping and filling the database were uncertain, we studied the periods between 1962 and 2006 (*Fig. 1*), and 1974 and 2006 (*Fig. 2*) separately.

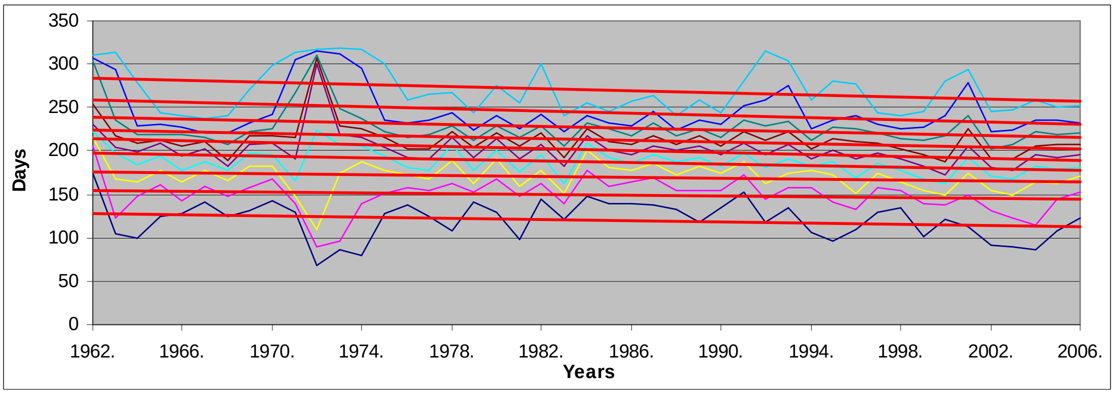

Figure 1. *The linear quantile regressions of the number of individuals between 1962 and 2006*

Table 1. *Analyses of the regression lines*

Quotient	Coefficient	Standard error
10%	-0.349	0.245
20%	-0.215	0.227
30%	-0.268	0.190
40%	-0.476	0.150
50%	-0.557	0.208
60%	-0.486	0.206
70%	-0.544	0.229
80%	-0.622	0.298
90%	-0.594	0.296

The regression coefficient of all regression lines are negative, which shows that the captures were getting earlier.

10% of the annual sum of the number of individuals was reached 15 days earlier in 2006 than in 1962, while 90% of it was reached 26 days earlier. The ratios shifted 20 days earlier on average.

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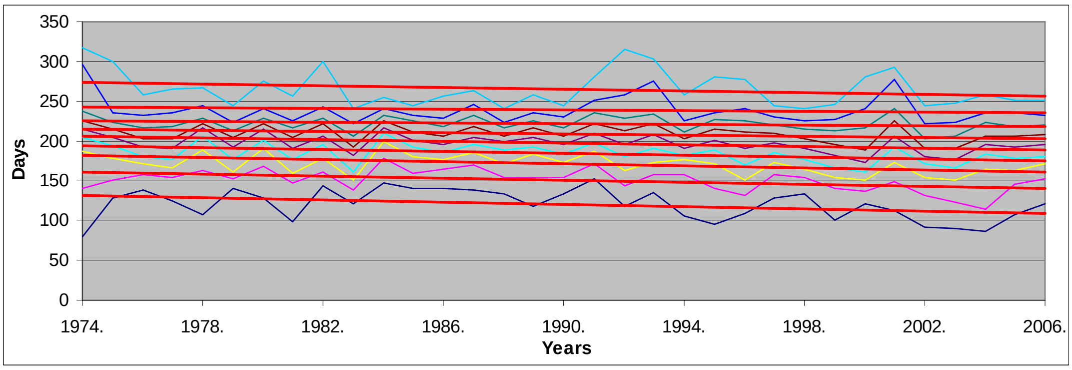

Figure 2. *The linear quantile regressions of the number of individuals between 1974 and 2006*

Table 2. *Analyses of the regression lines*

Quotient	Coefficient	Standard error
10%	-0.658	0.337
20%	-0.691	0.227
30%	-0.627	0.213
40%	-0.580	0.206
50%	-0.516	0.187
60%	-0.391	0.186
70%	-0.194	0.170
80%	-0.223	0.310
90%	-0.540	0.407

Negative steepness (coefficient) of the regression lines was observed here as well. 10% of the annual sum of the number of individuals was reached 21 days earlier in 2006 than in 1974, while 90% of it was reached 18 days earlier. The ratios shifted 16 days earlier on average.

Investigation of the heat sum

The heat sum was investigated as a phenological indicator. The days of the year, on which the heat sum reached 10, 20…90% of the annual sum of daily mean temperatures (annual heat sum) were analysed.

As the minimum temperature in the examined period was -12.6°C data needed to be transformed, when calculating the heat sum, in a way that 13°C was added to each temperature value. This way we could avoid counting with negative values. The quantile regression lines received after the calculation is shown in *Figure 3*.

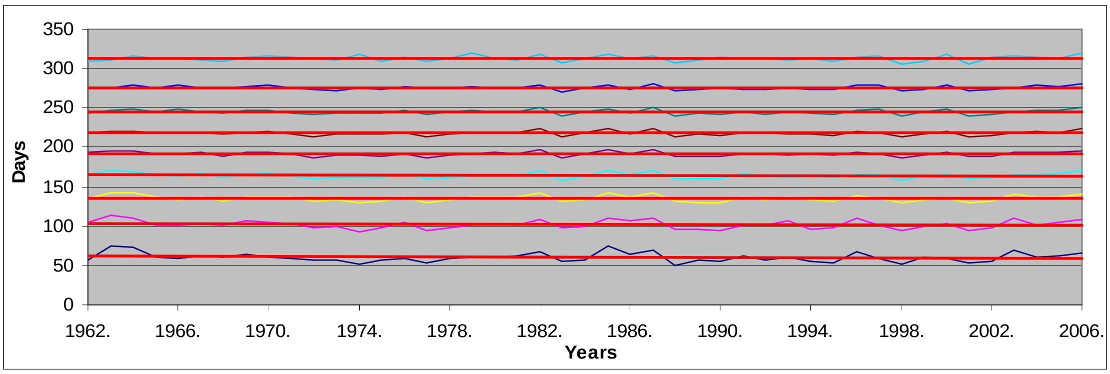

Figure 3. *The linear quantile regressions of the heat sum between 1962 and 2006*

Table 3. *Analyses of the regression lines:*

Quotient	Coefficient	Standard error
10%	-0.059	0.068
20%	-0.046	0.060
30%	-0.031	0.045
40%	-0.024	0.039
50%	-0.013	0.033
60%	0.002	0.030
70%	-0.014	0.033
80%	-0.006	0.031
90%	0.009	0.039

At the lower percentages of heat sum a negative steepness was observed, meaning that it started getting warm earlier. In case of the higher percentages the steepness is or is around zero (sometimes positive, sometimes negative). The above different tendencies of the regression lines indicate that the warm period was extending while the cold (winter) one was getting shorter.

10% of the annual heat sum was reached 2.5 days earlier in 2006 than in 1962, while 90% of it was reached on the same day.

Connection between heat sum and the number of captured individuals

That heat sum was investigated, at which the number of captured individuals reached 1, 2…10% of the total annual captures (*Fig. 4*), as well as 10, 20…90% of it (*Fig. 5*).

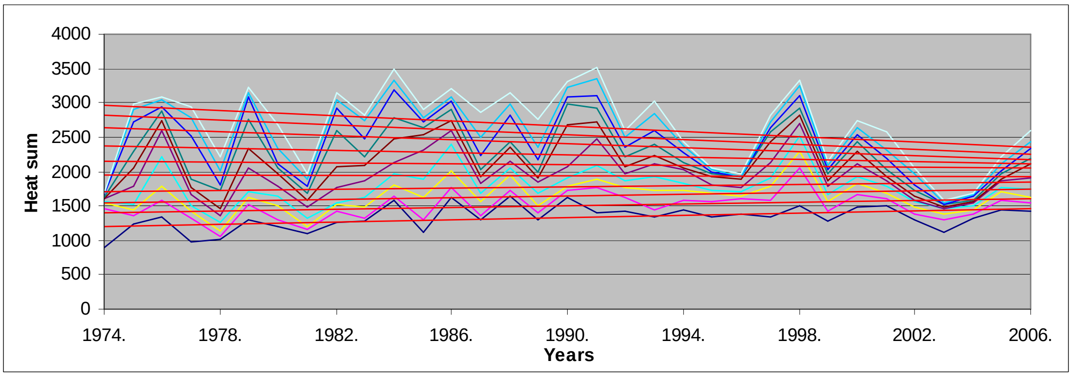

Figure 4. *The 1, 2…10% linear quantile regressions of heat sum – number of captured individuals between 1974 and 2006*

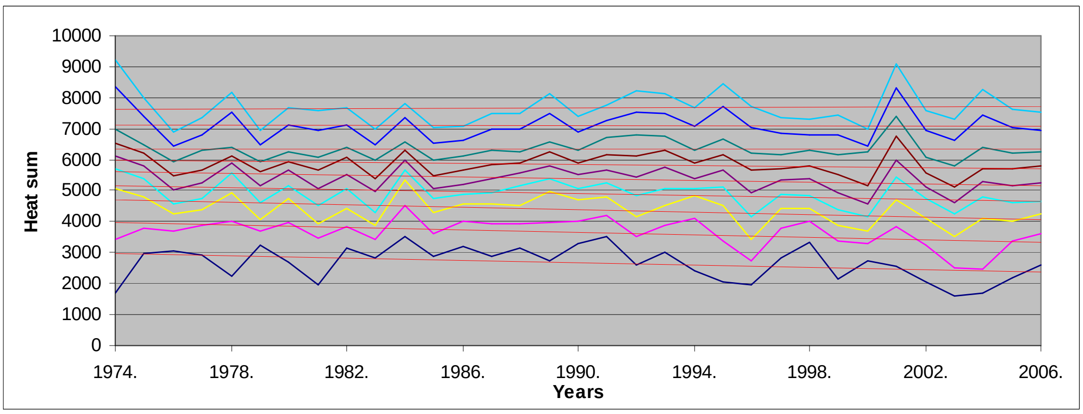

Figure 5. *The 10, 20…90% linear quantile regressions of heat sum – number of captured individuals between 1974 and 2006*

Table 4. *Analyses of the regression lines:*

Quotient	Coefficient	Standard error	Quotient	Coefficient	Standard error
1%	8.272	3.054	10%	-18.875	9.538
2%	6.440	3.521	20%	-20.878	7.588
3%	6.147	4.085	30%	-18.993	7.477
4%	4.286	5.099	40%	-16.287	7.180
5%	-0.552	6.162	50%	-13.623	6.653
6%	-3.372	7.239	60%	-8.013	6.686
7%	-8.401	8.072	70%	0.497	6.143
8%	-14.992	8.848	80%	-0.993	8.934
9%	-17.744	9.195	90%	2.193	10.329

In the analyses it is seen that for the appearance of Lepidoptera, under the 4% quantile greater and greater heat sum was needed over the years (see the 4 lowest lines in *Fig.4*). However, for further captures less and less heat sum was required (*Fig.5*).

Investigation of a diversity index

The days on which the Shannon-diversity index reached 10, 20…90% of the maximum value in the given year (between 1974 and 2006) were examined (*Fig. 6*).

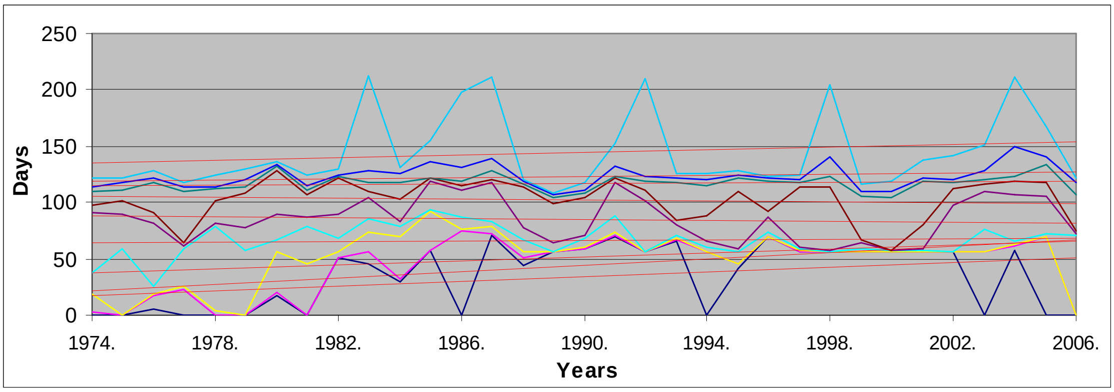

Figure 6. *The 10, 20…90% linear quantile regressions of the diversity index between 1974 and 2006*

Table 5. *Analyses of the regression lines*

Quotient	Coefficient	Standard error
10%	1.068	0.481
20%	1.447	0.384
30%	0.884	0.421
40%	0.110	0.266
50%	-0.233	0.361
60%	-0.227	0.333
70%	0.090	0.137
80%	0.240	0.184
90%	0.594	0.605

Diversity index increased until the 50 and 60% quantiles, where the steepness became negative indicating a decrease in the diversity index. After this an ascending tendency can be seen again.

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Seasonal dependence

The averages of daily mean temperatures, diversity indices, the numbers of individuals and species concerning the same days of the years within the period between 1974 and 2006 are shown in Figure 7. It presents how different values changed as a function of the day of the year (for the sake of better visualisation, values were weighted to obtain similar scaling).

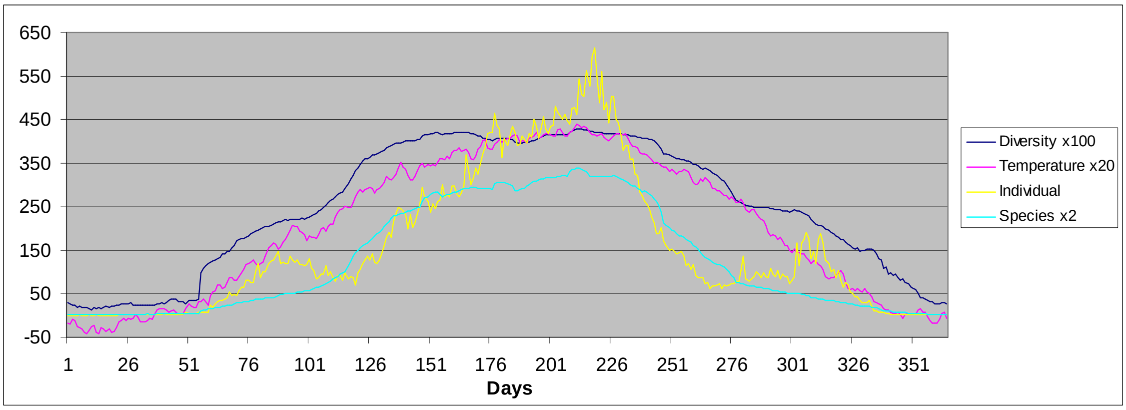

Figure 7. *The averages of temperatures, diversity indices, the numbers of individuals and species concerning the same days of the years within the period between 1974 and 2006*

Although temperature increased till the end of February as it is shown in *Figure 7,* diversity index was low and relatively constant during the winter period. After that (at spring) it started increasing significantly and stayed relatively constant during the summer, apart from a decline in the middle of summer. After September the diversity index started decreasing.

According to *Fig.7* there were two smaller peaks in the number of individuals in the early spring and late autumn, besides the regular summer maximum. These two rises were caused by species appearing only in these periods. The number of individuals reached its maximum when the temperature had already begun to decline.

The graph of the number of species begins to rise more significantly towards the end of spring. It reaches its maximum in the second half of July, after a small increase, where it begins to decline sharply. The maximums of the number of species and the temperature approximately coincided.

Figure 8 shows the values of daily mean temperature of 33 years as a function of the days of the year, and *Figure 9* shows diversity values in the same way.

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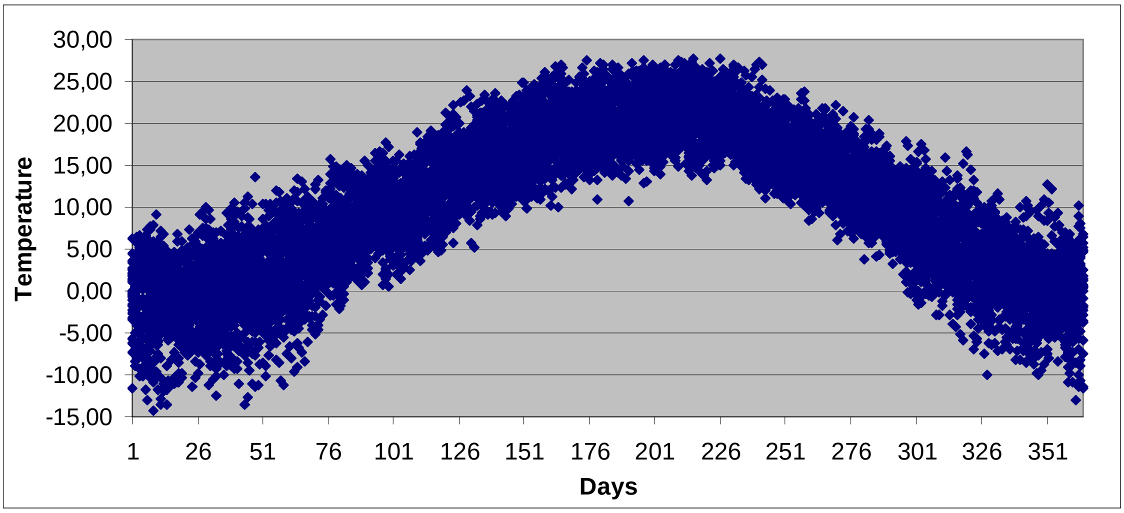

Figure 8. *Daily mean temperatures in the years between 1974 and 2006*

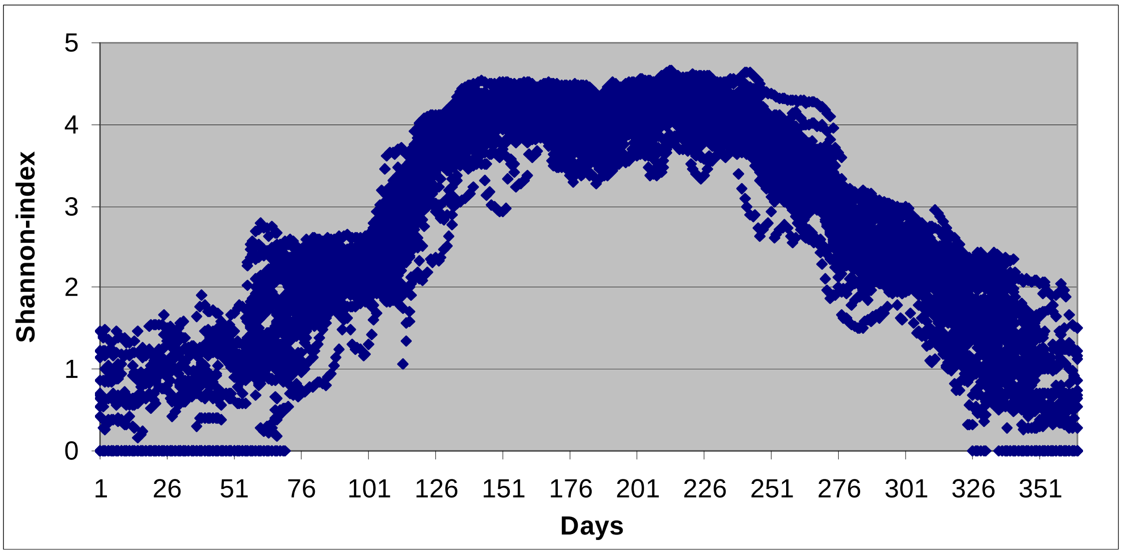

Figure 9. *Daily diversity indices in the years between 1974 and 2006*

It can be seen that when the standard deviation of temperature is greater (at winter, early spring and late fall), the standard deviation of the diversity index is more significant as well. The correlation between these two values is 0.6953. *Figure 10* shows the standard deviations of the temperature and of the diversity index.

It can be seen in *Figure 9* that there were days in January, February and December, when the diversity index was zero, which means that there were no captures on those days. This cannot be observed on the other days of the year.

Furthermore, it can also be seen that the value of diversity was smaller at a lower temperature, and greater when the temperature was higher, although diversity only rised with temperature until a certain point. At the beginning of July even a decline was observed.

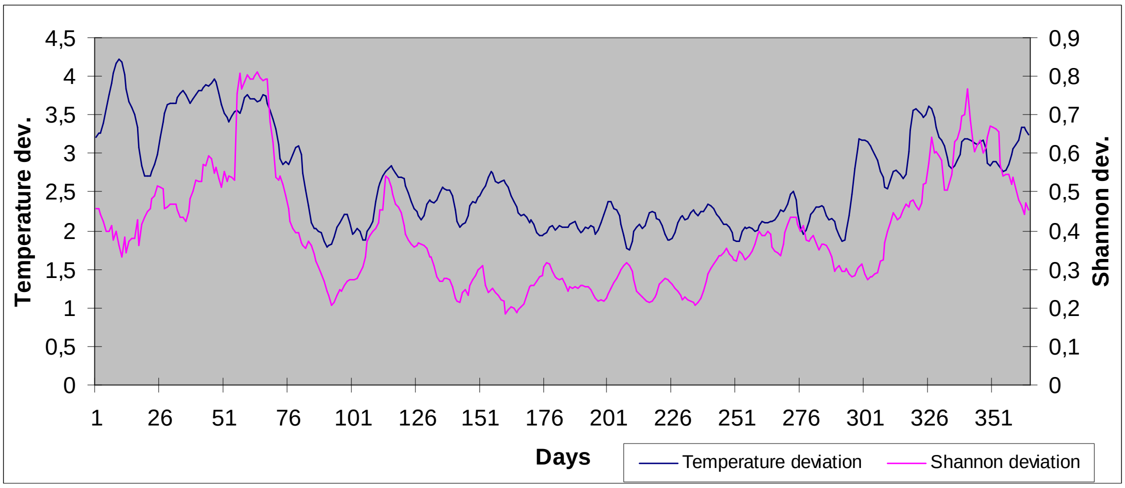

Figure 10. *The standard deviations of temperature and diversity index in the years between 1974 and 2006*

Figure 11 shows the connection between temperature and diversity. It is obvious that there is a significant (0.9815) correlation between them.

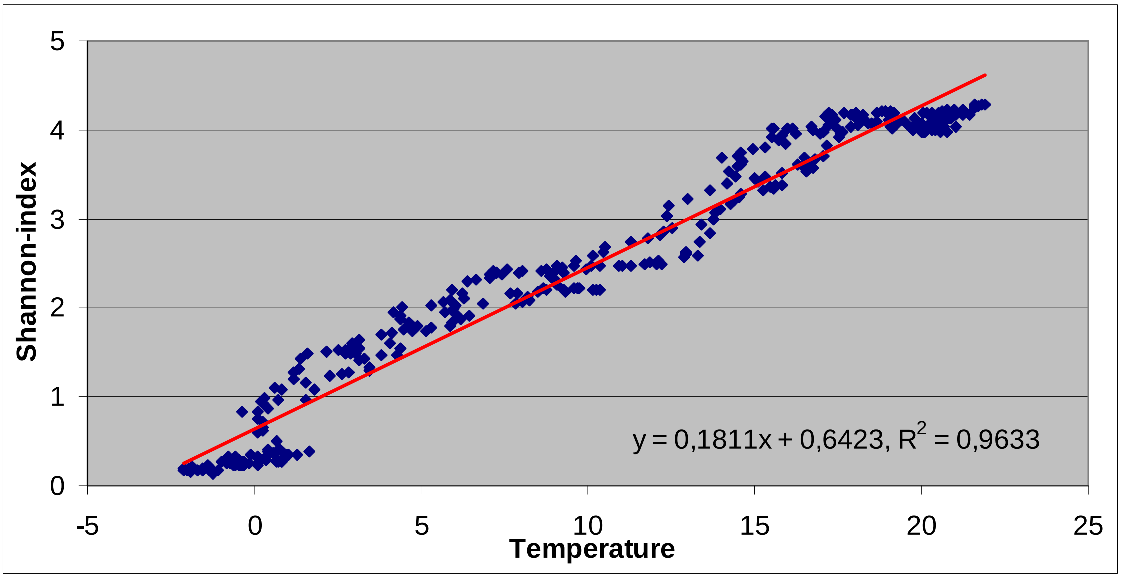

Figure 11. *The change of diversity index as a function of temperature calculated with the averages of the years between 1974 and 2006*

Examining the standard deviation of temperature and the diversity index on *Figure 12*, it can be seen that as the standard deviation of temperature increased, the diversity index decreased. These two values are negatively correlated (-0.7949).

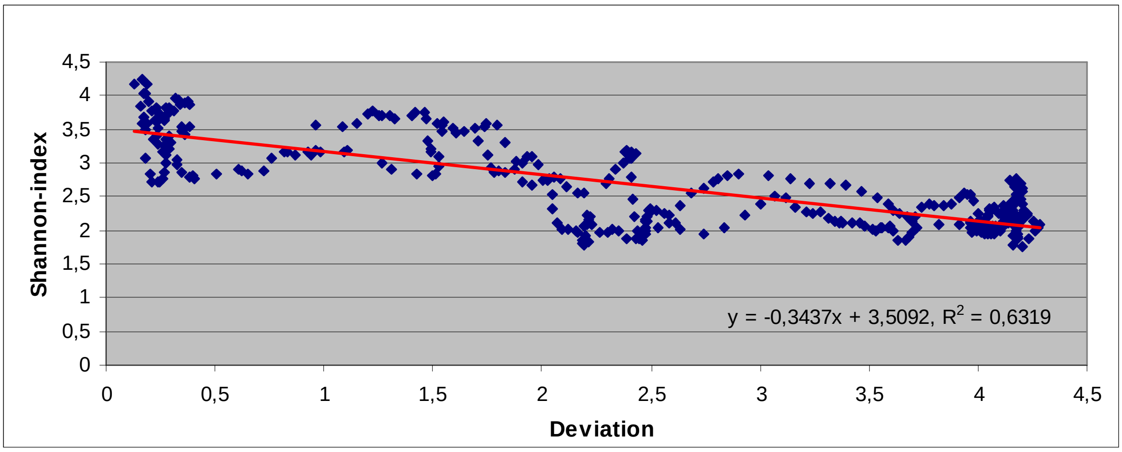

Figure 12. *Change of diversity index as a function of the standard deviation of temperature calculated with the averages of the years between 1974 and 2006*

Discussion

The database compiled by us from the data of the Hungarian Plant Protection and Forestry Light Trap Network is suitable for application in important research areas of the national light trapping summarized by Szentkirályi (2002), for fauna, zoogeographical, taxonomic, zoocoenological, ethological, phenological, ecological, etc. examinations (Nowinszky, 2003b).

In our present study the long-term seasonal changes of the data were examined. Investigating the change in the number of individuals we found that the appearance of species is approximately 2-3 weeks earlier than 30 years ago. Similar changes were observed by Kovács et al. (2009), who, examining the migration of birds, experienced an almost one week shift earlier over 24 years.

No significant changes happened during the investigated period of the exceeding days of the 10, 20…90% of the annual heat sum, however it was found that the exceeding days of the lower percentages had negative tendency, while the exceeding days of higher percentages had a tendency around zero, which altogether refers to the extension of the growing season. This is consistent with the forward shift of the seasonal pattern of the total number of Lepidoptera individuals.

However, our analyses of the diversity indices showed that the increase of the different diversity index values shifted forward.

Examining the correlation between the capturing data of Lepidoptera and the daily mean temperatures we found that the values of daily mean temperature and the daily diversity closely correlated (*Fig. 11*), which corresponds to what Ferenczy et al. (2010) observed. However, diversity and the standard deviation of temperature showed negative correlation (*Fig. 12*).

Some authors reported an increase (e.g. Bazzaz, 1975), while others a decrease in the number of individuals (e.g. Nowinszky et al., 2003) as a function of temperature. Taylor, (1963) found that flying and, as a consequence, capturing had both lower and upper temperature threshold values. This statement was supported by our investigations (*Fig. 8*), i.e. that in the warmest summer period the diversity value did not increase, even a slight decrease could be observed. Relying upon these findings we came to the conclusion that as an effect of climate change, the number of Lepidoptera and consequently their diversity would decrease. This is also supported by the long-range model developed by Drégelyi-Kiss et al. (2010) and Hufnagel et al. (2008).

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NATURAL REGENERATION STATUS OF CHILGOZA PINE (*PINUS GERARDIANA* WALL.) IN HIMACHAL PRADESH, INDIA: AN ENDANGERED PINE OF HIGH EDIBLE VALUE

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Abstract. *Pinus gerardiana* Wall. locally known as ‘chilgoza’ is near endemic and endangered to Himalayan region. The species has aptly been described as the “*Champion of Rocky Mountains*” as it grows under extremely rough site conditions. Being a flavorsome wild edible, the chilgoza pine nuts have high demand in local, national and international markets and fetch very high prices. Due to rampant harvesting of cones, natural regeneration of this species is being fatally hampered. In present study, the natural regeneration assessment of the species was conducted in the forests of Kinnuar of Himachal Pradesh, India. The whole distribution area of the species was divided into three zones, *viz*., Dry temperate, Semi arid and Arid. The sampling plots of 20m × 20m were selected in which the regeneration sampling quadrates of 2m × 2m were laid in the eastern and western bank of river Sutlej. The results revealed that the semiarid zone on the western aspect of the area have maximum established regeneration of the species (291.66 plants/ha). Overall, the mean natural regeneration status of the species was very poor (15%). Thus, the species is facing higher risk of extinction and needs to be considered as ‘Critically Endangered’ in Indian Himalayan Region. It is suggested here that a suitable strategy and action plan including sustainable harvesting methods should be prepared and implemented on priority basis.
Keywords: *Pinus gerardiana, natural regeneration, status, endangered, Himachal Pradesh*

Introduction

Conifers are valuable natural resources of India, which contribute substantially to its socio-economic development by providing goods and services to the people and industries. They generate considerable revenue and also play a major role in enhancing the quality of environment by influencing the basic life support system. One of them, *Pinus gerardiana* Wall. named after its discoverer, Captain Gerard, is a small to medium sized evergreen tree. Its branches are short and horizontal forming compact habit, while bark is thin, glabrous, silver grey, having mottled appearance and often exfoliating in irregular, thin scales (Gupta and Sharma, 1975). Cones are oblong, ovoid and glaucous when mature while scales are thick, woody and reflexed (Gamble, 1902). The species is commonly known as “chilgoza or neoza pine”. Its distribution is very sparse in the world, confined only to mountains of eastern Afghanistan, Pakistan, India and other scattered localities in the Hindu Kush Himalaya (30^o to 37^o N latitude and 66^o

to 80^o E longitude). In India, it occurs in North-west Himalaya ranges from 31^o 55' to 32^{o}05' N latitude and 77^o 45' to 79^{o}35' E longitude (Chib, 1978) and grows between 1600 and 3300 m amsl. It is the only conifer in India which provides edible nuts/kernels rich in carbohydrate, proteins, fat, moisture, fiber and mineral matter. The chilgoza pine nuts fetch very high price ranging from Rs. 400-650/kg in the open market (*personal survey, 2005-07*) (Malik, 2007) and play an important role in socio-economic uplift of the people in tribal areas of Himachal Pradesh and Jammu and Kashmir (Sehgal and Khosla, 1986). The species has aptly been described as the "*Champion of Rocky Mountains*" as it grows under difficult sites conditions as prevailing in the inner Himalaya. The species occurs in dry temperate region experiences low temperature and scanty precipitation received mostly in the form of snow during winter. Besides, the species is an excellent soil binder and prevent large scale soil erosion from the otherwise lose and fragile strata in the region. The area under chilgoza forest has already shrunk to about 2000 hectares in Himachal Pradesh because each and every cone is lopped by local peoples as they have the right of chilgoza collection from natural forest, leaving very little for natural regeneration (Tandon, 1963; Singh *et al*. 1973; Sehgal and Sharma, 1989). The species is facing higher risk of extinction and therefore, categorized as Endangered in the Himalayan region (Sehgal and Sharma, 1989) and also listed in Red Data Book (Dogra, 1964). A detailed perusal of literature indicated that the information on propagation, storage condition, biochemical attributes of seeds and nursery development of the species is available (Malik, 2007; Singh and Chaudhary, 1993; Singh *et al*. 1992; Sharma, 2005; Malik *et al*. 2008; Malik and Shamet, 2008; Malik *et al*. 2009). However, there is very little information available on natural regeneration of the species (Singh et al. 1973; Sehgal and Sharma, 1989; Reddy, 1963). The understory plant regeneration is important and has increasingly been the subject of research in recent years (Leoppold *et al*. 2001; Smale *et al*. 2001; Hardwick *et al*. 2004; Carnus et al. 1979). Keeping this in view, need is being felt to study the present status of natural regeneration of the species so that effective steps can be taken for its conservation and sustainable utilization. The main objective of this study is therefore, to assess whether or not there is adequate regeneration (seedling or established growth) of this species in Kinnuar, Himachal Pradesh, India.

Figure 1. *Map of Kinnaur district distribution zone of Pinus gerardiana*

Description of study area

The present study was carried out in chilgoza forest in district Kinnaur covering the whole distribution zone of species. The area lies in the dry temperate zone which is located in the north-eastern part of Himachal Pradesh, India (*Fig. 1*).

The study area exhibits dry temperate climate characterized by long winters from October to April and short summers from June to August. Though rain is scanty, precipitation is received mostly in the form of snow during winter. The data pertaining to meteorological conditions of the area during the study period are presented (*Fig. 2a & 2b*).

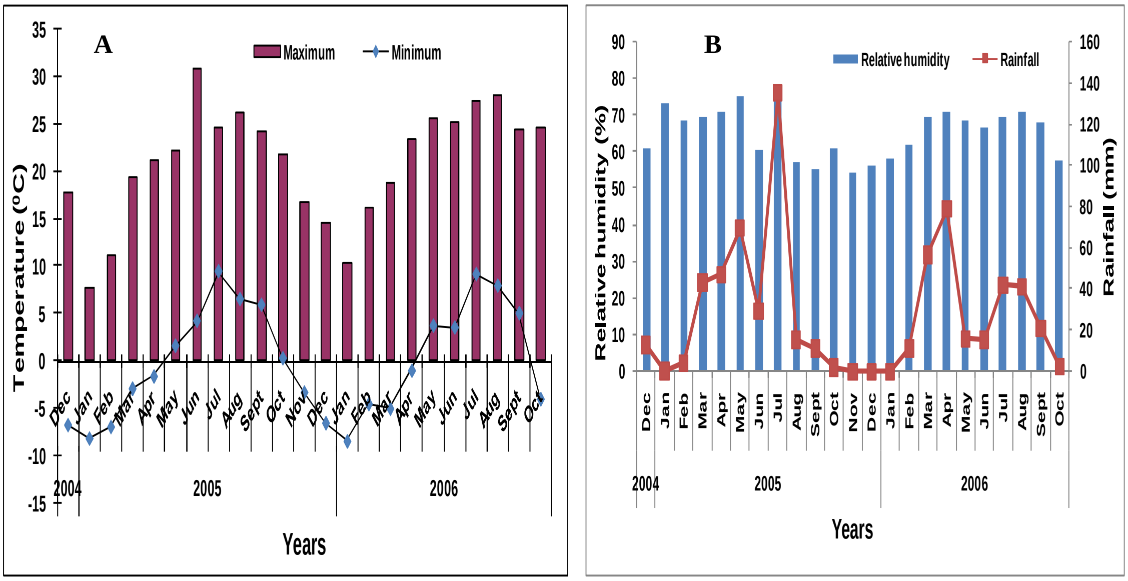

Figure 2. *Meteorological data of Kinnaur during study period from December 2004 to October 2006 [A=Temperature (^{0}C); B=Relative humidity (%) and Rainfall (mm)]*

Materials and Methods

The regeneration status of *P. gerardiana* was assessed by dividing the whole distribution area of the species into three zones, *viz*., lower dry temperate zone (Tapri and Shongthong), mid semi-arid zone (Peo, Kalpa, Pangi, Powari upper and Ribba) and upper arid zone (Akpa, Jangi, Leepa, Moorang and Purbani). Each zone was further divided into sub zones based upon elevation and aspect by laying quadrates on both sides, *i.e*., eastern/right and western/left banks of the river Sutlej. The following methodology was adopted to assess regeneration status of the species:

Sr. No.	Parameters	Nos.
a.	Zones	3
b.	Sub-zone per zone	2
c.	Number of quadrates per zone (20m x 20m)	2
d.	Total number of quadrates	12
e.	Number of regeneration plots per quadrate (2m x 2m)	10
f.	Total number of regeneration plots on one side of Sutlej	60
g.	Total number of regeneration plot	120

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The sampling units of 20m × 20m each were selected. Each sampling unit had ten regeneration units of 2m × 2m quadrate as per Cleark (1979). The survey was conducted for counting number of recruits (r), which may be defined as current year's seedlings, unestablished regeneration (u) seedlings other than recruits which has not yet established and with height less than 2 meter and established regeneration (e) plants with height of more than 2 meter. Here four (4) unestablished plants were taken equivalent to one established regeneration for calculating the regeneration per cent. The height of unestablished plants was also measured for the assessment of regeneration.

Regeneration assessment

The data was analyzed in each sampling plot for the assessment of regeneration status of the species. The established plants of 2500 per hectare were considered satisfactory regeneration. The quadrate was considered fully stocked when it contained one established plant. The data thus collected was analyzed using the formulae given by Chacko (1965).

- Weight Average height (m) $= \dfrac{\text{Total height of unestablished regeneration + (No. of established plants x establishment height)}}{\text{Total unestablished plants + total established Plants}}$

- Recruits (r) /ha $= 2500 \sum_{i=1}^{n} r\, i / m$

- Unestablished regeneration (u)/ha $= 2500 \sum_{i=1}^{n} u\, i / m$

- Established regeneration (e)/ha $= 2500 \sum_{i=1}^{n} e\, i / m$

Where

n - Number of sampling units
m – Total number of recording units in survey
ri – Total number of recruits in each sampling unit
ui – Total number of unestablished plants in each sampling unit
ei – Total number of established plants in each sampling unit

- Establishment index (I_1) $= \dfrac{\text{Weighted average height}}{\text{Establishment height}}$

- Stocking index (I_2) $= 1/2500 \times \dfrac{\text{Unestablished regeneration/ha}}{4} + \text{Established regeneration/ ha}$

- Established stocking per cent = 100 (I_1 x I_2)

A method was developed to understand the regeneration per cent of the species on the basis of stocking index as: Regeneration per cent = Stocking index × 100

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Results and Discussion

The assessment of natural regeneration is an important aspect one has to undertake for initiating silvicultural treatments under forest management systems in forest crops. In present study, *Cedrus deodara, Quercus ilex, Celtis australis, Daphne oleoides, Artemisia maritime, Rosa webbiana, Berberis* spp., *Desmodium* spp., *Indigofera gerardiana*, etc., were the major associated species of *P. gerardiana*.

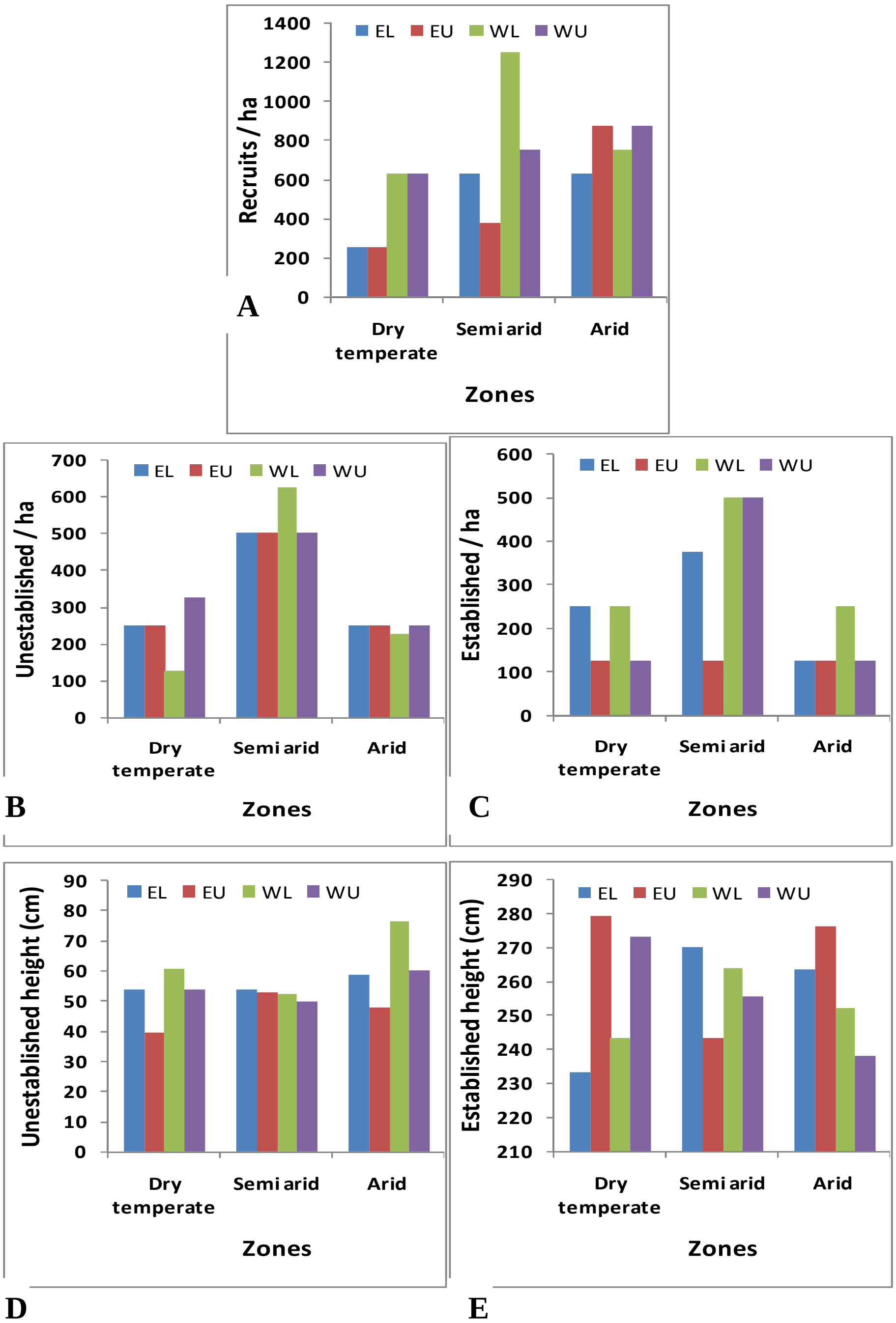

Figure 3. *Natural regeneration status of Pinus girardiana in different zones of Kinnuar, Himachal Pradesh*

(A= Number of recurits/ha; B= Unestablished/ha; C= Established/ha; D= Unestablished height (cm), E= Established height (cm); EL= Eastern Aspect Lower Elevation; EU= Eastern Aspect Upper Elevation; WL= Western Aspect Lower Elevation; WU= Western Aspect Upper Elevation)

The maximum number of recruits, unestablished and established regeneration was recorded in mid zone II (Semi arid) with elevation ranging from 2180 to 2500 m amsl on the left bank (western aspect) of the river Sutlej (*Fig. 3*). However, the maximum unestablished regeneration and established regeneration height was noticed in zone III (Arid) on the left bank with elevation of 2580-2900 m amsl. As far as aspect is concerned, the number of recruits (812.5/ha; *Fig. 3A*), unestablished regeneration (341.66/ha; *Fig. 3B*), established regeneration (291.66/ha; *Fig. 3C*), unestablished height (58.77 cm; *Fig. 3D*) and established height (254.15 cm; *Fig. 3E*) were greater on the left/western aspect as compared to the right/eastern aspect of the area. Further, the zone-III at lower elevation (2580 m amsl) resulted in maximum weighted average height (169.05) and establishment index (0.84) while as zone-II at lower elevation (2180 m amsl) showed maximum stocking index (0.265), establishment stocking (16.04 %) and regeneration (26.50 %) on the left bank, *i.e.*, western aspect of the area (*Table 1*).

Table 1. *Natural regeneration status of Pinus gerardiana in different zones of district Kinnuar*

Zones/Elevations	Weighted average height (cm)	Establishment index (I_1)	Stocking index (I_2)	Establishment stocking per cent ($100\ I_1 \times I_2$)	Regeneration (%)
Right bank /eastern aspect					
Zone –I (Tapri, Shongtong)					
Lower (1700 m amsl)	151.23	0.75	0.125	8.82	12.50
Upper (2020 m amsl)	78.37	0.35	0.075	3.96	7.50
Zone-II (Peo, Kalpa, Pangi)					
Lower (2100 m amsl)	144.45	0.56	0.200	11.62	20.00
Upper (2420 m amsl)	75.26	0.37	0.100	4.55	10.00
Zone-III (Akpa, Jangi, Leepa)					
Lower (2500 m amsl)	91.52	0.45	0.075	4.42	7.50
Upper (2820 m amsl)	123.95	0.61	0.075	5.57	7.50
Mean	**110.79**	**0.51**	**0.108**	**6.49**	**10.83**
Left bank/western aspect					
Zone –I (Tapri, Shongtong)					
Lower (1780 m amsl)	165.18	0.82	0.115	9.06	11.50
Upper (2100 m amsl)	126.91	0.63	0.080	5.97	8.00
Zone-II (Powari upper, Ribba)					
Lower (2180 m amsl)	131.76	0.65	0.265	16.04	26.50
Upper (2500 m amsl)	115.85	0.57	0.250	15.92	25.00
Zone-III (Akpa, Moorang, Purbani)					
Lower (2580 m amsl)	169.05	0.84	0.115	9.31	11.50
Upper (2900 m amsl)	90.79	0.45	0.075	4.57	7.50
Mean	**133.26**	**0.66**	**0.150**	**10.14**	**15.00**

Singh (2004), while working on *Cedrus deodara* revealed that the maximum number of recruits and established plants were recorded on northern aspect than southern aspect (Singh, 2004). The finding also gets support from a number of reports as in ponderosa pine (Barrett et al., 1983), fir and spruce in Narkanda forest (Gupta, 1996) and fir and spruce in Shimla forest range (Yashpal, 2006). The overall mean maximum regeneration (15%) was observed to be higher on the western aspect as compared to that in eastern aspect in the area (10.83 %). This is probably due to more conducive

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microclimate, optimum moisture content, less human interference as well as grazing pressure on the left than right bank of the river Sutlej. The findings have also been supported by many researchers working on different tree species, *i.e*., as in *Taxus baccata* (Shamet and Gupta, 2005), *Abies concolor* and *A. magnifica* (Gordon, 1970), silver fir (Singh, 1973) and Englmann spruce (Noble and Alexander, 1977). In long years back, Singh *et al.* (1973) and Reddy (1963) have reported low natural regeneration potential in *Pinus gerardiana* in Himachal Pradesh. The scanty/failure of natural regeneration has been attributed to collection of almost each and every cone of the species by the right holders in district Kinnaur as the nuts have high market value and demand. Further, inhospitable climate, summer drought, poor edaphic conditions and deficit soil moisture collectively hamper natural regeneration of this species (*Plate 1*). At present, roughly 80-120 tones of chilgoza nuts are collected by the right holders and exported to plains every year from the Kinnuar (Malik, 2007).

Plate 1. Old stand of ***Pinus gerardiana*** showing unscientific lopping and meagre established regeneration at Pangi (near Kalpa)

Conclusions

The study concludes that chilgoza forest characterized as semi arid zone (mid zone) at elevation ranging between 2180 and 2500 m amsl showed better regeneration status of this species as compared to other zones of the study area. Overall, the maximum mean regeneration of 15 per cent and establishment stocking of 10.14 per cent was recorded which is considered as failure of natural regeneration in the area. If proper silvicultural measures will not be applied, the further decline in the status of the species will occur in near future. The status of the species is categorized as Near Threatened Red List (2008). In view of poor natural regeneration status and stand condition, the species deserves to

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be included in Critically Endangered species category in Indian Himalayan region. There is an immediate need to protect this species from over harvesting, grazing and other destructive activities. Besides in-situ conservation and management, mass scale afforestation of this species with the participation of local communities on potential zone need to be done. For that, a suitable strategy and action plan including sustainable harvesting methods should be prepared and executed, effectively. Local communities need to be made aware about conservation and sustainable utilization of P. gerardiana.

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GEOCHEMICAL ANALYSIS OF GROUNDWATER QUALITY IN AGBARA AND ENVIRONS

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Abstract. This study examines the geochemical compositions of groundwater from three communities viz: Agbara, Igbesa and Lusada to determine their suitability for human consumption. A total of seventeen (17) boreholes were sampled and analyzed using standard procedures. The parameters determined include: pH, temperature, electrical conductivity (EC), redox potential (RP), biochemical oxygen demand (BOD), anions (Cl^-, PO_4^{3-}, NO_3^-, SO_4^{2-}, HCO_3^- and metals (As^{2+}, Ca^{2+}, Mg^{2+}, K^+, Na^+, Mn^{2+} and Cd^{2+}). Results showed variations in the values of water quality parameters from one community to another. pH of water samples from Agbara and Igbesa were acidic (4.1 - 5.8) and generally fell below World Health Organization (WHO) limit of 6.5 – 8.5 while that of Lusada was within the WHO range. Chloride, phosphate, bicarbonate and BOD values were generally high in all the three study areas greater than the acceptable limits in drinking water. Metal concentrations in all the sampled groundwater were very low while cadmium and arsenic were not detected in Igbesa and Lusada samples. Since most of the parameters with abnormal values have no (WHO) health-based guidelines, groundwater in these study areas may be suitable for direct consumption.
Keyword*: groundwater resource, geochemistry, parameters, consumption, Agbara*

Introduction

Groundwater exploitation - for the purpose of supplying good quality drinking water is widely practiced in many developing nations of the world. This is achieved by well sinking, which could be shallow (hand dug or tube wells), or deep as in the case of boreholes. Well sinking is not a new phenomenon but an ancient craft that had been in practice for thousands of years (Todd, 1980). It is an essential resource that supplies drinking water to a large number of people in Nigeria in the urban and rural areas. The study of geochemical compositions of groundwater is therefore, important due to the attached significance for potable water-supply. Besides, it is an alternative against the epileptic supply of pipe-borne water by the water authority. However, the quality of any groundwater resource in terms of physical, chemical and biological compositions determines its fitness for human consumption and diverse usage. These qualities, in turn, are functions of groundwater ionic species like Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, NO_3^-, SO_4^{2-}, and Cl^- (Sadashivaiah et al., 2008). Human activities such as sewage disposal, industrial waste products, agriculture are as well capable of altering groundwater quality (Adekunle et al., 2007). The influence of water ions on groundwater chemistry depends on their concentrations in the aquifer. Groundwater quality also relies on the

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compositions of water recharged into the ground, the interaction between it and media of the aquifer; the reactions that take place in the aquifer and the overlying soil (Rahaman, 1976). International standards have been set as criteria for drinking water quality by relevant bodies such as World Heath Organization (WHO), European Community, (EC) and United State Environmental Protection Agency (USEPA). The essence of this is to safeguard the public health against waterborne diseases. However, there are no water standard guidelines for drinking water in Nigeria. The defunct Federal Environmental Protection Agency, FEPA (1991) had only successfully set guidelines for effluents discharge into Nigeria's waterways, in which compliance was at the moment at zero level. It is therefore, necessary to assess the quality of some of these aquifers and compared it with the international standards. This will however, create a public awareness on the possible health risks attached to abnormal geochemical values of the groundwater especially in the study communities.

Many works have been done on the groundwater quality from the southwestern Nigeria. Adekunle et al. (2007) had analyzed groundwater quality of hand-dugs wells from Igbora in Oyo state. Results showed pollutions of wells located close to dumping sites. High water parameters such as nitrate, BOD, Cd, Pb and Coliforms were recorded higher than WHO standard indicating organic pollution. Groundwater resource from Lagos state has been studied by Yusuf (2007) and Longe and Balogun (2009). Gbadebo et al. (2010) had also observed high nitrate concentrations in groundwater aquifer from Sango and Abeokuta in Ogun state. Taiwo et al. (2011) had reported the physico-chemical characteristics of groundwater quality of wells sampled in Abeokuta. The metal contents of boreholes sampled in Sango has been studied by Ayedun et al (2011), while a comprehensive geochemical analysis of groundwater aquifers in Sango had been carried out by Gbadebo and Taiwo (2011). The major concern of groundwater in southwestern Nigeria is the issue of high nitrate concentration. High nitrate concentrations in these groundwater aquifers might be connected to seepage of leachates from septic tanks, dumping sites and agricultural activities. Nitrate value greater than permissible standard in drinking water could pose a health problem to infants less than 6 months of age for blue baby syndrome (Taiwo, 2010). Faecal pollution of the groundwater in southwestern Nigeria has also been documented (Adekunle et al., 2007, Orebiyi et al., 2009).

Materials and methods

Study areas

The study areas, which include Agbara, Igbesa and Lusada is situated within longitude 3°04′ and 3°10′ E, and latitude 6°31′ and 6°34′ N in Ado Odo/ Ota Local Government, Ogun state (*Fig. 1*), Nigeria. Agbara is noted for industrial activities in Ogun state while the Igbesa and Lusada are civil communities characterized by peasant farming with little or no viable industrial activities. *Table 1* shows the sampling locations and their coordinates.

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Figure 1. *The map of Ogun State showing the Local Governmnet Areas (red arrow pointing to the study areas)*

Table 1. *Description of the groundwater locations*

Borehole Serial number	Location	Latitude N	Longitudes E
1	Joak Block	06° 34′ 319″	003° 04′ 897″
2	Omiata Block	06° 33′ 701″	003° 04′ 758″
3	Car Wash	06° 31′ 951″	003° 04′ 537″
4	Dive Primary School	06° 31′ 545″	003° 04′ 566″
5	Opic estate1	06° 31′ 431″	003° 04′ 493″
6	Baptist church	06° 31′ 042″	003° 04′ 737″
7	Opic estate 2	06° 31′ 090″	003° 04′ 769″
8	Crawford University1	06° 30′ 553″	003° 04′ 690″
9	Crawford University2	06° 30′ 682″	003° 04′ 708″
10	Crawford University3	06° 30′ 731″	003° 04′ 605″
11	Ketu-Adie Owe1	06° 30′ 853″	003° 04′ 424″
12	Ketu-Adie Owe2	06° 30′ 935″	003° 04′ 164″
13	Crawform Guest house	06° 30′ 688″	003° 04′ 599″
14	GRA Igbesa	06° 30′ 561″	003° 04′ 733″
15	Lusada1	06° 35′ 176″	003° 04′ 892″
16	Grace court hotel	06° 35′ 010″	003° 04′ 123″
17	Lusada2	06° 35′ 009″	003° 04′ 055″

Geology of the study area

The study areas (Agbara, Lusada and Igbesa) form part of Dahomey basin, a very extensive sedimentary basin on the continental margin on the Gulf of Guinea, which extends from Volta River Delta, Southeastern Ghana in the west, to the Western flank of the Niger Delta (Jones and Hockey, 1964). This Formation known as Coastal Plain Sand is made up of poorly sorted sands, which are in parts cross-bedded and shows transitional to continental characteristics like Ilaro and Abeokuta Formations. The thickness of coastal plain sand ranges from 10m to 100m while the age falls under Pleistocene and Oligocene (Jones and Hockey, 1964).

Methodology

To determine the chemical constituent of the groundwater, a total of 17 borehole water samples were analyzed in this study in for physico-chemical parameters using standard procedures (APHA, 1989). The parameters evaluated were pH, temperature, electrical conductivity (EC), redox potential (RP), biochemical oxygen demand (BOD), chloride, phosphate, nitrate, sulphate, bicarbonate and metals (arsenic, calcium, magnesium, potassium, sodium, manganese and cadmium). Parameters like pH, temperature, EC and RP were measured in-situ using probe methods while BOD was determined by dilution method. However, chloride, sulphate and bicarbonate were determined in the laboratory by titrimetric method while phosphate and nitrate were assayed using spectrophotometric method. Analysis of metals was carried out by Atomic Absorption Spectrophotometry (AAS) except sodium and potassium which were determined by flame photometric method.

Table 2. *Summary of analytical data of groundwater from Agbara*

Parameters	Mean ± SD	Range	WHO (2008)
Temperature (^oC)	29.2 ± 17.2	27.7-32.2	
pH	4.34 ± 0.54	4.10-4.81	6.5-8.5
Electrical conductivity ($\mu S\ cm^{-1}$)	349 ± 9.74	195-721	
Redox Potential (RP) (mV)	350.4 ± 18.51	346-380	
BOD ($mg\ L^{-1}$)	11.14 ± 18.51	10.43-12.67	
Chloride ($mg\ L^{-1}$)	937.5 ± 569.40	531.5-1455.5	200-300
Phosphate ($mg\ L^{-1}$)	7.83 ± 0.32	7.03-9.43	
Sulphate ($mg\ L^{-1}$)	15.7 ± 0.89	14.0-18.05	< 250
Nitrate ($mg\ L^{-1}$)	0.01 ± 0.00	0.01-0.02	50
Bicarbonate ($mg\ L^{-1}$)	1804 ± 250.24	1022-2628	
Arsenic ($mg\ L^{-1}$)	0.01 ± 0.00	0.01-0.02	0.01
Calcium ($mg\ L^{-1}$)	15.0 ± 0.04	10.0-23.0	100-200
Potassium ($mg\ L^{-1}$)	3.0 ± 0.04	2.0-4.0	
Sodium ($mg\ L^{-1}$)	15.0 ± 0.04	14.0-16.0	200
Magnesium ($mg\ L^{-1}$)	3.0 ± 0.01	2.0-13.0	250
Iron ($mg\ L^{-1}$)	0.96 ± 0.39	0.00-2.00	0.30
Manganese ($mg\ L^{-1}$)	0.01 ± 0.05	0.00-0.10	0.40
Cadmium ($mg\ L^{-1}$)	ND		0.003

SD- Standard Deviation, ND-Not Detected, BOD-Biological Oxygen Demand

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Results

Geochemical values of groundwater samples in Agbara, Igbesa and Lusada were shown in Table 2-4 respectively. Results of geochemical parameters follow similar trends in the three communities with results range: temperature (27.7-33.2 ^oC), pH (4.10-7.77), EC (195-721 µS cm^{-1}), RP (344-390 mV), BOD (9.47-15.3 mg L^{-1}), chloride (531.5-3088.5 mg L^{-1}), phosphate (6.94-9.43 mg L^{-1}), sulphate (10.75-18.05 mg L^{-1}), nitrate (0.01-0.02 mg L^{-1}), bicarbonate (1022-2628 mg L^{-1}), As (ND-0.02 mg L^{-1}), Ca (2.0-120.0 mg L^{-1}), K (1.0-20 mg L^{-1}), Na (14.0-38.0 mg L^{-1}), Mg (1.0-13.0 mg L^{-1}), Fe (0.0-2.0 mg L^{-1}), Mn (0.0-0.30 mg L^{-1}) and Cd (ND-0.02 mg L^{-1}).

Table 3. *Summary of analytical data of groundwater from Igbesa*

Parameters	**Mean ± SD**	**Range**	**WHO (2008)**
Temperature (^oC)	30.32 ± 1.72	28.1-33.2	
pH	4.78 ± 5.04	4.28-5.84	6.5-8.5
Electrical conductivity (µS cm^{-1})	377.5 ± 9.74	345-410	
Redox Potential (RP) (mV)	350.86 ± 18.51	344-385	
BOD (mg L^{-1})	11.41± 19.82	9.47-15.3	
Chloride (mg L^{-1})	1887.64 ± 569.40	1132-3088.5	200-300
Phosphate (mg L^{-1})	7.55 ± 0.32	6.94-8.05	
Sulphate (mg L^{-1})	13.27 ± 0.89	11.93-14.4	< 250
Nitrate (mg L^{-1})	0.01 ±0.00	0.01-0.02	50
Bicarbonate (mg L^{-1})	1585.1 ± 250.24	1153-1826	
Arsenic (mg L^{-1})	0.01 ±0.00	0.01-0.02	0.01
Calcium (mg L^{-1})	30.0 ± 5.04	20-120	100-200
Potassium (mg L^{-1})	3.0 ± 1.50	1.0-10.0	
Sodium (mg L^{-1})	21.0 ± 2.04	15.0-26.0	200
Magnesium (mg L^{-1})	2.0 ± 1.01	1.0-4.0	250
Iron (mg L^{-1})	0.46 ± 0.39	0.00-1.10	0.30
Manganese (mg L^{-1})	0.06 ± 0.05	0.00-0.20	0.40
Cadmium (mg L^{-1})	0.01 ± 0.02	0.0-0.02	0.003

SD- Standard Deviation, ND-Not Detected, BOD-Biological Oxygen Demand

Table 4. *Summary of analytical data groundwater from Lusada*

Parameters	Mean ± SD	Range	WHO (2008)
Temperature (^oC)	29.3 ± 0.33	28.9-29.4	
pH	6.74 ± 0.10	6.52-7.77	6.5-8.5
Electrical conductivity (µS cm^{-1})	253.3 ± 5.19	308-417	
Redox Potential (RP) (mV)	360.67 ± 5.44	359-390	
BOD (mg L^{-1})	10.31 ± 6.89	9.40-11.06	
Chloride (mg L^{-1})	1275.59 ± 51.21	1203.5-1312.25	200-300
Phosphate (mg L^{-1})	6.89 ± 0.19	6.70-7.03	
Sulphate (mg L^{-1})	11.60 ± 0.78	10.75-12.63	< 250
Nitrate (mg L^{-1})	0.01 ±0.00	0.00-0.01	50
Bicarbonate (mg L^{-1})	1349.40 ± 185.30	1095-1531	
Arsenic (mg L^{-1})	ND		0.01
Calcium (mg L^{-1})	6.0 ± 0.06	2.0-15.0	100-200
Potassium (mg L^{-1})	10.0 ± 2.05	7.0-20.0	
Sodium (mg L^{-1})	28.0 ± 1.08	21.0-38.0	200
Magnesium (mg L^{-1})	4.0 ± 0.02	2.0-7.0	250
Iron (mg L^{-1})	1.50 ± 0.08	1.40-1.60	0.30
Manganese (mg L^{-1})	0.17 ± 0.09	0.10-0.30	0.40
Cadmium (mg L^{-1})	ND		0.003

SD- Standard Deviation, ND-Not Detected, BOD-Biological Oxygen Demand

Discussions

Water pH values of groundwater samples collected in Agbara and Igbesa were acidic while the pH of Lusada sample was slightly alkaline. Agbara and Igbesa water samples fell below the WHO limit for pH in drinking water given as 6.5 to 8.5 (WHO, 2008). The implication of the low pH may include corrosion, solubility of heavy metals and impartation of bitter and metallic taste in water (USEPA, 2007). However, the water hardness was very high in all the sampled groundwater, and might reduce the dissolution of metals (Adeyeye and Ayejuyo, 2002) against metal toxicity. Similarly, low pH in these boreholes may be attributed to dissociation of carbonic acid in the aquifer due to their unconfined nature (Zhou, 2006). Chemical contaminants from Agbara Industrial Estate seeping into groundwater might also have resulted into low water pH. Similar low pH values have been observed by Efe et al. (2005) in groundwater samples from Niger-Delta of Nigeria.

Redox potential is a measure of a water system’s capacity to either release or gain electrons in chemical reactions (Eaton et al., 1995). The essence of RP measurement in

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water is to evaluate corrosions of metals in drinking water. The range of RP in this study will not cause corrosion but may be considered in normal range of 350 mV-390 mV (EnviroEquip, 2007). Positive RP in the samples indicated that the boreholes are oxidizing with appreciably high dissolved oxygen.

Owen and Blair (1996) have defined Electrical conductivity as a measure of dissolved ions or salinity in water. EC values obtained in this study could be described as good according to Sadashivaiah, et al. (2008) classification of EC in water (*Table 5*). WHO desirable limit for EC in drinking water is 1000 µS cm^{-1}. All the observed mean values of EC fell within this limit. High salinity in groundwater may be associated with alluvial aquifers (Geetha et al., 2008).

Table 5. *Salinity Standard (Sadashivaiah, et al., 2008)*

EC (µS cm^{-1})	**Class**
100-250	Excellent
250-750	Good
750-2250	Doubtful
> 2250	Unsuitable

High BOD in groundwater is a signal of organic pollution. The extreme high values of BOD recorded across the borehole in the three communities indicated that the groundwater resources were polluted. Water with BOD greater than 4 mg L^{-1} is considered a rejectable source by Kothandaraman and Ewing (1969) as depicted in Table 6. Environmental Canada (1977) and (1979) has classified water BOD values greater than 10 mg L^{-1} as polluted. Elevated values of BOD in these groundwater samples might probably be from infilteration of leachates from nearby dumpsites and septic tanks into the groundwater resource. Adekunle et al. (2007) reported a high value of BOD (35.0 mg L^{-1}) in groundwater close to defecating sites in Igbora, Nigeria. Abnormal values of BOD in groundwater could harbour pathogenic organisms which may cause water-borne diseases.

Table 6. *BOD Standard for Raw Water: (Source: Kothandaraman and Ewing, 1969)*

Average BOD value (mg L^{-1})	**Class**
0.25 – 1.5	Excellent source
1.5 – 2.5	Good source
2.5 – 4.0	Porous source
Greater than 4.0	Rejectable source

Sulphate and nitrate values were generally low in the groundwater samples with very slim possibility of initiating any health problems when the water is consumed. However, the mean values of phosphate in the groundwater was high, and could be attributed to weathering of underlain phosphate rocks in the aquifers and from other point sources. High phosphate values have been reported in groundwater near landfill site (Longe and Balogun, 2009). The greatest threats to groundwater quality and quantity include nutrient loading from agricultural fields, urban run-off, industrial effluents (and other contaminants that could infiltrate the groundwater systems), climate change, and high groundwater use (Danielopol *et al*., 2003).

Mean values of chloride in this study exceeded WHO recommended maximum limit of 250 mg L^{-1} in drinking water. High chloride and electrical conductivity in groundwater might be attributed to the intrusions of saline water from the coastal water (Department of National Health and Welfare (Canada), 1978). The studied areas fall under coastal plain Formation (Jones and Hockey, 1964). The chloride concentration of these study areas was very high with possible effect on health (Wesson, 1969) and impairment of water taste. Other environmental considerations of high chloride concentration are increases pitting corrosion of metal pipes, elevation of metals in drinking water and galvanic corrosion (Gregory, 1990).

Bicarbonate values of the groundwater samples are generally high, which may due to the underlain sedimentary rock. Groundwater samples in the study areas follow the geochemical trend in terms of abundance of ions as follows: Agbara (HCO_3^-, > Cl^- > SO_4^{2-} > Na^+ / Ca^{2+} > K^+ > NO_3^-); Igbesa (Cl^- > HCO_3^-, > Ca^{2+} > Na^+ > SO_4^{2-} > K^+ > NO_3^-) and Lusada (HCO_3^-, > Cl^- >Na^+ > SO_4^{2-} > Na^+ > K^+ > Ca^{2+} > NO_3^-). The rock types of these aquifers were mainly bicarbonate and chloride types.

All the metals data collected from Agbara and Lusada groundwater samples except iron were low and met the permissible standards in drinking water (WHO, 2008). In Igbesa water samples, only the values of Fe and Cd were higher than the WHO permissible limits. Cadmium toxicity may include cancer (JECFA, 2000), hypertension, renal failure (Krajnc et al., 1987), proteinura and itai-itai disease (WHO, 2008; JECFA, 1989). Iron has no health effect on healthy individual as it is required by the body as essential trace metal (Finch, 1972). However, it could impart taste and offensive odour in water at concentration greater than 0.3 mg L^{-1}. (Lemley et al., 1999). Iron and Manganese in the samples from Lusada were higher than WHO standard. Manganese has similar effects as iron in drinking water. Ayedun et al. (2011) and Gbadebo and Taiwo (2011) had previous reported high concentrations of Fe, Mn and Cd in boreholes sampled in Sango, which is about 10 km away from the study areas.

Conclusion

The geochemical compositions of the groundwater of the study areas showed that the water quality was poor in terms of chloride concentration and this would definitely affect the taste of the water. The higher values of iron, greater than WHO standard in the groundwater from the study areas could also affect the taste of the water. The presence of considerable value of cadmium in Igbesa groundwater samples is a major concern to public health. However, other parameters were within the permissible standard. In terms of abundance of ions, the predominant ions in the aquifers are: bicarbonate, chloride, sulphate, calcium, sodium, potassium while nitrate level was very low. Chloride removal from the groundwater samples in the study areas could be expensive since it cannot be done by ordinary filtration or boiling. However, the visible method of its removal could be achieved by reverse osmosis, deionization and distillation. This paper recommends proper and routine monitoring of groundwater resources in Agbara and environs.

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