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Assessing the Heavy Metal Content in Forest Dormouse (Dryomys nitedula Pallas, 1778) from an Agricultural Region in Bulgaria

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Abstract. The heavy metals load in the forest dormouse (*Dryomys nitedula*), inhabiting in forest shelter belts in the agricultural region was assessed. The concentrations of Cd, Co, Cu, Ni, Pb and Zn (expressed in mg/kg of dry tissue) were established in the liver, using an atomic-absorption analysis. The fact that the highly toxic metals (Cd and Pb) were found in considerable concentrations together with other metals with concentration dependent toxic effect (Cu, Ni, Zn and Co) in the liver of forest dormice, suggests that it is necessary to carry out regular assessment and forecasting of accumulation of these metals in species, which are not direct targets of cultivation and control activities in agricultural ecosystems. The obtained values were used to create a baseline for estimation of heavy metal accumulation in the internal organs of the forest dormouse, both in anthropogenically transformed habitats and natural biotopes, as well as for using this species as a monitor of environmental status.

Key words: heavy metals, forest dormouse, forest shelterbelts, *Dryomys nitedula.*

Introduction

Due to the typical for the species high degree of ecological adaptation (AIRAPETYANTZ, 1983) the forest dormouse (*Dryomys nitedula* Pallas, 1778) is able to inhabit biotopes in the forest ecosystems throughout much of Eurasia (BATSAIKHAN *et al.*, 2008). Its area covers the territory of Europe from the Alps southward, including the Balkan Peninsula, northward to the Baltic Sea, and eastward to Volga and the Ural Mountains. The distribution of the forest dormouse inside the area is sporadic (KRYŠTUFEK, 1999).

In Bulgaria, as well as in the biggest part of its European area (KRYŠTUFEK, 1999) the forest dormouse occurs mainly in deciduous and coniferous woodlands, wherever suitable conditions are present. The presence of bushes and thick undergrowth are the main habitat requirements, determining the present distribution of this species in Bulgaria too (MARKOV, 1959). Under these conditions the forest dormouse is able to inhabit biotopes in many natural mountainous and hilly forest ecosystems throughout Bulgaria (MARKOV, 2003), which are quite variable in

their physical geographical conditions (TISHKOV, 1976). The forest dormouse usually avoids human dominated habitats such as agricultural areas (BATSAIKHAN *et al.*, 2008).

During the second half of the 20th century in the fields of North-eastern Bulgaria artificial forest plantations of basic and concomitant vegetation (trees and bushes), forming the forest shelter belts have been created. These artificial forest plantations have created new, potentially suitable favourable biotopes for the forest dormouse in Bulgaria. They provide various plots of deciduous forest with shrubby layer and dense undergrowth, which are the most optimum stations in the forest dormouse habitats. Nowadays the forest dormouse is settled permanently there and develops vital populations. The newly established populations of the forest dormouse in the agricultural regions of the country exist under ecological conditions that are influenced by the surrounding anthropogenic agricultural activities (MARKOV *et al.*, 2009).

Given the growing human impact on the biosphere, the heavy metals represent a special danger for the agricultural biocoenoses. Getting into the soil and plants they accumulate in the agricultural ecosystems and get involved in the metabolic cycles of living organisms; forming highly toxic carcinogenic compounds and causing negative reactions, they create unfavorable conditions for the existence of the living organisms (WHO, 1992).

Because of the ability of wild mammals to accumulate heavy metals in their internal organs such as liver and kidney (GOYER, 1986), they have been recognized as valuable biological monitors of xenobiotic pollutants present in the ecosystem (VENOGOPAL & LUCKEY, 1978; WREN, 1986; TALMAGE & WALTON, 1991). The evaluation of accumulating contaminants of heavy metals levels, which belong to the group of the most dangerous inorganic toxic substances (LUCKEY *et al.*, 1974) in wild animals is important not only for assessing the potential effects of pollutants on their health status, but also for obtaining information about the quality of the ecosystem, an essential part of which are the animals (TATARUCH & KIERDORF, 2003).

Forest dormice are omnivores and their diverse food spectrum includes both animal foods, such as birds, eggs, insects, terrestrial non-insect arthropods, and plant foods: leaves, seeds, grains, nuts and fruit (AIRAPETYANTS, 1983; LOZAN, 1970). They have small home ranges (about 65–100 m long), strongly associated with tree and shrub habitats, good reproductive potential (one female gives birth to 2-9 cubs each year, most often 4-5 cubs), relatively short lifespan (3-5 years in wild, more often 4 years) (BATSAIKHAN *et al.*, 2008; ŚCIŃSKI & BOROWSKI, 2006; LOZAN, 1970; 1979; NOWAKOWSKI, 2001; SLUDSKII, 1977; AIRAPETYANTS, 1983). These biological features together with the fact that it is widely spread in Bulgaria (MARKOV, 1959; 2001) and Europe (KRYŠTUFEK, 1999) make the forest dormouse an useful species for the risk assessment of heavy metals accumulation in the wild animals and environmental monitoring.

The lack of knowledge about the actual values of the concentrations of heavy metals in natural populations of forest dormice prompted the present investigation. The aim was to evaluate the concentrations of residues of priority pollutants of the heavy metal group (Cu, Ni, Zn, Co, Pb and Cd) in samples from the target organ (liver) of forest dormice, inhabiting the main agricultural region of North-eastern Bulgaria and to provide a basis for their future monitoring in natural and agricultural ecosystems in the Eurasian range of the species.

To assess the specificity of heavy metal bioavailability in the forest dormouse, the heavy metals residuals in the liver samples were compared with the residuals in the liver of specimens from two other species: one related – the fat dormouse (*Glis glis*) and one differing by biological and ecological characteristics – the common vole (*Microtus arvalis*). The data were obtained during previous studies on heavy metal levels in small mammals in the same agricultural

region (MARKOV, 2012). Both dormice species are sympatric and occupy permanently the tree-shrub layer in the artificial forest shelterbelts. They differ by their lifespan – shorter in the forest dormouse (GOLODUŠKO & PADUTOV, 1961; PILASTRO *et al*., 2003) and diet – the animal component is more strongly represented in the food spectrum of the forest dormouse (NOWAKOWSKI & GODLEWSKA, 2006). The common vole lives underground in colonies across open agricultural lands, which are intensively treated with different chemicals for increasing crop yields. This species is territorially conservative. The average lifespan of the common vole is too short – about 4.5 months. The mean litter size is close to 5 cubs. The common vole is a typical herbivorous rodent with a food spectrum including more than 80 plant species and preferences to cereals, *Asteraceae* and *Fabaceae* (SOKOLOV & BASHENINA, 1994).

Material and Methods

Specimens of the forest dormouse were obtained from forest shelterbelts during a study of the presence and abundance of small mammal pests (*Microtus* spp., *Apodemus* spp. and *Mus* spp.) in an agricultural region in the Shumen District of North-eastern Bulgaria. The study plot covered an alfalfa field, the adjacent corn field and forest shelterbelt, as well as the nearby undeveloped area of the primary steppe biotope.

To avoid the potential influence of the specimens' age and gender on the quantitative accumulation of heavy metals in their bodies, only adult males (> 3 years) was analysed. Age determination of the forest dormouse was based on the degree of the wear of tooth enamel (LOZAN, 1961); gender was determined by external appearance and confirmed by dissection.

In the liver samples from 10 adult males forest dormouse the residual amounts of the studied elements (Cd, Co, Cu, Ni, Pb and Zn) were established using an atomic-absorption analysis. The heavy metal concentrations were determined with a Perkin-Elmer Model 3030B atomic absorption spectrophotometer with an air/acetylene flame, and expressed as mg/kg of dry analysed tissue. Before analysis, all samples were dried to constant weight at 60 ºC (HAVEZOV & TSALEV, 1980). The homogenization of each sample was performed by crushing in a porcelain mortar. Subsamples of 1 g were removed and transfer to an iodination flask. There it was wetted with distilled water and a 15 ml of a concentrated $HClO_4$ and HNO_3 acids were added. After the sample stayed for 24 hours at room temperature, it was heated in a sand bath to wet residue. The solutions were made up to 10 ml with 1n HNO_3. The digestion of samples was carried out in duplicate to ensure the reproducibility of the method. Analytical grade reagents were used to make up the relevant blanks and calibration curves

The basic statistical parameters: the mean (X), the standard deviation (SD), the standard error of the mean (SE) and the ± 95% confidence interval of the mean values were calculated for each investigated residual concentration of heavy metals in the liver of forest dormouse**.**

The significance of differences between the residuals found in the forest dormouse liver during the present investigation and the data for the fat dormouse and common vole obtained by MARKOV (2012) was tested in the Mann - Whitney U-test. The difference is insignificant since $p>0.05$

All calculations were performed using the statistical package STATISTICA version 8.0 (STATSOFT INC., 2008).

Results

The mean values of the residual heavy metals (Cd, Pb, Ni, Zn and Cu) found in the liver of the adult male forest dormice inhabiting forest shelter belts in the main agricultural region of North-eastern Bulgaria and their statistical estimation are presented in Table 1.

According to the empirically obtained mean values of the heavy metals residuals the elements studied in the liver could be arranged as follows: Zn > Cu > Pb > Ni > Co > Cd. This sequence of the heavy metals residuals in the liver of the forest dormouse corresponds to the relative concentrations of

these metals found in rodents inhabiting non-loaded or extremely loaded environments or obtained during experimental investigations under laboratory conditions (ŠUMBERA *et al.*, 2003).

Table 1. Heavy metals residuals X [mg/kg dry weight], their standard deviation (SD) and ±95 % confidence limits (C. L.) in liver of forest dormice (*Dryomys nitedula*) from the forest shelterbelt in an agricultural region in North-eastern Bulgaria

Metal	Mean	S.D.	-95 % C. L.	+95 % C. L.
Zn	63,102	6,833	46,128	80,075
Cu	12,387	1,536	-	16,202
Ni	0,533	0,145	0,174	0,892
Co	0,529	0,121	0,230	0,828
Cd	0,310	0,153	-	0,690
Pb	2,381	0,525	1,078	3,685

The highest absolute variation (SD) was found in the mean values of Zn and Cu residuals in the liver. The absolute variation of the mean value of Pb was also high. The absolute variation of the mean values of the remaining three elements – Ni, Co and Cd was of similar magnitude. The mean values of Cd, Ni, Co and Pb showed high relative variation with the highest rate in Cd – almost 50%. In Zn and Cu it was about 10%. The mean values of the residuals of all metals were obtained with a relatively low rate of the arithmetic mean: from 29 % in Cd to 6% in Zn.

The bioavailability comparison of the studied heavy metals in the forest dormouse liver with those in the fat dormouse liver and the common vole liver (Fig. 1) showed:

(i) The mean values of the Zn, Cu, Pb, Ni and Co concentrations in both dormice species were similar and the differences between them were statistically insignificant. The mean concentration of Cd in the forest dormouse was higher than the concentration found in the liver of the fat dormouse, where it was less than <0.001 mg/kg. Probably the absence of statistical significance in the empirical differences in the mean values of the heavy metals residuals in both closely related dormouse species was due to the similar habitat structures they were using (shrubby layer) in the forest shelterbelts, i.e. they lived under equal ecological conditions. This habitat was not directly used for farming and the dormice were non-target species for anthropogenic activities there.

(ii) The empirically found mean values of the residuals of the studied heavy metals in the liver of the common vole were higher than in the forest dormouse, but still statistically insignificant. Because of the high individual variation of the studied metals concentrations in the liver of the common vole, the mean values of the residuals found in the liver of the forest dormouse fall within their wide bounds and the 95% confidence intervals of their mean values in both species overlapped.

The common vole is a target species of human activities associated with the usage of xenobiotics in soil treatment, aiming to reduce pest numbers in agricultural areas. It is also directly affected by tillage. Probably, the different level of impact of these two anthropogenic factors determines the degree (from low to extremely high), in which substances, imported into the agricultural ecosystem and containing heavy metals, affect the common vole individuals.

Discussion

Toxic metals are ubiquitous in the environment (POHL *et al.*, 2011). Because of human activities, animals can be exposed to abnormal amounts of toxic metals. This exposure can lead to the accumulation of pharmacologically significant concentrations of metals in animal tissues.

The present work gives a warning about the bioavailability of heavy metals in forest dormouse in the country's plain regions. For the first time in this study initial norms of their variation in typical agricultural landscapes are given. The found concentrations of priority pollutant residues from the metal group – elements with a concentration dependant toxic effect (Cu, Ni, Zn, Co) and microelements with a proven highly toxic effect on living organisms (Cd, Pb) provide information on the actual problems

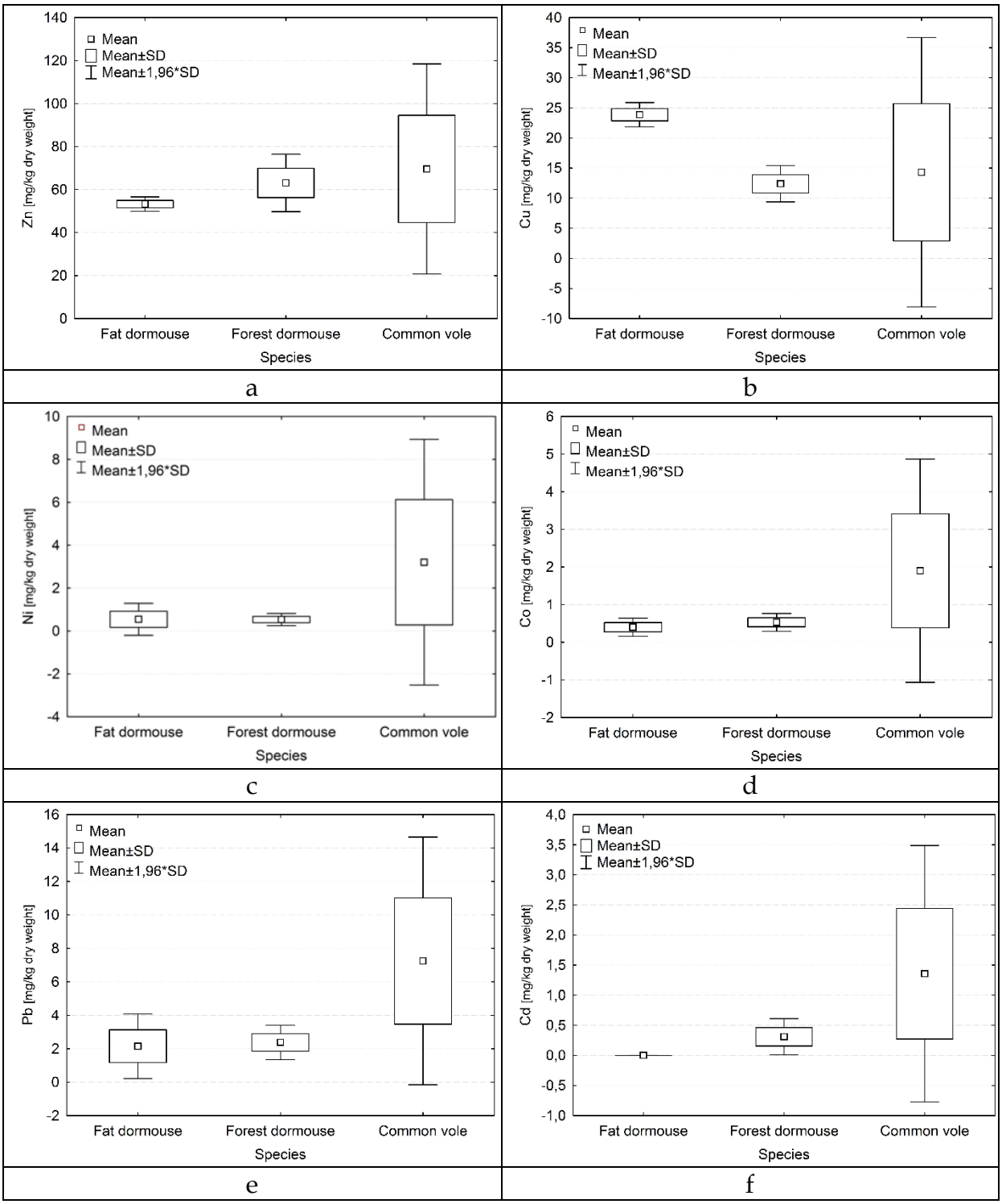

Fig. 1. Heavy metals residuals Mean [mg/kg dry weight and their statistical evaluation in liver samples of forest dormouse (*Dryomys nitedula*), fat dormouse (*Glis glis*) and common vole (*Microtus arvalis*) from the forest shelterbelt in North-eastern Bulgaria. Combined results of this study (liver form forest dormouse) and the reference data for fat dormouse and common vole (after MARKOV, 2012).

associated with potentially increasing anthropogenic pollution of the environment and the quality of the agricultural ecosystems of which the forest dormouse is an intrinsic part. They show that highly toxic elements, such as lead and cadmium

(KABATA-PENDIDAS & PENDIDAS, 1979; LUCY & VENUGOPAL, 1986), could be found in considerable concentrations in the liver of forest dormouse. The increased presence of these elements in the bodies of wild animals is commonly accepted because of the anthropogenic pollution of the environment (SAWICKA-KAPUSTA, 1979).

If the concentrations of essential elements, such as Cu, Ni, Zn, Co, are increased, they could turn into toxic agents when ingested in excess (CLEMENS, 2006; SINGH *et al*., 2011), and their accumulation should be traced in pollution bio-monitoring and environment hazard assessment.

Conclusions

As the residuals of microelements with a proven highly toxic effect and essential elements with a possible toxic effect (when their normal physiological concentration is exceeded) were found in the forest dormouse, their accumulation in the bodies of these animals should be tracked out as a component of the biomonitoring of agricultural ecosystems. Clearly, the survey of the heavy metals residues in the forest dormouse tissues, together with finding out of their harmless levels in future should aid in expanding our knowledge of the anthropogenic impact on agricultural lands, because this species represents an intermediate stage between low and high trophic levels in the ecosystems, where it inhabits.

The development of this task could provide new opportunities for using this species as a monitor of environmental status, both in new anthropogenically transformed habitats and in natural biotopes over its European range. A regular monitoring of heavy metal burdens in forest dormouse, together with monitoring of various physical, chemical and biological components of an ecosystem will provide important data regarding the bioavailability of contaminants within natural ecosystems and the health status of the species.

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Bioaccumulation of Cadmium and Lead in Rodent Species from the Region of Lead-Zinc Smelting Factory – Plovdiv (South Bulgaria)

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Abstract. The levels of the toxic metals, Cd and Pb, were measured in liver of yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834), Mediterranean mouse *(Mus macedonicus* Petrov & Ružić, 1983) and common vole (*Microtus arvalis* Pallas, 1778) from the vicinity of Plovdiv (South Bulgaria), where the lead-zinc smelting factory is the main source of pollution. The study was carried out at three sites located along a pollution gradient. An unpolluted region, the Strandzha Natural Park was used as a background region. MANOVA analysis revealed significant differences by species ($F=9.61$, $p=0.003$), site ($F=24.12$, $p=0.0001$) and exposure ($F=3.79$, $P=0.013$) effects. Significant increase of Pb and Cd bioaccumulation was found along the pollution gradient. Cd and Pb mean concentrations were highest at the site closest to the smelter and decreased with increasing the distance from them. The bioaccumulation of Pb was significant highest in the individuals of the yellow-necked mouse, followed by Mediterranean mice and common voles, whereas the common voles accumulated more Cd in comparison with the yellow-necked-, and Mediterranean mice. However, there is little evidence of adverse cadmium-mediated effects in yellow-necked- and Mediterranean mice and this species may be tolerant to Cd exposure. High Cd concentrations in body organs may simply reflect an ability to store the metal in a nontoxic, metallothionein-bound state. Liver Pb and Cd concentration did not differ significantly among sexes.

Key words: Bioaccumulation, lead, cadmium, *Apodemus flavicollis*, *Mus macedonicus, Microtus arvalis*

Introduction

Industrial pollution has become a new environmental factor that has essentially influenced the normal functioning of ecosystems. Therefore, it is necessary to analyze, in detail, the entry of various substances as a result of human activity into the environment and their interaction with living organisms at different levels. An essential stage in the overall ecological risk assessment is the establishment of residue content and distribution of specific pollutants of anthropogenic origin such as heavy metals in animal organisms that are especially sensitive to the change of the quantitative content of xenobiotics in the environment (SÁNCHEZ-CHARDI *et al.*, 2007).

Over the last decades the production of lead (Pb) and cadmium (Cd) in the industrial areas increased 2- and 15-fold,

respectively, and the subsequent release of these metals into the environment is of some concern (NRIAGU, 1988). The ecosystems seem to offer an effective filter by retaining contaminants in soil profiles, transferring them into aquatic (NÉGREL & ROY, 2002) and/or terrestrial systems, and thereby increasing the bioavailability and poisoning risk both to humans and the environment (AL SAYEGH PETKOVŠEK *et al.*, 2015). Lead and cadmium, two non-essential elements that are widely distributed, are well known for their highly toxic effects on biological systems (WOLFE *et al.*, 1998; LEWIS *et al.*, 2001).

The pollution status at the area of the Plovdiv (Bulgaria) lead-zinc smelting factory is well documented, particularly for certain heavy metals present in freshwaters and soils. In this region the industrial polymetallic dust emission of lead, cadmium and zinc microaggregates from the smelting factory remain the primary sources of *in situ* metal pollution. In the first quarter of 2014 the concentration of polymetallic dust in the air pool over the town Plovdiv increased from 0.75 to 1.0 mg/m^3 or was 1.5 to 2 times higher than the TLV (Threshold Limit Value) (source PRIEWS, 2014).

The intake and bioaccumulation of pollutants by mammals is known to occur (TALMAGE & WALTON, 1991; SHORE, 1995; KOMARNICKI, 2000; SÁNCHEZ-CHARDI *et al.*, 2007; AL SAYEGH PETKOVŠEK *et al.*, 2015). Several studies have shown that rodents and voles are mammals suitable for eco-toxicological research, especially due to their widely distribution, large number, r-type reproductive strategy, relatively small home range, high trophic chain position and metabolic rate (MA & TALMAGE, 2001). Small mammals respond to stress effects in the environment, such as the intensity of changes in their organism correlates with the intensity of stress factors (MITKOVSKA *et al.*, 2012a). The fact that concentrations of heavy metals found in natural rodent populations regularly correlates with environmental pollution makes it possible to regularly use small mammals in eco-monitoring studies (METCHEVA *et al.*, 2001; IERADI *et al.*, 2003; MARKOV, 2012; MITKOVSKA *et al.*, 2012a, b). Moreover, the pattern of heavy metals distribution and the levels of heavy metals found in their tissues are similar to those found in humans (DAMEK-POPRAVA & SAVICKA-KAPUSTA, 2003). Furthermore, consumers at higher trophic levels in terrestrial ecosystems may be useful in predicting risks to human health (KOMARNICKI, 2000). In several studies rodent species have shown to be relevant zoomonitors (ABRAMSON-ZETTERBERG *et al.*, 1997; METCHEVA *et al.*, 2001; IERADI *et al.*, 2003; TOPASHKA-ANCHEVA *et al.*, 2003; VELICKOVIC, 2004; TOPASHKA & YORDANOVA, 2008; AL SAYEGH PETKOVŠEK *et al.*, 2015).

The main goal of this study was to quantify the bioaccumulation of non-essential metals (Pb and Cd) in rodent species along the pollution gradient in the area of the Plovdiv lead-zinc smelting factory and to evaluate the exposure-, species-, and gender- related effects.

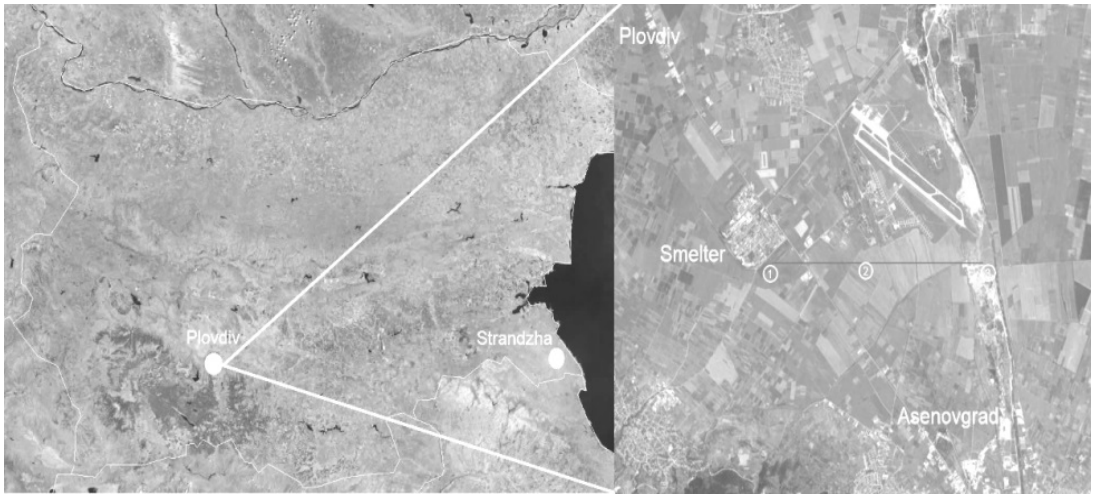
Material and Methods

Study area. The area of study covers two regions determined by "NATIONAL BIOMONITORING PROGRAM OF BULGARIA" (PEEV & GERASSIMOV, 1999) as impact (polluted – the area of the lead-zinc smelting factory near Plovdiv) and background (unpolluted – Strandzha Natural Park) (Fig. 1). The lead-zinc smelting factory is located in the Thracian valley, at 230 m asl. The natural forest vegetation has been completely destroyed, only fractions of scattered mosaic mixed deciduous forests, bushy and grassy components are still preserved. The areas around the smelting factory are agricultural ecosystems. The industrial pollution is presented by SO_2, NO_2, Pb, Cd, Zn and other toxic substances. Microaggregates of Pb, Cd and Zn are emitted in the atmosphere by aerosols in the torch of pollution. They accumulate in the ground, spreading over vegetation and aquatic areas. Larger Zn aggregates (from 250 to 370 microns) fall in the vicinity of the factory, while the heavier particles of Pb, which have a minimum size of 30 to 70 microns, are blown away along the direction of the

prevailing wind. Extensive investigations indicate that the polymetal pollution appears in soil samples. The heavy metals spread in the shallow plow layer of the soil and penetrate in downward direction to a limited extent (SENGALEVICH, 1998). By the end of 2010 the content of heavy metals and metalloids in soil samples from the factory area was: 4077.40 mg/kg Zn (by THV of 360 mg/kg); 3414.10 mg/kg Pb (by THV of 80 mg/kg) and 68.32 mg/kg Cd (by THV of 3.0 mg/kg). The average annual concentrations of Pb aerosols in the atmosphere remain below the corresponding average rate of 0.5 $\mu g/m^3$ over the last few years, while the concentrations of Cd aerosols increase (source PRIEWS, 2010).

The sites of study inside the area of the smelting factory cover locations along a pollution gradient. Pollution is highest on the eastern side. Three study sites were selected: site 1, adjacent to the smelter, where a green belt of vegetation exists; site 2, located 2 km east of the smelter and site 3, located 4 km east, respectively (Fig. 1). The Strandzha Mountain is located in the southeastern part of Bulgaria (Fig. 1a). This clean and uninhabited area also suffers from global air pollution caused by industrial emissions in this part of South-East Europe. However, the yearly average concentrations of pollutants are considerably lower and there are no important local sources of industrial pollutions and the animals are not directly exposed to environmental pollution (PEEV & GERASSIMOV, 1999).

Material. In total, 84 specimens of 3 rodent species were collected (Table 1). The common voles and Mediterranean mice were not catch in enough numbers in the sampling area of the Strandzha Natural Park to perform statistical analysis. To avoid intraspecific differences related to age, only adult specimens were examined. The age was determined according to criteria of molar root development and growth (FELTEN, 1952; GUSTAVSSON *et al.*, 1982).



a **b**

Fig. 1. Topographic location of the investigated regions and sites: a) location of the impact (lead-zinc smelting factory) and background (NP "Strandzha") regions and b) location of the investigated sites (1, 2 and 3) along the pollution gradient in the impact region.

Table 1. Number of investigated species in the studied areas.

Species	Lead-zinc smelting factory - Plovdiv			NP "Strandzha"	Total
	Site 1	Site 2	Site 3		
Apodemus flavicollis	8	10	7	16	41
Mus macedonicus	6	8	11	-	25
Microtus arvalis	6	7	5	-	18
				Total number:	**84**

Sampling. Trapping sessions were carried out from the second half of September 2012 until the end of October 2013. Sherman live traps were placed at dusk, left active overnight, and collected the next morning. The rodents were brought to the laboratory, where they were sexed and weighed. The animals were sacrificed by cervical dislocation after deep anesthesia. The liver was dissected for heavy-metal analyses. The liver tissue was stored at -20° C until further analysis.

The liver samples were dried for 24 h at 60°C until dry mass was obtained and then weighed. Afterward, a mixture of 5 ml of HNO_3 (70%) and 250 µl of H_2O_2 (30%), both ultrapure grade, was added to the dried samples to start digestion. Pb and Cd concentrations were determined using atomic absorption spectrophotometry (Perkin-ElmerISP-7000). The determination of lead was carried out in a graphite furnace. All metal concentrations were expressed on a dry weight basis in mg/kg.

The data were checked for both normal distribution (D'Agostino and Pearson omnibus normality test) and homogeneity of variance (Levene, F-test). Initially, an overall measure of the effect of sex, species and site effects was obtained by a three-way analysis of variances (MANOVA). Site and species divergences in the concentrations of heavy metals were performed using one-way analyses of variance (ANOVA), followed by Tuky's multiple comparison post-test. Intraspecies differences between both sexes and regions (impact and background) were calculated by Student's t-test. The differences in metal concentration distributions were statistically tested at $P<0.05$. All calculations were performed with the software program PRISM, version 4.02.

Results

Cd and Pb concentrations in the liver of the animals from the impact region were above the detection threshold (in mg/kg, Pb: 0.05; Cd: 0.05). MANOVA revealed significant differences by species ($F=9.61$, $P=0.003$), by different sites ($F=24.12$, $P=0.0001$) and exposure ($F=3.79$, $P=0.013$). Therefore, basic descriptive statistic (sample size, arithmetic mean, standard error, and range) of heavy metal concentrations are shown in Table 2.

Gender effect. The one-way analysis of variance (ANOVA) showed a lack of statistically significant sex differences in the accumulation of Pb and Cd in the liver of the studied species ($F=0.51$, $P=0.76$). Although, the average values of Pb were higher in females of the yellow-necked mice (Table 2), no statistically significant differences were found ($t=0.8923$; $df=13$; $P=0.3885$). Both males and females voles have approximately the same amount of Pb, while the values for the females are slightly higher (Table 2). No statistically significant differences were observed between both sexes of common voles ($t=0.3554$; $df=2$; $P=0.7562$) and Mediterranean mouse, either ($t=0.7908$; $df=78$; $P=0.4550$).

The average liver concentrations of Cd were higher in females in all studied species. In voles this trend is particularly emphasized as females show a substantially higher concentration of Cd compared to that of males, although no statistically significant differences between both sexes were observed ($t=1.434$, $df=2$, $P= 0.2881$). The samples result of the Mediterranean mice were more or less the same ($t=1.198$, $df=7$, $P=0.2698$).

The liver concentrations of Pb and Cd showed no gender effect and this enable data for both sexes to be combined in general samples in order to evaluate the species and exposure effects.

Exposure effect. The differences in mean concentrations of Pb and Cd between the impact and background regions were tested only by yellow-necked mice samples, because they inhabit both regions. The common voles and Mediterranean mice were catch in enough numbers to perform statistical analysis only in the region of lead-zinc smelting factory. In general, yellow-necked mice from the polluted region showed a significant increase in Pb ($t=2.193$, $df=18$, $P=0.0417$) and Cd concentrations ($t=2.764$, $df=19$, $P=0.0123$) than in the individuals from the background region (Fig. 2).

Table 2. Sample size (N), mean concentration in mg/kg (X), standard deviation (SD), minimal (Min) and maximal (Max) values of Pb and Cd by region and species

Region	Metal	Species	Male					Female				
			N	X	SD	Min	Max	N	X	SD	Min	Max
Lead-zinc smelting factory	Pb	*Apodemus flavicollis*	14	15.5	19.4	3.63	53.3	11	24.2	24.0	4.8	77.6
		Mus macedonicus	13	17.1	10.8	3.39	31.4	12	11.6	6.50	4.43	17.0
		Microtus arvalis	10	7.53	2.95	5.44	9.62	8	8.59	2.97	6.48	10.7
NP "Strandzha"		*Apodemus flaicollis*	8	0.47	0.35	0.22	0.71	8	0.79	0.66	0.39	1.77
Lead-zinc smelting factory	Cd	*Apodemus flavicollis*	14	12.5	10.3	4.46	27.6	11	16.7	15.1	2.39	49.7
		Mus macedonicus	13	11.8	9.69	2.53	30.0	12	21.6	15.1	9.14	38.4
		Microtus arvalis	10	12.2	4.27	9.15	15.2	8	34.6	21.7	19.3	49.9
NP "Strandzha"		*Apodemus flavicollis*	8	0.05	0.03	0.03	0.07	8	0.11	0.05	0.04	0.15

In the impact region, liver concentration of Pb differed among the sites. All rodents inhabiting site 1 accumulated significant more Pb (F=8.61, P=0.006) in comparison with these from sites 2 and 3 (Fig. 3). The mean liver concentration of Pb in the yellow-necked mice from site 1 was significant higher (F=20.46, P=0.0001) than that in individuals from sites 2 and 3 (Fig. 3).

In Mediterranean mice the mean amount of Pb did not differ significantly among sites (F=0.53, P=0.75) along the pollution gradient. Lack of statistically significant differences were also observed between the common voles from the different sites (F=2.53, P=0.07).

Significant differences in the mean values of cadmium accumulated in livers of the species from the three different sampling sites were observed (Fig. 4). The yellow-necked mice accumulated significantly more cadmium in the liver in the green belt area, less in the site 2 and at least in site 3 (F=13.92, P=0.0007). The average liver concentration of Cd in Mediterranean mice also differed significantly between individuals from the different sampling sites (F=6.48, P=0.009).

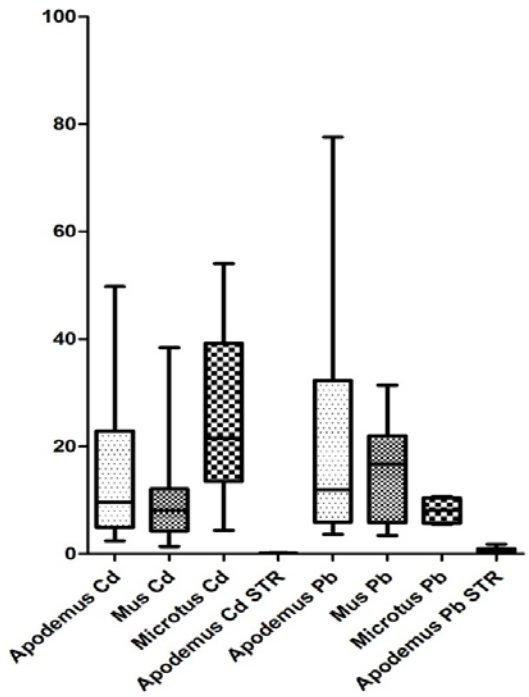


Fig. 2. Mean liver concentrations of Cd and Pb (mg/kg) in the investigated rodent species from the impact (lead-zinc smelting factory) and background regions (STR – Strandzha Natural Park). Bottom and top of the box represent 25 and 75% percentile values, respectively, with median values within the box. Error bars indicate minimum and maximum values.

More cadmium was accumulated in individuals from site 1. Such pattern of Cd accumulation was observed also in the common voles. Individuals of common vole accumulate significantly more cadmium in the green belt area (F=14.28, P=0.0003).

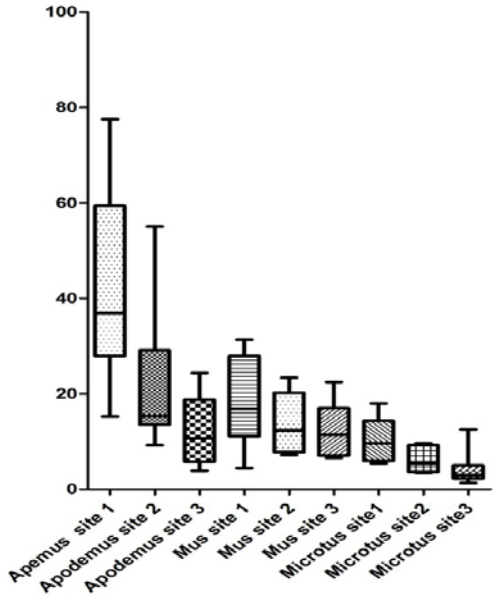


Fig. 3. Mean liver concentration of Pb (mg/kg) in the investigated rodent species along a pollution gradient in the impact region (lead-zinc smelting factory near Plovdiv). Bottom and top of the box represent 25 and 75% percentile values, respectively, with median values within the box. Error bars indicate minimum and maximum values.

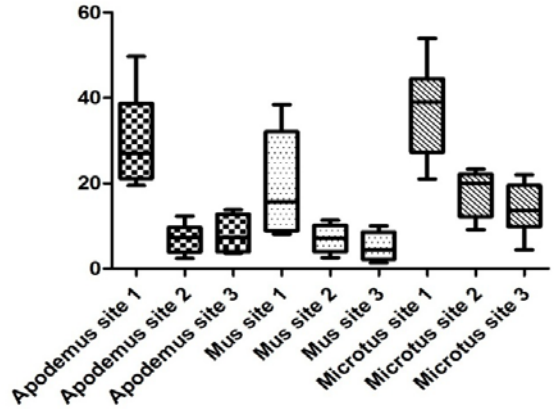


Fig 4. Mean liver concentration of Cd (mg/kg) in the investigated rodent species along a pollution gradient in the impact region (lead-zinc smelting factory near Plovdiv). Bottom and top of the box represent 25 and 75% percentile values, respectively, with median values within the box. Error bars indicate minimum and maximum values.

Species effect

In the impact region the mean concentration of Pb was highest in yellow-necked mice (20.48±21.77), followed by Mediterranean mice (15.26±9.54), and lowest in common voles (8.06±2.5) (Fig. 2). Outlined differences were not statistically significant (F=2.53, P=0.077).

The average concentrations of Cd in the livers of the investigated rodents differ statistically significant between the investigated species (F=5.82, P=0.006). The mean concentration of Cd was higher in voles (25.22±14.49), followed by yellow-necked mice (14.75±12, 79), and lowest concentrations were observed in the Mediterranean mice (10.55± 9.64) (Fig. 2).

Discussion

The obtained results revealed that the accumulation of Pb and Cd in the analyzed rodents varied according to site, species and exposure gradient, arranged in descending order of importance, whereas the sex remains statistically invariably for all three species.

Exposure related effect in metal bioaccumulation

The high levels of Pb and Cd found in the rodents from the polluted region can be easily explained as a result of intense industrial activities carried out in this area. According to data from the Plovdiv regional inspectorate of environment and waters (PRIEWs, 2012) the concentrations of Pb and Cd in the soil were 3108.18 mg/kg by TLV (Threshold Limit Value) of 80 mg/kg and 63.99 mg/kg by TLV of 3.0 mg/kg, respectively. Such anthropogenic impact had led to an increase of bioavailability of non-essential heavy metals by biota, and consequently to a chronic exposure for small mammals inhabiting the area of the smelter. Liver Cd concentrations in rodents from the polluted region were comparable to levels observed in other studies of rodents from polluted sites. The highest liver Cd concentrations observed until now in rodents were reported by HUNTER *et al.* (1989) for *Microtus agrestis* (mean, 22.7 mg/g) living near a copper-cadmium refinery. This liver concentration is

comparable to those observed in the present study. High values for yellow-necked mice also were observed in the latter study (mean, 18.2 mg/g) and in a study of yellow-necked mice living on a mining waste site (range, 10.3–39.7 mg/g) (JOHNSON *et al.*, 1978). The obtained in our study liver levels of Pb were far below the extremes reported from other polluted sites (JOHNSON *et al.*, 1978; ROBERTS *et al.*, 1978). The concentrations of metals in yellow-necked mice from the background region were consistent with those reported for the same species and other rodents inhabiting non-polluted sites (TALMAGE & WALTON, 1991; SHEFFIELD *et al.*, 2001; BEERNAERT *et al.*, 2007; ROGIVAL *et al.*, 2007). As can be deduced from the obtained data, the range of variation in metal concentration is generally wider in polluted areas than in un-contaminated sites (Table 1). This circumstance may indicate an individual response due to a different metal exposure and/or to particular ecological, genetic, and physiological factors in chronically exposed animals (TALMAGE & WALTON, 1991). Additionally, the tissular turnover that occurs in liver causes changes in metal concentration, particularly in lead, over the life of an animal, which may also partially explain the great variation observed at the population level. In contrast, in non-polluted sites, the animals do not need detoxification mechanisms to control the intake of metals and to prevent toxic effects to their organs.

The decrease in Pb and Cd levels with increasing distance from the smelter indicates that heavy-metal exposure decreased with increasing distance from the smelter, which agrees with the findings of previous studies (BERCKMOES *et al.*, 2005).

Gender related effects in metal bioaccumulation

Data on the effect of biotic factors, such as sex, on metal bioaccumulation in the investigated species are scarce. Those available do not show clear patterns as the effect of this factor vary greatly between populations. Although several authors have reported sex-dependent variation of metal concentrations in wood mice (e.g. LOPES *et al.*, 2002; SCHEIRS *et al.*, 2006; BEERNAERT *et al.*, 2007), we did not detect these differences in yellow-necked mice. This discordant result may be attributed mainly to inter-population variation caused by differences in exposure and uptake of elements.

Species related effect in metal bioaccumulation

The long-term pollutant activities from smelting factories may disturb or destroy ecosystems, thereby making them less suitable for wildlife. In fact, these polluted sites may produce an increase in the levels and bioavailability of toxic compounds, such as heavy metals, which stress populations during multiple generations as a result of extended bioavailability. Therefore, continuous biomonitoring of pollutants by means of selected species is required. In our study, *A. flavicollis* did indeed show the highest lead concentrations of the three small mammal species in the green belt site of the lead-zinc smelting factory. In this context, our results corroborate other studies that reported the mice from *Apodemus* genus to an effective bioindicator of non-essential metals and the effects of environmental pollution (TALMAGE & WALTON, 1991; BARGAGLI *et al.*, 1997; SHEFFIELD *et al.*, 2001; GONZÁLEZ *et al.*, 2006; SCHEIRS *et al.*, 2006; WIJNHOVEN et al., 2007; ROGIVAL *et al.*, 2007; AL SAYEGH PETKOVŠEK *et al.*, 2015). The fact that a particular small-mammal species accumulates larger amounts of heavy metals does not necessarily mean that this is the species most at risk of toxic effects from pollutants. Some species could be more sensitive to heavy metals than others, and storage and transformation of heavy metals to less harmful products in organs, such as the liver, could be a good mechanism to cope with toxicants (SHORE & DOUBEN, 1994).

The significant difference between Cd concentration in mice and voles might be particularly linked to their food preferences. The species share similar levels in the trophic chain (primary consumers), but are specialized to take different plant foods – mice are weevils and voles – phyllophagous. According to POKARZHEVSKIJ (1985) the concentration of a given element in animal organisms is practically directly

proportional to its content in the food. The yellow-necked mice feed mainly on seeds and fruits. The green parts of the plants, which are more dusted, are present in lower degree in their feeding. Mice supplement their diet with invertebrates – up to 20% of all food consumed (MARTINIAKOVA *et al.*, 2012). However, voles feed mainly on the green parts of the plants. They are polluted in the greatest degree and more accessible to precipitation and atmospheric dust. Particularly, with respect to Cd exposure, it was established that Cd is more readily taken up by plants than other metals, such as Pb (GOYER, 1996). This could be the reason for the leading position of *M. arvalis* in mean liver Cd bioaccumulation. According to the Pb concentration, voles were less loaded. They are characterized by good excretion and therefore, lower contaminant retention in the organism.

Biomonitoring of pollution through wild animals is crucial for the assessment of environmental quality and to improve our understanding of the response capacity of natural populations to pollution. The requirement for this systematic control is greater in protected areas inhabited by endangered species.

Conclusions

Based on the obtained results the following conclusions could be made:

1. The established bioaccumulation and the increased concentrations of Cd and Pb in the liver of the studied small rodents prove the existence of industrial pollution in the area of lead-zinc smelting factory - Plovdiv and testify to the presence of environmental risk.

2. The bioaccumulation of Cd and Pb in the liver of the studied small rodent species showed no gender effect.

3. Different degrees of bioaccumulation of Cd and Pb in the liver of the studied species were established:

- The investigated species of the Muridae family accumulate more Pb in the liver compared to the voles from the Arvicolinae subfamily;
- The investigated species of the Arvicolinae subfamily accumulate more Cd in the liver compared to species of the Muridae family.

4. Different levels of bioaccumulation in relation to the exposure of industrial pollution were registered. The highest concentrations of Cd and Pb were detected in species of green belt in the area of lead-zinc smelting factory, i.e. accumulation of heavy metals decreases with increasing distance from the smelter.

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Streptomyces levoris Immobilized on Silica Gel 60 as a Novel Biosorbent for Copper (II) Preconcentration

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Abstract. In the present study dead *Streptomyces levoris* biomass loaded on silica gel 60 was applied as an eco-friendly solid phase extractor for copper (II) preconcentration prior to its determination by flame atomic absorption spectrometry. The influences of different parameters such as pH of the sample solution, amount of solid phase, type and concentration of eluent, flow rate of sample solution, sample volume, and interfering effect of diverse ions on the preconcentration procedure were evaluated. An enrichment factor of 25 was achieved under optimum experimental conditions. The obtained results showed that *Streptomyces levoris* immobilized on silica gel can be considered as a promising new biosorbent for solid phase extraction of trace amounts of copper (II).

Key words: *Streptomyces levoris*, preconcentration, trace metal, atomic absorption spectrometry.

Introduction

Copper has received considerable attention because it is the most widely used chemical element in mechanical engineering, electronics, construction, metallurgy and chemical industry. Moreover, it is a trace element present in all tissues and is required for cellular respiration, peptide amidation, neurotransmitter biosynthesis, pigment formation, and connective tissue strength. Copper is a cofactor for numerous enzymes and plays an important role in the central nervous system development. Low concentrations of copper may result in incomplete development, however excess copper intake leads to serious health problems such as severe mucosal irritation, damages of capillary, hepatic, renal and central nervous system (DESAI & KALER, 2008; KALAVATHY *et al.*, 2005). For these reasons the precise determination of copper at low concentrations in natural water and biological samples is very important.

Various instrumental techniques like spectrophotometry, voltammetry, inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), X-ray fluorescence (XRF), electrothermal atomic absorption spectrometry (ETAAS) and flame atomic absorption spectrometry (FAAS) are used and continuously employed for determination of traces of heavy metal ions and copper, respectively. The ease of operation, high precision, selectivity, and low cost make FAAS one of the most

frequently used instrumental technique for determination of trace metals (SVRAKA *et al.*, 2014; NGEONTAE *et al.*, 2009). However, because of the high detection limits of FAAS (or low instrumental sensitivity), as well as the interfering effect of the sample matrix the determination of trace metals in water samples is usually associated with a step of preconcentration of analytes (SOYLAK *et al.*, 2005; BUDIZAK *et al.*, 2003). The most widely used preconcentration techniques include precipitation and co-precipitation (DURAN *et al.*, 2014; FEIST & MIKULA, 2014), liquid-liquid extraction (SARAN *et al.*, 1992; ABKENAR *et al.*, 2010), membrane filtration (SOYLAK *et al.*, 2004; DIVRIKLI *et al.*, 2007), ion exchange (KENEWY *et al.*, 2000), electrochemical methods (BULSKA, 2001), solid phase adsorption (SAÇMACI *et al.*, 2011; CHALAPATHI, 2012), etc.

The solid phase extraction is the most frequently used preconcentration technique and can be easily used with FAAS without laborious procedures. It offers several advantages such as flexibility in solid phase selection, low cost due to the minimal reagent consumption, high preconcentration factor, improved sensitivity, no requirements of toxic solvents, speed, simplicity and the ability for automatisation.

Inactive or dead microbial biomass can serve as a new biosorbent material capable to concentrate and recover heavy metals even when they are in concentrations less than 100 ppm. Different types of microorganisms (fungi, yeast, bacteria) and algae, immobilized on natural and synthetic adsorbents have been used for preconcentration, matrix separation, and speciation analysis of heavy metals in trace levels. These new biosorbents are not only selective, efficient and cheap, but they can be regenerated for multiple use or reutilized and are competitive with artificial resins and sorbents for metal concentration (PEREZ-CORONA *et al.*, 1997; BĂG *et al.*, 1998; BAYTAK & TÜRKER, 2004, 2005a; BAYRAMOGLU *et al.*, 2005; BAYTAK *et al.*, 2006; DOGRU *et al.*, 2007).

The *Streptomyces* genus belongs to Gram positive bacteria (EL-SAYED *et al.*, 2011). Three main components – peptidoglycan, teichoic acids and surface proteins, consist the *Streptomyces* cell wall. All of these polymers could play an important role in metal ions sequestration (CHOJNACKA, 2010; SAURAV & KANNABIRAN, 2011). The use of *Streptomyces* biomass as a sorbent in solid phase extraction is poorly studied. The first study is reported by YILDIZ *et al.* (2013). Their research proposes the use of *Streptomyces albus* immobilized on sepiolite as a biosorbent for preconcentration of Cd, Zn and Ni prior their analysis by flame atomic absorption spectrometry.

The aim of this study was to use *Streptomyces levoris*, loaded on silica gel, as an eco-friendly biosorbent for preconcentration of copper in water samples prior to its determination with FAAS.

Materials and Methods

Reagents and solutions

Deionized water was used to prepare all solutions. All solutions and chemicals used were of analytical reagent grade. The copper (II) working solutions were prepared daily by diluting a corresponding 1000 μg dm^{-3} solution (Merck). The pH values of the sample solutions were adjusted to a range of 2–9 with HCl or NH_3. The HCl and HNO_3 solutions used as eluents were prepared by direct dilution from the concentrated solutions. The laboratory glassware was kept overnight in a 5% (v/v) HNO_3. Afterwards, it was rinsed thoroughly with deionized water and dried.

Instrument

A Perkin-Elmer PinAAcle 900 T atomic absorption spectrometer was used. The apparatus was run in flame mode under the conditions suggested by the manufacturer, i.e.: wavelength, 324.8 nm; bandwidth of the slit, 0.7 nm; air/acetylene flow rates, 10 and 3.3 dm^3 min^{-1}. A pH meter model WTW inoLab pH 720 was used to measure the pH of the solutions.

Microorganism and growth conditions

Levorin producing strain *Streptomyces levoris* was provided by Department of Biochemistry and Microbiology, Plovdiv University "Paisii Hilendarski". Liquid culture medium was used for development of the culture with the following

composition in g dm^{-3}: glucose – 2.0; starch – 1.0; $(NH_4)_2SO_4$ – 0.8; KH_2PO_4 – 0.01; KCl – 0.1; $MgSO_4$ – 0.25; $CaCO_3$ – 0.3. For inoculation 2% (v/v) spore inoculum was used with 2.10^9 cm^{-3} concentration of the spores. The cultivation was conducted in 500 cm^3 Erlenmeyer flasks containing 50 cm^3 of the culture medium at pH 7.2, at 28±2°C on a rotary shaker at 220 rpm for 96 h. After 96 h of incubation, at the end of the exponential growth phase, the biomass was separated from the medium by vacuum filtration and heat inactivated at 120°C for 20 min at a pressure of 1 atm (STANCHEV *et al.*, 2010). The biomass was washed several times with deionized water until pH 6 was obtained then was treated with 0.1 mol dm^{-3} HCl solution for 30 min. The mixture was centrifuged at 4000 rpm, the resulting biomass was dried to constant weight at 80°C. The obtained biomass was stored at 4°C until further use.

Immobilization procedure

Streptomyces levoris was immobilized in Silica gel 60 (Merck 35–70 mesh). Before use the surface of the silica gel was activated – 4 g of silica gel and 30 cm^3 HCl (6 mol dm^{-3}), were heated at reflux while stirring for 8 hours, then the silica gel was filtered, washed with distilled water until neutral pH was achieved and dried under vacuum at 70°C for 8 h. Then 1g of the activated silica gel was mixed with 300 mg dry and dead biomass powder. The biomass was immobilized by the procedure proposed by MAHAN & HOLCOMBE (1992). The silica gel and biomass mixture was wetted with 2 cm^{-3} of water and thoroughly mixed. After mixing, the resulting paste was heated in an oven at 105°C for 1 h to dry the mixture. In order to accomplish maximum contact between the *Streptomyces levoris* biomass and the silica gel, and to improve the immobilization efficiency the wetting and drying steps were repeated several times. The resulted immobilized biosorbent was marked as SL.

Column preparation

The column used for the preconcentration was 1 cm in diameter and 10 cm long. A small amount of glass wool was placed at the both ends of the column in order to hold the immobilized biomass. The bed height of the SL biosorbent in the column was approximately 10 mm. The column was connected with a peristaltic pump (Masterflex, Cole-Parmer), which provide a continuous flow of the liquid samples. Before use, 1 mol dm^{-3} HCl solution and deionized water were passed through the column in order to clean and activate it. The column was conditioned to the studied pH by passing aqueous solutions of HCl or NH_3 with the same pH as the sample.

General preconcentration procedure

An aliquot of a sample solution containing 10 µg of Cu (II) was taken and the pH was adjusted to the optimum value. The resulting solution was passed through the column at a flow rate of 3 cm^3 min^{-1}. Then, the retained metal ions were eluted with suitable eluent, determined experimentally at flow rate of 1 cm^3 min^{-1}. The copper concentration was determined by FAAS. The biosorbent was used repeatedly after washing with 1 mol dm^{-3} HCl and deionized water. The analyte recovery was calculated from the ratio of the concentration found by FAAS to that calculated theoretically.

Results and Discussion

To obtain the maximum recoveries and to determine the applicability of the method, different parameters such as pH of sample solution, amount of biosorbent, type and concentration of eluent, sample volume and flow rate of sample solution were optimized. Interfering effects of common coexisting metal ions were also studied.

Effect of pH on recovery of copper (II)

The first variable optimized was the pH of the sample solution, because pH is the major factor influencing the metal biosorption process, the surface charge of the biosorbent and the solution chemistry of the metals. The effect of pH of the solution on the copper (II) recovery was studied at pH ranging from 2.0 to 9.0 and the obtained results are shown in Fig. 1.

Quantitative recoveries (>95%) were obtained at pH 6.0 – 7.0. At pH 6.0 the calculated recovery was 98±2.4%.

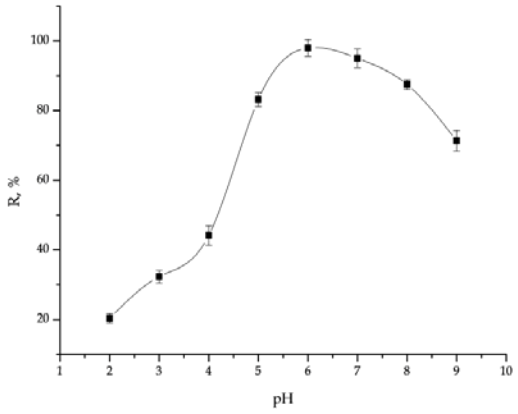

Fig. 1. Effect of pH on copper (II) recovery (sample volume –50 cm^3; amount of copper – 10 µg; eluent – 10 cm^3 of 1 mol dm^{-3} HCl solution; flow rate of sample – 3 cm^3 min^{-1}; flow rate of eluent – 1 cm^3 min^{-1}).

At lower pH, the copper recovery decreased, due to the electrostatic repulsion of the protonated active sites of the biosorbent with the positive charged copper species. The increasing of the pH caused the deprotonation of these groups and formed negatively charged sites for electrostatic attraction of the positively charged metal ions. For higher pH values, the retention decrease and at pH 9.0 the calculated recovery was 71.3±3%. This result could be explained with the competition between the hydroxylated complexes of the metal and active sites of the cell. Similar pH dependences were obtained with *Saccharomyces cerevisiae* immobilized on sepiolite for column preconcentration of Fe (III) and Ni (II) (BĂG *et al.*, 1998), and *Agrobacterium tumefacients* immobilized on Amberlite XAD-4 for iron (III), cobalt (II), manganese (II) and chromium (III) preconcentration (BAYTAK & TÜRKER, 2005b).

Effect of biosorbent amount

The amount of SL biosorbent is another important parameter that affects the metal recovery. A quantitative retention could not be obtained when the amount of solid phase is less, but an excess amount of sorbent prevents the quantitatively elution of the retained analyte by the small volume of the eluent (INKAYA *et al.*, 2010).

With the increasing of the mass of the SL biosorbent in the range from 100 to 400 mg, the recoveries of the metal ion also increased from 73±3.0 to 98.4±1.8%, respectively (Table 1). Therefore, 300 mg of immobilized biosorbent was used for subsequent experiments.

Table 1. Effect of amount of the immobilized biosorbent on copper (II) recovery (n=3).

Amount of biosorbent, mg	Recovery, %
100	73.0±3.0
200	85.2±2.2
300	98.0±2.4
400	98.4±1.8

Type and concentration of eluent

The effectiveness of the solid phase extraction depends on the type and the concentration of the eluent. The most frequently used eluents are acids and to prevent the biomass degradation it is preferable to be used solutions with lower concentrations (BAĞ *et al.*, 2000). Two inorganic acids, HCl and HNO_3, were examined as a suitable eluents. The effect of different volumes and concentrations of HNO_3 and HCl were tested to remove the absorbed copper (II) ions from the bacterial biomass loaded into the column. The obtained results (Table 2) showed that copper ions could be eluted efficiently with 10 cm^3 of 1 mol dm^{-3} HCl and this eluent was chosen in subsequent experiments.

Effect of flow rate

The flow rate of the sample solution through the column is another important parameter, since does not only affectthe recovery of the analyte, but also influences the analysis time. The recovery of the copper (II) ions was studied at flow rates from 2 to 6 cm^3 min^{-1}. The obtained results (Fig. 2) showed that flow rates in range from 1 to 4 cm^3 min^{-1} had no significant effect on the recoveries of the copper; this could indicate that the copper (II) sorption is a rapid process. At flow rate equal and higher than 5 cm^3 min^{-1}, the recovery decrease, because the copper ions cannot be sorbed sufficient-

ly. Optimal flow rate 3 cm^3 min^{-1} was determined. The obtained results are in accordance with other researches (BĂG *et al.*, 1998; BAYTAK & TÜRKER, 2005a, 2005b; BAYTAK *et al.*, 2005).

Table 2. Effect of type and volume of eluent on the recovery of copper (II) (n=3)

Type and concentration of elution solution	Volume, cm^3	Recovery, %
HCl, 0.5 mol dm^{-3}	5	44.3±2.2
	7	52.7±2.2
	10	74.1±1.8
HCl, 1 mol dm^{-3}	5	80.1±1.6
	7	93.6±1.4
	10	98.0±2.4
HNO_3, 0.5 mol dm^{-3}	5	38.1±1.4
	7	42.3±2.6
	10	58.0±4.2
HNO_3, 1 mol dm^{-3}	5	56.3±2.8
	7	60.5±2.2
	10	76.8±3.2

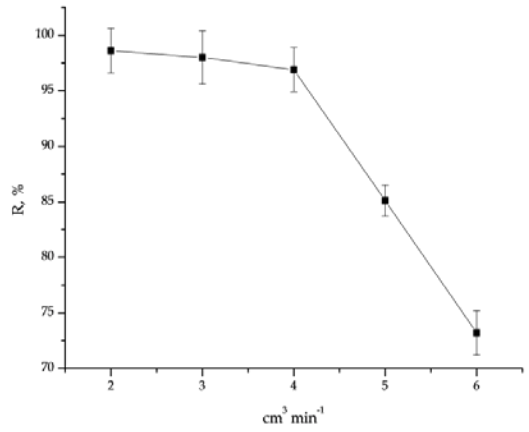

Fig. 2. Effect of sample flow rate on copper (II) recovery (pH 6.0; amount of copper – 10 µg; eluent – 10 cm^3 of 1 mol dm^{-3} HCl solution; flow rate of eluent – 1 cm^3 min^{-1}; sample volume –50 cm^3)

Effect of sample volume

The influence of the sample volume on the recoveries of the copper ions was also examined in order to determine the maximum applicable sample volume and the minimal analyte concentration. Copper (II) ions were preconcentrated, under optimal conditions (pH, eluent type, flow rate, etc.), from the sample solutions that containing 10 µg of copper in 50, 100, 250, 500 and 1000 cm^3, which corresponds to copper concentrations of 0.2, 0.1, 0.04, 0.02 and 0.01 µg cm^{-3}, respectively. The obtained results are shown in Fig. 3.

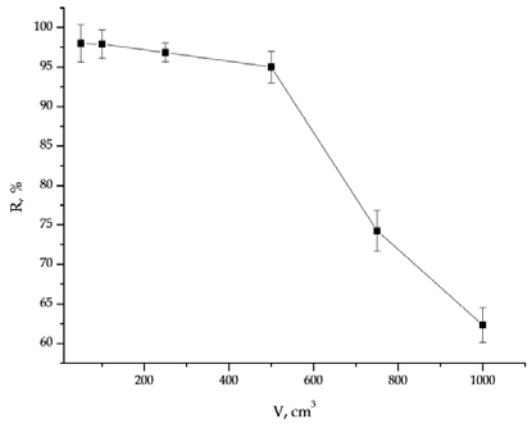

Fig. 3. Effect of sample volume on copper (II) recovery (pH 6.0; amount of copper – 10 µg; eluent – 10 cm^3 of 1 mol dm^{-3} HCl solution; flow rate of sample – 3 cm^3 min^{-1}; flow rate of eluent – 1 cm^3 min^{-1}).

The recovery of copper ions was quantitative (>95%) when the sample volumes were below 250 cm^3. At higher volumes, the recoveries decreased. Since the original sample volume and final volume of solution (after preconcentration) are 250 cm^3 and 10 cm^3, and an enrichment factor of 25 was achieved. These results showed that

copper could be determined even in concentration of 0.04 μg cm^{-3} by the proposed method, which could not be determined directly by FAAS with satisfactory precision.

Interference studies

One of the main problems in the atomic absorption spectrometric determination of heavy metal ions is the matrix interference. In water analysis the most common coexisting metal ions are Na^+, Ca^{2+}, Mg^{2+} and often their concentrations exceed 2-5 orders the concentration of the toxic metal. Therefore, the influence of interfering ions on the solid phase extraction of copper (II) ions was studied. Metal ions (Na^+, K^+, Ca^{2+}, Mg^{2+} and Zn^{2+}) were added individually to copper solution as their nitrate or chloride salts (NaCl, KCl, $CaCl_2$, $MgCl_2$, $Zn(NO_3)_2$) and the proposed preconcentration method was applied. No interfering effect was found on the recovery of the copper up to 250 mg dm^{-3} of Na^+, 5 mg dm^{-3} of Mg^{2+}, 100 mg dm^{-3} of K^+, 20 mg dm^{-3} of Ca^{2+} and 10 mg dm^{-3} of Zn^{2+} ions. At these concentrations of the coexisting metal ions the copper recoveries were 96±3, 95±2, 96±4, 95±3, 96±3, respectively. These data showed that the proposed preconcentration method could be applied to natural water samples that contain such ions at the tolerable levels listed above.

Column stability

At optimum experimental conditions the stability of the biosorbent loaded column was tested with several cycles of adsorption and desorption, done by passing 100 cm^3 of copper solutions through the column. Testing the column stability showed (Fig. 4) that the sorption capacity after 10 cycles of sorption and desorption does not vary more than 2.0%. Therefore, repeated use of the resin is possible.

Analytical figures of merit

To determine the detection limit (LOD) of the proposed method, 50 cm^3 of a blank solution (distilled water) was passed through the column and the retained metal ions were eluted with 50 cm^3 of 1 mol dm^{-3} HCl solution. This procedure was repeated 10 times.

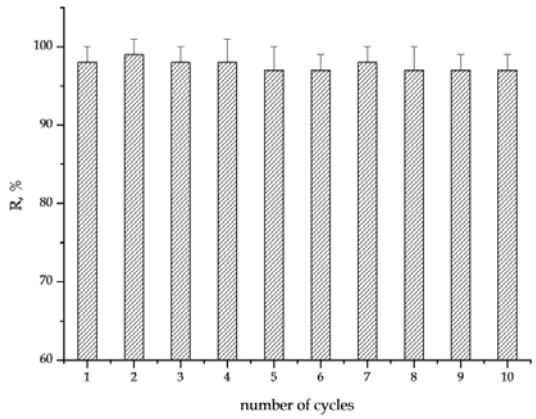

Fig. 4. Stability of SL column for preconcentration of copper (II) ions (pH 6.0; amount of copper – 10 μg; eluent – 10 cm^3 of 1 mol dm^{-3} HCl; flow rate of sample – 3 cm^3 min^{-1}; flow rate of eluent – 1 cm^3 min^{-1}; sample volume – 100 cm^3).

The LOD and the limit of quantification (LOQ) were calculated by using equations (1) and (2) (BĂG *et al.*, 1998; BAYTAK *et al.*, 2005):

$$LOD = [3\,\sigma / m] / PCF \quad (1)$$

$$LOQ = 3LOD \quad (2)$$

where: σ – standard deviation of the blank signal; m – slope of calibration curve; PCF – preconcentration factor.

The linear range for copper determination, without the preconcentration system was between 0.1 and 5 μg cm^{-3}. After preconcentration of the copper (II) ions a LOD of 1.39 μg dm^{-3} was found. The limit of quantification (LOQ) was calculated as 4.17 μg dm^{-3} by considering 3 times the LOD value. These concentrations of copper cannot be determined directly by FAAS with sufficient accuracy and precision.

For the determination of the repeatability of the method, six successive retention and elution cycles were performed with 100 cm^3 of sample solution containing 10 μg Cu (II) at the optimum conditions. The recovery of Cu (II) is quantitative – 98.0±2%, the calculated relative standard deviation was 2.04%.

Application

The proposed method was applied to preconcentrate and determine the copper (II)

ions content in spiked samples of mineral water. Water sample – a commercially available mineral water, was filtered through a Millipore cellulose membrane filter with 0.45 μm pore size. The pH of the filtered water sample was adjusted to 6.0 with 1 mol dm^{-3} HCl solution and the sample was passed through the column. The copper adsorbed on the SL biosorbent was eluted with 1 mol dm^{-3} HCl and the concentration of the analyte was determined by FAAS. The results are shown in Table 3.

Table 3. Determination of copper (II) in mineral water (sample volume 250 cm^3; mean of five determinations at 95% confidence level).

Added, μg dm^{-3}	Found, μg dm^{-3}	Recovery, %
-	7.9± 0.5	-
10	17.7± 0.2	99
20	27.6± 0.4	99

Conclusions

The proposed SPE procedure provides a simple, selective, accurate, precise and eco-friendly method for preconcentration and determination of copper in large volumes of various sample solution. The main advantages of the proposed procedure is the minimal consumption of reagents, low limits of LOD and LOQ, high tolerance to interfering ions quick and easy preparation of the extraction system, and a good preconcentration factor of 25. The operating stability of the system is good and no organic reagents are used. Disadvantages of the proposed preconcentration procedure are the narrow pH range for the quantitative recovery of the copper ions and the time needed to reach the preconcentration factor of 25, is in the order of 120 min.

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Composition and Abundance of Phytoplankton in Boggy Freshwater Lake, Turkey: In Relation to Physical and Chemical Variables

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Abstract. Lake Çalı (41° 12′ N, 43° 13′ E, Kars, Turkey) is a small (171273 m², 1 m depth), shallow and macrophyte-dominated semi-arid Mediterranean lake. Although it is called a Lake, in reality it is a small pond. The phytoplankton of Lake Çalı was studied from May to September 2010 at three sampling stations. Eighty-two phytoplankton taxa were determined, consisting of Cyanophyta (15), Chlorophyta (30), Euglenophyta (13), Bacillariophyta (19), Cryptophyta (2), Dinophyta (1) and Chrysophyta (2). Total phytoplankton density increased from May to August. The dominant phytoplankton group was Cyanophyta during the study period, followed by Bacillariophyta and Chlorophyta. Temparature, pH and dissolved oxygen range from 14.6 to 21.6 ^{0}C, 7.62 to 8.08, 5.96 to 7.46 mg/L, respectively. Chlorophyll *a* level ranged from 0.0035 to 0.0059 mg/L. On the other hand both phosphate and nitrogen levels tended to increase from May to July. The maximum and minimum densities of phytoplankton were 14034 org/mL (August) and 11885 org/mL (September), respectively, and this variable correleted significantly with temperature, pH, DO, SRP, TP, Cyanophyta, and Bacillariophyta.

Key words: Lake Çalı, phytoplankton, physico-chemical variable, boggy environment, pond.

Introduction

Throughout the world small, shallow ponds exist in different shapes, depths and sizes. They can occur seasonally, temporarily, or permanently. The environmental conditions that affect their trophic status and biota may also create small ponds (FAIRCHILD *et al.*, 2005; PETERYATKO *et al.*, 2007; SOINIEN *et al.*, 2007). There is a strong interrelation between number of species and habitat diversity. There is also high species diversity in shallow lakes and ponds because they are found in a large number of ecological niches (REYNOLDS, 1984; WETZEL, 2001; DUELLI & OBRIIT, 2003). Continuous mix in shallow ponds promotes algal growth, allow to algae to remain in suspension and exposing them to light (MESSYASZ *et al.*, 2005). In shallow ponds, phytoplankton communities generally exhibit a horizontal band close to the surface. The dense phytoplankton growth in this band appears in depths between 5-8cm and 45-50cm. The depth of the band depends on many factors such as water turbidity, nutrient supply and light intensity (MESSYASZ & JUGONSKA, 2003).

Although Turkish research on phytoplankton communities has focused predominantly on lakes, no phytoplankton studies have been conducted using data from Lake Çalı. The present study, therefore, is the first attempt to describe the seasonal abundance of phytoplankton in this

ecological system. The effects of physico-chemical parameters such as temperature, DO, pH, SRP, TP, NH_4-N, NO_3-N and chlorophyll *a* on the phytoplankton community have also been evaluated.

Material and Methods

Lake Çalı (41°12'N, 43°12'E) is a small (171273 m², 1 m depth), shallow and macrophyte-dominated (particularly *Myriophyllum spicatum* L. and *Potamogeton pectinatus* L.) semi-arid Mediterranean lake (Kars, Turkey), adjacent to the road between Kars and Digor that bisects the site (Fig.1). On the south there is a 15 ha permanent lake with reach submersed flora. The macrophytes cover may reach up to 50% in the growing season (summer). The area to the north of the road (approximately 10 ha) is seasonal. Although it is called a Lake, in reality it is a small pond. This small wetland lies in a vast elevated plateau, largely covered by extensively grazed steppe grassland. The site is one of the important bird habitats in Turkey. The lake is fed by seasonal springs. There is one small settlement on the shore and drinking water for Kars is pumped from a well nearby the lake (MAGNIN & YARAR; 1997).

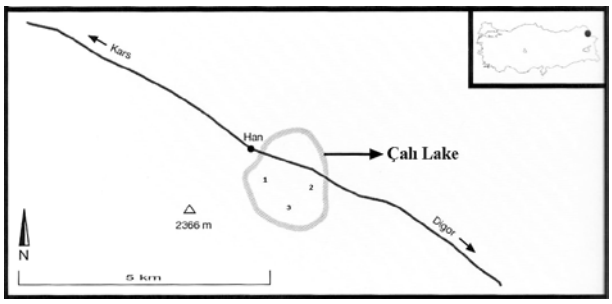

Fig.1. Map of the study area and sampling station.

Samples were collected monthly from May to September 2010 at three sampling stations scattered along the lake. In October, the Lake surface was completely covered by floating leaved macrophytes, leaves and debris, and it was not possible to take samples. Sampling was also not carried out during the winter because of the severe weather conditions. At each sampling station, water temperature, dissolved oxygen (DO), and pH were measured *in situ* with commercial meters. Water samples (1 litre) were also collected for analysis of soluble reactive phosphorus (SRP), total phosphate (TP), ammonium-nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N), following MACKERETH *et al.* (1978). Single subsurface phytoplankton samples were taken with a Van Dorn sampler and preserved with Lugol's solution immediately after sampling. Subsamples were examined and enumerated with an inverted microscope at a magnification of ×400, according to the method described by LUND *et al.* (1958). Standard texts were used for identification of phytoplankton species (LIND & BROOK, 1980; HARIS, 1986; KRAMER & LANGE-BERLATOT, 1991; CANTER-LUND & LUND, 1996). Chl*a* was extracted with acetone, and the concentration was calculated from the absorbance reading at 663 nm (TALLING & DRIVER, 1961). The Shannon-Wiener species diversity index (H') was calculated as follows:

$$H' = -\Sigma P_i (\ln P_i)$$

where P_i represents the proportion of each species in the sample.

The dominance (%) values were calculated for each species as mean density of species divided by mean total density of phytoplankton and multipled by 100. Avarage values were used for all variables in results section. Principal Component Analysis (PCA) was performed using SPSS 22.0 to assess the influence of the physico-chemical variables on the abundance of phytoplankton.

Results

A total of 82 phytoplankton taxa were identified in Lake Çalı. Throughout the study period, the dominant group was Cyanophyta, followed by Chlorophyta and Bacillariophyta. The 11 taxa that made up more than 5% of the total phytoplankton biomass were members of seven functional groups (FGs) (Table 1). Within Cyanophyta, three species exceeded 10% dominance: *Anabaena munitissima*, *Nostoc sp.* and *Lyngbya concorta.* For the Chlorophyta, global

dominance > 20% for most of the study period, with only three taxa (*Monoraphidium irregulare, Pediastrum simplex, Scenedesmus communis*) exceeding 5% dominance. Another important group was Bacillariophyta, where only one taxa (*Fragilaria crotonensis*) reached the 10% dominance value. The Shannon-Wiener species diversity index, which is based on the number of individuals, ranged from 3.11 (May) to 3.89 (August) (Table 2).

The physico-chemical variables of Lake Çali ranged from 7.62 (May) to 8.08 (August) for pH, from 14.6 ˚C (September) to 21.6 ˚C (June) for temperature, from 5.96 (June) to 7.46 (May) mg/L for DO, from 0.0035 (May) to 0.0059 (June) mg/L for Chl *a*, from 0.145 (September) to 0.442 (June) mg/L for NO_3-N, from 29.18 (May) to 63.76 (June) µg/L for NH_4-N, from 58.2 (September) to 234.3 (July) µg/L for TP, and from 49.1 (September) to 147.1 (July) µg/L for SRP (Fig. 2).

The density of phytoplankton ranged from 11885 (September) to 14034 (August) org/mL (Fig. 3). This variable correlated significantly with temperature ($p<0.05$), pH ($p<0.05$), DO ($p<0.05$), SRP ($p<0.05$), TP ($p<0.005$), Cyanophyta ($p<0.05$), and Bacillariophyta ($p<0.001$); however, the density of phytoplankton did not correlate significantly with the other variables ($p>0.05$ in all cases).

Table 1. Main phytoplankton taxa with the corresponding functional groups in Lake Çalı.

Functional group	Taxon
C	*Cyclotella meneghiniana*
H_1	*Anabaena flos-aquae, Anabaena minutissima*
J	*Pediastrum simplex, Scenedesmus communis*
S_1	*Lyngbya concorta, Planktothrix sp.*
S_2	*Nostoc sp., Spirulina sp.*
P	*Fragilaria crotonensis*
X_1	*Monoraphidium irregulare*

Among the three most abundant groups in the lake, Cyanophyta strongly correlated with temperature, Chlorophyta strongly correlated with pH, and Bacillariophyta strongly correlated with SRP ($p<0.01$ in all cases). Correlation with NH_4-N ($p<0.05$), and TP ($p<0.05$) was also significant for Bacillariophyta. On the other hand, no significant association with NO_3-N was evident for any of the three most abundant groups. Likewise, no significant relation with NH_4-N was found for either Chlorophyta or Cyanophyta (Fig. 4).

Table 2. Species composition, dominance values and divesity (H': Shannon-Wiener diversity index) of phytoplankton in Lake Çalı.

	Dominance (%)					
	Taxa	**May 2010**	**June 2010**	**July 2010**	**Aug. 2010**	**Sept. 2010**
	CYANOPHYTA	56.07	51.31	43.86	43.55	46.12
1	*Anabaena minutissima*	20.58	8.12	3.87	3.27	2.17
2	*Anabaena flos-aquae*	3.71	13.46	6.03	6.00	9.93
3	*Anabaena oscillariodes*	1.52	1.52	1.26	2.69	2.67
4	*Anabaena spiroides*	1.63	2.15	1.67	2.83	2.50
5	*Anabaenopsis elenkinii*	2.71	1.90	1.02	1.56	0.87
6	*Chroococcus minor*	0.85	1.19	2.45	1.60	1.59
7	*Chroococcus turgidus*	0.71	0.49	0.43	0.96	0.94
8	*Gloeocapsa sp.*	1.52	0.62	0.48	1.63	1.57
9	*Lyngbya concorta*	2.49	6.74	6.47	6.94	16.67
10	*Lyngbya sp.*	2.94	1.39	1.44	3.57	3.24
11	*Nostoc sp.*	14.98	6.13	4.56	3.68	1.99
12	*Oscillatoria brevis*	2.41	1.63	1.27	1.24	1.14

13	*Phormidium sp.*	0.60	0.31	0.49	1.41	1.56
14	*Planktothrix sp.*	3.47	1.04	0.91	5.12	2.86
15	*Spirulina sp.*	1.07	2.26	1.49	5.86	1.15
	CHLOROPHYTA	19.80	20.15	26.80	33.75	27.95
16	*Ankistrodesmus falcatus*	0.02	0.07	0.23	0.31	0.40
17	*Ankistrodesmus gracilis*	0.002	0.06	0.25	0.33	0.66
18	*Botryococcus branuii*	0.05	0.006	0.20	0.40	0.74
19	*Chlamydomonas regularis*	0.17	0.34	0.09	0.20	0.57
20	*Chlamydomonas vulgaris*	0.002	0.02	0.05	0.10	0.06
21	*Chlamydomonas sp.*	2.11	0.99	1.04	2.00	1.31
22	*Cladophora sauteri*	0.22	0.27	0.70	1.77	1.31
23	*Closterium calosporum*	0.42	0.15	0.96	1.61	1.19
24	*Closterium ehrenbergii*	0.08	0.24	0.34	0.38	0.56
25	*Closterium sp.*	0.008	0.05	0.06	0.12	0.08
26	*Coelastrum cambricum*	0.05	0.04	0.25	0.55	0.56
27	*Cosmarium subcrenatum*	0.07	0.11	0.11	0.15	0.08
28	*Elakatothrix gelatinosa*	0.005	0.009	0.02	0.02	0.009
29	*Gloeocystis sp.*	0.17	0.04	0.52	0.37	0.69
30	*Lagerheimia sp.*	0.05	0.15	0.32	0.35	0.61
31	*Monoraphidium irregulare*	3.56	3.10	4.50	7.61	6.36
32	*Oocystis elliptica*	0.00	0.05	0.24	0.36	0.58
33	*Oocystis sp.*	0.04	0.28	0.29	0.56	0.47
34	*Pediastrum duplex*	0.05	0.02	0.04	0.07	0.04
35	*Pediastrum simplex*	6.33	4.39	3.61	6.72	3.97
36	*Scenedesmus acuminatus*	0.42	0.31	0.25	0.26	0.13
37	*Scenedesmus arcuatus*	0.69	0.59	0.51	0.61	2.69
38	*Scenedesmus armatus*	0.36	0.36	0.15	0.43	0.47
39	*Scenedesmus communis*	4.39	4.11	3.67	7.94	2.92
40	*Scenedesmus falcatus*	0.45	0.40	0.08	0.21	0.30
41	*Scenedesmus quadricauda*	0.63	0.59	0.51	0.62	1.65
42	*Schroederia setigera*	0.15	0.10	0.11	0.12	0.09
43	*Staurastrum cingulum*	0.00	0.004	0.01	0.03	0.10
44	*Staurastrum denticulatum*	0.02	0.03	0.03	0.06	0.04
45	*Tetrastrum triangulare*	0.93	0.53	0.14	0.33	0.48
	EUGLENOPHYTA	0.11	0.11	0.12	0.19	0.21
46	*Euglena gracilis*	0.05	0.05	0.06	0.06	0.05
47	*Euglena oxyuris*	0.04	0.02	0.03	0.06	0.09
48	*Euglena spirogyra*	0.00	0.00	0.001	0.005	0.003
49	*Euglena tripteris*	0.00	0.002	0.003	0.002	0.00
50	*Euglena sp.*	0.00	0.002	0.001	0.007	0.003
51	*Lepocinclis sp.*	0.00	0.00	0.001	0.005	0.006
52	*Phacus caudata*	0.00	0.002	0.001	0.002	0.006
53	*Phacus granum*	0.00	0.00	0.001	0.07	0.006
54	*Phacus longicauda*	0.00	0.002	0.001	0.01	0.006
55	*Phacus sp.*	0.00	0.00	0.001	0.007	0.006
56	*Trachelomonas armata*	0.01	0.02	0.001	0.01	0.003
587	*Trachelomonas caudata*	0.00	0.002	0.003	0.005	0.01
58	*Trachelomonas sp.*	0.002	0.00	0.001	0.005	0.006
	BACILLARIOPHYTA	22.98	27.35	27.79	21.10	24.94
59	*Amphora ovalis*	0.00	0.00	0.007	0.01	0.01
60	*Asterionella sp.*	0.008	0.01	0.02	0.01	0.01
61	*Cyclotella meneghiniana*	1.00	9.60	4.35	2.31	0.77

62	*Cyclotella kützingiana*	0.71	0.45	1.18	2.42	0.97
63	*Cymbella microcephala*	0.13	0.13	0.23	0.37	0.45
64	*Cymbella minuta*	0.25	0.24	0.28	0.54	0.56
65	*Diatoma vulgare*	0.03	0.05	0.05	0.11	0.11
66	*Fragilaria crotonensis*	10.32	2.84	2.79	4.96	14.41
67	*Gomphonema angustatum*	2.95	5.05	5.17	2.47	1.71
68	*Gomphonema truncatum*	3.24	4.00	3.24	2.68	1.83
69	*Navicula lanceolata*	1.07	0.73	0.71	1.69	1.18
70	*Navicula minuscula*	0.52	0.31	0.54	1.19	0.89
71	*Navicula sp. 1*	0.49	0.41	0.38	0.84	0.80
72	*Navicula sp. 2*	0.80	0.47	0.49	0.89	0.53
73	*Nitzschia sp.*	0.40	0.36	0.32	0.45	0.52
74	*Stephanodiscus hantzschii*	0.36	0.48	0.39	0.60	0.81
75	*Surirella sp.*	1.55	0.17	0.28	046	0.53
76	*Synedra capitata*	0.77	0.41	0.47	0.70	0.75
77	*Tabellaria sp.*	0.43	0.32	0.50	0.69	0.61
	CRYPTOPHYTA	0.83	0.71	0.55	0.70	0.35
78	*Chroomonas sp.*	0.58	0.52	0.45	0.61	0.35
79	*Cryptomonas ovata*	0.25	0.18	0.09	0.08	0.00
	CHRYSOPHYTA	0.28	0.28	0.47	0.84	0.49
80	*Dinobryon sp.*	0.19	0.18	0.34	0.70	0.40
81	*Tribonema sp.*	0.08	0.09	0.12	0.13	0.08
	DINOPHYTA	0	0.04	0.02	0.01	0.01
82	*Peridinium sp.*	0.00	0.004	0.02	0.01	0.01
	Diversity index H'	3.11	3.43	3.71	3.89	3.26

Discussion

In the present study, total phytoplankton density increased from May to August in Lake Çalı, then decreased during September (Fig. 2). Throughout the study period, the dominant phytoplankton group in Lake Çalı was Cyanophyta. This disagrees with a number of studies performed in Turkish lakes that have identified diatoms (Bacillariophyta) as a dominant group (AYKULU *et al.*, 1983; OBALI, 1984; ÜNAL, 1984; ALTUNER, 1984; GÖNÜLOL & OBALI, 1986; ELMACI & OBALI, 1992; 1999; TEMEL, 1997; ŞAHIN, 1998; KILINÇ, 1998; AKBULUT & AKBULUT, 2000; ŞAHIN, 2000). On the other hand, results similar to the present study have been reported by ÖZEMSI (1987) and YILDIZ *et al.* (1999) who found Cyanophyta to be a dominant group in Sultan Sazlığı and Hotamış Sazlığı, which are both Turkish wetlands that are as important as Lake Çalı. In both studies, within Cyanophyta, Anabaena, Nostoc, Oscillatoria, Lyngbya and Spirulina species were similar to those of Lake Çalı. Similarly, in Lake Aktaş (ÖZBAY & KILINÇ, 2008) and the River Kars (ÖZBAY, 2011), which are both in the same region as Lake Çalı, Cyanophyta have been found as a dominant phytoplankton.

It is well known that phytoplankton density increases due to eutrophication (SEABORN, 1997; PETR *et al.*, 2004). High concentrations of cyanobacteria, chlorophytes, cryptophytes, and diatoms are frequently associated with the eutrophic condition in ponds (OLADIPO & WILLIAMS, 2003; HARSHA & MALAMMANAVAR, 2004; PERETYATKO *et al.*, 2007). In general, Lake Çalı had sufficient nutrients for phytoplankton growth during the study period. Nutrient levels in the lake increased from May to July for TP and SRP and from May to June for NH_4-N and NO_3-N due to the uninterrepted mixing of the lake, which moves the nutrients from the bottom to the surface. After June and July, nutrient levels decreased due to the increasing growth of both macrophytes and phytoplankton. In this study, the total phytoplankton density tended to increase according to rising nutrient levels in the Lake from May to July.

This is in agreement with the majority of studies that indicate that phytoplankton density increases due to seasonal succession (AYKULU & OBALI, 1981; CIRIK-ALTINDAĞ, 1982; GÖNÜLOL & OBALI, 1986; CIRIK *et al.*, 1989; CIRIK & CIRIK, 1989; GÖNÜLOL & ÇOMAK, 1992; ŞEN *et al.*, 1994; YILDIZ *et al.*, 1999; ÖZBAY & KILINÇ, 2008) in Turkish freshwater systems. Similarly, some studies have found low phytoplankton numbers in low-nutrient concentration waters (CHESSMAN, 1985; DIMITROVIC *et al.*, 2007).

LAUREN (2007, USA, pers. comm.) suggested that nitrate was the limiting factor for phytoplankton growth in the Duwamish River, USA. In this study, however, no nutrient limitation has been identified, but it is likely that phosphate was the primary nutrient for phytoplankton growth. This is in line with studies that have suggested that phosphorus rather than nitrogen is the limiting factor in freshwater ecosystems (WHEELER & NEUSHAL, 1981; HECKY & KILHAM, 1988). Similarly, FAIRCHILD *et al.* (2005), in a study of 13 eutrophic ponds, reported that the phytoplankton biomass was directly correlated with the total phosphorus and negatively correlated with the Secchi depth. MCMASTER *et al.* (2005) also associated phytoplankton abundance and composition with total phosphorus, total nitrogen and conductivity in Alpine ponds.

Dissolved oxygen yielded negative scores for all the variables tested in this study. Although Chl *a* levels increased with the increase in nutrients, the lack of correlation that was demonstrated between the chlorophyll *a* level and the biomass of phytoplankton or any of the phytoplankton groups may be explained by other factors.

The structure of phytoplankton communities not only depends on grazing pressure but also on nutrient and light availability (REYNOLDS, 1988). As a result, species belonging to similar functional groups can in turn be classified as having one of three basic adaptive strategies: (1) C (colonist-invasive), (2) S (stress-tolerant) and (3) R (ruderal). The succession of phytoplankton in Lake Çalı began with S-strategist in May, and they remained dominant at the end of the study. Since the lake macrophytes dominated, strong competition should be take place for nutrient and light between phytoplankton and macrophytes during the study period. Therefore, because of the S-strategist (Cyanophyta in this study), as a stress-tolerant group, might be compete with the macrophytes better than others, and became dominant group in the lake from the beginning to the end of the study. This group was followed by R-Strategists (Bacillariophyta). Cyanophyta, Bacillario-phyta and Chlorophyta were the most abundant groups of phytoplankton in Lake Çalı. Within these three groups, 11 species exceeded the 5-20% dominance: *Anabaena minutissima*, *Nostoc sp.*, *Lyngbya concorta, Monoraphidium irregulare, Pediastrum simplex, Scenedesmus communis, Fragilaria crotonensis, Anabaena flos-aquae*, *Spirulina sp.*, *Planktothrix sp.*, and *Cyclotella meneghiniana.*

KEMP (2009, USA, pers. comm.) indicated that in the Cyanophyta community, the abundance of non-heterocytic (non N-fixing) species decreases with reduced inorganic N. This is in contrast to heterocytic (N-fixing) species such as *A. munitissima* and *A. elenkinii* in both the Yangebup and Bibra Lakes. In this study, there was also an abundance of *A. munitissima* in May when the inorganic N content was low.

Field studies indicate that large colonies of *A. flos-aquae* are associated with an increase in water transparency (HOLM *et al.*, 1983). In Lake Çalı, *A. flos-aquae* reached a high density in June and September because the high submersed macrophyte density served to increase water transparency in both months. Although there was no Secchi depth measurement in this study, AKPINAR (2011, Turkey, pers. comm.) reported the Secchi depth as 74 cm for June and 63 cm for September in Lake Çalı, both values are high enough to reach the light to the bottom of the lake.

The non N_2 fixing blue green algae, especially those that belong to the Oscillatoriaceae family, are usually a nuisance in shallow eutrophic waters (SCHIFFER, 2004). Members of Oscillatoriaceae

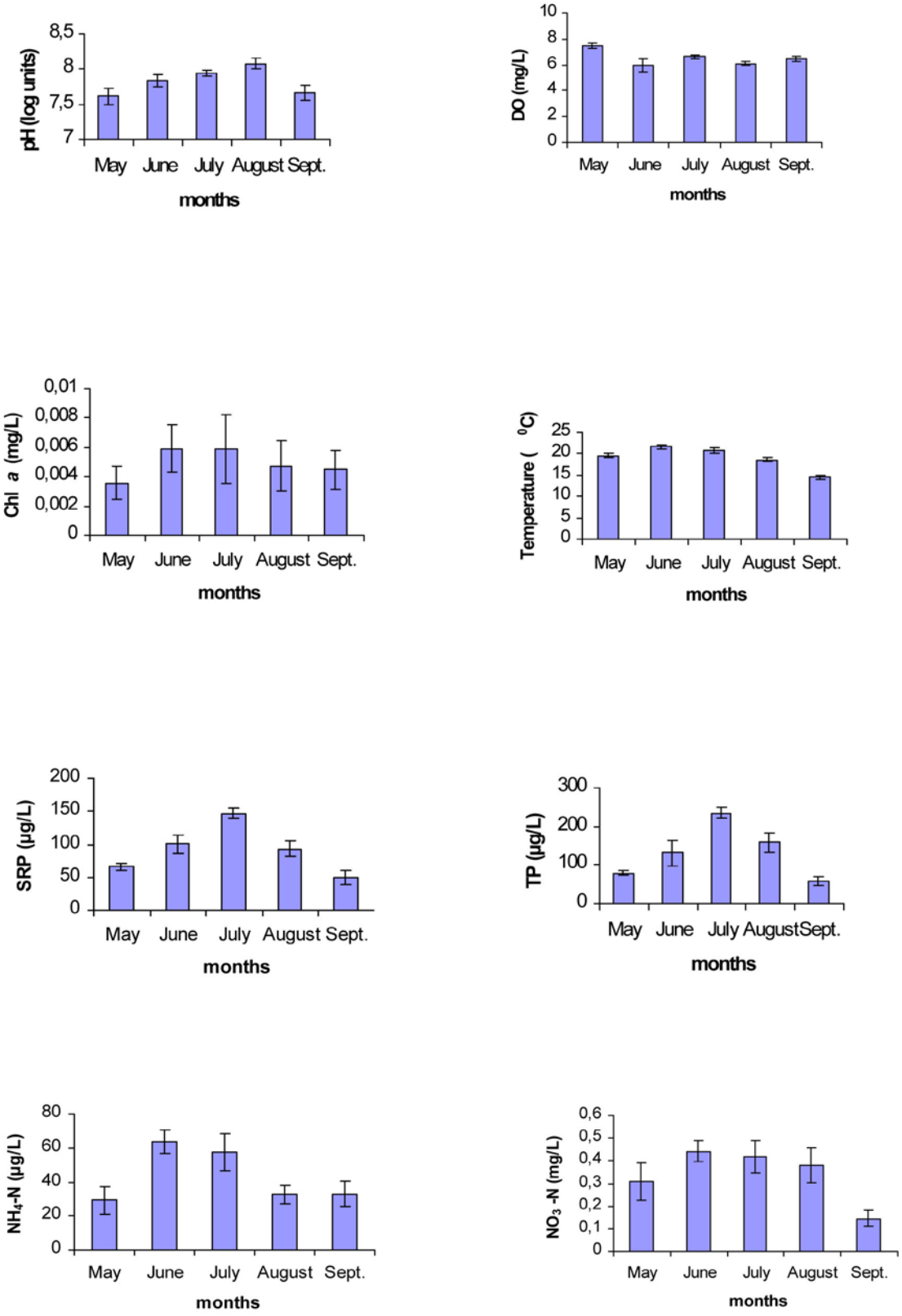

Fig. 2. Avarage values ±SD of physico-chemical variables and chl *a* concentration in lake water during the study. See text for abbreviations.

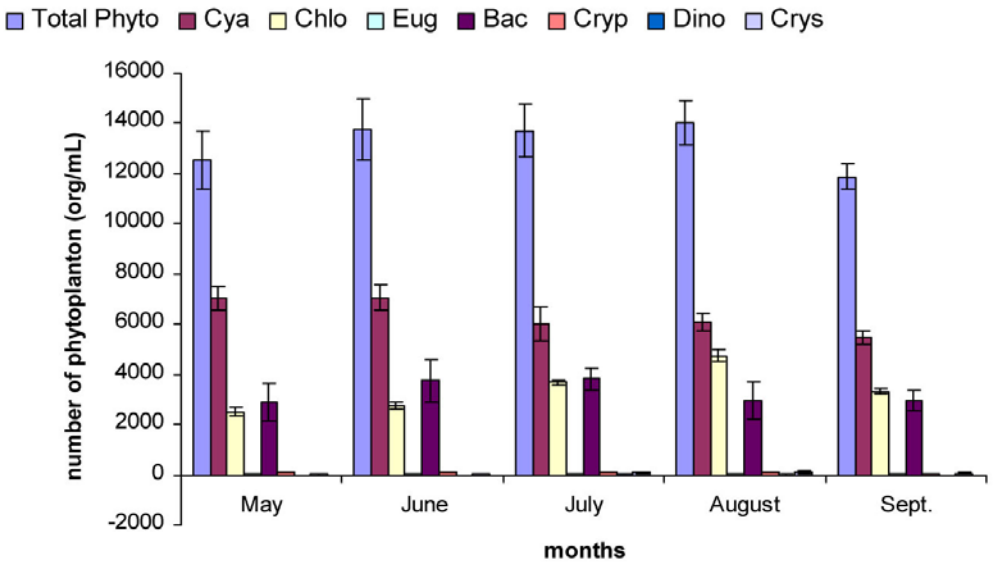

Fig. 3. Average density of phytoplankton groups (org/mL) in Lake Çalı during the study.

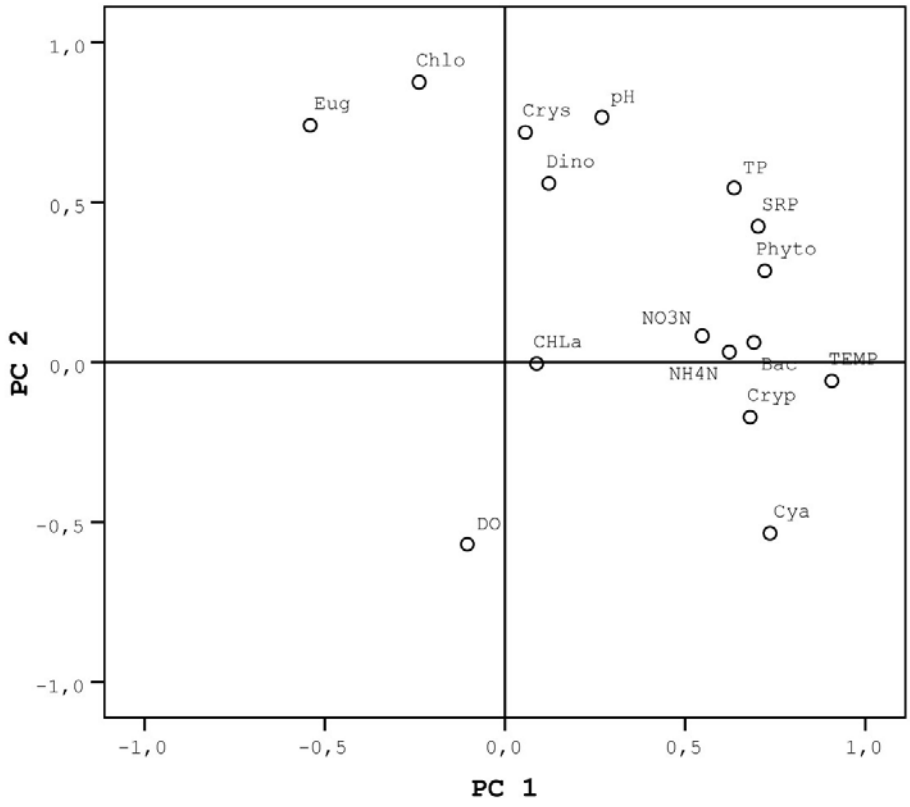

Fig. 4. Principal component analysis (PCA) of physico-chemical variables and main phytoplankton groups. *Abbreviations*: CHL a, chlorophyll *a*; DO, dissolved oxygen; NH_4-N, ammonia nitrogen;NO_3-N, nitrate nitrogen; TP, total phosphate; SRP, soluble reactive phosphorus; TEMP, temperature; Cya, Cyanophyta; Chlo, Chlorophyta; Eug, Euglenophyta; Bac, Bacillariophyta; Cryp, Cryptophyta; Dino, Dinophyta; Cry, Chrysophyta; Phto, total phytoplankton.

may reach a high biomass in eutrophic water, but they cannot be densely abundant on the water surface. This is attributed to their ability to maintain high growth rates at low light conditions (LOEB & RUETER, 1981). Therefore, it is possible that they become dominant phytoplankton in spring and fall when there is wind-drive mixing in shallow waters. This might be the cause in Lake Çalı, because *L. concorta, Planktothrix sp.,* and *Spirulina sp.,* all species belonging to the Oscillatoriaceae family, reached maximum densities in August and September. Research on Lake Shira (Siberia) suggested that the photoheterotrophic capability of *L. concorta* might help to explain its development in deeper waters, where light availability is near the light compensation point (QUESADA *et al.,* 2002). The photoheterotrophic communities are able to assimilate organic compounds, thus supplementing their carbon and energy requirements. A negative relationship has been found between the uptake of organic compounds and light intensity in Lake Shira (DEGERMENDZY & GULATI, 2002). In Lake Çalı, *L. concorta* reached the maximum in September (16.67%), likely due to the increase in organic compounds (e.g., macrophyte decay). Although there is no data for COD (Chemical Oxygen Demand) and BOD (Biochemical Oxygen Demand) in this study, AKPINAR (2011, Turkey, pers. comm.) measured highest conductivity, 126 μScm^{-2}, in September for Lake Çalı, probably because of the increasing decomposition process.

Dominance % of *Cyclotella meneghiniana* was found to be high in June (9.60%), when both the NH_4-N and NO_3-N levels were high in Lake Çalı. Correlation with NH_4-N ($p< 0.05$) was also found significant for Bacillariophyta in Lake Çalı. The Cyclotella species have been commonly found in some other eutrophic lakes in Turkey (AKBAY *et al.,* 1999; KIVRAK & GÜRBÜZ, 2010; FAKIOĞLU & DEMIR, 2011).

Fragilaria crotonensis was found to be dominant in August (7.61%) in Lake Çalı. Species of Fragilaria are tolerant of carbon dioxide depletion and tend to be present more often in eutrophic waters (REYNOLDS *et al.,* 2002). High nutrient levels in Lake Çalı in July might be the reason for finding dominant *Fragilaria crotonensis* in August.

Non-gelatinous, non-motile Chlorococcales is predominant in shallow highly enriched systems represented by *Scenodesmus*, *Pediastrum* and *Coelastrum* (REYNOLDS *et al.,* 2002). In Lake Çalı, both *Pediastrum simplex* and *Scenedesmus communis* became dominant in August following high nutrient levels in July. *Monoraphidium* species also associate with eutrophic water, and in Lake Çalı *Monoraphidium irregulare* was found dominant in August, like the *Pediastrum simplex* and *Scenedesmus communis* species. The initial addition of nutrients shifted the algal composition toward rapid-growing algae from functional groups X_1, J and Y, all presenting characteristic of shallow enriched freshwaters, and as Lo described for stratified water (REYNOLDS *et al.,* 2002). According to ROMO & VILLENA (2005), small, rapidly growing species belonging to the functional grous X_1, Y, J and Lo showed direct increment with higher biomass related to higher nutrient levels in a shallow Mediterranean lake (Spain). This result agrees with the present studies. According to REYNOLDS *et al.* (2002), X_1, Y, and J are potentially more highly grazed by zooplankton, but not all species showed the same response. According to ROMO & VILLENA (2005), the *Scenedesmus* species (J group) was significantly more abundant in mesocosms with presence of larger zooplankton, although the small *S. ecornis* is better developed with microzooplankton. Unfortunately, there is no opportunity to adopt this idea into the present study because of the lack of studies on zooplankton and fish species in Lake Çalı.

In conclusion, Cyanophyta was the dominant group in the phytoplankton of Lake Çalı; however, in contrast, of most of the studies have that found Bacillariophyta was the dominant group in phytoplankton in Turkish lakes. On the other hand, Lake Çalı is a boggy environment and there is limited information about phytoplankton diversity in such environments in Turkey. Thus, more studies are needed to

understand and compare the structure and ecology of the phytoplankton in boggy systems within Turkey.

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Phenolic Profile and Antioxidant Activity of Methanolic Extract of Carduus acicularis Bertol. (Asteraceae)

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Abstract. Phenolic acid and flavonoid profiles of *Carduus acicularis* were investigated for the first time. Eleven phenolic acids and eight flavonoids were identified and quantified in the inflorescences, by high performance liquid chromatography. The main phenolic compounds were found to be: sinapic acid (930.41 ± 21.72 μg/g dw), chlorogenic acid (582.66 ± 13.60 μg/g dw), rutin (545.65 ± 12.82 μg/g dw), apigenin (478.75 ± 11.38 μg/g dw), luteolin (288.46 ± 6.86 μg/g dw) and myricetin (276.32 ± 5.21 μg/g dw). The antioxidant activity of methanolic extract of inflorescences has been investigated, employing four different established testing systems: scavenging activity on 2,2-diphenil-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonate (ABTS) radical cation decolorization assay, ferric reducing antioxidant power (FRAP) and copper reduction antioxidant assays (FRAP). The highest antioxidant activity values were measured by the ABTS assay, among all performed methods.

Key words: *Carduus acicularis,* phenolic acids, flavonoids, HPLC analysis, antioxidant activity.

Introduction

Carduus acicularis Bertol. is one of the Bulgarian representatives of the genus *Carduus* from family *Asteraceae*. This species is an annual plant, 20-60 cm high, growing in grassy areas up to 800 m altitude. Its distribution in Bulgaria covers the Black Sea Coast, Mountain Strandzha, Valley of Struma River, Thracian Lowland and Tundzha Hilly Valley (DELIPAVLOV & CHESHMEDZHIEV, 2003). Some species of genus *Carduus* are medicinal plants with different applications mainly in folk medicine (PETKOV, 1982).

Previous phytochemical studies on the genus *Carduus* show the presence of different groups of substances - phenolics, terpenoids, sterols, volatiles, alkaloids and few other compounds (JORDON-THADEN & LOUDA, 2003). Phenolics are the most studied secondary metabolites, due to their role as antioxidants and positive influence on the human health (BECKMAN, 2000; GRAF *et al.*, 2005). That was the reason for the beginning of phytochemical studies on Bulgarian representatives of the genus *Carduus* for examining the presence of phenolic compounds (ZHELEV *et al.*, 2011; 2013; SLAVOV *et al.*, 2014). Moreover, the initial screening of several *Carduus* species, which grow in Bulgaria, for radical scavenging and antioxidant activity,

revealed that they could be evaluated as a rich source of antioxidants (ZHELEVA-DIMITROVA *et al.*, 2011).

The objective of the present study was to identify and quantify some of the widespread phenolic compounds (flavonoids and phenolic acids) by HPLC analysis, and to evaluate the related total antioxidant potential of methanolic extract from *Carduus acicularis*.

Material and Methods

Plant material

Inflorescences (flower heads) of *C. acicularis* were collected from a natural habitat (*Black Sea Coast* floristic region) in Bulgaria, during the 2014 vegetative season and then they were air-dried in darkness at room temperature. Species identification was carried out at the Department of Botany of the University of Plovdiv "Paisij Hilendareski", according to TUTIN *et al.* (1976) and DELIPAVLOV & CHESHMEDZHIEV (2003). Voucher specimen of this species was deposited in the Herbarium at the Agriculture University of Plovdiv, Bulgaria (Herbarium SOA - 059650).

Preparation of plant extracts

Dried plant material was grounded and 0.5 g of the accurately weighed sample was refluxed exhaustively three times with 70 % (v/v) methanol at 70ºC for 30 min. The extracts were combined and made up to 30 ml with methanol in a volumetric flask.

HPLC analysis

The HPLC analysis were performed by a Waters HPLC system, (Milford, MA, USA) equipped with binary pump (Waters 11525), a UV-VIS detector (Waters 2487) and Breeze 3.30 SPA software. Detailed conditions of HPLC analyses are reported previously (MARCHEV *et al.*, 2011). Concentration of each individual compound was calculated, basing on external standard method, and converted to µg compound per g dry weight (dw).

DPPH free-radical scavenging activity

This assay is based on the bleaching of purple colored methanol solution of DPPH. The DPPH radical scavenging activity was determined, following the method of BRAND–WILLIAMS *et al.* (1995). Freshly prepared $4x10^{-4}$ M methanolic solution of DPPH was mixed with the sample in a ratio of 2:0.5 (v/v). The reaction was performed at 37°C in a dark place. The light absorption was measured at 517 nm and the antiradical activity of samples was calculated and represented as function of the concentration of Trolox. The unit of Trolox equivalent antioxidant capacity (TEAC) was defined as the concentration of Trolox having equivalent antioxidant activity expressed as mMTE/g dry plant material.

ABTS radical cation decolorization assay

The total antioxidant activity of the sample was measured by improved ABTS radical cation decolorization assay, according to the method of RE *et al.* (1999). TEAC was defined as the concentration of Trolox having equivalent antioxidant activity expressed as mMTE/per gram dry weight (dw).

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to BENZIE & STRAIN (1999). The FRAP reagent was prepared fresh daily and was warmed to 37°C prior to use. 150 µl of plant extracts were allowed to react with 2850 µl of the FRAP reagent for 4 min at 37°C and the absorbance was recorded at 593 nm. The results were expressed as mM/TE g dw.

Copper reduction antioxidant assay (CUPRAC)

CUPRAC assay was performed according to the method of AK & GÜLÇIN (2008). 1 ml of $CuCl_2$ solution (1.0×10^{-2}M), 1 ml of neocuproine methanolic solution (7.5×10^{-3} M), and 1 ml NH_4Ac buffer solution (pH 7.0) were added to a test tube and then mixed; 0.1 ml of herbal extract (sample) followed by 1 ml of water were added (total volume = 4.1 ml), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of extracts was expressed as mM/TE g dw.

Statistical analysis

The presented results are average from two independent experiments carried out in triplicates. The results were expressed as

Mean ± SD and statistically analyzed using MS Excel 2003 software.

Results

Flavonoid and phenolic acid profiles

Six flavonoid aglycons and two glycosides were identified in *Carduus acicularis* (Table 1). Rutine was better represented glycoside (545.65 µg/g dw) compared with hyperoside 211.48 µg/g dw). Among all established aglycons, apigenin (478.75 µg/g dw), luteolin (288.46 µg/g dw) and myricetin (276.32 µg/g dw) showed the highest concentration.

Eleven phenolic acids were identified in the investigated methanolic extracts (Table 1). The highest concentration was found for sinapic acid (930.41 dw), followed by chlorogenic acid (582.66 µg/g). Ferulic and caffeic acids were comparatively well represented (Table 1). The presence of p-coumaric acid, 2-hydroxybenzoic acid, vanillic acid, 3,4-dihydroxybenzoic acid, syringic acid, cinnamic acid and gallic acid was also found.

Table 1. Content of flavonoids and phenolic acids in inflorescences of *Carduus acicularis* (µg/g dw).

Phenolic acids	**Mean ± SD**
Caffeic acid	112.97 ± 2.64
Cinnamic acid	29.66 ± 0.69
Chlorogenic acid	582.66 ± 13.60
p-Coumaric acid	84.77 ± 1.98
3,4-Dihydroxybenzoic acid	67.85 ± 1.58
Ferulic acid	188.45 ± 4.40
Gallic acid	19.08 ± 0.45
2-Hydroxybenzoic acid	82.59 ± 1.93
Sinapic acid	930.41 ± 21.72
Syringic acid	36.52 ± 0.85
Vanillic acid	66.37 ± 1.55
Flavonoid glycosides	**Mean ± SD**
Hyperoside	211.48 ± 5.03
Rutin	545.65 ± 12.82
Flavonoid aglycones	**Mean ± SD**
Apigenin	478.75 ± 11.38
Hesperedin	75.83 ± 1.81
Kaempferol	78.42 ± 1.86
Luteolin	288.46 ± .6.86
Myricetin	276.32 ± 5.21
Quercetin	6.08 ± 0.15

Antioxidant activity

For determination of antioxidant activity of prepared methanolic extract of inflorescences were conducted experiments with two stable radicals DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, but the ferric reducing antioxidant power (FRAP) and copper reduction (CUPRAC) assays were also performed. The results were expressed as Trolox equivalent antioxidant capacity - TEAC (Table 2). TEAC values were in range of 10.69 mM/TE g dw (CUPRAC**)** to 32.28 mM/TE g dw (ABTS)**.** The highest antioxidant activity were measured by the ABTS assay, followed by FRAP assay.

Discussion

The results obtained suggested that phenolics (phenolic acids, flavonoid aglycones and glycosides) were important components of *C. acicularis.*

Table 2. In vitro antioxidant activity in inflorescences of *Carduus acicularis* (mM/TE g dw). *Legend:* TEAC - Trolox equivalent antioxidant capacity; DPPH: 2,2-diphenil-1-picrylhydrazyl; ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; FRAP: Ferric reducing antioxidant power; CUPRAC: Copper reduction antioxidant assay; dw- Dry weight.

Method / **Sample**	TEAC $_{DPPH}$	TEAC $_{ABTS}$	TEAC $_{FRAP}$	TEAC $_{CUPRAC}$
methanolic extract	11.59±0.52	32.28±0.08	22.19±0.65	10.69±0.36

The established qualitative composition of flavonoids confirms previously data for the genus *Carduus* flavonoid aglycones and glycosides - apigenin, kaempferol, luteolin, quercetin, and rutin (BAIN & DESROCHERS, 1988; JORDON-THADEN & LOUDA, 2003; TERENTJEVA & KRASNOV, 2003; KOZYRA, 2013), as well as the myricetin and hyperoside, referred for the first time in the *C. thoermeri* from SLAVOV *et al.* (2014). Main flavonoid components in *C. acicularis* were rutin and apigenin, accompanied with luteolin, myricetin and hyperoside. The measured amounts of rutin and myricetin were higher (eight and three times, respectively) than those established for *C. thoermeri* (SLAVOV *et al.*, 2014). Many studies have suggested that flavonoids (especially abovementioned) exhibit biological activities, including antiallergenic, antiviral, antihypertensive, antiinflammatory and anticancerogenic properties (NIJVELDT *et al.*, 2001; ODONTUYA *et al.*, 2005; SANGUINE *et al.*, 2010; SHEN *et al.*, 2013). It is known that apigenin is protectant against cardiotoxic agents (BREINHOLT *et al.*, 1999).

Phenolic acids have been poorly studied in the genus *Carduus*. First data for their presence in *Carduus acanthoides* was pointed out by LIU *et al.* (2013) and SLAVOV *et al.* (2014) - in *Carduus thoermeri*. The present study showed that from eleven of the identified phenolic acids in *C. acicularis* sinapic and chlorogenic acids prevailed, accompanied by ferulic, caffeic, p-coumaric, 2-hydroxybenzoic acids.

Acid-phenols and their esters are known for their antioxidative properties. Caffeic, sinapic, ferulic and p-coumaric acids have antioxidative potency (CUVELIER *et al.*, 1992). Antioxidant $ONOO^-$scavenging ability of sinapic acid was indicated in the study of ZOU *et al.* (2002) and for anxiolytic-like effects in mice (YOON *et al.*, 2007). Other studies mention that ferulic acid and caffeic acid derivatives may have antitumor activity (LI *et al.*, 2012). Chlorogenic acid possesses pharmacological activities as antihypertensive effect (ZHAO *et al.*, 2011), anti-diabetic and anti-lipidemic effects (ONG *et al.*, 2013).

Good antioxidant capacity of the methanolic extract of C. *acicularis* were established in this study, especially towards $ABTS^{\bullet+}$and FRAP-assay. For comparison, ethanolic extract of the species were also found to have antioxidant activity (ZHELEVA-DIMITROVA *et al.*, 2011).

Conclusion

Phenolic acids and flavonoid profiles of *Carduus acicularis* were investigated for the first time. The present study reported eight flavonoids and eleven phenolic acids in the methanolic extract of flower heads. Exhibited antioxidant activity, as well as predominance of sinapic and chlorogenic acids, also rutin and apigenin from flavonoids, determine the species as a natural source of antioxidants.

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Forest Habitats in Natura 2000 Protected Zone BG0000211 „Tvardishka planina" – Floristic Composition and Conservation Status

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Abstract. The publication deals with the results of floristic study in Natura 2000 protected zone BG0000211 „Tvardishka planina". The objects of investigation are forest habitats: 9110, 9130, 9150, 91G0, 9170, 9530, 9180, 91M0, 91E0, 91AA. The floristic composition of the habitats, abundance of each species by Braun-Blanquet scale and plant relevès are presented. The assessment of nature conservation status is made applying the developed methodology under the project "Mapping and determining of the natural conservation status of the nature habitats and species – phase I" that was adopted by Ministry of Environment and Water. As a result of conducted inventory the conservation status of all habitats was assessed as unfavourable-unsatisfactory.

Key words: forest habitat, floristic composition, conservation status, protected zone.

Introduction

„Tvardishka planina" protected zone is part of the European ecological network Natura 2000, declared according to Directive 92/43/EEC for conservation of natural habitats and of wild fauna and flora. The zone is declared mainly for the conservation of forest habitats such as "9150 - Termophilous beech forests (*Cephalanthero-Fagion*)" and "9130 - Beech forests *Asperulo-Fagetum*". The habitats 9110, 91G0, 9170, 9530, 9180, 91M0, 91E0, 91AA, as well as many non-forest (grass, shrub and rock) habitats can be also observed in the zone. The protected zone is with big importance, because of the large number of species with conservation status - rare, endangered and endemic animals and plants such as *Fritillaria pontica* Wahlenb., *Verbascum adrianopolitanum* Podp., *Taxus baccata* L., *Anemone sylvestris* L., *Aquilegia nigricans* Baumg., *Crocus veluchensis* Herb., etc. (NATURA 2000 Standard Data Form, 2013)

The main aim of the study is to present the floristic composition and characteristics of the forest habitats in protected zone „Tvardishka planina" and to assess their contemporary conservation status.

Currently there is no floristic data available for the studied territory, but such habitats are researched in other areas by MICHALIK (1990), DZWONKO & LOSTER (2000), STOJANOV (1941), PENEV *et al.* (1969), BONDEV (1991), GARELKOV & STIPTCOV (1995), PAVLOV & DIMITROV (2003), TZONEV *et al.* (2006).

Materials and Methods

The site is located northern from Sliven Town with longitude E 26° 50' 36"

and latitude - N 42° 50' 25" (Fig.1). The altitude of the lowest point is 319 m and of the highest -1501 m a.s.l., the average altitude of the site is 892 m. The site covers 3864.95 ha. The area is related to continental and alpine bio geographic region.

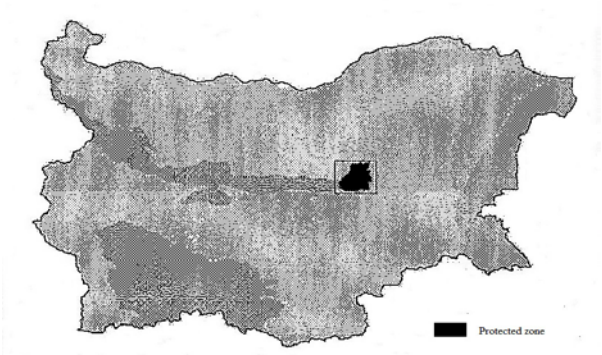

Fig.1. Location of the the study site - protected zone "Tvardishka planina" in Bulgaria.

The habitats were identified by KAVRAKOVA *et al.* (2009). The methodology for the assessment of habitats conservation status was developed under the realized project (ZINGSTRA *et al.*, 2009; GANEVA, 2013).

Plant relevès with area 350 m² were made in typical places of sample plots. They included: floristic composition of main horizons of community; total cover of the horizons; species abundance; data about environmental conditions (altitude, exposition, slope, soil). Species identification was carried out according to DELIPAVLOV *et al.* (2003) and IORDANOV (1973).

The species abundance in plant community was assessed through abundance and cover scale of BRAUN-BLANQUET (1964) – Table 1.

Table. 1. The Braun-Blanquet scale, used in the current study.

Abundance	Number	Cover, %
r	1 individual	under 1
+	2-5 individuals	under 1
1	6-50 individuals	under 5
2	over 50 individuals	6 - 25
3	without significance	26 – 50
4	without significance	51 – 75
5	without significance	over 75

Results and Discussion

Totally ten forest habitats were registered in the zone as we present the collected floristic data, plant communities' characteristics and conservation status assessments. The abundance of each species is given in brackets.

Nature habitat "9150 Thermophilous beech forests (Cephalanthero-Fagion)" (Fig. 2).

The habitat is one of well-presented in the zone (19% from the zone area). It can be observed between 500 and 900 m a.s.l. at slopes of 6-25° on east, northwest and southwest exposures on calcareous soils. The stand age is between 30 and 70 years. The tree layer is with cover 90%. Shrub and herbaceous layers are not well formed. The herbaceous layer has 10% cover. The plant community is formed from 6 tree, 1 shrub and 8 herbaceous species. The tree species are: *Fagus sylvatica* L. (4-5), *Sorbus torminalis* (L.) Crantz (+), *Quercus petraea* (Matt.) Liebl. (+), *Carpinus betulus* L. (1-2), *Populus tremula* L. (2) and *Prunus avium* L. (2). *Ligustrum vulgare* L. (2) is the only shrub species**.** The established herbaceous species are as follows: *Physospermum cornubiense* (L.) DC (1-2), *Luzula luzuloides* (Huds.) Gaud. (+), *Mycelis muralis* (L.) Dumort (+), *Brachypodium pinnatum* (L.) Beauv (1-2), *Poa nemoralis* L. (1), *Cephalanthera* sp. (+), *Galium odoratum* (L.) Scop. (1), *Carex sylvatica* Huds (1).

„Tvardishka planina" protected zone has favorable conditions for development of habitat 9150. Most of the parameters are at favorable conservation status. The habitat has characteristic species at all layers. Incorrectly planned and carried out cuttings, dead wood removal, non-regulated

gathering of non-wood forest resources, threat by construction and infrastructure, recreation and tourism, using of the habitat for grazing, as well as reforestation with exotic and non-native species were not observed.

The parameters that have unfavourable valuation are: low average age of tree species, lack of old age forests, insufficient quantity of dead wood, as well as lack of old trees. This reflects to the assessment of conservation status as unfavourable-unsatisfactory.

Fig. 2. Nature habitat "9150 Thermophilous beech forests (*Cephalanthero-Fagion*)".

Nature habitat "9130 Beech forests Asperulo-Fagetum" (Fig.3)

The habitat occupies around 9% in the zone and can be observed at 1100 m a. s. l. on 20° slopes with northern exposures. The stand age is 90 years. The tree layer cover is 80%. Shrub and herbaceous layers are not well formed. The species of herbaceous layer are with cover 10- 20%. The plant community is formed from 1 tree and 6 herbaceous species which are presented below. The tree species is *Fagus sylvatica* L. (5). The established herbaceous species are as follows: *Galium odoratum* (L.) Scop. (2), *Dryopteris filix-mas* (L.) Schott (1), *Aegopodium podagraria* L. (+), *Salvia glutinosa* L. (+), *Lamiastrum galeobdolon* L. (+) and *Brachypodium sylvaticum* (Huds.) Beauv (+).

Most of the parameters such as cover of the first tree layer, composition of the first tree layer and others have favourable assessment.

The parameters which assessment is unfavourable are: lack of old age forests, insufficient quantity of dead wood, as well as absence of old trees. Although, that habitat occupies one of the highest parts of the zone because of a well-developed road system it has been a subject of cuttings during the last 100 years. As a result the old forests have been demolished and the tree layer is with coppice origin. The natural conservation status is assessed as unfavourable-unsatisfactory.

Fig. 3. Nature habitat "9130 Beech forests *Asperulo-Fagetum".*

Nature habitat "9110 Luzulo-Fagetum beech forests" (Fig. 4)

The habitat occupies only 0.01% of the zone area and it is located at 1500 m a. s. l. on 11-15° slopes at northern expositions. The stand age is between 60 and 70 years. The tree layer cover is 90%. Shrub layer is not well formed but species are with 10% cover. The herbaceous layer is with cover 30%. The plant community is formed from 2 tree, 1 shrub and 3 herbaceous species which are presented below. Tree species are *Fagus sylvatica* L. (5) and *Sorbus aucuparia* L. (+). *Rubus hirtus* W. et K. (2) is the only shrub species**.** The established herbaceous species are as follows: *Luzula luzuloides* (Lam.) Dandy (2), *Dryopteris filix-mas* (L.) Schott (2) and *Geranium macrorrhizum* L. (2).

Most of the parameters of habitat 9110 have favourable assessment. The floristic composition is characteristic for the habitat.

Nature disturbances and threats include incorrectly planned and carried out cuttings, non-regulated gathering of non-wood forest resources, threat by construction and infrastructure, recreation and tourism, fires, using of the habitat for grazing, as well as by reforestation with exotic and non-native species.

The parameters which classify the assessment as unfavourable are: low average age of tree species, lack of old age forests, insufficient quantity of dead wood as well as old trees. All these negative assessments of habitat status are a result by the anthropogenic impact in the past and inappropriate management. This is the reason this habitat status to be finally assessed with unfavourable-unsatisfactory assessment.

Fig. 4. Nature habitat "9110 *Luzulo-Fagetum* beech forests".

Nature habitat "91M0 Pannonian-Balkanic turkey oak-sessile oak forests" (Fig. 5)

The habitat occupies 4.4% of the zone area. It is located from 489.57 to 626.07 m a.s.l. on 6-20° slopes at east and west expositions. The stand age is between 30 and 50 years. The tree canopy cover is 50%-70%. Shrub layer is not well formed and the species are with cover between 10-20%. The herbaceous layer cover is between 40 and 20%. The plant community is formed from 7 tree, 6 shrub and 15 herbaceous species which are presented below. The tree species are: *Quercus frainetto* (3-4), *Quercus petraea* (Matt.) Liebl. (1), *Quercus cerris* L. (2), *Fagus sylvatica* L. (+), *Sorbus torminalis* (L.) Crantz (+), *Acer campestre* L. (1) and *Fraxinus ornus* L. (+). The shrub species are: *Crataegus monogyna* Jacq. (2), *Carpinus orientalis* Miller (1-3), *Chamaecytisus supinus* (L.) Link (+; 1), *Genista tinctoria* L. (+), *Rubus caesius* L. (+), and *Genista carinalis* Griseb. (+).The established herbaceous species are as follows: *Prenanthes purpurea* L. (+), *Festuca heterophylla* Lam. (1-2), *Luzula sylvatica* (Huds.) Gaud. (1), *Galium pseudoaristatum* Schur (+; 1), *Hieracium hoppeanum* Schleiher (+), *Euphorbia amygdaloides* L. (1), *Dactylis glomerata* L. (2), *Poa nemoralis* L. (2), *Lychnis coronaria* (L.) Desr. in Lam. (+), *Mycelis muralis* (L.) Dumort (+), *Anthoxanthum odoratum* L. (+;1), *Silene viridiflora* L. (+), *Fragaria vesca* L. (+), *Cruciata glabra* (L.) Ehrend. (+) and *Hieracium racemosus* W. et K. (+).

Most of the parameters are at favorable conservation status. The study shows that the habitat has characteristic species at all layers. The cover of the first tree layer, average age of the first tree layer and ground cover are also appropriate. Incorrectly planned and carried out cuttings, gathering of non-wood forest resources, threat by construction and infrastructure, recreation and tourism, fires, using of the habitat for grazing, as well as by reforestation with exotic and non-native species were not observed.

The parameters which assessment is unfavourable are: lack of old age forests, insufficient quantity of dead wood, and old trees. Some indications for succession processes because of *Carpinus orientalis* Miller high abundance at some parts of the habitat and nature disturbances as windfalls were observed. That is why the natural conservation status is assessed as unfavourable-unsatisfactory.

Nature habitat "91AA East pubescent oak forest" (Fig. 6)

The habitat occupies insignificant area of the zone (0.02%). It can be observed on 450 m a.s.l. at 6-10° slopes on east exposures. The stand age is between 30 and 40 years. The tree layer is with cover 70%.

Fig. 5. Nature habitat "91M0 Pannonian-Balkanic turkey oak-sessile oak forests".

Fig. 6. Nature habitat "91AA East pubescent oak forest".

Shrub layer is not well formed - species are with cover 20%. The herbaceous layer is with cover 30%. The plant community is formed from 2 tree, 3 shrub and 5 herbaceous species which are presented below.The tree species are *Quercus pubescens* Willd. (4) and *Fraxinus ornus* L. (1). The shrub species are: *Syringa vulgaris* L. (1), *Cornus mas* L. (1), *Carpinus orientalis* Miller (2). The established herbaceous species are as follows: *Poa nemoralis* L. (1), *Festuca heterophylla* Lam. (2), *Fragaria vesca* L. (+), *Dactylis glomerata* L. (1) and *Brachypodium pinnatum* (L.) Beauv (1).

The habitat has characteristic trees, shrub and herbaceous species. Unfavorable are the parameters related to the age and the presence of dead wood in the stands, most probably due to incorrect management in the past expressed with cuttings and subsequent difficult regeneration of the *Quercus pubescens* Willd. There were not dead wood observed, which can be due to the fact that the stands are near to the settlements and the fallen branches are gathered for firewood. It can be pointed using the habitats territory for grazing and high abundance of *Carpinus orientalis* Miller which is indication for succession processes from the treats for good nature conservation status for this habitat. This leads to unfavourable-unsatisfactory assessment of the habitat status.

Nature habitat "91G0 Pannonic woods with Quercus petraea and Carpinus betulus" (Fig. 7)

The habitat occupies 5.4% of the zone and is distributed at 400 m a.s.l. at 6-10° slopes on northwest expositions. The stands age is 60 years. The tree layer is with cover 70%. Shrub layer is not well formed - species are with coverage 10%. The herbaceous layer is with coverage of 40%. The plant community is formed from 8 tree, 2 shrub and 11 herbaceous species. The tree species are: *Prunus avium* L. (1), *Carpinus betulus* L. (2), *Quercus frainetto* Ten. (2), *Quercus cerris* L. (3), *Quercus petraea* (Matt.) Liebl. (3), *Acer campestre* L. (1), *Tilia cordata* Miller (1) and *Sorbus torminalis* (L.) Crantz (+). The shrub species are *Crataegus monogyna* Jacq. (1) and *Cornus mas* L. (2). The established herbaceous species are as follows: *Euphorbia amygdaloides* L. (+), *Clinopodium vulgare* L. (+), *Potentilla micrantha* Ramond ex DC (+), *Dactylis glomerata* L. (2), *Prenanthes purpurea* L. (+), *Physospermum cornubiense* (L.) DC (2), *Poa nemoralis* L. (1), *Fragaria vesca* L. (1), *Digitalis viridiflora* Lindley (+), *Cruciata glabra* (L.) Ehrend. (+) and *Galium pseudoaristatum* Schur (+).

The conservation status of most parameters of habitat 91G0 is assessed as favorable. The investigation shows that the habitat has characteristic species in tree, shrub and herbaceous layers. Indications for succession processes, threats by nature

disturbances, incorrectly planned and carried out cuttings, gathering of non-wood forest resources, treat by construction and infrastructure, recreation and tourism, fires, using of the habitat for grazing, as well as by reforestation with exotic and non-native species were not observed.

The parameters which assessment is unfavourable are: low average age of tree species, lack of old age forests, insufficient quantity of dead wood, as well as lack of old trees. This is the reason for unfavorable conservation status.

Fig. 7. Nature habitat "91G0 Pannonic woods with *Quercus petraea* and *Carpinus betulus".*

Nature habitat "91E0 Alluvial forests with Alnus glutinosa and Fraxinus excelsior (Alno-Pandion, Alnion incanae, Salicion albae)" (Fig. 8)

The habitat occupies insignificant part of the zone (0.005%). It can be observed on 400-500 m a.s.l. at 1-5° near rivers. The stand age is 40 years. The tree layer is with 60% cover. Shrub layer is not formed. The herbaceous layer is with cover 80%. The plant community is formed from 2 tree and 6 herbaceous species. The tree species are *Salix alba* L. (3) and *Alnus glutinosa* (L.) Gaertner (3). The established herbaceous species are as follows: *Mentha longifolia* (L.) Hudson (1), *Heracleum verticillatum* Pančі (2), *Sambucus ebulus* L. (1), *Valeriana officinalis* L. (3), *Equisetum arvense* L. (3) and *Circaea luteciana* L. (1).

The conservation status of most parameters of habitat 91E0 is assessed as favorable. The study shows that the habitat has characteristic species at all layers. Penetrating of invasive species, changes in hydrological regime, cleaning of river beds, using of the habitat for grazing, threats by construction and infrastructure, recreation and tourism, as well as by reforestation with exotic and non-native species were not observed.

The parameters which classify the assessment as unfavourable are: low average age of tree species, lack of old age forests, old trees and insufficient quantity of dead wood. All these negative parameters are a result of anthropogenic impact in the past but nowadays they lead to unfavorable-unsatisfactory assessment of the habitat.

Fig. 8. Nature habitat "91E0 Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior (Alno-Pandion, Alnion incanae, Salicion albae".*

Nature habitat "9180 Tilio-Acerion forests of slopes, screes and ravines" (Fig. 9)

The habitat occupies 7.2% of the zone "Tvardishka planina". It is located about 500 m a.s.l. at 11-15° slopes at north expositions. The stand age is between 50 and 60 years. The tree layer is with cover 80%. Shrub layer is not well formed - species are with cover 10%. The herbaceous layer is with cover 30%. The plant community is formed from 5 tree, 1 shrub and 16 herbaceous species. The

tree species are *Tilia plathyphyllos* Scop (2), *Acer pseudoplatanus* L. (2), *Carpinus betulus* L. (3), *Fraxinus excelsior* L. (2) and*Prunus avium* L. (2). *Cornus mas* L. (2) is the only shrub species**.** The established herbaceous species are as follows: *Athyrium filix-femina* (L.) Roth (2), *Galium odoratum* (L.) Scop. (2), *Lamiastrum galeobdolon* L. (2), *Geranium robertianum* L. (+), *Euphorbia amygdaloides* L. (+), *Dryopteris filix-mas* (L.) Scott. (2), *Polygonatum latifolium* (Jacq.) Desf. (+), *Campanula rapunculoides* L. (+), *Viola riviniana* Reich. (+), *Aremonia agrimonoides* (L.) DC (+), *Circaea luteciana* L. (+), *Poa nemoralis* L. (1), *Carex pilosa* Scop. (+), *Salvia glutinosa* L. (1), *Sanicula europaea* L. (2) and *Lathyrus vernus* (L.) Bernh. (+).

The conservation status of most parameters of habitat 9180 is assessed as favorable. The investigation shows that the habitat has characteristic species in tree, shrub and herbaceous layers. Incorrectly planned and carried out cuttings, removal of dead wood, using the habitat for grazing, threats by construction and infrastructure, recreation and tourism, as well as by reforestation with exotic and non-native species were not observed.

The parameters which classify the assessment as unfavourable are: low average age of tree species, lack of old age forests and insufficient quantity of dead wood. This is the reason for unfavorable-unsatisfactory conservation status of the habitat.

Nature habitat "9170 Galio-Carpinetum oak-hornbeam forests" (Fig. 10)

The habitat occupies 11% of the zone. It is located between 600 and 850 m a.s.l. at 6-30° slopes on south, east and west expositions. The stand age is between 50 and 80 years. The tree layer is with cover 60-80%. Shrub layer is not well formed - the species are with cover 10%. The herbaceous layer is with cover 40 - 70%. The plant community is formed from 7 tree, 4 shrub and 17 herbaceous species. The tree species are: *Quercus dalechampii* Ten. (3-5), *Carpinus betulus* L. (1-2), *Acer platanoides* L. (1), *Fagus sylvatica* L. (1-3), *Fraxinus excelsior* L. (+), *Sorbus torminalis* (L.) Crantz (+) and*Acer campestre* L. (1). The shrub species are**:** *Crataegus monogyna* Jacq. (1), *Corylus avellana* L. (1), *Cornus mas* L. (2) and *Rubus hirtus* W. et K. (1). The established herbaceous species are as follows: *Luzula luzuloides* (Huds.) Gaud. (+; 3), *Lathyrus niger* (L.) Bernh. (2), *Festuca drymeja* Mert et Koch. (2-3), *Vicia sativa* L. (1), *Fragaria vesca* L. (+), *Galium pseudoaristatum* Schur (+; 1), *Festuca valesiaca* Schleich. ex Gaud. (1-3), *Lathyrus laxiflorus* (Desf.) Kuntze (1), *Euphorbia amygdaloides* L. (+), *Platanthera bifolia* (L.) Rich (+), *Poa nemoralis* L. (2-4), *Helleborus odorus* W. et K. (+), *Stellaria holostea* L. (3), *Melica uniflora* Retz (1), *Dactylis glomerata* L. (+), *Vincetoxicum hirundinaria* Medik. (+) and *Festuca heterophylla* Lam. (1).

Fig. 9. Nature habitat "9180 *Tilio-Acerion* forests of slopes, screes and ravines".

Most of the parameters of habitat 9170 are at favorable conservation status. The observations show that the habitat has characteristic species at tree, shrub and herbaceous layers. Incorrectly planned and carried out cuttings, removal of dead wood, gathering of non-wood forest resources, threat by construction and infrastructure, recreation and tourism, using the habitat for grazing, as well as by reforestation with exotic and non-native species was not observed.

The parameters which are unfavourable for the habitat are: threat by fires, insufficient quantity of dead wood, lack of old age forests, old trees and low average age of first tree layer. The assessment of conservation status is unfavorable-unsatisfactory.

Fig. 10. Nature habitat "9170 *Galio-Carpinetum* oak-hornbeam forests".

Nature habitat "9530 SubMediterranean pine forests with endemic black pines" (Fig. 11)

The habitat occupies insignificant part of the zone (0.08%) or several compartments southwest from the Tvarditca town near to the main ridge of Balkan Range. It can be observed at 700 m above sea level on stony 40° slopes at southwest expositions. The soils are shallow, poor and dry. The stand age is around 30 years. The tree layer cover is 40%. There is no shrub and herbaceous layer while species are with cover 10 and 20% respectively. The plant community is formed from 1 tree, 3 shrubs and 2 herbaceous species. The tree species is *Pinus nigra* Arn. subsp. *pallasiana* (3). The shrub species are *Cotinus coggygria* Scop. (2), *Carpinus orientalis* Miller (1) and *Clematis vitalba* L. (1). The established herbaceous species are *Poa nemoralis* L. (2) and *Trifolium alpestre* L. (1).

Most parameters of habitat 9530 in protected zone „Tvardishka planina" testify to a favourable conservation status.

The observations show that it has characteristic species in the tree layer composition and also in the ground layer. Incorrectly planned and carried out cuttings, dead wood removal, and threat by fires, construction and infrastructure, recreation and tourism, using of the habitat for grazing, as well as by reforestation with exotic and non-native species have not been observed. Lots of seeding and undergrowth presence which appears very often in cracks and almost soil devoid terrains were observed. It is necessary to be set up total prohibition for all types of cuttings in the habitat because the steep terrains which the habitat occupies and its negligible participation in the total area of the protected zone. It is also desirable to be planned additional protection measures as creation of mineralization strips around passing asphalt road near the stands.

The parameters which are unfavourable for the habitat are: low density, low average age of first tree layer; lack of old age forests and old trees, insufficient quantity of dead wood and because of this the assessment is unfavourable-unsatisfactory status.

Fig. 11. Nature habitat "9530 Sub Mediterranean pine forests with endemic black pines".

Conclusions

In sum, it was established that the habitats have a typical floristic composition. The most of natural conservation status parameters have favorable assessment. The parameters with unfavorable valuation almost for all habitats are: lack of old forests, old trees and insufficient quantity of dead

wood trees. So the final status assessment of all habitats in the site is unfavourable-unsatisfactory. The habitat management has to be directed not only to the wood utilization but also to the biodiversity maintaining. These will lead to the natural status improving of habitats: 9110, 9130, 9150, 9170, 9180, 91AA, 91G0, 91M0. The forests managemant has to be oriented to maintaining the composition and mixed age stand structure through leaving of old trees in quantity 10 numbers.ha^{-1} and dry and fallen trees in amount of 8% from the stand stock.

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Inventory of Bryophytes in the "Bulgarka" Nature Park

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Abstract. This study reports data on the diversity of bryophytes in the Bulgarka Nature Park. The registered 55 species belonged to 23 families and 46 genera. Six species were with conservation status; 2 were assessed as Not Evaluated. The main threats were assessed and measures towards bryophyte conservation were proposed.

Key words: Bulgarka Nature Park, bryophytes, conservation status, threats.

Introduction

Recently, conservation biologists alarm about mass extinction of biodiversity caused by human influences (GOOD & RODRÍGUEZ, 2009). However, preserved territories still protect habitats, species and genotypes. Among them is Bulgarka Nature Park, located on the ridges and northern slopes of Shipka and Trevnenska mountains and partly of the adjacent Pre-Balkan region (Bulgaria) with total area 21 772.2 ha. The territory includes the Yantra River springs and its main tributaries in the upper reaches. The park covers settlements located within the municipalities of Gabrovo, Tryavna and Muglizh. Hristo Smirnenski dam also belongs to the territory of the park.

Bryophytes are among the best-adapted plants in habitats with extreme conditions, such as Arctic and Alpine Tundra, marshes and bare rocks, etc. (DIERSSEN, 2001). Bryophytes have an important role in natural ecosystems. Their most important contribution is related to the water cycle, primary production and carbon fixation (HALLINGBÄCK & HODGETTS, 2000). Some moss species are valuable for their medicinal properties and contain chemical components that are active against certain cell lines of cancer cells. Others have antibacterial, antimicrobial and antifungal action (RAYMUNDO *et al.*, 1989; ASAKAWA, 1995; ASAKAWA *et al.*, 2003; NAGASHIMA *et al.*, 2003). Because of their small size, they largely reflect micro-conditions of the environment, especially the microclimate, soil development and its chemical composition.

About 754 bryophyte species were registered in Bulgaria (PETROV, 1975;

NATCHEVA & GANEVA, 2009). At present the Red List of bryophytes in Bulgaria includes 251 species, 228 of them being Threatened (28 Critically Endangered, 42 Endangered and 158 Vulnerable) (NATCHEVA *et al.*, 2006). Four species are included in Annex 2 of the Biological Diversity Act (2002): *Buxbaumia viridis*, *Dicranum viride*, *Hamatocaulis vernicosus* and *Mannia triandra*.

Nowadays it is obvious that the landscape in most of the world has changed to such an extent that the environment is already unfavorable for the existence and development of a number of plant and animal species (VIÉ *et al.*, 2009). Bryophytes particularly suffer from various threats, but they have received significantly less conservation attention in comparison with vascular plants (SABOVLJEVIĆ *et al.*, 2014). Reduction of biodiversity may be caused directly by deforestation, urbanization, road construction, construction of dams, mining, production of peat and many other human activities. Changes of habitats caused by the humans entail changes in the conditions of the abiotic environment, thus creating additional negative effects to bryophytes. Habitat loss is the fastest growing threat to the survival of the species and this will probably continue to be the dominant risk factor in the coming decades (BROOKS *et al.*, 2002; FAHRIG, 2002).

Bulgarka Nature Park aims to secure habitats and species, thus species that are under threat must first be registered. This is the first study describing the bryophyte flora in Bulgarka Nature Park with a focus on threaten species.

Materials and Methods

Inventorization of bryophytes was done in the period May-October 2012 and in the spring of 2013 on the territory of Bulgarka Nature Park (Figure 1). The route transect method was used. Samples of bryophyte species were collected from different substrates: rocks, stones of different sizes, trunks of beech trees, dead wood, soil, mostly in rocky ridges, grasslands, and beech forests. Portable GPS receiver GARMIN 530 was used for studying the localities of the species.

Taxonomic composition was determined by microscopic identification following PETROV (1975) and SMITH (2004). Herbarium samples were used to confirm the species identification.

HILL *et al.* (2006) were followed for moss nomenclature and GROLLE & LONG (2000) for liverworts. The threat status was assessed according to NATCHEVA *et al.* (2006) and European working list of mosses (2014).

Results and Discussion

Fifty-five species belonging to four classes were described after the field inventory in Bulgarka Nature Park: Marchantiopsida, Jungermanniopsida, Polytrichopsida and Bryopsida (Table 1). They usually develop on rock substrate and on the trunks of trees in beech forest communities. The analysis of the described species shows that class Marchantiopsida was represented by two species belonging to genus *Conocephalum* and *Conocephalum conicum* (L.) Dumort being among the most common. Class Jungermanniopsida is represented by only two species. Class Polytrichopsida was also represented by two species and several localities of *Polytrichum juniperinum* Hedw. were found. Class Bryopsida comprised 49 species, grouped in 42 genera of 20 families referring to 7 orders. Order *Hypnales* was represented by the highest diversity of species (30 species) and the most common species belonging to class Bryopsida were: *Hypnum cupressiforme* Hedw., *Homalothecium lutescens* (Hedw.) H.Rob., *Plagiomnium undulatum* (Hedw.) T. J. Kop., *Platyhypnidium riparioides* (Hedw.) Dixon and *Rhytidiadelphus loreus* (Hedw.) Warnst.

Table 2 presents identified species, their conservation status according to NATCHEVA *et al.* (2006) and European working list of mosses (2014). Six of the registered moss species have a conservation value. The species *Dicranum viride* var. *papillosum* (Sull. et Lesq.) Lindb. is endangered (EN) and *Anomodon rugelii* (Müll.Hal.) Keissl., *Grimmia torquata* Drumm., *Lescuraea mutabilis* (Brid.) Lindb. ex I.Hagen, *Philonotis caespitosa* Jur., *Sciuro-hypnum glaciale* (Schimp.) Ignatov & Huttunen are vulnerable (VU). *Pseudoleskea*

radicosa (Mitt.) Macoun & Kindb. belongs to the category near threatened (NT). *Conocephalum salebrosum* Szweykowski et al. and *Dichelyma falcatum* (Hedw.) Myrin are underexplored and respectively, not evaluated (NE). Finding and registering new habitats of the latter species in Bulgarka Nature Park, in the region of Kozyata River in particular, will help future evaluation of their conservation status.

Fig. 1. Topographic map of the study area.

Table 1. Distribution (in number) by taxonomic categories of the moss taxa identified in Bulgarka Nature Park.

Class	Order	Family	Genus	Species
Marchantiopsida	1	1	1	2
Jungermanniopsida	1	1	1	2
Polytrichopsida	1	1	2	2
Bryopsida	7	20	42	49

The major threats to the bryophytes were related to the habitat loss and fragmentation in the studied territory, mainly from construction works. In recent years grassland habitats are really endangered by the growing interest in renewable energy sources (wind generators and photovoltaics). A wind farm has been constructed at the park's border in the regions of Buzloudzha peak, Atovo padalo (Atovo fal) peak, Karadzhova kula (Karadzhova tower) peak, and Bedek peak. The construction of the ski slope from Ispolin peak down to Uzana locality, situated to the northwest of the peak, also has a negative effect on the vegetation.

Recent review of peer-reviewed studies on the effects of construction of structures by humans, stated that the alteration of a landscape through the removal of vegetation changes the characteristics of the environment in a way that affects wildlife (LOVICH & ENNEN, 2011). Increasing habitat fragmentation and land-use changes in surrounding areas form nature preserves as habitat "islands" (GOOD & RODRÍGUEZ,

2009). In addition, activities leading to fragmentation within the nature park itself, may cause its decrease in size and increases isolation between threaten species.

Few actions, taken in order to fight the decline in bryophyte diversity in the last 30 years were documented (SABOVLJEVIĆ et al., 2014). Due to the limited experience worldwide and based on the highlighted threats within the studied protected area, the following measures should be taken for protecting threaten species' habitats. Of high importance are further studies on the so-called 'hot spots' of distribution. In parallel, activities dedicated to the popularization and conservation of these bryophyte species should be taken also.

Conclusion

Predominantly high abundance of bryophytes was registered in all studied sites, which confirms their important environmental role. Thus their conservation worldwide and particularly in Bulgaria, is crucial. Based on the data obtained, bryophyte diversity on the territory of Bulgarka Nature Park can be considered high. The list of bryophytes found in the protected area includes 55 species, 6 of them being of conservation importance. Two of the species are underexplored in the studied region.

The major threat assessed to the bryophytes in the studied territory was habitat loss and fragmentation.

This is the first bryophyte study within the protected area, and it could be a basis for further conservation measures to ensure the long-term threaten species survival. Phytocenological studies including species composition and their quantitative assessment should be carried out with the aim of determine detailed environmental characteristics of the areas, especially with species of conservation significance.

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Table 2. Taxonomic composition, conservation status and GPS coordinates of the localities of the identified bryophytes on the territory Bulgarka Nature Park. Legend: EN - Endangered; VU - Vulnerable; NT - Near Threatened; LC - Least Concern; NE - Not Evaluated.

Class MARCHANTIOPSIDA					
Order	**Familia**	**Genus**	**Species**	**Conservation value**	**GPS coordinates**
Marchantiales	Conocephalaceae	*Conocephalum*	*Conocephalum conicum* (L.) Dumort	LC	N 42°48`30.0″ E 25°18`15.4″ N 42°46`89.3″ E 25°15`31.0″ N 42°46`13.3″ E 25°18`02.6″ N 42°49`57.1″ E 25°12`85.0″ N 42°48`16.5″ E 25°15`39.6″ N 42°48`07.8″ E 25°15`28.7″ N 42°46`07.0″ E 25°23`07.1″ N 42°48`23.2″ E 25°34`49.0″
			Conocephalum salebrosum Szweykowski et al.	NE	N 42°46`13.3″ E 25°18`02.6″
Class JUNGERMANNIOPSIDA					
Order	**Familia**	**Genus**	**Species**	**Conservation value**	**GPS coordinates**
Porellales	Porellaceae	*Porella*	*Porella laevigata* (Schrad.) Pfeiff.	Unknown	N 42°48`23.2″ E 25°34`49.0″
			Porella platyphylla (L.) Pfeiff.	Unknown	N 42°48`23.2″ E 25°34`49.0″
Class POLYTRICHOPSIDA					
Order	**Familia**	**Genus**	**Species**	**Conservation value**	**GPS coordinates**
Polytrichales	Polytrichaceae	*Polytrichastrum*	*Polytrichastrum formosum* (Hedw.) G.L.Sm.	LC	N 42°48`23.2″ E 25°34`49.0″
		Polytrichum	*Polytrichum juniperinum* Hedw.	LC	N 42°45`98.9″ E 25°14`45.9″ N 42°45`00.6″ E 25°25`59.5″ N 42°45`51.8″ E 25°28`28.8″ N 42°46`05.8″ E 25°29`06.2″
Class BRYOPSIDA					
Order	**Familia**	**Genus**	**Species**	**Conservation value**	**GPS coordinates**
Encalyptales	Encalyptaceae	*Encalypta*	*Encalypta streptocarpa* Hedw.	LC/status unknown	N 42°48`23.2″ E 25°34`49.0″
Grimmiales	Grimmiaceae	*Schistidium*	*Schistidium agassizii* Sull. & Lesq.	LC	N 42°45`08.4″ E 25°14`20.7″
		Grimmia	*Grimmia hartmanii* Schimp.	LC/status unknown	N 42°45`98.6″ E 25°14`38.0″

			Grimmia pulvinata (Hedw.) Sm.	LC/status unknown	N 42°48`23.2″ E 25°34`49.0″
			Grimmia torquata Drumm.	VU	N 42°45`89.2″ E 25°15`13.6″
		Racomitrium	*Racomitrium canescens* (Hedw.) Brid.	LC	N 42°48`55.9″ E 25°29`19.6″
Dicranales	Dicranaceae	*Dicranella*	*Dicranella heteromalla* (Hedw.) Schimp	LC/status unknown	N 42°45`98.9″ E 25°14`45.9″
		Dicranum	*Dicranum viride* var. *papillosum* (Sull. et Lesq.) Lindb.	EN	N 42°45`98.6″ E 25°14`38.0″
			Dicranum flexicaule Brid	LC	N 42°48`23.2″ E 25°34`49.0″
		Paraleucobryum	*Paraleucobryum longifolium* (Hedw.) Loeske	LC/status unknown	N 42°48`23.2″ E 25°34`49.0″
Pottiales	Pottiaceae	*Tortella*	*Tortella tortuosa* (Hedw.) Brid.	LC/status unknown	N 42°48`23.2″ E 25°34`49.0″
		Weissia	*Weissia* sp.		N 42°48`23.2″ E 25°34`49.0″
Orthotrichales	Orthotrichaceae	*Orthotrichum*	*Orthotrichum* sp.		N 42°48`23.2″ E 25°34`49.0″
Bryales	Bartramiaceae	*Philonotis*	*Philonotis caespitosa* Jur.	VU	N 42°45`54.2″ E 25°22`03.4″
	Bryaceae	*Bryum*	*Bryum turbinatum* (Hedw.) Turner	LC	N 42°45`54.2″ E 25°22`03.4″
			Bryum pseudotriquetrum (Hedw.) P. Gaertn. et al.	LC	N 42°46`13.3″ E 25°18`02.6″
		Rhodobryum	*Rhodobryum roseum* (Hedw.) Limpr.	LC	N 42°45`98.6″ E 25°14`38.0″
	Cinclidiaceae	*Rhizomnium*	*Rhizomnium punctatum* (Hedw.) T. J. Kop.	LC	N 42°46`07.0″ E 25°23`07.1″
	Plagiomniaceae	*Plagiomnium*	*Plagiomnium affine* (Blandow ex Funck) T.J.Kop.	LC	N 42°48`23.2″ E 25°34`49.0″
			Plagiomnium rostratum (Schrad.) T.J.Kop.	LC	N 42°48`23.2″ E 25°34`49.0″
			Plagiomnium undulatum (Hedw.) T. J. Kop.	LC	N 42°48`39.6″ E 25°16`06.0″
					N 42°45`54.2″ E 25°22`03.4″
					N 42°46`07.0″ E 25°23`07.1″
					N 42°48`23.2″ E 25°34`49.0″
					N 42°48`23.2″ E 25°34`49.0″
Hypnales	Fontinalaceae	*Dichelyma*	*Dichelyma falcatum* (Hedw.) Myrin	NE	N 42°46`13.3″ E 25°18`02.6″
	Amblystegiaceae	*Cratoneuron*	*Cratoneuron filicinum* (Hedw.) Spruce	LC	N 42°48`23.2″ E 25°34`49.0″
		Amblystegium	*Amblystegium serpens* (Hedw.) Schimp.	LC	N 42°45`08.4″ E 25°14`20.7″
		Hygroamblystegium	*Hygroamblystegium varium* (Hedw.) Mönk.	LC	N 42°49`08.5″ E 25°29`56.6″
		Palustriella	*Palustriella commutata* (Hedw.) Ochyra	LC/status unknown	N 42°46`13.3″ E 25°18`02.6″
	Leskeaceae	*Leskea*	*Leskea polycarpa* Hedw.	LC	N 42°46`89.3″ E 25°15`31.0″
		Pseudoleskeella	*Pseudoleskeella catenulata* (Brid. ex Schrad.) Kindb.	LC	N 42°45`98.6″ E 25°14`38.0″
		Pseudoleskea	*Pseudoleskea radicosa* (Mitt.) Macoun & Kindb.	NT	N 42°45`98.6″ E 25°14`38.0″
		Lescuraea	*Lescuraea mutabilis* (Brid.) Lindb. ex I.Hagen	VU	N 42°46`25.7″ E 25°33`48.3″
	Brachytheciaceae	*Eurhynchium*	*Eurhynchium striatum* (Hedw.) Schimp	LC	N 42°48`55.9″ E 25°29`19.6″
			Eurhynchium praelongum (Hedw.) Schimp	LC	N 42°47`35.1″ E 25°28`13.5″
		Platyhypnidium	*Platyhypnidium riparioides* (Hedw.) Dixon	LC	N 42°48`30.0″ E 25°18`15.4″
					N 42°49`57.1″ E 25°12`85.0″

				N 42°47`59.9″ E 25°14`26.8″
				N 42°48`23.2″ E 25°34`49.0″
				N 42°44`57.0″ E 25°31`44.6″
	Plasteurhynchium	*Plasteurhynchium striatulum* (Spruce) M.Fleisch.	LC	N 42°48`23.2″ E 25°34`49.0″
	Oxyrrhynchium	*Oxyrrhynchium speciosum* (Brid.) Warnst.	LC	N 42°48`23.2″ E 25°34`49.0″
				N 42°46`13.3″ E 25°18`02.6″
	Sciuro-hypnum	*Sciuro-hypnum glaciale* (Schimp.) Ignatov & Huttunen	VU	N 42°46`55.6″ E 25°14`01.2″
	Brachythecium	*Brachythecium rivulare* Schimp.	LC	N 42°47`35.1″ E 25°28`13.5″
	Homalothecium	*Homalothecium lutescens* (Hedw.) H.Rob.	LC	N 42°46`89.3″ E 25°15`31.0″
				N 42°48`16.5″ E 25°15`39.6″
				N 42°48`07.8″ E 25°15`28.7″
				N 42°46`00.5″ E 25°29`19.0″
		Homalothecium philippeanum (Spruce) Schimp.	LC	N 42°48`23.2″ E 25°34`49.0″
Hypnaceae	*Campylophyllum*	*Campylophyllum sommerfeltii* (Myrin) Hedenäs	Unknown	N 42°45`98.9″ E 25°14`45.9″
	Calliergonella	*Calliergonella cuspidata* (Hedw.) Loeske	LC/status unknown	N 42°48`39.6″ E 25°16`06.0″
	Hypnum	*Hypnum cupressiforme* Hedw.	LC	N 42°45`98.6″ E 25°14`38.0″
				N 42°45`98.6″ E 25°14`38.0″
				N 42°48`23.2″ E 25°34`49.0″
Hylocomiaceae	*Pleurozium*	*Pleurozium schreberi* (Willd. ex Brid.) Mitt.	LC/status unknown	N 42°45`00.6″ E 25°25`59.5″
	Rhytidiadelphus	*Rhytidiadelphus loreus* (Hedw.) Warnst.	LC/status unknown	N 42°45`74.4″ E 25°15`14.6″
				N 42°46`89.3″ E 25°15`31.0″
				N 42°46`03.7″ E 25°29`12.0″
				N 42°44`57.0″ E 25°31`44.6″
	Hylocomium	*Hylocomium splendens* (Hedw.) Schimp.	LC/status unknown	N 42°45`98.9″ E 25°14`45.9″
Lembophyllaceae	*Isothecium*	*Isothecium alopecuroides* (Dubois) Isov.	LC/status unknown	N 42°48`23.2″ E 25°34`49.0″
Leucodontaceae	*Leucodon*	*Leucodon sciuroides* (Hedw.) Schwägr.	LC	N 42°46`55.6″ E 25°14`01.2″
				N 42°49`68.1″ E 25°12`73.4″
Anomodontaceae	*Anomodon*	*Anomodon rugelii* (Müll.Hal.) Keissl.	VU	N 42°48`23.2″ E 25°34`49.0″
		Anomodon viticulosus (Hedw.) Hook. & Taylor	LC	N 42°48`23.2″ E 25°34`49.0″
Neckeraceae	*Neckera*	*Neckera complanata* (Hedw.) Huebener	Unknown	N 42°48`23.2″ E 25°34`49.0″
Plagiotheciaceae	*Plagiothecium*	*Plagiothecium cavifolium* (Brid.) Z. Iwats.	LC	N 42°48`23.2″ E 25°34`49.0″
				N 42°49′68.1′′E 25°12′73.4′′

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Study of Plant Species Composition of Grasslands in Mugla Village Region (Western Rhodopes, South Bulgaria)

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Abstract. The study presents data on the diversity of grass species in the region of the village of Mugla (the Western Rhodopes). One hundred forty-one species of higher plants belonging to 40 families were registered. (Apiaceae, Aspleniaceae, Asteraceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Cistaceae, Cyperaceae, Dipsacaceae, Equisetaceae, Ericaceae, Euphorbiaceae, Fabaceae, Gentianaceae, Geraniaceae, Gesneriaceae, Hypericaceae, Juncaceae, Lamiaceae, Lemnaceae, Liliaceae, Linaceae, Menyanthaceae, Oleacea, Onagraceae, Orchidaceae, Parnassiaceae, Plantaginaceae, Plumbaginaceae, Poaceae, Polygalaceae, Primulaceae, Ranunculaceae, Rosaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Valerianaceae and Violaceae). Their conservation status was presented, as well as medicinal plants.

Key words: Mugla, Western Rhodopes, higher plants, conservation status.

Introduction

The Rhodope Mountains are located in the central part of the Balkan Peninsula and they have a total area of 18000 km², over 14000 km² of them being in the territory of the Republic of Bulgaria. Morphographically they are divided into two major parts – the Western and the Eastern Rhodopes Mts. The border between them runs along the river Kayaliyka, Kitka saddle, the Valley of Borovitza River, the eastern slopes of Zhalty Dyal Hill and reaches the saddle Tri Kamaka, southwest of Zlatograd Town (BRAMBAROV, 2001).

The Western Rhodope Mountains are the higher part of the mountain massif. They are of a medium mountain type, with a highly indented, thick and deeply incised river network. The average altitude of this part of the mountains is 1150 meters. The Western Rhodopes Mts. are divided into two parts along the Valley of Vacha River: Batak-Dabrava and Perelik-Prespa. The major peaks in the Perelik-Prespa part are Perelik, Prespa and Chernatitsa Peaks. The highest peak of the Rhodopes is in the Perelik Peak, named "Golyam Perelik" Peak (2 191 m). A large number of tributaries of the rivers Vacha, Chepelarska and Arda rise in that part of the Rhodopes. Smolyan and Hvoyna Valleys are also located in that part of the Western Rhodopes Mts. They fall in the Southern Bulgarian mountain-and-valley region, sub-region of the Western Rhodopes Mts. (PETROV, 1997).

Materials and Methods

According to the administrative division of the Republic of Bulgaria, the studied area includes the territories of the

villages of Mugla and Chamla, a small part of the villages of Gela, Gyovren and Trigrad and Nastan quarter of the town of Devin (the border areas with the village of Mugla). Administratively they all belong to the municipalities of Smolyan and Devin Towns, Smolyan District. Smolyan District belongs to South Central region of Bulgaria according to the National Plan for Regional Development (Fig. 1). Inventory of the grass plants was carried out from June to October 2015. A routing method with transect transitions was used. Transects were selected according to the specificities of different floristic groups, peculiarities of the terrain and altitude, aiming to cover maximum area and all typical and representative of the floristic diversity areas from the studied territory (Table 1). A GPS receiver GARMIN530 was used for inspection of the localities.

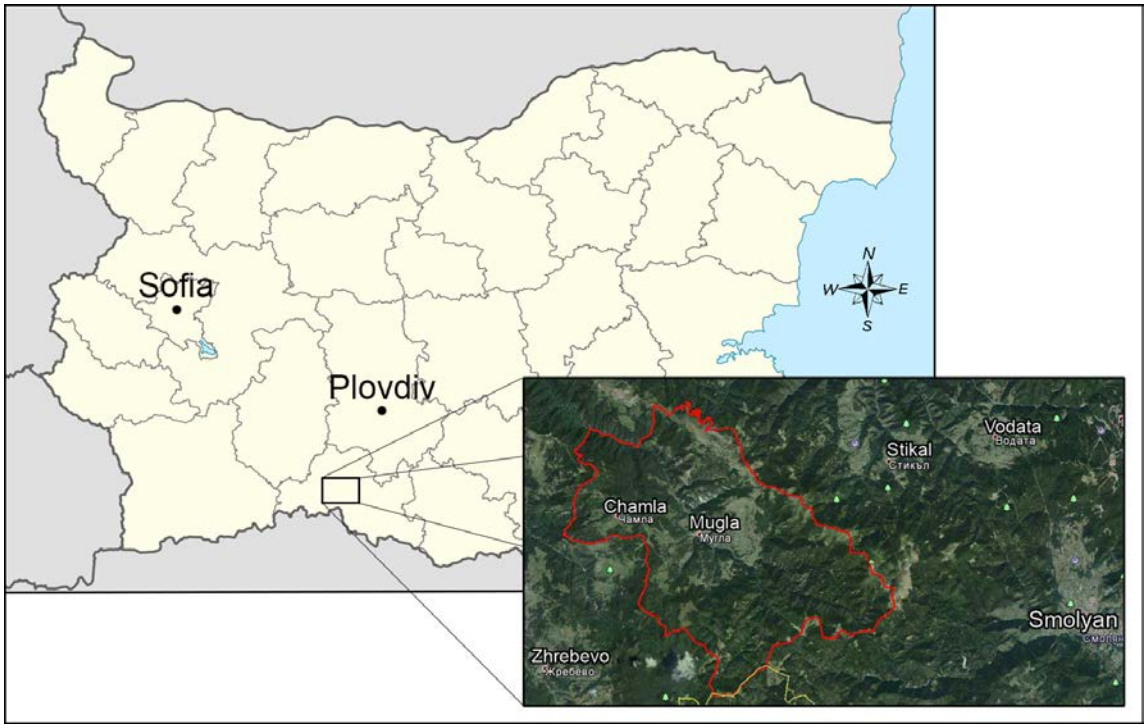

Fig. 1. Indicative map of the studied area in Bulgaria.

Table 1. Geographical coordinates of the studied areas.

№	Locality	Altitude	GPS coordinates
01.	Novak	1790 m	N 41°34.913`; E 024°32.419`
02.	Kanarata roud	1590 m	N 41°35.487`; E 024°31.363`
03.	Kasaka (swamp)	1573 m	N 41°36.856`; E 024°31.223`
04.	Ravna gora	1647 m	N 41°37.286`; E 024°30.715`
05.	Syulmenitsa	1714 m	N 41°38.106`; E 024°30.066`
06.	Roud toward Chaeva chuka	1829 m	N 41°38.563`; E 024°30.376`
07.	Chaeva chuka	1925 m	N 41°38.881`; E 024°30.031`
08.	Chaeva chuka (peak)	1841 m	N 41°39.086`; E 024°29.758`
09.	Mursalitsa (cattle-shed)	1774 m	N 41°39.840`; E 024°27.693`
10.	Village of Mugla	1452 m	N 41°37.156`; E 024°29.133`
11.	Kazandzhi dere	1368 m	N 41°37.526`; E 024°29.459`
12.	Kazandzhi dere (falls)	1398 m	N 41°37.606`; E 024°29.677`
13.	Golyamata dzhinupa	1559 m	N 41°35.853`; E 024°29.543`
14.	Yozere	1616 m	N 41°35.880`; E 024°28.483`
15.	Chamla (Usaykata)	1680 m	N 41°37.497`; E 024°26.915`
16.	Chairski ezera (hut)	1413 m	N 41°35.682`; E 024°26.711`
17.	Mechata polyana	1693 m	N 41°34.442`; E 024°30.788`
18.	Chetraka	1771 m	N 41°34.249`; E 024°30.352`

The nomenclature follows DELIPAVLOV & CHESHMEDJIEV (2003). Localization and characterization of taxa populations with conservation status was carried out parallel to the route research.

Results and Discussion

One hundred forty-one plant species belonging to 40 families were found during the field inventory of the area (Apiaceae, Aspleniaceae, Asteraceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Cistaceae, Cupressaceae, Cyperaceae, Dipsacaceae, Equisetaceae, Ericaceae, Euphorbiaceae, Fabaceae, Gentianaceae, Geraniaceae, Gesneriaceae, Hypericaceae, Juncaceae, Lamiaceae, Lemnaceae, Liliaceae, Menyanthaceae, Oleacea, Onagraceae, Orchidaceae, Parnassiaceae, Plantaginaceae, Plumbaginaceae, Poaceae, Polygalaceae, Primulaceae, Ranunculaceae, Rosaceae, Rubiaceae, Saxifragaceae, Scrophulariaceae, Valerianaceae and Violaceae). The full list of the plants is presented in Table 2.

Figure 2 shows that out of the 141 identified species, *Asteraceae* family is represented by the richest species diversity (18 species), followed by *Lamiaceae* family (13 species). *Caryophyllaceae* and *Fabaceae* families are represented by 9 species each and *Rosaceae* and *Poaceae* – by 8 species each. The rest of the families are under-represented.

Medicinal plants and species with conservation status

Seventy-five (50,34%) of all the 141 described plants are under a special regime (Table 3). 64 species are included in the Medicinal Plants Act (2000), which provides various activities for their conservation and sustainable use, including the collection and buy-out of herbs obtained thereof.

Eight species are included in the Biological Diversity Act (2002). Two of them (*Carex limosa* and *Menyanthes trifoliata*) belong to Annex 2a of the Act and it is stated that the conservation of their habitats is intended by designating protected areas. Five species (*Cortusa matthioli*, *Gentiana lutea ssp. symphyandra*, *Geum rhodopaeum*, *Haberlea rhodopensis* and *Potentilla palustris*) are included in Annex 3 of the same Act and they are announced protected on the territory of the whole country. *Primula veris* species is included in Annex 4 of the Biological Diversity Act under a regime of protection and of regulated use from nature.

There are 4 protected species included in the Red Book of the Republic of Bulgaria, Volume 1. Plants and fungi (PEEV *et al.*, 2015) – *Carex limosa*, *Gentiana lutea* ssp. *symphyandra*, *Menyanthes trifoliate* and *Sideritis scardica*. They all fall in the category Endangered (EN).

The Red List of Bulgarian vascular plants (PETROVA & VLADIMIROV, 2009) includes 12 species. *Gentianella praecox* species is included under the categpry Data Defficient (DD). *Haberlea rhodopensis* is under the category Least Concern (LC), the species *Geum rhodopaeum*, *Jasione bulgarica* and *Saxifraga stribrnyi* are under Near Threatened (NT) category. Three species: *Angelica pančičii*, *Cortusa matthioli* and *Potentilla palustris* and under Vulnerable (VU) and *Carex limosa*, *Gentiana lutea* ssp. *symphyandra*, *Menyanthes trifoliata* and *Sideritis scardica* are Endangered (EN).

Haberlea rhodopensis is included in Appendix 1 "Strictly protected flora species" of the Convention on the Conservation of European Wildlife and Natural Habitats (CEST, 1979).

Seventeen of the species described in the studied area are included in the Red List of Threatened Plants of the International Union for Conservation of Nature (IUCN, 2015). 16 of them – *Caltha palustris*, *Carex limosa*, *Dactylorhiza cordigera*, *Dactylorhiza sambucina*, *Equisetum palustre*, *Filipendula ulmaria*, *Haberlea rhodopensis*, *Juniperus communis*, *Lemna minor*, *Mentha longifolia*, *Menyanthes trifoliata*, *Nasturtium officinale*, *Parnassia palustris*, *Potentilla palustris*, *Scirpus sylvaticus* and *Trifolium pratense* – are under the category of Least Concern (LC), and the species *Sideritis scardica* – under Near Threatened (NT).

The endemic plants listed in the "Atlas of Bulgarian Endemic Plants" (PETROVA, 2006) are 7. Those are the species *Geum rhodopaeum*, *Jasione bulgarica*, *Sideritis scardica*, *Saxifraga stribrnyi*, *Haberlea*

rhodopensis, *Armeria rumelica* and *Chamaecytisus absinthioides*.

Balkan endemics in the Bulgarian flora (PETROVA & VLADIMIROV, 2010) are: *Achillea ageratifolia*, *Chamaecytisus calcareus*, *Pastinaca hirsute*, *Cirsium appendiculatum* and *Knautia midzorensis*. The species *Haberlea rhodopensis* is also a Tertiary relict and the species *Cortusa matthioli*, *Gymnadenia conopsea* and *Parnassia palustris* are glacial relicts.

Table 2. List of the registered plants species in the studied area of Mugla (Western Rhodopes, South Bulgaria).

№	Familia	Species
1.	Apiaceae	*Angelica pančičii* Vandas
2.	Apiaceae	*Chaerophyllum aureum* L.
3.	Apiaceae	*Pastinaca hirsute* Pančiči
4.	Aspleniaceae	*Asplenium trichomanes* L.
5.	Asteraceae	*Achillea ageratifolia (Sibth. & Sm.)* Boiss.
6.	Asteraceae	*Achillea millefolium* L.
7.	Asteraceae	*Antennaria dioica* (*L.*) Gaertner
8.	Asteraceae	*Anthemis arvense* L.
9.	Asteraceae	*Carlina acanthifolia* All.
10.	Asteraceae	*Centaurea nervosa* Willd.
11.	Asteraceae	*Centaurea stenolepis* A. Kerner
12.	Asteraceae	*Cirsium appendiculatum* Griseb.
13.	Asteraceae	*Doronicum austriacum* Jacq.
14.	Asteraceae	*Hieracium hoppeanum* Schultes
15.	Asteraceae	*Hieracium pilosella* L.
16.	Asteraceae	*Leontodon crispus* Vill.
17.	Asteraceae	*Leucanthemum vulgare* Lam.
18.	Asteraceae	*Petasites albus (L.)* Gaertner
19.	Asteraceae	*Scorzonera laciniata* L.
20.	Asteraceae	*Solidago virgaurea* L.
21.	Asteraceae	*Taraxacum officinale* Weber
22.	Asteraceae	*Tussilago farfara* L.
23.	Boraginaceae	*Echium vulgare* L.
24.	Boraginaceae	*Myosotis sicula* Guss.
25.	Boraginaceae	*Myosotis sylvatica* Ehrh. ex Hoffm.
26.	Boraginaceae	*Nonea pulla (L.)* DC.
27.	Boraginaceae	*Symphytum officinale* L.
28.	Brassicaceae	*Arabis alpine* L.
29.	Brassicaceae	*Cardamine rivularis* Schur
30.	Brassicaceae	*Nasturtium officinale* R. Br.
31.	Brassicaceae	*Roripa sylvestris* (*L.*) Besser
32.	Brassicaceae	*Thlaspi sp.*
33.	Campanulaceae	*Campanula rapunculoides* L.
34.	Campanulaceae	*Campanula rapunculus* L.
35.	Campanulaceae	*Campanula sparsa* Friv.
36.	Campanulaceae	*Jasione bulgarica* Stoj. & Stefanov
37.	Caryophyllaceae	*Dianthus deltoides* L.
38.	Caryophyllaceae	*Dianthus pinifolius* Sm.
39.	Caryophyllaceae	*Lychnis flos-cuculi* L.
40.	Caryophyllaceae	*Moenchia mantica* (*L.*) Bartl.
41.	Caryophyllaceae	*Paronychia kapela* (*Hacq.*) A. Kerner
42.	Caryophyllaceae	*Scleranthus polycarpos* L.
43.	Caryophyllaceae	*Silene roemeri* Friv.
44.	Caryophyllaceae	*Silene vulgaris* (*Moench*) Garcke
45.	Caryophyllaceae	*Stellaria graminea* L.
46.	Cistaceae	*Helianthemum nummularium* (*L.*) Miller
47.	Cyperaceae	*Carex limosa* L.
48.	Cyperaceae	*Eriophorum vaginatum* L.
49.	Cyperaceae	*Scirpus sylvaticus* L.
50.	Dipsacaceae	*Knautia arvensis* (*L.*) Coulter
51.	Dipsacaceae	*Knautia midzorensis* Form.

52.	Equisetaceae	*Equisetum arvense* L.
53.	Equisetaceae	*Equisetum palustre* L.
54.	Ericaceae	*Bruckenthalia spiculifolia* (*Salisb.*) Reichenb.
55.	Euphorbiaceae	*Euphorbia myrsinites* L.
56.	Fabaceae	*Anthyllis montana* L.
57.	Fabaceae	*Anthyllis vulneraria* L.
58.	Fabaceae	*Astragalus monspessulanus* L.
59.	Fabaceae	*Chamaecytisus absinthioides* (*Janka*) Kuzmanov
60.	Fabaceae	*Chamaecytisus calcareus (Velen.)* Kuzmanov
61.	Fabaceae	*Genista lydia* Boiss.
62.	Fabaceae	*Trifolium pratense* L.
63.	Fabaceae	*Trifolium repens* L.
64.	Fabaceae	*Vicia incana* Gouan
65.	Gentianaceae	*Gentiana lutea* ssp. *symphyandra* (*Murb.*) Hayek
66.	Gentianaceae	*Gentiana utriculosa* L.
67.	Gentianaceae	*Gentianella praecox* (*A. & J. Kerner*) Dostal
68.	Geraniaceae	*Geranium macrorrhizum* L.
69.	Geraniaceae	*Geranium sanguineum* L.
70.	Gesneriaceae	*Haberlea rhodopensis* Friv.
71.	Hypericaceae	*Hypericum maculatum* Crantz
72.	Hypericaceae	*Hypericum perforatum* L.
73.	Juncaceae	*Juncus effuses* L.
74.	Juncaceae	*Luzula sp.*
75.	Lamiaceae	*Ajuga genevensis* L.
76.	Lamiaceae	*Ajuga laxmanii* (*L.*) Bentham
77.	Lamiaceae	*Betonica officinalis* L.
78.	Lamiaceae	*Clinopodium vulgare* L.
79.	Lamiaceae	*Coronilla varia* L.
80.	Lamiaceae	*Galeopsis speciosa* Miller
81.	Lamiaceae	*Mentha longifolia* (*L.*) Hudson
82.	Lamiaceae	*Nepeta nuda* L.
83.	Lamiaceae	*Onobrychis montana* DC
84.	Lamiaceae	*Origanum vulgare* L.
85.	Lamiaceae	*Prunella vulgaris* L.
86.	Lamiaceae	*Sideritis scardica* Griseb.
87.	Lamiaceae	*Thymus sp.*
88.	Lemnaceae	*Lemna minor* L.
89.	Liliaceae	*Muscari botryoides* (*L.*) *Miller*
90.	Liliaceae	*Ornithogalum umbellatum* L.
91.	Liliaceae	*Veratrum album* L. *ssp. lobelianum (Bernh.)* Reichenb.
92.	Linaceae	*Linum capitatum* Kit. ex Schultes
93.	Menyanthaceae	*Menyanthes trifoliata* L.
94.	Oleacea	*Thesium alpinum* L.
95.	Onagraceae	*Chamaenerion angustifolium* (*L.*) Scop.
96.	Orchidaceae	*Dactylorhiza cordigera* (*Fries.*) Soò
97.	Orchidaceae	*Dactylorhiza sambucina* (*L.*) Soò
98.	Orchidaceae	*Gymnadenia conopsea* (*L.*) R. Br.
99.	Parnassiaceae	*Parnassia palustris* L.
100.	Plantaginaceae	*Plantago lanceolata* L.
101.	Plantaginaceae	*Plantago media* L.
102.	Plumbaginaceae	*Armeria rumelica* Boiss.
103.	Poaceae	*Agrostis cappilaris* L.
104.	Poaceae	*Alopecurus myosuroides* Hudson
105.	Poaceae	*Apera spica-venti* (*L.*) Beauv.
106.	Poaceae	*Arrhenatherum elatius* (*L.*) Beauv. ex J. & C. Presl
107.	Poaceae	*Briza humilis* Bieb.
108.	Poaceae	*Calamagrostis epigeios* (*L.*) Roth
109.	Poaceae	*Festuca nigrescens* Lam.
110.	Poaceae	*Festuca pratensis* Hudson
111.	Polygalaceae	*Polygala anatholica* Boiss. & Heldr.
112.	Polygonaceae	*Rumex acetosa* L.
113.	Polygonaceae	*Rumex acetosella* L.
114.	Primulaceae	*Cortusa matthioli* L.

115.	Primulaceae	*Primula veris* L.
116.	Ranunculaceae	*Caltha palustris* L.
117.	Ranunculaceae	*Ranunculus acris* L.
118.	Ranunculaceae	*Ranunculus polyanthemos* L.
119.	Ranunculaceae	*Thalictrum aquilegifolium* L.
120.	Rosaceae	*Filipendula ulmaria* (*L.*) Maxim.
121.	Rosaceae	*Fragaria vesca* L.
122.	Rosaceae	*Geum revale* L.
123.	Rosaceae	*Geum rhodopaeum* Stoj. & Stefanov
124.	Rosaceae	*Potentilla argentea* L.
125.	Rosaceae	*Potentilla erecta* (*L.*) Räuschel
126.	Rosaceae	*Potentilla palustris* (*L.*) Scop.
127.	Rosaceae	*Sanguisorba minor* Scop.
128.	Rubiaceae	*Cruciata laevipes* Opiz
129.	Rubiaceae	*Galium rivale* (*Sibth. & Sm.*) Griseb.
130.	Rubiaceae	*Galium verum* L.
131.	Saxifragaceae	*Saxifraga adscendens* L.
132.	Saxifragaceae	*Saxifraga stribrnyi* (*Velen.*) Podp.
133.	Scrophulariaceae	*Euphrasia minima* Jasq. Ex DC.
134.	Scrophulariaceae	*Melampyrum sylvaticum* L.
135.	Scrophulariaceae	*Rhinanthus rumelicus* Velen.
136.	Scrophulariaceae	*Verbascum sp.*
137.	Scrophulariaceae	*Veronica sp.*
138.	Valerianaceae	*Valeriana officinalis* L.
139.	Valerianaceae	*Valerianella sp.*
140.	Violaceae	*Viola kitaibeliana* Schultes
141.	Violaceae	*Viola tricolor* L.

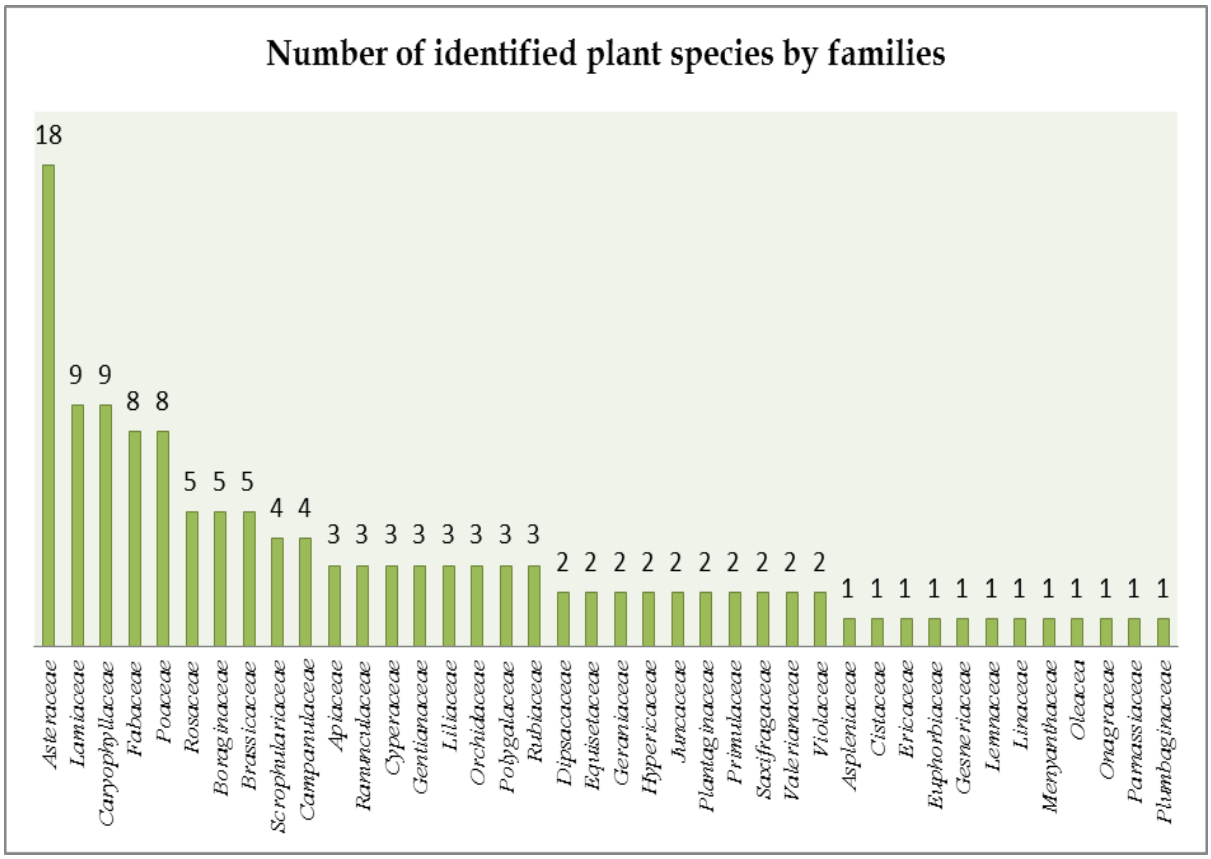

Fig. 2. Number of identified plant species by families.

Table 3. Plants of conservation importance and medicinal plants, registered in the studied area of Mugla (Western Rhodopes, South Bulgaria). *Legend:* IUCN - IUCN Red List of Threatened species; BC - Bern Convention; RLBVP - Red List of Bulgarian vascular plants; RB - Red Book of the Republic of Bulgaria; BDA - Biological Diversity Act; MPA - Medicinal Plants Act (explanations and citations are in the text).

№	Species	IUCN	BC	RLBVP	RB	BDA	MPA
1.	*Achillea millefolium*	-		-	-	-	+
2.	*Angelica pančičii*	-		VU	-	-	-
3.	*Antennaria dioica*	-		-	-	-	+
4.	*Anthyllis vulneraria*	-		-	-	-	+
5.	*Asplenium trichomanes*	-		-	-	-	+
6.	*Betonica officinalis*	-		-	-	-	+
7.	*Caltha palustris*	LC		-	-	-	+
8.	*Carex limosa*	LC		EN	EN	+	-
9.	*Clinopodium vulgare*	-		-	-	-	+
10.	*Coronilla varia*	-		-	-	-	+
11.	*Cortusa matthioli*	-		VU	-	+	-
12.	*Cruciata laevipes*	-		-	-	-	+
13.	*Dactylorhiza cordigera*	LC		-	-	-	-
14.	*Dactylorhiza sambucina*	LC		-	-	-	-
15.	*Echium vulgare*	-		-	-	-	+
16.	*Equisetum arvense*	-		-	-	-	+
17.	*Equisetum palustre*	LC		-	-	-	+
18.	*Eriophorum vaginatum*	-		-	-	-	+
19.	*Euphorbia myrsinites*	-		-	-	-	+
20.	*Filipendula ulmaria*	LC		-	-	-	+
21.	*Fragaria vesca*	-		-	-	-	+
22.	*Galeopsis speciosa*	-		-	-	-	+
23.	*Galium verum*	-		-	-	-	+
24.	*Gentiana lutea* ssp. *symphyandra*	-		EN	EN	+	+
25.	*Gentianella praecox*	-		DD	-	-	-
26.	*Geranium macrorrhizum*	-		-	-	-	+
27.	*Geranium sanguineum*	-		-	-	-	+
28.	*Geum rhodopaeum*	-		NT	-	+	-
29.	*Gymnadenia conopsea*	-		-	-	-	+
30.	*Haberlea rhodopensis*	LC	+	LC	-	+	+
31.	*Hieracium pilosella*	-		-	-	-	+
32.	*Hypericum maculatum*	-		-	-	-	+
33.	*Hypericum perforatum*	-		-	-	-	+
34.	*Jasione bulgarica*	-		NT	-	-	-
35.	*Juniperus communis*	LC		-	-	-	-
36.	*Knautia arvensis*	-		-	-	-	+
37.	*Lemna minor*	LC		-	-	-	+
38.	*Leucanthemum vulgare*	-		-	-	-	+
39.	*Lychnis flos-cuculi*	-		-	-	-	+
40.	*Mentha longifolia*	LC		-	-	-	+
41.	*Menyanthes trifoliata*	LC		EN	EN	+	+
42.	*Nasturtium officinale*	LC		-	-	-	+
43.	*Origanum vulgare*	-		-	-	-	+
44.	*Parnassia palustris*	LC		-	-	-	+
45.	*Petasites albus*	-		-	-	-	+
46.	*Plantago lanceolata*	-		-	-	-	+
47.	*Plantago media*	-		-	-	-	+
48.	*Potentilla argentea*	-		-	-	-	+
49.	*Potentilla erecta*	-		-	-	-	+
50.	*Potentilla palustris*	LC		VU	-	+	+
51.	*Primula veris*	-		-	-	+	+
52.	*Prunella vulgaris*	-		-	-	-	+
53.	*Ranunculus polyanthemos*	-		-	-	-	+
54.	*Rumex acetosa*	-		-	-	-	+

55.	*Rumex acetosella*	-	-	-	-	+
56.	*Salix caprea*	-	-	-	-	+
57.	*Salix purpurea*	-	-	-	-	+
58.	*Sambucus racemosa*	-	-	-	-	+
59.	*Sanguisorba minor*	-	-	-	-	+
60.	*Saxifraga stribrnyi*	-	NT	-	-	-
61.	*Scirpus sylvaticus*	LC	-	-	-	-
62.	*Sorbus aucuparia*	-	-	-	-	+
63.	*Sideritis scardica*	NT	EN	EN	-	+
64.	*Solidago virgaurea*	-	-	-	-	+
65.	*Stellaria graminea*	-	-	-	-	+
66.	*Symphytum officinale*	-	-	-	-	+
67.	*Taraxacum officinale*	-	-	-	-	+
68.	*Thalictrum aquilegifolium*	-	-	-	-	+
69.	*Trifolium pratense*	LC	-	-	-	+
70.	*Trifolium repens*	-	-	-	-	+
71.	*Tussilago farfara*	-	-	-	-	+
72.	*Vaccinium myrtillus*	-	-	-	-	+
73.	*Valeriana officinalis*	-	-	-	-	+
74.	*Veratrum album* L. *ssp. lobelianum*	-	-	-	-	+
75.	*Viola tricolor*	-	-	-	-	+

Conclusions

In the present study was found a high abundance of plants in all investigated territories. This could be a hallmark for their importance of environmental issues. The list of plants includes 141 species, 24 of them being of conservation importance. Phytocenological studies including species variety and quantity should be carried out in order to determine more detailed characteristics of the areas. This would be of great importance to be done for species with conservation significance.

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Food Composition of the Snake-Eyed Lizard, Ophisops elegans Ménétriés, 1832 (Reptilia: Sauria: Lacertidae) from Gökçeada (Imbros), Turkey

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Abstract. The study presents data on the food composition of the snake-eyed lizard (*Ophisops elegans*), from Gökçeada (Imbros), Çanakkale, Turkey. A total of 94 preys were determined in the digestive systems of 20 individuals (10 males, 10 females) examined in the study. Insects (67%) constitute most of its food composition. Major prey groups in the food composition are included in Aranea (13%), Lepidoptera (13%), Coleoptera (19%), and Homoptera (20%) in numeric proportion. No significant difference was observed between sexes considering food composition.

Key words: Snake-eyed lizard, Lacertidae, Fecundity, Food composition, Turkey.

Introduction

The genus *Ophisops* includes 8 valid species distributing from southeastern Europe to North Africa and Asia (KYRIAZI *et al.*, 2008). The snake-eyed lizard, *Ophisops elegans* Ménétriés, 1832, is a small sized lacertid and considered as a Mediterranean species. The species is widely distributed across the eastern Mediterranean region, Southwestern Asia, and North Africa (SCHLEICH *et al.*, 1996; ANDERSON, 1999, SINDACO *et al.*, 2000, ANANJEVA *et al.*, 2006; KYRIAZI *et al.*, 2008). It has been classified as LC category in IUCN Mediterranean Basin Red List (COX *et al.*, 2006) and included in the Appendix II (Strictly Protected Fauna Species) at the Bern Convention (CETS, 1979). The snake-eyed lizard typically inhabits open and arid plains, agricultural fields and stony hillsides with sparse vegetation or low shrubs at elevations of up to 2000 m (BARAN & ATATÜR, 1998; ANDERSON, 1999).

Most of the studies on the snake-eyed lizard in Turkey are concerned with taxonomy of this species (e.g. TOK, 1992; TOK *et al.*, 1997; KYRIAZI *et al.*, 2008); however, there is little research on its ecology, e.g. age structure, (TOK *et al.*, 1997) and feeding biology (AKKAYA & UĞURTAŞ, 2006). The aim of the present study is to present the food composition of the snake-eyed lizard, *Ophisops elegans*, from Gökçeada (Imbros), Çanakkale, Turkey.

Materials and Methods

In the study 20 preserved specimens of the snake-eyed lizard (10 males, 10 females) were examined, which were collected between 4 April and 5 May 2009 from

Gökçeada (Imbros), Çanakkale, Turkey. The material was registered in the Museum of the Faculty of Arts and Sciences, Çanakkale Onsekiz Mart University and incorporated into the collection of ZDEU-ÇOMU (Zoology Department Ege University-Çanakkale Onsekiz Mart University), Turkey.

The snout-vent length (distance from the tip of the snout to the cloaca, SVL) and total length (from the cloaca to the tip of the tail, TL) of the specimens were measured using a caliper to the nearest 0.1 mm and recorded. In addition, the secondary sexual characters were determined. After these procedures, they were dissected and their digestive tracts were removed. The obtained food contents were preserved in 70% ethanol for further 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 thus not considered as food.

The food contents were presented both in numeric proportion (the number of a particular prey item in all preys, N %), frequency of occurrence (the frequency of lizard stomachs containing a particular prey type, F %) and volumetric proportion (the volume of a particular prey item in all preys, V %). The prey volume was calculated using ellipsoid formula (DUNHAM, 1983): $V= 4/3\pi (L/2) (W/2)^2$ [V: prey volume; L: length of prey; W: width of prey]. Trophic niche overlap was measured using Pianka's index (PIANKA, 1973). This index ranges from 0 (no similarity) to 1 (totally similar). Food-niche breadth was determined using Shannon's index (H, , 1948). All niche calculations were made using "EcoSimR vers. 1.0" package (GOTELLI & ELLISON, 2013) in R vers. 3.2.2. Sexes were compared by t-test, and Mann-Whitney U tests performed using Deducer statistical package (FELLOWS, 2012) in R vers. 3.2.2. The alpha level was set at 0.05. The mean values are provided with their standard deviations.

Results

The mean body length (SVL) was 51.3±2.19 (46.0–70.0) mm for males and 51.5±1.94 (49.0–55.0) mm for females. The mean total length (TL) was determined as 147.1±10.25 (131.0–160.0) mm in males and 142.3±8.26 (130.0–150.0) mm in females. No statistically significant difference was observed between sexes in terms of their sizes (SVL, t=0.94 P=0.926; TL, t=0.86, P=0.413).

In the stomach contents of 20 individuals, 94 prey items, with body lengths ranging from 2 to 15 mm, were determined with a median (±SD) number of 5±1.81 (range=2-9). The number of median prey items was 3.5±1.27 (2-6) in males, and 5±1.45 (5-9) in females. There was a significant difference between males and females (Mann-Whitney U test, Z=10.0, P=0.002). Males consumed fewer preys than females did. Aranea (n%=13%), Homoptera (20%), Coleoptera (19%) and Lepidoptera (14%) were important prey groups in the food content. Among the prey taxa shown in Table 1, Coleoptera (f%=60%), Aranea (55%), and Homoptera (50%) were frequently consumed by the lizards. More active preys like non-formicid Hymenoptera, Orthoptera and Diptera were less encountered in the food content (Table 1). The larval preys were 18% in number, 35% in frequency and 33% in volume of the food contents. The largest volume in the food composition belonged to Coleoptera (v%=37%), Aranea (21%), Homoptera (18%) and Orthoptera (11%). The contribution of the remaining groups was less than 10%.

According to the Pianka's niche overlap index, food compositions of sexes were mostly similar (males vs. females = 0.87). This indicates that feeding habit does not change with sex and both sexes use similar microhabitat for foraging. Food niche breadth (Shannon's index) was 1.65 in males and 1.63 in females. Both sexes have similar niche breadth and food spectrum of the species is rather limited according to the index value.

Table 1. Food composition of 20 (10 males and 10 females) Snake eyed lizard, *Ophisops elegans* from Gökçeada. M: males, F: females, N (%): Numeric proportion, N (%): Frequency of occurrence, V (%): Volumetric proportion.

	N (%)			F (%)			V (%)		
Prey taxa	**M**	**F**	**Overall**	**M**	**F**	**Overall**	**M**	**F**	**Overall**
Arachnida	5 (0.14)	7 (0.12)	12 (0.13)	4 (0.40)	7 (0.70)	11 (0.55)	112.73 (0.11)	366.44 (0.29)	479.16 (0.21)
Aranea	5 (0.14)	7 (0.12)	12 (0.13)	4 (0.40)	7 (0.70)	11 (0.55)	112.73 (0.11)	366.44 (0.29)	479.16 (0.21)
Insecta	30 (0.86)	33 (0.56)	63 (0.67)	9 (0.90)	9 (0.90)	18 (0.90)	909.96 (0.89)	895.84 (0.71)	1805.81 (0.79)
Heteroptera	1 (0.03)	2 (0.03)	3 (0.03)	1 (0.10)	1 (0.10)	2 (0.10)	16.96 (0.02)	33.91 (0.03)	50.87 (0.02)
Pentatomidae, *Pentatoma* sp.	1 (0.03)	2 (0.03)	3 (0.03)	1 (0.10)	1 (0.10)	2 (0.10)	16.96 (0.02)	33.91 (0.03)	50.87 (0.02)
Homoptera	12 (0.34)	7 (0.12)	19 (0.20)	6 (0.60)	4 (0.40)	10 (0.50)	294.85 (0.29)	115.87 (0.09)	410.71 (0.18)
Cicadellidae, *Cicada* sp.	12 (0.34)	7 (0.12)	19 (0.20)	6 (0.60)	4 (0.40)	10 (0.50	294.85 (0.29)	115.87 (0.09)	410.71 (0.18)
Hymenoptera	2 (0.06)	2 (0.03)	4 (0.04)	1 (0.10)	2 (0.20)	3 (0.15)	28.26 (0.03)	28.26 (0.02)	56.52 (0.02)
Formicidae	2 (0.06)	2 (0.03)	4 (0.04)	1 (0.10)	2 (0.20)	3 (0.15)	28.26 (0.03)	28.26 (0.02)	56.52 (0.02)
Coleoptera	7 (0.20)	11 (0.19)	18 (0.19)	4 (0.40)	8 (0.80)	12 (0.60)	270.35 (0.26)	575.25 (0.46)	845.59 (0.37)
Larvae	2 (0.06)	-	2 (0.02)	1 (0.10)	3 (0.30)	4 (0.20)	130.62 (0.13)	433.32 (0.34)	563.94 (0.25)
Carabidae	-	3 (0.05)	3 (0.03)	-	1 (0.10)	1 (0.05)	-	16.96 (0.01)	16.96 (0.01)
Coccinellidae, *Coccinella* sp.	1 (0.03)	3 (0.05)	4 (0.04)	1 (0.10)	2 (0.20)	3 (0.15)	11.30 (0.01)	33.91 (0.03)	45.22 (0.02)
Curculionidae	2 (0.06)	4 (0.07)	6 (0.06)	1 (0.10)	1 (0.10)	2 (0.10)	17.89 (0.02)	35.80 (0.03)	53.69 (0.02)
Tenebrionidae	2 (0.06)	1 (0.02)	3 (0.03)	1 (0.10)	1 (0.10)	2 (0.10)	110.53 (0.11)	55.26 (0.04)	165.79 (0.07)
Diptera	1 (0.03)	-	1 (0.01)	1 (0.10)	-	1 (0.05)	-	16.96 (0.01)	16.96 (0.01)
Tabanidae	1 (0.03)	-	1 (0.01)	1 (0.10)	-	1 (0.05)	-	16.96 (0.01)	16.96 (0.01)
Lepidoptera	5 (0.14)	8 (0.14)	13 (0.14)	3 (0.30)	-	3 (0.15)	184.00 (0.18)	-	184.00 (0.08)
Larvae	5 (0.14)	8 (0.14)	13 (0.14)	3 (0.30)	-	3 (0.15)	184.00 (0.18)	-	184.00 (0.08)
Orthoptera	2 (0.06)	3 (0.05)	5 (0.05)	2 (0.20)	2 (0.20)	4 (0.20)	115.55 (0.11)	125.60 (0.10)	241.15 (0.11)
Total number of prey items	35	59	94				1022.7	1262.3	2285.0

Discussion

Our study revealed that snake-eyed lizardmostly consumed spiders (f%=55%) and insects (90%), especially Coleoptera and Homoptera. The food content consists mainly (n%>10%) of Aranea, Homoptera, Coleoptera and Lepidoptera. The flying preys including non-formicid Hymenopetara, Diptera and Orthoptera were less encountered in the food composition. In previous studies on the species, Isopoda, Opilionida, Aranea, Pseudoscorpionida, Chilopoda, Colembolla, Orthoptera, Blattodea, Mantodea, Homoptera, Heteroptera, Hymenoptera (especially Formicidae), Diptera and Lepidoptera were reported in the food content (PÉREZ-MELLADO *et al.*, 1993; ANDERSON, 1999; AKKAYA & UĞURTAŞ, 2006). Aranea and insect larvae are particularly important food sources for the snake-eyed lizard (PÉREZ-MELLADO *et al.*, 1993; AKKAYA & UĞURTAŞ, 2006).

The snake-eyed lizard is considered as an opportunistic predator which eats any prey abundant in its environment (AKKAYA & UĞURTAŞ, 2006). Actively foraging predators encounter and consume mostly non-moving types of prey items (PIANKA, 1966). PERRY & PIANKA (1997) stated that actively foraging species used their visual and smelling senses while foraging; and food niche breadth is rather narrow. The snake-eyed lizard actively searches for suitable prey (PÉREZ-MELLADO *et al.*, 1993), which generally includes insects and other arthropods (PÉREZ-MELLADO *et al.*, 1993; ANDERSON, 1996; AKKAYA & UĞURTAŞ, 2006). Our results confirm that due to the limited prey range of the snake-eyed lizard and less active preys in the food composition, it could be included in the active foragers.

In conclusion, the food composition of the snake-eyed lizardis mostly composed of slow-moving arthropods. Therefore, more active and flying preys were less encountered in the food composition. The species mainly feed on spiders, homopterans and coleopterans.

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Initial Study of the Ground Beetles (Coleoptera: Carabidae) and Other Invertebrates from "Leshnitsa" Nature Reserve (Central Stara Planina Mountains, Bulgaria)

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Abstract. The invertebrate fauna of the "Leshnitsa" nature reserve was studied, with particular consideration to the ground beetles. During the study altogether 394 specimens of carabid beetles belonging to 32 species and subspecies were captured, as well as 23 other invertebrate species, some of which are with a conservation significance (protected, Bulgarian and Balkan endemics). Ground beetles were characterized and classified according to their zoogeographical belonging, degree of endemism and the life forms they refer to. Threats for the invertebrate fauna and negative factors of anthropogenic origin were determined and measures for diminishing of their effect were proposed. So far the invertebrate fauna in this part of the mountain has been insufficiently studied. The real state of the diversity of this group in the area will be revealed only after future investigations and discovery of additional new species for the region.

Keywords: ground beetles, Carabidae, Invertebrata, "Leshnitsa" reserve, Stara Planina Mts., conservation.

Introduction

The investigation of the biota of the protected natural areas is an important component of their functioning and allows the assessment of the value of the given territory and its representativeness as a repository for the gene pool of the particular ecosystems. The strict nature reserve "Leshnitsa" keeps typical ecosystems in the lower upland belt of the Shipka mountain massif in Central Stara Planina Mts., near the village of Yasenovo, Municipality of town of Kazanlak (42°42'47"N, 25°13'5"E). Its area is 388.95 ha. The habitats are relatively preserved due to their toilsome accessibility. The territory of the reserve includes century-old beech forests, which are mostly unaffected by anthropogenic activities. They relate to Natura 2000 Habitats with code 9110 and 9130 and in general are characterized by good self-sustaining capability and a specific set of coexisting animal species (SSYMANK *et al.*, 1998; LESHNITSA, 2015).

However, intensive wood felling was observed adjacent to the boundaries of the reserve. This fact greatly reduces the buffer role of these border areas. The area has not been subjected to detailed faunistic investigations. Previous studies on the insect fauna of the reserve are partial and insufficient, and particularly in terms of the carabid fauna the data are missing. The lack of faunistic studies in "Leshnitsa" impedes

the overall assessment of the species abundance, size of the populations, nature of their spatial distribution, biodiversity and extent of anthropogenic influence.

For the middle parts of the Stara Planina mountain chain where the natural reserve "Leshnitsa" is located, but not exactly for the territory of the reserve, about 230 species of ground beetles are known (Coleoptera: Carabidae), and among them there are great percentage of rare and endemic forms, as especially peculiar is the cave carabid fauna. For example, only in the area of the Mount of Shipka there are three endemic species – the local Bulgarian endemites *Duvalius balcanicus* (J. Frivaldszky, 1879) and *Laemostenus plasoni* (Reitter, 1885), as well as the Balkan endemic *Amara municipalis bischoffi* Jedlicka, 1946 (GUÉORGUIEV & GUÉORGUIEV, 1995; GUÉORGUIEV *et al*., 1997).

Up to this point purposive investigations on the species composition of the invertebrate fauna as a whole in the territory of the "Leshnitsa" reserve have not been conducted, which premises the aim of this study. The main objective of the present study is to determine the species composition of Carabidae, existing in the research area. The study will be a preliminary step in order to detect the Coleoptera fauna of Central Stara Planina Mts., where the group is poorly studied.

Material and Methods

Field work was carried out in the periods: 8 – 12 May, 9 – 14 June and 1 – 5 September 2014. It included: 1) transect method with observations *in situ* or collection of material; 2) stationary method with „pitfall" traps (DAHL, 1896; HERTZ, 1927; BARBER, 1931) made of plastic bottles, buried at the level of the ground surface, with a 4% solution of formaldehyde as a fixation fluid; this method is suitable for ecological research on adult beetles, and mainly reflects their activity (LÖVEI & SUNDERLAND, 1996); there are no reasonable alternatives to this type of traps in the study of epigeic arthropod communities (SPENCE & NIEMELA, 1994); it is considered that the application of this method allows approximately 95% of the species active in radius of 50 m around the traps to be caught (BAARS & VAN DIJK, 1984); 3) handpicking and shaking of branches, capturing with a standard entomological sack.

Sampling areas were three: (I) Humid forest and ecotone with White Butterbur; (II) Mesophilous beech forest and ecotone with a brook; (III) Humid beech forest and ecotone with the river of Leshnitsa. Captured animals were determined with the help of several main literary sources: LINDROTH (1974); TRAUTNER & GEIGENMÜLLER (1987); HŮRKA (1996); HARDE (2000); REITTER (2006); ARNDT *et al*. (2011); KRYZHANOVSKIJ (pers. com.), and are deposited in the collection of the Institute of Biodiversity and Ecosystem Research (BAS, Sofia).

The systematic list of Carabidae follows KRYZHANOVSKIJ *et al*. (1995). According to their zoogeographical belonging ground beetles were separated in zoogeographical categories and faunal types according to VIGNA TAGLIANTI *et al*. (1999) with some changes (KODZHABASHEV & PENEV, 2006).

Categorization of the species in respect of their life forms follows the classification of SHAROVA (1981). The following codes were used: Life form class 1. Zoophagous. Life form subclass: 1.2 – Epigeobios; 1.3 – Stratobios;. Life form groups: 1.2.2 – large walking epigeobionts; 1.2.2(1) – large walking dendroepigeobionts; 1.3(1) – series crevice-dwelling stratobionts; 1.3(1).1 – surface and litter-dwelling; 1.3(1).2 – litter-dwelling; 1.3(1).3 – litter and crevice-dwelling; 1.3(1).4 – endogeobionts; 1.3(2) – series digging stratobionts; 1.3(2).1 – litter and soil-dwelling. Life form class 2. Mixophytophagous. Life form subclass: 2.1 – Stratobios; 2.3 – Geohortobios. Life form groups: 2.1.1 – crevice-dwelling stratobionts; 2.3.1 – harpaloid geohortobionts.

Results

During the study 55 invertebrate species under 3 classes, 9 orders and 18 families were established. Altogether 394 specimens of ground beetles were captured. They belong to 32 species and subspecies under 17 genera and 10 tribes (Table 1).

In Table 2 is given the list of the other 23 invertebrate species, established during the field studies: three gastropod species; one lumbricid worm; 19 insects, mostly coleopteran species.

Table 1. Systematic checklist of the carabid beetles, found in "Leshnitsa" natural reserve (codes for the life forms and sampling sites are given in the Material and Methods section).

№	Species	Range type	Life form	Material
	Tribe Nebriini			
1.	*Leistus (Pogonophorus) magnicollis* Motschulsky, 1866	Balkan endemic	1.3(1).2	2♀, 1♂ (II)
2.	*Leistus (Pogonophorus) rufomarginatus* (Duftschmid, 1812)	European	1.3(1).2	1♂ (II)
3.	*Nebria (Nebria) brevicollis* (Fabricius, 1792)	European-Neareastern	1.3(1).2	1♂ (I)
	Tribe Carabini			
4.	*Calosoma (Calosoma) sycophanta* (Linnaeus, 1758)	Palearctic	1.2.2(1)	1♂ (hand)
5.	*Calosoma (Acalosoma) inquisitor* (Linnaeus, 1758)	Palearctic	1.2.2(1)	1♂ (III)
6.	*Carabus (Morphocarabus) versicolor versicolor* E. Frivaldszky, 1835	Bulgarian endemic	1.2.2	1♀, 2♂ (I)
7.	*Carabus (Chaetocarabus) intricatus intricatus* Linnaeus, 1761	European	1.2.2	1♂ (hand); 2♀, 1♂ (II)
8.	*Carabus (Megodontus) violaceus azuresens* Dejean, 1826	Balkan endemic	1.2.2	2♀, 1♂ (II)
9.	*Carabus (Procerus) gigas gigas* (Creutzer, 1799)	Central and Eastern European	1.2.2	1♂ (I)
	Tribe Cychrini			
10.	*Cychrus semigranosus balcanicus* Hopffgarten, 1881	Balkan endemic	1.2.2	1♂ (II)
	Tribe Trechini			
11.	*Trechus cardioderus balcanicus* Jeannel, 1927	Balkan endemic	1.3(1).2	5♀, 2♂ (I); 1♂ (III)
	Tribe Bembidiini			
12.	*Bembidion (Metallina) lampros* (Herbst, 1784)	Holarctic	1.3(1).1	2♂ (I)
13.	*Bembidion (Peryphus) andreae bualei* Duval, 1852	European	1.3(1).1	1♀, 1♂ (III)
	Tribe Pterostchini			
14.	*Myas chalybaeus* (Palliardi, 1825)	Balkan-Carpathian	1.3(1).4	17♀, 28♂ (II); 1♀, 2♂ (III)
15.	*Pterostichus (Cryobius) vecors* (Tschitschérine, 1897)	Bulgarian endemic	1.3(2).1	1♀, 7♂ (I); 1♀, 4♂ (II)
16.	*Pterostichus (Platysma) niger* (Schaller, 1783)	Euroasiatic	1.3(2).1	3♀, 1♂ (II)
17.	*Pterostichus (Melanius) nigrita* (Paykull, 1790)	Palearctic	1.3(2).1	1♂ (hand)
18.	*Pterostichus (Phonias) ovoideus* (Sturm, 1824)	Eurosiberian	1.3(2).1	1♀ (II)
19.	*Pterostichus (Pterostichus) brucki* Schaum, 1859	Northmediterranean	1.3(2).1	1♀ (II)
20.	*Abax parallelus* Duftschmid, 1812	European	1.3(2).1	22♀, 16♂ (II); 4♀, 2♂ (III)
21.	*Abax carinatus* (Duftschmid, 1812)	Central and Eastern European	1.3(2).1	1♂ (I); 2♂ (III)
22.	*Molops alpestris kalofericus* Mlynar, 1977	Bulgarian endemic	1.3(2).1	1♂ (hand); 4♀, 3♂ (I); 11♀, 14♂ (II); 9♀, 14♂ (III)
23.	*Molops dilatatus angulicollis* G. Müller, 1936	Bulgarian endemic	1.3(2).1	1♀, 3♂ (I); 9♀, 6♂ (II); 24♀, 21♂ (III)
24.	*Molops rufipes klisuranus* Apfelbeck, 1902	Balkan endemic	1.3(2).1	2♀, 1♂ (II); 6♀, 9♂ (III)
25.	*Molops piceus bulgaricus* Maran, 1938	Balkan endemic	1.3(2).1	18♀, 22♂ (II); 3♀, 16♂ (III)
26.	*Tapinopterus (Tapinopterus) cognatus kalofirensis* Mařan, 1933	Bulgarian endemic	1.3(2).1	1♀, 6♂ (II); 1♂ (III)
	Tribe Platynini = syn. Agonini			
27.	*Limodromus assimilis* (Paykull, 1790)	Palearctic	1.3(1).1	1♀ (I); 6♀, 2♂ (II); 7♀, 3♂ (III)
28.	*Agonum (Platynus) proximum* (Frivaldszky, 1879)	Bulgarian endemic	1.3(1).1	3♀, 2♂ (II); 1♂ (hand)
	Tribe Amarini = syn. Zabrini			
29.	*Amara (Amara) aenea* (De Geer, 1774)	Holarctic	2.3.1	1♀, 1♂ (II); 1♂ (III)
	Tribe Harpalini			

30.	*Harpalus (Pseudoophonus) rufipes* (De Geer, 1774)	Palearctic	2.1.1	1♂ (III)
31.	*Harpalus (Harpalus) rubripes* (Duftschmid, 1812)	Euroasiatic	2.3.1	1♀ (III)
	Tribe Brachinini			
32.	*Aptinus (Aptinus) bombarda* (Illiger, 1800)	European	1.3(1).3	7♀, 6♂ (II); 1♀ (III)

Table 2. Species of invertebrates, other than Carabidae, found at the territory of the "Leshnitsa" nature reserve.

Species	Family	Order
Phylum Mollusca		
Class Gastropoda		
Helix pomatia Linnaeus, 1758	Helicidae	Sigmurethra
Fruticicola fruticum (O. F. Müller, 1774)	Bradybaenidae	Sigmurethra
Aegopinella nitidula (Draparnaud, 1805)	Oxychilidae	Sigmurethra
Phylum Annelida		
Class Clitellata		
Lumbricus terrestris Linnaeus, 1758	Lumbricidae	Haplotaxida
Phylum Arthropoda		
Class Insecta		
Calopteryx splendens Harris, 1780	Calopterygidae	Odonata
Perla sp.	Perlidae	Plecoptera
Saga pedo (Pallas, 1771)	Tettigoniidae	Orthoptera
Panorpa communis (Linnaeus, 1758)	Panorpidae	Mecoptera
Anthelephila caeruleipennis LaFerté-Senéctère, 1847	Anthicidae	Coleoptera
Morimus funereus Mulsant, 1862	Cerambycidae	Coleoptera
Prionus (Prionus) coriarius (Linnaeus, 1758)	Cerambycidae	Coleoptera
Rosalia alpina Linnaeus, 1758	Cerambycidae	Coleoptera
Anoplotrupes stercorosus (Hartmann, 1791)	Geotrupidae	Coleoptera
Geotrupes vernalis (Linnaeus, 1758)	Geotrupidae	Coleoptera
Dorcus parallelipipedus (Linnaeus, 1758)	Lucanidae	Coleoptera
Lucanus cervus (Linnaeus, 1758)	Lucanidae	Coleoptera
Potosia aeruginosa (Drury, 1770)	Scarabaeidae	Coleoptera
Nicrophorus vespillo Linnaeus, 1758	Silphidae	Coleoptera
Oiceoptoma thoracicum (Linnaeus, 1758)	Silphidae	Coleoptera
Silpha obscura Linnaeus, 1758	Silphidae	Coleoptera
Tipula sp.	Tipulidae	Diptera
Formica rufa Linnaeus, 1761	Formicidae	Hymenoptera
Vespa crabro Linnaeus, 1758	Vespidae	Hymenoptera

Zoogeographical peculiarities of the ground beetles

Endemic complex prevails, consisting of 12 (38%) taxa (5 Bulgarian and 7 Balkan endemics). European faunal type (mostly forest dwelling species connected to the middle and southern part of Europe) consists of 9 (28%) taxa. Representatives of the Northern Holarctic and European-Siberian faunal complex (distributed mainly in the northern regions of the Holarctic, mostly in Europe and Siberia) are 8 species (25%) and the European-Asiatic type (species ranges lie between the Eurosiberian and Mediterranean zones) includes only 2 species (6%) (Table 3).

Life forms of the ground beetles

The 32 ground beetle species and subspecies, established for the area of "Leshnitsa" reserve, relate to two classes of life forms proposed by SHAROVA (1981), with clear predominance of class Zoophaga, presented by 29 species. Mixophyito-phagous were only 3 species. The most numerous are the digging litter and soil-dwelling stratobionts from class Zoophaga, typical forest dwellers from the genera *Pterostichus*, *Abax*, *Molops* and *Tapinopterus* (Fig. 1).

Species with conservation and biogeographical significance

Twenty five species with conservation and biogeographical significance were found, including endemic, protected, rare or species with limited distribution (Table 4). Eleven species are included in the IUCN Red List (IUCN, 2015) with the categories: LC – Least Concern (5 species), NT – Near

Threatened (3 sp.) and VU – Vulnerable (3 sp.); five species are protected by the Biological Diversity Act (2002): Annex II – Species for which conservation are declared protected areas for protection of their habitats, Annex III – Species protected on the territory of the whole country and

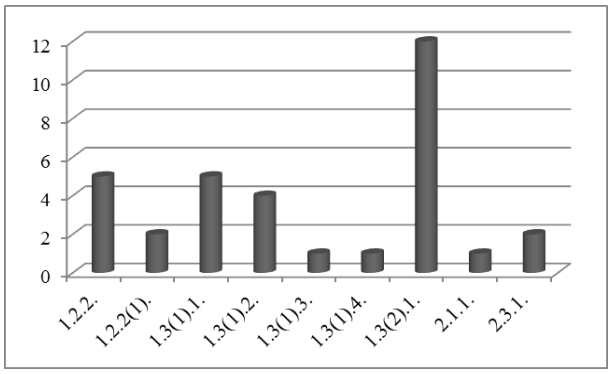

Fig. 1. Life forms of the ground beetles from "Leshnitsa" nature reserve (descriptions of the life form codes are given in the Material and Methods section).

Annex IV – Species under regime of protection and regulated use from the nature; five Natura 2000 species were found; four species are protected under the Directive 92/43 (DIRECTIVE 92/43/EEC, 1992): Annex II – Animal and plant species of community interest whose conservation requires the designation of Special Areas of Conservation. Annex V – Animal and plant species of community interest, whose taking from the wild can be restricted by European Law; three species are included in the check list of the threatened invertebrates of the CORINE biotopes project (COUNCIL OF THE EUROPEAN COMMUNITIES, 1991); three are in the Appendices of the Bern Convention (CETS, 1979): two in Appendix II – Strictly protected fauna species and one in Appendix III – Protected fauna species; *Calosoma sycophanta* is included in the ESC Red List of threatened animals and plants in Europe of the Economic and Social Council (ESC) of the United Nations. Five Bulgarian and seven Balkan endemics are recorded as well as one Balkan subendemic and one Tertiary relict. This study represents the first exact note about the distribution of *Leistus magnicollis* in the mountain, which was cited so far simply as "Stara Planina" (GUÉORGUIEV & GUÉORGUIEV, 1995; GUÉORGUIEV *et al.*, 1997).

Table 3. Zoogeographical categories of the ground beetles (Coleoptera: Carabidae) in "Leshnitsa" natural reserve.

Faunal type	Zoogeographical element	Number of taxa
Northern Holarctic and European-Siberian	Holarctic	2
	Palearctic	5
	European-Siberian	1
European	European-Neareastern	1
	European	5
	Central and Eastern European	2
Euroasiatic	Balkan-Carpathian	1
	Euroasiatic steppe complex	2
Mediterranean	Northmediterranean	1
Endemic	Balkan endemic	5
	Bulgarian endemic	7

Table 4. Species with conservation significance, found at the territory of the "Leshnitsa" nature reserve.

№	Trivial name	Latin name	Conservation status
1.	Roman snail	*Helix pomatia*	IUCN –LC; Directive 92/43(V); LBD (IV); CORINE
2.	Banded demoiselle	*Calopteryx splendens*	IUCN – LC
3.	Predatory bush cricket	*Saga pedo*	IUCN – VU; Natura 2000; Bern (II)
4.	Common scorpionfly	*Panorpa communis*	IUCN – LC
5.	Blue ground beetle	*Carabus intricatus*	IUCN – NT; Natura 2000
6.	Forest caterpillar hunter	*Calosoma sycophanta*	ESC Red List; CORINE
7.		*Carabus versicolor versicolor*	Bulgarian endemic; rare
8.		*Molops alpestris kalofericus*	Bulgarian endemic
9.		*Molops dilatatus angulicollis*	Bulgarian endemic
10.		*Tapinopterus cognatus kalofirensis*	Bulgarian endemic
11.		*Agonum proximum*	Bulgarian endemic
12.		*Leistus magnicollis*	Balkan endemic
13.	Violet ground beetle	*Carabus violaceus azuresens*	Balkan endemic
14.		*Cychrus semigranosus balcanicus*	Balkan endemic
15.		*Trechus cardioderus balcanicus*	Balkan endemic
16.		*Pterostichus vecors*	Balkan endemic
17.		*Molops rufipes klisuranus*	Balkan endemic
18.		*Molops piceus bulgaricus*	East-Balkan endemic
19.		*Myas chalybaeus*	Balkan subendemic; Tertiary relict
20.	Beech longhorn beetle	*Morimus funereus*	IUCN – VU; Natura 2000; Directive 92/43 (II); CORINE; LBD (II)
21.	Rosalia longicorn	*Rosalia alpina*	IUCN – VU; Natura 2000; Directive 92/43 (II); Bern (II); LBD (II, III)
22.	Tanner beetle	*Prionus coriarius*	IUCN – LC
23.	Stag beetle	*Lucanus cervus*	IUCN – NT; Natura 2000; Directive 92/43 (II); Bern (III); LBD (II, III)
24.	Red wood ant	*Formica rufa*	IUCN – NT; LBD (III)
25.	European hornet	*Vespa crabro*	IUCN – LC

Discussion

Forest environment is definitely the most ancient on the Balkans (POPOV & DELTSHEV, 1997), which is the reason the predominant arboreal morphoecotype to be formed by cryptobionts with lower mobility: geobionts, stratobionts, and subterranean species. Trophic structure of carabid beetles in woodland habitats is usually characterized by predominance of zoophagous beetles (SHAROVA, 1981; AYDAMIROVA, 2009; LÖVEI, 2008; etc.). Same predominance was established during the study. It evidences for the relative evolutionary completion of the researched area, as far as the typical predators are characteristic for the final stages of the successional development (e.g. LÖVEI, 2008).

Degradation of the forest communities in large parts throughout Europe is the reason for the displacement of the typical European nemoral complex by more adaptable European-Asian species in many otherwise forest or mountain areas (DESENDER & TURIN, 1989; KODZHABASHEV & PENEV, 2006; ALEKSANDROWICZ, 2011). The old nemoral carabid complex (Carabini, Pterostichini, Platynini) is shifted to plots with preserved forest biotopes, such as the studied nature reserve. The remnants of preserved woodlands treasure those forest elements in the denuded and

anthropogenized territories and their conservation is essential for maintaining a diverse fauna of mostly European and European-Siberian forest species (VARVARA, 2005; KODZHABASHEV & PENEV, 2006).

Some of the species (e.g. *Carabus intricatus* and *Leistus rufomarginatus*) have become rare under the influence of anthropogenic pressures and changes in their primary habitats. *Calosoma inquisitor*, *Calosoma sycophanta* and some of the *Carabus* species are usually highly sensitive to chemical agents, which affects their range and numbers (HUUSELA-VEISTOLA, 2000). Most of the species are stenotopic forest inhabitants, attached to mesophilous vegetation. Only three eurybionts are established (*Bembidion lampros*, *Harpalus rufipes*, *Harpalus rubripes*), represented with single specimens. A large part of the priority species are directly linked to the presence of dead wood. Such are *Rosalia alpina*, *Morimus funereus, Lucanus cervus*, etc., whose larvae develop in dead deciduous wood.

Threats and negative factors for the invertebrate fauna in "Leshnitsa" natural reserve

The primary threat to the biodiversity of the invertebrate fauna in the forests is the systematic removal of the fallen dead wood, which affects the invertebrates of all trophic levels taking part in the circle of the wood, and it was observed in the border areas mainly in the northern parts of the reserve. The primary purpose of protected areas is the maintenance of model biodiversity, which is unthinkable in such anthropogenic interventions. Felling, destruction of old trees and trees with hollow holes, removal of deadwood may lead to deterioration, narrowing or destruction of the forest habitats, and as a result – to the distortion of the structure of the communities and banishing or destruction of the populations of different species. Most of the insect species included in the international conservational regulations and agreements develop in the deadwood. Nearly one-third of all forest species are dependent on the presence of dead wood and old trees (DUDLEY & VALLAURI, 2004).

In the immediate vicinity of the borders of the reserve was observed intensive wood felling. This fact greatly reduces the buffer role of these border areas. Given the fact that the main threatening factors for the beech forests are: intensive forestry, too short cultivation of logging, uprooting, depositing of harmful substances in the air, damage from wild game (SSYMANK *et al.*, 1998), and that the forestry practices are the major threat for the red-listed species (RASSI *et al.*, 2000), serious attention to this problem should be paid.

Afforestation with conifer species (found in the southern part of the reserve) also has a strong negative impact on the populations of a number of species. The disappearance of old beech forests limits the food base for the development of the beetles.

Collection by collectors can greatly influence the abundance of some of the rare or priority species (e.g. the large and attractive longhorn or ground beetles, butterflies, dragonflies, etc.).

Conclusions

The fauna of the ground beetles in the reserve has not been studied so far. As a result of the present study 32 taxa were captured. Twelve endemics were found. Twenty three other invertebrates were established.

Twenty five invertebrate species with conservation and biogeographical significance were found. Monitoring of the populations of all protected, endemic, relict and rare species, which have been identified so far, is to be carried out and the preservation of their natural habitats undisturbed or not altered by human activity is recommended. The real state of the diversity of this group in the area could be revealed only after future investigations and discovery of additional new species for the region.

It is necessary the abiding of all restrictions and prohibitions currently in force within the territory of the reserve to be ensured, and the conservation of the natural

habitats in unaltered state, which would provide a possibility for fulfilment of the natural successional changes.

Habitat fragmentation and deterioration are one of the most important causes of species declines and extinctions across the world. Therefore it is necessary to provide for the limitation of the intensive economic (forestry) activities, observed during the study in the buffer zones of the reserve.

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A Survey of Plant Species Diversity and Ecological Species Group from the Coastal Zone Of Boujagh National Park, Guilan, Iran

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Abstract. The aim of this study was to identify the ecological species groups and investigate the diversity among them. The research area comprises in a coastal system of Boujagh National Park, in Northern of Guilan Province, Iran. Vegetation sampling was carried out along 6 shore perpendicular transects, ween minimum 153 m and maximum 5562 m long. A total of 52 plot of 25 square meters were taken in transects. In each sampled plot, the cover percentage value of each species was estimated using Bran-Blanquet scales. Vegetation classified using Two-Way Indicator Species Analysis (TWINSPAN). Classification of plots showed four vegetation groups: *Convolvulus persicus - Crepis foetida, Argusia sibirica, Eryngium caucasicum - Juncus acutus, Rubus sanctus.* Plant diversity in these vegetation groups have been evaluated. The comparison of diversity indices among groups were performed with ANOVA test. Results of analysis of variance in species diversity indices showed significant differences among the groups in terms of biodiversity indices. The survey of variation in the groups showed that group 3 had the highest and group 2 had the lowest Shannon-Wiener's, Simpson's and Fisher's diversity indices respectively. In Menhinink's and Margalef's richness indices group 2 and 3 had the highest and group 1 had the lowest measure. In Sheldon's evenness index group 2 had the highest and group 3 had the lowest measure. Finally, the overall survey of indices showed that groups 1 and 2 had less diversity but had more evenness than groups 3 and 4.This shows that despite suitable living conditions for the growth and development of vegetation in the groups 3 and 4, the abundance of species has declined Because of the destruction done in this section.

Key words: Boujagh National Park, Plant species diversity, Caspian Sea, Iran.

Introduction

Progression in natural resources sciences and the need to conserve biodiversity and manage resources of life, the survey of biological diversity by using different indicators to describe and compare the ecological diversity in natural resource management is highly regarded (KOLONGO *et al.,* 2006). Phytosociological surveys are important tools of ecologists to assess and evaluate the vegetation types of given ecosystem. These surveys ultimately help in planning, management and exploitation of natural resources since important components of food chain viz., human, livestock, wildlife and soil fauna are closely associated with specific plant assemblages of the area (MASHWANI *et al.,* 2011). Species diversity and richness or biodiversity are good indicators which

determine the health of an ecosystem. Diversity and richness of plants are reduced by abiotic (slope, feature, altitude, latitude, soil properties, etc.) and biotic (animal and human) factors along the time (JOURI *et al.*, 2011).

Coastal vegetation is interface region between land and sea, including habitat sand and plain is constantly affected by natural permanent changes and human interference (BEATLEY, 2002). Despite to endangered state of coastal vegetation in Iran, some fragmented sandy areas are still natural. Some of these separated sandy patches often constitute parts of Caspian coastal ecosystems designed in Ramsar checklist of International Wetlands (Alagol, Ulmagol and Ajigol Lakes, Amirkelayeh Lake, Anzali Mordab (Talab) complex MR., Bujagh National Park, Gomishan Lagoon, Miankaleh Peninsula, Gorgan Bay and Lapoo-Zaghmarz Ab-bandan) and others are considered as part of protected areas, no hunting areas, wildlife refuges, biosphere reserves (RAVANBAKHSH *et al.*, 2015). Boujagh National Park (BNP) is the first founded land-marine National Park and one of nineteen National Parks in Iran located in Caspian coastline (NAQINEZHAD *et al.*, 2006). BNP is very important ecosystem complex because of the fact that this area serves as a very valuable resting, nesting and wintering place for a wide variety of waterfowls particularly Siberian Crane, an endangered migratory bird (NAQINEZHAD, 2012b). Some studies were conducted on the Flora and identification of species groups of this national park. The floristic study of this unique ecosystem investigated for the first time by NAQINEZHAD *et al.* (2006). They identified 248 vascular plants and 10 bryophytes out of which six taxa are endemic for the flora of Iran. Then in 2007 they recorded 4 species for Iran and Flora Iranica from these collected species. *Melilotus polonicus* L. (Fabaceae), as psammophyte plant on the Caspian coast is reported as new noteworthy record for the flora of Iran. *Apium leptophyllum* (Apiaceae), *Sisyrinchium exile* (Iridaceae) and *Tagetes minuta* (Asteraceae) are recorded for the first time from Iran/Flora Iranica area. NAQINEZHAD (2012a) recognized nine vegetation types in the area based with physiognomic-ecologic approach. This study was carried out to identify ecological species groups of the coastal zone of Boujagh National Park by phytosociological analysis of existing vegetation and inventory plant species diversity in this part of BNP.

Materials and Methods

Study area. Boujagh National Park located on the coast of Caspian Sea. This national park is located in Guilan Province, about 2 km away from north of Kiashahr city, and 35 km from northwest of Rasht city. It is 21 m below sea level and has an area of 3177 ha. Its geographical coordinates are 49° 51′ 40″- 49° 59′ 50″E and 37° 25′ 00″-37° 28′ 50″N. Boujagh and Kiashahr Lagoons are located within this national park (Fig. 1) (NAQINEZHAD, 2012b; REIHANIAN *et al.*, 2015).

Sampling methods. Prior to the commencement of fieldwork a short reconnaissance survey was undertaken to get an overview of the area (MASHWANI *et al.*, 2011). Vegetation sampling was carried out along 6 shore perpendicular transects between minimum 153 m and maximum 5562 m long (Fig. 2). The length of transects was variable depended on the strip of the natural vegetation. Size of sampling plots was determined using nested plot sampling and species/area curve (MULLER-DOMBOIS & ELLENBERG, 1974) A total of 52 plot of 25 square meters were selected in stands of vegetation that were homogeneous to the eye in floristic composition and structure (MONSERRAT *et al.*, 2012). In each sampled plot, the cover percentage value of each species was estimated using Braun-Blanquet scale (BRAUN-BLAUNQUET, 1964; MULLER-DOMBOIS & ELLENBERG, 1974).

Vegetation analysis. The phytosociological data were collected during 2014-2015, and using the cover-abundance scales. A divisive classification of 52 relevés was carried out, using the modified TWINSPAN embedded in a JUICE program (TICHÝ, 2002). Pseudospecies cut levels were set to seven and the values of cut levels to 1, 2, 3, 4, 5, 6, 7. Five relevés were selected as a minimum group size for division. The

fidelity of species to clusters and diagnostic species for particular vegetation units was calculated with the help of presence/ absence data using the phi-coefficient. Threshold value of phi = 0.25 was selected (TICHÝ & CHYTRÝ, 2006).

Measuring plant diversity. To quantify the diversity of the plant species, The Shannon-Wiener diversity index (H'), Simpson diversity index (1-D), Fisher's alpha - a diversity index (a), Menhinink richness index (DMn), Margalef richness index (DMg) and sheldon (Buzas and Gibson) eveness index (E3) were used. The formulas are shown in Table 1.

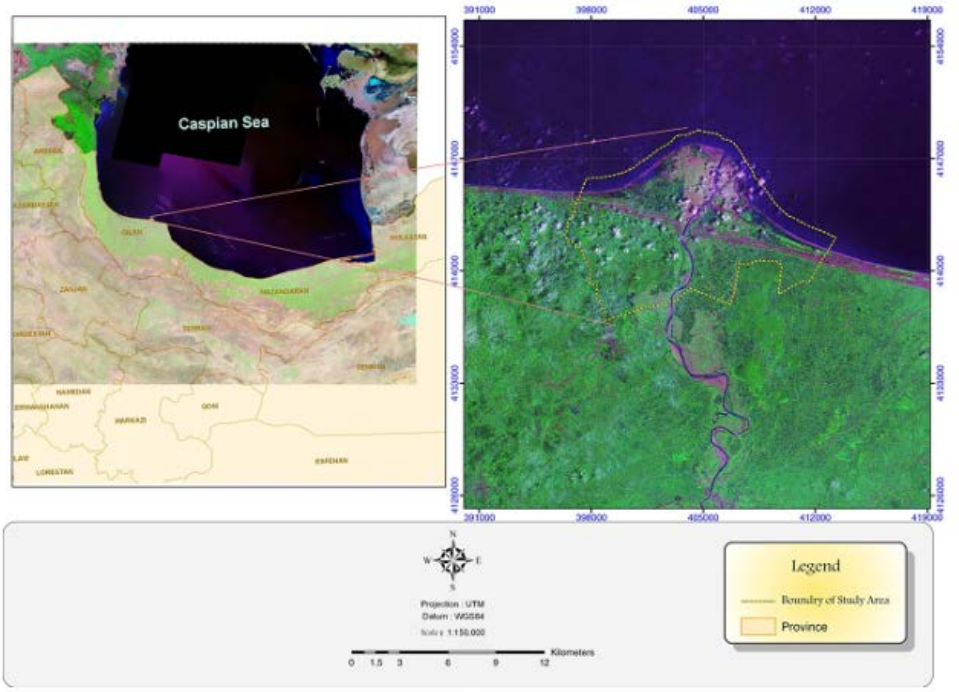

Fig. 1. Location of Boujagh National Park

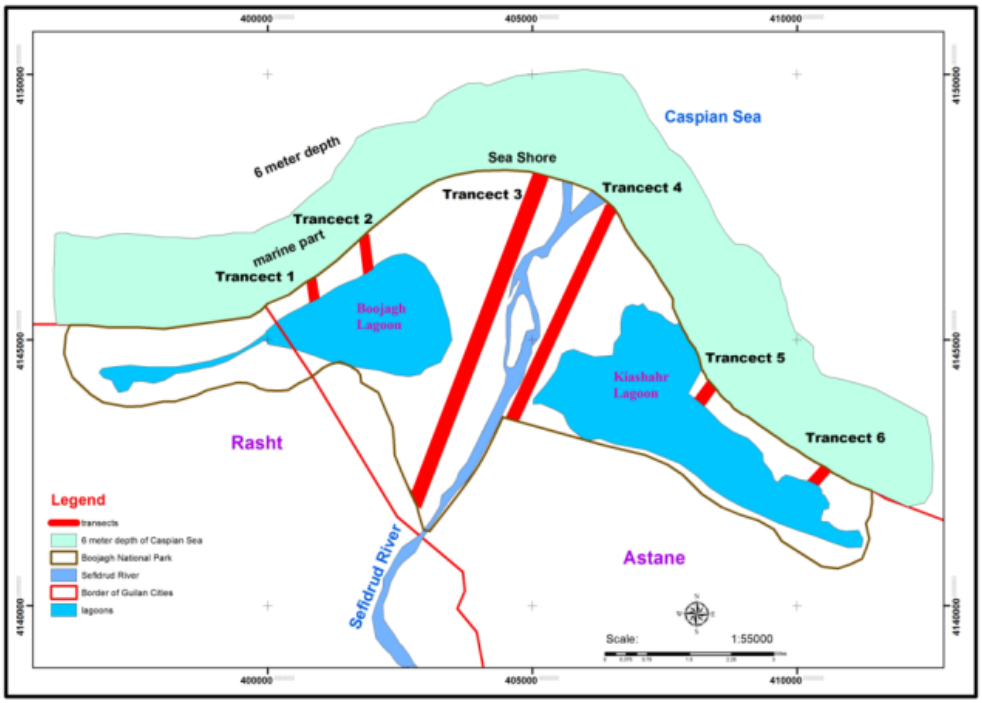

Fig. 2. Location of vegetation sampling in study area

Table 1. Richness, diversity and evenness indices used in this study (after EJTEHADI *et al.*, 2009). *Legend:* Pi = relative frequency of ith species, S = number of species (taxa), n is number of individuals, N = Total individual of species.

Diversity index	Richness index	Eveness index
$H' = -\sum_{i=1}^{s} Pi \ln Pi = -\sum_{i=1}^{s} (Pi)(\log pi)$	$D_{Mg} = \frac{S-1}{LnN}$	$E_3 = \frac{e^H}{S}$
$1 - D = \sum_{i=1}^{s} P_i^2 \qquad Pi = \frac{ni}{N}$	$D_{Mn} = \frac{S}{\sqrt{n}}$	
$S = a^* \ln(1 + {}^{n}/_{a})$		

Normality of the data distribution was checked by Kolmogorov- Smirnov test, and Levene's test was used to examine the equality of the variances. One-way analysis (ANOVA) of variance were used to compare groups with normal distribution data. Duncan test was used to test for significant differences in the species richness, diversity and evenness indices among the groups. This analysis was conducted using SPSS 16.0.

Results

Modified TWINSPAN analysis based on 52 plots were classified coastal area of Boujagh National Park. Four distinct groups of species were identified (Fig. 3, Table 2). Details of each group are as follows:

Group I (*Convolvulus persicus - Crepis foetida* subsp. *foetida*): This group with 12 plots, including annual plants which mostly formed a variable width strip in sand dune. Plants adapted to periodically disorder, heterogeneous and intolerable conditions such as strong winds, Waves, Waterlogging, severe storms and sand movement and can grows by seed and underground stems. Most plants of this group are obligatory psamophytes. The diagnostic species include *Convolvulus persicus, Crepis foetida* subsp. *foetida, Xanthium spinosum, Medicago minima.*

Group II (*Argusia sibirica*): This group with 5 plots including plants growing in dunes along the coastline of the Caspian Sea. Plants are resistant to high acidity and some of the species able to build colonies in this harsh environment by producing abundant seed or strong root. This plant species is resistant to low nutrients, high temperature, erosion and burying in the sand. Indicator species are *Argusia sibirica, Senecio vernalis, Cynodon dactylon*.

Group III (*Eryngium caucasicum - Juncus acutus*): This group of 16 plots were established in established sand around Sefidrood River and humid coastal areas. The most plants are ruderal, perennial and don't sensitive to soil acidity. Mostly have hemicryptophyte life form and help to soil stabilization. This group had most richness. Indicator species are *Eryngium caucasicum, Juncus acutus, Anagalis arvensis, Plantago lanceolata, Lolium perrene, Briza minor, Cerastium semidecandrum.*

Group IV (*Rubus sanctus*): this group with 19 plots has distance from the coastal line, therefore water salinity is lowed sand structure is relatively stable and soil is almost plainly. This group is suitable habitat for phanerophyte life form. Indicator species are *Rubus sanctus, Lotus krylovii, Medicago lupulina, Poa trivialis.*

Species diversity among groups. First of all, based on Kolmogorov-Smirnov test it should be approved that the data are normal. For analyzing the diversity among the groups, one-way Analysis of variance (ANOVA) was used. ANOVA results of diversity indices among groups and mean and standard error of diversity indices were

listed in Table 2. ANOVA showed that there were significant differences among groups in terms of biodiversity indices ($P<0.05$).

Duncan's test of groups showed in fig. of 4-9. Figure 4, 5 and 6 shows the changes of diversity indices (Shannon-Wiener, Simpson and Fisher). Group 3 and group 2 had maximum and minimum of these indices respectively. The measurement of these indices indicated that Groups 3 and 4 also Groups 1 and 2 had closer value to each other. In Simpson index is not significant difference between groups 3 and 4.

Fig. 7 and 8 shows the changes Menhinink and Margalef's richness indices among ecological groups. Group 1 had the lowest value of these indices and the highest value belong to Group 2 and Group 3 in these indices. Figure 9 shows the changes of Sheldon's evenness index evenness index among ecological groups. The highest value of Sheldon's evenness index was in group 2 and group 3 had the lowest value of this richness index. In this index is not significant difference between groups 3 and 4 also, the value of group 1 and 2 are close together. In total the indices showed group 1 and 2 had more evenness but lower richness and diversity in compared to group 3 and 4.

Discussion

Conversation of plant species diversity is one of the goals of ecosystems management. Plant species diversity is used in vegetation studies and environmental assessments as one of the important and rapid indices of determining ecosystem status (SHARAFATMANDRAD *et al.*, 2014).

Coastal sand dune habitat as an ecological niche between onshore and offshore basins is used to create a major natural conservation sites. However, these habitats have been severely affected by natural and human activities resulting in its fauna and flora have been destroyed (SAYE & PYE, 2007). The coastal parts of the South Caspian Sea are severely degraded because of intensive human activities. Nearly all parts of the area are occupied by villas, hotels, industry or are under cultivation. Some fragmented dunes can be seen in the eastern parts between Anzali and Astara, which they are invaded by many ruderal plants (AKHANI, 2003).

Seventeen vegetation types respectively from the coast to the mountains in the Caspian area identified. In the first was Sand dune vegetation. Large parts of sandy dunes along the coast have been degraded and fragmented due to intensive urbanization, transforming into agricultural lands and industry (AKHANI *et al.*, 2010). NAQINEZHAD (2012b) introduced least three vegetation bands (zones) around the south Caspian coastline. first band is related to sand dune habitats covered by psammophytes. The second band covered by wet sandy soils, has a higher water table and possesses many plant species. The most important species dominated often in these habitats are *Juncus* species. The third belt is characterized with many depressions, big holes, wetlands or lagoons.

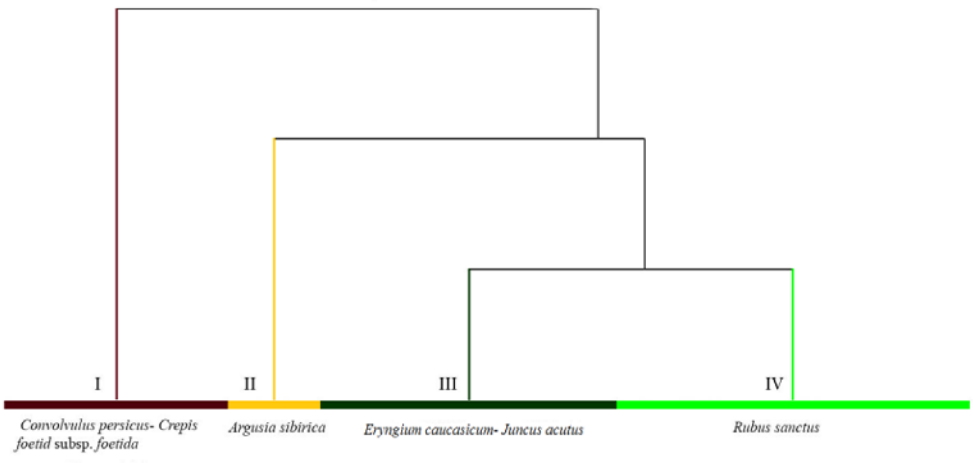

Fig. 3. The cluster analysis to classify samples by Modified TWINSPANS.

Table 2. Synoptic table with the frequency of each species in each group by Modified TWINSPANT analysis.

Group	**1**	**2**	**3**	**4**
Number of plots	**12**	**5**	**16**	**19**
Crepis foetida subsp. *foetida*	83	.	6	5
Convolvulus persicus	67	.	.	5
Xanthium spinosum	58	.	31	16
Poa annua	42	.	25	32
Medicago minima	42	.	25	11
Argusia sibirica	25	80	.	11
Senecio vernalis	.	40	6	11
Xanthium brasilicum	8	40	13	.
Eryngium caucasicum	.	.	69	26
Anagalis arvensis	17	20	56	21
Plantago lanceolata	.	.	50	5
Lolium perrene	.	20	50	26
Briza minor	.	.	44	26
Cerastium semidecandrum	17	.	44	32
Cerastium glomeratum	8	.	44	11
Equisetum ramosissimum	.	.	31	47
Juncus acutus	17	.	63	68
Rubus sanctus	17	.	63	89
Lotus krylovii	8	.	13	37
Medicago lupulina	17	20	6	37
Poa trivialis	.	.	.	32
Cynodon dactylon	25	80	75	42
Geranium dissectum	.	.	31	26
Alopecurus myosuroides	8	.	6	26
Euphorbia helioscopia	.	.	31	26
Plantago major	.	.	38	26
Veronica persica	.	.	31	21
Torilis leptophylla	.	.	6	21
Galium gilanicum	.	.	38	21
Geranium molle	.	.	6	16
Mentha aquatica	.	.	.	16
Conyzanthus squamatus	.	20	13	16
Parentucellia viscosa	.	.	.	16
Lophochloa phleoides	.	.	19	16
Tamarix ramosissima	.	.	.	16
Setaria glauca	8	.	25	16
Sisymbrium officinale	.	.	38	16
Echinochloa crus-galli	.	.	.	16
Rumex sanguineus	.	.	6	11
Hydrocotyle vulgaris	.	.	.	11
Trifolium resupinatum	.	.	38	11
Vulpia myuros	8	.	6	11
Geranium purpureum	.	.	25	11
Alnus glutinosa subsp. *barbata*	.	.	.	11

Lotus corniculatus	8	.	13	11
Myosotis palustris	.	.	.	11
Digitaria sanguinalis subsp. *pectiniformis*	.	.	.	11
Conyza canadensis	.	.	38	11
Sonchus oleraceus	.	20	.	11
Ranunculus scleratus	.	.	25	11
Veronica arvensis	.	.	.	11
Vicia tetrasperma	.	.	19	11
Cirsium vulgare	.	.	19	11
Alnus subcordata	.	.	.	11
Eleusine indica	.	.	19	11
Galium elongatum	.	.	25	11
Lolium loliaceum	33	.	6	11
Potentilla reptans	.	20	25	11
Medicago polymorpha	.	.	6	11
Conyza bonariensis	25	.	6	11
Spergularia marina	.	.	.	11
Calamagrostis pseudophragmites	.	20	.	5
Daucus carrota	.	.	.	5
Polypogon fugax	.	.	6	5
Lathyrus aphaca		.	.	5
Stellaria media	.	.	.	5
Juncus gerardii	.	.	.	5
Iris pseudacorus	.	.	.	5
Paspalum paspaloides	.	.	.	5
Vicia sativa	.	.	.	5
Phragmites australis	17	.	.	5
Minuartia hybrida subsp. *hybrida*	.	.	6	5
Centaurium pulchellum	.	.	6	5
Lythrum hyssopifolia	.	.	6	5
Amaranthus viridis	.	.	6	5
Urtica urens	.	.	.	5
Salicornia europaea	.	.	6	5
Plantago psyllium	.	.	6	5
Arenaria leptoclados	25	20	19	5
Samolus valerandi	.	.	19	5
Artemisia annua	8	.	19	5
Polygonum patulum	.	.	19	5
Daucus litoralis subsp. *hyrcanus*	33	.	.	5
Catapodium rigidum	8	20	13	5
Eclipta prostrata	.	.	.	5
Chondrilla juncea	.	.	.	5
Phytolacca americana	.	.	.	5
Ranunculus marginatus	8	.	25	5
Trifolium repens	8	.	38	5
Capsella bursa-pastoris	.	.	.	5
Agriophyllum squarrosum	.	.	.	5
Punica granatum	.	.	.	5
Trachomitum venetum	.	.	.	5

Lolium persicum	.	.	25	5
Parapholis incurva	8	20	13	5
Maresia nana	.	.	.	5
Trifolium micranthum	.	.	13	5
Sonchus asper subsp. *glaucescens*	17	.	6	5
Silene conica	.	.	.	5
Melilotus indicus	8	.	.	5
Polycarpon tetraphyllum	17	.	.	5
Trifolium scabrum	.	.	6	5
Juncus bufonius	.	.	13	5
Potentilla supina	8	.	6	5
Silybum marianum	.	.	13	5
Lycopus europaeus	.	.	13	5
Silene gallica	8	.	.	.
Cakile maritima	8	.	.	.
Mulgedium tataricum	25	.	.	.
Salsola kali	.	20	.	.
Typha caspica	25	.	.	.
Rorippa islandica	.	.	13	.
Epilobium hirsutum	.	.	13	.
Ranunculus muricatus	.	.	13	.
Veronica polita	.	20	13	.
Bidens tripartita	.	.	6	.
Veronica anagaloides	17	.	13	.
Paspalum dilatatum	.	.	13	.
Carex divisa	.	.	19	.
Verbena officinalis	.	.	25	.
Trifolium campestre	.	.	25	.
Mentha pulegium	.	.	38	.
Hypericum perforatum	.	.	19	.
Ranunculus ophioglossifolius	.	.	19	.
Phyla nodiflora	.	.	19	.
Prunella vulgaris	.	.	19	.
Calystegia sepium	.	.	6	.
Crypsis schoenoides	.	.	6	.
Erodium cicutarium	.	.	6	.
Aster tripolium	.	.	6	.
Solanum nigrum	.	.	6	.
Sambucus ebulus	.	.	6	.
Nasturtium officinale	.	.	6	.
Bromus brachystachys	.	.	6	.
Rumex pulcher	.	.	6	.
Raphanus raphanistrum subsp. *raphanistrum*	.	.	6	.
Lolium rigidum	25	.	6	.
Linum bienne	.	.	6	.
Centaurea iberica	.	.	6	.
Trifolium striatum	.	.	6	.
Atriplex sp.	.	.	6	.

Table 2. ANOVA results of diversity indices among groups and mean and standard error of diversity indices.

Diversity index		F	P	Mean square	df	Mean and standard error
Diversity index	Shanon diversity index	7.534	0.000*	1.242	3	1.532 ±0.654
	Simpson diversity index	4.752	0.05*	0.101	3	0.688 ±0.221
	Fisher's diversity index	3.793	0.016*	25.391	3	3.707 ±0.382
Richness index	Menhinink 's richness	3.056	0.037*	0.980	3	1.104 ±0.822
	Margalef richness index	7.702	0.000*	8.628	3	2.481 ±0.171
Evenness index	sheldon's evenness index	4.248	0.010*	0.121	3	0.443 ±0.252

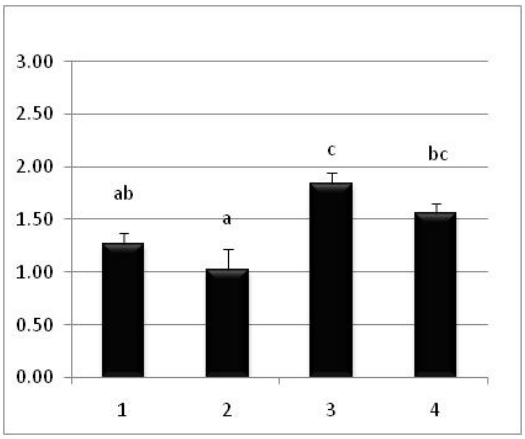

Fig. 4. Changes in in Shannon-Wiener's diversity index among ecological groups.

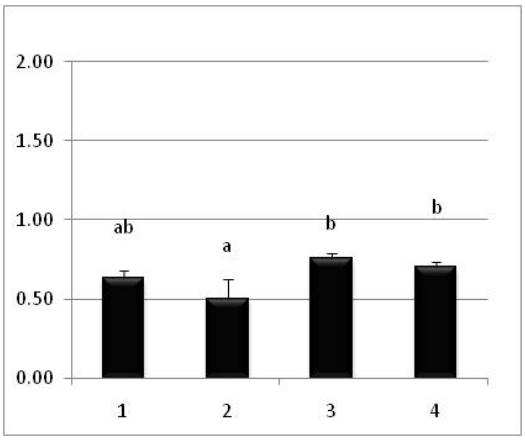

Fig. 5. Changes in in Simpson's diversity index among ecological groups.

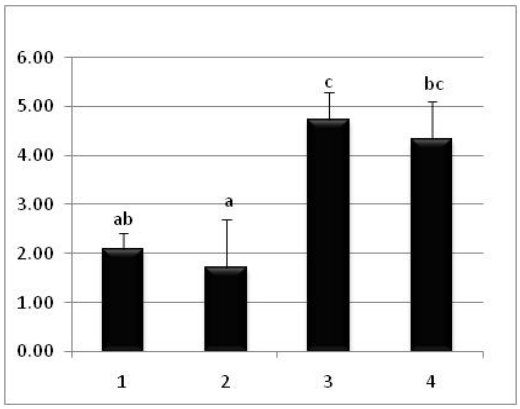

Fig. 6. Changes in Fisher's diversity index among ecological groups**.**

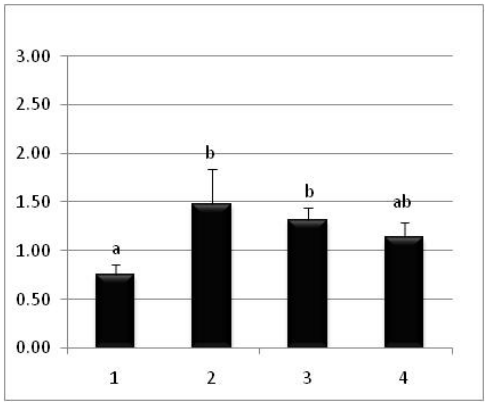

Fig. 7. Changes in Menhinink 's richness index among ecological groups.

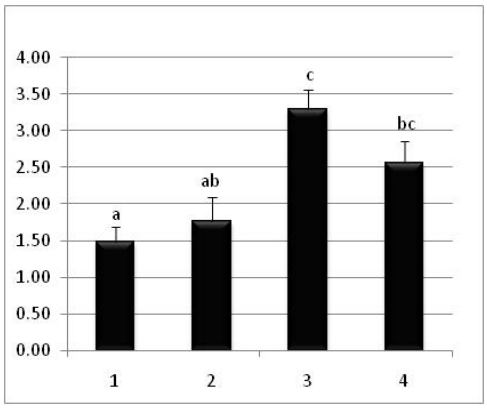

Fig. 8. Changes in in Margalef 's richness index among ecological groups.

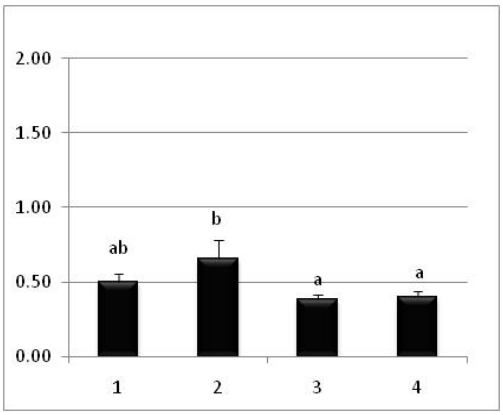

Fig. 9. Changes in in sheldon's evvennes index among ecological groups.

This study for the first time introduced ecological species group in coastal zone of Boujagh national park (BNP) by floristic method and multivariate analysis. Modified TWINSPAN analysis was identified four species groups. Other studies presented vegetation type and species group in coastal area of southern Caspian Sea are belong to SHOKRI *et al*. (2004), EJTEHADI *et al*. (2009), ASRI *et al*. (2007) and RAVNABAKHSH *et al*. (2015).

NAQINEZHAD (2012a) introduced four vegetation unites in coastal area of BNP Based on the field observations and physiognomic-ecologic method. Comparing our study results to this investigation showed that two groups *Convolvulus persicus* and *Juncus acutus* are similar and groups *Rubus sanctus* and *Argusia sibirica* as new groups of study area are introduced. Anova analysis results indicated that groups 1 and 2 (*Convolvulus persicus* - *Crepis foetida* and *Argusia sibirica*) had less diversity but had more evenness than groups 3 and 4. The survey of geographical location in these groups showed them located in unstable sand dunes. In more distance from the coastline is situated wet and stabilized sand dunes where is located groups 3 and 4 (*Eryngium caucasicum* - *Juncus acutus* and *Rubus sanctus*). In this habitat, ecological species groups with more diversity and richness and less evenness occupant. This shows that despite suitable living conditions for the growth and development of vegetation, the abundance of species has declined Because of the destruction done. The most important destruction reason in this habitat are grazing, recreation, the release of waste, commercial port construction, harvesting sand from the beaches and Sefidrud River. RAVNABAKHSH *et al*. (2015) surveyed plant diversity in ecological species group in Caspian Sea coastal sand dune. Checking of the group's position with high diversity in comparison with other groups indicated the group settled on the coastal land with stabilized soil and proper distance from the sea had higher diversity indices.

Conclusion

This study assessed coastal area of BNP by using diversity indices as an indicator for evaluating vegetation. Coastal area of BNP remain as fragmented strips lying parallel to the Caspian Sea coastline and are largely occupied by anthropogenic impacts including grazing, deposition of solid wastes, Construction and development of commercial port, Fishing, tourism and agriculture that result in drastic changes in plant species diversity. Strict enforcement of rules and monitoring of their implementation can be an effective step in reducing the damage caused by unsustainable development activities implemented in the study area.

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Impact of Lead Acetate on Quantitative Consumption and Utilization of the Cotton Leaf Worm, Spodoptera littoralis (Boisduval, 1833) (Lepidoptera: Noctuidae)

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Abstract. The 3rd, 4th, 5th, and 6th instars of the cotton leaf worm *Spodoptera littoralis* (Boisduval, 1833) were treated with lead acetate, 100 mg lead [Pb $(C_2H_3O_2)_4$]/kg, using the leaf-dip method, to evaluate the effect of Pb on nutritional indices. The consumption index was significantly increased at the 3rd and 6th instars. The growth rate significantly increased in 4th instars. The reverse was true in 6th instars. The absorptive capacity, in terms of approximate digestibility, was insignificantly changed in the entire instars. The food utilization efficiencies, in terms of the conversion of ingested (ECI) and digested food (ECD) to biomass, were significantly increased in 4th instars. However, the ECD was significantly decreased in the 5th and 6th instars. In conclusion, treatment with lead may adversely affect the population biomass of *S. littoralis* due to the gross reduction in the final weight gain of larvae approaching to pupation. This might lead to reduced level of population size.

Key words: *Spodoptera littoralis*, lead, food consumption, food utilization, heavy metals.

Introduction

The cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) is a polyphagous insect, and it is one of the major cotton pests. Accordingly, it causes a considerable damage to many important vegetables and crops in the Mediterranean and Middle East countries (SHONOUDA & OSMAN, 2000; MAGD EL-DIN & EL-GENGAIHI, 2000; KHAWAS & ABD EL-GAWAD, 2002; ADHAM *et al.*, 2005a; TIESSEN, 2012). Lead (Pb) is widely used in industries, and it is considered as a significant environmental pollutant that contaminates food, water, urban soil, and air (BOGDANOV, 2005; HAQ *et al.*, 2012). It is listed at number two on the Comprehensive Environmental Response, Compensation, and Liability Act Priorities List of Hazardous substances (US EPA, 2011).

Several studies have been carried out on the biological effects of Pb, however, its toxic potential against insects remains unestablished. Due to the possible hazardous effects of this metal, its presence in the environment is, therefore, a matter of urgent concern. The poor accumulation of Pb in the organisms could be one cause to its less toxicity. Nevertheless, Pb is considered to be an important toxic waste. Consequently, insects could be influenced by such metal. Few studies have been carried out to demonstrate the biological effects of lead and its toxic potential against insects (MARGIM, 2005).

The present investigation aims to determine the effect of lead on the growth and food utilization of 3rd, 4th, 5th and 6th instar larvae of the cotton leaf worm, *S. littoralis*.

Materials and Methods

Insect rearing. The stock colony of *S. littoralis* was started as egg masses obtained from a standard laboratory colony maintained at the Department of Entomology, Faculty of Science, Cairo University, Giza, Egypt at 25 ± 2°C, 65 ± 5% R. H. and 12:12 hours (D:L) photoperiod (ABU ELELA & ELSAYED, 2015a; b). The larvae were fed fresh castor-bean leaves, *Ricinus communis* (Linnaeus, 1753), whilst the adults were fed on 15% sugar solution.

Bioassay protocol. Twenty newly moulted 3rd, 4th, 5th and 6th instar larvae were treated by Pb acetate using the leaf dipping method technique (BAGHBAN *et al.*, 2014; ABU ELELA & ELSAYED, 2015a; b). Fresh castor bean leaves, *Ricinus communis* (Linnaeus, 1753), were dipped in Pb acetate solution [100 mg $Pb(C_2H_3O_2)_4$/kg] for 20 seconds and then left to dry in room air for 10 minutes (MOADELI *et al.*, 2014). The dried leaves were placed singly in clear and clean plastic boxes (18 w x 10 h x 25 l cm). Newly moulted 3rd, 4th, 5th and 6th instar larvae were allowed to feed randomly on Pb-treated leaves. Three replicates of each instar group with 20 larvae each were setup. A parallel control of non-treated instars was also conducted.

Nutritional indices. Nutritional indices were calculated using standard gravimetric procedures described by WALDBAUER (1968) as follows:

1) Consumption index (CI) measures the amount of food eaten per unit time relative to mean weight of larvae during the feeding period, CI=C/ [(T) (A)].

Where C - fresh weight of leaf consumed, T - duration of feeding period and A - mean fresh weight of the larvae during the feeding period.

2) Growth rate (GR) measures the amount of weight gained per unit time relative to the mean weight of the larvae during the feeding period; GR= G/[(T)(A)].

Where G - fresh weight gain of the larvae.

3) Efficiency of conversion of ingested food to body tissue (ECI) is an overall measure of the larvae's ability to utilize ingested food for growth, ECI= (G/C) x (100%).

4) Efficiency of conversion of digested food to body tissue (ECD) is an overall measure of the larvae's ability to utilize digested food for growth, ECD= [G/(C-F)] x (100%). Where F - faeces weight during the feeding period.

5) Approximate digestibility (AD) measures the larvae's ability to digest the introduced food, AD= [(C-F)/C] x (100%).

Statistical analysis. Data were given as mean ± SE, and they were analyzed with one way analysis of variance (ANOVA). All statistical computations were carried out by PAST ver. 2.17 software (HAMMER *et al.*, 2001).

Results and Discussion

Figure 1 shows that treatment with Pb significantly enhanced ($p<0.05$) the CI in 3rd and 6th instars. However, 4th and 5th instars did not elicit any appreciable change ($p>0.05$) in CI due to such treatment. It appears that CI decreased gradually with advancing instars in both non-treated and Pb-treated instars. The CI was steadily decreased through the studied instars. HARE (1992) reported that Cd, Pb, and Hg, even at low concentrations, are toxic for the test organisms.

GR of Pb-treated larvae significantly increased in 4th instars. In contrast, exposure of 6th instar to Pb significantly ($p<0.05$) decreased the GR (Fig. 2). In the present study, the enhanced GR could be due to the increased efficiency of food eaten (ECI and ECD), as evident in this study (Fig. 4 and 5). This result is in agreement with that of BAGHBAN *et al.* (2014) who reported that treatment of the cotton boll worm, *Helicoverpa armigera* with Cd, Cu, and Zn enhanced the GR.

The pattern of the change in GR, due to exposure to Pb, was similar to that of the CI, i.e. GR decreased gradually with advancing instars. The present results indicates that Pb does not necessarily have a negative impact on the organism, where treatment with this heavy metal increased the GR in 3rd and 4th instars (Fig. 2). This finding is confirmed by WOODRING *et al.* (1978) who indicated that the amount of growth reduction was proportional in general to reduced food consumption.

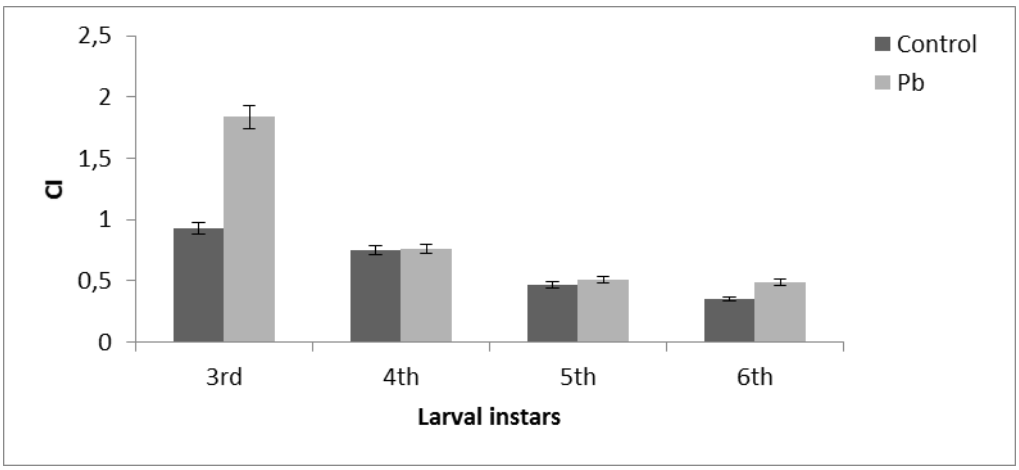

Fig. 1. Consumption index (CI) of the cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833), fed on Pb-treated castor leaves. Bar on the top of column represents SE.

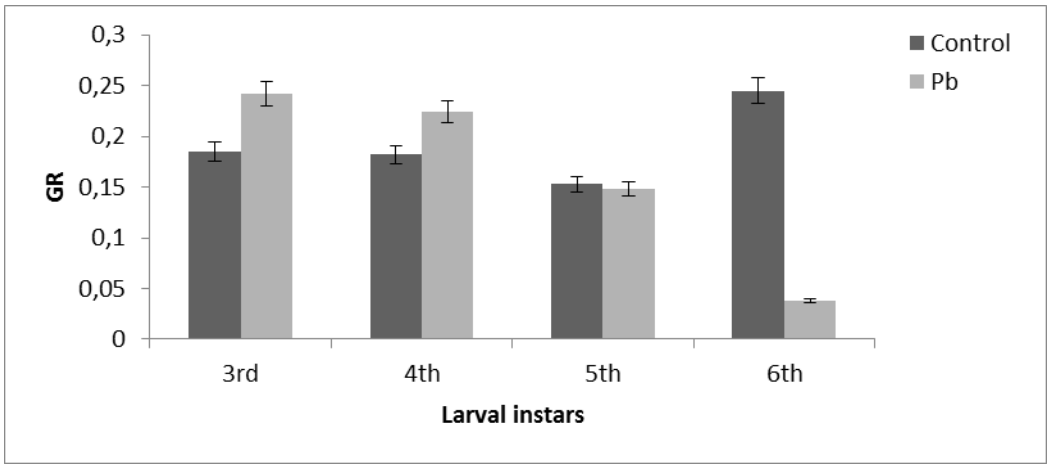

Fig. 2. Growth Rate (GR) of the cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833), fed on Pb-treated castor leaves. Bar on the top of column represents SE.

The absorptive capacity of larvae, expressed as AD, did not significantly change ($p>0.05$) due to treatment with Pb compared to the control (Fig. 3). AD constantly declined with advancing instars. The same pattern was true for CI and GR (Fig. 1 and 2). The AD is inversely proportional to ECD and ECI, as stated by WALDBAUER (1968). This statement agrees with our results (Fig. 4 and 5).

ECD showed significant ($p<0.05$) increase due to treatment with Pb compared to the control in the 4th instar larvae (Fig. 4). It appears that the 4th instar larvae were more selective feeders and choose more digestive foliage from the inter-vein regions of the leaf. Also, their metabolic rate was higher than older ones and hence more of the digested food is available for conversion to body substance (ECD) (ABU ELELA & ELSAYED, 2015a).

Treatment with Pb significantly declined the ECI in 5th and 6th instars. In contrast, significant increase was achieved for 4th instars (Fig. 5). EMRE *et al.* (2013) and BAGHBAN *et al.* (2014) attributed the increase in ECI under the stress of heavy metal treatment to the fact that insect requires a lot of energy to deal with the metal toxicity. This explanation may extend to our results.

Conclusions

In conclusion, the Pb treatment may adversely affect the population biomass of *S. littoralis* due to the gross reduction in the final weight gain of larvae approaching to pupation. This might also lead to reduced level of population size.

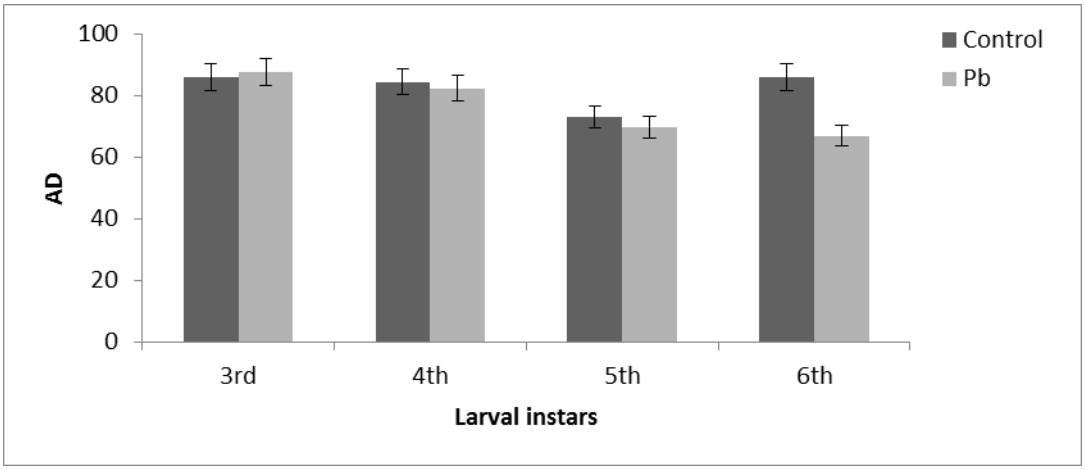

Fig. 3. Approximate Digestibility (AD) of the cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833), fed on Pb-treated castor leaves. Bar on the top of column represents SE.

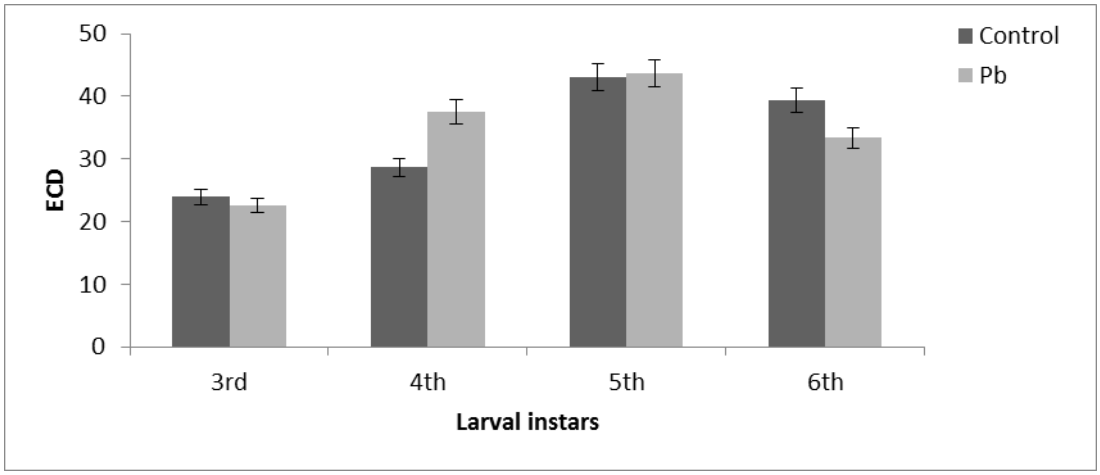

Fig. 4. Efficiency Conversion of Digested food (ECD) of the cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833), fed on Pb-treated castor leaves. Bar on the top of column represents SE.

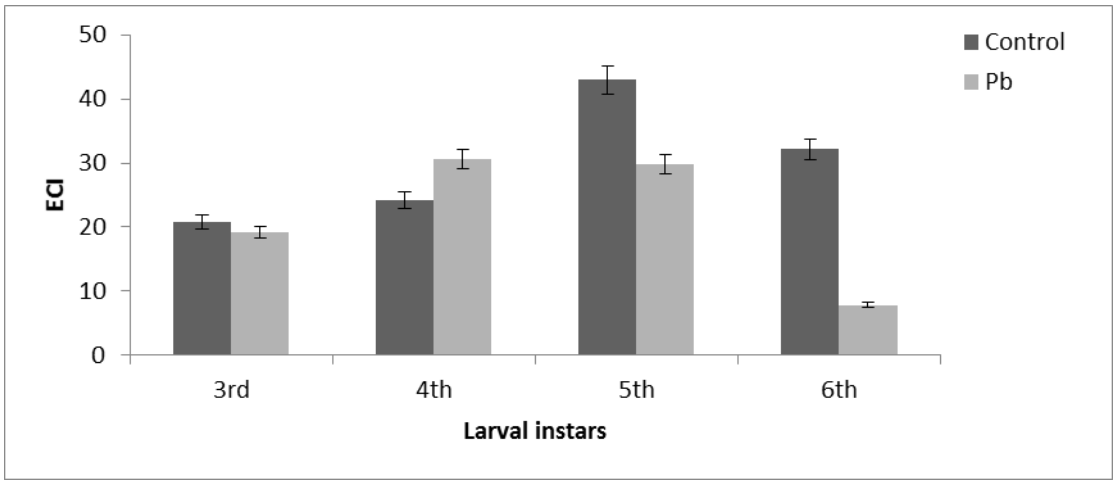

Fig. 5. Efficiency Conversion of ingested food (ECI) of the cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833), fed on Pb-treated castor leaves. Bar on the top of column represents SE.

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Influence of Soil Organic Matter Content on Abundance and Biomass of Earthworm (Oligochaeta: Lumbricidae) Populations

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Abstract. The current study explores the influence of soil organic matter content on abundance and biomass of earthworm communities. The observation was carried out on three type of soils: Pellic Vertisols (very fine texture), Cromi-Vertic Luvisols (fine texture) and Calcaric Fluvisols (medium texture) from the Balkan Peninsula (Bulgaria). The field experiment was provided on uncultivated plots. In the studied area earthworm fauna comprises of four species: *Aporrectodea rosea*, *Aporrectodea caliginosa*, *Lumbricus terrestris* and *Octolasion lacteum*. We found peregrine lumbricid taxa, which are widely distributed in European soils. Our study demonstrated that soil organic matter has a positive effect on lumbricid populations. It was revealed that augmentation of soil organic matter favours characteristics of earthworm communities. The soil organic matter content and earthworm abundance are in strong positive correlation ($r > 0.981$). The same relationship was revealed between the biomass of lumbricid fauna and amount of soil organic matter ($r > 0.987$). In sum, the soil organic matter could be used as an indicator for earthworm communities in uncultivated soils.

Key words: earthworms, Lumbricidae, Oligochaeta, soil organic matter, *Aporrectodea rosea*, *Aporrectodea caliginosa*, *Lumbricus terrestris*, *Octolasion lacteum*.

Introduction

Earthworms are considered as ecosystems engineers with great impact on physical, chemical and biological soils (LAVELLE *et al.*, 2007). Lumbricid abundance is considered major actors in the delivery of ecosystem services by soils. Earthworms feed and live in the soil, so their communities and their abundance are determined by soil properties and soil environmental conditions (CURRY, 1998). Earthworms incorporate plant residues into the soil and decompose organic matter (LAVELLE & MARTIN, 1992), thus affecting availability for plant and microbial growth (EDWARDS & BOHLEN, 1996). Through their burrowing activities, they create habitats for soil mesofauna. Earthworms often form the major part of the soil fauna biomass, representing up to 50% of the soil fauna biomass in some temperate grasslands, and up to 60% in some temperate forests (TURBÉ *et al.*, 2010).

Materials and Methods

The study was carried out over the 2011 - 2013 year period on uncultivated soils: Pellic Vertisols from Bozhurishte town, Cromi-Vertic Luvisols from Chelopechene village and Calcaric Fluvisols from Negovan village in Sofia Plain (Bulgaria). Earthworms were collected by the diluted formaldehyde method (RAW, 1959) complemented with digging 0.5 × 0.5 m quadrates, hand sorting and searching under stones and the bark of fallen logs. The biomass of

aclitellat and clitellat exemplars was estimated. The abundance of all collected earthworms was adjusted to one square meter. The specimens were killed in 70% ethanol, fixed in 4% formalin solution and 96% ethanol, then transferred into 75% ethanol. The organic matter content was estimated by the method of TURIN (1937). The statistical data were presented with correlation analyses and mean ± standard deviation.

Results and Discussion

The lumbricid density ranged between 75 – 32 exemplars/m². The earthworm abundance explored in study area revealed that a high value was measured in Pellic Vertisols (very fine soil texture) – 75 exemplars/m². Lower density was observed in Cromi-Vertic Luvisols (fine soil texture) 45 exemplars/m² and in Calcaric Fluvisols (medium soil texture) was observed - 32 exemplars/m² (Table 1).

Table 1. Earthworm abundance, biomass and soil organic matter content in studied soils.

Soil	Abundance (n/m²)	Biomass (g/m²)	Soil organic matter (%)
Pellic Vertisols	75±9	48±6	11.87
Cromi-Vertic Luvisols	45±4	32±3	3.0
Calcaric Fluvisols	32±3	33±2	1.8

The abundance of earthworms (Lumbricidae) increased proportionally with augmentation of soil organic matter content. The data showed a strong correlation R= 0.981008 (Fig. 1). The biomass of lumbricid communities ranged in the studied soils between 48 and 32 g/m². High biomass was estimated in Pellic Vertisols - 48 g/m². Calcaric Fluvisols and Chromi-Vertic Luvisols had earthworm populations with a similar biomass amount - 33 and 32 g/m².

Influence on earthworm biomass have not only organic matter, but and species biodiversity. In Calcaric Fluvisols, beside of lower abundance of earthworms in comparison with Chromi-Vertic Luvisols, the biomass is similar, because of high density of *Lumbricus terrestris*. This species is a large size earthworm with high biomass. Correlation analyses showed a strong relationship between the soil organic matter and earthworm biomass R= 0.987259 (Fig. 2).

Similarly, HENDRIX *et al.* (1992) reported a strong correlation between earthworm abundance and soil organic carbon. Earthworms play a major role for the accumulation and transformation of organic matter, while they ingest plant residues and soil enriched of organic litter. Earthworm populations could produce 1t/ha casts per year (TURBE *et al.*, 2010) and form vermic horizon (NIELSEN & HOLE, 1964). Lumbricid species produce soil macroaggregates (casts), which can last years and are important for conservation of soil organic matter (MARTIN, 1991). Earthworms increase the turnover of soil organic carbon (STOUT, 1983), as they enhance humification in the soil and transformation of mor and moder type humus in mull humus (LANGMAID, 1964).

Conclusions

The field experiment revealed that the soil organic matter content favours abundance and biomass of earthworm (Lumbricidae) populations. Soil organic matter content and earthworm abundance and biomass are in a strong positive correlation in all explored types of soil. Influence of humus on lumbricid populations is very high, nevertheless of different soil texture. Overall, the soil organic matter could be used as indicator for earthworm communities in uncultivated soils.

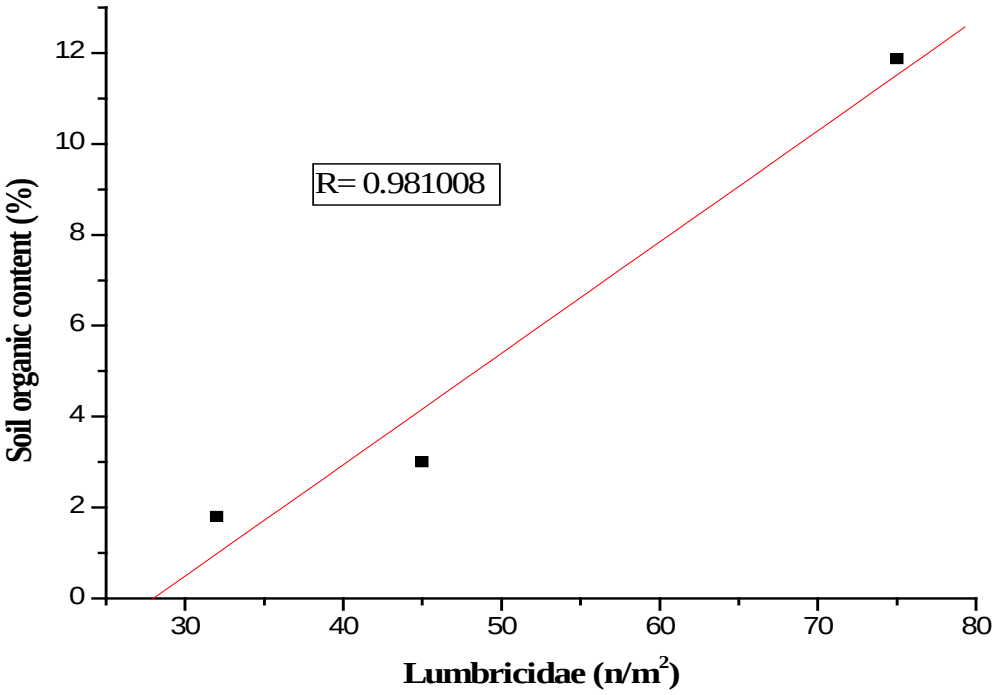

Fig. 1. Correlation analysis between the earthworm abundance and soil organic matter content.

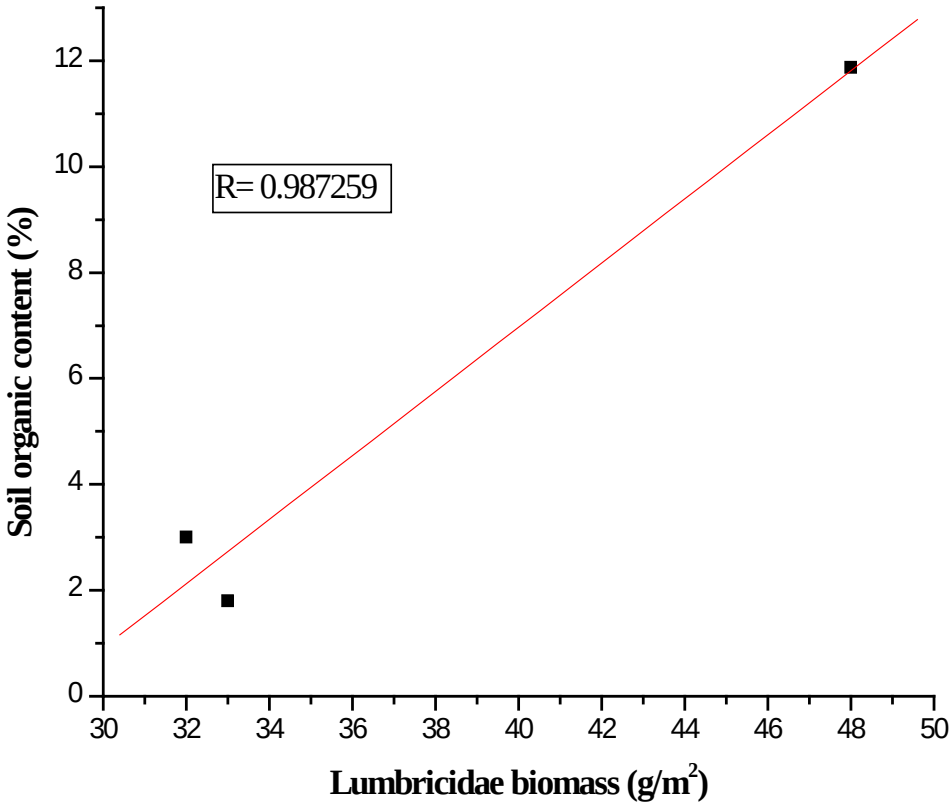

Fig 2. Correlation analysis between earthworm biomass and soil organic matter content.

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Short note

Gastropod Shells from Excavations of the "Antic Forum" Complex in the City Of Plovdiv (Bulgaria): IV-VI Century

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Abstract. Total of 35 shells were found and identified during the archeological excavations 2012-2014, studied Slavic dwellings-dugouts which reuse earlier structures of the roman forum in Plovdiv, Bulgaria. From both localities shells from 7 species of snails were found. Most of the shells were from two species: *Zebrina detrita* and *Helix philibinensis* – total of 28 shells, other were represented by 1-3 specimens per species. The lack of shell materials from *Eobania vermiculata* supports the theory of later introduction of this species in the city of Plovdiv.

Key words: archeology, Mollusca, late antic period, Plovdiv.

Introduction

During the archeological excavations 2012-2014, it is studied Slavic dwellings-dugouts which reuse earlier structures of the roman forum. Slavonic ceramics made by hand was found from home № 2 and was discovered a coin from Justin II and Sofia (565–578), which allows us to date the Slavic presence in the city in the third quarter of the VI century. Last emissions coins found the Roman Forum of Philippopolis minted during the reign of Emperor Flavius August Phocas (602-610). Upon the ruins of Slavic settlement was established a sediment layer about 20-25 cm thick, which contain river's sand and gravel, probably spill basin, where shells of molluscs were found. This situation is recorded in other sectors of the ancient Philippopolis, which gives us reason to assume that the city regions are spilled from the river Maritsa (KESYAKOVA, 2014). The shell material collected during these excavations is described in this short note.

Material and Methods

Shells of gastropods were collected from two late antic layers (IV-VI century) and two localities at the archeological area of the "Antic Forum" complex in the city of Plovdiv (Bulgaria) in 2013 from the first author. Species were identified using comparative shell collection of the second author and deposited in his collection.

Results and Discussion

Total of 35 shells were found and identified:

I. "Forum-West", late antic layer, IV-V century (so called "Magazin №1 - south"):

Helix philibinensis Rossmässler, 1839: 1 ad. well preserved shell;

Zebrina detrita (O. F. Müller, 1774): 2 ad. (1 fragmented shell);

Planoris planorbis (Linnaeus, 1758): 1 ad. well preserved shell;

II. "Forum-West", late antic layer, V-VI century:

Cepaea vindobonensis (Férussac, 1821): 1 ad. fragmented shell;

Monacha cartusiana (O. F. Müller, 1774) - species complex: 3 ad. (2 fragmented, 1 well preserved shell);

Chondula microtragus (Rossmässler, 1839): 1 ad. well preserved shell;

Zebrina detrita (O. F. Müller, 1774): 18 ad. well preserved shells, 2 ad., and 10 juv. fragmented;

Helix philibinensis Rossmässler, 1839: 1 ad. well preserved shell, 1 ad., and 5 juv. fragmented;

Planorbarius corneus (Linnaeus, 1758): 1 juv. well preserved shell;

From both localities shells from 7 species of snails were found. Most of the shells were from two species: *Zebrina detrita* and *Helix philibinensis* – total of 28 shells, other were represented by 1-3 specimens per species.

The lack of shell materials from *Eobania vermiculata* supports the theory of later introduction of this species in Plovdiv city.

The presence of freshwater species could be one of the evidences of periodical floods on the human settlements at the area of the "Antic Forum" complex.

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Corrigendum

Corrigendum to "Species Diversity and Distribution of Amphibians and Reptiles in Nature Park "Sinite Kamani" in Stara Planina Mt. (Bulgaria)" [Ecologia Balkanica, 6(2): 83-92]

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The authors would like to report an error, they made indetifying one of the amphibian species, reported in the article, published in volume 6, issue 2, 2014. The species *Rana graeca* is wrongfully identified and is actually a subadult *Rana temporaria*. The mistake was made, because not all photographic evidence was present at the time of publication, which lead to the wrong identification of the species. Now all photographs made by A. Mechev are available and it is clear that the discovered frog is in fact subadult *Rana temporaria*, judging by the length of the hind legs and the size of the tympanic membrane (tibiotarsal joint does not exceed the tip of the snout and the tympanic membrane has a large diameter) – features that entirely confirm *R. temporaria* (Fig. 1-3).

Fig. 1. The original photograph of the discovered frog, published in the article in 2014, Appendix 5, page 91.

Fig. 2. Second photograph made the same day, right after the first one, showing that the tibiotarsal joint does not exceed the tip of the snout.

Fig. 3. The third photograph showing the dorsal side of the discovered frog.

The common frog (*Rana temporaria*) is actually new, previously unreported species for the studied area and all other information provided in the article about the discovered frog is correct.

The authors would like to apologize for this error.

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

Reviewers Acknowledgment

Peer reviewers are key to advancing scholarship and contributing to the quality of scholarly journals. We would like to thank the following reviewers who have taken part in the peer-review process for vol. 8, iss. 1, 2016 of Ecologia Balkanica.

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Galina Kolganihina - Moscow State Forest University, Department Botany and Plant physiology (RUSSIA)
Gana Gecheva - Plovdiv University, Faculty of Biology, Department of Ecology and EC (BULGARIA)
Ivelin Mollov - Plovdiv University, Faculty of Biology, Department of Ecology and EC (BULGARIA)
Leena Luís - University of Lisbon, Center of Geology (PORTUGAL)
Mariyana Lyubenova - University of Sofia, Department Ecology and Environment Protection (BULGARIA)
Mete Misirlioğlu - Eskisehir Osmangazi University, Department of Biology, Eskişehir (TURKEY)
Nasko Atanasov - Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (BULGARIA)
Oguz Turkozan - Adnan Menderes University, Department of Biology (TURKEY)
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Manuscripts must conform strictly with the instructions to authors and sent to the Editor. **All manuscripts must be accompanied with a cover letter,** which can be downloaded from here. All fields from the cover letter form must be filled out and the cover letter must be sent along with the full text of the manuscript to the journal's e-mail. Incoming manuscripts are initially judged by the Editor. *Manuscripts may be rejected without peer review if they do not comply with the instructions to authors or are beyond the scope of the journal.* If the manuscript is acceptable, it will be forwarded to referees for evaluation. All manuscripts are peer-reviewed by 2 or 3 independent reviewers. After final edition and approval by the Editorial Board, the manuscript will be accepted for publication. The Editor reserves the right to make editorial changes. Authors agree, after the manuscript's acceptance, with the transfer of copyright to the publisher.

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The manuscripts must be prepared in English. *Contributors who are not native English speakers are strongly advised to ensure that a colleague fluent in the English language*, if none of the authors is so, *has reviewed their manuscript.* Spelling should be British or American English and should be consistent throughout. All abbreviations and acronyms should be defined at first mention. To facilitate reader comprehension, abbreviations should be used sparingly.

Technical information

Manuscripts must be submitted in **electronic version only**, as well as the original figures and tables, implemented in the text. The manuscript text should be **MS-Word** processed (all versions are acceptable), justified, font size 11, font "Book Antiqua", without footnotes, column or page breaks, single spaced (about 60 lines per page), on A4 (210 x 297 mm) paper, with margins of exactly 2.5 cm on each side. Pages should not be numbered.

The manuscripts should conform to the following format:

Title: Provide a title that is concise, but also an informative synthesis of the study. Where appropriate, include mention of the family or higher taxon.

Author(s): Full first name(s), middle initials and surname(s) in ***bold italic.*** The corresponding author should be marked with the *-symbol.

Address(es): As complete as possible. E-mail address is given only for the corresponding author.

Abstract: Maximum length 300 words. The abstract should state briefly the objective of the research, the primary results and major conclusions, with no description of methods, discussions, references and abbreviations.

Key words: Usually 3–10 words suitable for information-retrieval system.

The standard order of sections should be: Abstract, Key words, Introduction, Material and Methods, Results, Discussion (or Results and Discussion), Conclusions (optional), Acknowledgements (optional) and References.

The *Introduction* has to explain the actuality of the researched problem and give the aim of the study.

Materials and Methods have to provide sufficient information to permit repetition of the experiment and/or fieldwork. The technical description of study methods should be given only if such methods are new; otherwise a short presentation is enough.

The *Results* section must be a concise presentation of the finding of the study. **Avoid presentation of the same information as text and/or figure and/or table.**

Discussion section should be separate from the results section at full-length papers and should deal with the significance of the results and their relationship to the aims of the paper. Also include how the

findings of the paper will change or influence the state of our knowledge about the topic at hand. In separate cases a joint section "Results and Discussion" is allowed, but not preferable.
Conclusions should shortly describe the main contributions and recommendations of the study without including citations and statistics.
In the *Acknowledgements* section all persons and organizations that helped during the study in various ways, as well as the organization that financed the study must be listed.

Short Notes (generally less than four-five manuscript pages) should be produced as continuous text, preceded by an abstract of no more than 150 words.

Tables: The tables must not repeat information already presented in the figures or in the text. Each table must be self-explanatory and as simple as possible. No fold-outs are accepted. Tables must be numbered consecutively. **They should be placed within the text at the desired position by the author(s).** An explanatory caption, located on the top of the table, should be provided.

Example:

Table 1. Shannon-Wiener indexes in the burned ($\mathbf{H}_{burned}$) and control ($\mathbf{H}_{control}$) territory for the total duration of the study (2004–2006).

Figures: They must not repeat information already presented in the tables or in the text. Lines and letters in figures must be able to be enlarged or reduced without reduction in quality. They should conform to the size of the type area (16 × 24 cm) which is the limit for all illustrations. Magnification should be shown by scale bars. Colour illustrations are accepted, but will appear only in the electronic version of the journal (PDF). The illustrations in the hardcopy printed version will be greyscale. All illustrations must be sharp, of high quality with at least 300 dpi. The following formats are acceptable: JPEG, GIF, TIFF, EPS. Figures must be numbered consecutively and should be provided with an explanatory legend below them. **They must be placed within the text at the desired position by the author(s).**

Example:

Fig. 1. Indicative map of the study area.

All tables and figures must be referred to in the text!

Citations and references

Literature citations in the text should indicate the author's surname in SMALL CAPITALS (for more information on SMALL CAPITALS and how to make them in Word) with the year of publication in parentheses, e.g. CARLIN (1992); BROOKS & CARLIN (1992); SHAPIRO *et al.* (1968). Citations in brackets should be divided with semicolons and the author's name and the year of publication with comma (*example:* CARLIN, 1992; BROOKS & CARLIN, 1992; SHAPIRO *et al.,* 1968). If there are more than two authors, only the first should be named, followed by "*et al.*" in *italic*. References at the end of the paper should be listed in alphabetical order by the first author's family name and chronologically. If there is more than one work by the same author or team of authors in the same year, a, b, etc. is added to the year both in the text and in the list of references. Each citation in the text must be accompanied by a full reference in the list of references and vice versa. **Please provide DOI numbers for all electronic resources (journal articles, online articles, books), if they have DOI, when cited in the references list!**

Examples:

A journal article:

AUTHOR A. 1990. Title of the article. - *Full title of the journal*, 56(3): 35-105.

AUTHOR A., B. AUTHOR. 1990. Title of the article. - *Full title of the journal*, 56(2): 35-105.

AUTHOR A., B. AUTHOR, C. AUTHOR. 1990. Title of the article. - *Full title of the journal*, 56(1): 35-105.

A book:

AUTHOR A. 2000. *Title of the book.* Vol. I. Place of publication. Publishing house.

Proceedings or book chapter:

AUTHOR A., B. AUTHOR 1990. Title of the contribution. - In: Author A. (Ed.): *Title of the book or proceedings.* Place of publication. Publishing house, pp. 235-265.

Software:

STATSOFT INC. 2004. *STATISTICA (Data analysis software system)*, Vers. 7. Computer software. Available at: [http://www.statsoft.com].

GARMIN LTD. 2007. *MapSource*, Vers. 6.12. Computer software. Available at: [http://www.garmin.com]

Website:

FAUNA EUROPAEA. 2007. *Invertebrates. Fauna Europaea.* Vers. 1.1. Available at: [http://www.faunaeur.org]. Accessed: 12.10.2009.

In case of papers written in other than Latin letters, if there is an English (or German, or French) title in the summary, it is recommended to be used. If there is not such a summary, the author's names must be transcribed and the title of the paper must be translated into English and put in square brackets. If the name of the journal is also not in Latin letters it also should be transcribed (not translated). This should be noted in round brackets at the end of the paragraph, for instance: (In Bulgarian, English summary).

Example:

ANGELOV P. 1960. Communications entomologiques. I. Recherches sur la nourriture de certaines espèces de grenouilles. – *Godishnik na muzeite v grad Plovdiv*, 3: 333-337. (In Bulgarian, Russian and French summary).

KOROVIN V. 2004. [Golden Eagle (*Aquila heliaca*). Birds in agricultural landscapes of the Ural]. Ekaterinburg, Published by Ural University, 57 p. (In Russian).

Names of persons who provided unpublished information should be cited as follows: "(ANDERSSON, 2005, Stockholm, pers. comm.)".

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