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Biomonitoring Study of Air Pollution with Betula pendula Roth., from Plovdiv, Bulgaria

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Abstract. The present study is a small part of a program for application the methods of passive and active biomonitoring with tree, herbaceous, moss and lichen species for assessment of the anthropogenic factor in urban conditions. All reported results here are preliminary. *Betula pendula* was studied as a possible biomonitor of air pollution in Plovdiv. Eight sampling sites in the urban roadside, city center and suburban areas were investigated. Chlorophyll content was determined as essential and sensitive physiological parameter. The concentrations of 26 micro- and macroelements were analyzed by FAAS and ICP-MS. Maximum for chlorophyll was found in the birch leaves from west part of the town, minimum – in these from north part. More significant variations were detected for Ni, Mn, B, Cr, Co, Fe, Bi, Cd, Al, Zn. Highest concentrations of 12 elements were found in the samples, collected from the central area of Plovdiv.

Key words: biomonitor, *Betula pendula,* chlorophyll *a* and *b*, macroelements, microelements.

Introduction

Urbanization is one of the most drastic changes that can be imposed on an environment (MOLLOV & VALKANOVA, 2009). Urban ecosystems are comprised of diverse land uses including commercial, industrial, residential, transport, recreational, agricultural and nature areas, resulting in different habitats for plants, animals and human within urban landscape. Urban habitat quality results the integration of different abiotic and biotic components, such as air, soil and water quality, microclimate and the presence of vegetation.

The use of plants as passive biomonitors to complete the information on trace elements deposition from fully or semi-automatic gauges, commonly used in current pollution monitoring programs, obtain increasing attention. This reliable, versatile and inexpensive method can assist us on the subject of health and environmental protection against potentially hazardous trace elements. Providing a high density of sampling points, the biomonitors are very effective for tracing maps of airborne metal contamination in the urban environments (KLUMPP *et al.*, 2009; BAYCU *et al.*, 2006).

An advantage of plants as biomonitors is that they are effective collectors which reflect the accumulated effect of environmental pollution and accumulation of toxicants from atomspheric pollution (deposition, binding and solubility of metals on the leaf surface) and soil pollution (concentration and bioavailability of elements in soil).

Different biomonitors have been used for evaluation of the distribution of heavy metal pollution: mosses and lichens (ANIČIĆ *et al.*,

2009; CULICOV & YURUKOVA, 2006; GONZALEZ *et al.*, 1996; STEINNES, 1993), grasses (KLUMPP *et al.*, 2009), many trees as chestnut (YILMAZ *et al.*, 2006), maple, linden, willow, birch (PICZAK *et al.*, 2003), poplar (DJINGOVA *et al.*, 1995), oak (MONACI *et al.*, 2000).

Betula pendula (Betulaceae) was successfully used as a biomonitor in many studies for assessment of the pollution level in different countries like Russia (KOZLOV *et al.*, 1995), England (MAHER *et al.*, 2008; MATZKA & MAHER, 1999; REY & JARVIS, 1998), Germany (FRANZARING *et al.*, 2006), Finland (RIIKONEN *et al.*, 2005; JUUROLA, 2003; PÄÄKKÖNEN *et al.*, 1998). It had been widely planted all over the town of Plovdiv as a ruderal ornamental tree and fulfills all basic criteria about selection of a species as a biomonitor, given by WITTIG (1993).

The aim of this study was to evaluate the reliability of *Betula pendula* as biomonitor by quantifying inorganic leaf content (26 trace elements, toxic and heavy metals) and concentrations of chlorophylls *a* and *b* as essential and sensitive physiological parameters.

Material and methods

Study area and sampling sites

The town of Plovdiv (42° 9' N, 24° 45' E), one of the most populated city of Bulgaria (over 365 000 inhabitants on 102 km²), was selected as a study area. It includes several industrial zones, densely populated central area, some moderately populated areas around it, wide network of busy streets and train tracks, big parks and other green yards.

The climate in Plovdiv is temperate with mild influence from the Mediterranean Sea and a huge temperature range between summers and winters. The average annual temperature is 12.3°C with maximum in July (32.3°C) and minimum in January (6.5°C). The average relative humidity is 73%. It is highest in December (86%) and lowest in August (62%). The total precipitation is 540 mm – the wettest months of the year are May and June with an average precipitation of 66.2 mm, while the driest is August with an average of 31 mm. Gentle winds (0 to 5 m s^{-1}) are predominant in the city, winds with speed of up to 1 m s^{-1} represent 95% of all winds during the year. The prevailing wind direction is from west, rarely from east.

The sampling sites was selected as follows: 1 – Vegetable Crops Research Institute "Maritsa", 2 – Park "Lauta", 3 – Railway station "Trakiya", 4 – Ruski Boulevard (sidewalk), 5 – Nature monument Bunardzhik, 6 – Housing estate "Smirnenski", 7 – Park "Otdih i kultura", 8 – near Foreign language school, at the NW end of the town (Fig. 1).

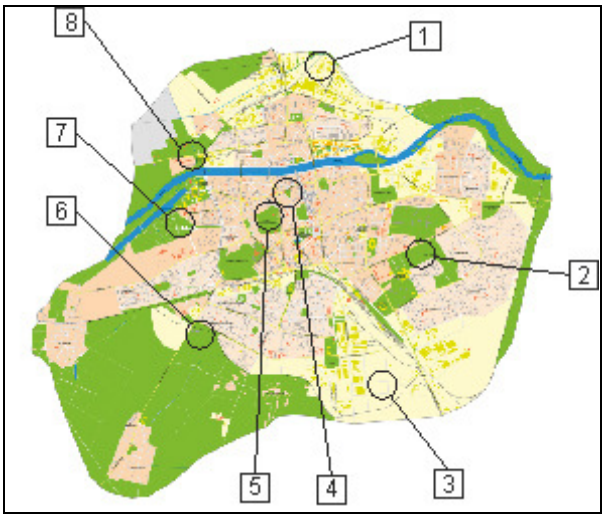

Fig. 1. Sampling sites in Plovdiv (Bulgaria)

Sampling and sampling preparation

At each sampling site were chosen at least two birch trees (diameter 20-25 cm), growing at similar light conditions and mineral nutrition. They must have been from 5 to 10 m away from intense traffic, except site 4 – Ruski Bul., where trees were on the sidewalk, up to 1 m from the roadway. Sampling period was 13-14 June 2010. Leaves were sampled from the lower part of the tree crown at the 2.5–3 m height in all directions. Usually 20-40 fully expanded leaves were collected and a composite sample was prepared for analyses. All the samples were stored in clean, labeled, polyethylene bags, closed tightly to avoid contamination during transportation. Determination of chlorophyll content at laboratory conditions was carried out immediately after sampling. Plant

material for additional analyses was air dried for two weeks, ground to a powder and homogenized.

Chlorophyll analysis

Pigment analysis followed SHLYK (1965). Spectrophotometric reading of photosynthetic pigments was performed after extraction with 90% acetone (SPECOL 11 absorption spectrophotometer) at the Faculty of Biology, University of Plovdiv "Paisii Hilendarski". All the analyses were conducted in three replications.

Concentrations of chlorophyll (chl) *a* and *b* were calculated as follows:

$C_a = 9.784 \times E_{662} - 0.990 \times E_{644}$

$C_b = 21.426 \times E_{644} - 4.650 \times E_{662}$

$C_{a+b} = 5.134 \times E_{662} + 20.436 \times E_{644}$,

where C_a – concentration of chl *a* in mg l^{-1}, C_b – concentration of chl *b* in mg l^{-1}, C_{a+b} – total content of chlorophylls in mg l^{-1}.

The received results were recalculated in mg g^{-1} fresh weight:

$C_a' = (C_a \times V \times R) \times g^{-1}$

$C_b' = (C_b \times V \times R) \times g^{-1}$

$C_{a+b}' = (C_{a+b} \times V \times R) \times g^{-1}$,

where C_a – concentration of chl *a* in mg l^{-1}, C_b – concentration of chl *b* in mg l^{-1}, C_{a+b} – total content of chlorophylls in mg l^{-1}, V – volume of extract in l, R – dilution (if it was necessary), g – starting fresh weight of the sample.

Chemical analysis

About 1 g ground plant material was treated with 5 ml 65% nitric acid (Merck) for 24 h at room temperature. The wet-ashed procedure was assisted by a Microwave Digestion System CEM MDS 81D. Samples were treated for 5 min at maximum power (600 W) in closed vessels. After cooling for 1 h at room temperature, vessels were opened and 2 ml nitric acid and 3 ml 30% hydrogen peroxide were added and were left to react for another 1 h. Vessels were closed and treated by the Microwave Digestion System for 10 min again at 600 W for full digestion of the organic matter. The filtrate was diluted with double distilled water up to 50 ml.

The elements Zn, Fe, K, Mg, Mn, Na, Cu and Ca were determined by FAAS method using Atomic Absorption Spectrometer PERKIN-ELMER 4000 (flame air - acetylene). The sample solutions with dilution factors from 50 till 250 were additionally spiked with La as releasing agent - 0,05% La for Zn, Fe, K, Mg, Mn, Na, Cu, and 1% La for Ca. Calibration standards Multy VI (MERCK) from 0.5 up to 40 ppm were used for different elements.

The content of Be, B, Al, S, P, Cr, V, Co, Ni, As, Se, Sr, Mo, Cd, Hg, Pb, Bi and U were determined by inductively coupled plasma mass spectrometry (ICP-MS) using instrument Agilent 7700 ICP-MS (2009), DF 1000. All samples, blanks and standards were spiked with internal standards - Ge 50 ppb and Rh 5 ppb final concentration in the solutions. Calibration standards Multy VI (MERCK) were freshly prepared from 1 to 1000 ppb in 0.05 volume% HNO_3 (p.a.) Monostandard of Hg 100 ppt was also used in the calibration. Signals of suitable isotopes for the tested elements have been measured twice in both modes - without and with helium gas collision cell.

Statistical analysis

For evaluation of determined concentrations a descriptive statistical analysis was applied. For grouping the studied elements a cluster analysis was used (Unweighted pair-group average linking and Pearson's index distance measure) and the relationships between the contents of individual elements in collected leaf samples were tested using Spearman rank correlation coefficients. For all statistical analysis the STATISTICA 7.0 statistical package was used (STATSOFT INC., 2004).

Results and Discussion

Chlorophyll content

Chlorophyll *a* content varied between 1.32 and 1.89 mg g^{-1} within the study areas, whereas, chl *b* content varied from 0.54 to 1.52 mg g^{-1} (Fig.2). Maximums of chl *a*, chl *b* and total chlorophyll content have been observed in the sample from site 7. Chlorophyll *a/b* ratio varied from 1.41 to 2.44 (Table 1). It had highest value in leaves from site 1 as a result of minor anthropogenic

impact and lowest in leaves from site 7, in negative correlation with chlorophyll content.

The lowest concentrations of chlorophyll have been observed in the sample from site 1, the sampling site situated in the NE end of Plovdiv and being most distant from the central part of the town. The higher concentrations in other seven sites are due to the intensive anthropogenic activity.

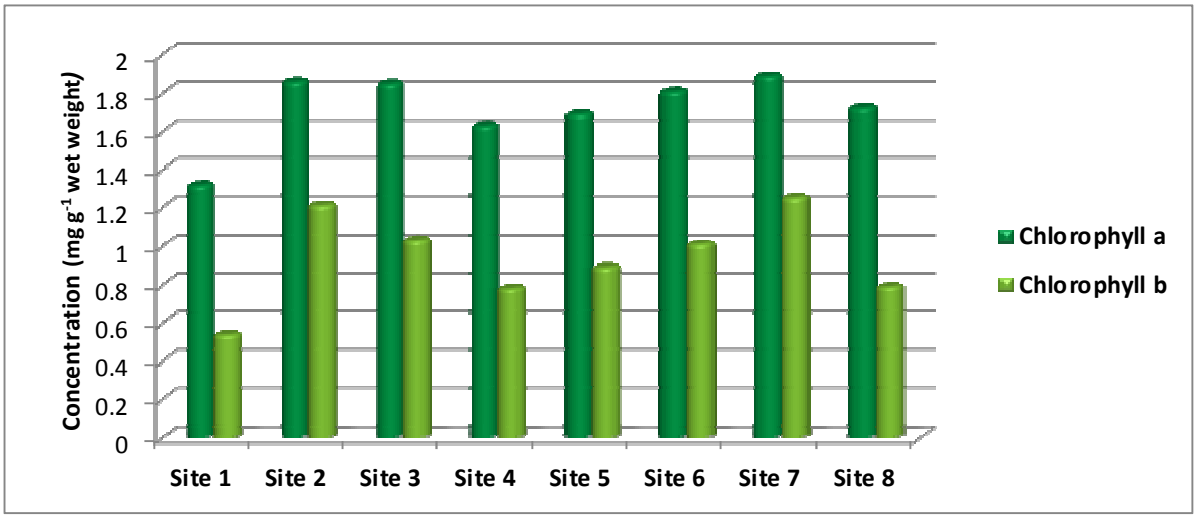

Fig.2. Comparison of chlorophyll concentrations in birch leaves from different sampling sites

Table 1. Levels of chlorophyll in birch leaves (mg g^{-1} wet weight**).**

Sampling site	Chl *a*	SD	Chl *b*	SD	Chl *a+b*	SD	Ratio chl *a/b*	SD
1	1.32	0.22	0.54	0.14	1.86	0.13	2.44	0.19
2	1.86	0.22	1.21	0.35	3.07	0.13	1.53	0.36
3	1.85	0.05	1.03	0.12	2.88	0.03	1.79	0.15
4	1.63	0.60	0.78	0.31	2.42	0.04	2.09	0.05
5	1.69	0.10	0.89	0.10	2.58	0.06	1.90	0.10
6	1.81	0.04	1.01	0.07	2.83	0.02	1.79	0.09
7	1.89	0.09	1.25	0.21	3.14	0.06	1.51	0.21
8	1.72	0.12	0.79	0.14	2.51	0.07	2.18	0.21

The birch leaves from roadside trees (sample from site 4) showed quite low content of pigments, followed by the leaves from site 5. That can be explained with stress reaction of plant and the degradation of chlorophyll (ALI, 1991). The studied pigment had increased levels as a compensatory mechanism towards enhanced concentrations of air pollutants. It could be supposed that reaching of determined pollution level interrupted photosynthesis process, followed by chlorophyll degradation.

Similar results about pigment concentrations in birch leaves, sampled in June from 7 years old trees (in Sofia, Bulgaria), had been reported by IVANOVA & VELIKOVA (1990). In cited publication the average chlorophyll *a* and *b* concentrations were 1.65 mg g^{-1} wet weight and 0.92 mg g^{-1} wet weight, respectively. The average chl *a* content, found in this study, was 1.72 mg g^{-1} (4% higher) and the average chl *b* content was 0.94 mg g^{-1} (2% higher).

Inorganic content

The results from the chemical analyses of collected leaved samples are presented in Table 2. Under detection limits were some elements like Be, As, Se, Mo, Hg (0.1, 0.5, 0.6,

Table 2. Mean values and RSD of micro- and macroelements in birch leaves.

Sampling site	B mg kg^{-1}	RSD %	Al mg kg^{-1}	RSD %	V mg kg^{-1}	RSD %	Cr mg kg^{-1}	RSD %	Co mg kg^{-1}	RSD %
1	36	2.4	40	0.8	0.11	2.6	0.31	7.2	0.11	3.8
2	31	1.4	54	9.5	0.14	2.1	0.44	2.3	0.14	4.0
3	29	1.1	57	14.6	0.14	3.3	0.43	3.9	0.14	2.1
4	61	1.4	75	14.9	0.17	9.1	0.58	18.2	0.11	9.4
5	13	2.9	75	1.2	0.27	1.1	1.2	2.6	0.13	3.0
6	20	2.4	102	6.0	0.26	2.6	0.91	15.6	0.11	3.0
7	44	1.2	36	16.5	0.12	6.3	0.27	6.1	0.39	1.5
8	51	3.2	36	0.6	0.11	2.3	0.39	7.0	0.22	2.1

Table 2. Continued…

Sampling site	Ni mg kg^{-1}	RSD %	Sr mg kg^{-1}	RSD %	Cd mg kg^{-1}	RSD %	Pb mg kg^{-1}	RSD %	Bi mg kg^{-1}	RSD %
1	0.48	3.6	81	0.4	0.17	3.5	1.17	0.8	0.72	-
2	0.61	1.2	73	0.4	0.36	2.3	3.34	0.7	0.68	-
3	0.59	4.7	72	0.2	0.34	1.7	3.27	0.7	0.66	-
4	2.3	1.1	39	1.7	0.12	2.4	1.31	1.3	1.73	15.3
5	0.65	4.7	43	0.2	0.25	5.5	2.66	1.1	0.71	-
6	1.4	1.4	32	1.3	0.28	0.6	3.04	0.7	0.54	-
7	1.7	1.9	34	0.8	0.18	3.6	1.36	1.5	0.67	-
8	0.41	7.1	41	1.3	0.13	5.2	1.24	0.9	0.70	-

Table 2. Continued…

Sampling site	Zn mg kg^{-1}	RSD %	Cu mg kg^{-1}	RSD %	Na mg kg^{-1}	RSD %	Fe mg kg^{-1}	RSD %	Mn mg kg^{-1}	RSD %
1	104	0.9	4.5	4.6	30.2	1.0	89.3	1.8	85	1.0
2	102	1.0	5.1	4.1	29.8	0.8	97.9	2.0	44	0.7
3	142	0.8	4.9	3.9	27	2.7	103.4	1.8	44	0.6
4	85	1.5	3.8	4.6	26.6	0.7	115.3	1.2	56	0.9
5	140	0.9	5.3	8.4	47.3	1.4	248.8	2.5	187	1.6
6	122	0.9	4.7	8.3	25.9	1.0	177.4	3.3	34	1.1
7	122	0.9	3.4	11.8	31.7	1.5	77.7	1.9	44	0.8
8	237	0.7	6.1	9.7	27.7	1.0	97.9	2.6	57	1.1

Table 2. Continued…

Sampling site	P %	RSD %	S %	RSD %	Mg %	RSD %	K %	RSD %	Ca %	RSD %
1	0.21	1.0	0.29	5.2	0.31	0.5	1.62	0.5	1.5	0.8
2	0.19	0.8	0.29	5.6	0.26	1.6	1.25	1.2	1.7	1.6
3	0.23	0.4	0.30	4.0	0.45	1.9	1.00	1.2	1.3	0.9
4	0.15	1.6	0.27	4.9	0.23	1.1	1.31	3.2	1.1	0.8
5	0.25	0.8	0.28	2.6	0.26	1.6	1.10	0.6	1.2	0.7
6	0.16	0.4	0.25	3.6	0.29	1.2	1.01	1.4	1.1	1.5
7	0.25	0.7	0.28	2.9	0.37	0.7	1.10	1.6	1.2	0.7
8	0.28	0.9	0.27	4.5	0.32	1.1	1.55	1.5	1.1	0.9

0.4, 0.05 mg kg^{-1}, respectively) and they are not showed in this table. Exceptions were determined for As (0.5 mg kg^{-1}) in site 4, Hg (0.05 mg kg^{-1}) in sites 2 and 3, also for Mo (0.45 mg kg^{-1}) in site 7 and (0.86 mg kg^{-1}) in site 4. The concentrations of uranium were between 0.01 and 0.02 mg kg^{-1} in all cases, higher in sites 4, 5 and 6, and are not presented too.

The average concentration of macro- and microelements in the *Betula pendula* leaves, collected in the town of Plovdiv, was in the descending order as follows: Ca (1.3%)>K (1.24%)>Mg (0.31%)>S (0.28%)>P (0.22%)>Zn (132 mg kg^{-1})>Fe (126 mg kg^{-1})>Mn (69 mg kg^{-1})>Al (60 mg kg^{-1})>Sr (52 mg kg^{-1})>B (36 mg kg^{-1})>Na (30.8 mg kg^{-1})>Cu (4.7 mg kg^{-1})>Pb (2.18 mg kg^{-1})>Ni (1.01 mg kg^{-1})>Bi (0.80 mg kg^{-1})>Cr (0.56 mg kg^{-1})>Cd (0.23 mg kg^{-1})>V (0.16 mg kg^{-1})>Co (0.17 mg kg^{-1})>U (0.01 mg kg^{-1}).

The maximums of 6 elements were measured in birch leaves from the sidewalk of Ruski Boulevard (B, Ni, Bi, As, Mo and U) and from the Nature monument "Bunardzhik" (V, Cr, U, Na, Fe and Mn). These two sampling sites, situated along one of the major traffic arteries in Plovdiv, differed only by the greenbelt which separated "Bunardzhik" from the road.

The maximums of 4 elements (Cd, Hg, Pb and Ca) were detected in sample from site 2 (Park Lauta). This highest content of pointed elements could be due to a carting speedway (open about 3 years ago). Three highest concentrations were obtained in site 3 – near the Railway station Trakiya (S, Mg, Hg) and in site 8 (Zn, Cu, P).

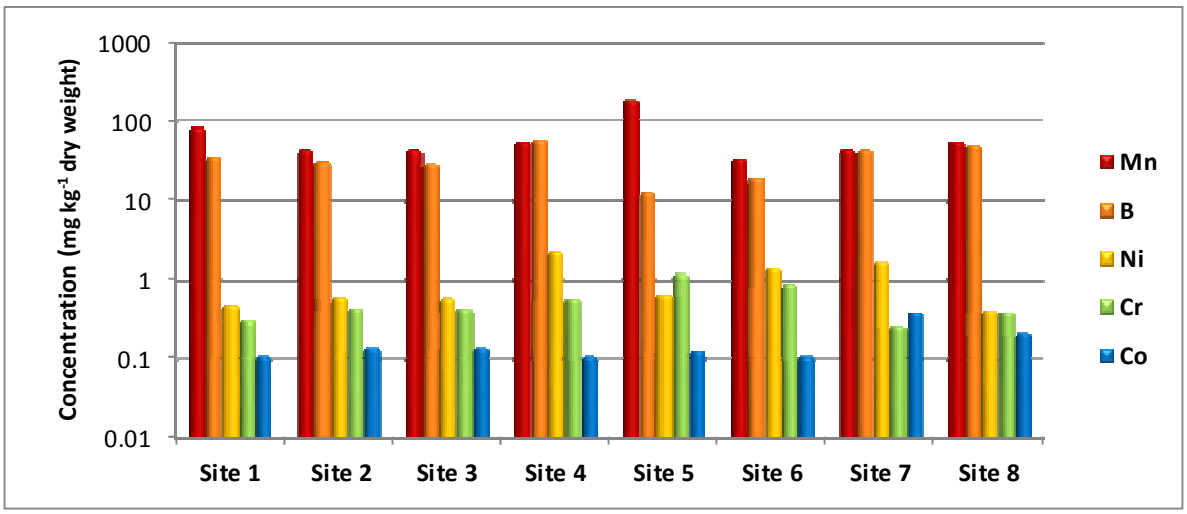

Fig. 3. Comparison of Ni, Mn, B, Cr and Co concentrations in the birch leaves from different sampling sites

Most significant variation (6-fold) were detected for nickel - from 0.41 mg kg^{-1} at site 8 to 2.3 mg kg^{-1} at site 4 and for manganese - from 34 mg kg^{-1} at site 6 to 187 mg kg^{-1} at site 5. Boron, chromium and cobalt varied 4-5 times: B - from 13 mg kg^{-1} at site 5 to 61 mg kg^{-1} at site 4, Cr – from 0.27 mg kg^{-1} at site 7 to 1.2 mg kg^{-1} at site 5, and Co - from 0.11 mg kg^{-1} at sites 1 and 6 to 0.39 mg kg^{-1} at site 7 (Fig. 3).

Sulphur was the biogenic element which varied insignificantly in the birch leaves from the selected sampling sites. PICZAK *et al*. (2003) studied element content, analyzed by ICP-AES method, in birch leaves, collected in June in Wrocław, Poland. The content of Cr (0.50 μg g^{-1}), Al (45.9 μg g^{-1}), Ca (12300 μg g^{-1}), Mg (2240 μg g^{-1}) are similar with the concentrations found by us. Lead found in our study is slightly higher while cadmium is 5 times lower in comparison with the birch Polish leaves.

Statistical evaluation

The cluster analysis divided the studied elements in two major groups (Fig. 4). The

first group consisted of only three elements – B, Ni and Bi, which appeared to have similar deposition levels in the leaves. The second major group was further separated into two subgroups. The first one clustered Al, V, Cr, Fe, Na and Mg and the second group was segmented into two smaller clusters – (Co, Mg, Zn, P, Cu and K) and (Sr, Ca, S, Cd and Pb).

The elements Ca, Mg, K, P and S (together with N and Cl, the concentrations of which were not determined) represented the main inorganic components in plants. For birch leaves this fact was confirmed by the statistical analysis, which clustered them into one subgroup (according the uniformity and similarity of distributions of these elements). Zinc, being a key functional microelement in plant physiology, also had similar accumulation

The Spearman's rank correlation analysis revealed some significant correlations between the analysed chemical elements (Table 3). The content of Fe, which resulted primarily from the abrasion of vehicle brake linings, was closely correlated with Al, Cr and V concentrations. High correlation coefficient was found between Pb and Cd, which are released during the combustion of fuel. Therefore, motor vehicle traffic may represent the main emission source for these groups of pollutants.

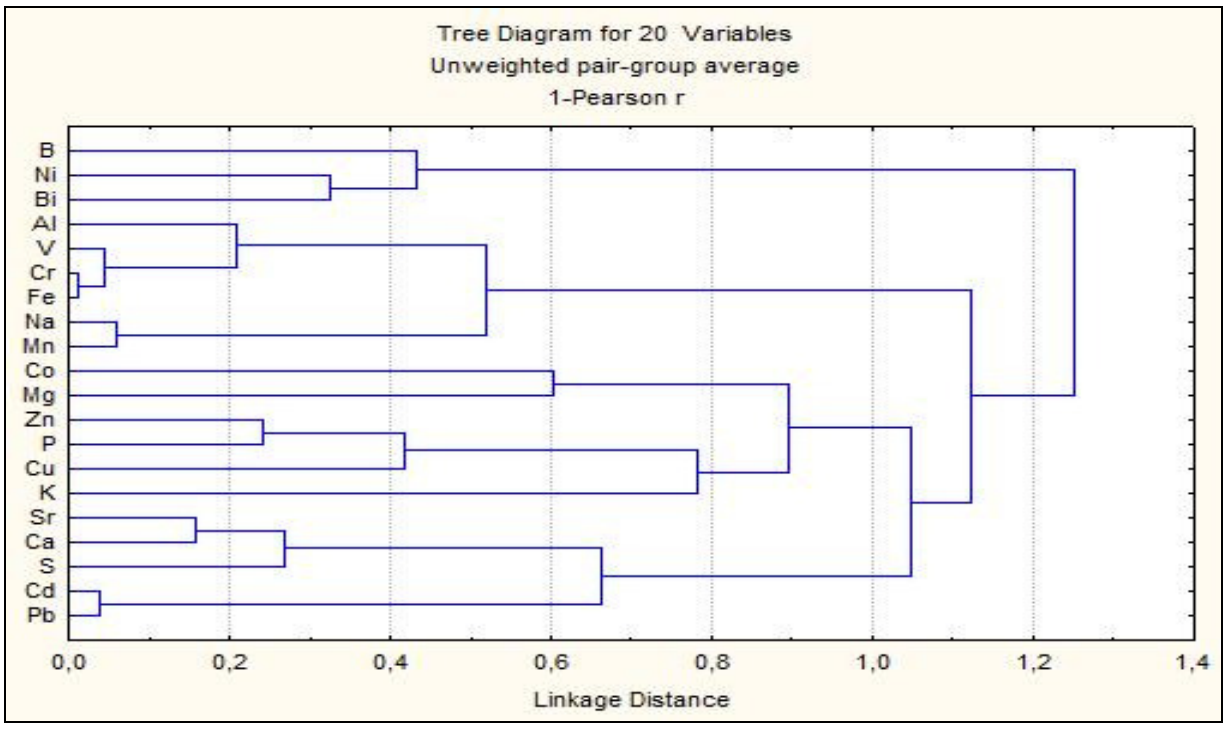

Fig. 4. Tree diagram for analyzed chemical elements
(Cluster analysis, Unweighted pair-group average, Pearson's index)

Based on this correlation analysis, it could be supposed the existence and the effect of different synergistic and antagonistic relationships between chemical elements. That was clearly observed for Cd and B, K and Pb, as well as Co and Al, where a negative correlations were strongly expressed ($r>0.7$) Close positive correlations ($r>0.8$) were observed with the plant macroelements Ca and S, Ca and Sr, as well as S and Sr, P and Zn, P and Co (in this case $r>0.74$).

Conclusions

Pigment response to urban air pollution was studied in birch leaves from 8 sampling sites in Plovdiv; highest chlorophyll values were observed at medium level of urbanization and road traffic pressure, probably as a result of enhanced protection against stress, which seemed to be not

efficient in conditions of strong anthropogenic impact.

Inorganic characteristic of leaf samples tended to indicate high values in dense traffic and industrialized areas and low values in the less urbanized areas. Correlation coefficients were calculated for each element-element combination; many positive and three negative relationships were found.

Table 3. Spearman correlations between micro- and macroelements in birch leaves (positive significant correlations are marked in grey, negative – in pink), p<0,05.

Element	B	Al	V	Cr	Co	Sr	Cd	Pb	Bi	Zn	S
Al	-0.530										
V	-0.590	0.896									
Cr	-0.548	0.892	0.916								
Co	0.184	-0.721	-0.422	-0.528							
Sr	-0.119	-0.265	-0.349	-0.190	-0.061						
Cd	-0.738	0.241	0.313	0.238	0.160	0.238					
Pb	-0.643	0.446	0.530	0.405	0.037	0.095	0.929				
Bi	0.476	-0.096	-0.120	0.024	-0.344	0.357	-0.690	-0.595			
Zn	-0.287	-0.236	-0.145	-0.096	0.506	-0.084	0.096	-0.120	-0.407		
Fe	-0.539	0.891	0.885	0.958	-0.513	-0.252	0.144	0.299	-0.012	0.096	
Mn	0.098	-0.198	-0.136	0.024	-0.126	0.464	-0.512	-0.586	0.805	0.123	
P	-0.036	-0.642	-0.400	-0.395	0.741	0.084	-0.084	-0.323	-0.084	0.801	
S	-0.206	-0.307	-0.288	-0.352	0.231	0.812	0.473	0.376	-0.012	0.018	
K	0.635	-0.473	-0.582	-0.347	-0.148	0.371	-0.659	-0.731	0.778	-0.319	-0.085
Ca	-0.282	-0.273	-0.248	-0.282	0.127	0.822	0.577	0.454	-0.012	-0.216	0,900

The passive biomonitoring with *Betula pendula* proved to be a simple and reliable tool for assessing and monitoring the air pollution in Plovdiv. In this way, biomonitoring with tree leaves will contribute additional information to the routine monitoring program in urban areas. Data of this investigation represented the pollution situation in a specific growing season. It will be necessary to make repetitions for obtain information about temporal trends and to demonstrate the increasing or decreasing environmental relevance of air pollutants.

In conclusion, our study fully supports the view that *Betula pendula* can be a usefull biomonitor of air pollution as it is a commonly distributed species, the leaves are easy to sample and show clear response to differences in air quality. Therefore, future studies are needed to establish such correlations, and to find out if other tree species are more sensitive to air pollution, and thus are better suited for passive biomonitoring.

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On the Communities of Freshwater Gastropods on Aquatic Macrophytes in Some Water Basins of Southern Bulgaria

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Abstract. The research was conducted through the period 2008 - 2009 in the Upper Thracian Lowland: Maritsa River in the city of Plovdiv, flood area near the bridge at UFT; Eastern Rhodopes: Varbitsa River at around 3 km south of the town of Kardzhali; Perperek River, within the village of Perperk; a pond in the village of Chernoochene. The material was collected from total of 3427 g herbage biomass from 7 plant species. On the aquatic macrophytes we generally found approximately the same diversity of species of freshwater gastropods during the cold and during the warm seasons. During the warm period we found a total of 6 species, and during the cold - 7 species. Most species we found on *C. demersum*, and *E. canadensis*. Overall for the studied water basins and seasons, the species *R. auricularia, Ph. acuta* and *G. albus* were most numerous and prefer to live on *C. demersum*. We calculated a narrow ecological niche of the species in most cases, where slightly wider ecological niches were registered for *R. auricularia* and *G. albus*. Largest diversity of snail communities we found on *C. demersum* and *E. canadensis*. The value of the diversity index was very low for the other species of macrophytes. We calculated low values of Sörensen's index between most of the freshwater macrophytes in relation to communities of gastropods. High similarity between the communities we indicated for *C. demersum* and *P. pussilus*, and *C. muricatum*, and *Lemna* sp., and very high between *P. pussilus*, and *Lemna* sp. We found an aggregated distribution on the macrophytes of the following species of gastropods: *R. auricularia, Ph. acuta, P. corneus, P. planorbis, G. albus* and occasional one for *V. piscinalis, A. lacustris,* and *L. stagnalis*.

Key words: snails, Pulmonata, Prosobranchia, habitats, South Bulgaria.

Introduction

In Bulgaria there is lack of detailed investigations regarding the ecology of the freshwater molluscs, while in the same time the foreign literature is quite rich on such kind of researches. Some of the most significant aspects of the ecology of freshwater gastropods are their relations with the aquatic plants and the biotope as a basin.

Studies on the relationship between freshwater gastropods and aquatic plants show several major features: freshwater macrophytes as a food for the gastropods (LACH *et al*., 2000), as a substratum on which the algae are growing, serving a food for the gastropods (HIGGINS & HANN, 1995; SEMENCHENKO *et al*., 2008) and as a micro habitats for the gastropods (BRÖNMARK, 1985).

The aim of our study was to examine the role of the aquatic vegetation as a habitat of the freshwater snails in several basins of south Bulgaria.

Material and methods

The research was conducted through the period 2008 - 2009 in the Upper Thracian Lowland: Maritsa River in the city of Plovdiv, flood area near the bridge at UFT, N42˚09` E24˚43`; Eastern Rhodopes: Varbitsa River at around 3 km south of the town of Kardzhali, N41˚34` E25˚23`; Perperek River, within the village of Perperk, N41˚45` E25˚21`; a pond in the village of Chernoochene, N41˚40` E25˚32`.

The field trips were made from 19.02.2009 until 12.11.2009. The molluscs were collected by hand or with a sack, along with the aquatic vegetation and were transported to the laboratory. For determination of the plants species the guide of DELIPAVLOV & CHESHMEDJIEV (2003) was used.

The material was collected from total of 3427 g herbage biomass from the following plant species: *Ceratophyllum demersum* L. – Rigid Hornwort (1123 g), *Ceratophyllum muricatum* Cham. (100 g), *Elodea canadensis* Michx. – Pondweed (809 g),), *Lemna* sp. – Duckweed (110 g), *Myriophyllum spicatum* L. - Water Milfoil (350 g), *Potamogeton natans* L. – Floating Pondweed (685 g), *Potamogeton pussilus* L. – Small Pondweed (250 g).

The material was separated from the plants by hand and by running water. The shells of the molluscs were measured and determined by GLÖER & MEIER-BROOK (2003) and a reference collection.

Data obtained was mathematically processed with the program BioDiversity Professional (McAleece *et al.*, 1997). Qualitative and quantitative analyzes were performed according to the different seasons (warm season - spring and summer, cold season - autumn and winter), habitat and collecting areas. The following indices were calculated: the index of quantitative diversity of Simpson, quantitative similarity of Sörensen, breadth of ecological niche of Levin, and preference index of Ivlev. Cluster analysis of Brey-Curtis was also used to investigate the relations between snail communities.

The reciprocal value of the Simpson's Diversity index is calculated by the following formula (MAGGURAN, 1988):

$$S = \frac{1}{\sum p_i^2},$$

where S – Simpson's Diversity index; p_i – proportion of species i.

The Sørensen similarity index was calculated by the following formula (SØRENSEN, 1948):

$$QS = \frac{2C}{A+B},$$

where A and B are the number of species in samples A and B, respectively, and C is the number of species shared by the two samples.

The ecological niche breadth was calculated using Levin's formula (HURLBERT, 1978):

$$B = \frac{1}{R\sum P_i^2},$$

where B – ecological niche breadth; R – number of species in the complex; P_i – proportion of species i.

The cluster analysis was performed using the Bray-Curtis index and group average linking. Bray-Curtis index is calculated by the following formula (BRAY & CURTIS, 1957):

$$BC_{ij} = \frac{S_i - S_j + 2C_{ij}}{S_i + S_j},$$

where C_{ij} is the sum of minimum abundances of the various species (abundance at the site where the species is the rarest). S_i and S_j are the total number of specimens captured at both sites.

We used Ivlev's electivity index (JACOBS, 1974) to indicate habitat selection. Electivity varies from -1.0 to +1.0, where -1.0 indicates avoidance and +1.0 preferences for a particular habitat. The formula is:

$$Ei = \frac{r_i - p_i}{r_i - p_i - 2r_i p_i},$$

where r_i is the proportion of snails observed over habitat i and p_i is the proportion of habitat i in the study area.

Results and Discussion

Qualitative analysis - composition of freshwater gastropods species

Communities of plants, basins and seasons, in which have not been found any freshwater gastropods were not included in the statistical analysis. During the study these were: 02.07.2009, 210 g P. *natans* and 455 g *C. demersum* from Perperek River, 05.09.2009, 100 g *M. spicatum* and 245 g *C. demersum*, 07.11.2009, 76 g *M. spicatum* of the pond in the village of Chernoochene and 91 g *P. natans* from Perperek River.

Overall, we found approximately the same diversity of species of freshwater gastropods during the cold and the warm seasons. During the warm period we registered total of 6 species (*Physella acuta, Planorbarius corneus, Radix auricularia, Planorbis planorbis, Acroloxus lacustris, Gyraulus albus*), and in the cold one - 7 species (*Lymnaea stagnalis, Physella acuta, Planorbarius corneus, Radix auricularia, Planorbis planorbis, Valvata piscinalis, Gyraulus albus*) on various species of aquatic plants.

Regarding the water basins, most species were found in the flood area of Maritsa River (6 species), followed by Varbitsa River (4 species), and Perperek River (2 species) and poorest was the pond at Chernoochene Village (1 species).

Most species were found on *C. demersum* (6 species: *L. stagnalis*, *Ph. acuta*, *P. corneus*, *R. auricularia*, *P. planorbis*, *A. lacustris*, *G. albus*), followed by *E. canadensis* (5 species: *Ph. acuta, P. corneus, R. auricularia, P. planorbis, V. piscinalis*). On *P. natans* and *P. pusillus* the same number of species was registered (2 species) as on *P. natans* were found *R. auricularia* and *A. lacustris*, and on *P. pusillus* - *R. auricularia* and *G. albus*. On the other species of aquatic vegetation one species on each was found, as follows: on *C. muricatum* - *G. albus*, *Lemna* sp. - *G. albus*, and *M. spicatum* - *R. auricularia*.

In Varbitsa River due to lack of study during the cold seasons we present only the species diversity during the warm period (4 species: *Ph. acuta*, *R. auricularia*, *A. lacustris*, *G. albus*). The species *Ph. acuta* was collected from *C. demersum* and *P. natans*, as well as *R. auricularia* and *A. lacustris* from the same plant species. Only on *C. demersum* we found *G. albus*. In the pond in the village of Chernoochene we found only *R. auricularia* on *C. demersum* and *M. spicatum* during the warm season and no any gastropods were collected in the cold one because the pond was drained by by the owners. In Perperek River we found the species *G. albus* and *R. auricularia* during the warm season and only *G. albus* in the cold season. *G. albus* was collected from *C. demersum* and *Lemna* sp. during the cold season. It was found together with *R. auricularia* during the warm season on *P. pusillus* and alone on *C. muricatum*. In the flood part of Maritza in Plovdiv 5 species were found during the cold season (*L. stagnalis, Ph. acuta, P. corneus, R. auricularia, V. piscinalis*) and 4 during the warm (*P. corneus, Ph. acuta, R. auricularia, P. planorbis*). During the warm season from *E. canadensis* the species *P. corneus, Ph. acuta, R. auricularia, P. planorbis* were collected and the same species were collected from *C. demersum*. During the cold season from *E. canadensis* were found *Ph. acuta, P. corneus, R. auricularia, V. piscinalis*, and on *C. demersum* the species *L. stagnalis*, *Ph. acuta, P. corneus, R. auricularia* have been identified.

Quantity and ecological niche breadth of the gastropods on the freshwater macrophytes.

Overall for seasons and collection sites by single or small-numbered specimens were represented the species *V. piscinalis* (N = 1), *A. lacustris* (N = 4), *L. stagnalis* (N = 1), *P. corneus* (N = 7) and *P. planorbis* (N = 8). Two of the numerous species included in the statistical analysis were on *C. demersum*: *R. auricularia* - 52.54% of all identified individuals and *Ph. acuta* - 86.76%. The first species was found in large numbers also on *P. natans* (42.39%), and the second with much lower numbers on *E. canadensis* (13.24%). *G. albus* had a higher percentage on *P. pussilus* (36.46%) compared with *Ceratophyllum* (31.25%). In summary we found a narrow ecological niche (index of Levin) of all the species close to one, in most cases with value lower than 1.5. Slightly wider ecological niches were calculated for *R. auricularia* and *G. albus* with values of 2.19 and 3.43, respectively (Table 1).

Table 1. Number of gastropods registered and their ecological niche breadths on the freshwater macrophytes collected.

	V. piscinalis	*A. lacustris*	*R. auricularia*	*L. stagnalis*	*Ph. acuta*	*P. corneus*	*P. planorbis*	*G. albus*
C. demersum	0	3	176	1	177	6	7	30
C. muricatum	0	0	0	0	0	0	0	9
E. canadensis	1	0	11	0	27	1	1	0
M. spicatum	0	0	3	0	0	0	0	0
P. natans	0	1	142	0	0	0	0	0
P. pussilus	0	0	3	0	0	0	0	35
Lemna sp.	0	0	0	0	0	0	0	22
Total	**1**	**4**	**335**	**1**	**204**	**7**	**8**	**96**
Levin	**1,00**	**1,60**	**2,19**	**1,00**	**1,30**	**1,32**	**1,28**	**3,43**

Table 2. Quantitative index of Simpson's diversity, calculated according to seasons and macrophytes.

Species	***C. demersum***	***C. muricatum***	***E. canadensis***	***M. spicatum***	***P. natans***	***P. pussilus***	***Lemna sp.***	**Total**
Cold season								
Total 6 species								
Valvata piscinalis	0	0	1	0	0	0	0	1
Radix auricularia	19	0	9	0	0	0	0	28
Lymnaea stagnalis	1	0	0	0	0	0	0	1
Physella acuta	24	0	15	0	0	0	0	39
Planorbarius corneus	1	0	1	0	0	0	0	2
Gyraulus albus	0	0	0	0	0	0	22	22
Total	45	0	26	0	0	0	22	93
Simpson div. index	2.21	0	2.3	0	0	0	1	3.16
Warm season								
Total species 6								
Acroloxus lacustris	3	0	0	0	1	0	0	4
Radix auricularia	157	0	2	3	142	3	0	307
Physella acuta	153	0	12	0	0	0	0	165
Planorbarius corneus	5	0	0	0	0	0	0	5
Planorbis planorbis	7	0	1	0	0	0	0	8
Gyraulus albus	30	9	0	0	0	35	0	74
Total	355	9	15	3	143	38	0	563
Simpson div. index	2.58	1	1.57	1	1.01	1.16	0	2.50
As a whole								
Total species 8								
Valvata piscinalis	0	0	1	0	0	0	0	1
Acroloxus lacustris	3	0	0	0	1	0	0	4
Radix auricularia	176	0	11	3	142	3	0	335
Lymnaea stagnalis	1	0	0	0	0	0	0	1
Physella acuta	177	0	27	0	0	0	0	204
Planorbarius corneus	6	0	1	0	0	0	0	7
Planorbis planorbis	7	0	1	0	0	0	0	8
Gyraulus albus	30	9	0	0	0	35	22	96
Total	400	9	41	3	143	38	22	656
Simpson div. index	2.54	1	2.02	1	1.01	1.18	1	2.65

Index of variety among seasons and macrophytes.

We found similar values of the Simpson's index for the two periods of the year, from which slightly higher diversity was calculated during the cold season than the warm one (values of 3.16 and 2.50).

The largest diversity we found on the species *C. demersum* (Simpson index = 2.54) and *E. canadensis* (S = 2.02). The index value

of diversity was very low for other species of macrophytes and was close to one (Table 2).

Quantitative index of faunistic similarity between communities on different types of macrophytes.

We calculated the low values of Sörensen index between most freshwater macrophytes in relation to gastropods communities (values lower than 25%). High similarity between the communities we found for *C. demersum* and *P. pussilus* (52.67%), and *C. muricatum*, and *Lemna* sp. (58.06%), and very high between *P. pussilus* and *Lemna* sp. (73.33%) (Table 3). In cluster analysis of communities as a whole we found the formation of two large cluster groups including three taxa of plants: *Lemna* sp.; *P. pussilus* and *C. muricatum*, and *C. demersum*, *E. canadensis* and *P. natans*. The community on *M. spicatum* was specific and was separated from the two other cluster groups (Fig. 1).

Table 3. Quantitative index of faunistic similarity (index of Sörensen) between communities of gastropods on different species of macrophytes represented as a percentage.

	C. demersum	*C. muricatum*	*E. canadensis*	*M. spicatum*	*P. natans*	*P. pussilus*	*Lemna sp.*
C. demersum	*	4.40	18.14	1.49	52.67	15.07	10.43
C. muricatum	*	*	0.00	0.00	0.00	38.30	58.06
E. canadensis	*	*	*	13.64	11.96	7.59	0.00
M. spicatum	*	*	*	*	4.11	14.63	0.00
P. natans	*	*	*	*	*	3.31	0.00
P. pussilus	*	*	*	*	*	*	73.33
Lemna sp.	*	*	*	*	*	*	*

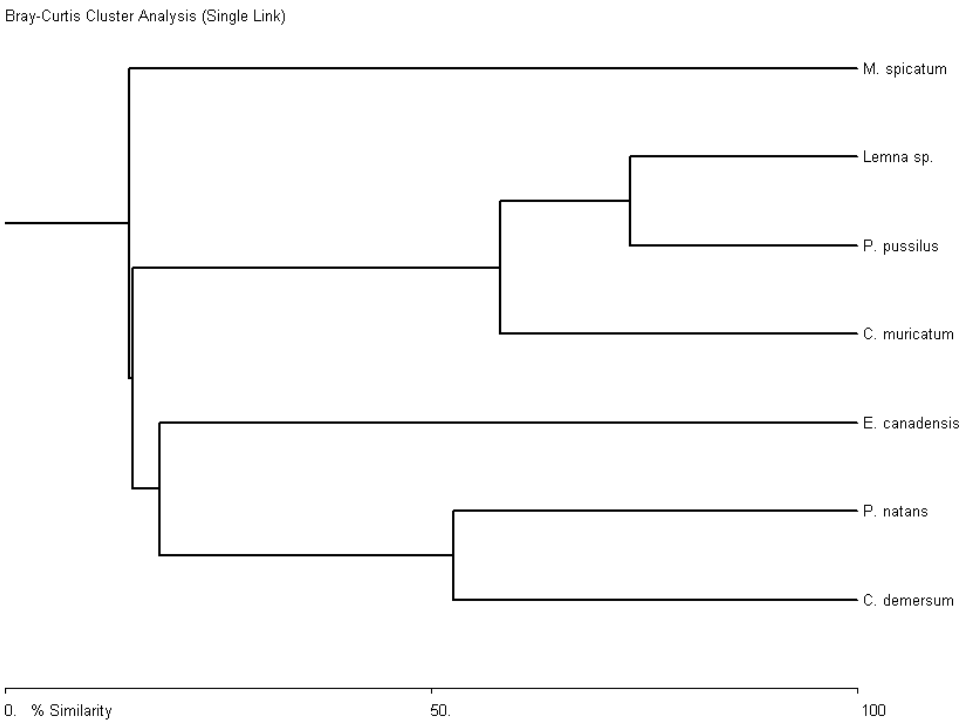

Fig. 1. Cluster analysis of gastropod communities on different species of aquatic macrophytes.

Distribution of the species of gastropods on the species of freshwater macrophytes

We determined an aggregated distribution on the macrophytes of the following gastropods: *R. auricularia, Ph. acuta, P. corneus, P. planorbis, G. albus* and occasional one for *V. piscinalis, A. lacustris*, and *L. stagnalis*.

Conclusion

On the aquatic macrophytes we found approximately the same diversity of species of freshwater gastropods during the cold and during the warm seasons. During the warm period we found a total of 6 species (*Ph. acuta, P. corneus, R. auricularia, P. planorbis, A. lacustris, G. albus*), and during the cold - 7 species (*L. stagnalis, Ph. Acuta, P. corneus , R. auricularia, P. planorbis, V. piscinalis, G. albus*) on various species of aquatic macrophites.

Most species we found on *C. demersum* (6 species: *L. stagnalis, Ph. acuta, P. corneus, R. auricularia, P. planorbis, A. lacustris, G. albus*), followed by *E. canadensis* (5 species: *Ph. acuta, P. corneus, R. auricularia, P. planorbis, V. piscinalis*).

Overall for the studied water basins and seasons, the species *R. auricularia, Ph. acuta* and *G. albus* were most numerous and prefer to live on *C. demersum*.

We calculated a narrow ecological niche (index of Levin) of the species in most cases with a value lower than 1.5. Slightly wider ecological niches were registered for *R. auricularia* and *G. albus*, respectively, with values of 2.19 and 3.43.

Largest diversity of snail communities we found on *C. demersum* (S = 2.54) and *E. canadensis* (S = 2.02). The value of the diversity index was very low for the other species of macrophytes.

We calculated low values of Sörensen's index between most of the freshwater macrophytes in relation to communities of gastropods (values lower than 25%). High similarity between the communities we indicated for *C. demersum* and *P. pussilus* (52.67%), and *C. muricatum*, and *Lemna* sp. (58.06%), and very high between *P. pussilus*, and *Lemna* sp. (73.33%).

We found an aggregated distribution on the macrophytes of the following species of gastropods: *R. auricularia, Ph. acuta, P. corneus, P. planorbis, G. albus* and occasional one for *V. piscinalis, A. lacustris*, and *L. stagnalis*.

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Heavy Metals Content in Soil Near Non-ferous Metals Production Facility and Domestic Wastes Landfill in the Area of Kardzhali Town

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Abstract. The predominant soil types in the region of Kardzhali are maroon forest soils - highly leached and poorly podzolized. Most of the soils have medium to high erosion ability - II-III degree, and the pH is neutral to slightly alkaline (7-7,8). Few are weak and moderate acidic pH (6,7-5). Soils are mostly shallow to moderately deep, poor in nutrients. In the region of Kardzhali main pollutants are heavy metals from mining-processing and metallurgical business activities of "Gorubso-Kardzhali" - AD and LZC - AD, Kardzhali. In the paper are presented results of soil contamination mainly with lead, cadmium, copper and zinc, showing concentrations of lead in the soil over the MAC, according to Ordinance № 3/2008 Standards for acceptable content of harmful substances in soil.

Key words: soil, heavy metals, pollution, Kardzhali. Bulgaria.

Introduction

The predominant soil type in District Kardjali is maroon forest soils - highly leached and poorly podzolized. There are also smaller brown forest soils rendzinas (humus-carbonate), alluvial, maroon-podzolic, delluvial and insignificant amount other soil types. The most fertile soils are in the valleys of the Arda River and its tributaries in the northern area of the Haskovo region border.

Most of the soils have medium to high erosion - II-III degree, and the pH is neutral to slightly alkaline (7-7,8). Few are weak and moderate acidic pH (6,7-5). Soils are mostly shallow to moderately deep, poor in nutrients. Arable lands are scattered and small. Characteristic in this region is that the arable area does not form compact areas, as in other regions of the country and is divided into many units, different size, shape and position (YANCHEV *et al.*, 2005).

In the region of Kardzhali main pollutants are heavy metals from mining and metallurgical processing business of "Gorubso-Kardjali" - AD and LZC-AD Kardzhali (STAYKOVA, 2009).

In this publication are presented the results of soil contamination mainly with lead, cadmium, copper and zinc.

Material and methods

Sampling of soil samples was performed in accordance with the requirements of ISO 10381-1 and BSS 17.4.5.01-85 for analysis of heavy metals. Depending on the accepted practice in hygienic criteria are defined representative sampling points. Their number is 21 and are located near sources of pollution, to the over traffic road junctions away from pollutants and near kindergartens and schools.

Soil samples were taken according to "Method of diagonals", with conditional splitting territory for sampling of plots of 0,5 ha, with an analysis of 5 samples from each plot. Samples from each sampling point are mixed with spot shaping random sample of 1 kg. Average samples consist of 5 single samples taken uniformly from the area of the fixed site. Sampling was conducted from a depth 0-10 cm.

Determination of toxic elements in mineralizatite was conducted through the methods of atomic absorption spectroscopy using flame AAS according to ISO 11047:1998, after appropriate sample preparation - mineralization by BSS 17.4.4.01-79 (MECHKUEVA, 2003).

Preparation and analytical determination of the studied elements was made according to standardized methods (Table 1).

Table 1. Used laboratory methods for analysis of heavy metals.

Element	Method of analysis	Analytical technique	Detection Limit, mg/kg
Cadmium	BSS 17.4.4.04-80, ISO 11047	FAAS	0,25
Copper	BSS 17.4.4.03-80, ISO 11047	FAAS	0,30
Zinc	BSS 17.4.4.05-80, ISO 11047	FAAS	0,32
Lead	BSS 17.4.4.02-80, ISO 11047	FAAS-2	2,5

Results and Discussion

The main industrial pollutants in soil near the town of Kardzhali are: Lead-zinc complex (LZC AD), "S & B Industrial Minerals" AD (ex Bentonit AD) and tailings of "Gorubso-Kardzhali" AD. The heavy metals fall in the soil through the emitted into the air emissions from those industries.

Anthropogenic pollution from industrial activity depends on the direction of prevailing winds, which are for the region north and northwest of the city.

Besides the direction of prevailing winds and the ambient elevation, soil acidity is of great importance, since it depends on the MAC of heavy metals in soils (Ordinance № 3/01.08.2008 for the standards of acceptable content of harmful substances in the soil).

Soils in the region of Kardzhali are calcareous humus-resinous, average power and light clay. Soil composition and reaction factors are relatively active migration of heavy metals in soil vertical profile.

In Table 2 are presented data on the content of heavy metals (cadmium, copper, zinc and lead) in soil, averaged over the period 2008-2010. From those data in Table 2 and Figure 1-4 was found that the cadmium content in the soil into a research area does not exceed the MAC, under Ordinance 3/2008 (MEW, MH, MAF, 2008). The highest cadmium concentration (2,40 mg/kg) was recorded in the sampling point № 7 - Vocational School "Vasil Levski", which is located near the factory for the extraction and processing of lead-zinc ores – "Gorubso-Kardzhali" - AD.

The content of copper and zinc is bellow the MAC at all locations. Limit of copper were specified in sampling point № 9 - square "Studen Kladenets" Street "Dospat" № 1A (182,66 mg/kg), and zinc (387,27 mg/kg) again at sampling point № 7 - VS "Vasil Levski". Approximately in 50% of the sampling points a lead content is above the MAC (200 mg/kg) from 1.10 to 3.44 times. The most massive lead contamination of soil samples was examined in the region of VS "Vasil Levski". High average values for the period are recorded in sampling point - the

Railway station. The probable accumulation of lead is not only LZC-AD, and from the tailings pond of "Gorubso-Kardzhali"-AD by the transport of lead concentrate near this point and traffic from the adjacent boulevard. The average total lead concentration for the 21 sampling points does not exceed the MAC.

Table 2. *Heavy metal content, averaged over the period 2008-2010 in (mg/kg).*

№	Sampling Point	Heavy metals content, mg/kg			
		Cd	Cu	Zn	Pb
1	Kardzhali, Baykal District, 2 "Gen. Vladimir Stoychev"Street	1,38	9,87	127,85	222,56
2	Kardzhali, exit to Haskovo	0,67	11,64	122,59	114,65
3	Kardzhali, Belomorski Blvd., yard of Kindergarten "M. Gorki"	0,91	25,78	198,25	289,50
4	Kardzhali, Studen Kladenets District, "Veslets" Street	0,59	42,09	99,58	31,11
5	Kardzhali, Studen Kladenets District, RRM	1,43	41,64	200,12	405,31
6	Kardzhali, Railway Station, Block 81	1,74	22,89	205,23	441,28
7	Kardzhali, Vocational School "Vasil Levski", the yard	2,40	27,68	387,28	688,67
8	Kardzhali , MSCH	1,82	22,28	115,20	221,03
9	Kardzhali , Studen Kladenets District, 1A "Dospat" Str.	1,06	182,66	103,38	239,54
10	Kardzhali , Studen Kladenets District, "St. Kliment Ohridski" High School	1,21	19,51	113,82	293,95
11	Kardzhali , FHL Kindergarten	0,79	17,79	116,72	49,58
12	Kardzhali, Vazrozhdentsi District, P. R. Slaveykov High School	1,33	17,36	95,73	132,36
13	Kardzhali, Veselchane District, 9 "Victory" Street	0,80	21,05	115,42	38,67
14	Kardzhali,Gledka District, 4 "Tina Kirkova" Street	0,53	21,08	99,64	27,08
15	Kardzhali, Gorna Gledka District, 17 "K. Lyatifova" Street	0,86	15,33	78,85	35,94
16	vil. Ostrovitsa, municipality Kardzhali	1,66	24,98	164,27	64,27
17	Kardzhali – exit to Ardino	0,59	10,15	78,06	22,88
18	Kardzhali, Prileptsi District opposite PWPS	0,74	12,97	51,33	21,61
19	vil. Vishegrad, municipality Kardzhali	0,67	23,98	54,30	25,83
20	vil. Gluhar, municipality Kardzhali	0,81	13,98	193,07	36,15
21	vil. Enchets, municipality Kardzhali	0,50	16,13	134,88	375,00
Toatal Average value		**1,35**	**1,07**	**28,61**	**135,98**

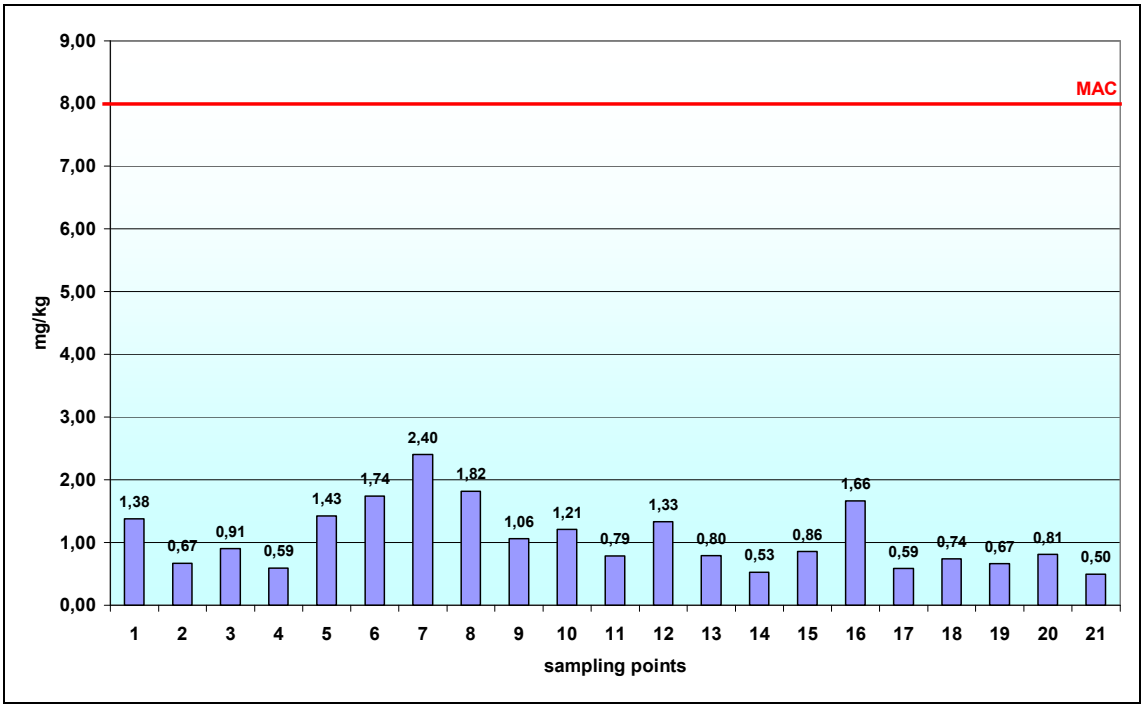

Fig.1. Cadmium content in soils – average to the period 2008-2010.

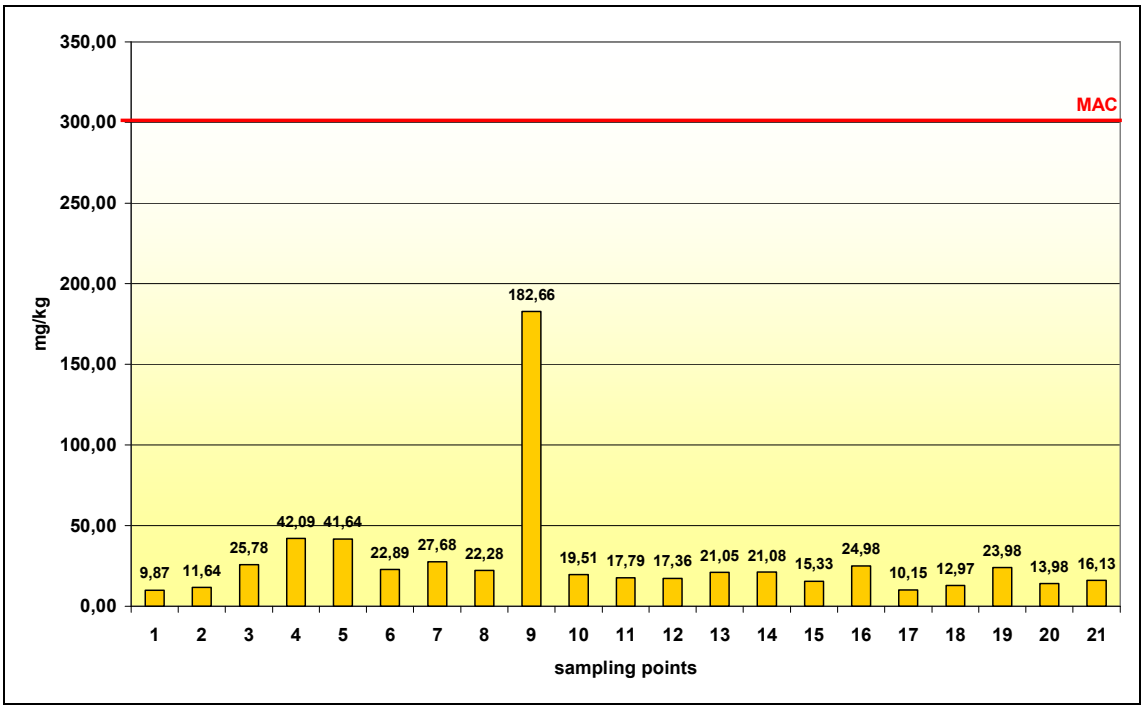

Fig. 2. Copper content in soils – average to the period 2008-2010.

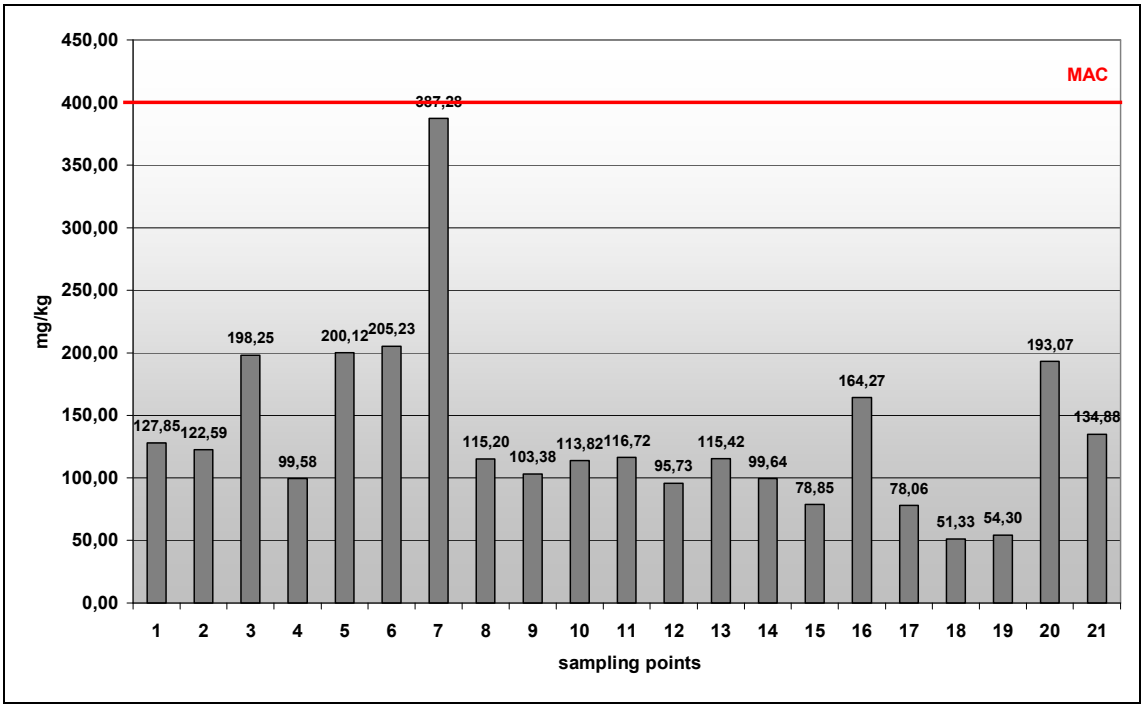

Fig. 3. Zinc content in soils – average to the period 2008-2010.

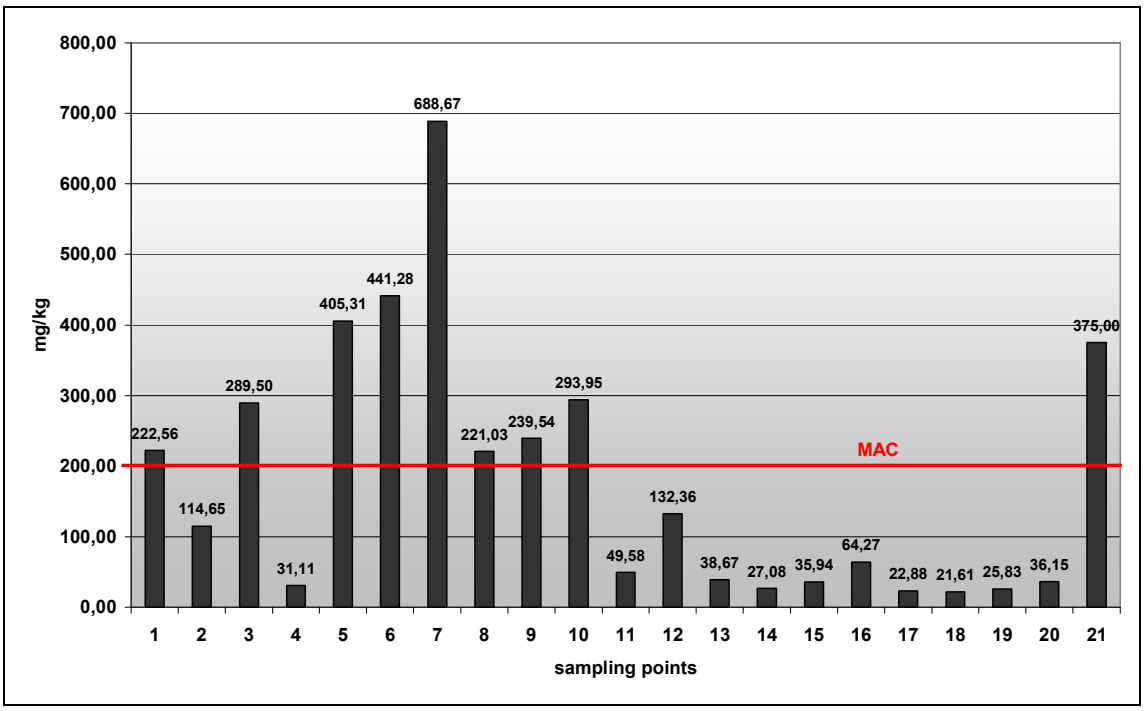

Fig. 4. Lead content in soils - average to the period 2008-2010.

Index of variety among seasons and Comparing the data content of heavy metals (cadmium, copper, zinc and lead) in the soil of the region of Kardzhali for the period 2008-2010 was found significant higher concentration at the end of the study, which confirms the conservative properties and cumulative soil as a media. The results are presented in Table 3.

Table 3. Averages, medians and standard deviations of heavy metals in soils for the period 2008-2010

Heavy metals	Year	Statistical indicator		
		Average value	Median	Standard deviation
Cd	2008	0,500	0,400	0,138
	2009	0,465	0,280	0,631
	2010	1,516	1,250	0,868
Cu	2008	44,150	39,500	20,559
	2009	7,102	0,870	15,533
	2010	17,143	14,000	9,873
Zn	2008	168,600	173,000	32,107
	2009	42,143	16,880	60,520
	2010	201,357	180,000	133,312
Pb	2008	36,950	38,000	5,889
	2009	19,853	2,750	42,232
	2010	70,626	48,000	52,256

Conclusions

• The soil secondary contaminated by the atmosphere is a depot facility for heavy metals exceeding the statutory limits could be a potential source of groundwater contamination, plant and animal products with a local origin.

• For the observed period (2008-2010) was found that approximately 50% in the sampling points a lead content of soils over the MAC (200 mg/kg) from 1,10 to 3,44 times. Cadmium, copper and zinc is bellow the MAC.

• At the regional level institutions with responsibilities for the management of environmental quality and public health in the region need to fit together to successfully achieving of effective solutions to issues concerning:

- Aerosol pollution from tailings pond of "Gorubso – Kardzhali" AD;

- Optimization of treatment facilities and LZC – AD and "S & B Industrial Minerals" AD in their proceedings;

- Promotion among private farmers growing crops with little potential for accumulation of heavy metals and appropriate planting.

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Comparative Data on the Trophic Spectrum of Syntopic Bombina variegata and Rana temporaria (Amphibia: Anura) Populations from the Iezer Mountains, Romania

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Abstract: In August 2009, we compared the trophic spectrum of syntopic *Bombina variegata* and *Rana temporaria* populations from the Bratia Valley in the Iezer Mountains. The separation of the trophic niche observed between the juveniles and the adults of *R. temporaria* is caused by the differences in size between them, differences that influence their jump. The main consumed prey taxa by *Rana temporaria* is represented by Arahnida, Gastropoda and Hymenoptera. Furthermore, the differences between the sizes of their mouths separate the trophic niche between the two species. Our results indicate a trophic selectiveness for *B. variegata,* which prefers ants. Despite the fact that the two species share the same habitat, the ants were consumed mostly by *B. variegata*. This is probably a consequence of the fact that *B. variegata* is a poisonous species, the toxins being able to originate from their preys – as are ants - as well. The lack of competition for the two species indicates a rich trophic offer, enough to satisfy the energy needs of both species in contact in a limited space.

Key words: *Bombina variegata, Rana temporaria,* food composition, trophic niche, Romania.

Introduction

The trophic niche represents the entire trophic relations of a species, of its relationships with its prey and with its enemies (ELTON, 1927). The overlapping of this niche means partially exploring the same resources in the same physical space by two or more species, each niche comprising an own domain and an overlapping domain with at least one related species (STUGREN, 1994). Organisms may have a high degree of liberty in terms of their physiological capacities in order to exist under the influence of various environment factors, especially the biotic ones – weather they are trophic or just the interaction with other organisms with which they share their special niche (COHEN, 1977). A variety of factors may cause community-wide patterns such as resource partitioning, and the relative importance of these factors may differ among taxa or communities at different geographical locations (TOFT, 1985).

Comparative studies between two related species that occupy the same habitat were done before in the specialized literature (e.g. JONES *et al*., 2006; STOJANOVA & MOLLOV, 2008; etc.). Comparison of the trophic spectrum of two species offers the possibility to study the feeding behavior in a more accurate way, in relation with the other species. We compared the trophic spectrum of *Rana temporaria* and *Bombina variegata*, species for which the trophic spectrum was analyzed separately (e.g.

BLACKITH & SPEIGHT, 1974; FERENTI *et al*., 2010 etc.) or together with many other species (e.g. KUZMIN, 1990).

Material and methods

We analyzed the feeding of a *Bombina variegata* and *Rana temporaria* populations respectively, from the Bratia Valley (Candesti locality, Arges County, Romania). The study took place in August 2009. We analyzed 54 individuals (males and females) of *B. variegata* and 62 individuals of *R. temporaria* (22 adults (males and females) and 40 juveniles). The habitat is represented by a large wet area in the Candestului meadow. This pool system is made out of smaller puddles with 30-40 cm deep waters and with variable surface. In some areas the puddles are connected and form a temporary canal. Larger pools directly linked with the river, which – at this stage – already has a high and fast flow, represent the upstream of this zone.

For taking our samples we used a method recommended by the specialized literature (SOLE *et al*., 2005): namely stomach flushing. This way our study does not affect the sizes of each population. The stomach contents were later analyzed and identified in the laboratory, with the help of the specialized literature.

In analyzing our results we used the following parameters. For the intensity of the feeding we used the feeding rate, the maximum and the average numbers of preys/individual. Other parameters used are the amounts of each prey taxa, the frequency of the prey taxa, their origin, and for the diversity of the preys we also calculated the Shannon–Wiever index. In order to estimate the similarity of the feeding among the individuals of the studied population, we determined the Sørensen index (CHAO *et al*., 2005) using EstimateS 7.0 (COLWELL, 2005). Also, for analyzing the significance of the differences between the trophic spectrums of the two species we used Statistica 6.0 to calculate the Mann Whitney U-test (STATSOFT INC., 2004).

Results

The feeding rate does not differ a lot between the two species, the values of empty stomachs for *R. temporaria* being 4.84% and for *B. variegata* 3.7% respectively. The frequency of stomachs with vegetal remains is high for both species. However, the higher values are met in the case of *R. temporaria*. That aside, we can observe a parallel between the terrestrial preys and the presence of vegetal debris in the stomach samples.

The frequency of stomachs with shed skin and minerals shows very clearly the place where both species hunt. For shed skin, the higher frequency is observed in the case of *B. variegata*. The intensity of the feeding differs a lot between these two species. We observed that the yellow-bellied toad has a much higher intensity for the case of each parameter (Table 1).

Table 1. Number of studied individuals, empty stomachs and the consumed preys, the feeding intensity and the origin of preys

	Rana temporaria	*Bombina variegata*
No. of the studied individuals	62	54
% Empty stomachs	4,83	3,70
The total number of the preys	370	410
The max. no. of prey/individual	14	25
The average no. of prey/individual	5,96	7,59
% Aquatic preys	1,89	10,48
% Terrestrial preys	98,10	89,51

From the prey taxa percentage abundances' point of view, we can detect the same differences mentioned above. For *R. temporaria* the highest amount is represented by Araneida, followed then by Hymenoptera, Coleoptera, Gastropoda, Muscidae, etc. For *B. variegata* the first place is taken by the Formicidae, followed after by the same taxa as those recorded for *R. temporaria* (Table 2). It is easy to observe the presence in relatively high amounts of preys like Formicidae and Collembola in the diet of this species. For the case of *R. temporaria*, the preys with the greatest amounts (especially for the adults) are the bigger sized ones as

well (Coleoptera, Araneida, Gastropoda, *Limax sp*., etc). On the other hand, small preys are present in the diet of *R. temporaria* juveniles, but the Formicidae do not represent an important amount. Collembola and Aphida are present in the stomach contents of *B. variegata*, too, but most likely because of their opportunistic feeding behavior. The composition of the trophic spectrum of both species and the presence of taxa of different prey and way of life respectively suggest that the selection of preys is not voluntary for neither of the two species (except for the Formicidae in the case of *B. variegata*).

In the case of the frequencies of occurrence of the prey taxa, a parallel between them and amounts of those prey taxa can be observed. Formicidae not only appear with a high amount, but their frequency is also very high, a fact that suggests that *B. variegata* consumes ants by actually selecting them. We also noticed the presence of small-sized preys for *R. temporaria* juveniles – due to their smaller size – but the Formicidae did not represent a high amount in this case (Table 2). The biggest difference between the trophic spectrums of these two species can be observed when it comes to the origin of the prey taxa. While in the case of *R. temporaria* the aquatic preys represent only 1.89 %, for *B. variegata* they have a value of 10.49% from all preys (Table 1).

In terms of diversity, the differences aren't very considerable. For *R. temporaria* the values of the diversity are H=2.95 where as for *B. variegata* H=2.97. Also, for the similarity of the feeding we calculated the Sørensen index. Its values are again close: S=0.25 for *R. temporaria* and S=0.26 for *B. variegata*. While comparing the differences between the trophic spectrums after applying the Mann-Whitney U-test, the values are not significant ($p>0.05$, $p=0.59$).

Discussion

The presence of a small number of individuals that had no stomach contents suggests that the habitat had the necessary conditions for feeding, almost optimally, being thus favorable for both species.

The different foraging areas of the two species can cause the dissimilarities between the feeding rates. Even if *B. variegata* is considered a more terrestrial species than its congener one – *Bombina bombina* (NEČAS *et al.*, 1997), it hunts in both terrestrial and aquatic environments. Meanwhile, *R. temporaria* uses the aquatic environment almost exclusively for reproducing (COGĂLNICEANU *et al*., 2000), hunting only on land (KOVACS *et al*., 2010). Thus, the higher feeding rate of *B. variegata* can be caused by its ability to hunt in both environments, a fact that doubles its chances of capturing preys. On the other hand, though, these values suggest less optimal conditions in the terrestrial environment. However, the parallel between the frequency of ingested vegetation fragments and the presence of terrestrial food leads to the idea that vegetation was swallow on land. The higher value for the frequency of vegetal remains in the case of *R. temporaria* is a consequence of the fact that it hunts strictly in the terrestrial environment.

For amphibians, the specialty literature indicates cases where the amounts of aquatic preys are in positive correlation with the frequency of shed skin, which in turn suggests that these were in fact consumed in the water (CICORT-LUCACIU *et al*., 2007; FERENTI *et al*., 2008). For *R. temporaria*, the absence of aquatic preys from their trophic spectrum demonstrates that shed skin weren't consumed from the water. Such is also the case of other terrestrial species where shed skin consumption was recorded (KOVACS *et al*., 2007; KOVACS *et al*., 2010) and the explanation of the phenomenon is similar.

The differences between the behaviors of the two species deduced from the differences of their feeding intensities can appear due to the size of both preys and predator. Thus, the size of the mouth is a limiting factor for selecting preys, being considerably smaller for *B. variegata*. Hence, it resorts to smaller preys, which in turn, in order to meet the energy needs, have to be consumed in greater numbers. Thus, the common brown frogs don't have to consume a great number of these preys in order to

Table 2. Trophic spectrum of both studied amphibian species. **Legend:** *R. t. –Rana temporaria, B. v. – Bombina variegata,* ad. – adults, J.-juveniles, ter. - terrestrial, L. – larvae, aq. – aquatic.

Prey taxa	**Percentage abundance**			**Frequency of occurrence**		
	R. t.		***B. v.***	***R. t***		***B. v.***
	Ad.	***J.***		***Ad.***	***J.***	
Oligocheta - Lumbricidae	2.59	1.18	1.12	13.64	7.5	22.22
Gastropoda (ter.)	10.34	7.09	1.95	27.27	35	14.81
Gastropoda - *Limax* sp.	0	1.57	1.95	0	10	11.11
Lamellibranhiata	0	0.79	0	0	2.5	0
Arahnida - Pseudoscorpionidae	0.86	0.79	0.48	4.55	5	3.70
Arahnida - Araneae	12.07	16.54	11.70	54.55	67.5	57.40
Arahnida - Acari	0	9.06	0.73	0	35	5.55
Arahnida - Opiliones	0.86	0.79	0	4.55	5	0
Crustacea –Isopoda(ter.)	0	0	0.48	0	0	3.70
Crustacea - Gamaridae	0	0	4.63	0	0	12.96
Myriapoda – Diplopoda	1.72	1.18	0.73	9.09	7.5	5.55
Myriapoda – Chilopoda	0.86	1.18	0.24	4.55	7.5	1.85
Thysanura	0	0	0.24	0	0	1.85
Collembolla	1.72	7.48	2.19	4.55	30	7.40
Ephemeroptera (L.)	0	0	0.24	0	0	1.85
Ephemeroptera (ad.)	0	0	0.24	0	0	1.85
Odonata (L.)	0	0	0.48	0	0	3.70
Plecoptera (L.)	2.59	0	0.97	9.09	0	3.70
Plecoptera (ad.)	0	0	0.24	0	0	1.85
Orthoptera	11.21	0.79	0.73	54.55	5	3.70
Dermaptera	3.45	0.39	1.70	18.18	2.5	12.96
Homoptera - Cicadillidae	2.59	2.36	2.92	13.64	15	16.66
Homoptera - Afidinidae	0	1.18	3.17	0	5	16.66
Heteroptera (ter.)	7.76	0.79	1.21	31.82	5	7.40
Coleoptera (L. ter.)	0.86	0.39	0.97	4.55	2.5	5.55
Coleoptera (ad. ter.)	6.90	9.84	6.82	31.82	45	40.74
Coleoptera - Dytiscidae (L. aq.)	0	0.79	3.90	0	2.5	20.37
Coleoptera - Dytiscidae (ad. aq.)	0	0	0.24	0	0	1.85
Coleoptera - Carabidae	7.76	2.76	2.43	31.82	15	14.81
Coleoptera - Stafilinidae	1.72	1.18	2.68	4.55	7.5	12.96
Coleoptera - Elateridae	0	0	0.24	0	0	1.85
Coleoptera – Coccinelidae	0	0	0.24	0	0	1.85
Coleoptera - Curculionidae	0.86	0.39	0	4.55	2.5	0
Coleoptera - Crizomelidae	0	0.39	0.24	0	2.5	1.85
Neuroptera	0	0	1.21	0	0	7.40
Lepidoptera (L.)	7.76	7.09	2.19	40.91	30	14.81
Lepidoptera (ad.)	0	1.18	0	0	7.5	0
Diptera - Nematocera - Typulidae	0.86	0.39	0	4.55	2.5	0
Diptera - Nematocera - Culicidae	1.72	1.97	3.17	9.09	12.5	22.22
Diptera – Brahicera (L. ter.)	0	0	0.97	0	0	5.55
Diptera – Brahicera (ad. ter.)	0	0	0.48	0	0	1.85
Diptera – Brahicera- Muscidae	5.17	5.91	6.34	22.73	25	31.48
Hymenoptera	3.45	12.99	3.17	18.18	40	20.37
Hymenoptera - Formicidae	3.45	1.57	21.95	9.09	5	66.66
Hymenoptera - Apidae	0	0	0.24	0	0	1.85
Hymenoptera - Vespidae	0.86	0	0	4.55	0	0
Vegetal fragments	-	-	-	77.27	67.5	61.11
Shed skin	-	-	-	13.64	22.5	22.22
Minerals	-	-	-	-	5	7.40

obtain the same quantity of energy. The differences brought by the size of their mouths separate thus, invariably, the trophic niches of these two species, as well as dividing the niches between those of adults and juveniles. *R. temporaria* juveniles and *B. variegata* adults present very little differences due to their similar sizes. Such a phenomenon of trophic niche overlap between one species' juveniles and another's adults was documented in the specialty literature before (KUZMIN, 1990). This is how the ontogenetic evolutionary state plays an important role in trophic niche overlapping for other amphibians as well (SOLE *et al*., 2009; FRANCA *et al*., 2004).

The separation of the trophic niche observed between adults and juveniles of *R. temporaria* also appears between the two species and is founded on their difference in size. *R. temporaria*, being a bigger species and having larger leaps, has a facile access to preys that are, for example, on higher plants, whereas *B. variegata* does not. The influence of amphibians` leap distance in the composition of their trophic spectrum was recorded for other amphibian populations from the Carpathian Mountains (KUZMIN, 1990). The separation of their trophic niches is consequently done vertically in space.

R. temporaria captures preys from the substratum as well, but only larger preys and not at all Formicidae – a taxon that holds very high amounts in the trophic spectrum of *B. variegata*. This fact strengthens the statement of some authors according to which insects rich in alkaloids, formic acid etc. are consumed more intensively by species with a toxic skin secretion, these alkaloids being discovered in their toxin (MEBS *et al*., 2005; DALY, 1998).

Due to its way of life, the common brown frog consumes aquatic preys only accidentally and these are represented only by Plecoptera larvae. It is possible that these larvae were consumed shortly after they left the aquatic environment or in a time when the water level dropped. A similar explanation was given not long ago in regards to another terrestrial amphibian - *Salamandra salamandra* – and its aquatic prey consumption (COVACIU-MARCOV *et al*., 2002). As of *B. variegata* the aquatic preys are represented by various taxa, which indicates that the food acquired from this environment completes its trophic spectrum. The consumption of about 10% aquatic preys was pointed out by other authors for other populations, but each time it depended on the environment's conditions (SAS *et al*., 2004; PETER *et al*., 2005; GHIURCĂ & ZAHARIA, 2005, SZEPLAKI *et al*., 2006).

The lack of competitiveness between the two species that occupy the same habitat seems to be a general rule for amphibian species. Thus, situations in which two species residing in the same habitat did not compete for food and did not bother each other were previously documented for newts (COVACIU-MARCOV *et al*., 2010), two species of brown frogs (KOVACS *et al*., 2010) and even some snake species (METZGER *et al*., 2009). Such a fact may seem surprising, because in many cases the habitat occupied by those species was limited in surface, which only brought them more frequently in contact. The absence of competition between synoptic amphibian species, despite using the same trophic base, can only suggest a rich trophic offer that those species exploit. As a result, the trophic offer is probably sufficient to meet the trophic needs of more amphibians, despite the fact that they are aiming for the same target prey groups and have relatively similar hunting methods. As such, the differences between their trophic niches (vertical and horizontal) that appear between the trophic spectrum of some species that occupy the same ecological niche look like the result of limits caused by the morphologic particularities of each species (KUZMIN, 1990).

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Comparison of growth, nutrition and soil properties of pure stands of Quercus castaneifolia C. A. Mey. and mixed with Carpinus betulus L. in the Hyrcanian forests of Iran

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Abstract. In the present study, *Quercus castaneifolia* (Oak, as target species) and *Carpinus betulus* (Horn beam, as native component species) were planted in five proportions (100Q, 70Q:30C, 60Q:40C, 50Q:50C, 40Q:60C) in the Noor region (North Iran). After 12 years, the effects of the species on the growth of the trees, nutrient concentrations in the live and senescent leave and on soil properties were assessed. The results showed the survival and diameter at breast height (dbh) of the individual Oak trees were positively affected by the presence of Horn beam. Percent retranslocation of the nutrients in *Quercus* trees was: K>N>P. Leaf-litter fall production ranged from 4.70 to 6.80 Mg ha^{-1} $year^{-1}$. N concentration in fully expanded leaves, N and Ca concentrations in the senescent leaves of *Quercus* trees and N concentration in topsoil were higher in some of the mixed plantations than in the monocultures of the *Quercus* trees. N fluxes, N and P retranslocation, and soil P concentrations in the monocultures were intermediate relative to mixed plantations. The obtained results somewhat indicated that the mixing with hornbeam increased the productivity and sustainability of the oak sites. Within the framework of this experiment, it appeared that production was maximized when these two species were grown together in the proportion of 50% *Quercus castaneifolia* and 50% *Carpinus betulus*.

Keywords: Tree growth, Mixed plantation, Nutrition, Nutrient flux, Nutrient retranslocation, Soil property.

Introduction

The development of human societies often has caused an overexploitation of forests and a decrease in their area (AUGUSTO *et al.*, 2002). Over the last few years, interest in establishing reforestation projects with native species in forest plantations as a means to supplement existing wood markets and as a way to detain the overexploition of natural resources has increased (PIOTTO *et al.*, 2004). While plantations proliferate, local

communities are looking for suitable native species to cultivate in plantations (PETIT & MONTAGNINI, 2004).

The growth of dominant species is faster in mixed than in pure plantation, and that mixed plantations have high volume and biomass production in comparison with pure stands (MONTAGNINI & PORRAS, 1998; MONTAGNINI, 2000). However, the success of the establishment of mixed forest plantations depends on plantation design and an appropriate definition of the species to be used, taking into consideration ecological and silvicultural aspects (WORMALD, 1992; PIOTTO *et al.*, 2004).

To assess the growth performances of a species, it is important to study the productivity and nutrient dynamics (SHANMUGHAVEL & FRANCIS, 2001). Repeated harvesting of planted trees may deplete site nutrients. Heavy nutrient drain is an adverse impact on long-term site quality and sustained production (HOPMAN *et al.*, 1993; KHANNA, 1997; SWAMY *et al.*, 2006). Forest productivity is limited by the supplies of one or more nutrients in almost all forests, and forest nutrition management is a key issue in the management of commercial forests (STAPE *et al.*, 2006).

Within the same community, foliar nutrient concentrations vary largely among different species and among different individuals of the same species despite similar soil conditions (NIINEMETS & KULL, 2003), but the plant and species characteristics responsible for this variability are still poorly understood. Degree of retranslocation or resorption of nitrogen and phosphorus within the plants is also an important factor to be considered. Within-Plant cycling of these elements decreases plantation dependence on the biogeochemical processes to fulfill nutrient requirements after the initial exponential phase of tree growth (MILLER, 1984). It also decreases potential losses from the system due to leaching and erosion, as resorption maintains the nutrients within the living biomass (CUEVAS & LUGO, 1998).

In Iran, Hyrcanian vegetation zone is a green belt stretching over the northern slopes of Alborz mountain ranges and covers the southern coasts of the Caspian Sea. It has a total area of 1.84 million ha comprising 15% of the total Iranian forests and 1.1 % of the country's area (KHOSROSHAHI & GHAVVAMI, 2006). Hyrcanian or northern forests of Iran stretch out from the sea level up to an altitude of 2800 m and encompass different forest types thanks to their 80 tree and shrub species (SAGHEB-TALEBI *et al.*, 2004). Among these species, *Quercus castaneifolia* C. A. Mey. (Fagaceae) is the most commercial species after beech (*Fagus orientalis* Lipsky), which includes 6.6 % of the area and 8.01 % of the standing volume of these forests and it can reach 50 m in height and 2 m in dbh.

The potential for maintenance and expansion of the oak forests by natural regeneration appears to be at best limited (WATT, 1919; LINHART & WHELAN, 1980, LANGBEIN, 1997; MIRKAZEMI, 1997; MOHAJER, 1999; PALMER *et al.*, 2004). In order to promote expansion and rehabilitation of the oak forests, a program of oak seedling planting may be required (TRUSCOTT *et al.*, 2004). Oaks can be established as pure or mixed plantations on the old fields or other open areas largely devoid of forest vegetation (JOHNSON *et al.*, 2002).

It should be noted that many native Hyrcanian tree species are also potentially valuable commercially and that using such species, aside from satisfying economic objectives, could be more acceptable ecologically and socially. We examined oak growing in mono-specific and two-specific plantations with *Carpinus betulus* L. (Corylaceae). We chose these species as they are native species of Hyrcanian Forests in the north of Iran and have potential value in wood production. There are considerable surfaces of Querco-carpinetum communities in the adjacent natural forests and other parts of the Hyrcanian forests (SABETI, 1994). These species were mixed by four planting ratios in 1995. The main objectives of the present study were to increase our understanding of the nutrient fluxes associated with litter fall and undertaken to assess the influence of plantations on soil fertility parameters, the influence of *Carpinus* on *Quercus* growth and nutrient

concentrations of the fully expanded and senescent leaves and differences in the degree of internal cycling of N, P and K (retranslocation) among these species in monoculture and mixed plantations.

Material and methods

Site characteristic

The study area is located at the Chamestan experiment station in Mazandaran province, Iran input the map (36°29/ N, 51°59/ E). The experimental plots were located at an altitude of 100 m above the sea level. The area is on flat and uniform terrain with gentle slope (0-3%). Annual rainfall averages 803 mm, with wetter months occurring between September and February, and a dry season from April to August usually monthly rainfall averages <40 mm. Average monthly temperature ranges from 11.7 °C in February to 29.5 °C in August.

The soils are deep, moderately well drained and stone-free with organic matter of 1-3% prior to planting. They have a silty clay loam and clay loam textures with a pH of 6.0-7.5 and $CaCO_3$ of 0-15%. The soil order name is Alfisols. Approximately 50 years ago, this area has been dominated by the natural forests containing native tree species such as *Quercus castaneifolia*, *Zelkova carpinifolia*, *Gleditschia caspica* Desp., *Carpinus betulus* L., etc. The surrounding area is dominated by agricultural fields and commercial buildings.

Experimental design

Experimental plantations were established in 1995 using a randomized complete block design that included three replicate 25 m × 25 m plots of each of the following treatments:

(i) *Quercus castaneifolia* (100Q);
(ii) 70% *Q.castaneifolia* + 30% *Carpinus betulus* (70Q:30C);
(iii) 60% *Q.castaneifolia* + 40% *C. betulus* (60Q:40C);
(iv) 50% *Q.castaneifolia* + 50% *C. betulus* (50Q:50C);
(v) 40% *Q.castaneifolia* + 60% *C. betulus* (40Q:60C);
(vi) Unplanted control (grass).

Tree spacing within the plantations was 1m×1m and two species were systematically mixed within the rows. The stands were never fertilized.

Site preparation and plantation of the seedlings

Site preparation for all the plantation and control plots consisted of disk harrowing to a depth of 10-15 cm. Containerized seedlings were used for transplanting in February, 1995. The seedlings were obtained from the nursery of Chamestan experiment station. The seedlings of both species were transplanted simultaneously in monoculture of oak and mixed (oak + hornbeam) plantations with different ratios.

Tree survival and growth measurements

In each plot, diameter at breast height (dbh) and total height were measured for each tree in the 21 m × 21 m area (excluding the outer two tree rows) of each plot. The averages of the total height, dbh, top height, basal area and survival were calculated for each plot. To quantify mean top height at each stand we considered the maximum height of the dominant trees by averaging the four highest trees for each species (LEWIS *et al*., 1976; ROMANYA & VALLEJO, 2004).

Nutrition and nutrient return by the leaves

Foliage samples were collected from the stands in July 2006 (peak month of leaf maturity). The leaves were collected from the bottom one-third of the tree by clipping two small distal twigs located on the opposite sides of the crown (minimum 30 leaves). Six representative trees (two near the center of the sub-plot and one in each corner of it) of each species were sampled for the fully expanded mature leaves. In addition, senescent (soon to be shed) and freshly fallen leaves were collected from each species in each sub-plot in September, 2006 (leaf senescing period). In the mixed plots, the foliage samples were collected to compare nutrient concentrations between the trees growing in the mixed and in pure plantations. These foliar nutrient

concentrations were used in subsequent calculations for species in the mixed plots.

The samples were oven dried at 70°C for at least 48 h and ground in a Willey mill to pass through a 2 mm sieve before chemical analysis. The powdered leave material of each species was analyzed for macro bioelements such as total nitrogen, phosphorous, potassium, calcium and magnesium. N was analyzed after digesting the sample in concentrated H_2SO_4 using a catalyst mixture (potassium sulphate and cupric sulphite in ratio 9:1) with a quick digestion unit. The total N was estimated following micro-kjeldhal method (JACKSON, 1967). P was estimated after digesting the samples in triple acid mixture (HNO_3 , H_2SO_4 and $HClO_4$ in 10:1:3 ratios). The total P was determined by vanado-molybdate phosphoric yellow color procedure (JACKSON, 1967). K, Ca and Mg were determined using an atomic absorption spectrophotometer after wet digestion of a 1 gram sample with triple acid mixture (10 ml of conc. HNO_3 , 4 ml of $HClO_4$ and 1 ml of conc. HCl). The digested samples were filtered through Whateman No. 42 filter paper and made up to 100 ml with distilled water and the obtained solution was stored and used for analysis (ISSAC & JOHNSON, 1975).

Litters collected in three litter traps were randomly distributed among the trees and away from the border in each stand in January, 2007 (the end of leave fall season). The size of each trap was 1×1 m^2 with 30 cm high wooden sides fitted with a nylon net bottom, horizontally placed 40cm above the forest floor in September 2006 (first autumn and beginning of litter falling). The amount of branch litter was negligible. This allowed estimating the flux of litter and through it also the real amount of nutrients which reached the soil with litter. Leave litter from each litter trap was oven-dried at 70°C to constant weight and separated by species. To determine mean nutrient contents of floor leaf litter, biomass values for each treatment (n=3) were multiplied by their average nutrient concentrations.

Net retranslocation is determined in two successive years was quantified as the total amount of an element in year n, minus leaching intervening months and the total amounts retained in older tissues in the year n + 1 (SWITZER & NELSON, 1972; LIM & COUSENS, 1986). Percentage net retranslocation was calculated as Equation 1 (PARROTTA, 1999; SALIFU & TIMMER, 2001; LODHIYAL & LODHIYAL, 2003):

$$\% \text{ Re} = [1 - (A / B)] \times 100$$

Equation 1

where, Re is nutrient retranslocation percent, A is the nutrient mass in senesced leaves, and B is the nutrient mass in mature green leaves. The A and B were calculated on the basis of nutrient per unit weight of mature green and senesced leaf, respectively, multiplied by total amount of leaf litter fall. Since rainfall is negligible in the region when leaves senesce, leaching is likely to have only a minimal effect on nutrient loss from the leaves (MILLER *et al.*, 1976; LIM & COUSENS, 1986; LODHIYAL & LODHIYAL, 2003).

Soils

In the experimental area, the soil investigations were carried out with the aim of estimating the effect of plantation type on soil fertility. Composite samples were taken for every plantation and control treatment in each of the three replicate plots at 0-20 cm (A layer) and 21-60 cm (B layer) depths during February 2006 using a 7.6 cm diameter core sampler (n=3 cores/plot). The air-dried soil samples were sieved (aggregates were broken to pass through a 2 mm sieve) to remove roots prior to chemical analyses.

Soil texture was obtained by the Bouyoucos hydrometer method (BOUYOUCOS, 1962). pH was determined using an Orion Ionalyzer Model 901 pH meter in a 1: 2.5 mixture of soil and deionized water. EC (electrical conductivity) was determined using an Orion Ionalyzer Model 901 EC meter in a 1: 2.5 mixture of soil and water solution. Soil organic carbon was measured with the Walkley-Black technique (ALLISON, 1975). The total nitrogen was measured using a semi Micro-

Kjeldhal technique (BREMNER & MULVANEY, 1982). The available P was determined by spectrophotometer using Olsen method (HOMER & PRATT, 1961). The available K, Ca and Mg (by ammonium acetate extraction at pH 9) were determined with Atomic absorption spectrophotometer (BOWER *et al*., 1952).

Statistical analyses

The data on the growth, biomass and nutrients among the experimental treatments were analyzed following the randomized block design. Normality of the variables was checked by Kolmogorov-Smirnov test and Levene's test was used to test the equality of the variances. One- way analysis of variance (ANOVA) was used to compare the data among the experimental treatments. Tukey-HSD and Duncan tests were used to separate the means of the dependent variables significantly affected by the treatment. The soil parameters were analyzed following two-way analysis (ANOVA) procedure, treating plantation and soil depth as factors with interaction. Within each layer (soil horizon), the effects of plantation type were also tested using one-way analysis of variance (ANOVA). Significant difference between treatment means for different parameters were tested at $P \leq 0.05$ using least significant difference (LSD) test. SPSS v.11.5 software was used for all the statistical analyses.

Results

Tree survival and growth

Measurements in the experimental plantations (at 12 years of age) indicated that *Quercus* had the higher survival rate in the 50Q:50C treatment than in the 100Q, 70Q:30C and 60Q:40C treatments (P<0.05, Tukey-HSD). For *Carpinus*, no significant differences were observed among all the mixed-treatments with oak (Fig.1a).

The stem basal diameter of *Quercus* was positively affected by the presence of Carpinus in all the planting ratios (Fig.1b). In the 50Q:50C, the stem basal diameter of *Quercus* was higher than 70Q:30C treatment and pure oak treatment. (P<0.05, Tukey-HSD). For *Carpinus*, this parameter in the 40Q:60C was greater than in the 60Q:40C and 50Q:50C treatments (P<0.05, Tukey-HSD).

The mean of the total tree heights for *Quercus* (Fig.1c) in the 50Q:50C were higher than in the 70Q:30C treatment (P<0.05, Tukey-HSD). The mean of the top height, as a reliable indicator of site quality of forest stands (ASSMANN, 1970; LEWIS *et al*., 1976; OLIVER & LEARSON, 1990; ZINGG, 1994), for oak ranged from 11.34 to 11.75 m which did not show any significant differences in monocultures compared to mixed plantations in our study (Fig.1d). For *Carpinus*, this parameter in the 40Q:60C was greater than in the 60Q:40C and 50Q:50C treatments (P<0.05, Tukey-HSD).

In the stand basal area trends, as shown in Fig.1e, total basal-area development ranged from 12.25 to 14.10 m^2/h which was generally unaffected by the presence of *Carpinus* with different proportions. But, this parameter for oak was higher in the 100Q and 50Q:50C treatments than in the 40Q:60C treatment (P<0.05, Tukey-HSD).

Litter fall production, nutrient content and nutrient fluxes

Fully expanded and senescent leave nutrient concentrations showed significant differences in the two species among the different planting ratios (Table 1). Nitrogen concentrations in the fully expanded leaves of *Quercus* were higher in the 40Q:60C than in the pure oak treatments (p<0.05, Duncan). Mg concentrations of fully expanded leaves of *Quercus* in the 70Q:30C and 40Q:60C treatments were different (P<0.05, Duncan) from those of 60Q:40C treatment. The other macronutrient elements (P, K and Ca) did not show any significant differences for *Quercus* leaves among the different planting ratios with *Carpinus.* Phosphorus concentrations in the fully expanded leaves of *Carpinus* were higher in the 60Q:40C than in the 50Q:50C treatment (p<0.05, Duncan). For other macro elements, leaf concentrations of *Carpinus* were not found any significant differences among plantations.

For *Quercus*, senescent leaf concentrations for N were highest in the 40Q:60C among all

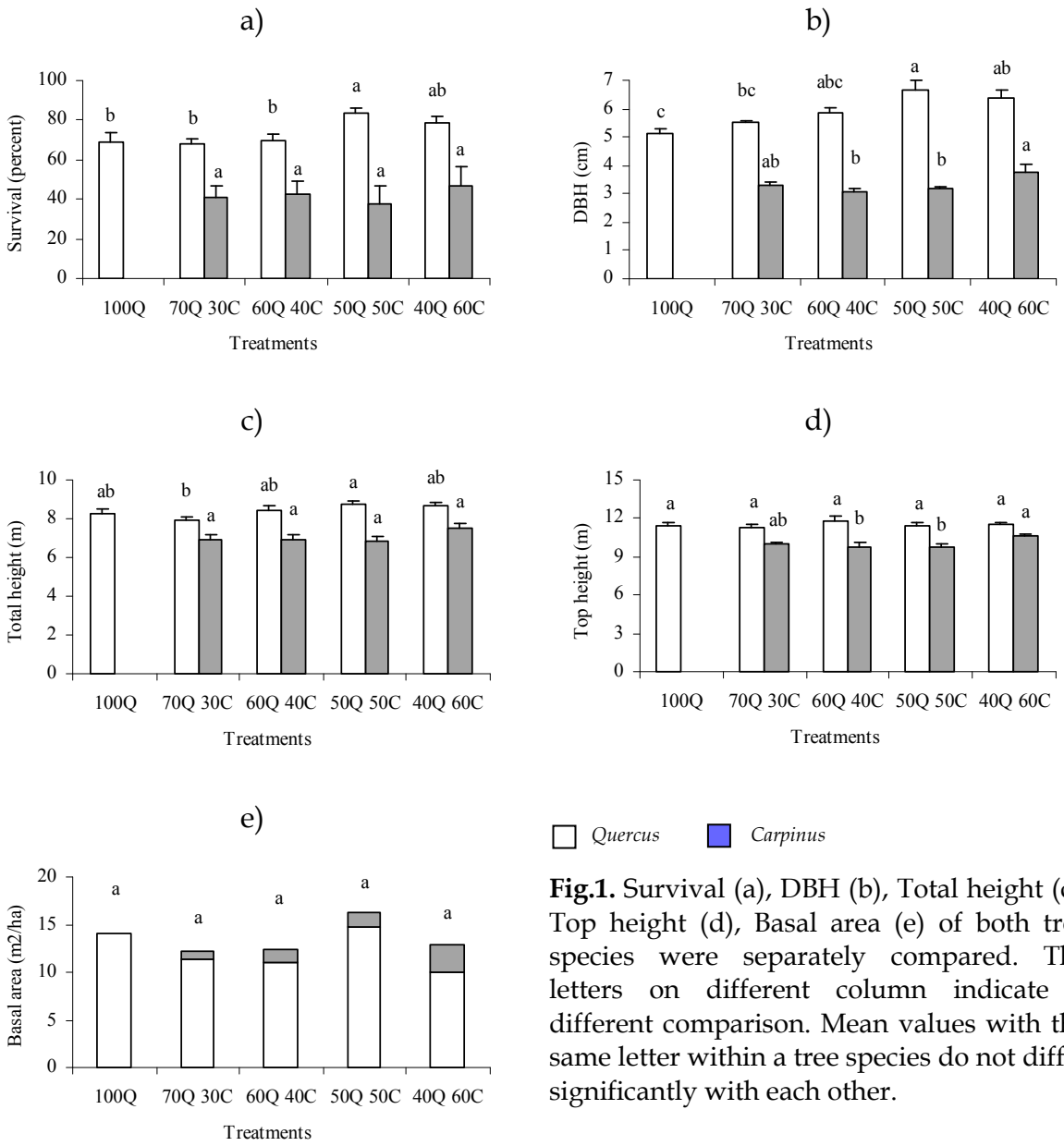

Fig.1. Survival (a), DBH (b), Total height (c), Top height (d), Basal area (e) of both tree species were separately compared. The letters on different column indicate a different comparison. Mean values with the same letter within a tree species do not differ significantly with each other.

plantation treatments (P<0.05, Duncan). Ca return by senescent leaves of *Quercus* in the monoculture plantations was significantly lower than in 50Q:50C plantations (P<0.05, Duncan). P, K and Mg returns by senescent leaves of *Quercus* did not show any significant differences among the different treatments. Senescent leave nutrient concentrations of *Carpinus* showed significant differences among the different planting ratios only for P which was higher in the 60Q:40C treatment than in the 40Q:60C treatment (P<.05, Duncan).

Significant differences in nutrient retranslocation .i.e. the percentage of N, P, or K withdrawn from the leaves prior to leaf-fall, were observed for N and P in each species among the treatments (Table 2). In *Quercus,* N retranslocation was significantly greater in the 60% than the 40% proportion with hornbeam (P<0.05, Duncan) and P retranslocation in the 50Q:50C was higher than in the 70Q:30C (P<0.01, Duncan). In contrast, N retranslocation in *Carpinus* was significantly greater in the 40Q:60C treatment than in the 60Q:40C treatment (P<0.05, Duncan). P retranslocation for this species in the 60Q:40C was higher than in the 50Q:50C and 40Q:60C treatments (P<0.01, Duncan). However, retranslocation

percent of the nutrients (NPK) in *Quercus* was in order: K (48-68%) > N (22-42%) > P (20-37%) while in *Carpinus*, it was in order: N (22-44%) > K (19-42%) > P (18-40%).

Leaf-litter fall production ranged from 4.70 to 6.80 Mg ha^{-1} $year^{-1}$, showing no significant differences among the planting ratios (Table 2). Due to the account of the treatment differences in litter production and species differences in litter-nutrient concentrations, the leaf-litter fall fluxes were significantly different among the treatments only for N (P<0.05, Duncan) (Table 2). This element, ranged from 89.05 to 120.47 Kg ha^{-1} $year^{-1}$, was higher in the 60Q:40C than in the 70Q:30C and 50Q:50C treatments. No obvious differences were found in leaf-litter flux rates for P (3.86-5.77 Kg ha^{-1} $year^{-1}$), K (25.61-15.70 Kg ha^{-1} $year^{-1}$), Ca (59.40-85.63 Kg ha^{-1} $year^{-1}$) and Mg (9.26-12.57 Kg ha^{-1} $year^{-1}$) among the treatments.

Table1. Nutrient concentrations in live and senescent leaves with their standard error in the parenthesis.

	Pure	70 *Q* + 30 *C*		60 *Q* + 40 *C*		50 *Q* + 50 *C*		40 *Q* + 60 *C*	
	Quercus	*Quercus*	*Carpinus*	*Quercus*	*Carpinus*	*Quercus*	*Carpinus*	*Quercus*	*Carpinus*
live leaves									
nitrogen (mg/g)	27.42 (0.64)	28.47 (1.59)	30.13 (1.36)	28.23 (0.63)	28.27 (0.92)	28.57 (0.50)	28.67 (0.59)	30.47 (0.52)	32.23 (1.52)
phosphorous (mg/g)	1.26 (0.09)	1.57 (0.03)	1.24 (0.05)	1.23 (0.03)	1.29 (0.05)	1.21 (0.00)	0.99 (0.06)	1.22 (0.09)	1.18 (0.05)
potassium (mg/g)	9.13 (1.00)	6.80 (1.78)	6.97 (1.02)	5.75 (0.91)	5.62 (1.83)	8.20 (0.68)	7.87 (3.02)	8.52 (0.47)	8.53 (1.09)
calcium (mg/g)	4.15 (0.62)	4.73 (0.91)	5.98 (0.52)	4.59 (0.87)	4.28 (1.06)	4.10 (1.45)	6.46 (1.70)	5.77 (1.20)	3.80 (1.07)
magnesium (mg/g)	2.13 (0.28)	2.63 (0.18)	2.75 (0.38)	2.13 (0.12)	2.17 (0.46)	2.33 (0.50)	2.67 (0.88)	2.46 (0.15)	2.17 (0.58)
Senescent leaves									
nitrogen (mg/g)	17.35 (0.65)	18.53 (0.60)	21.20 (2.00)	16.37 (1.52)	22.07 (1.82)	18.10 (0.72)	17.10 (1.28)	23.67 (0.88)	17.80 (1.15)
phosphorous (mg/g)	0.93 (0.08)	0.94 (0.04)	0.81 (0.02)	0.88 (0.01)	0.77 (0.04)	0.76 (0.02)	0.83 (0.06)	0.83 (0.05)	0.82 (0.01)
potassium (mg/g)	2.83 (0.22)	2.86 (0.30)	5.54 (0.68)	3.72 (0.03)	6.87 (0.70)	3.49 (0.2)	5.08 (0.29)	2.87 (0.09)	4.69 (0.57)
calcium (mg/g)	11.85 (0.23)	12.55 (1.18)	12.94 (0.46)	12.81 (0.06)	12.02 (0.59)	14.72 (0.96)	12.92 (0.32)	12.91 (1.71)	10.05 (1.50)
magnesium (mg/g)	1.65 (0.07)	1.84 (0.22)	2.53 (0.19)	1.60 (0.13)	2.52 (0.31)	2.19 (0.45)	2.73 (0.11)	1.95 (0.20)	2.23 (0.59)

Soil properties

There were few differences in soil properties among the experimental treatments after 12 years. The soils were characterized as fine-textured (Table 3). Soil pH, ranging from 6.31 to 7.24, did not show any significant differences between the treatments. Likely, soil EC (0.38 – 0.73 ds/m), organic carbon (1.38-3.76%), available K (310-450 mg/kg), available Ca (120-215 mg/kg) and available Mg (21.0-58.5 mg/kg) exhibited no significant differences between the soil horizons of treatments (Table 3). Total nitrogen ranging from 0.17% to 0.29%, in the upper soil layer was higher in the 50Q:50C treatment than in the pure oak and 60Q:40C treatments and no significant differences were found in the 21-60 cm depth of soil (Table 3). Carbon: Nitrogen ratio was significantly different, which was the highest in pure *Quercus* (15.33) among the

Table2. Leaf-litter fall production, nutrient fluxes and retranslocation in 11 year-old plantation stands with their standard error in the parenthesis.

	Pure	70 *Quercus* + 30 *Carpinus*			60 *Quercus* + 40 *Carpinus*			50 *Quercus* + 50 *Carpinus*			40 *Quercus* + 60 *Carpinus*			ANOVA
	Quercus	*Quercus*	*Carpinus*	total	*Quercus*	*Carpinus*	total	*Quercus*	*Carpinus*	total	*Quercus*	*Carpinus*	total	
Litter fall production (Mg/ha/year)	6.14 (0.58)	3.87 (0.12)	0.83 (0.02)	4.70 (0.14)	5.01 (0.80)	1.79 (0.28)	6.80 (1.09)	3.67 (0.14)	1.32 (0.05)	4.99 (0.19)	4.07 (0.23)	1.15 (0.05)	5.23 (0.30)	n.s.
Litter fall nutrient flux (Kg/ha/year)														
Nitrogen	105.21ab (7.35)	71.67 (1.65)	17.58 (2.12)	89.25b (2.90)	81.95 (9.95)	38.52 (3.56)	120.47a (13.36)	66.52 (4.51)	22.53 (0.85)	89.05b (3.66)	95.92 (2.11)	21.72 (0.72)	117.64ab (2.22)	*
Phosphorous	5.70 (0.82)	3.65 (0.20)	0.66 (0.00)	4.31 (0.21)	4.39 (0.73)	1.38 (0.24)	5.77 (0.96)	2.77 (0.02)	1.09 (0.08)	3.86 (0.11)	3.42 (0.41)	1.01 (0.05)	4.43 (0.46)	n.s.
Potassium	17.71 (2.45)	11.13 (1.46)	4.57 (0.59)	15.70 (1.46)	13.59 (2.05)	12.02 (1.47)	25.61 (3.26)	12.77 (0.64)	6.71 (0.20)	19.48 (0.82)	11.65 (0.62)	5.75 (0.76)	17.40 (1.31)	n.s.
Calcium	72.82 (7.17)	48.74 (5.68)	10.66 (0.04)	59.40 (5.64)	64.09 (10.15)	21.54 (3.50)	85.63 (8.46)	53.96 (3.87)	17.12 (0.75)	71.08 (3.80)	53.32 (9.83)	12.11 (1.34)	66.43 (7.87)	n.s.
Magnesium	10.13 (1.08)	7.18 (1.03)	2.08 (0.09)	9.26 (0.97)	8.22 (1.90)	4.35 (0.40)	12.57 (2.15)	8.04 (1.72)	3.61 (0.15)	11.65 (1.62)	7.96 (1.02)	2.65 (0.55)	10.61 (1.13)	n.s.
Percent retranslocation														
Nitrogen	36.55ab (2.88)	34.41ab (4.90)	29.53ab (6.72)		41.79a (6.56)	22.17b (4.11)		36.62ab (2.52)	40.46a (3.44)		22.38b (1.54)	44.48a (4.92)		*
Phosphorous	26.97ab (1.44)	19.56 b (4.18)	34.35ab (0.80)		28.46ab (1.30)	40.46 a (2.75)		37.28 a 2.39)	17.65 c (5.66)		31.77ab (6.84)	29.87 b (1.86)		**
Potassium	68.00 (3.00)	47.74 (9.79)	26.25 (8.35)		49.75 (9.05)	18.96 (8.96)		56.33 (6.38)	37.12 (10.89)		66.03 (2.36)	41.55 (13.42)		n.s.

ANOVA results: n.s. –treatment effect not significant; * - $p < 0.05$; ** - $p < 0.$ 0.01. Similar letters within a row indicate that means were similar (Tukey HSD).

Table 3. Soil properties in plantations and control plots in two soil layers (A and B horizons) with their standard error.

Soil properties	Depth cm	100Q	70Q:30C	60Q:40C	50Q:50C	40Q:60C	Control	ANOVA[b]
Clay (%)	0-20	38.0 a (1.08)	26.5 b (3.00)	28.5 ab (5.50)	27.5 ab (4.50)	24.5 b (2.50)	31.5 ab (0.95)	*
	21-60	35.5 (2.17)	31.5 (0.50)	23.5 (0.50)	28.5 (9.50)	24.5 (1.50)	29.5 (2.62)	ns
Silt (%)	0-20	38.25 (4.73)	40.00 (7.00)	45.50 (9.50)	40.50 (4.50)	48.00 (10.00)	37.50 (3.30)	ns
	21-60	42.25 (6.81)	40.00 (12.0)	56.50 (9.50)	36.00 (13.00)	41.00 (18.00)	48.50 (8.42)	ns
Sand (%)	0-20	23.75 (5.46)	33.50 (5.50)	31.00 (8.00)	32.00 (6.00)	26.50 (8.50)	31.00 (3.91)	ns
	21-60	22.25 (6.20)	28.50 (10.50)	20.00 (8.00)	35.50 (20.50)	34.50 (12.50)	22.00 (6.55)	ns
pH (1:2.5 H2o)	0-20	6.84 (0.28)	7.04 (0.08)	7.00 (0.33)	6.82 (0.09)	7.24 (0.66)	7.12 (0.09)	ns
	21-60	6.88 (0.27)	7.07 (0.11)	6.82 (0.19)	6.31 (0.19)	6.60 (0.09)	7.02 (0.26)	ns
EC (ds/m)	0-20	0.45 (0.02)	0.73 (0.14)	0.54 (0.05)	0.50 (0.20)	0.64 (0.05)	0.60 (0.03)	ns
	21-60	0.60 (0.04)	0.50 (0.20)	0.47 (0.09)	0.38 (0.02)	0.42 (0.03)	0.40 (0.05)	ns
Organic Carbon (%)	0-20	2.90 (0.27)	2.85 (0.02)	2.21 (0.25)	3.76 (0.66)	2.52 (0.45)	2.22 (0.14)	ns
	21-60	1.97 (0.27)	1.55 (0.15)	1.38 (0.18)	2.15 (0.19)	1.48 (0.28)	1.60 (0.42)	ns
Total N (%)	0-20	0.20 b (0.02)	0.30 ab (0.04)	0.24 b (0.11)	0.41 a (0.03)	0.25 ab (0.00)	0.24 ab (0.04)	*
	21-60	0.18 (0.02)	0.18 (0.02)	0.20 (0.03)	0.23 (0.07)	0.17 (0.02)	0.21 (0.04)	ns
P available (mg/kg)	0-20	23.38 ab (3.49)	26.25 ab (3.75)	14.00 b (1.00)	31.00 ab (6.00)	32.50 a (4.50)	29.75ab (2.42)	*
	21-60	28.31abc (1.74)	23.50 bc (1.50)	21.90 c (4.10)	35.00 a (5.00)	33.00 ab (3.00)	29.75abc (2.24)	*
K available (mg/kg)	0-20	320.0 (14.14)	410.0 (45.00)	335.0 (40.00)	450.0 (30.00)	375.0 (25.00)	417.5 (41.70)	ns
	21-60	315.0 (15.00)	325.0 (25.00)	355.0 (25.00)	365.0 (15.00)	310.0 (10.00)	317.5 (23.93)	ns
Ca available (mg/kg)	0-20	200.0 (63.90)	170.0 (10.00)	155.0 (25.00)	165.0 (10.00)	120.0 (25.00)	215.0 (30.95)	ns
	21-60	165 (25.33)	155 (25.00)	125 (15.00)	125 (5.00)	125 (15.00)	180 (12.24)	ns
Mg available (mg/kg)	0-20	42.0 (16.79)	42.0 (6.00)	27.0 (10.00)	42.0 (13.00)	21.0 (3.00)	58.5 (15.75)	ns
	21-60	39.0 (14.14)	42.0 (12.00)	21.0 (3.00)	27.0 (3.00)	24.0 (6.00)	40.5 (6.65)	ns

a Based on three composted 7.6 cm diameter core samples per plot

b ANOVA results: ns = treatment effect not significant ; *, $p<0.05$ Duncan. Mean values with the same letter within the soil layer do not differ significantly with each other.

all treatments in 0-20 cm (P<0.05, Duncan). Also, it was the highest in the 70Q:30C treatment (12.73) followed by the 40Q:60C (11.73), pure *Quercus* (10.74), 50Q:50C (9.00), control plots and 60Q:40C treatments (7.64 and 7.80, respectively) in the 20-60cm depth (P<0.01, Duncan). Available P in 0-20 cm depth of the 40Q:60C (32.50 mg/kg) was higher than in the 60Q:40C (14.0 mg/kg) treatment and in 21-60 cm depth of the 50Q:50C (35.00 mg/kg) was the highest and in the 60Q:40C (21.90 mg/kg) treatment was the lowest among the treatments (P<0.05, Duncan) (Table 3).

Discussion

In the study, trees in the mixed plantations of *Quercus* with *Carpinus* in 50% proportions exhibit faster rates of growth than trees in monocultures. Our results are similar to those obtained by ASSMANN (1970), PROKOPEV (1976), REUKEMA & BRUCE (1977), FRY & POOLE (1980), POLENO (1981), KAWAHARA & YAMAMOTO (1982, 1986), LAMPRECHT (1986), HAGGAR & EWEL (1995), MENALLED *et al*., (1998), BERGQVIST (1999), PARROTTA (1999), MONTAGNINI (2000), SIMARD & HANNAM (2000), LAMB & GILMOUR (2003), PIOTTO *et al.* (2004), PETIT & MONTAGNINI (2004, 2006), FAHLVIK *et al*. (2005), KANOWSKI *et al*. (2005), SHEIL *et al*. (2006), SAYYAD *et al.* (2006).

Kelty (2006) suggested that higher stand-level productivity in mixtures has been found with two kinds of species interactions: (1) complementary resource use between species that arises from development of a stratified canopy (and possibly root stratification); (2) facilitative improvement in nutrition of a valuable timber species growing in mixture with a nitrogen-fixing species (but only if combined with complementary resource use as well). The mixed-species plots may have been more productive than the monocultures because they were able to take advantage of the available growing space, by growing in strata (LAMPRECHT, 1986; WHYTE & WOOLLONS, 1990).

A key concept for designing highly productive mixed-species stands is the need to combine species that differ in characteristics such as shade tolerance, height growth rate, crown structure (particularly leaf area density), foliar phenology, and root depth and phenology (KELTY, 1992). *Quercus* and *Carpinus* differ substantially in these characteristics; they may capture site resources more completely and/or use the resources more efficiently in producing biomass, resulting in greater total stand biomass production than would occur in monocultures of the component species. Such species are said to have complementary resource use (HAGGAR & EWEL, 1997) or good ecological combining ability (HARPER, 1977). This a question that why oak with 50% mixture rate showed better growth than other mixture rates?

AUGUSTO *et al.* (2002) suggested that in temperate forests, the chemical composition of foliar is dependent on tree species and site. In the present study, none of the plantations showed foliar macro-elements concentrations lower than the limit for possible deficiency. Full expanded leave N and senesced leave Ca concentrations of *Quercus* in the pure plantations were lower than the mixed plantations. These trends can be due to different leaf- litter fall mass and quality of both two species regarding the different planting ratios. Also, AUGUSTO *et al.* (2002) reported that the effect of overstory species on tree nutrition is strongly influenced by forest management (e.g. low density stands or mixed stands can promote litter decomposition).

In temperate forests, the annual amount of litter fall of a mature stand is only slightly influenced by the species of the overstory because the major influences are latitude, that is climate (VOGT *et al*., 1986), and stand management (AUGUSTO *et al*., 2002). We found that the litter fall production of plantations did not have any significant differences, and so their levels were higher than the average annual litter fall in European temperate forests (between 3.5 and 4.0 t ha^{-1} yr^{-1}; 3.7 and 3.8 t ha^{-1} yr^{-1} for *Quercus petraea* and *Quercus robur*, respectively; AUGUSTO *et al*., 2002) and the 30 years old plantations of *Quercus serrata* in India (419.9 g cm^{-2} $year^{-1}$; PANDEY *et al*.,

2007). These trends may be due to low plant spacing in our study.

There were few differences in the nutrient fluxes among the treatments. These trends can be due to different leaf- litter fall mass and quality of both two species regarding the different planting ratios. For instance, CUEVAS & LUGO (1998) detected relationship between litter and nutrient fluxes among contrasting species stands. Differential resources uptake, and release of carbon and nutrients are important factors at the stand level, because they represent major fluxes through the system (MILLER, 1984). The rate of fall and subsequent decay dynamics of litter affect soil organic matter formation and nutrient storage (LUGO *et al.*, 1990). Knowledge of the timing of nutrient and mass return through litterfall to the forest floor is important for plantation management (CUEVAS & LUGO, 1998). Internal differences between species on the basis of nutrient flux may cause effects on soil fertility, due to this case the species selection for plantation is very important (STANLEY & MONTAGNINI, 1999) .

Degree of retranslocation or resorption of nutrients within the plants is also an important factor to be considered (MILLER, 1984). Retranslocation may be regulated by soil nutrient supply, nutrient uptake rates, the size of plant nutrient reserves, and age of trees (MUNSON *et al.*, 1995; MALIK & TIMMER, 1998; HAWKINS *et al.*, 1999). Furthermore, the relative contributions of nutrients from retranslocation and from external uptake to skins of new growth are unknown (HAWKINS *et al.*, 1999; SALIFU & TIMMER, 2001). Similar to our study in the case of *Quercus*, LODHIYAL & LODHIYAL (2003) found that the percent retranslocation of nutrients (NPK) in tree layers of Bhabar Shisham forests in central Himalaya was in order K > N > P. The present estimates of the percent of retranslocation of the nutrients from senesced leaves are higher than those from *Quercus rubra* (23-39%; GRIZZARD *et al.*, 1976). LODHIYAL & LODHIYAL (1997) argued that the higher is the leaf tissue nutrient level, the greater would be the percent of retranslocation capacity. However, CHAPIN & KEDROWSKI (1983) pointed out that both percent of retranslocation and concentrations of nutrients are positively correlated. This trend is consistent with our results about P for *Carpinus*.

CUEVAS & LUGO (1998) reported the species that return the most mass are not necessarily the ones that return the most P and N. Moreover, the species can adjust their performance though differences in retranslocation rates before leaf fall. Translocation of nutrients during the ageing of tissues especially in foliage of trees during senescence is an important mechanism for maintaining tree growth (LIU *et al.*, 2004).

There were few differences in soil properties among the experimental treatments at 12 years. This might be a result of fairly short time frame, i.e., longer time spans are required for an influence of the tree species to develop in the mineral soil (VESTERDAL *et* al., 2002). No statistically significant differences were observed in soil pH between the treatments. MONTAGNINI (2000) and GIARDINA *et al.* (1995) reached the same results as ours, whereas RHOADES & BINKLEY (1992), BINKLEY (1996) and PARROTTA (1999) found lower soil pH in mixed plantations. Higher planting density and lower age of our plantations might be the main reasons for lack of significant differences in soil pH. AUGUSTO *et al.* (2002) reported *Quercus* spp. as the second tree species that reduced topsoil pH.

No significant differences were observed in soil organic carbon content in both soil layers between the treatments. PARROTTA (1999) and SAYYAD *et al.* (2006) came to the same conclusion. In contrast to our results, GARCIA-MONTIEL & BINKLEY (1998) found that organic carbon content in the 0-20 cm depth of the soil under NFT *Albizia* was higher than in the soil under *Eucalyptus*. AUGUSTO *et al.* (2002) suggested that the soil carbon content and the soil organic weight are dependent on the canopy species.

Soil total nitrogen in the 50Q:50C treatment was higher than in the pure oak plantation. The effect of tree species on total nitrogen stocks in the soil is inconsistent (AUGUSTO *et al.*, 2002). PARROTTA (1999), and to some extent, MONTAGNINI (2000) did not

observe any significant differences in soil nitrogen between monoculture and mixed plantations.

Due to lack of litter, the control plots had lower values in the C/N ratio than the other treatments. Pure oak and 70Q:30C treatment had the highest C/N ratio in A and B layers, respectively. PARROTTA (1999) and SAYYAD *et al.* (2006) did not found any significant differences in monoculture and mixed plantations. The impact of an overstory species on soil varies significantly with factors like climate, geology and silvicultural management. Thus, the soil carbon stock and the C/N ratio depend on the species of over story but they also depend strongly on soil type and climate (MEENTEMEYER & BERG, 1986).

LUGO *et al.* (1990) suggested that amounts of nutrients return to the forest floor have a measurable impact on soil nutrient characteristics, especially in the surface layers. Mixed plantations have intermediate values of soil N, P and K, but lower soil Ca and Mg relative to pure plantations (STANLEY & MONTAGNINI, 1999; MONTAGNINI, 2000). This trend is consistent with our results about soil P. In our study, no significant differences were observed between the monoculture and mixed plantations in concentrations of K, Ca and Mg in soil. MONTAGNINI (2000) came to the same results in monoculture and mixed plantations as we did. For some nutrients, like phosphorous, it is difficult to show a constant influence of overstory species on soil nutrient content because of inconsistent results (AUGUSTO *et al.*, 2002).

The mixed designs provided intermediate to fast decomposition rates, releasing nutrients to the soil and allowing a litter layer to protect the soil (BYARD *et al.*, 1996). Nutrient cycling characteristics must also be taken into account when assessing the potential impacts of plantation species on site nutrients. To ensure the sustainable management of forests, resiliency of the ecosystems should be estimated to determine the most appropriate tree species and silvicultural management (AUGUSTO *et al.*, 2002).

Conclusions

Although the plantations are still young and it may be too soon to determine the behavior of the species studied, it is evident that the best growth for oak was somewhat demonstrated in the mixed systems. Introduction of one mixture as the best one is somewhat difficult but 50Q:50C treatment could be the most productive and sustainable one. In the present study, 50Q:50C treatment obtained a significantly higher values for survival, diameter at breast height, Ca foliage concentration, total N (in A horizon) and available P (in B horizon) than the 100Q treatment. Thus, for the purposes of forest rehabilitation and restoration in the Hyrcanian forests, mixed-plantations such as with *Quercus* and *Carpinus* under appropriate can serve a dual role by accelerating natural recovery of the species-rich forest ecosystems while providing required wood products to populations facing with shortages of fuel wood, timber and other forest products.

There exists very little information on the growth of tree species native to the Hyrcanian forests and information on experiences comparing pure and mixed forest plantation with different mixturing degrees is limited. These results would be helpful in understanding the nutrient behavior in a highly productive forest plantation and thereby providing decisive information for their sustainable management. Moreover, this information may be used in the selection of species, planting ratios and harvest regimes in order to conserve or replenish plantation soil fertility.

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Chemical Elements in Mulch and Litterfall of Beech Ecosystems and Their Total Turnover

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Abstract. The beech communities on the territory of Bulgaria had been objects of regional, local as well as large scale national investigations aiming their classification, determination of their ecological characteristics, conservation status, habitats etc. They are included as objects of the intensive monitoring of forest ecosystems in Bulgaria also. The investigations of chemical content of the litter – fall in these forests were conducted until now. The novelty of the present research is investigation of these elements in the mulch and the ratio between the established quantities calculation. The main goal is the biological turnover special features characterization of the investigated elements which give us a chance to define the investigated ecosystems state and functioning. The indexes as litter – mulch and acropetal coefficients were used for this aim. The content of macroelements as N, Ca and K and microelements as Pb, Zn, Mn and Fe in soils, mulch and in different litter- fall fractions have been calculated. The investigation was carried out on three sample plots.

During the investigation was established that the soils are characterized with acid reaction, high content of Fe, N and Mn and low content of Ca and K. The concentration of Zn and Pb are high also.

The calculated average store of investigated elements in litter – fall is 81.312 kg.ha^{-1} and in the mulch - 314 kg.ha^{-1}. According to the acropetal coefficient N is accumulated mainly in the acorns, K – in the annual phytomass fractions and Ca – in the perennial fractions. The leaves and the acorns fraction accumulate Mn, and cupolas - Fe.

The litter – mulch coefficient vary from 1,6 (Mn) to 4,2 (Pb). The tendencies of Zn and Ca turnovers acceleration are discovered, while the turnover of more investigated elements is inhibited. The litter – mulch coefficient for Zn and Ca is 0,8 and 1,4 accordingly, i.e. corresponding to the intensive type of turnovers which is not typical for the broadleaved forest communities.

The determination of cause factors and the functioning of beech communities in the future have a great importance for the environment and the economy of the country.

Key words: Bulgaria, beech ecosystem, macro- and microelements, total turnover.

Introduction

The beech communities on the territory of the Petrohan experimental and educational forest enterprise (EEFE "Petrohan") and State hunting range "Vitinia" had been objects on regional, local (GARELKOV, 1967; PAVLOV, 1978; PAVLOV *et al.*, 2003) as well as large scale national investigations (PENEV *et al.*, 1969; TZONEV *et al.*, 2006 etc.) aiming their classification, determination of their ecological characteristics, conservation status, habitats etc. (DJANKOVA *et al.*, 2003; MIHOV *et al.*, 2008). They are included as object of the intensive

monitoring of forest ecosystems in Bulgaria (KOLAROV *et al*. (2002).

The investigations of some of the main functional characteristics as turnover of the chemical elements, quality and quantity of the litter-fall and the processes of decomposition in these forests were also a subject of many investigations in Bulgaria (KOCHEV & GORUNOVA, 1975; LALOVA, 1994; LYUBENOVA & ALEXANDROV, 1999; LYUBENOVA & DIMOVA, 2000; LYUBENOVA *et al*., 2002; MIHOV *et al*., 2008) as well as in abroad (SANTA REGINA & TARAZONA, 2001; KAVVADIAS *et al*., 2001; LEBRET *et al*., 2001 and others).

Investigations of the litter – fall chemical content in these forests have been conducted recently. The aim of present research is to characterize the biological turnover special features of the investigated elements and to define ecosystems status and the functioning rate. The novelty is investigation of these elements in the mulch and finding the ratio between the established quantities.

Material and methods

Object of investigation

The objects of investigation are beech communities in the region of West Balkan Mountains. Sample plot 1 (SP 1) is situated in a region of State hunting range "Vitinia". It is representative for the high stem beech forests. The territory belongs to the West Balkan Region of the temperate continental forest area (PAVLOV, (1995) and to the Europe broadleaves forest area (BONDEV, 1991). The investigated region gets into the Mountainous climatic district, West Balkan Mountain sub district, and characterizes with annual average precipitations (AP) around 900 mm and prevailing winds with south component (VELEV, 2002). The soils are *Cambisols*, CM (NINOV, 2002).

Sample plots 2 and 3 are situated in the region of the Petrohan experimental and educational forest enterprise. The area belongs to North Bulgarian climatic zone with temperate-continental climate. The average annual precipitations are between 700-1000 mm (VELEV, 2002). The soils are *Cambisols*, CM (NINOV, 2002). The region is slightly impacted by different forms of environmental pollution.

The investigated beech communities are related to associations *Festuco drymejae-Fagetum sylvaticae* and *Asperulo odoratae-Fagetum sylvaticae* (habitat *Asperulo-Fagetum* - 9130) in the region of Petrohan and association *Aremonio agrimonoidis-Fagetum sylvaticae* (habitat *Moesiacus beech forests*, 91W0) in the region of Vitinia (MIHOV *et al*., 2008).

Methods

The annual litter-fall was gathered with five 1 m^2 litter-catchers from each sample plot. The stores of mulch were calculated according methodical leads of Smolianinov (by LYUBENOVA, 2009).

Chemical analyses were made with samples (1 g) which are average for the respective phytomass fraction and the investigated communities. They have been dried to absolutely dry weight (85° C, 48 h) and grinded.

The soil samples were cleared from big roots, stones and other mixtures, dried, grinded and sift out through 2 mm sieve.

The pH was determined in soil suspense in relation soil: water - 1:2.5 (according to standards) using the potentiometric method (ISO 10390).

The total N was determined by Kjeldahl. The analytical determining of other elements was made on PERKIN-ELMER 310A spectrometer in the Institute of Biodiversity and Ecosystem Investigations after wet mineralization of samples according to the requirements of ISO 11466.

The ratio between the contents of investigated elements in the litter-fall fractions and between their contents in the mulch and litter-fall (acropetal and litter-mulch coefficient) were calculated (by LYUBENOVA, 2009).

Results and Discussion

Content of macro- and microelements in the soil and the mulch in studied communities

The content of macro- and microelements in soil and mulch of the beech communities are presented in Table 1. The soils are characterized with acid reaction, high

content of N, Fe and Mn and low content of Ca and K. The content of Zn and Pb is also high. The A/B ratio shows that the soils are the main reservoir of Pb and Fe, respectively about 200 and 82 times higher soil content of these elements than the mulch content. The soil content of Zn, Mn and N also is higher (respectively 5, 3 and 1,3 times) than the mulch content. The A/B ratio for Ca and K is the same.

According to the established average total amount (mg.kg^{-1} a.d.w.) of macroelements in the litter-fall the investigated fractions are arranged as follows: Acorns>Branches>Leaves>Cupolas (Table 2). According to the quantity proportions in the litter-fall the investigated elements are in the following order: N>Ca>K, i.e. N with 73.10^3 mg.kg^{-1} average sum was prevailing in the investigated fractions. The average content of N and K in the acorns, and of Ca – in the branches is biggest then the content of these elements in the other fractions (respectively 30.5.10^3, 4.1.10^3 and 7.5.10^3 mg.kg^{-1} a.d.w.).

The total sum of macroelements varies for the different investigated beech communities from 96.5.10^3 (I) to 110.9.10^3 mg.kg^{-1} a.d.w. (III). The sum of macroelements in the acorns and in the cupolas was more variable in comparison with the other fractions.

According to the calculated average sum of microelements (in mg.kg^{-1} a.d.w.) the investigated fractions are arranged as follows: Leaves > Acorns > Branches >Cupolas (Table 2).

According to the quantity proportions in the litter-fall the investigated microelements are in the following order: Mn>Fe>Zn>Pb, i.e. Mn with 2.1.10^3 mg.kg^{-1} average sum is prevailing in the investigated fractions. The contents of: Mn in the leaves (1.0.10^3 mg.kg^{-1} a.d.w.), Fe in the cupolas (0.6.10^3 mg.kg^{-1} a.d.w.) and Zn and Pb in the branches (0.05.10^3 and 1 mg.kg^{-1} a.d.w.) are biggest than the contents of these elements in the other fractions.

The total sum of microelements varies for the different investigated beech communities from 3.1.10^3 (III) to 4.4.10^3 mg.kg^{-1} a.d.w. (I). The microelements content varying in the litter – fall fractions of studied communities is better expressed in comparison with this of macroelements.

The average stores of investigated macro- and microelements in the litter - fall are 81.312 kg.ha^{-1} (Table 3) and in the mulch – 170.314 kg.ha^{-1} (Table 1).

Table. 1. Average storage of macro- and microelements in soil and mulch

Elements	Soil, kg.ha^{-1} (A)	Mulch, kg.ha^{-1} (B)	A/B
Ph	4.2	-	-
N	145.350	108.940	1,33
Ca	0.260	28.421	0,01
K	0.240	27.004	0,01
Pb	0.790	0.004	197,50
Zn	0.960	0.171	5,61
Mn	11.040	3.576	3,09
Fe	179.300	2.198	81,57

According to the established average total amount (mg.kg^{-1} a.d.w.) of macroelements in the litter-fall the investigated fractions are arranged as follows: Acorns>Branches>Leaves>Cupolas (Table 2). According to the quantity proportions in the litter-fall the investigated elements are in the following order: N>Ca>K, i.e. N with 73.10^3 mg.kg^{-1} average sum was prevailing in the investigated fractions. The average content of N and K in the acorns, and of Ca – in the branches is biggest then the content of these elements in the other fractions (respectively 30.5.10^3, 4.1.10^3 and 7.5.10^3 mg.kg^{-1} a.d.w.).

The total sum of macroelements varies for the different investigated beech communities from 96.5.10^3 (I) to 110.9.10^3 mg.kg^{-1} a.d.w. (III). The sum of macroelements in the acorns and in the cupolas was more variable in comparison with the other fractions.

According to the calculated average sum of microelements (in mg.kg^{-1} a.d.w.) the investigated fractions are arranged as follows: Leaves > Acorns > Branches >Cupolas (Table 2).

According to the quantity proportions in the litter-fall the investigated microelements are in the following order: Mn>Fe>Zn>Pb,

i.e. Mn with $2.1.10^3$ mg.kg^{-1} average sum is prevailing in the investigated fractions. The contents of: Mn in the leaves ($1.0.10^3$ mg.kg^{-1} a.d.w.), Fe in the cupolas ($0.6.10^3$ mg.kg^{-1} a.d.w.) and Zn and Pb in the branches ($0.05.10^3$ and 1 mg.kg^{-1} a.d.w.) are biggest than the contents of these elements in the other fractions.

The total sum of microelements varies for the different investigated beech communities from $3.1.10^3$ (III) to $4.4.10^3$ mg.kg^{-1} a.d.w. (I). The microelements content varying in the litter – fall fractions of studied communities is better expressed in comparison with this of macroelements.

The average stores of investigated macro- and microelements in the litter - fall are 81.312 kg.ha^{-1} (Table 3) and in the mulch – 170.314 kg.ha^{-1} (Table 1).

Table 2. Content of macro- and microelements (mg.kg^{-1} a.d.w.) in the litter – fall fractions

Macroelements						Microelements					
Plot/ Fraction	**Leaves**	**Branches**	**Acorns**	**Cupolas**	**Sum**	**Plot/ Fraction**	**Leaves**	**Branches**	**Acorns**	**Cupolas**	**Sum**
I						**I**					
N	13440.0	15520.0	28700.0	10670.0	**68330.0**	**Pb**	0.3	2.1	0.8	0.7	**3.9**
Ca	5299.3	6080.4	3966.4	1345.3	**16691.4**	**Zn**	23.2	75.0	15.9	9.8	**123.9**
K	2703.9	1834.0	3903.2	3065.9	**11507.0**	**Mn**	1310.3	516.5	698.3	164.0	**2689.1**
						Fe	162.3	372.4	282.5	756.0	**1573.2**
Sum	**21443.2**	**23434.4**	**36569.7**	**15081.2**	**96528.4**	**Sum**	**1496.1**	**966.0**	**997.6**	**930.5**	**4390.2**
II						**II**					
N	16210.0	14830.0	27900.0	16210.0	**75150.0**	**Pb**	0.2	0.2	0.6	0.7	**1.7**
Ca	4102.6	6488.1	2181.3	1377.3	**14149.3**	**Zn**	24.1	44.3	30.3	10.5	**109.2**
K	2922.1	1793.0	4574.9	3886.9	**89299.3**	**Mn**	1176.0	388.4	245.3	116.5	**1926.1**
						Fe	336.0	252.3	200.0	604.1	**1392.3**
Sum	**23234.7**	**23111.1**	**34656.2**	**21474.2**	**102476.2**	**Sum**	**1536.3**	**685.2**	**476.2**	**731.7**	**3429.4**
III						**III**					
N	12750.0	16490.0	34900.0	11360.0	**75500.0**	**Pb**	0.8	0.7	0.3	0.7	**2.5**
Ca	9037.6	9991.7	2530.4	1734.4	**23294.1**	**Zn**	23.4	42.9	15.6	11.2	**93.1**
K	2733.9	1909.4	3725.7	3734.8	**12103.7**	**Mn**	656.0	192.2	896.0	69.0	**1813.2**
						Fe	148.0	552.6	68.0	452.1	**1220.7**
Sum	**24521.5**	**28391.1**	**41156.1**	**16829.2**	**110897.8**	**Sum**	**828.2**	**788.4**	**979.9**	**532.9**	**3129.4**
Average						**Average**					
N	14133.3	15613.3	30500.0	12746.7	**72993.3**	**Pb**	0.4	1.0	0.6	0.7	**2.7**
Ca	6146.5	7520.1	2892.7	1485.6	**18044.9**	**Zn**	23.6	54.1	20.6	10.5	**108.8**
K	2786.6	1845.5	4067.9	3562.5	**12262.6**	**Mn**	1047.4	365.7	613.2	116.5	**2142.8**
						Fe	215.4	392.4	183.5	604.1	**1395.4**
Sum	**23066.5**	**24978.9**	**37460.6**	**17794.8**	**103300.8**	**Sum**	**1286.9**	**813.2**	**817.9**	**731.7**	**3649.7**

Table 3. Average stores of investigated elements in litter-fall, kg.ha^{-1}

Elements	Leaves	Branches	Acorns	Cupolas	Total	%
N	28.200	6.516	6.867	5.420	47.003	57.81
Ca	16.200	3.021	0.523	0.727	20.471	25.18
K	7.441	0.780	0.861	1.648	10.730	13.20
Pb	0.001	0.000	0.000	0.000	0.001	0.001
Zn	0.044	0.149	0.004	0.005	0.202	0.25
Mn	1.878	0.158	0.140	0.037	2.213	2.72
Fe	0.305	0.133	0.026	0.228	0.692	0.85
Total	54.069	10.757	8.421	8.065	81.312	100.00
%	66.50	13.23	10.36	9.92	100.00	

The percent participation of elements in litter-fall stores is shown on Fig. 1 and is preserved in the established stores of mulch. The distribution of the investigated elements in separate fractions is shown on Fig. 2. The content in the leaves fraction is the highest one – 66,50%, while in the other fractions it varies between 10 and 13%.

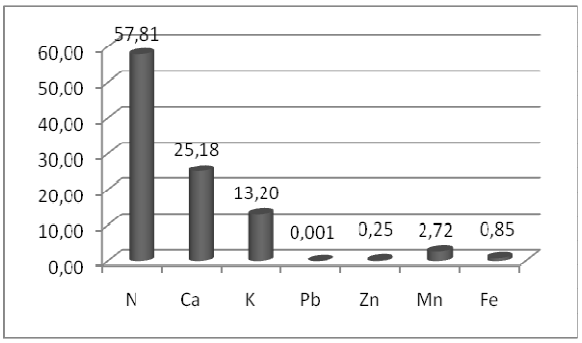

Fig. 1. Average participation of investigated elements in the litter - fall (%)

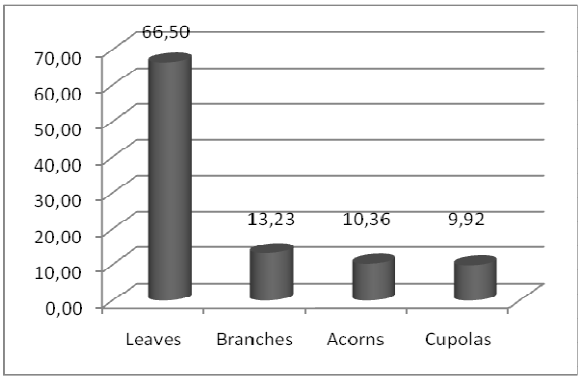

Fig.2. Average fraction distribution of investigated elements in the litter - fall (%)

The obtained results in this investigation are comparable with other investigations for Bulgarian beech forests.

Acropetal and litter-mulch coefficient

According to the acropetal coefficient N is accumulated mainly in the acorns, K – in the annual phytomass fractions and Ca – in the perennial fractions (Table 4). The leaves and the acorns accumulate Mn, and the cupolas - Fe. The investigated micro-elements have higher quantities in the perennial fractions as a whole.

Table 4. Acropetal and Litter-mulch coefficients

Elements/ Fractions	Acropetal coefficient			Litter-mulch coefficient
	Leaves	Acorns	Cupolas	
N	0.9	2.0	0.8	2,3
Ca	0.8	0.4	0.2	1,4
K	1.5	2.2	1.9	2,5
Pb	0.4	0.6	0.7	4,2
Zn	0.4	0.4	0.2	0,8
Mn	2.9	1.7	0.3	1,6
Fe	0.5	0.5	1.5	3,2

The turnover of the more investigated elements is inhibited. The litter – mulch coefficient vary from 1.6 (Mn) to 4.2 (Pb). The tendencies of accelerating of Zn and Ca turnover are established. The litter – mulch coefficients are 0.8 and 1.4 accordingly, i.e. corresponding to the intensive type of turnovers which are not typical for the broadleaved forest communities. The prevailing element is N followed by Ca and K.

Conclusions

The soils of the investigated beech communities are assessed as slight to average stored with nutrients. The content of Zn, Cu and Pb is below the level of maximum admissible concentrations for these elements in soils. The soil is the main reservoir of Pb and Fe while the mulch – of Ca and K. The soil content of Zn, Mn and N is also higher than the content in mulch.

The average stores of investigated elements in litter – fall are 81.312 kg.ha^{-1} and in mulch – 170.314 kg.ha^{-1}.

The acorn fraction is the richest with nitrogen, the annual fractions - with K and the perennial fractions – with Ca. The leaves and acorns accumulate Mn, and the cupolas - Fe. The biggest quantities of investigated microelements are measured in the leaves fraction.

According to the established capacity the biological turnover of investigated beech communities corresponds to the category of the average capacity turnovers (0,26 - 0,8 t.ha-^{1}) (LYUBENOVA, 2009).

According to the established chemistry the turnover belongs to the group of Na-Ca

turnovers with average mineral capacity which is characteristic for the average productive broadleaved forests (class of calcium-sub boreal turnovers) (LARCHER, 1978; LYUBENOVA, 2009). The prevailing of Ca over N in the phytomass is typical for this type of turnovers. For the investigated communities the N content prevailing have been observed and deviation of turnover to nitrogen - subtropical class of turnovers.

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Using Analytical Hierarchy Process (AHP) for Prioritizing and Ranking of Ecological Indicators for Monitoring Sustainability of Ecotourism in Northern Forest, Iran

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Abstract. Ecotourism has been identified as a form of sustainable tourism which is expected to contribute to both conservation and development. Unfortunately, due to inadequate environmental assessment, many ecotourism destinations tend to be both hazardous and self-destructive. Indicators are an important tool to provide a means toward sustainability. Among all different aspects of indicators, ecological indicators are significant for monitoring and evaluating sustainable management of ecotourism. In this study criteria and indicators were identified by using the Delphi approach through an expert panel from different fields. At the end of the process, a consensus of 9 criteria and 61 indicators was reached. For prioritization and ranking the Analytical Hierarchy Process (AHP) and Expert choice software was used. The 9 criteria include identified :1- Conservation of Natural resources & biodiversity2- Maintenance of sceneries ,natural &physical features 3-Conservation of soil & water resources 4- Maintenance of heritage & cultural diversity 5- existence of legal, institution, legislation and policy frameworks for empowering Ecotourism 6- promoting economic benefits & poverty alleviation7- Educational affairs and public awareness 8- Maintenance of hygiene& tourist safety 9- Tourists & local people satisfaction. The results showed that, out of the 9 criteria, the first three, which we labeled as Ecological criteria and comprised 21 indicators, stood as the top highest priority. We also continued the ranking of indicators with related criterion and then all of the indicators were ranked and prioritized by AHP method and using of expert choice software.

Key words: Ecological Indicator, Analytical Hierarchy Process (AHP), Monitoring, Sustainable Ecotourism.

Introduction

Northern forest of Iran is blessed with rich biological diversity, endemic and endangered species, spectacular panorama and sceneries landscape and its masterpieces of natural creative forms of an ancient forest. This forest contains the most important and significant natural habitats for in-situ conservation of biological diversity. Ten thousand domestic tourists visit this area annually, and if their presences are not accompanied by sound management and assessment, it may cause of the deterioration and devastation of the environment (WHINAM & CHILCOTT, 2003). Hence, there is a need for prioritizing and ranking of criteria and indicators for a sustainable management of ecotourism. According to KOTWAL *et al.* (2008) one of the most important indicators for ecotourism

monitoring is the ecological indicators (KOTWAL *et al.*, 2008).

Since the 1990s after the introduction of the concept of sustainable development by the Brundtland commission (WCED, 1987) environmental protection became a major issue all over the world. Tourism as an economic activity has an unavoidable effect on the environment of tourism destinations. As the environment is an essential asset to the tourism industry, the protection and conservation of environmental resources should be the top priority in the tourism industry (LIM & MCALEER, 2003). Sustainable development is seen as a tool for social equity and a procedure for achieving balance between natural resources conservation and development (LIM & MCALEER, 2004). Ecotourism has been recognized as a form of sustainable tourism which is expected to contribute to both conservation and development (TSAUR & LIN, 2005). Widespread and global concern over the state of the environment and the impact of human activities on natural ecosystem calls for long-term and high quality, datasets for detecting and understanding environmental changes (PARR *et al.*, 2003).

Unfortunately, due to inadequate environmental assessment and audits, many ecotourism destinations tend to be both hazardous and self destructive (TSAUR & LIN, 2005) thus it is necessary to identify a set of indicators for monitoring ecotourism sustainability. The criteria and indicators can become useful tools to determine parameters of a sustainable management (GOUGH *et al.,* 2008; RAISON *et al.,* 2001). In reality, the criteria and indicators must try to simplify the complexities of the world by providing manageable information to help understand the decisions and management of activities in the field (PENG *et al.,* 2002). Chapter 40 of agenda 21 urges all countries, governmental and non-governmental organization to identify effective indicators at the national and international level for sustainable development (BARRERA-ROLDAN & SALDIVAR-VALDES, 2002). In a relatively short period of time about 150 countries had adopted specific criteria and indicators for sustainable management (HICKEY & INNES, 2008). These criteria and indicators are important because 150 of the countries with a total of 97.5% of forest area were involved in the processes of formulating regional and international criteria and indicators (WIJEWARDANA, 2008).

Nowadays, ranking and prioritizing of criteria and indicators have turned into a serious debate in the world, the technique of prioritization are used together with the criteria and indicators under the general title of multi-criteria decision making methods (MENDOZA & PRABHU, 2006). The Analytical Hierarchy Process (AHP) is the most important and widespread decision making tool (OMKARPRASAD, 2004). The AHP method which was developed by SAATY (1980), has been used extensively in almost all the applications related to the multiple criteria decision making (MCDM) in the last 30 years. VAIDYA & KUMAR (2006) found that there were more than 150 articles studying the AHP combined with general applications. Besides being applied to the finance sector (STEUER & NA, 2003), the AHP was adopted in the education, engineering, government, industry, management, manufacturing, personal, political, social, and sports (VAIDYA & KUMAR, 2006). The wide application of AHP is due to its simplicity, ease of use, and great flexibility. In recent years, the idea of sustainable ecotourism management has attracted a lot of attention but, in spite of the existence of high ecological, economic and social values of forests, forest management in Iran does not take advantage of criteria and indicators (GOUSHEGIR *et al.,* 2009). Thus this study emphasizes prioritization and ranking of ecological indicators that can monitor ecotourism sustainability in protected watershed.

Material and methods

*Study Area***.** The study area located in western part of Mazandaran province in Northern of Iran (Fig. 1). The area lies between 36°19′22″ to 36°45′25″ Northern latitude and 50°21′06″ to 50°23′30″Eastern longitude. The whole area is 77563 hectares, which includes 32761 ha designated as core

zone (biosphere reserve) and 44802 ha as a buffer zone. The altitude at the lowest point is 100 m and the highest point about 4851 m and the entire region endowed with natural resources. This watershed is a protected area and it is under consideration to be registered as a biosphere reserve by the Forest, Range and Watershed Department of Iran (AMIRI, 2008). The region is very attractive and has a potential for recreational and ecotourism due to beautiful sceneries, spectacular landscapes, lush and rolling rivers, streams, different plant communities, religious and historical monuments, snow-capped mountains, natural glacier and blooming valleys and is a paradise for nature lovers, conservationists, botanists, zoologists and environmentalists. The area has attracted large numbers of tourists in the peak in the season from June to September.

Delphi technique. KAYNAK & MACAULEY (1984) described the Delphi method as a unique technique for eliciting and refining group judgment. This technique, developed in early 1950's by RAND corporation, is a method for structuring a group communication process in a way that allow individuals to deal with a complex problem (LINSTONE & TUROFF, 1975).

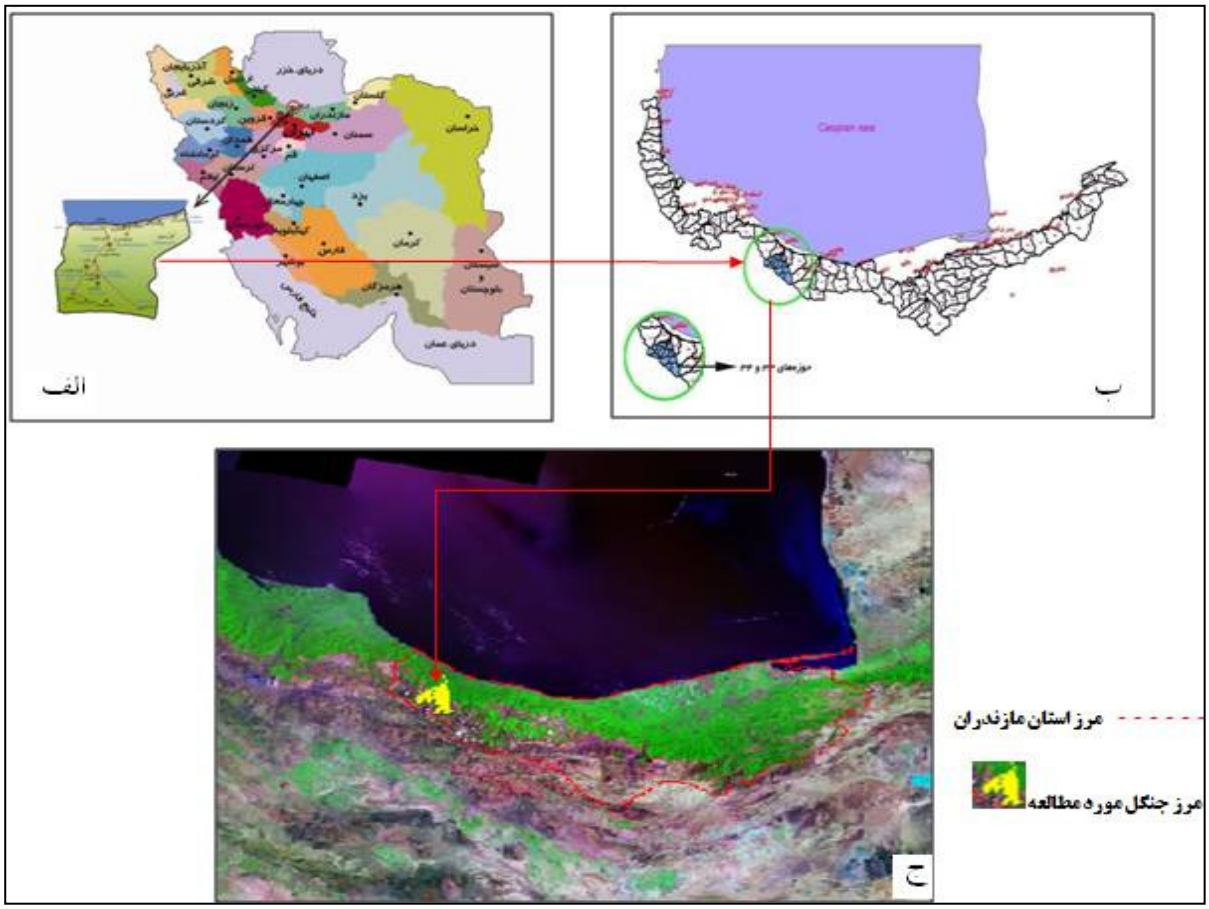

Fig. 1. Study area - Dohezar & Sehezar watersheds in Iran

The aim of Delphi surveys is to obtain the advice of panel members ,and whenever possible to reach a consensus (RICHERY *et al.*, 1985).the carefully selected experts answer questionnaire in two or more rounds. At the end of each round the researcher provides an anonymous summary of panel member's suggestions from the previous round. Finally the process is stopped after reaching stability of result by determining mean or median scores.

The Delphi technique was used to identify criteria and indicators for ecotourism sustainability and it was completed in two rounds. For this purpose the assessment team organized which

consisted of ten members. The members of team included 5 experts with PhD degree and 5 others with MS and BS degree. All team members are very familiar with field of research and also to study area.

Analytical hierarchy process. The Analytic Hierarchy Process (AHP), a well-known approach, was applied to solve MCDM problems (SAATY, 1980). AHP is a scoring model that depends on subjective managerial entered data on Multiple Criteria. These inputs are changed into scores that are used to assess each of the possible alternatives (HANDFIELD *et al.,* 2002). POH *et al*. (2001) stated that AHP as a qualitative and quantitative approach can be used to determine the priority and weight of each performance criteria and indicators through paired comparison of attributes. Weighting of the criteria and indicators was carried out via the analytical hierarchy process (AHP). This process is one of the most renowned and famous techniques in multi-criteria decision making, which was innovated and established by SAATY (1970).

There are different methods to measure the importance of coefficient (weight) for criteria and indicators; one of the traditional ones is the pair-wise comparison. In this method criteria and indicators are compared with each other and the degree of importance for each criterion or indicator is specified with respect to each other. For this purpose we can use the standard manner which is proposed by SAATY (1970). The procedure of this method focuses on two factors at a time and their relation to each other. The relative importance of each factor is rated by a measurement scale to provide numerical judgments corresponding to verbal judgments. The instrument used in this study was adopted from SAATY (1980), and the scale of the pairwise comparison is showed in Table 1.

For weighting of C&I, all of the tables related to criteria and indicators for comparison were prepared and then distributed among panel members and they were requested to rank the C&I based on Table 1.

Inconsistency rate is a mechanism through which the validity of respondent's responses was evaluated using a matrix comparison mechanism. This mechanism specified the reliability of response gained from respondents with respect to the comparison of criteria and indicators. For computing inconsistency ratio, duo to number of respondents is more than one; we computed geometric means. Inconsistency ratio in AHP method must be less than 0.1 (TZENG *et al.,* 2002). If the inconsistency ratio is more than 0.1, the process may warrant recomputed by user (CHANGA *et al.,* 2007).

Table 1. Saaty's Pairwise comparisons for Criteria and Indicators (C&I)

Intensity of importance	Verbal Judgment of preference
1	Equally importance
3	Moderate importance
5	Strong importance
7	Very strong importance
9	Extreme importance
2,4,6,8	Intermediate values between adjacent scale values

Results and Discussion

Nine criteria and 61 indicators were identified by experts through two rounds of the Delphi process. These criteria and indicator were then ranked and prioritized by expert panel member through AHP. The weight of the criteria determines the importance of the criteria against each other leading to the attainment of the goal of sustainable management on ecotourism. The geometrical means was entered into the tables related to pair wise comparison of the C&I. After finishing the above procedure the C&I was ranked by Expert Choice software.

There were 9 criteria for sustainable management of ecotourism was identified and the weight and inconsistency ration is showed in Table 2.

Table 2. List of criteria for sustainable management of ecotourism.

Criteria	Weight
1. Conservation of Natural resources & biodiversity	0.278
2. Conservation of soil & water resources	0.180
3. Educational affairs and public awareness	0.036
4. Tourists & local people satisfaction	0.023
5. promoting economic benefits & poverty alleviation	0.058
6. Existence of legal, institution, legislation and policy frameworks	0.079
7. Maintenance of heritage & cultural diversity	0.116
8. Maintenance of hygiene& tourist safety	0.034
9. Maintenance of sceneries, natural &physical features	0.197
Inconsistency ratio:	*0.06*

With regard to the results of AHP method and using expert choice software, three criteria (criteria number 1, 2 and 9) which encompass ecological indicators; have been arranged to the amount of relative importance. Criterion 1: Conservation of natural resources & biodiversity with 27.8%; Criterion 9: Maintenance of sceneries, Natural & physical features with 19.7% and Criterion 2: Conservation of soil & water resources with 18% occupied the top priority among other criteria. This procedure continued for indicators which belong to three of the above criteria and the prioritizing & ranking have been set for them based on the above mentioned manner and the result is illustrated as the following: Criterion 1: Conservation of natural resources & biodiversity and constitute 9 indicators which show in Table 3. Criterion 2: conservation of soil & water resources, and constitute 7 indicators which show in Table 4. Criterion 3: Maintenance of sceneries, natural & physical features and constitutes 5 indicators which show in Table 5.

As show in Table 6, based using AHP and expert choice software,the first 3 criteia which are the environmental criteria stood as the top priority among all 9 criteria.

Table 3. Indicators related to Criterion 1

Criterion 1: Conservation of natural resources and biodiversity	Weight
1.1. Extent of protected area	0.295
1.2. No of protected water resource (rivers, marsh, streams, etc…)	0.044
1.3. No of rare, threatened, vulnerable & endangered species	0.147
1.4. Existence & implementation of Action plan for conservation	0.109
1.5. Existence of different plant types (forest and range)	0.061
1.6. Diversity of plants and wildlife	0.085
1.7.Existence of zoning and comprehensive management system	0.202
1.8. Extent of damaged area duo to human activities	0.031
1.9. Existence & implementation of EIA program in recreational zones	0.025
Inconsistency ratio:	*0.05*

Table 4. Indicators related to Criterion 2

Criterion 2: Conservation of soil and water resources	**Weight**
2.1. Amount of erosion & sediment	0.378
2.2. Amount of contamination materials in waters	0.210
2.3. Amount of fluctuation water resources	0.153
2.4. Extent and percentage of uncovered lands	0.075
2.5. Control of domestic (dairy cattle) animal in range & forest	0.056
2.6. Extent and percentage of afforested area	0.034
2.7. Amount of density for road and pedestrian in watershed	0.095
Inconsistency ratio:	*0.05*

Table 5. Indicators related to Criterion 3

Criterion 3: Maintenance of sceneries, natural & physical features	**Weight**
3.1. Existence of management plan for protection of spectacular landscape	0.429
3.2. Extent and no of specific natural plant communities	0.289
3.3. Existence of management plans for conservation of riparian zones	0.083
3.4. Growth rate of incompatible construction in region	0.49
3.5. Existence of plan for protection of topography & geological features	0.151
Inconsistency ratio:	*0.06*

Table 6. Weight and rank of criteria related to sustainable management of ecotourism

Criteria's title	**Weight**	**Ranking**
Conservation of natural resources & biodiversity	0.278	1
Maintenance of Sceneries, natural & Physical features	0.197	2
Conservation of soil and water resources	0.180	3
Maintenance of heritage and cultural diversity	0.116	4
Existence of Legal, institutional, legislation and policy framework	0.079	5
Economic benefits and poverty alleviation	0.058	6
Educational affairs and public awareness	0.036	7
Maintenance of hygiene & tourist safety	0.034	8
Tourist & local people satisfaction	0.023	9

Table 7 to 9 shows the prioritized ecological indicators in relation to the different environmental criterion. The 21 ecological indicators, 9 indicators belong to Criterion 1: Conservation of natural resources & biodiversity, 7 indicators to Criterion 2: Conservation of soil & water resources and 5 indictors to criterion 3: Maintenance of Sceneries, natural and physical features.

Conclusion

Northern forests of Iran have high ecological, economics and social values, but no definite criteria and indicators have been developed to monitor these forests in order to assess it, especially in ecotourism dimension. The absence of factors has prevented managers from understanding whether the forest is experiencing sustainability or not (GOUSHEGIR *et al*., 2009).

The studies of KOTWAL *et al*. (2008) and GOUGH *et al.* (2008) indicate that ecological indicators play a crucial role in sustainability and need to be covered by social and economical indicators.

Table 7. Weight and rank of indicators related to Criterion 1: Conservation of natural resources & biodiversity

Indicator's title	Weight	Ranking
Extent of protected area	0.295	1
Existence of zoning and comprehensive management system	0.202	2
No of rare, threatened, vulnerable & endangered species	0.147	3
Existence & implementation of Action plan for conservation	0.109	4
Diversity of plants and animals	0.085	5
Existence of different plant types (forest and range)	0.061	6
No of protected water resource (rivers, marsh, streams, etc…)	0.044	7
Extent of damaged area duo to human activities	0.031	8
Existence & implementation of EIA program in recreational zones	0.025	9

Table 8. Weight and rank of indicators related to Criterion 2: Conservation of soil & water resources

Indicator's title	Weight	Ranking
Amount of erosion & sediment	0.378	1
Amount of contamination materials in waters	0.210	2
Amount of fluctuation water resources	0.153	3
Amount of density for roads and pedestrian in watershed	0.095	4
Extent and percentage of uncovered lands	0.075	5
Control of domestic (dairy cattle) animal in range & forest	0.056	6
Extent and percentage of afforested area	0.034	7

Table 9. Weight and rank of indicators related to Criterion 3: Maintenance of Sceneries, natural and physical features

Indicator's title	Weight	Ranking
Existence of institutional & policy framework for ecotourism in region	0.429	1
Existence of legal obligations, incentives for promoting ecotourism	0.289	2
Existence of legal frameworks for participation of all stakeholders	0.151	3
Existence of collaboration among different related organization	0.083	4
Existence of approved national plan for sustainable tourism	0.049	5

Identification, ranking and prioritizing of criteria with related indicators can provide this opportunity for us to monitor and evaluate ecotourism sustainability precisely for forest watersheds of Northern Iran. Though the Analytical Hierachy Process is based on the knowledge and experience of experts (KUSWANDARI, 2004). It can still be a good choice because it is a quantitative method and can be modified regarding the charachterestics of Northern forest of Iran (GOUSHEGIR *et al.,* 2009). MENDOZA & PRABHU (2000) made use of multiple criteria decision making techniques (rating, ranking and pairwise comparison) a decision tools for assessing criteria and indicators designed to evaluate sustainable forest management.

The results of this survey showed that the applied technique for ranking and

prioritizing was very effective and impressive. The ranking and prioritizing of ecological indicators provide us with an opportunity with regard to the pivotal and crucial role they play in the sustainable management of ecotourism in The Northern forest of Iran. Among the 9 criteria which have been distinguished ,three of them which are: (1) Conservation of natural resources and biodiversity; (2) Maintenance of sceneries, natural and physical features and (3) Conservation of soil and water resources were the top priority among the criteria. This indicated that the importance of these criteria which encompass affiliated ecological indicators which are suitable for monitoring and evaluating ecotourism's sustainability in the Northern forest watershed of Iran. The ecological resources are the basic resources for attaining sustainable deveopment in economical, social and cultural dimensions; it is essential and vital to attain a precise and effective indicators for monitoring of sustainable management of ecotourism. Ranking and prioritizing provides opportunities to monitor ecotourim sustainability, trend of tourists activities and sustainable management and prevent damage and irreversiable alteration to ecotourism resources.

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Data on food composition of Phrynocephalus horvathi Méhely, 1894 (Reptilia: Agamidae) in Mount Ararat (Northeastern Anatolia, Turkey)

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Abstract. The study presents data on the food composition of Horváth's toad-headed agama, *Phrynocephalus horvathi,* in northern slopes of Mount Ararat (Aralık, Iğdır). A total of 294 prey items were determined in the digestive systems of 36 (8 males, 11 females, and 16 juveniles) individuals examined in the study. Prey groups in the food composition are included in Aranea (1.4%), Orthoptera (1.0%), Hymenoptera (73.5%), Coleoptera (23.1%) and Diptera (1.0%). No significant difference was observed between sexes regarding food composition. Consequently, *Phrynocephalus horvathi* is partially myrmecophagous (73.5%) and an active predator.

Key words: *Phrynocephalus horvathi,* Food composition, Northeastern Anatolia, Turkey.

Introduction

The toad-headed agamas of the genus, *Phrynocephalus* Kaup, 1825 include 37 species distributed in the arid zones of south and southeastern Europe, Middle Asia, north-western China, Iran, Afghanistan, Pakistan, northern Africa and Arabian Peninsula (ANDERSON, 1999; ANANJEVA *et al.*, 2006; BARBANOV & ANANJEVA, 2007). Together with the lizards of the genus *Eremias*, *Phrynocephalus* represents a core of the Palearctic fauna of deserts (ANANJEVA *et al.*, 2006). *Phrynocephalus horvathi* occurs in the Araks River valley of Armenia, Turkey, Nakhichevan (Azerbaijan), and north-western Iran (MELNIKOV *et al.*, 2008).

The Horváth's toad-headed agama generally lives in open desert landscapes, saltwort and wormwood semi-desert with sparse xerophytic herbaceous vegetation and takyr-like (clay desert) soils (ANANJEVA *et al.*, 2006; ANANJEVA & AGASYAN, 2008). Declining population of *P. horvathi* in Armenia requires special protection programs as its habitat is significantly fragmented by land conversion, intensive agriculture and urbanization (ANANJEVA & AGASYAN, 2008). On the other hand, there is no important anthropogenic pressure on the population in Turkey.

There are some studies on the taxonomic status of the species of genus *Phrynoephalus* (e.g. BARABANOV & ANANJEVA, 2007; MELNIKOV *et al.*, 2008), as well as on their distribution (ANANJEVA *et al.*, 2006), population dynamics and ecology (e.g. SHAMMAKOV, 1981; SHENBROT, 1987; ANANJEVA & SHAMMAKOV, 1985), age structure and life history (SMIRINA &

ANANJEVA, 2001); however, there is still lack of information on feeding habits of the species in Turkey.

The aim of the present study is to present the food composition of Horváth's toad-headed agama from northeastern Anatolia (Iğdır, Turkey).

Material and methods

In the study, 36 preserved specimens of *P. horvathi* (8 males, 11 females, and 16 juveniles) were examined, collected between 25 June and 27 August 2010 from sand dunes in Aralık (Lat: 39.863483°N, Long: 44.505245°E, 826 m a.s.l.), and Iğdır Province of Turkey [collected as a priority for determining the herpetofauna of Iğdır]. The material was registered in the Museum of Faculty of Art and Science, Onsekiz Mart University and incorporated into the collection of ZDEU-ÇOMU (Zoology Department Ege University-Çanakkale Onsekiz Mart University), Turkey. The species syntopically inhabits with *Eremias pleiskei*, *E. strauchi*, *Laudakia caucasica*.

Body length (SVL) and total length (TL) of the specimens were measured using calipers to the nearest 0.1 mm and recorded; in addition, sexes were determined. After these procedures, they were dissected and their digestive tracts were removed. The obtained food contents were preserved in 70% ethanol for later analysis. Food contents were identified to the lowest possible taxa. Vegetal materials, sand and little pebbles were also encountered in the food content. However, these materials were most likely ingested accidentally during foraging and not considered as food.

The food contents were presented both in numeric proportion (n%) and frequency of occurrence (f%). Sexual food niche overlap was measured using PIANKA'S overlap index (1973). This index varies between 0 (no similarity) and 1 (totally similar). Food-niche breadth was determined using SHANNON'S index (1948). All niche calculations were done using the "EcoSim 700" program (GOTELLI & ENTSMINGER, 2010). T-test and Mann-Whitney U test were used to compare the sexes; statistical analyses were performed using SPSS 10.0, and the alpha level was set at 0.05. In the results section, the mean values are given with their standard deviations.

Results

In the study, 36 individuals of *P. horvathi* (8 males, 11 females, and 17 juveniles) from Aralık (Iğdır) were examined. The mean body length (SVL) was 27.8±3.02 (22.0 – 33.3) mm for juveniles, 42.7±5.63 (34.3 – 49.4) mm for males and 45.6±4.88 (36.7 – 54.0) mm for females. The mean total length (TL) was determined as 57.9±6.38 (45.6 – 68.3) mm in juveniles, 93.9±12.45 (77.2 – 110.3) mm in males and 93.9±8.88 (76.8 – 108.7) mm in females. No statistically significant difference was observed between sexes in terms of their sizes (SVL: $t=1.254$ $p\leq0.225$; TL: $t=1.109$, $p\leq0.281$).

In the stomach contents of 36 individuals, 294 prey items, with body lengths ranging from 2 to 30 mm, were determined with a median (±SD) number of 8.0±5.09 (range= 1-20). The number of median prey items was 5.0±4.63 (1-20) in juveniles, 11.0±4.60 (1-16) in males, and 11±4.72 (2-18) in females. No statistically significant difference was detected between sexes regarding the number of prey items in the stomach (Mann-Whitney U test, $Z=0.000$, $p\leq1.000$); however, there was a significant difference between juveniles and adults (Mann-Whitney U test, $Z=2.991$, $p\leq0.003$). Juveniles consume smaller (1-20 mm) and fewer prey objects than do adults.

Aranea (n%=1.4%), Orthoptera (1.0%), Hymenoptera (73.5%), Coleoptera (23.1%) and Diptera (1.1%) are the prey groups included in the food content. Among the prey taxa shown in Table 1, Formicidae (n%=73.5, f%=88.9%) and Coleoptera (23.1%, 66.7%) were frequently consumed by Horváth's toad-headed agama. More active prey species like Aranea, Orthoptera, and Diptera were encountered less frequently in the food content. According to the Pianka's niche overlap index, food compositions of sexes are mostly similar (males vs. females = 0.99, males vs. juveniles = 0.99, females vs. juveniles = 1.00). This indicates that feeding habit is not changed with age and that the same microhabitat is used for foraging. Food

niche breadth (Shannon's index) was 1.06, 0.80 and 0.67 in males, females and juveniles, respectively. No difference was observed among the sexes. Moreover, food spectrum of the species is rather limited according to the index value.

Table 1. Food composition of the Horváth's toad-headed agama from Northeastern Anatolia. n%: numeric proportion, f %: frequency of occurrence.

Prey Taxa	n (%)				f (%)			
	Juvenile	Male	Female	Total	Juvenile	Male	Female	Total
ARACHNIDA		4 (4.8)		4 (1.4)		4 (50.0)		4 (11.1)
Aranea, Lycocidae		4 (4.8)		4 (1.4)		4 (50.0)		4 (11.1)
			116	290				
INSECTA	94 (100)	80 (95.2)	(100)	(98.6)	17 (100)	8 (100)	11 (100)	36 (100)
Orthoptera		3 (3.6)		3 (1.0)		2 (25.0)		2 (5.6)
Hymenoptera,				216				
Formicidae	73 (77.7)	56 (66.7)	87 (75.0)	(73.5)	14 (82.4)	7 (87.5)	11 (100)	32 (88.9)
Coleoptera (total)	21 (22.3)	21 (25.0)	26 (22.4)	68 (23.1)	8 (47.1)	7 (87.5)	9 (81.8)	24 (66.7)
Coleoptera, Larvae	14 (14.9)	11 (13.1)	13 (11.2)	38 (12.9)	8 (47.1)	5 (62.5)	5 (45.5)	18 (50.0)
Coleoptera, Carabidae	2 (2.1)	2 (2.4)	6 (5.2)	10 (3.4)	1 (5.9)	1 (12.5)	4 (36.4)	6 (16.7)
Coleoptera, Coccinellidae	5 (5.3)	5 (6.0)	6 (5.2)	16 (5.4)	3 (17.6)	3 (37.5)	4 (36.4)	10 (27.8)
Coleoptera, Scarabaeidae		3 (3.6)	1 (0.9)	4 (1.4)		3 (37.5)	1 (9.1)	4 (11.1)
Diptera, Muscidae			3 (2.6)	3 (1.0)			3 (27.3)	3 (8.3)
vegetal material						1 (12.5)	1 (9.1)	2 (5.5)
sand and little pebbles e.g.					12 (70.6)	6 (75.0)	9 (81.1)	27 (75.0)
Number of prey items	**94**	**84**	**116**	**294**				

Discussion

Our study results demonstrated that Horváth's toad-headed agama mostly consumed slow insects, especially Formicidae and Coleoptera. In contrast, more active and flying prey species included in Aranea, Diptera and Orthoptera were encountered less frequently in the food composition. Ants, coleopterans and other small insects (*Locusta* sp.), larvae, and spiders were reported to form the food composition of the species (TERENTEV & CHERNOV, 1965; BAŞOĞLU & BARAN, 1977; ANDERSON, 1999).

Widely-foraging predators encounter and consume mostly non-moving types of prey items (PIANKA, 1966). PERRY & PIANKA (1997) stated that external, internal and evolutionary factors were important for determining the foraging behaviour; widely foraging species used their visual and smelling senses while hunting, and food niche breadth is rather narrow. Due to the limited prey range of *P. horvathi* and less active prey species in the food composition, it could be included in the widely foraging species.

Ants and termites are frequently encountered in the food composition of agamids (e.g. PIANKA & PIANKA, 1970; HUEY & PIANKA, 1981; DÜŞEN & ÖZ 2001; HUEY *et al*., 2001). *Phrynocephalus versicolor* commonly consumes ants in summer months (84%), and carabids (11%) are their other important prey objects (QUAN *et al*., 2006). Important food overlap was observed in *Eremias multiocellata*, *E. argus*, and *P. frontalis* which live syntopically (CHEN, 1997).

DÜŞEN & ÖZ (2001) determined that Hymenoptera (72.21%) and especially the Formicidae (49.83%) were mostly present in the food composition of *Laudakia stellio.* On the other hand, Formicidae were also important prey of *Uma paraphygas* and *U. exsul* (GADSDEN & PALACIOS-ORONA, 1997; GADSDEN *et al*., 2001). In addition, agamids

have positive energy balance, and there is always food in the stomach of ant-eating agamids (HUEY *et al.*, 2001). None of the individuals examined in the study had an empty stomach, and there was at least one prey object. This finding supports the hypothesis.

In conclusion, the food composition of Horváth's toad-headed agama, *P. horvathi*, is mostly composed of slow-moving prey items. However, more active and good flying prey species were less encountered in the food composition. The species is partially myrmecophagous and a widely foraging hunter that consumes slow-moving prey items, especially ants and coleopterans.

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Data on Hatchlings of Caucasian Rock Lizard, Darevskia valentini (Boettger, 1892) in Northeastern Anatolia

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Abstract. During our field work in the Tepeler Village (Ardahan, Turkey) on 27th of August, 2010, we observed hatchlings of *Darevsika valentini*. The mean body length of was found as 28.06±1.14 (25.3 - 30.4) and the total length as 70.81±3.92 (61.9 - 81.0). Juveniles were generally observed under stones and those portions of the roots of annual herbaceous plants which remain under stone are also used as shelters for juveniles. Neonates on the southern slopes of the hills could be individually seen and mostly, individuals aggregated [median number of individuals, 6 (1-15)] and lived in groups.

Key words: Darevskia valentine, hatchlings, Northeastern Anatolia, Turkey.

Introduction

Darevskia valentini, Caucasian rock lizard, ranges from the mountain-steppe zones of Armenia to southern Azerbaijan, eastern Turkey, southwestern Georgia and northwestern Iran (DAREVSKY, 1967; BAŞOĞLU & BARAN, 1977; BANNIKOV *et al*., 1977; ANDERSON, 1999; SINDACO *et al*., 2000; ANANJEVA *et al*., 2006). In Turkey, the distributional range of the species is eastern Anatolia, eastern part of Black Sea (west to Sinop province) and mountains of central and eastern Anatolia (provinces of Kayseri, Niğde and İçel), and it is recorded from 1,300 to 3,000m a.s.l. (BAŞOĞLU & BARAN, 1977; ANDERSON, 1999; SINDACO *et al*., 2000; ILGAZ, 2004; Ananjeva *et al*., 2006; TOK *et al*., 2008). It is classified as the least concern on the IUCN Red List (TOK *et al*., 2008) and in the list of Annex III of Bern Convention.

The species inhabits large rock blocks and small stony places on the hills in the subalpine zone. Moreover, it is also observed in areas with intensive vegetation at the border of sloping stony or rocky places (DAREVSKY, 1967; BANNIKOV *et al*., 1977; Ilgaz, 2004). The adult morphology of *D. valentini* has been studied in detail (DAREVSKY, 1967; BANNIKOV *et al*., 1977; Eiselt et al., 1992; Ilgaz, 2004) in its distribution site; however, no sufficient information is available about juvenile morphology and ecology. In this study, hatchlings of *D. valentini* were observed and our observations on the nests, dispersion, and some morphometric and color-pattern

features of these individuals were presented.

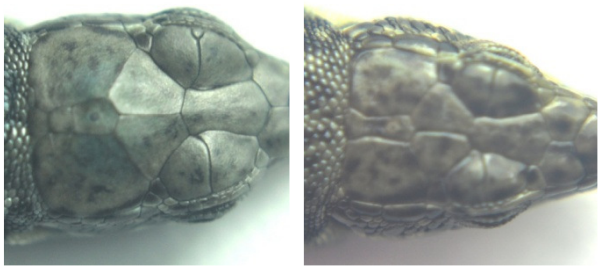
Materials and methods

In our field studies in Ardahan (Northeastern Anatolia, Turkey), we observed hatchlings of *D. valentini* in Tepeler Village (lat.: 41.060104° N, long.: 42.575360°E, 1866 m a.s.l, Fig. 1) 15 km southwestern of Ardahan on August 27, 2010. We noted some habitat characteristics and approximately density of individuals. Fifty-three hatchlings were measured and its color pattern characteristics were recorded. Following morphometric measurements were taken using dial calipers with an accuracy of 0.01 mm: Tail length (TaL): Anal cleft to the tip of the tail; Head width (HW): At the widest point of the head; Head length (HL): Tip of snout to the posterior margin of the ear opening: Pileus length (PL): Tip of the snout to the posterior margins of the parietals; Pileus width (PW): At the widest point between the parietal plates; Snout-vent length (SVL): Tip of the snout to the anal cleft; Total body length (TL): Tip of the snout to the tip of the tail. Furthermore, some morphometric indexes and ratios were calculated: Head index: (HW/HL)*100; Pileus index: (PW/PL)*100; SVL/TaL. Authors have to explain how to determine of body length for hatchlings in material and method section.

Fig. 1. Habitat of hatchlings from Tepeler Village (Ardahan, Turkey).

Results and Discussion

In the hatchlings of *D. valentini*, interparietal and occipital plates are well-developed and the parietal plates found to be laterally pushed are trapezoidal (Fig. 2). The pileus of the specimens is convex upwards. The morphological characteristics of the specimens are given in Table 1.



(A) (B)

Fig. 2. The view of pileus in hatchling (A) and adult (B) Caucasian rock lizards

Table 1. Morphometric measurements of hatchlings from Tepeler Village (Ardahan, Turkey). [N: number of specimens; SD: Standard deviation; SE: Standard error of the mean]

Characters	N	Mean	Min -Max	SD	SE
HL	53	7.44	7.0 - 8.1	0.29	0.04
HW	53	4.70	4.2 - 5.1	0.19	0.03
PL	53	7.08	6.6 - 7.7	0.28	0.04
PW	53	4.02	3.4 - 4.5	0.21	0.03
SVL	53	28.06	25.3 - 30.4	1.14	0.16
(HW/HL)*100	53	158.53	146.1 - 173.5	6.82	0.94
(PW/PL)*100	53	56.81	52.0 - 62.5	2.53	0.35
TL	32	70.81	61.9 - 81.0	3.92	0.69
SVL/TaL	32	0.66	0.6 - 0.7	0.03	0.01

Furthermore, a vitellus cleft occupying 2 or 3 transverse ventral rows manifests itself in the individuals and starts at ventral rows 16 and 18. In 53 individuals, the dorsal ground color is green, and maculation over the head (the pileus always has clear maculation) is very clear in 22 (41.5%), scarcely clear in 28 (52.8%) and unclear in 3 (5.7%) of all the specimens (Fig. 3). No occipital stripe is observed in 27 (51%) of the specimens showing a reticular pattern, whereas there is a clear occipital stripe in the rest of them. The ventral part is dirty white and the outermost ventral plates have blackish dots. The tail is lighter green relative to the dorsum. The maculation of the juveniles is thinner (primarily the head region) and

large and integral dots cover less area as compared to the adults.

Fig. 3. Egg-laying site and shelters of juveniles.

TERENTEV & CHERNOV (1965) reported that the oviposition period of *D. saxicola* is from late June to early August and that the females lay 2 to 4 eggs (mean 3). In the Armenian population of *D. valentini*, adults come out of hibernation at about the end of April and at the beginning of May and lay eggs at around the end of June and in the midst of July (DAREVSKY, 1967). ILGAZ (2004) stated that May and June are the breeding period of *D. valentini*. According to DAREVSKY (1967), the average clutch size of females is 5.2 (range= 3 – 8) and its egg size is 14.5x8.5mm. In the Crimean population of *Darevskia saxicola*, juveniles hatch from late August to early September and the size of neonates is 25 mm and reaches 31 mm by the next spring (TERENTEV & CHERNOV, 1965). Hatchlings start to appear at the end of August and at the beginning of September, and their body lengths range from 26 to 27 mm after 55-to-65-day incubation depending on temperature (DAREVSKY, 1967). In the hatchlings of the Tepeler Village population, the mean body length was found as 28.06±1.14 (25.3 - 30.4) and the total length as 70.81±3.92 (61.9 - 81.0). Our observations also support the fact that oviposition period resembles that of the Armenian population. They hatch at around the end of August and at the beginning of September in Armenia, while slightly mature individuals were observed in the Tepeler Village population on August 27. As observed in some populations (DAREVSKY, 1967), this might be due to the quicker development in cold regions or early mating and oviposition in order to complete the development.

DAREVSKY (1967) observed that Caucasian rock lizards lay their eggs into rock cracks, under stones and into soil clefts and that female has egg-laying site fidelity. In Tepeler Village population as well, juveniles were generally observed under stones and remnants of egg shells were encountered under some stones. This indicates that the species lay its eggs under stones in the place concerned. In addition, those portions of the roots of annual herbaceous plants which remain under stone are also used as both egg-laying sites and shelters for juveniles (Fig. 3).

During the fieldwork, it was observed that neonates under stones on the southern slopes of the hill could be individually seen and mostly, individuals aggregated [median number of individuals, 6 (1-15)] and lived in groups (Fig. 4). In this way, individuals can more easily perform their daily functions, such as finding a prey, balancing the body temperature, and protection from their potential enemies. It was striking that these sites were at the same time egg-laying sites.

Fig. 4. Aggregation of five Caucasian rock lizards from Tepeler Village (Ardahan, Turkey).

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Range Expansion of Metcalfa pruinosa (Homoptera: Fulgoroidea) in Southeastern Europe

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Abstract. The citrus flatid planthopper *Metcalfa pruinosa*, a Nearctic species of Fulgoroidea: Flatidae, was accidentally introduced in Europe, first in Italy in the late 1970's. In a few decades, *Metcalfa pruinosa* has spread over most of Europe, finally reaching the Black Sea coast in 2009. Hundreds of individuals of different life stages were observed for the first time in the south-eastern part of Romania throughout the summer of 2009 on several host plants: *Hibiscus syriacus, Ligustrum vulgare, Robinia pseudoacacia, Evonymus japonicus, Spirea x vanhouttei, Aesculus hippocastanum, Philadelphus coronarius, Ficus carica, Vitis vinifera, Fraxinus pennsylvanica.* The number of individuals observed and the area covered increased dramatically in 2010 as well as the number of host plants (110 species in 49 families), suggesting the planthopper is in the expansion phase of the invasion process.

Key words: alien species, *Metcalfa pruinosa,* Flatidae, Romania.

Introduction

Human-driven biotic invasions have caused major alteration of the Earth's biota, representing a major threat to global biodiversity and habitat loss (MACK *et al.*, 2000). Globalization and increasing trade leads to higher risks of alien species arriving accidentally to new areas (MOONEY, 2005). Many studies exist concerning the economic value of the damage produced by invasive species, their impact on agriculture, forestry and fisheries is considerable; several billions of dollars are spent each year on prevention, eradication, or control of such species (e.g. PERRINGS *et al.*, 2010).

The citrus flatid planthopper *Metcalfa pruinosa,* Say 1830 is a North-American species that was accidentally introduced in Italy, near Treviso in 1979 (ZANGHERI & DONADINI, 1980). From northern Italy, *Metcalfa pruinosa* has rapidly spread throughout Europe (Table 1). The possible ways of spreading were accidental by pasive transport of adults or eggs with infested plant material, or by beekeepers that deliberately introduce it for the secretion of honeydew (MIHAJLOVIĆ, 2007). Active flight of adults seems to be an important way of dispersal only at small spatial scales; for example estimated rate of natural spread of *Metcalfa pruinosa* in Vienna is of 0.2-0.5 km/year (KAHRER *et al.*, 2009).

Metcalfa pruinosa is gregarious, univoltine, overwinters in egg stage and has five larval instages. The body of adults is brown to grey in color and is covered by a waxy powder. In their native range, adults of *Metcalfa pruinosa* measure 5.5 to 8 mm in length (MEAD, 2004). However dimensions vary and may even reach 15 mm (COLOMBO,

2009). Its presence is easily observed due to the white waxy filaments that cover the plants and to their characteristic display on branches.

In 2009 juvenile specimens were observed on *Fraxinus pennsylvanica* leaves in Constanța Harbor enclosure and later on, adults were observed and identified as *Metcalfa pruinosa* using MEAD (2004) (PREDA & SKOLKA, 2009). The aim of this study was to investigate and quantify the range expansion of *Metcalfa pruinosa,* Say 1830 in this newly colonized area.

Table 1. Reports of *Metcalfa pruinosa* in Europe since 1979.

Country	Year of first report	Status	Source
Italy	1979	established	ZANGHERI & DONADINI, 1980
France	1986	established	DELLA GIUSTINA, 1987
Spain	1988	established	PONS *et al*., 2002
Slovenia	1990	established	SIVIC, 1991
Great Britain	1994	eradicated	MALUMPHY *et al*., 1994
Switzerland	1993	established	JERMINI *et al*., 1995
Croatia	1993	established	MACELJSKI *et al*., 1995
Austria	1996	established	HOLZINGER *et al*., 1996
Czech Republic	2001	eradicated	LAUTERER, 2002
Greece	2002	established	DROSOPOULOS *et al*., 2004
Turkey	2003	established	KARSAVURAN & GÜÇLÜ, 2004
Hungary	2004	established	PÉNZES *et al*., 2005
Bulgaria	2004	established	TRENCHEV *et al*., 2007
Serbia	2006	established	MIHAJLOVIĆ, 2007
Bosnia and Herzegovina	2006	established	GOTLIN ČULJAK *et al*., 2007
Netherlands	2006?	unclear	STRAUSS, 2009
Romania	2009	established	PREDA & SKOLKA, 2009

Material and methods

Between April and October 2009 and 2010, we monitored every fortnight alien insect species in Constanța County, in 15 localities along the Black Sea coast (Fig. 1a). In 2009, 32 sites covered by semi natural vegetation and ornamental plants such as city parks, etc. were surveyed in the city of Constanța and surroundings, to assess the presence of *M. pruinosa* and identify host plants (Fig. 1a). We considered the planthopper's preference for shaded areas with dense vegetation (PONS *et al*., 2002) and focused our surveys on these types of potential habitats. Host plant species were identified using CIOCÂRLAN (2000). We considered a plant species as "host" when several individuals (juveniles and/or adults) were present and signs of their activity were evident on the plant (white waxy filaments, exuviae and honeydew secretions as traces of feeding). In 2010, we repeated the survey performed in the city of Constanța and made additional observations in similar areas south of Constanța city, along the Romanian Black Sea coast. We investigated in total 81 sites represented by green urban areas in 15 localities, 50.6% located in Constanța city (including Mamaia) and 49.4% south of Constanța city, up to the last Romanian locality next to the Bulgarian border (Fig. 1a). We recorded the presence or absence of *Metcalfa pruinosa*, the host plants and the level of infestation of the investigated site. The level of infestation was assigned considering the abundance of individuals (juveniles and adults), which was visually estimated using a scale from 1 to 5 as follows: level 1 – fewer than 10 individuals; level 2 – between 11 and 20; level 3 – between 21 and 50; level 4 – between 51 and 100; level 5 – over 101 individuals. Geographic coordinates were measured with a Garmin eTrex Summit GPS

receiver; the maps were realized with Garmin MapSource Version 6.15.6.

Results

In 2009 *Metcalfa pruinosa* was observed in three localities from Constanța County along the Romanian Black Sea coast (Constanța, Eforie and Tuzla). Its presence was noticed in 81% of the 32 surveyed sites, in parks, public gardens and on vegetation along the roads. The host plants on which *Metcalfa pruinosa* was encountered were *Aesculus hippocastanum* L., *Fraxinus pennsylvanica* Marsh., *Robinia pseudoacacia* L., *Hibiscus syriacus* L., *Philadelphus coronarius* L., *Evonymus japonicus* L. f., *Spirea x vanhouttei* (Briot) Zabel, *Ligustrum vulgare* L., *Ficus carica* L., *Vitis vinifera* L. We did not record its presence on a number of common species in Constanța like *Tilia cordata* Mill., *Acer negundo* L., *Thuja orientalis* L., *Thuja occidentalis* L., *Juniperus communis* L., *Ailanthus altissima* Mill., *Eleagnus angustifolia* L., *Populus x canadensis* Moench although more than 200 species are mentioned in the literature as host plants (BAGNOLI & LUCCHI, 2000; PONS *et al.*, 2002). The highest abundance of individuals of different life stages was observed on *Hibiscus syriacus* hedges in sheltered green spaces.

The next year, we noticed the presence of *Metcalfa pruinosa* in several other localities along the Black Sea coast. If we compare the average levels of infestation estimated for the same period in 2010 in the surveyed localities, the highest abundance of planthoppers can be observed south of Constanța city, in Comorova forest (Fig. 1b). In Constanța city, the extent of the area covered increased compared to 2009, as well as the abundance of the individuals (Fig. 2). The number of recently infested sites multiplied in the second year (Fig. 3). In total, *Metcalfa pruinosa* was present in 76 sites along the Romanian Black Sea coast, about 94% of the surveyed sites. Not surprisingly, the number of host plants increased as well, including among others the common plant species previously mentioned as not infested in 2009. In 2010, we identified 110 host plants belonging to 49 families. Rosaceae and Asteraceae comprised almost 26% of the species; again the most frequented species was *Hibiscus syriacus* (Fig. 4). Analyzing the native ranges of the host plants identified, only 14% are north-American, while most of them are originated in Eurasia (Fig. 5).

Discussion

Preliminary data suggest that *Metcalfa pruinosa* has a similar life cycle in the south eastern part of Romania, as in the southern part of Europe (PONS *et al.*, 2002; SOULIOTIS *et al.*, 2008). The planthopper develops one generation per year, adults can be observed from late June until late September. The increase in the abundance of individuals, of the area covered and of the number of host plants observed in the two successive years suggest that *Metcalfa pruinosa* is in the expansion phase of the invasion process. The successful range expansion of *M. pruinosa* can be related to its great polyphagy and to the lack of specialized predators in the newly colonized areas (STRAUSS, 2009). In Europe, *Metcalfa pruinosa* reaches higher densities than in the United States and the number of host plants is also higher (BAGNOLI & LUCCHI, 2000; WILSON & LUCCHI, 2001). We assume that the expansion of *Metcalfa pruinosa* in our study area is also favored by its great polyphagy thus the geographical origins of the available plant species should not have a negative influence on their potential use as hosts. We noticed that the planthopper is capable of living on plants that originate in its native area as well as on a high number of plant species native to Europe and/or Asia (Fig. 5). *Hibiscus syriacus* L., a species of Asian origin, was the most frequented plant by juveniles and adults of *Metcalfa pruinosa* (Fig. 4). A high number of individuals of different life stages were observed on *Campsis radicans* (L.) Seemann, a species of North-American origin, but also on two Mediterranean species, *Aesculus hippocastanum* L. and *Ligustrum vulgare* L. Its wide range of host plants and gregarious behavior gives it a competitive advantage enabling it to successfully reproduce and spread.

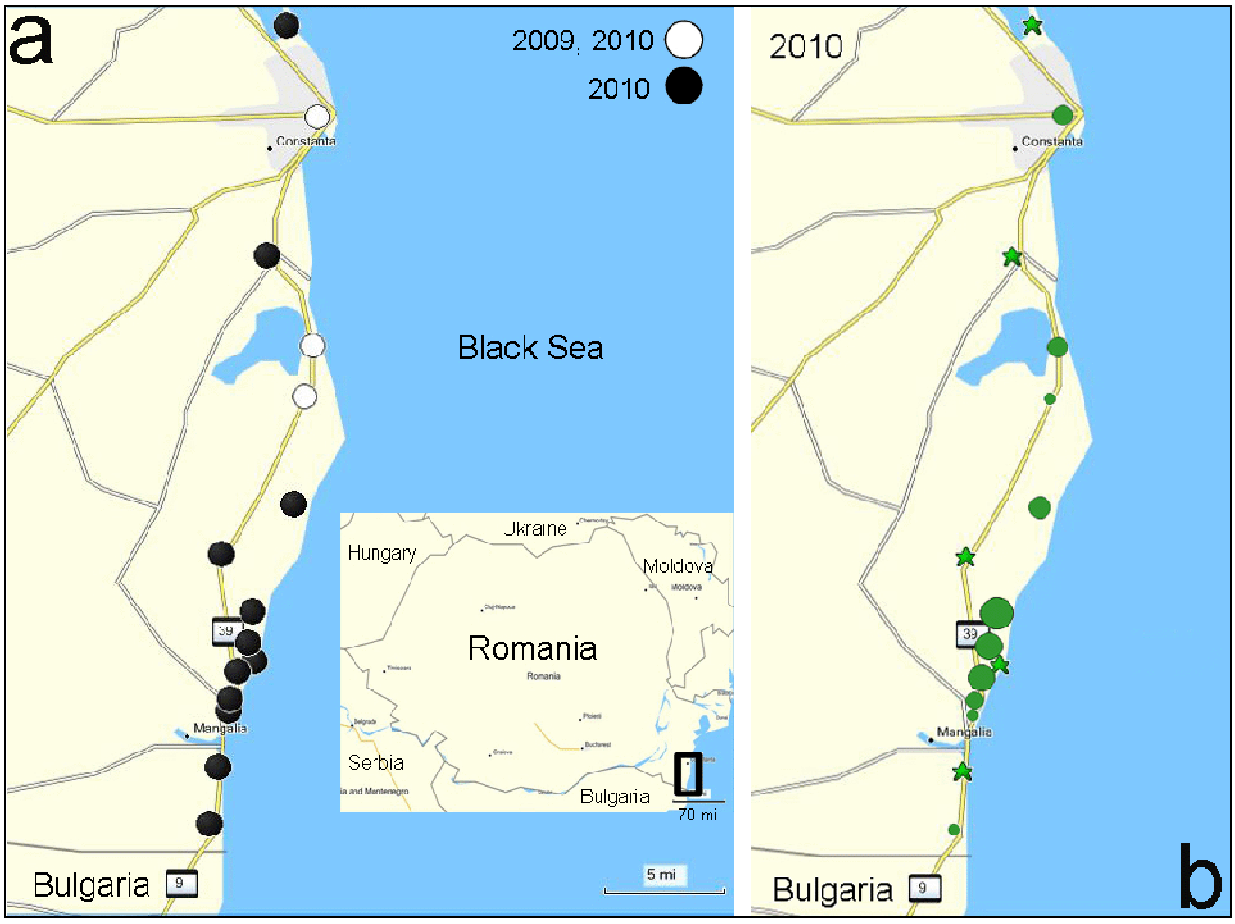

Fig. 1. The study area along the Romanian Black Sea coast (mini map) (a.) indicates time of investigation, dark circles represent new localities surveyed only in 2010 (b.) average levels of infestation estimated for the surveyed localities; values are ascending from 1 (smallest circle) to 4.2 (biggest circle), stars represent value 0.

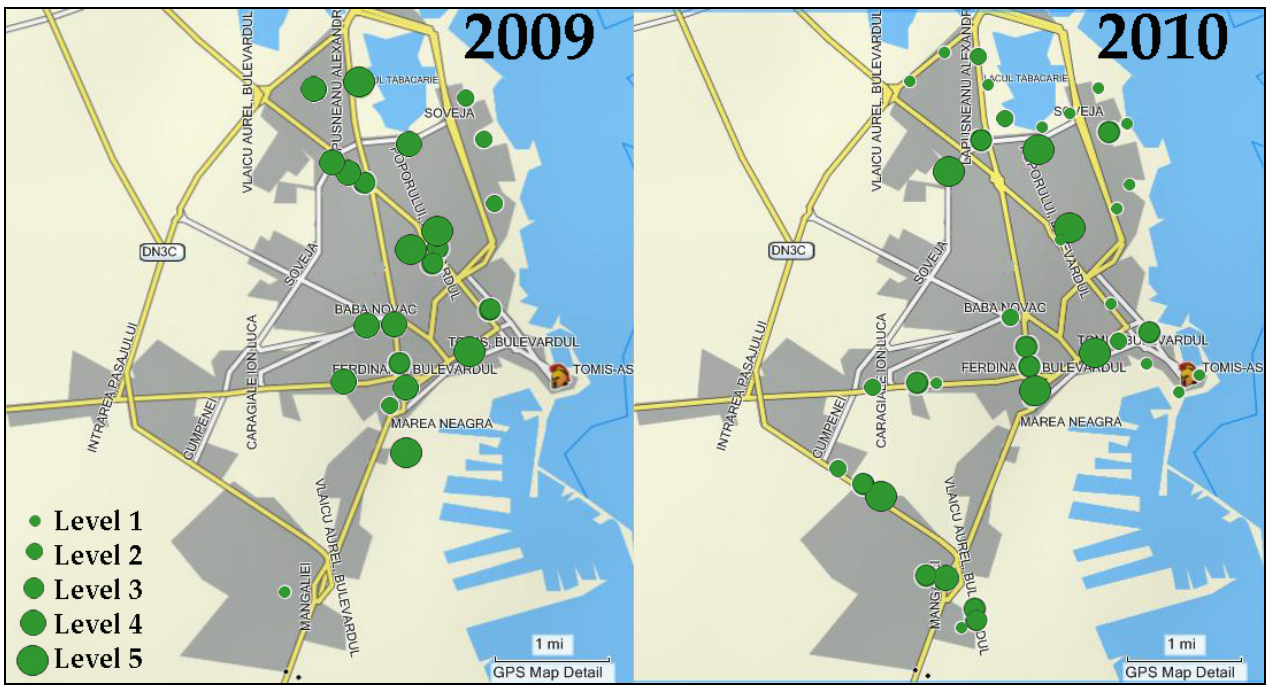

Fig. 2. Occurence of *Metcalfa pruinosa* in Constanța city in two succesive years (estimated levels of infestation are described in Methods section).

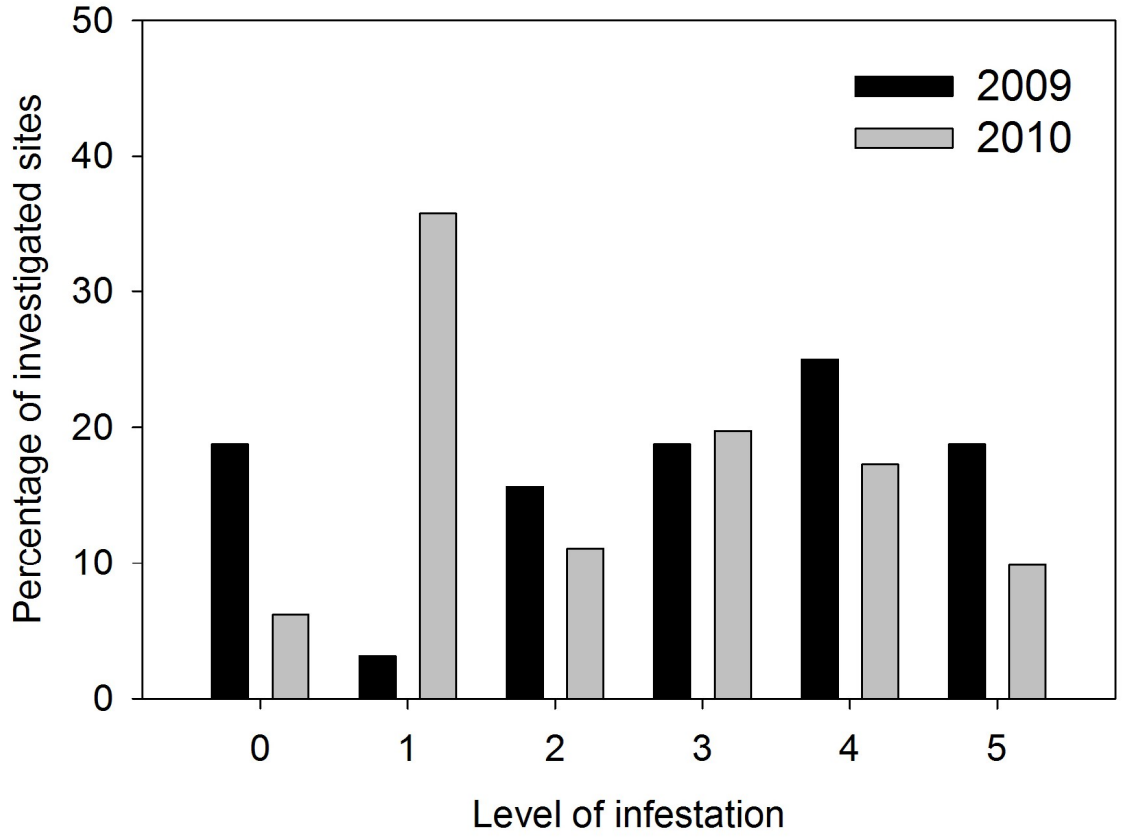

Fig. 3. Estimated levels of infestation by *Metcalfa pruinosa* in south-eastern part of Romania (n=32 for 2009; n=81 for 2010).

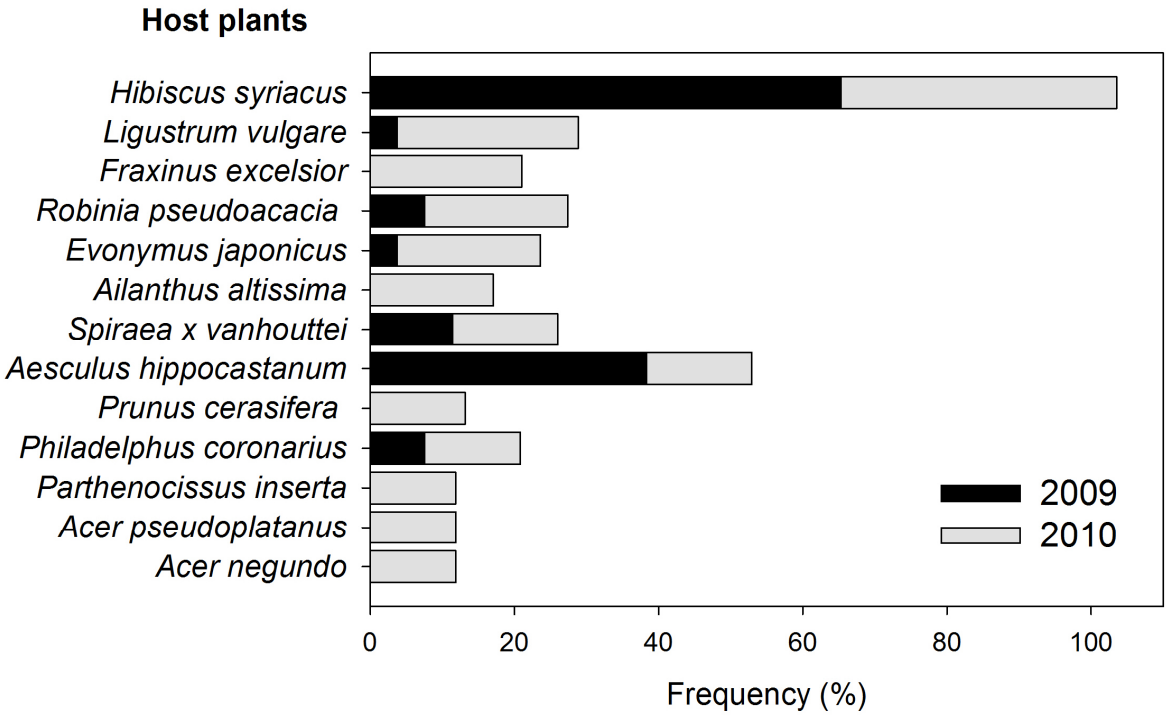

Fig. 4. Host plants on which *Metcalfa pruinosa* was most commonly encountered (n=26 sites in 2009; n=76 sites in 2010).

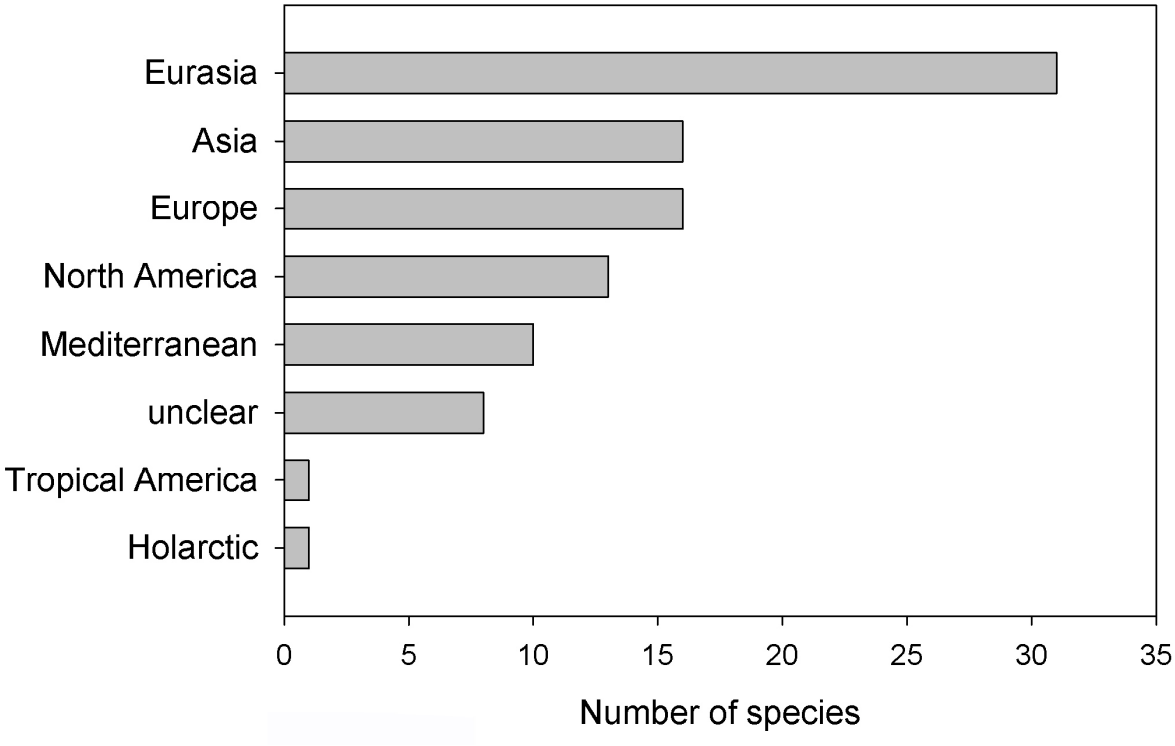

Fig. 5. Native range of the species of host plants identified in the south-eastern part of Romania (n=96).

Another contributing factor might be the climate of the region. According to LIEBHOLD & TOBIN (2008) low density populations such as newly founded invading populations are strongly influenced by abiotic factors, among others. *Metcalfa pruinosa* is less found in dry regions and occurs mainly in humid regions with annual average precipitation between 600 and 1625 mm (STRAUSS, 2010). By comparing climographs based on average monthly temperature and precipitation data in areas from native range and Europe, WILSON & LUCCHI (2000) concluded that *Metcalfa pruinosa* is capable of overcoming this barrier and occupy even microhabitats in areas considered unsuitable like Italy and southern France. Another example is the south-eastern part of Romania where the average annual precipitation registered in the period 1965-2000 ranged between 300 and 500 mm (TORICĂ, 2008).

Based on the large number of planthoppers observed, of different life stages, and on the extension of the area covered, we hypothesized that *Metcalfa pruinosa* arrived in the south eastern part of Romania at least two years prior to our observations. Several possibilities exist in terms of the planthopper's arrival in this part of Romania: directly introduced from Italy with imported plant material as is the case of Czech Republic (LAUTERER, 2002) or natural spread from Bulgaria. We also considered possible the colonization from western or central Romania (PREDA & SKOLKA, 2009). In the summer of 2010, the presence of *Metcalfa pruinosa* was reported from Timişoara, in the western part of the country (GOGAN *et al.*, 2010). This is not surprising since the planthopper was already present in Hungary for several years. However, we believe that we are dealing with multiple separate introductions and stratified dispersal of *Metcalfa pruinosa*. Since the abundance of *Metcalfa pruinosa* was scarce close to the Bulgarian border, we believe that *Metcalfa pruinosa* was introduced

in the south-eastern part of Romania with plant material. Point introductions might act as source populations and contribute to the rapid range expansion of the planthopper. Field observations in Constanța city reveal the same pattern. In 2009, several sites with high abundance of individuals were observed mainly in city parks and unsupervised terrains covered with vegetation located in the central area of the city and in Constanța harbor enclosure. The next year, the expansion of the covered area was easily noticed through the increased number of recently infested sites, almost 40%, towards the outskirts of the city (Fig.2). The impact of *Metcalfa pruinosa* can be both detrimental on natural plants and beneficial for bee keepers due to the secretion of honeydew. Although interactions between *M. pruinosa* and native species need further investigations, it is safe to assume that *Metcalfa pruinosa* competes with native phytophagous species for resources and causes structural changes in their community. So far, the only predator on *M. pruinosa* we observed was *Araneus diadematus* Clerck, 1757. The risks of economic losses due to *M. pruinosa* are high since vineyards and orchards (mostly peach and apricot) occupy a large surface of south-eastern Romania. In some cases seedlings or fruits become unfit for sale. In northern Italy *Metcalfa pruinosa* was responsible for the loss of about 40% of the soybean crop (CIAMPOLINI *et al.*, 1987). The presence of *Metcalfa pruinosa* in Romania requires extensive monitoring to determine the breadth of its spread and estimate its impact and potential control measures.

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Bryophytes in Protected Territories of Plovdiv City (Bulgaria): Preliminary Species List and First Data of Air Pollution Monitoring

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Abstract. The study comprises preliminary results of survey on bryophyte diversity in protected areas in the city of Plovdiv and biomonitoring urban air pollution with moss *Hypnum cupressiforme*. The finding of 2 species with conservational value (*Isothecium myosuroides* Brid., *Grimmia crinita* Brid.) suggests that surveys of bryophyte biodiversity across protected landscapes should be continued. The determined concentrations of 15 elements in *Hypnum cupressiforme* revealed no serious pollution by airborne heavy metals and toxic elements contaminants. The applied biomonitor and PCA distinctly separated anthropogenic influenced city sites and reference one.

Keywords: Bryophytes, Biodiversity, Monitoring of air pollution, *Hypnum cupressiforme*

Introduction

First account of Bulgarian bryophyte flora had been accomplished by PETROV (1975). Approximately 754 species are registered up to now (NATCHEVA & GANEVA, 2009). The present Red List of Bulgarian Bryophytes includes 251 species, of which 228 are Threatened (28 Critically Endangered, 42 Endangered, and 158 Vulnerable) (NATCHEVA *et al.*, 2006). Researches on species composition in urban conditions in Bulgaria are limited to bryoflora of Sofia (GANEVA, 2004), due to science interests focusing on anthropogenic undisturbed regions. Bryophyte diversity conservation worldwide and particularly in Bulgaria is of great importance.

Bryophyte species composition could enlighten micro-habitat conditions (GLIME, 2007). Morphological and anatomical characteristics (i.e. lack of epidermis and cuticle or their reduction, single layered leafs, absence of roots, etc.) result in considerable tolerance against contamination. Mapping of atmospheric pollution on the basis of mosses sensitivity appeared to allow precise determination of air quality (ANISHCHENKO, 2009).

Moss monitoring of atmospheric pollution was applied first by RÜHLING & TYLER (1968). Carpet-forming moss species have a number of advantages as biomonitors: wide geographical distribution; mineral supply obtained mainly by wet and dry precipitation; ability to accumulate elements in concentrations higher than the medium; fast uptake due to the lack of epidermis and cuticle, and the large surface-to-weight ratio,

alive tissues of 3-4 years old and evergreen; easy and cheap technique (GRODZIŃSKA & SZAREK-ŁUKASZEWSKA, 2001; RÜHLING & STEINNES, 1998; TYLER, 1990). Bulgaria was included in the project Atmospheric Heavy Metal Deposition in Europe using Mosses in 1995 (YURUKOVA, 2000). During the second moss sampling, the project was transferred in UN/ECE ICP Vegetation (United Nations Economic Commission for Europe International Co-operative Programme on Effects of Air Pollution on Natural Vegetation and Crops) - European Heavy Metals in Mosses (BUSE *et al*., 2003; YURUKOVA, 2006). The third one took place in 2005/2006 (YURUKOVA, 2007, 2010). Recently more than 28 countries were involved in the UNECE ICP Vegetation - Heavy Metal Accumulation in Mosses in Europe (HARMENS *et al*., 2007, 2008). According to the EMEP data (Co-operative Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutant in Europe) Bulgaria is one of the main sources of heavy metals in the Southeastern part of Europe. The contribution to the total 2005 emissions (including anthropogenic, natural and historical) of Bulgarian manufacturing industries and constructions for Pb was 77% and 91% for Cd (ILYIN *et al*., 2005; 2007).

The city of Plovdiv comprises area of 10198 ha, located at Upper Thracian Lowland and is the only city in Bulgaria with protected areas (PAs) at urbanized environment. There are 4 PAs with total area of 84556 ha – Nature monuments "Youth Hill" (DzhendemTepe) – 285.5 m above sea level, "Hill of the Liberators" (Bunardzhik) – 265 m a. s. l., "Danov Hill" (Sahat Tepe) – 227 m a. s. l. and Protected site "A roosting site of Pygmy Cormorant - Plovdiv". The main factor influencing the vegetation on the hills, is the anthropogenic. In the past the hills had been directly and indirectly subjected to human impact which resulted in the loss of primary vegetation due to the large-scale invasion of ruderal and weed species.

The city's passenger transport system consists mainly of private automobiles, taxis and buses. As of 2008, there were approximately 163400 private motor vehicles in the city, about 1500 taxis and 400 buses. Virtually buses are diesel powered and most taxis are gasoline powered. In recent years, automobile use has increased rapidly. One of the major negative effects of Plovdiv's current transportation system is high level of air pollution throughout much of the year. Plovdiv city has been classified as a region in which levels of total dust, coarse particles (also known as particulate matter PM_{10}) and Cd are above established norms according REGULATION 7 (1999). Much of Plovdiv's pollution problem stems from the city's climate and topography. A thermal inversion acts as a cap over the city during the fall and winter, inhibiting the dispersion and diffusion of pollutants. Pollutant dispersion is further obstructed by the city's location in a river valley.

After the analysis of 221 different European cities in 2008, the city of Plovdiv ranked first in the classification of the 30 worst cities in terms of air quality (ITALIAN NATIONAL INSTITUTE OF STATISTICS, 2010). With 208 days in 2008, it also ranked first for the number of days on which the PM_{10} limit value of 50 $\mu g/m^3$ was exceeded.

The aim of this study was to present preliminary results on the bryophyte species composition and the atmospheric pollution represented by bioaccumulation in the moss *Hypnum cupressiforme*.

Material and Methods

Bryophytes were recorded and sampled during 2010 from various substrates (rocks, stones, dead wood, and soil) in the area of three Nature monuments. The nomenclature accepted in HILL *et al.* (2006) is followed. Categories of threat are according NATCHEVA *et al.* (2006). The region of Kazandji Dere, Central Rhodopes was chosen as reference site for control sample of the biomonitor *Hypnum cupressiforme*.

Samples of *H. cupressiforme* were air-dried, cleaned carefully and age separated. About 1 g moss material was treated with 5 ml 65% nitric acid (Merck) overnight. The wet-ashed procedure was followed by Microwave Digestion System CEM MDS 81D, duration 5 minute at 600 W. After 3 ml 30% hydrogen peroxide addition, vessels

were cooled for 1 hour at room temperature. Vessels were treated by the Microwave Digestion System for 10 min again at 600 W for full digestion of the organic matter. The filtrate was diluted with double distilled water up to 50 ml.

The elements Pb, Cd, Co, Ni, As, Cr, Se, B, Sr, Be, V, Mo, Bi, U and Hg were determined by inductively coupled plasma mass spectrometry (ICP-MS) via Agilent 7700 ICP-MS, DF 1000. All samples, blanks and standards were spiked with internal standards - Ge 50 ppb and Rh 5 ppb final concentration in the solutions. Calibration standards Multy VI (MERCK) were freshly prepared from 1 to 1000 ppb in 0.05 volume% HNO_3 (p.a.) Monostandard of Hg 100 ppt was also used in the calibration. Signals of suitable isotopes for the tested elements have been measured twice in both modes - without and with helium gas collision cell.

Data analyses using package Canoco ver. 4.5 were conducted (TER BRAAK & SMILAUER, 2002). Linear method Principal Component Analysis (PCA) was carried out on data on analyzed 15 elements in moss tissues at 3 sampling sites. Scaling was focused on sample distances. The data were transformed ($x' = \log (x+1)$), automatically centered and standardized.

Results and Discussion

The species list of studied urban protected areas in Plovdiv includes 7 mosses, 2 of which with conservational value (Table 1). These preliminary results compared to the total of 19 species registered in parks, gardens and meadows in Sofia (GANEVA, 2004), suggest high bryophyte diversity in the city of Plovdiv could be expected. Despite numerous pressures in urban environment, bryophyte flora in cities appeared to be rich. For example in the metropolitan city of Cologne (W. Germany) were accounted 143 taxa (SABOVLJEVIĆ & SABOVLJEVIĆ, 2009).

Table 1. List of registered species in the protected territories of the city of Plovdiv. Categories (according to IUCN, 2010): CR - *Critically Endangered*; LR - *Lower risk*/Not *threatened*; VU – Vulnerable.

Species	Locality	**Category of threat**
***Bryum dichotomum* Hedw.**	**Danov Hill**	LR
***Bryum moravicum* Podp.**	**Dzhambaz Hill***	LR
***Dicranella schreberiana* (Hedw.) Schimp.**	**Dzhambaz Hill**	LR
***Hypnum cupressiforme* Hedw.**	**Youth Hill, BunardzhikHill**	LR
***Isothecium myosuroides* Brid.**	**Danov Hill**	CR
***Grimmia crinita* Brid.**	**Youth Hill**	VU
***Tortula modica* R.H.Zander**	Dzhambaz Hill	LR

* part of the "Three Hills" (also known as "Trimontsium") comprising Teksim Hill, Dzhambaz Hill and Nebet Hill)

Among the above species *Isothecium myosuroides* showed poor or restricted growth during the maximum phase of SO_2 pollution (ADAMS & PRESTON, 1992). Species growth assessed in the monitored site could be related to the summer season without use of fossil fuels for heating.

Measured content of 15 elements in selected biomonitor are presented at Table 2. The results for five elements: Pb, Cd, Cr, V and Ni showed increased level of pollution at the NM "Youth Hill". The prevailing winds in the city of Plovdiv move in west-to-east direction and thus industrial contamination could be excluded. Moreover after the assessment of emissions dispersion from main industrial sources, the contribution of these sources to the air pollution was found to be negligible in comparison with the air pollution caused by the other sources (ATANASSOV *et al.*, 2006). The immediate proximity of railways could be assumed as a factor exerting greatest influence on the polluted site.

Table 2. Analyzed concentrations of 15 elements in moss *Hypnum cupressiforme.*

Index	Reference site		NM "Bunardzhik Hill"		NM "Youth Hill"	
	mg kg^{-1}	RSD %	mg kg^{-1}	RSD %	mg kg^{-1}	RSD %
Pb	4.03	0.5	9.12	0.7	21.6	0.6
Cd	0.35	0.9	1.26	1.1	2.5	1.4
Co	2.2	0.6	0.8	0.6	0.93	1.1
Ni	1.7	0.9	2.8	1.2	4.4	1.5
As	<0.5		0.57	12	0.52	12
Cr	1.9	2.6	1.7	2	3	1.5
Se	<0.6		<0.6		<0.6	
B	<10		<10		<10	
Sr	11	1.5	57	0.9	54	0.6
Be	<0.1		0.17	14	<0.1	
V	1.6	0.4	4	0.5	7.7	0.5
Mo	<0.4		<0.4		<0.4	
Bi	0.8		0.6		0.7	
U	0.05	3.8	0.19	0.7	0.64	1.2
Hg	0.08	13.5	0.17	10.5	0.13	11.6

Relationship between analyzed elements in *Hypnum cupressiforme* at selected sites was assessed by PCA (Fig. 1). The first axis with eigenvalue 0.722 explained 72.2% of data variation. First and second axes together explained 100% of the variation. The ordination plot represents a gradient between sites located at the city of Plovdiv and normally subject to anthropogenic pressure (left part of the plot) to locality at the reference region of the Central Rhodopes Mountain characterized by higher values only of Bi and Co. NM Bunardzhik Hill with elevated values of Be, Hg, As and Sr lied in the top-left quadrant of the diagram above more impacted NM Youth Hill represented by higher loads of 6 elements (Cr, U, Pb, Ni, V, Cd).

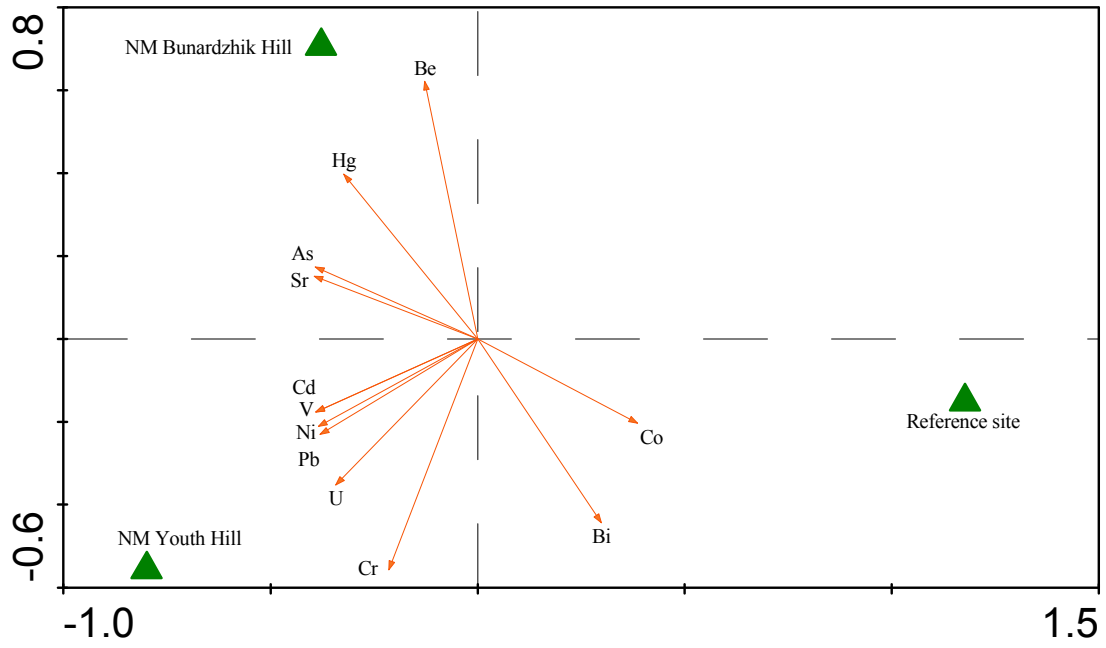

Fig. 1. PCA ordination diagram with data for reference and 2 city sites.

Maximum concentration of cadmium measured at NM "Youth Hill" was 2 times lower and close to the Bulgarian and European maximum of 5.23 mg kg^{-1} (YURUKOVA & GECHEVA, 2009). Arsenic had similar levels at the 2 protected territories which were about 20 times lower compared to the maximum concentration of 12.6 mg kg^{-1} for Bulgaria and approximately 40 times lower than European maximal value of 21.6

mg kg^{-1}. Lead showed average load at the selected sites, holding position between minimal and maximal values for the country. Levels of nickel and chromium in both city sites were close to the minimum concentrations assessed at Bulgaria and Europe. Mercury was approximately 2.5 times lower as compared to the maximum values analyzed in FYR of Macedonia.

Conclusion

According to our preliminary results, the list of moss species in protected territories of Plovdiv city could be characterized by high diversity in future detail studies.

Comparatively low content of assessed 15 elements in biomonitor *Hypnum cupressiforme* confirmed tendency towards diminishing air pollution in the city. Results of the PCA clearly opposed selected two city sites under anthropogenic pressure to reference region.

The bryomonitoring of total air pollutants certainly plays a role in moss protection and conservation.

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Nesting Activity of Loggerhead Turtles (Caretta caretta) at Göksu Delta, Turkey during 2004 and 2008 nesting seasons

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Abstract. Göksu Delta is one of the most important nesting beaches in Turkey for the endangered loggerhead turtle (*Caretta caretta*). This paper provides information on the nesting activities of loggerhead turtles, the spatial and temporal distribution of nesting, nesting success, nesting density, hatching success, incubation duration and clutch size over two nesting seasons. A total of 902 emergences occurred over two seasons, of which 239 (26.5%) nests were deposited (137 nests in 2004 and 102 nests in 2008) and the overall mean nesting density was 3.4 nests/km. The peak of nesting emergences takes place mainly in June. Of the overall nests, 226 (94.6%) were excavated and 16044 eggs were counted. Of these eggs, 3680 (22.9%) hatchlings emerged and 2695 (73.2% of hatchlings) of them were able to reach the sea. The mean number of eggs per clutch was 71 (range: 15 – 143). The shortest and longest incubation duration in these 2 seasons ranged from 46 to 62 days with a mean of 53 days. The main problems are negatively affecting loggerhead turtle population at Göksu Delta are dense jackal predation both adult and eggs and inundation in nests. The average nesting effort here (mean: 119.5 nests/season) confirms that Göksu Delta is one of the most important nesting sites for loggerhead turtles in Turkey.

Key words: *Caretta caretta,* sea turtle, reproduction, nesting, conservation, Göksu Delta, Turkey.

Introduction

Two endangered sea turtle species, *Caretta caretta*, loggerhead turtles and *Chelonia mydas*, green turtles (IUCN, 2007), nest regularly in the Mediterranean. While the nesting zones of *C. caretta* is widely distributed in the eastern basin of Mediterranean (BRODERICK *et al.*, 2002; MARGARITOULIS *et al.*, 2003), sparse nesting activities were reported from the western basin (LLORENTE *et al.*, 1992; TOMAS *et al.*, 2002; DELAUGUERRE & CESARINI, 2004). In eastern Mediterranean the major nesting grounds for loggerhead turtles, are Greece, Turkey and Cyprus with smaller numbers recorded in Egypt, Libya, Tunisia, Israel, Syria and Lebanon (BRODERICK *et al.*, 2002; NEWBURRY *et al.*, 2002; MARGARITOULIS *et al.*, 2003).

Three species of marine turtle – *C. caretta, C. mydas* and *Dermochelys coriacea* – have been reported from Turkish waters (BARAN & KASPAREK, 1989; BARAN *et al.*, 1998). Only the first two are known to nest on the Turkish coast of the Mediterranean,

whereby green turtle nesting is mostly limited to a few eastern beaches. The first nesting records of *C. caretta* and *C. mydas* from Turkey were by HATAWAY (1972). BAŞOĞLU (1973) and BAŞOĞLU & BARAN (1982) provided information on the carapace plates of *C. caretta* measured in İzmir, Köyceğiz and Fethiye. GELDIAY *et al.* (1982) described marine turtle populations and their protection along the Mediterranean coast of Turkey. BARAN & KASPAREK (1989) conducted the first comprehensive survey of the Turkish Mediterranean coast for turtle nesting sites. Their primary objective was to locate nesting sites and to assess their relative importance. More recently, a series of population studies have been carried out on selected beaches, and problems affecting the turtles there were determined (BARAN *et al.*, 1992; BARAN & TÜRKOZAN, 1996; CANBOLAT, 2004; ERK'AKAN, 1993; ILGAZ & BARAN, 2001; KASKA *et al.*, 2010; SAK & BARAN, 2001; TAŞKIN & BARAN, 2001; TÜRKOZAN & BARAN, 1996; TÜRKOZAN, 2000; TÜRKOZAN & YILMAZ, 2008; YALÇIN-ÖZDILEK, 2007; YERLI, 1990).

A total of 13 beaches were determined to represent the main nesting areas for marine turtles in Turkey (BARAN & KASPAREK, 1989; BARAN *et al.*, 1992; GROOMBRIDGE, 1990). A number of nesting beaches with fewer turtles were also identified (BARAN & KASPAREK, 1989), and four of these were also indicated by Groombridge (1990). Previous studies of sea turtles at Göksu Delta confirmed that this beach is one of the few sites where both loggerhead and green turtles nest in the Mediterranean (PETERS & VERHOWVEN, 1992; PIGGELEN & STRIJBOSCH, 1993; YERLI & DEMIRAYAK, 1996; GLEN *et al.*, 1997; YERLI & CANBOLAT, 1998; AKÇINAR, 2006; CANBOLAT, 2006).

The fundamental goal of the present study was to provide information on the nesting activities of loggerhead turtles, the temporal and spatial distribution of nesting, hatching success, hatchling emergence period, incubation duration and clutch size over two nesting seasons at Göksu Delta, Turkey. Moreover various threats affecting sea turtle populations were discussed.

Material and Methods

The current investigation was carried out over varying periods of the breeding seasons (early June to mid September) – 2004: 01 June to 20 September; 2008: 15 June to 19 September. The nesting records of the May were provided from a local volunteer. The following methodology was utilized: Teams of 2-3 persons conducted night patrols (21:00 – 2:00) and morning patrols (6:00 – 8:00). When the opportunity arose, we counted eggs as they were laid without disturbing the turtle at night patrols. During morning patrols, the shape and pattern of tracks were noted and those tracks that resulted in nests were marked. The nest locations were confirmed with probes and then marked. Tracks with no nests were counted as non-nesting emergences. The number of successful nesting attempts can be called nesting success. The nesting success (NS) percentage was calculated with the following formula: NS (%) = [(N)/(N + T)] X 100 where N is the number of nesting emergence, and T is the number of non-nesting emergence. In cases of partial animal predation, the egg chambers and surrounding area were cleared of damaged eggs and covered with moist sand to the original level. Intact eggs were not moved. Damaged eggs and eggshells were counted and reburied elsewhere. In beach sections with known high pressure from land predators (e.g. jackal, *Canis aureus*), a protective metal grating (72x72 cm, mesh size 9 cm) was placed over the eggs at the center of the egg chamber.

During the hatchling emergence season from July to late September, the numbers of hatchling tracks coming from each nest were counted daily, and the numbers of hatchlings reaching the sea were determined by following the tracks. These tracks were then raked to avoid confusing old and new tracks on consecutive days. Those tracks that were interrupted by predator tracks such as jackals or ghost crabs (*Ocypode cursor*) were categorized as "predated". Between 8 and 10 days after the first emergence of hatchlings, nests were excavated. The numbers of remaining hatchlings, empty eggshells,

yolkless eggs and developmentally interrupted eggs (embryo) were counted. These categories were then added up to calculate the total number of eggs in the clutch. The latter two categories were distinguished by their color: shortly after oviposition, those eggs containing embryos turn white as the mucus dries and the embryo takes oxygen. The other eggs do not whiten and tend to remain creamy-white or to become slightly yellowish: when opened they show no sign of development, indicating they were infertile (MILLER, 1985). Hatching success was the percentage of eggs that produced hatchlings. This was ascertained by counting hatched eggshells (fragmented eggshells were pieced together to represent one egg). Incubation duration was defined as the number of days from the date of egg deposition to the date of first hatching.

For practical fieldwork reasons, the beaches at Göksu Delta were subdivided into 7 subsections: 760-766 subsections. These subsections also partly reflect different beach morphologies and when relevant the results are differentiated according to beach subsection. Göksu Delta is one of the important nesting sites for sea turtles on the Mediterranean shoreline of Turkey (Fig. 1). Göksu Delta (36º17'N-33º39'E), is located at 80 km west of Vilayet Mersin and almost 35 km in length. The beach was divided into 8 subsection beaches by BARAN & KASPAREK (1989). Göksu Delta in Turkey is among the most important nesting grounds for *C. caretta* and the beach is designated as Special Environmental Protection Area (SPA). The importance of this area is enhanced because it is also used as 'Reproduction and Conservation Zone for Water Birds' as well as included in RAMSAR and 1st degree Natural Site. The 767 subsection beach was not included into calculate such as nest density etc because it is not used as nesting zone by sea turtles.

Subsection 760 (10.7 km): starts from the canal in Kum district at the east of Seka paper factory and lies till İncekum cape. This part is an important marine turtle nesting zone (BARAN & KASPAREK, 1989). The first 200 m of the beach consists of pebbles and the remainder fine sand. Secondary constructions are located just behind the beach and this part is being used touristic purposes. Sand junes are started after the entrance gate of private security.

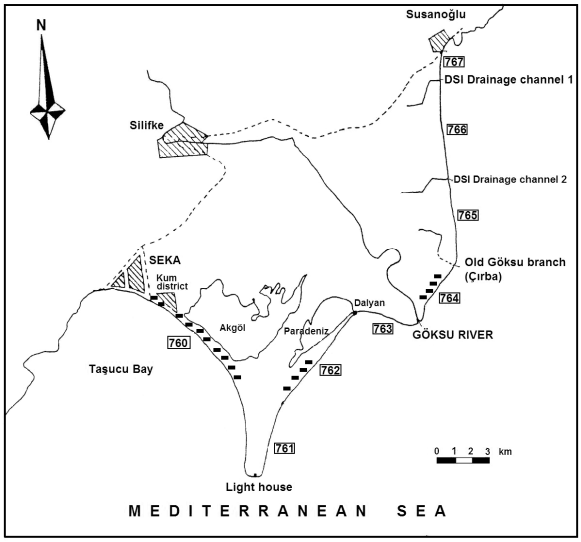

Fig. 1. A sketch map of Göksu Delta showing subsection beaches and spatial distribution of dense nesting sites.

Thorny bushes and herbaceous plants were dominant structure on the dunes. The sea floods during the strong winds constituted small ponds on the beach. In general, this subsection consists of fine sand with scarce vegetation. Subsection 761 (3.5 km): starts from the İnce cape and lies till lighthouse. The altitude of this subsection is almost 0.5 m from the sea level with almost no vegetation. This subsection carries high risk of flood especially during storms. Subsection 762 (5.7 km): starts from light house and lies till Dalyan. The middle point of the beach almost located between the sea and Paradeniz and towards Dalyan a narrowing sand bank is located The sand dunes which lie parallel to the coast either posses almost 1 m elevation or at the sea level. The flood caused the compact sand type and small ponds on the beach towards Dalyan. The sandy plant vegetation starts after light house 15 m away from the coast. Subsection 763 (2.5 km): starts from Dalyan and lies till the mouth of Göksu River. The width of the beach sometimes reaches up to 100 m in some parts. The back of the beach is

covered with sandy vegetation. Subsection 764 (3.5 km): starts from the place where Göksu River reach the sea and ends at the old arm (Cırba district) Göksu River. The width of the beach reaches up to 20 m. Subsection 765 (4.2 km): Situated between Çırba and DSİ canal II. Sand dunes are located at the back of the beach. The hinterland of the beach was covered by wetlands and rice fields. Subsection 766 (4.6 km): starts from DSİ canal II and lies till DSİ canal I of Atayurt daily picnic area. The beach is completely designated as Specially Protected Area. In 760, 764-766 subsection beaches, secondary houses, holiday housing estates, daily picnic sites, pensions, guest house, camping place, desk chairs and umbrellas have been situated. There was no water sport except water bicycle in the area.

Results and Discussion

In total, 902 loggerhead turtle emergences occurred over the two nesting seasons. Of these, 239 (26.5%) resulted in a nest, with the number of nests ranging from 102 to 137 for per year (average number of nests 119.5). MARGARITOULIS *et al.* (2003) stated that average annual number of loggerheads nests throughout the Mediterranean reaches 5031 nests/season, and of these, 1366 nests/season (27.2%) occur in Turkish Mediterranean coasts. According to this value, Göksu Delta represents 2.4% of the total loggerhead nesting in the Mediterranean and 8.7% of the nesting in Turkey. Göksu Delta is thus an important nesting ground for loggerheads and substantially to the Mediterranean loggerhead population. CANBOLAT (2004) reviewed the both loggerhead and green turtle nesting in Turkey, resulted that 500-800 loggerhead turtles nest annually along the beaches of Turkey. He classified the Göksu Delta as a secondary important nesting ground holding 5% of the total nests laid annually on Turkish beaches. The comparative data on nesting and non-nesting activities of loggerhead turtles reported in earlier studies on Göksu Delta are given in Table 1. The second highest number of nesting emergence (137 nests) were recorded in 2004 nesting season over the ten nesting seasons.

Table 1. Previous studies result of nesting (N), non-nesting emergence numbers (NN) and nesting density (ND) at Göksu Delta, Turkey.

Period	N	NN	ND (nests/km)	References
1991 (June - August)	117	384	3.4	PIGGELEN & STRIJBOSCH (1993)
1992 (June – September)	89	271	2.6	PETERS & VERHOWVEN (1992)
1994 (June – September)	36	76	1.0	YERLI & DEMIRAYAK (1996)
1996 (June – August)	36	----	1.0	GLEN *et. al.*, (1997)
1998 (May – September)	94	214	2.7	YERLI & CANBOLAT (1998)
2004 (June – September)	137	393	3.9	THIS STUDY
2005 (June – September)	151	461	4.0	AKÇINAR (2006)
2006 (June – September)	107	149	3.1	CANBOLAT (2006)
2007 (July – September)	122	17	3.5	GÖKDOĞADER (2007)
2008 (June – September)	102	270	2.9	THIS STUDY

Using the assumption that each female nests on average three times in a season every 2-3 years (GROOMBRIDGE, 1990), approximately 40 (1/3 of the average number of nests) loggerheads nest on Göksu Delta. GROOMBRIDGE (1990) estimated 2000 *C. caretta* females nesting annually in the Mediterranean. BRODERICK *et al.* (2002) estimated 2280–2787 loggerhead turtles nesting annually in the Mediterranean using four different methods. Accordingly, almost 1.4-1.8 % of the *C. caretta* population of the Mediterranean nest in this region.

The overall nesting success (number of nests/total number of emergences) for two nesting seasons was 26.5%. The previous

reports at Göksu Delta in different nesting seasons were 23.4% (PIGGELEN & STRIJBOSCH, 1993), 24.7% (PETERS & VERHOWVEN, 1992), 30.5% (YERLI & CANBOLAT, 1998), 24.7% (AKÇINAR, 2006) and 41.8% (CANBOLAT, 2006). Moreover, nesting success at various nesting beaches in the Mediterranean has been reported as 33.7% at Dalyan (TÜRKOZAN & YILMAZ, 2008), 15.6% at Fethiye (TÜRKOZAN, 2000), 48.5% Kızılot (KASKA, 1993), 25.4% at Patara (TAŞKIN & BARAN, 2001), 24.6% at Dalaman (KASKA *et al.*, 2010), 19.4% at northern Karpaz, northern Cyprus (ILGAZ & BARAN, 2001), 25.7% at Laganas Bay, Greece (MARGARITOULIS, 2005) and 40.2% at El-Mansouri, Lebanon (NEWBURRY *et al.*, 2002). The value of nesting success determined over two nesting seasons is similar to other nesting sites in the Mediterranean except data given in CANBOLAT (2006) (for Göksu Delta) and NEWBURRY *et al.* (2002) (for El-Mansouri, Lebanon).

The peak of loggerhead turtle nesting occurred in June (Fig. 2). Of the overall number of nests recorded, 2.9% occurred in May, 59.8% in June, 36.4% in July and 0.8% in August. Temporal distribution of nesting shows that 62.7% of nests at Göksu Delta are completed in May and June as in other nesting beaches of Turkey and northern Cyprus (ILGAZ & BARAN, 2001; SAK & BARAN, 2001; TAŞKIN & BARAN, 2001; TÜRKOZAN & YILMAZ, 2008). However, the main nesting season covers in July and August in nesting beaches of Greece (64.5% of the total nests were completed in July and August at Laganas Bay and Kyparissia Bay, Greece in MARGARITOULIS 2005 and MARGARITOLIS & REES, 2001). We think that this difference could be caused from geographical variation of nesting beaches as

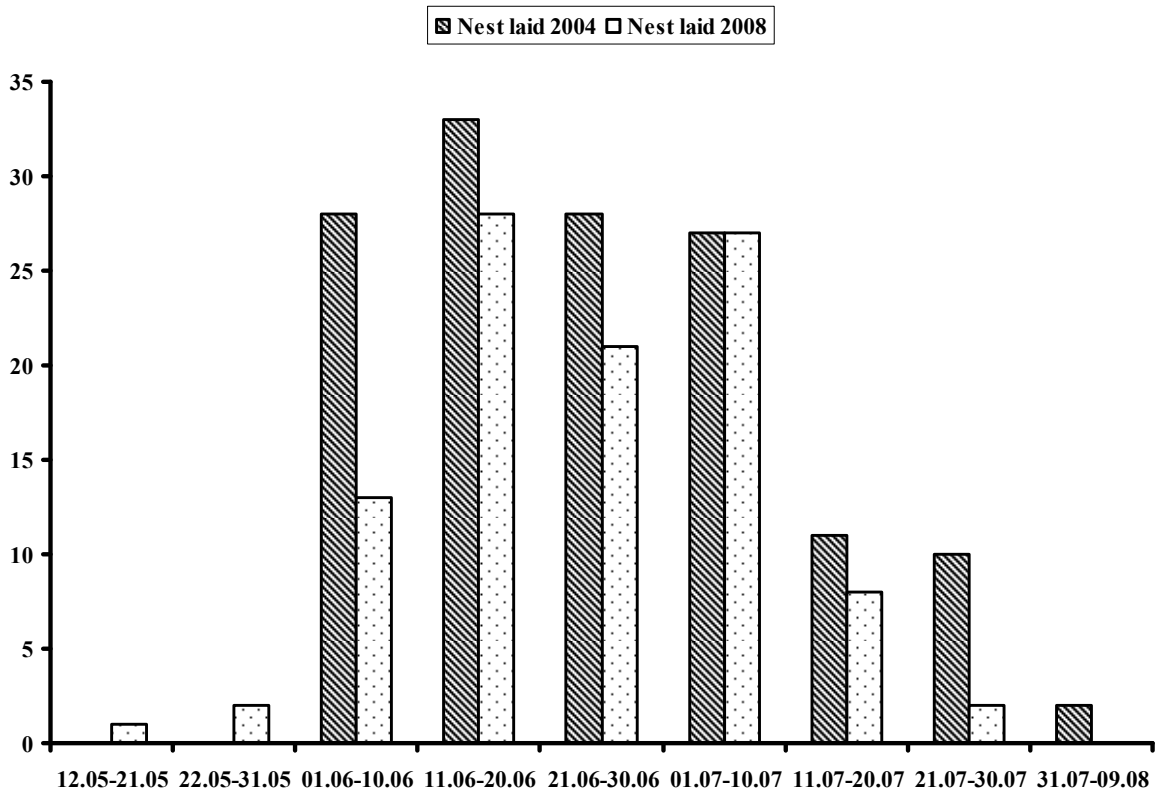

Fig. 2. Temporal distribution of nests during 2004 and 2008 nesting seasons at Göksu Delta.

in stated in MARGARITOLIS & REES (2001).

The overall nesting density was calculated as 3.4 nests/km at Göksu Delta (3.9 nests/km and 2.9 nests/km during the 2004 and 2005 nesting seasons, respectively). The comparative data on nesting density reported in earlier studies on Göksu Delta are given in Table 1. The presently reported value of 3.2 nests/km partly shows similarity with those previously reported

values for Göksu Delta except 1994 and 1996 nesting seasons. Nest densities in the Mediterranean vary widely. ILGAZ & BARAN (2001) reported a value for Dalyan beach of 28.7 nests/km and TAŞKIN & BARAN (2001) gave 7.4 nests/km for Patara beach. SAK & BARAN (2001) recorded 9.1 nests/km on Belek beach. TÜRKOZAN & YILMAZ (2008) found nest density for 47 nests/km over two nesting season at Dalyan beach. The nesting density in Greece was found to be 235.6 nests/km at Laganas Bay, 36.6 nests/km in Rethymnon (Crete), 8.9 nests/km at Chania Bay (MARGARITOULIS, 2000) and 46 nest/km at Kyparissia Bay (MARGARITOULIS & REES, 2001). In Israel, 1.0 nests/km was found by SILBERSTEIN & DMI'EL (1991) while it was 26.4 nests/km at El-Mansouri, Lebanon (NEWBURRY *et al.*, 2002). The nest density of *C. caretta* during two years monitoring period at Göksu Delta is relatively lower than that reported in the literature except Israel in the Mediterranean.

Nesting emergences varied greatly among beach subsections (Table 2). Of all nests, 69.0% concentrated on 760 subsection, 9.6% on 762 subsection, 8.4% on 764 subsection, 5.9% on 765 subsection, 3.8% on 763 subsection, 2.1% on 766 subsection and 1.3% on 761 subsection. The mean nesting densities were 7.7 nests/km for 760 subsection, 2.9 nests/km for 764 subsection, 1.8 nests/km for 763 subsection, 1.7 nests/km for 765 subsection, 0.6 nests/km for 766 subsection and 0.5 nests/km for 761 subsection. The spatial distribution of nests on the beach is given in Fig. 2. As understood from the values, 760 subsection is the most densely used by loggerhead at Göksu Delta as previously mentioned in the literature (PETERS & VERHOEVEN, 1992; PIGGELEN & STRIJBOSCH, 1993; YERLI & DEMIRAYAK, 1996). Most of the nesting and non-nesting emergences of loggerhead were occurred 4th, 5th and 6th km of 760 subsection. Thus, turtle nesting at Göksu Delta was concentrated in only 3 km of the total 34.7 km beach. This site of beach was named as core area by PETERS & VERHOEVEN (1992) for Göksu Delta.

Table 2. The number of nesting emergences, nest ratio (%) and nest density (nests/km) among beach subsection over two nesting seasons. (NN: Number of nesting emergences, NR: Nesting ratio, ND: Nest density)

		760	761	762	763	764	765	766	Total
2004	NN	100	3	14	1	9	8	2	137
	NR (%)	73.0	2.2	10.3	0.7	6.6	5.8	1.5	
	ND (nests/km)	9.3	0.9	2.5	0.4	2.6	1.9	0.4	
2008	NN	65	----	9	8	11	6	3	102
	NR (%)	63.7	----	8.8	7.8	10.8	5.9	2.9	
	ND (nests/km)	6.1	----	1.6	3.2	3.1	1.4	0.7	
Overall	NN	165	3	23	9	20	14	5	239
	NR (%)	69.0	1.3	9.6	3.8	8.4	5.9	2.1	
	ND (nests/km)	7.7	0.5	2.1	1.8	2.9	1.7	0.6	

Of the recorded nests per season, 129 (94.2% of recorded nests) and 97 (95.1%) were excavated and their contents examined in 2004 and 2008, respectively. A total of 16044 eggs were counted from 226 nests, with a mean clutch size of 71.0 eggs over two nesting seasons. The comparison of the total number of eggs, hatchlings and hatchlings reaching to sea with respect to different nesting seasons is given at Göksu Delta in Fig. 3. According to these values the higher hatching success was recorded in 2008 nesting seasons while the lower values was recoded in 2006 nesting seasons. In comparison with other Mediterranean nesting beaches, the hatching success of loggerhead turtles at Göksu Delta was lower than that of loggerhead turtles on Kızılot, Belek (1990–1996), Patara (1990–1996) and Dalyan (1988–1996) beaches in Turkey,

Laganas Bay in Greece and northern Cyprus (BRODERICK & GODLEY, 1996; TÜRKOZAN, 2000; TÜRKOZAN *et al.*, 2003; MARGARITOULIS, 2005; TÜRKOZAN & YILMAZ, 2008). It is clear that hatching success is changed not only nesting beach but also nesting seasons (MARGARITOULIS, 2005). The probable reason for the lower hatching success observed at Göksu Delta was from the high pressure of jackal predation and flooding of the nests as mentioned in previous studies (PETERS & VERHOEVEN, 1992; AKÇINAR, 2006). The hatching success and survival at Göksu Delta is presented in Table 3. According to this values, a total of 3680 hatchlings were emerged (hatching success: 22.9% of total number of eggs), of which 2695 hatchling were able to reach the sea over two nesting seasons. Expressed as a percentage of total egg number, this is equivalent to 16.8%

Table 3. Hatching success and survival at Göksu Delta, Turkey.

	2004		2008		OVERALL	
	N= 129	%	N=97	%	N = 226	%
Total number of eggs	9070		6974		16044	
Depredated eggs	3150	34.7	2532	36.2	5682	35.4
Unhatched eggs	250	2.8	172	2.5	422	2.6
Abnormal eggs	18	0.2	12	0.2	30	0.2
Dead embryos	3862	42.6	2368	34.0	6230	38.8
Hatchlings	1790	19.7	1890	27.1	3680	22.9
Remained in nest	334	18.7	115	6.1	449	12.2
Depredated or died on beach	264	14.7	272	14.4	536	14.6
Reach the sea	1192	66.6	1503	79.5	2695	73.2

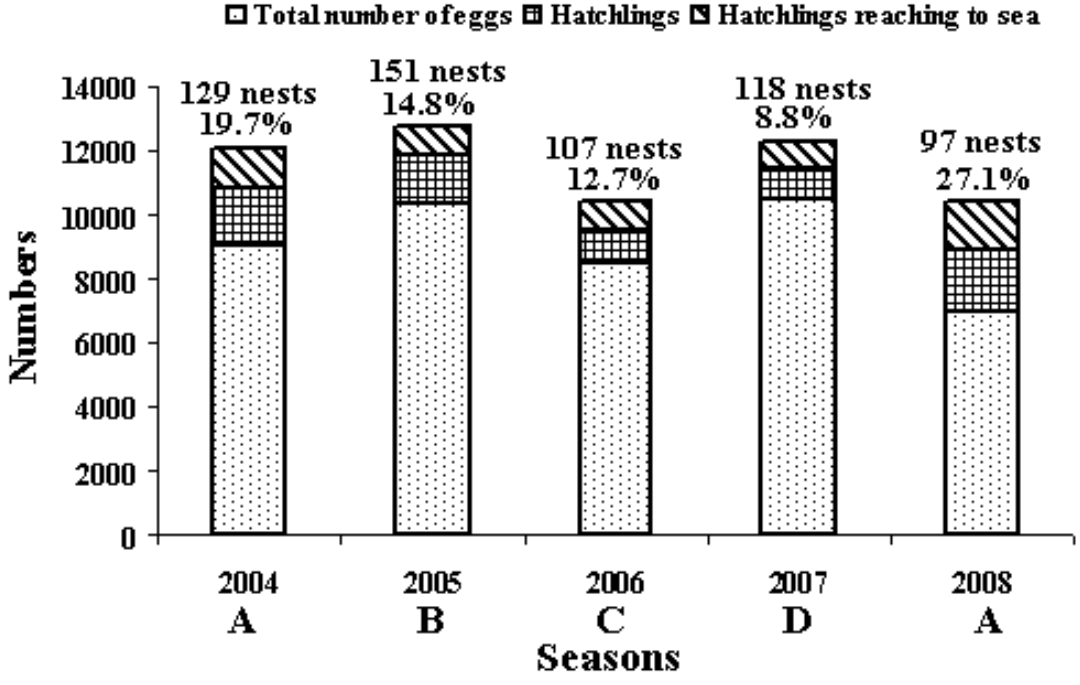

Fig. 3. The comparison of the total number of eggs, hatchlings and hatchlings reaching the sea at Göksu Delta. Percentages above the histograms indicate the mean hatching success according to years [A: This study, B: AKÇINAR (2006), C: CANBOLAT (2006), D: GÖKDOĞADER (2007)].

The mean clutch size at Göksu Delta was 71 eggs (range: 15-143). Mean clutch sizes vary greatly from year to year and from beach to beach. Thus, the 1992 study at Göksu Delta (PETERS & VERHOEVEN, 1992) reported a mean clutch size of 92 eggs. Canbolat (2006) recorded a value of 75 here in 2006. Values from elsewhere in the Mediterranean are 82 (SILBERSTEIN & DMI'EL, 1991) in Israel, 70 in northern Cyprus (BRODERICK & GODLEY, 1996), 117.7 in Greece (MARGARITOULIS, 1988), 64.7-64.3 in Egypt (CAMPELL *et al.,* 2001), 72.7 in Lebanon (NEWBURRY *et al.,* 2002) 76 in Dalyan (TÜRKOZAN & YILMAZ, 2008), 80.1 in Patara (TAŞKIN & BARAN, 2001) and 79 eggs (KASKA *et al.,* 2010). Outside of the Mediterranean, mean clutch sizes vary from between 101 to 126 eggs for the loggerhead turtles (HIRTH, 1980). Clutch size at Göksu Delta was lower than those documented in Greece (MARGARITOULIS,1988), Israel (SILBERSTEIN & DMI'EL, 1991) and Patara (TAŞKIN & BARAN, 2001) but is partly similar to Lebanon (Newburry *et al.* 2002) and Dalyan (TÜRKOZAN & YILMAZ, 2008). According to TIWARI & BJORNDAL (2000) there is a correlation between clutch size and latitude. MARGARITOULIS (2005) noted that variation in clutch size among nesting colonies of loggerhead in Greece, Cyprus and Turkey is probably results of body size difference.

In this study, the mean incubation periods were 52 (n= 23, range=46-61±3.26) and 54 (n= 17, range=48-62±3.79) days during the years 2004 and 2008 respectively. The shortest and longest incubation period in these 2 seasons ranged from 46 to 62 days with a mean of 53 days. In the previous studies, the mean incubation periods were reported as 57 (PIGGELEN & STRIJBOSCH, 1993), 55 (PETERS & VERHOEVEN, 1992), 52 (AKÇINAR, 2006) and 52 (CANBOLAT, 2006) days at Göksu Delta. GODLEY *et. al.,* (2001) derived field pivotal incubation duration for loggerheads turtles as 59.2 days. Upon this value, sex ratios on Göksu Delta are highly biased toward females in all years. MARGARITOULIS (1988) quotes a mean of 55.5 days in Greece, whereas CAMPELL *et al.* (2001) cite 48.1-53.5 days along the Mediterranean coast of Egypt. SILBERSTEIN & DMI'EL (1991) quote 54 days in Israel, whereas BRODERICK & GODLEY (1996) cite 48 days in northern Cyprus. MARGARITOULIS (2005) recorded incubation duration as 55.2 days at Laganas Bay, Greece. The mean incubation duration was ERK'AKAN (1993) cited 59.3 days on Dalyan beach, Turkey, whereas TÜRKOZAN & YILMAZ (2008) reported this value for 52.3 days for 2004 and 2005 nesting seasons. TÜRKOZAN (2000) give a mean of 56 and 49.8 days at Fethiye and Kızılot beaches, respectively. ILGAZ & BARAN (2001) give a mean of 51.8 and 52.4 days at northern Karpaz. According to KASKA *et al.* (2010), the mean incubate period is 49 days on Dalaman beach over the seven nesting seasons. Incubation period ranges between 48-58 days at Akrotiri Peninsula, Cyprus (MACLEAN *et al.,* 1998). The general range of incubation periods for sea turtle nests worldwide is quoted in the literature as 50-70 days (HIRTH, 1980). It is clear that there are substantial differences among the nesting beaches of loggerhead turtles in terms of incubation duration.

Early studies show that canids such as fox (*Vulpes vulpes*), jackal (*Canis aureus*) and feral dog are the main predators both hatchlings and eggs for loggerhead turtles in different nesting sites in the Mediterranean (MARGARITOULIS, 1988; MACDONALD *et al.,* 1994; BRODERICK & GODLEY, 1996). Previous studies show that jackal is the main predator at Göksu Delta (PETERS & VERHOEVEN, 1992; YERLI & DEMIRAYAK, 1996). Of the recorded nest, 33.9% were predated by jackal at Göksu Delta during 2004 and 2008 nesting seasons. A total of 5682 (35.4%) eggs were destroyed by jackals. The percentage of hatchlings depredated or died on the beach to the total number of eggs is 3.3%. The jackal predation was very intensive on 760-763 subsection beaches. The predation was occurred as soon as egg laying and nests were totally depredated.

During two nesting seasons, a total of 25 adult loggerhead turtles were killed by jackal (*Canis aureatus*). Of the killed adults, 66.7% were found on 760 subsections. The jackal predation on adults is most dense in June and July (93.3%). Furthermore, a total

of 10 loggerhead females washed ashore over two nesting seasons. One of the adverse effects on the beach was occurred during the stormy weather by flooding the nests. Of the recorded nests, 34.3% (82 nests) was flooded due to high tide line and 6230 eggs (38.8%) were not able to complete their embryonic development. According to ÖZDEMIR *et al.* (2008), embryonic mortality rate of loggerhead turtles is higher at Göksu Delta than at Fethiye beach. They also stated that lower slope at Göksu Delta is most important factors on higher value of embryonic mortality.

Protecting nests against predation with metal gratings is one of the effective conservation tools (YERLI *et al.*, 1997; TÜRKOZAN, 2000; TÜRKOZAN & YILMAZ, 2008). Throughout two breeding seasons, a total of 68 nests were protected against jackal predation by means of metal gratings (72X72) with a mesh opening of 9 cm was placed over the eggs (20 cm deep from the sand surface) centered around the egg chamber on 760 beach subsection. These gratings were very effective against jackal predation. Nevertheless 2 were totally depredated by jackals. These predations were resulted from the removal of metal gratings by the people before predation.

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Recent Vertebrate Animal Bones (Animalia: Vertebrata) from Yubileyna Cave (Rhodopes Mountain, South Bulgaria)

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Abstract. Excavations (area of 50 x 50 cm and 20 cm depth) at about 15 m from the cave entrance revealed various vertebrate fauna. As individual numbers the mammals and the frogs predominated as bone remains. All other taxa were with low percent of occurrence. The trogloxenic species dominated than the troglophilic. Considering the cave characteristics and the taxonomical identity of the bones we proposed two main ways of bone accumulation in this cave in recent times.

Key words: vertebrate faunal remains, bone findings, cave fauna, Yubileyna cave, Bulgaria.

Introduction

In the summary on the cave fauna of Bulgaria, BERON (1994) reported 48 species of vertebrate animals: 3 fish, 9 amphibian, 2 reptilian, 5 avian, and 9 mammalian species. Most of them are trogloxenes, occasionally met in the caves, as only the bats were troglophilic ones. Troglobiotic vertebrates are not known to live in the Bulgarian caves till now.

In spite of the long list of vertebrate animals found in caves in Bulgaria available many of the caves are still insufficiently studied according their recent and fossil fauna (BERON *et al.*, 2009). In the present paper we represent first data for recent vertebrates found as bone material in Yubileyna cave.

Material and methods

The study was carried out on 04.09.2010 in Yubileyna cave (West Rhodopes Mountain, south of Peshtera town). Excavations (area of 50 x 50 cm and 20 cm depth) at about 15 m from the cave entrance revealed various vertebrate fauna. Faunal remains were extracted through washing and sieving of recent soil deposits mixed with gravel. The bones were considered as recent by two criteria: 1. they were found in soil and humus deposits near the cave entrance, and 2. the organic components as for example fat and marrow of the bones were visibly not completely removed, and the bones were not filled with calcite. They were identified through a reference collection of skulls and bones of the Faculty of Biology, Plovdiv University. The minimal number of individuals was considered according the number of the left or right pair bones, specimen's size classes and their age groups. The animals were divided also according their ecological groups considering their relation with the life in caves: troglophilic and trogloxenic considering BERON (1994).

Results and Discussion

The list of the identified specimens is given in Table 1. As individual number the mammals predominated from which the rodent remains were mostly found (34.29% from all vertebrate individuals identified) followed by the bats (22.86%). The frogs also had high numbers as bone remains (11.43%) which were and the only amphibian representatives in the sample. All other taxa were with low numbers and occurrence below 10%.

The trogloxenic species dominated than the troglophilic (the bats) (N = 27, 77.4% and N = 8, 22.9%, respectively).

Considering the fact that Yubileyna cave is a horizontal one we accept that there were two main ways of accumulation of bones into it:

1. Bones from animals which used to live in the cave and died inside by different reasons. We suppose this way of bone accumulation was typical for the bats and for the wintering in the cave amphibians and reptiles.

2. Bringing bone material from outside by predators. During our research in the cave we found excrements of Rock Marten (*Martes foina* Erxl.) which is one of the most frequently registered carnivore in the Bulgarian caves (BERON, 1994), and possibly this species was contributed for some bone accumulations in Yubileyna as these one of fish, hare and rodents. Bringing of bones by owl pellets is not convincing because the cave has a relatively low entrance which is not suitable for nesting sites of such birds.

Table 1. Recent vertebrate animals found in the Yubileyna cave as bone remains.

Taxon	**MNI**	**%**	**Find**
Pisces, Osteichthyes sp.	2	5.71	*cranium* fragment
Total Pisces, Osteichthyes	**2**	**5.71**	
Ranidae sp.	3	8.57	*vertebrae*, long bones
Bufo bufo L., 1758	1	2.86	*os coxae*
Total Anura	**4**	**11.43**	
Serpentes sp.	2	5.71	*vertebrae*, *costae*
Total Serpentes	**2**	5.71	
Aves sp.	2	5.71	*falanx*, long bones
Total Aves	**2**	**5.71**	
Talpa europea (L., 1758)	2	5.71	*cranium* fragment, long bones
Crocidura sp.	1	2.86	*cranium* fragment
Insectivora	**3**	**8.57**	
Rhinolophus ferrumequinum (Schr., 1774)	2	5.71	*mandibulae*
Myotis myotis Bork., 1797	1	2.86	*cranium* fragment
M. myotis/oxygnatus (Mont., 1885)	3	8.57	*mandibulae*
Chiroptera sp.	2	5.71	*mandibulae*
Total Chiroptera	**8**	**22.86**	
Lepus europeus (Pall., 1778)	1	2.86	*molar*
Total Lagomorpha	**1**	**2.86**	
Muridae spp.	1	2.86	*mandibulae* fragments
Microtinae spp.	3	8.57	*mandibulae* fragments
Rodentia spp.	8	22.86	*incisivi*, various bones
Total Rodentia	**12**	**34.29**	
Martes foina (Erxl., 1777)	1	2.86	*femur* fragment
Total Carnivora	**1**	**2.86**	
Total Mammalia	**25**	**71.43**	
Total Vertebrata	**35**	**100**	

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Overwintered Hatchlings of Emys orbicularis from Lake Sülüklü (Western Anatolia, Turkey)

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Abstract. During our monitoring survey of amphibians and *Emys orbicularis* in Lake Sülüklü (Western Anatolia, Turkey), we observed four overwintered hatchlings of European pond turtle on May 4 and 10, 2010. The average straight-line maximum carapace length of the neonates captured was 26.48 mm and their average weight was 4.18 g. This observation is the second record for the Turkish population of *Emys orbicularis.*

Keywords: *Emys orbicularis*, neonate, overwintering, Western Anatolia, Turkey

Introduction

European pond turtle, *Emys orbicularis* (L., 1758), one of two representatives of the Emydidae family distributed in the Palearctic region, is one of the freshwater turtle species distributed from Northwest Africa in the West to the former Aral Sea in the East and from the Moscow Region in the North to the Turkish-Syrian border in the South (FRITZ, 2001, 2003). The European pond turtle is an endangered and protected species in many European countries and it is protected by the Bern Convention (1979) (Appendix II) and ANNEX II of the European Habitat and Species Directives (1992) and it is raised in the vulnerable category in the last IUCN report for the Mediterranean basin (COX & TEMPLE 2009).

Reproductive biology of the species has been well studied by many authors (e.g. ZUFFI & ODETTI, 1998; ZUFFI *et al.*, 1999, 2004, 2007). However, most of these papers focused on European populations (see reviews in FRITZ, 2001; 2003), and little information is available on Anatolian populations (AYAZ, 2003; AYAZ *et al.*, 2008). Overwintering behaviour is well known for many freshwater turtles (review ULTSCH, 2006). However, only little is known about the overwintering behaviour of *E. orbicularis* in many countries (e.g. PARDE *et al.*, 2000, MITRUS & ZEMANEK, 2003; THIENPONT *et al.*, 2004; NOVOTNY *et al.*, 2004) and whether overwintering occurs also at embryonic stages is still under debate. Here we present the second record of four hatchlings of *Emys orbicularis* found active in early May and also give new additional morphological data of overwintering hatchlings in Turkey.

Materials and Methods

Lake Sülüklü (Manisa, Western Anatolia) is situated on the north-eastern slope of Mt. Spil [38.565035° N, 27.532617° E, 612 m a.s.l.] and surrounded by a pine forest (*Pinus brutia*). The surface area of the lake is nearly 1.58 ha and its depth 2-4m. The lake is nourished by ground water, snow melt, and rain. The water level drops drastically, especially in summer (July and August), due to a decrease in spring waters and monthly rainfall.

During the monitoring survey of amphibians (*Pelophylax bedriagae*, *Lissotriton vulgaris* and *Triturus karelinii*) and turtles (*Emys orbicularis*), in the samplings of May 4 and 10, 2010, 4 *E. orbicularis* neonates, 2 on each date, were captured in the section on the lakeside in the depth of approximately 20-30 cm and with dense plant cover (*Typha angustifolius*, *Phragmites australis*, *Juncus* sp., *Carex* sp. and *Potamogeton* sp.). Air temperature was 27 and 30°C during the field studies, respectively.

The straight-line maximum carapace length (SCL), carapace width (CW), carapace height (CH), plastron length (PL), gular suture length (GuL), humeral suture length (HumL), pectoral suture length (PecL), abdominal suture length (AbdL), femoral suture length (FemL), anal suture length (AnL), and tail length (TaL) of the captured neonates were measured with digital callipers sensitive to 0.01mm. Colour pattern features were recorded and photographed, and then hatchlings were released into the place where they had been captured.

Results and Discussion

Mating takes place from the beginning of January to June as soon as after hibernation depending on latitude (ERNST & BARBOUR, 1989). Nesting starts in late May in the south, and in early July in the north (ZUFFI *et al.*, 1999; AYAZ *et al.*, 2007). The clutch number varies among different regions (ZUFFI *et al.*, 1999, 2007). In the southern European populations of *E. orbicularis*, females lay their first eggs in the first half of June, with the earliest time being the second half of May, while females in the Central and East European populations generally lay their first eggs in the first half of June (FRITZ, 2001, 2003). On the other hand, there is great variation in the Caucasian Region (FRITZ, 2001, 2003). The number of eggs in a clutch is 3-16, but usually 9-10 (ERNST & BARBOUR, 1989). After approximately 90 to 117 days of incubation, the young hatch from August to late October, depending on the latitude as well as seasonal conditions (LEBBORONI & CHELAZZI, 1991; ANDREAS & PAUL, 1998; SCHNEEWEISS *et al.*, 1998; SERVAN, 1998; SCHNEEWEISS & JABLONSKY, 2000). Hatchlings have carapaces of 20-25 mm, weigh about 5 g, and have large heads, long tails, and a carapace with a well-developed medial keel (ERNST & BARBOUR, 1989; FRITZ, 2001, 2003). Some neonates may overwinter in nest chambers or on land and emerge in the following spring (BANNIKOV, 1951; ZEMANEK & MITRUS, 1997; MITRUS & ZEMANEK, 1998, 2003; SERVAN, 1998; KOTENKO, 2000; SCHNEEWEISS & JABLONSKY, 2000; NOVOTNÝ *et al.*, 2004; THIENPONT *et al.*, 2004).

Our measurements for the specimens from Lake Sülüklü are presented in Table 1. The average body weight of the specimens was 4.18 (4.0-4.6) g and the median keel and a pair of low lateral keels were evident. Axillary and inguinal scutes are absent. Plastral formula is: Anale > Abdominale > Pectorale > Gularel > Femorale > Humorale. In all specimens, anal suture was the longest, whereas the femoral suture was the shortest. Limbs were covered with small- to medium-sized scales. No abnormalities were encountered regarding the keratin plates on carapace and plastron. Nuchal plates were parallel. Any carapacial horny plate was completely covered by granules, and no growth marks were present (Fig. 1a). The latter features along with the small body size and the relatively big head are clear indicators of a very early post-hatchling developmental stage. The carapaces of all studied hatchlings from Lake Sülüklü were olive brown. The edge of the marginal plates was slightly light yellow. The plastron was distinctly darker. This dark blotches cover at least around two-thirds of the plastron, and only the lateral rim was yellow (Figure 1b). Ground colour of soft parts was blackish with yellow dots.

For the last 20 years, the breeding biology of the European populations of *E. orbicularis* has been studied in detail (e.g. LEBBORONI & CHELAZZI, 1998; ZUFFI *et al.*, 1999, 2007). However, in Turkey, reproductive biology of the European pond turtle has not been investigated yet, except for a few observations made by AYAZ (2003). He observed the mating behaviour of *E.*

orbicularis in the Aegean Region at the beginning of May and discovered eggs in a nest built at a distance of about 16 meters from water in Turkey's Lake District on July 2, 2001, which was later destroyed by predators.

Table 1. Morphometric data of neonates of *Emys orbicularis* from Lake Sülüklü (Manisa/Turkey)

Characters	n	Mean+SE	Range	SD
SCL	4	26.48+0.40	25.82-27.58	0.80
PL	4	23.74+0.23	23.22-24.20	0.46
CH	4	18.24+3.19	12.15-24.14	6.38
CW	4	18.40+3.30	12.67-24.97	6.60
GuL	4	3.94+0.27	3.13-4.30	0.55
HuL	4	2.68+0.31	2.20-3.60	0.63
PecL	4	3.57+0.16	3.24-3.97	0.31
AbdL	4	3.49+0.23	2.85-3.86	0.45
FemL	4	2.10+0.18	1.77-2.58	0.36
AnL	4	6.52+0.13	6.30-6.90	0.27
TaL	3	23.13+0.29	22.67-23.66	0.50

Located among high and steep mountains in the north of western Taurus Region, Turkey's Lake District comprises a number of lakes of various sizes (e.g. Lake Beyşehir, Lake Eğirdir, etc.). He reported that turtles laid their eggs in captivity between early June and the first week of July. Moreover, AYAZ (2003) presented measurements for hatchlings from the Lake District in early July. Considering these findings, it can be concluded that hatching in Turkish populations starts in early July and continues till the end of summer. Mating was observed to be in April-May in Lake Sülüklü supporting the findings by AYAZ (2003).

In the present study, the average straight-line carapace length of the neonates captured from Lake Sülüklü was calculated as 26.48 mm and their average weight as 4.18 g. FRITZ *et al.* (2006) stated that in different subspecies of *E. orbicularis*, the values of carapace length ranged from 23.4 to 30.8 mm and the values of weight from 3.7 to 6.7 g. Likewise, DROBENKOV (2000) gave the average straight-line carapace length of hatchlings in the populations in the north of the distributional range of the species as 28.0 mm (25.9-29.0) and the average weight as 6.1 (5.65-6.45) g. At the same time, AYAZ *et al.* (2007) reported a neonate, captured from Lake Uluabat and having a straight-line carapace length of 22.3 mm, for the first time for Turkey. The results we obtained in our study remain within the limits of the values given in the above-mentioned studies, and the fact that no growth ring was observed in our specimens reveals that they were neonates (Figure 1b).

Related studies have reported that more than one egg-laying occur annually in many European populations and specimens that hatch at the end of summer spend their first winter in the nest (RÖSSLER, 2000a, b; also see the reviews in FRITZ, 2001, 2003). The neonate we captured probably hatched from an egg towards the end of the reproduction period and exhibited overwintering behaviour due to unfavourable climatic conditions.

The climate of the Aegean Region is partly continental and partly Mediterranean (TURKISH ECOLOGY FOUNDATION, 1993). In areas where the dominant climate is continental, environmental conditions can significantly change in a very short period of time. It is likely that Lake Sülüklü, located in the north-eastern slope of Mt. Spil (1,517 m a.s.l.), has an important impact on the changing environmental conditions. Probably, the bad weather conditions in some years stimulate the overwintering behaviour of hatchlings.

According to our observations in the study area, this area is convenient for both egg laying and the overwintering behaviour of neonates. Nevertheless, it is extremely difficult to state whether the overwintering behaviour is spent in the nest (e.g. KOTENKO & FEDORCHENKO, 1993; MITRUS & ZEMANEK, 2003) or on land after leaving the nest (e.g. BANNIKOV, 1951). Clarification of this dilemma will make a great contribution to the breeding behaviour of the species.

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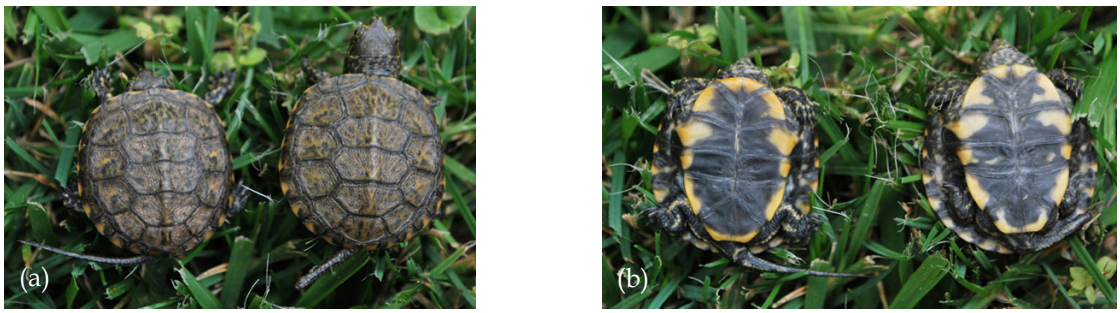

Fig. 1. Dorsal (a) and ventral (b) view of two neonates of *Emys orbicularis* from Lake Sülüklü (Manisa/Turkey)

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A New Seed Beetle Species to the Bulgarian Fauna: Bruchidius siliquastri, Delobel (Coleoptera: Chrysomelidae: Bruchinae)

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Abstract. A seed beetle *Bruchidius siliquastri* DELOBEL, 2007 (Coleoptera: Chrysomelidae) was reared from ripe pods of *Cercis siliquastrum* (Fabaceae) in Bulgaria and this is the first record of the species to the Bulgarian fauna. New host plants of the bruchid species were established on the basis of material collected in Hungary: *Cercis occidentalis*, *Cercis chinensis* and *Cercis griffithii*. A rich hymenopteran complex associated with the seed beetle was reared and comments on it are presented.

Key words: *Bruchidius siliquastri*, Bruchinae, Hymenoptera, Bulgaria, Hungary, new associations.

Introduction

Bruchids (Coleoptera: Chrysomelidae: Bruchinae) have a worldwide distribution, with the highest species diversity in tropical and subtropical zones (BOROWIEC, 1987). Seed beetles are of a great economic importance, because several species are serious pests of agricultural and stored products.

The genus *Bruchidius* SCHILSKY, 1905 consists of about 300 species, widespread in the Old World, but a few species were introduced outside their native area (KINGSOLVER, 2004). Host plants of most *Bruchidius* species in larval stage are legumes (Fabaceae) as well as Apiaceae and Asteraceae plants (BOROWIEC, 1987).

Bruchidius siliquastri DELOBEL, 2007 was found for the first time in Montpellier, France as a seed beetle of *Cercis siliquastrum* L. KERGOAT *et al*. (2007) described it, gave distributional information for China and Hungary and supposed presence of the species in other European countries also. Later, YUS RAMOS *et al*. (2009a) recorded the species in Spain. YUS RAMOS *et al*. (2009b, c, d, 2010) gave notes and comments on the biology and described the pre-imaginal stages of the species.

The genus *Cercis* L. (Fabaceae: Caesalpinioideae) consists of up to ten species, native to North America, China, Central Asia and Europe (KERGOAT *et al*., 2007). *C. siliquastrum* (Mediterranean redbud, Judas tree) is distributed in all temperate regions of the world as native or ornamental tree due to the beauty of its flowers. KERGOAT *et al*. (2007) discussed on the most likely origin of *Bruchidius siliquastri* and possible recent shift from Oriental species of *Cercis* as hosts toward *C. siliquastrum*.

In this paper, *Bruchidius siliquastri* is recorded for the first time from Bulgaria.

New host plants - *Cercis occidentalis* TORR. EX A. GRAY, *Cercis chinensis* BUNGE and *Cercis griffithii* BOISS are established. Comments on a parasitoid complex reared together with the seed beetle are presented.

Material and methods

Ripe pods of *C. siliquastrum* were collected in 2009 and 2010 in Plovdiv, Hissar and Kardzhali (Bulgaria). Pods of *C. siliquastrum*, *C. occidentalis*, *C. chinensis* and *C. griffithii* were collected from 2005 to 2007 from Budapest, Sopron, Kecskemét, Telki, Nagykanizsa, Szombathely and Vácrátót (Hungary). The material was stored at laboratory conditions (18–22° C) in plastic boxes until emergence of *Bruchidius siliquastri* and parasitoids.

Results and Discussion

Adults of *Bruchidius siliquastri* were reared from *Cercis siliquastrum* pods collected in Bulgaria and Hungary. The seed beetle infested three further *Cercis* species: *C. occidentalis* (4.X.2007, Sopron, University of West Hungary, Botanical Garden, leg. G. Lunk); *C. chinensis* (12.XI.2007, Sopron, University of West Hungary, Botanical Garden, leg. G. Lunk), and *C. griffithii* (12.III.2007, 9.X.2007, Vácrátót, Institute of Ecology and Botany of Hungarian Academy of Sciences, Botanical Garden, leg. G. Lunk).

During the study, we reared the following hymenopterans belonging to four families of Chalcidoidea (Pteromalidae, Eupelmidae, Eurytomidae and Eulophidae), and Ceraphronidae and Braconidae families, together with *Bruchidius siliquastri.*

Material:

Bulgaria: Plovdiv, 10.II.2010, *Dinarmus acutus* (THOMSON, 1878) (11 ♂♂, 6 ♀♀), ex *C. siliquastrum* (A. Stojanova).

Hungary: Budapest, 1.XII.2005, *Eupelmus urozonus* (DALMAN, 1820) (2 ♂♂, 10 ♀♀), *D. acutus* (5 ♂♂, 5 ♀♀), *Mesopolobus* sp. (1 ♂), *Bruchophagus* sp. (1 ♂), ex *C. siliquastrum* (Z. György); 23.III.2006, *E. urozonus* (4 ♂♂, 5 ♀♀), *D. acutus* (1 ♀), ex *C. siliquastrum* (Z. György); 26.IX.2006, *E. urozonus* (5 ♂♂, 8 ♀♀), *D. acutus* (34 ♂♂, 33 ♀♀), ex *C. siliquastrum* (Z. György); 2.II.2007, *E. urozonus* (9 ♂♂, 11 ♀♀), *D. acutus* (1 ♂, 3 ♀♀), ex *C. siliquastrum* (K. Kovács); Kecskemét, 18.VII.2006, Ceraphronidae gen. et sp. (1 ♀), ex *C. siliquastrum* (Á. Szentesi, M. Tuda, Z. György); Sopron, University of West Hungary, Botanical Garden, 12.XI.2007, *E. urozonus* (1 ♀), ex *C. chinensis* (G. Lunk); Telki, 28.IV.2006, *E. urozonus* (6 ♂♂, 6 ♀♀), *D. acutus* (30 ♂♂, 28 ♀♀), *Bruchophagus* sp. (1 ♂, 1 ♀), Braconidae gen. et sp. (1 ♀), Ceraphronidae et gen. sp. (1 ♂), ex *C. siliquastrum* (A. Bartha); Vácrátót, Institute of Ecology and Botany of Hungarian Academy of Sciences, Botanical Garden, 9.X.2007, *E. urozonus* (6 ♂♂, 4 ♀♀), *D. acutus* (10 ♂♂, 8 ♀♀), *Mesopolobus* sp. (1 ♂), Eulophidae gen. et sp. (1 ♀), ex *C. griffithii* (G. Lunk); Nagykanizsa, 2.IX.2007, *E. urozonus* (2 ♂♂, 3 ♀♀), *D. acutus* (8 ♂♂, 10 ♀♀), *Mesopolobus* sp. (1 ♀), Eulophidae gen. et sp. (1 ♂), Tetrastichinae gen. et sp. (1 ♀), ex *C. siliquastrum* (G. Lunk); Szombathely, 25.IX.2007, *E. urozonus* (2 ♂♂, 5 ♀♀), *D. acutus* (10 ♂♂, 14 ♀♀), ex *C. siliquastrum* (R. Dankovics, K. Vig).

Newly recorded host plant associations of *Bruchidius siliquastri* with *C. occidentalis* (California or Western Redbud, native to California), *C. chinensis* (Chinese redbud, native to Eastern Asia) and *C. griffithii* (Afghan Redbud, native to Southern Central Asia) reveal that the seed beetle is oligophagous on *Cercis* species.

The reared hymenopteran complex consists of *Dinarmus acutus* and *Mesopolobus* sp. (Pteromalidae), *Eupelmus urozonus* (Eupelmidae), *Bruchophagus* sp. (Eurytomidae), Eulophidae gen. et sp., Ceraphronidae gen. et sp. and Braconidae gen. et sp.

D. acutus is known to be a parasitoid of *Bruchus lentis*, *B. gilvus* and *B. lividimanus* in Algeria and Italy (GRAHAM, 1969). NOYES (2003) summarized the published data and gave six species of the genus *Bruchidius*, one species of *Acanthoscelides*, eight species of *Bruchus* and one species *Callosobruchus* as hosts. YUS RAMOS *et al*. (2009d) reported *D. acutus* and *Dinarmus italicus* (MASI, 1922) as parasitoids of *Bruchidius siliquastri* in Spain, where they found very high level of

parasitism (38%) of both species. Among all reared hymenopterans in our material, *D. acutus* represents 69% (219 specimens) and it could be a serious factor affecting the population dynamics of the seed beetle.

Eupelmus urozonus is a polyphagous parasitoid and hyperparasitoid of a considerable number of species of Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera (NOYES, 2003). This is the first record of its association of the species with *Bruchidius siliquastri*. Taking into consideration of the wide range of hosts of *E. urozonus*, its presence in our reared material is not unexpected.

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