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Fish Producer's Attitude to the Most Common Fish-Eating Birds in Central Bulgaria

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Abstract. In Bulgaria part of fish farming is through using extensive production technologies. Most of the dams used for fish production are located in the lowlands of the country and are the natural habitats of herons, cormorants and pelicans. Thus these birds are considered to be pests in extensive aqua production. To clarify whether in fact the owners and workers in fish farms obey the law with regard to fish-eating birds, an anonymous survey among 80 fish producers was conducted between January and August 2014. The positive and negative responses were expressed as a percentage. The economic factor determined the negative attitude of owners towards fish-eating birds. The lack of motivation for conservation of protected bird species was due to non-payment of compensations from the government.

Key words: conflict, herons, cormorants, pelicans, fish farming.

Introduction

The areas of fish farms in Bulgaria are controlled by concession holders or owners which are responsible for conservation of protected birds. The attitude of fish producers towards fish-eating birds as well as compliance to the Law, depends on their personal attitude, knowledge and the sanctions imposed on them. The attitude towards the game and its use are stipulated in the Law for hunting and protection of the game (SG, 2000), and the prohibited devices, methods and means of capture and killing of waterfowl are defined in Appendix 5 of the Biodiversity Act (SG, 2002). Most of investigated species are included in the Red Data Book of the Republic of Bulgaria (BOTEV & PESHEV, 1985). They are subject to European legislation.

Most studies concerning damage caused by fish-eating birds were focused on direct fish farms losses (MARION, 1990; OSIECK, 1991). Such studies have not been conducted in Bulgaria so far. Cormorants, herons and others piscivorous birds concentrate their fishing efforts on fish farms and gather nearby rivers and lakes similarly to marine fish-eating birds using the abundance of food (BARLOW & BOCK, 1984; DRAULANS, 1987; CALLAGHAN *et al.*, 1998; LEKUONA, 2002). Cormorants eat a large range of fish as they inhabit different type of habitats. Their average daily food intake is between 340 - 520 g fish (MARQUISS & CARSS, 1994). The damages from these birds were reported by TUCAKOV (2006) for Serbia, by LEKUONA (2002) for France, and other parts of Europe (IM & HAFNER, 1984; PERENNOU, 1987; MARION, 1990; OSIECK, 1991).

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Damages on fish should not be regarded as consumption only, but also as a worsening fish condition, injuries, transmitting parasites, anxiety etc. (CARRS, 1990; 1993). Cormorants attack fish in net cages and injure them fatally (RANSON & BEVERIDGE, 1983; CARRS, 1993). Regular attacks on fish stocks result in different extent of conditional stress which is associated to reduced production in farms (BERKA, 1989; ADAMEK, 1991). Fish-eating birds represent an important group of hosts of a wide range of parasite species using fish as intermediate hosts (SITKO *et al.*, 2006).

All fish-eating bird species are protected by the Law, and part of them are endangered. In recent years, an increase of anxiety from the effects of violations of fishermen and fish farmers on piscivorous bird populations was observed (TASKER *et al.*, 2000). The aim of the study was to clarify whether owners and workers in fish farms were obeyed the regulations with regard to fish-eating birds: herons (*Ardeidae*), pelecans (*Pelecanidae*), and cormorants (*Phalacrocoracidae*) and to analyze the reasons for this.

Material and Methods

To clarify the features of the conflict between owners and workers in fish farms and fish-eating birds a questionnaire with the following questions was composed (Table 1).

Table 1. Questionnaire for clarifying fish producer's attitude to the most common fish-eating birds.

1.	Have you seen cormorants in your fish farm?	Yes ○ No ○
2.	Have you seen herons in your fish farm?	Yes ○ No ○
3.	Have you seen pelicans in your fish farm?	Yes ○ No ○
4.	Do you think that cormorants cause damage to the fish?	Yes ○ No ○
5.	Do you think that herons cause damage to the fish?	Yes ○ No ○
6.	Do you think that pelicans cause damage to the fish?	Yes ○ No ○
7.	Do you consider that you should be compensated by the state because of damages caused by fish-eating birds in your fish farm?	Yes ○ No ○
8.	Do you think that cormorants should be exterminated?	Yes ○ No ○
9.	Do you think that herons should be exterminated?	Yes ○ No ○
10.	Do you think that pelicans should be exterminated?	Yes ○ No ○

In order to reveal the real attitude of fish producers towards piscivorous birds, an anonymous questionnaire was provided. The survey was conducted in the period January 2014 – August 2014 with 80 owners and workers in fish ponds from 115 registered fish farms in the region of Stara Zagora city, Yambol city and Sliven city.

The data were summarized as positive and negative responses for each question and were presented in table. The percentage values have been calculated.

Results and Discussion

The answers of the first and second questions of the survey pointed that cormorants and herons were present in all fish farms of the study area. Cormorants and herons concentrate their fishing efforts on fish farms and gather around rivers and lakes, just like marine fish-eating birds using the abundance of food (BARLOW & BOCK, 1984; CALLAGHAN *et al.*, 1998; LEKUONA, 2002). The additional conversation with respondents established that most of them were unable to distinguish the different species of cormorants, but they recognized the large herons (gray heron, purple heron and great egret). Pelicans were rare in the study area - only 5 reported cases (Table 2).

These taxonomic groups, however, include birds with different protection status. Most of studied species are endangered and with priority for habitat conservation. The great cormorant and gray heron are exceptions (Biodiversity act - SG, 2002). Piscivorous birds were treated as harmful, regardless of their protection status.

Table 2. Results from the survey.

Questions	Positive answers		Negative answers	
	n	%	n	%
1. Have you seen cormorants in your fish farm?	80	100	-	-
2. Have you seen herons in your fish farm?	80	100	-	-
3. Have you seen pelicans in your fish farm?	5	6.25	75	93.75
4. Do you think that cormorants cause damage to the fish?	80	100	-	-
5. Do you think that herons cause damage to the fish?	47	58.75	33	41.25
6. Do you think that pelicans cause damage to the fish?	80	100	-	-
7. Do you consider that you should be compensated by the state because of damages caused by fish-eating birds in your fish farm?	80	100	-	-
8. Do you think that cormorants should be exterminated?	64	80	16	20
9. Do you think that herons should be exterminated?	18	22.5	62	77.5
10. Do you think that pelicans should be exterminated?	11	13.75	69	86.25

All respondents considered that cormorants and pelicans caused damage to the cultivated fish (Table 1 – question 2-4). More than half (58.75%) of the respondents also mentioned herons as pests and the remaining 41.25% believed that these birds caused insignificant damage to the fish. The higher predation level by cormorants than by herons was recorded by GENARD *et al.* (1993). Further discussion made clear that this fact was also well-known to the fish producers. Pelicans were rarely found on the territory of the studied farms, the reason why they were not considered as pests.

Discussions with fish producers made clear that they reported direct and indirect damage from fish-eating birds. All of them pointed out to direct damage - eating and wounding the fish. Most of them, however, did not miss out the indirect damages – transport of weed fish eggs, stress leading to behavioral change, transmission of diseases, as in the studies of ADAMEK *et al.* (2007) and SITKO *et al.* (2006). Consumption of fish is a measurable damage and the most important circumstance that determined the attitude of the fish farms owners. Studies carried out in Southern France indicated that losses from cormorants were about 53% of fish yields, and those from gray herons - up to 10.8%. These are serious economic losses to fish production (LEKUONA, 2002).

All respondents considered that they should be compensated for the damage caused by the fish-eating birds from the government. Farm owners considered that they will not be compensated for their losses actually. In Bulgaria, there was a practice of paying compensations for the damage caused by fish-eating birds to fish farms, which was terminated. As a result, fish producers took intensive measures, including persistent persecution and extermination of the piscivorous birds, which also adversely affected small cormorants (PLACHIYSKI *et al.*, 2014). The number of great cormorants has increased as the species was protected from Council Directive 79/409/EEC of 2 April 1979 (EC, 1979), from the 1980s till now. This has exacerbated the conflict between fish producers and this bird species. Most of the respondents (80%) considered that the cormorant population should be reduced, even by means of extermination. The attitude towards herons and pelicans was more tolerant. Only 22.5% of respondents were willing to exterminate herons, and 13.75% of them - pelicans. A large number of respondents were tolerant to the presence of herons and considered that these birds consumed mostly weed fish. The scarce presence of the pelicans in the study area as well as the current legislation, were the reason why the main part of fish farmers (86.25%) did not exterminate them (Table 2).

Conclusions

Economic losses from eaten and damaged fish and the lack of compensation for fish producers from the government generate a negative attitude towards fish-eating birds in the study area, motivating fish farmers to exterminate piscivorous birds, opposing to nature conservation legislation.

Cormorants were the species with the highest potential threat for extermination from fish farmers.

References

ADAMEK Z. 1991. Food biology of great cormorant (*Phalacrocorax carbo* L.) on the Nove Mlyny reservoirs. - *Bulletin VURH Vodňany*, 27: 105-111.

ADAMEK Z., J. KORTAN, M. FLAJSHANS. 2007. Computer-assisted image analysis in evaluation of fish wounding by cormorant (*Phalacrocorax carbo sinensis* L.) attacks. - *Aquaculture International*, 15: 211-216. [DOI]

BARLOW C., K. BOCK. 1984. Predation of fish in farm dams by Cormorants, *Phalacrocorax spp.*. - *Australian Wildlife Research*, 11: 559-566. [DOI]

BERKA R. 1989. Avian predation in aquaculture (review). - *Bulletin VURH Vodňany*, 25(3): 18-32.

BOTEV B., Ts. PESHEV. 1985. *Red Data Book of the Republic of Bulgaria*. Vol. II. BAN. Sofia. (In Bulgarian)

CALLAGHAN D., J. KIRBY, M. BELL, C. SPRAY. 1998. Cormorant *Phalacrocorax carbo* occupancy and impact at Stillwater game fisheries in England and Wales. - *Bird Study*, 45(1): 1-17. [DOI]

CARSS D. 1990. Bird predation: correct species identification is often the key to successful damage reduction. - *Fish Farmer*, 13: 46-47.

CARSS D. 1993. Cormorants *Phalacrocorax carbo* at cage fish farms in Argyll, W. Scotland. - *Seabird*, 15: 38-44.

EC. 1979. Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds. - *Official Journal of the European Union*, L 103, 25/04/1979: 0001 – 0018. Available at: [eur-lex.europa.eu].

DRAULANS D. 1987. The effectiveness of attempts to reduce predation by fish-eating birds – A review. - *Biological Conservation*, 41(3): 219-232. [DOI]

GENARD M., J. MASSE, C. RIGAUD. 1993. Experimental approach to the predation by piscivorous birds in extensive water aquaculture. - *Bulletin Français de la Pêche et de la Pisciculture*, 329: 231-243. (In French) [DOI]

IM B., H. HAFNER. 1984. Impact of piscivorous birds and more particularly of the great cormorant *Phalacrocorax carbo sinensis* on fish farms in Camargue, France. - *Le Sambuc, StationBiologique de la Tour du Valat*, Rapport CEE Contrat Env-491-F (In French)

LEKUONA J. 2002. Food intake, feeding behavior and stock losses of cormorants, *Phalacrocorax carbo*, and grey herons, *Ardea cinerea*, at a fishfarm in Arcachon Bay (Southwest France) during breeding and non-breeding season. - *Folia Zoologica*, 51(1): 23-34.

MARION L. 1990. Piscivorous birds and fish activities: impact and protection. - *Secretariat d'Etat. Charge de l'environnement et Ministère de l'agriculture et de la forêt*, Paris, 28 p. (In French)

MARQUISS M., D. CARSS. 1994. Avian Piscivores: Basis for Policy. –Institute of Terrestrial Ecology, R&D Project Record 461/8/N&Y, *National Rivers Authority*, 95 p.

OSIECK E. 1991. Prevention of cormorant damage at the Lelystad fish farm. - In: Van Eerden, M. Ziljstra: *Proceedings Workshop 1989 on cormorants* (*Phalacrocorax carbo*). Lelystad. Rijkswaterstaat Directo-rate Flevoland, pp. 205-211.

PERENNOU C. 1987. The impact of grey heron *Ardea cinerea* on fish ponds. - *L'Oiseau et R.F.O.*, 57:262-265. (In French)

PLACHIYSKI D., D. DEMERDJIEV, G. POPGEORGIEV, N. PETKOV, Y. KORNILEV. 2014. *Action plan for conservation of the small cormorant (Phalacrocorax pygmeus) in Bulgaria.* Bulgarian Ministry of Environment and Water. (In Bulgarian)

RANSOM K., M. BEVERIDGE. 1983. Raiders from the skies. Bird predation at a freshwater cage farm. - *Fish farmer*, 6: 22-23.

SG. 2000. Law for hunting and protection of the game. – *State Gazette*, 78 of 26 September, 2000. Available at: [lex.bg] (In Bulgarian)

SG. 2002. Biodiversity Act. – *State Gazette*, 77 of August 9, 2002. Available at: [lex.bg] (In Bulgarian)

SITKO J., A. FALTYNKOVA, T. SCHOLZ. 2006. *Checklist of the Trematodes (Digenea) of Birds of the Czech and Slovak Republics*. Praha. Academia. 112 p.

TASKER M., C. CAMPHUYSEN, J. COOPER, S. GARTHE, W. MONTEVECCHI, S. BLABER. 2000. The impacts of fishing on marine birds. - *ICES Journal of Marine Science,* 57(3): 531-547. [DOI]

TUCAKOV M. 2006. Seasonal variations in numbers of Great cormorant (*Phalacrocorax carbo*) on the Kolut fish farm (NW Serbia). – *Archives of Biological Sciences,* 58(2): 83-86. [DOI]

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Bioaccumulation of Heavy Metals by Grasshoppers and a Mantid Along a Pollution Gradient

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Abstract. The concentrations of Cd, Cr, Cu, Fe, Ni, Pb, and Zn were studied in soil, plants, three grasshopper species (*Aiolopus thalassinus* (Fabricius, 1781), *Calephorus compressicornis* (Latreille, 1804), and *Acrotylus patruelis* (Herrich-Schäffer, 1838)), and a mantid (*Miomantis paykullii* Stål, 1871), to estimate the scale of spatial influence of a point pollution source, to investigate metal bioaccumulation up the soil–plant–grasshopper–mantid food chain, and to assess these insects as ecological bioindicators. Plant, insect, and soil sampling was undertaken simultaneously from grass margins of an arable farmland near an industrial complex in Al-Tebbin. Correlation analyses revealed a pollution gradient, with samples closest to the industrial activities having the highest concentrations of heavy metals. Sigmoid curves indicated that the influence radius of the industrial zone for heavy metal contamination in soil, plants, and grasshoppers was in the range of 3.0–4.0 km. The average concentrations of Cd, Cr, Cu, Fe, Ni, Pb, and Zn in the three grasshopper species were 4.9, 4.0, 73.9, 210, 5.2, 12.0, and 224.2 mg/kg, respectively, while those in the predaceous mantid were 9.0, 7.9, 106.4, 400, 14.8, 23.0, and 463.7 mg/kg, respectively. The metal content did not increase from soil to grasses, but at plant–primary consumer level, Cd, Cu, Pb, and Zn were biomagnified, and, in general, all metal concentrations increased with an increase in the trophic level (i.e., secondary consumer). In light of this site-dependent accumulation of heavy metals and their biomagnification patterns, the investigated acridids can be considered bioindicators of Cd, Cu, Pb, and Zn pollution.

Key words: bioaccumulation, bioindicator, food chain, grasshoppers, industrial activities, influence range.

Introduction

Environmental issues related to heavy metal pollution have become a widespread serious problem (SOFIANSKA *et al.*, 2013). Metals are released into the environment as a consequence of a wide range of industrial activities and combustion of fossil fuels. These different sources contribute to the load of metal pollutants in terrestrial and aquatic food chains (GALL *et al.*, 2015). Dust fall, bulk precipitation, and gas adsorption processes are responsible for the transportation of heavy metals from the atmosphere to the soil and plants (MALIZIA *et al.*, 2012; RAKIB *et al.*, 2014). In several localities, the emission of pollutants into the air by industrial activities has reached levels that are toxic to plant, animals, and humans. The measuring and monitoring of the environmental pollution in rural and urban ecosystems can help in the assessment of air quality and can be used to quantify the effective range of pollution sources (ROSS, 1987). Heavy metal inputs into agricultural soils have gradually increased

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over the last century, as a result of atmospheric deposition, disposal of sewage sludge, and agricultural practices (fertilizers, liming materials, pesticides, manures, and compost) (GALL *et al.*, 2015).

Element concentrations in living organisms are generally low, except in the vicinity of heavy metal pollution sources (ZHANG *et al.*, 2012). Nevertheless, because of the long retention times of toxic substances in the ecosystems, the transport of even small amounts through the food chain increase the concentration of these metals and can cause deterioration of the environment. Some heavy metals (e.g., Cu, Fe, Se, and Zn) are involved in biochemical and physiological functions in plants and animals, and therefore are essential in trace amounts (MARSCHNER, 2012). However, when present in excess, these become toxic to living organisms, and possibly dangerous to human health through their transfer via the food chain (CHAFFAI & KOYAMA, 2011). Other metals (e.g., Cd and Pb) are not known to have any essential function, and are toxic even at low concentrations (NORDBERG *et al.*, 2014). Heavy metals, such as Cd and Pb are responsible for certain diseases and cause a number of health hazards in humans (AGARWAL, 2009). Moreover, they have been classified as carcinogenic for humans and wildlife (BEYERSMANN & HARTWIG, 2008).

Pollution zones with high metal content in soil and biota are often found near industrial areas (DUDKA *et al.*, 1996). The ability of some animals to accumulate these metals, and establish a reliable relationship with the concentration of the metal in their surroundings, makes them useful indicators of environmental pollution.

Grasshoppers are herbivorous insects, feeding mainly on grass leaves, and account for 20–30% of the biomass of all arthropods. Therefore, they play a significant role in accumulating and transferring toxic metals to higher trophic levels through the food chain (DEVKOTA & SCHMIDT, 2000). Several studies have reported excessive concentrations of heavy metals in terrestrial food chains (HSU *et al.*, 2006; HECKEL & KEENER, 2007; ZHUANG *et al.*, 2009; JUNG & LEE, 2012; NICA *et al.*, 2012; BOSHOFF *et al.*, 2015; SIMON *et al.*, 2016; CONTI *et al.*, 2017; DAR *et al.*, 2017). However, few studies have focused only on grasshoppers. Some studies have been undertaken on the accumulation of metals in soil, plants, and grasshoppers, where the concentrations of metals are usually highest at the sites closest to the pollution sources (DEVKOTA & SCHMIDT, 2000; KARADJOVA & MARKOVA, 2009; NATH *et al.*, 2011; ZHANG *et al.*, 2012; ZHANG *et al.*, 2014). In addition, heavy metals could be potentially accumulated in predators that feed on grasshoppers (e.g., mantids), and transported to organisms higher up the food web (ZHENG *et al.*, 2008; ZHANG *et al.*, 2009, 2010).

The main purpose of this study was to specify the effective range of heavy metal contamination from industrial areas, and to investigate Cd, Cr, Cu, Fe, Ni, Pb, and Zn bioaccumulation and transportation up the soil–plant–grasshopper–mantid food chain in grass margins of arable farmland near an industrial complex in Al-Tebbin District of Egypt. We also assessed the grasshoppers (*Aiolopus thalassinus* (Fabricius, 1781), *Calephorus compressicornis* (Latreille, 1804), and *Acrotylus patruelis* (Herrich-Schäffer, 1838)) and a predator (*Miomantis paykullii* Stål, 1871) for suitability as ecological bioindicators for heavy metal contamination.

Materials and Methods

Study area. The study area was located east of the River Nile, 20 km south of Cairo, in Al-Tebbin region in the Helwan Province. The population of Al-Tebbin District is approximately 0.5 million people (2008); it is considered the biggest industrial zone in Egypt, with about 16.5% of the total industrial activities for the country. The main industrial activities include ferrous and nonferrous metallurgical work, coke factory, chemical and cement industry (MOHAMED *et al.*, 2011; SOLIMAN *et al.*, 2017). With the steady increase of industrial emissions, in addition to high temperatures, lack of rain, and predominantly low wind speed, this area has severe air pollution problems.

Sample collection and preparation. During the summers (July and August) of 2012 and 2013, soil, plant, and insect samples were collected from the grass strips along the edges of farmland in Al-Tebbin region.

Samples were taken from nine sites at different distances, up to 10 km downwind and 3.5 km upwind, from the main sources of industrial pollution (Fig. 1). *A. thalassinus* (356 specimens), *C. compressicornis* (219), *A. patruelis* (131), and *M. paykullii* (41) were collected, euthanized with alcohol, and preserved in a refrigerator at 4 °C until used. Three common grasses in the study area, *Paspalum distichum* L., *Brachiaria deflexa* (Schumach.) Robyns, and *Hyparrhenia hirta* (L.) Stapf, were sampled at the same sites and stored in polyethylene bags. The leaves and stems of the three species were washed with ultrapure water to remove metals attached to the surface. For each site, five soil samples were randomly collected from the upper soil layer (0–15 cm) and thoroughly mixed, with about 1 kg of soil used for lab analysis. The soil, plants, and arthropods were oven dried (70 °C) until they reached a constant weight, and were then ground to a homogenous powder and preserved in polythene bags. The sampling positions were recorded using GPS technology, and ArcGIS 9.3 was used to develop the location map.

Chemical analysis. The soil digestion was based on the protocol described by SOLIMAN *et al.* (2017). A soil sample (0.5 g, accurately weighed) was placed in a digesting flask and a pre-digestion step was run at room temperature for 24 hrs with 12 mL of 37% HCl: 65% HNO_3 (3: 1) mixture. Then, in a fume cupboard, the suspension was digested to near dryness on a thermostatically controlled hot plate at 90 °C. To the hot residue, 2.5 mL of 37% HCl and 2.5 mL of 30% H_2O_2 were added to complete the digestion, and the resultant mixture was heated again and then cooled to ambient temperature. The flask walls were washed with 10 mL of ultrapure water and the obtained suspension was filtered through Whatman filter paper (No. 41) in a volumetric flask, diluted to 50 mL, and stored in polyethylene bottles at 4 °C for analyses. The digestion of plant and insect samples were prepared in a similar way, but were allowed to stand for 24 hrs with 10.0 mL of 65% HNO_3, and only 2.0 mL of 30% H_2O_2 was added to aid the digestion of the organic matter. The filtered solutions were diluted up to 25 mL. The concentrations of Cd, Cr, Cu, Fe, Ni, Pb, and Zn were determined by inductively coupled plasma (ICP-AES-Jobin Yovin ultima2, France). Wavelengths and detection limits of the ICP for the analyzed metals were: 226.502 nm and 0.0023 mg L^{-1} for Cd; 220.353 nm and 0.028 mg L^{-1} for Pb; 324.754 nm and 0.0036 mg L^{-1} for Cu; 213.856 nm and 0.0012 mg L^{-1} for Zn; 231.604 nm and 0.01 mg L^{-1} for Ni; 205.552 nm and 0.0041 mg L^{-1} for Cr; and 259.940 nm and 0.0041 mg L^{-1} for Fe, respectively. Certified reference materials (NIST 2709, 1547, and 1577) and a reagent process blank were performed for each analytical batch to assess the analytical accuracy. In addition, mean values of three replicates were taken for each determination. Metal concentrations were expressed as mg/kg dry weight. Chemicals, stock solutions, and reagents were all of analytical grade and were obtained from Merck. All glassware and plastic materials were washed with distilled water before use, soaked in 2N nitric acid overnight, and then rinsed thoroughly with ultrapure water.

Statistical analyses. Only a limited number of data sets revealed a good fit to normal distribution and there were large differences in variance between treatment groups. Therefore, Kruskal-Wallis H-test was used to test for significant differences in metal concentrations between sites, while Mann-Whitney U-test was used to compare the total metal concentrations between the soil, plants, grasshoppers, and mantid, and mean metal bioaccumulation factors between trophic levels (plants, grasshoppers, and mantid) (SPSS STATISTICS, 2008). Correlation analyses of heavy metal concentrations with the distance from the industrial area, and between soil, plant, and grasshopper were conducted using Spearman's rank correlation (SPSS STATISTICS, 2008). In all tests $p \leq 0.05$ was considered significant. All treatments were replicated three times in the experiment. In this study, the bioaccumulation factor for plants was calculated as the ratio of the metal concentration in shoots to that in the soil (CUI *et al.*, 2007), and the bioaccumulation factor (B) for grasshoppers and the mantid was defined as:

$$B = Cn/Cn\text{-}1$$

where Cn is the total metal concentration in the organism from trophic level n, and Cn-1 is the total metal concentration in the food of this organism from trophic level n-1 (LASKOWSKI, 1991).

Results and Discussion

Metal content in the soil, plants, grasshoppers, and mantid. Metal concentrations in the soil–plant–insect food chain at different sampling sites are shown separately in Fig. 2 and collectively, with range of variation, in Table 1. The heavy metal content varied significantly among sample sites. Cu (H = 24.942, p = 0.002 for soil; H = 25.079, p = 0.002 for *C. compressicornis*; H= 24.335, p = 0.002 for *A. patruelis*) and Zn (H = 25.153, p = 0.001 for plant; H = 24.677, p = 0.002 for *A. thalassinus*) had the highest significant differences among the sites, while Fe (H = 22.783, p = 0.004 for soil) and Cr (H= 19.691, p = 0.012 for plant; H = 17.010, p = 0.030 for *A. thalassinus*; H = 19.744, p = 0.011 for *C. compressicornis*; H = 20.179, p = 0.010 for *A. patruelis*) had the lowest significant differences among the sites.

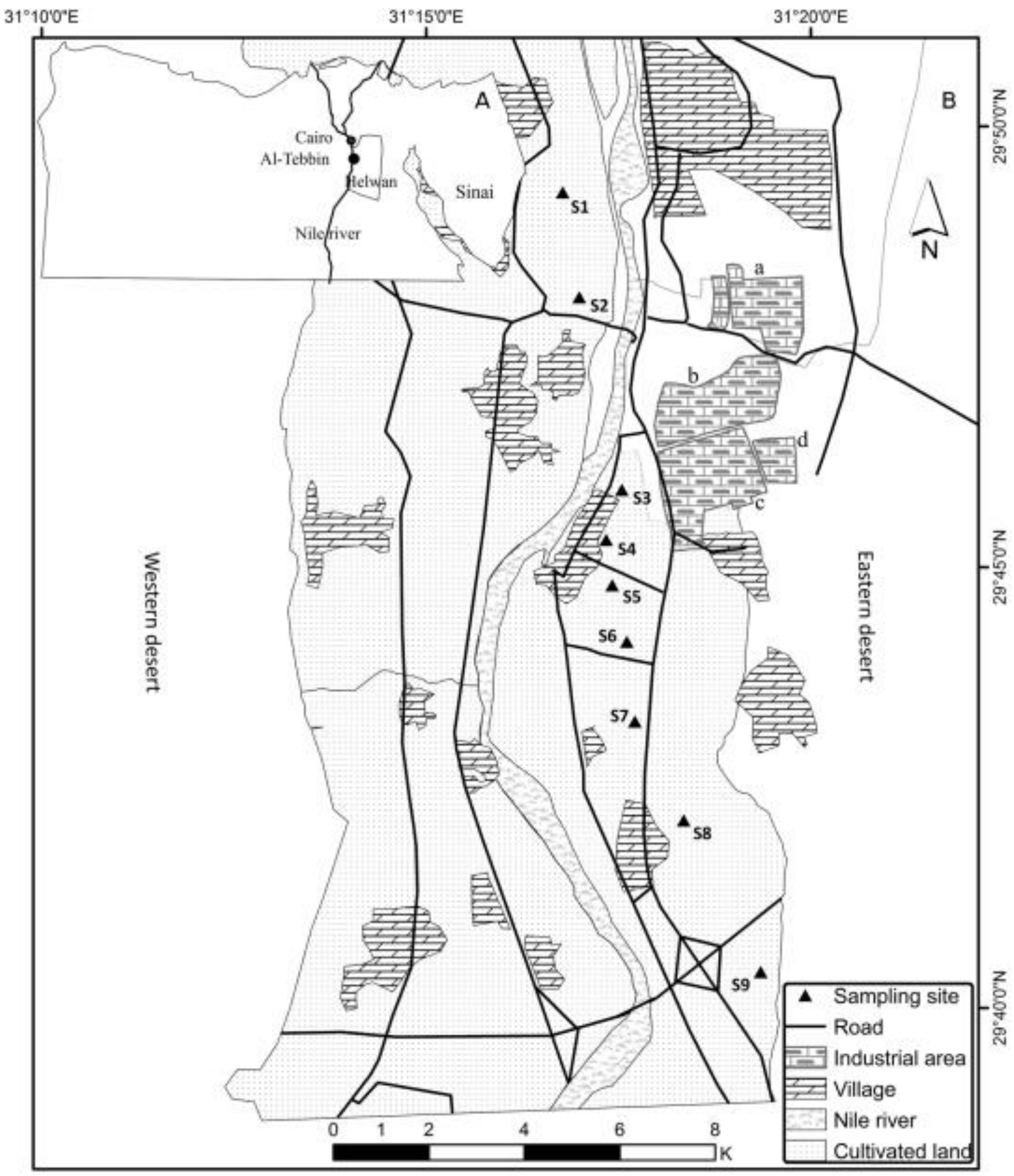


Fig. 1. A- Location of Al-Tebbin area southeast of Cairo. B- Location of industries and sampling sites within the Al-Tebbin area; (a) cement industry; (b) iron and steel industry; (c) coke and chemical industry; (d) metallurgical work.

The metal content in the soil, plants, and grasshoppers from sites S2, S3, S4, and S5 was higher than elsewhere, especially for S3, which was the site nearest the industrial area. In addition, Spearman's rank correlation analysis showed significant ($p < 0.05$) negative relationships between metal content in the soil, plants, and grasshoppers and the distance from the industrial area (Table 2) for Cd, Fe, Pb, Ni, and Zn in the soil and plants; Cd in the three grasshoppers; Pb in *A. thalassinus*; and Cu in *C. compressicornis*. The data showed that Cd, Fe, Pb, Ni, and Zn were the main heavy metal pollutants in this region, and, in general, the levels of heavy metals decreased with increasing distance from the pollution sources. This result is in agreement with HELIÖVAARA & VÄISÄNEN (1990), who found a continuous decrease in metal levels in the European pine sawfly adult, immature stages, larval food plants, and feces, with increasing distance from industrial plants. In our study, the relationships between distance from the industrial area and each of the heavy metals (except Cr) in the soils, plants, and grasshoppers were fit using a negative sigmoid model (Fig. 3); the metal concentrations decreased greatly with increasing distance until 3.0 km, when the curve began to flatten, with metal content stable after the distance exceeded 4.0 km. These results are similar to those of ZHANG *et al.* (2012), who found that the influence radius of zinc smelting on Cd and Pb contamination in soil, plants, and grasshoppers was in the range of 2.0–4.0 km. It should be noted that the predominant wind direction in our study area was northerly. This resulted in the contamination of the soil–plant–insect system in the area south of the industrial complex with excessive levels of heavy metals recorded in the soil: 67.5 mg/kg for Cd; 97.8 for Cr; 292 for Cu; 58900 for Fe; 160 for Pb; 80.0 for Ni; and 605 for Zn. S1 and S2, the areas mainly affected by the cement industry, showed relatively lower concentrations than S3, S4, and S5, which were the sites affected by the other emission sources. This may be because cement kiln dust does not contain high concentrations of heavy metals (ALI *et al.*, 1992). However, these areas were still enriched with relatively higher concentrations of toxic metals than those detected in the sites far from pollution sources. This might be explained by the southerly winds, which carried emissions from the metallurgical (iron and steel) and coke industries. When compared with the background levels of heavy metals in unpolluted soil in Egypt (ELSOKKARY & LAG, 1980; ELSOKKARY, 1996; ABOULROOS *et al.*, 1996; ABDEL-SABOUR & ABDEL-BASSET, 2002; ABDEL-SABOUR & ZOHNY, 2004), sites that were considered far away from industrial sources in our study were also found to be enriched with heavy metals. Our results indicated that this area is highly polluted by heavy metals, and the main source of pollution is the industrial complex.

In the present study, grasshoppers clearly showed site-dependent metal accumulation patterns for Cd, Cu, and Pb, and these results may support the thought that the body burden of heavy metals in animals reflects site pollution. Several studies have reported that there are physiological mechanisms that aid in the regulation of the essential elements in insect metabolism and prevent toxic levels (NEWMAN & UNGER, 2003; NFON *et al.*, 2009; BJERREGAARD *et al.*, 2014). This may explain the site-independent accumulation for Fe, Ni, and Zn in grasshoppers despite their significant inverse relation in soil and plants for the distances far from the industrial area. The soil Cd, Cu, and Pb concentrations showed significant positive correlations with those in plants and the herbivore insects, while the Ni was only correlated with *A. thalassinus* and *C. compressicornis*. Similarly, a strong relationship was also detected for metal concentrations in plants and grasshoppers, particularly for Cd, Pb, and Cu, which were significantly correlated with the three species of grasshoppers; in the case of Cr, Fe, Ni, and Zn, the relationship was only significant for one or two species (Table 3). The values for Zn in soil also showed significant relationships with plant Zn. The correlation analysis suggested that Cd, Cu, Pb, and Zn in the soil–plant–grasshopper system in Al-Tebbin District might originate from the same source; metal content in grasshoppers came mainly from their plant food.

Acridid grasshoppers are exposed to heavy metals via different paths, including from plants through their feeding habit and directly from the soil through oviposition behavior (Devkota & Schmidt, 2000). In the Biesbosch floodplains, Notten *et al.* (2005) suggested that metal transfer from plant leaves to the snail, *Cepaea nemoralis* (Linnaeus, 1758), is more important than is transfer from the soil. Likewise, in our study, strong relationships were found between the plants and grasshoppers for all metals, and this may confirm the expectation that food is the primary factor determining metal bioaccumulation in animals (Notten *et al.*, 2005; Chary *et al.*, 2008; Zhang *et al.*, 2012).

Three sites were selected for measuring total heavy metals in the mantid (S4, S5, and S8). Although Cd, Cu, Pb, and Zn content was comparatively higher in *M. paykullii* collected from S4 and S5 than in mantids collected from S8 (Fig. 2), metal levels were not significantly different at the different sites ($p > 0.05$, H-test). Apart from Cr and Ni, metal concentrations in soil, plants, grasshoppers, and mantids from all the sampling sites were in the following order: Fe > Zn > Cu > Pb > Cd (Table 1). Based on the average concentrations, there were no significant differences between grasshopper taxa in the concentrations of Cd, Pb, or Ni (Table 1). Cu and Zn concentrations were significantly lower, and the Cr concentration was significantly higher in *C. compressicornis* than in *A. thalassinus* and *A. patruelis*. The results also showed that significantly more Fe was accumulated in *A. thalassinus* than in the other two grasshoppers (Table 1).

Bioaccumulation of metals through the food chain. Table 4 shows the average and range of metal bioaccumulation factors for plants and arthropods. Significantly lower concentrations of heavy metals were noted for grasses than for soil (Table 1). The bioaccumulation factors for heavy metals in grasses were less than 1.0 (varied between 0.04 and 0.59), which indicated that metals did not accumulate in grasses. Metals that were present were in the following order: Cu > Zn > Cd > Pb ≈ Ni > Cr > Fe. From soil to plant leaves, the bioaccumulation factor was significantly higher for Cu and significantly lower for Fe than noted for the other heavy metals ($p < 0.05$, U-test). The bioaccumulation factors of the other metals (except Cr) were not significantly different ($p > 0.05$, U-test).

The bioaccumulation factors were higher for herbivorous insects than for plants. Although the trend of bioaccumulation factors for metals in each grasshopper was different, all three species had significantly higher Zn bioaccumulation factors (3.8, 2.7, and 3.4 for *A. thalassinus*, *C. compressicornis*, and *A. patruelis*, respectively) and lower Fe bioaccumulation factors (0.32, 0.19, and 0.20 for *A. thalassinus*, *C. compressicornis*, and *A. patruelis*, respectively). Cd, Cu, and Pb in the three grasshoppers were found in higher concentrations than in plants, and their bioaccumulation factors were > 1 and < 2. In the case of Cr, Fe, and Ni, the accumulation in grasshoppers never exceeded that of plants, and the bioaccumulation factors were < 1. Except for Cu, our results are broadly consistent with earlier reports on the European pine sawfly, *Neodiprion sertifer* Geoffroy, 1785, (Harjavalta, Finland) where Heliövaara & Väisänen (1990) found higher accumulation of Cd in *N. sertifer* than in pine needles, while the levels of Cu, Fe, and Ni were higher in plant food than in *N. sertifer*. Although Cd and Pb concentrations decreased along the soil–plant–grasshopper food chain in a study conducted by Zhang *et al.* (2014) in South China, their results also agreed with our data in the case of Cu and Zn. Our study showed that Cd, Cu, Pb, and Zn accumulated in the grasshoppers, with Zn having the highest bioaccumulation factor, while, Cr, Fe and Ni were not accumulated. The influence of homeostatic regulation on metal concentrations in organisms may explain the grasshoppers' low accumulation of Fe and Ni and the biomagnification of Cu and Zn. In general, uptake, accumulation, and excretion rates of these essential metals in terrestrial animals are regulated by metallothionein and metallothionein-like proteins (Newman & Unger, 2003; Nfon *et al.*, 2009; Bjerregaard *et al.*, 2014), and, based on the physiological requirements of these elements in the organisms, their bioaccumulation in the food chain is detected. Usually, naturally occurring concentrations of Ni seem to be sufficient to meet intake requirements because of their low physiological requirement (PHIPPS *et al.*, 2002). However, essential metals, such as Zn, are usually biomagnified up the food chain through invertebrates that are known to be efficient accumulators or that lack the necessary regulatory and detoxification mechanisms of higher-order animals (NFON *et al.*, 2009).

Of the three grasshoppers investigated, the highest metal concentrations were found in *A. thalassinus* and the lowest in *C. compressicornis* (Table 1), which suggested slightly larger metal absorption abilities for *A. thalassinus* than for *C. compressicornis* and *A. patruelis*. Even so, the highest accumulations of Ni and Cr were found in *A. patruelis* and *C. compressicornis*, respectively, and the lowest accumulation of Cr was found in *A. thalassinus*. Based on the aforementioned results, *A. thalassinus* may perform better as a test animal for the evaluation of metal pollution. In this sense, heavy metal accumulation in herbivorous insects may differ among metals and species. For example, in previous studies, Cd bioaccumulation factors for grasshoppers (DEVKOTA & SCHMIDT, 2000; ZHANG *et al.*, 2009, 2012) were more than 1.0 for all grasshoppers, but Pb accumulation factors were less than 1.0 for the same species; however, Pb concentration was 1.3 times higher in *Acrida chinensis* (Westwood, 1842) than in its food plant, *Echinochloa crusgall* (L.) P. Beauv (ZHANG *et al.*, 2009).

Praying mantids are predators feeding on other insects and arthropods (HURD, 1999), living solitary on trees, shrubs, and grasses. *M. paykullii*, the secondary consumer in the studied food chain, belongs to the group that prefers green grasses and small plants (SAWABY *et al.*, 2010), and thus, mainly feeds on *A. thalassinus*, *C. compressicornis*, and *A. patruelis*, the dominant grasshopper species in the study area. The metal concentrations were significantly higher in *M. paykullii* than in the three grasshoppers, particularly for Zn and Cu, which were high enough to exceed the concentrations of Zn and Cu in the soil (Table 1). Nonetheless, in *M. paykullii*, the bioaccumulation factors were comparatively lower in Zn and Cu than in the other elements. In the present work, Cd and Pb concentrations in mantid were, on average, 9.0 and 23.0 mg/kg, respectively. The concentrations for Cd and Pb were much lower in our mantid than in the mantids and spiders in a similar study from ZHANG *et al.* (2009). Cd, Cu, Pb, and Ni concentrations were much lower in the mantid than in other predators (waterstriders, antlions, ants, and dragonfly larvae) collected from sites close to an iron and steel factory in Koverhar, Hanko Peninsula, Finland (NUMMELIN *et al.*, 2007). In contrast, Fe concentrations were much higher in the other predators than in our mantid, while Zn concentrations were relatively the same between the two studies. Diverse biological factors and environmental conditions might contribute to these differences. Furthermore, organisms have various physiology and metabolic requirements, and consequently, methods for tolerating high concentrations of metals are different (PHIPPS *et al.*, 2002), encompassing avoidance of contaminated substrates, metabolic processes limiting metal uptake, and the uptake and compartmentalization of metals within the body (BENGTSSON & TRAVNIK, 1989; KABATA & PENDIAS, 2001).

The metal bioaccumulation factors of mantid differed among grasshopper species (Table 4), but as a whole (average), all metals had remarkably high accumulation in this predator: 5.0 for Cd; 4.6 for Cr; 4.1 for Fe; 3.5 for Pb; 2.3 for Ni; 2.2 for Zn; and 1.4 for Cu. Biomagnification was higher for Cd than for other metals, and thus biotransfer of Cd through the food chain, via the mantid, was probably more efficient for Cd than for the other metals. According to ROTH-HOLZAPFEL (1990), only Cd and Ni biomagnified along the food chain. In addition, HUNTER *et al.* (1987) stated that Cd and Cu were highly mobile in the invertebrate food web. Because of their related chemical properties, Cd can be accumulated by replacing Zn in the enzyme carbonic anhydrase, resulting in highly effective absorption of Cd into the bodies of animals (HOPKIN, 1989; ROTH-HOLZAPFEL, 1990; DEVKOTA & SCHMIDT, 2000).

Table 1. Mean heavy metal content in soil, plants, and arthropods near the industrial area in Al-Tebbin.

Metal	Soil	Plant	Grasshopper			*Miomantis*
			Aiolopus	*Calephorus*	*Acrotylus*	
Cd	16.2 ± 4.1a	3.5 ± 0.70b	5.4 ± 1.3b	5.0 ± 0.98b	4.4 ± 1.0b	9.0 ± 1.3a
	(1.9–67.5)	(0.34–10.8)	(0.47–19.6)	(0.40–14.1)	(0.28–14.3)	(5.4–14.0)
Pb	49.3 ± 9.6a	9.3 ± 1.6b	12.7 ± 1.7b	10.9 ± 2.2b	12.5 ± 3.0b	23.0 ± 5.1a
	(6.0–160.0)	(1.8–27.6)	(3.9–29.5)	(1.1–28.7)	(1.3–43.5)	(4.6–36.3)
Cu	104.8 ± 15.8ad	61.3 ± 9.2b	83.9 ± 7.1ad	62.4 ± 5.8bc	75.6 ± 7.2cd	106.4 ± 12.9a
	(29.0–292.0)	(22.1–160.4)	(33.6–146.0)	(29.3–112.7)	(30.0–142.0)	(63.0–137.0)
Zn	266.5 ± 36.1ac	96.2 ± 14.7b	288.5 ± 15.9a	153.6 ± 9.7c	230.4 ± 15.4a	463.7 ± 38.3d
	(98.0–605.0)	(20.0–256.0)	(136.7–410.0)	(106.0–263.2)	(123.0–389.0)	(326.2–556.0)
Ni	41.5 ± 4.7a	9.9 ± 1.6bd	5.2 ± 0.61bc	4.7 ± 0.74c	5.8 ± 0.97bc	14.8 ± 3.2d
	(12.0–80.0)	(2.7–23.4)	(1.0–8.3)	(1.3–12.7)	(0.92–17.0)	(4.8–23.6)
Cr	60.0 ± 5.5a	7.9 ± 1.3b	2.2 ± 0.30c	6.4 ± 4.2b	3.6 ± 0.58d	7.9 ± 1.6b
	(27.0–116.0)	(2.0–23.2)	(0.88–5.8)	(2.0–20.0)	(1.0–8.6)	(5.0–12.2)
Fe	29.9 ± 2.6a	1.3 ± 0.20b	0.32 ± 0.03c	0.15 ± 0.01d	0.16 ± 0.01d	0.40 ± 0.08e
	(11.5–58.9)	(0.35–3.5)	(0.14–0.63)	(0.07–0.23)	(0.09–0.27)	(0.11–0.66)

Data are presented as mean ± SE in mg/kg dry weight (mg/g for Fe) (range). Different letters within the same row denote significant differences (Mann–Whitney U test, $p < 0.05$).

Table 2. Spearman's rank correlation coefficients for comparisons between the distances from the industrial area and the concentration of each heavy metal in soil, plants, and grasshoppers.

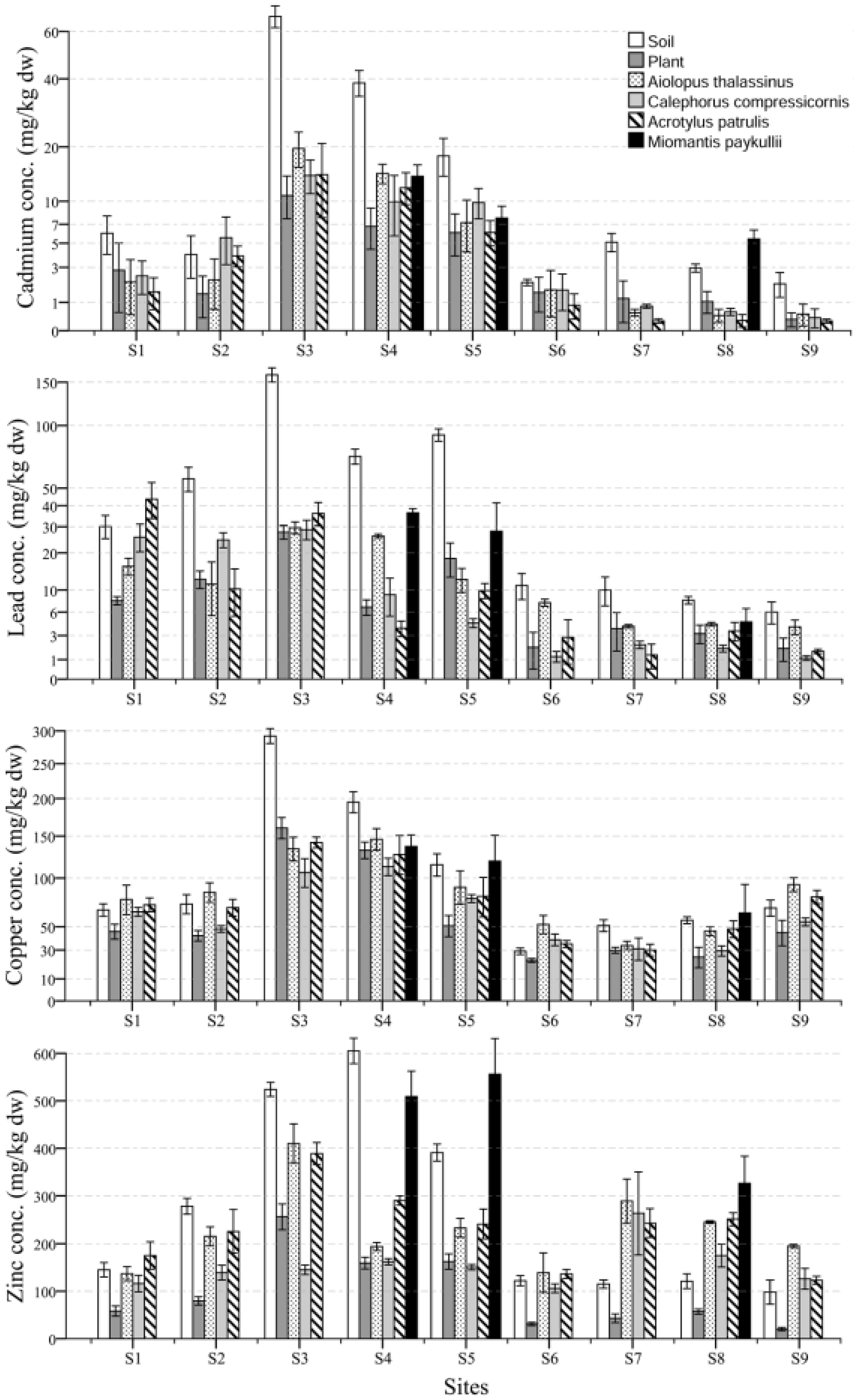
Taxon	Heavy metal						
	Cd	Pb	Cu	Zn	Ni	Cr	Fe
Soil	- 0.783*	- 0.933**	- 0.633	- 0.917**	- 0.433	- 0.159	- 0.767*
Plant	- 0.950**	- 0.700*	- 0.617	- 0.800**	- 0.667*	- 0.500	- 0.750*
Aiolopus	- 0.933**	- 0.867**	- 0.567	- 0.083	- 0.017	- 0.050	- 0.250
Calephorus	- 0.950**	- 0.617	- 0.717*	0.033	- 0.500	- 0.217	0.067
Acrotylus	- 0.933**	- 0.517	- 0.600	- 0.533	0.350	0.600	0.100

Statistically significant at $p < 0.05$ (*) or $p < 0.01$ (**).

Table 3. Spearman's rank correlation coefficients for comparisons between soil, plants, and grasshoppers for heavy metal concentrations.

Metal	Soil–Plant	Soil–Grasshopper			Plant–Grasshopper		
		Aiolopus	*Calephorus*	*Acrotylus*	*Aiolopus*	*Calephorus*	*Acrotylus*
Cd	0.883**	0.850**	0.883**	0.850**	0.933**	0.950**	0.933**
Pb	0.900**	0.900**	0.783*	0.700*	0.783*	0.883**	0.783*
Cu	0.917**	0.883**	0.850**	0.933**	0.833**	0.933**	0.933**
Zn	0.900**	0.000	0.050	0.583	0.333	0.250	0.700*
Ni	0.583	0.817**	0.733*	0.567	0.383	0.917**	0.233
Cr	0.602	0.502	0.368	0.377	0.683*	0.883**	0.150
Fe	0.367	- 0.100	- 0.350	- 0.150	0.667*	0.300	0.000

Statistically significant at $p < 0.05$ (*) or $p < 0.01$ (**).



Cadmium conc. (mg/kg dw)
Lead conc. (mg/kg dw)
Copper conc. (mg/kg dw)
Zinc conc. (mg/kg dw)
Soil
Plant
Aiolopus thalassinus
Calephorus compressicornis
Acrotylus patrulis
Miomantis paykullii
S1
S2
S3
S4
S5
S6
S7
S8
S9
Sites

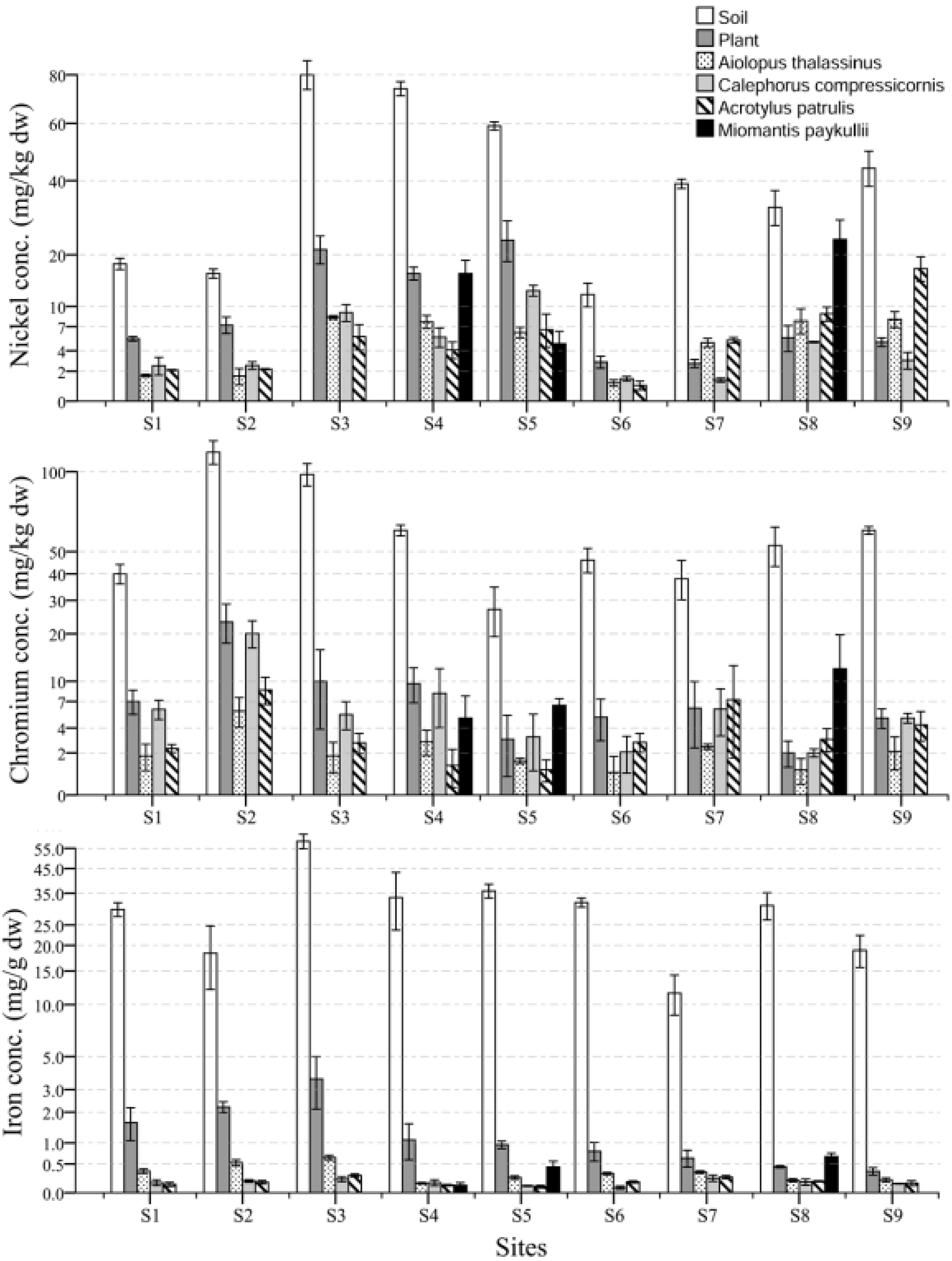


Fig. 2. Heavy metal concentrations in soil, plants, and arthropods at sampling sites near the industrial area in Al-Tebbin. The error bars indicate the standard error and each bar represents the mean of three replicates. (Miomantis was collected only from three sites: S4, S5, and S8).

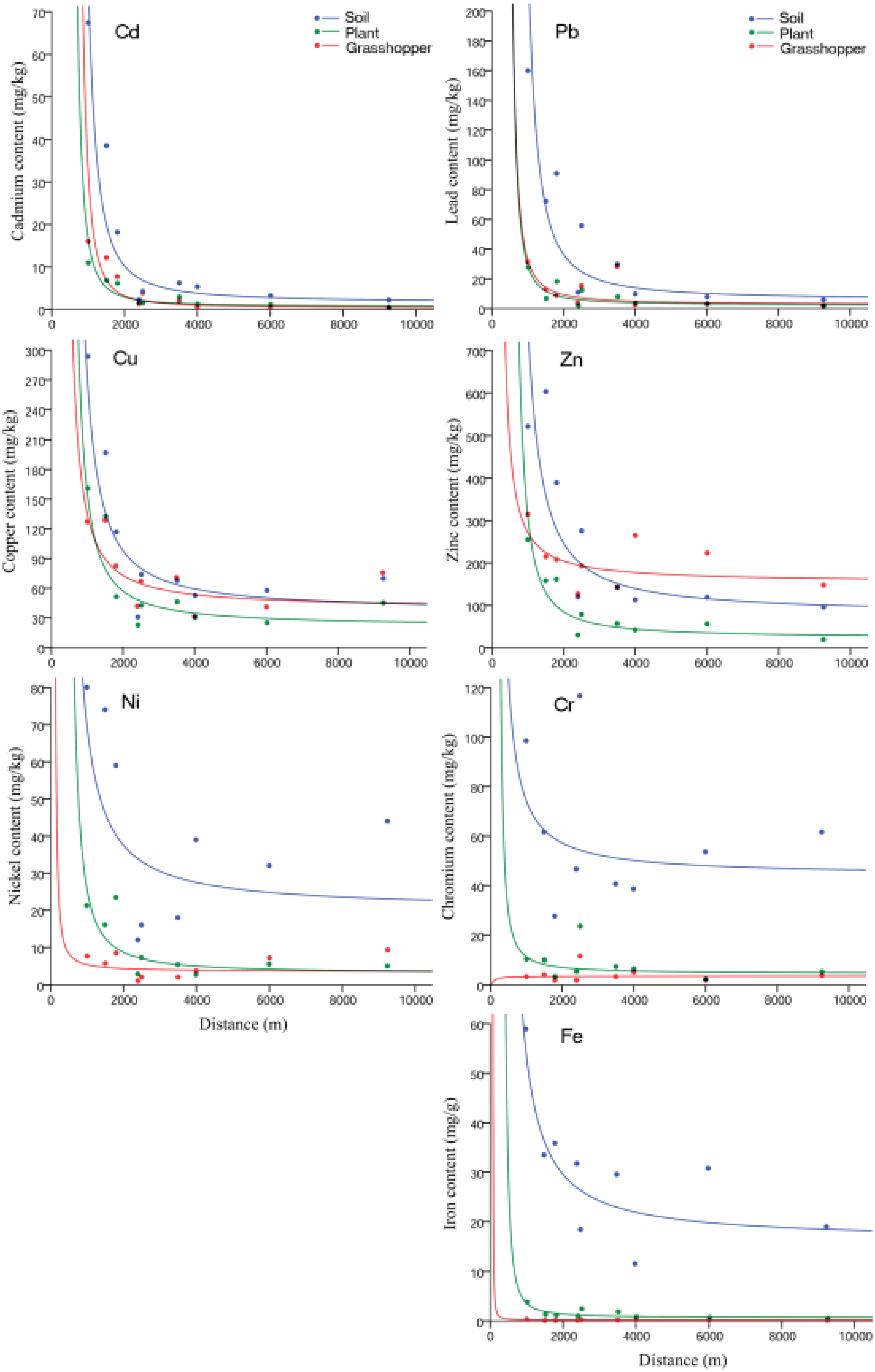


Fig. 3. Metal content in soil, plants, and grasshoppers against distance from the industrial area.

Table 4. Heavy metal bioaccumulation factors for plants, grasshoppers, and the mantid.

Metal	Plant/Soil	Grasshopper/Plant			Mantid/Grasshopper		
		Aiolopus	*Calephorus*	*Acrotylus*	*Aiolopus*	*Calephorus*	*Acrotylus*
Cd	0.33 ± 0.06$_{A}$	1.2 ± 0.19$_{AC}$	1.4 ± 0.33$_{AB}$	1.0 ± 0.27$_{AD}$	4.5 ± 3.4$_{A}$	3.7 ± 2.6$_{A}$	6.7 ± 5.4$_{A}$
	(0.16–0.72)	(0.46–2.1)	(0.59–3.9)	(0.25–2.7)	(0.96–11.4)	(0.79–8.8)	(1.1–17.6)
Pb	0.24 ± 0.03$_{A}$	1.9 ± 0.41$_{A}$	1.1 ± 0.31$_{AE}$	1.3 ± 0.52$_{AD}$	1.6 ± 0.35$_{A}$	4.3 ± 1.0$_{A}$	4.5 ± 2.5$_{A}$
	(0.09–0.40)	(0.68–3.9)	(0.24–3.2)	(0.35–5.5)	(1.0–2.2)	(2.5–6.3)	(1.3–9.5)
Cu	0.59 ± 0.03$_{B}$	1.6 ± 0.17$_{A}$	1.2 ± 0.11$_{AB}$	1.4 ± 0.12$_{D}$	1.2 ± 0.13$_{A}$	1.6 ± 0.27$_{A}$	1.3 ± 0.12$_{A}$
	(0.44–0.76)	(0.84–2.3)	(0.66–1.7)	(0.89–1.9)	(0.94–1.3)	(1.2–2.1)	(1.0–1.5)
Zn	0.35 ± 0.03$_{A}$	3.8 ± 0.94$_{B}$	2.7 ± 0.71$_{B}$	3.4 ± 0.58$_{B}$	2.1 ± 0.39$_{A}$	2.9 ± 0.54$_{A}$	1.8 ± 0.29$_{A}$
	(0.20–0.49)	(1.2–9.7)	(0.57–6.2)	(1.4–6.1)	(1.3–2.6)	(1.8–3.7)	(1.3–2.3)
Ni	0.24 ± 0.04$_{A}$	0.76 ± 0.21$_{CD}$	0.51 ± 0.05$_{C}$	0.97 ± 0.37$_{AD}$	1.9 ± 0.65$_{A}$	2.6 ± 1.2$_{A}$	2.4 ± 0.92$_{A}$
	(0.07–0.45)	(0.22–1.8)	(0.35–0.90)	(0.26–3.4)	(0.77–3.0)	(0.38–4.7)	(0.73–3.9)
Cr	0.12 ± 0.01$_{C}$	0.32 ± 0.04$_{D}$	0.83 ± 0.07$_{E}$	0.61 ± 0.15$_{A}$	6.1 ± 3.1$_{A}$	2.9 ± 1.6$_{A}$	4.8 ± 0.79$_{A}$
	(0.04–0.20)	(0.17–0.50)	(0.40–1.0)	(0.13–1.5)	(1.8–12.2)	(0.62–6.1)	(4.0–6.4)
Fe	0.04 ± 0.01$_{D}$	0.32 ± 0.05$_{D}$	0.19 ± 0.04$_{D}$	0.20 ± 0.05$_{C}$	3.0 ± 0.69$_{A}$	4.5 ± 1.2$_{A}$	4.8 ± 1.2$_{A}$
	(0.01–0.12)	(0.13–0.56)	(0.06–0.39)	(0.07–0.41)	(2.1–4.4)	(2.0–6.2)	(2.7–7.0)

Data are presented as the overall mean metal bioaccumulation factors in all sites ± SE (range). Means within each column followed by the same capital letter are not significantly different (Mann–Whitney U test, $p > 0.05$).

The results of this study indicate that the insectivorous *M. paykullii* can accumulate heavy metals in its body and can probably transfer them to higher trophic levels through the food web. On the whole, the bioaccumulation factors of the three trophic levels (primary producer, primary consumer, and secondary consumer) were: 0.33, 1.2, and 5.0 for Cd; 0.12, 0.58, and 4.6 for Cr; 0.59, 1.4, and 1.4 for Cu; 0.04, 0.23, and 4.1 for Fe; 0.24, 1.4, and 3.5 for Pb; 0.24, 0.74, and 2.3 for Ni; 0.35, 3.3, and 2.2 for Zn, respectively. The results showed that all heavy metals were biomagnified by the carnivorous insect when the terrestrial food chain extended to the secondary consumer. From the herbivorous grasshoppers to the predaceous mantid, average bioaccumulation factors for Cu remained constant, while values for Zn decreased. This was not surprising, as Cu and Zn are essential elements that are regulated by homeostasis, and are probably efficiently excreted by the mantid at the third trophic level.

Conclusions

In our study, different metals accumulated throughout the food chain, with differences between trophic levels. However, in general, the bioaccumulation factors of all metals increased along the food chain. Heavy metal concentrations did not increase from soil to grasses, but at the plant–primary consumer level, Cd, Cu, Pb, and Zn were biomagnified (i.e., metal concentrations were higher in the acridid grasshoppers than in the plants), and most metals were biomagnified with an increase in trophic level (i.e., secondary consumer). Thus, the present work provides some indications of the efficiency of the examined insect species in accumulating different trace elements, and provides additional evidence for metal biomagnification in terrestrial food chain, as has been previously reported (e.g., DEVKOTA & SCHMIDT, 2000; ZHENG *et al.*, 2008; ZHANG *et al.*, 2009; ZHANG *et al.*, 2014). In terms of this site-dependent accumulation of heavy metals and their biomagnification patterns, the investigated acridids can be considered bioindicators of Cd, Cu, Pb, and Zn pollution in polluted ecosystems such as Al-Tebbin region. Although the predator *M. paykullii* had higher levels for all metals than the herbivorous insects, its use as a bioindicator was not clear and needs more detailed research. Finally, we can conclude that the Al-Tebbin region has relatively high levels of potentially toxic metals in soils, plants, and arthropods. Therefore, we suggest that urgent action needs to be taken in order to control the anthropogenic impact.

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References

ABDEL-SABOUR M., N. ABDEL-BASSET. 2002. The affect of industrial activities on zinc in alluvial Egyptian soil determined using neutron activation analysis. - *Journal of Environmental Sciences*, 14(3): 330-332.

ABDEL-SABOUR M., E. ZOHNY. 2004. Impact of industrial activities on total chromium in alluvial Egyptian soils as determined by neutron activation analysis. - *Journal of radioanalytical and nuclear chemistry*, 260(1): 233-236. [DOI].

ABOULROOS S., S. HOLAH, S. BADAWY. 1996. Background levels of some heavy metals in soils and corn in Egypt. - *Egyptian Journal of Soil Science (Egypt)*, 43: 1-18.

AGARWAL S. 2009. *Heavy metal pollution*. New Delhi. APH publishing corporation.

ALI E., Y. IBRAHIM, M. NASRALLA. 1992. Contamination of the agricultural land due to industrial activities Southern of Greater Cairo. - *Journal of Environmental Science & Health Part A*, 27(5): 1293-1304. [DOI].

BENGTSSON G., L. TRANVIK. 1989. Critical metal concentrations for forest soil invertebrates. - *Water, Air, and Soil Pollution*, 47(3-4): 381-417. [DOI].

BEYERSMANN D., A. HARTWIG. 2008. Carcinogenic metal compounds: Recent insight into molecular and cellular mechanisms. - *Archives of Toxicology*, 82(8): 493-512. [DOI].

BJERREGAARD P., C. ANDERSEN, O. ANDERSEN. 2014. Ecotoxicology of metals-sources, transport, and effects on the ecosystem, -: *Handbook on the toxicology of metals fourth edition*. Elsevier Science.

BOSHOFF M., K. JORDAENS, S. BAGUET, L. BERVOETS. 2015. Trace metal transfer in a soil–plant–snail microcosm field experiment and biomarker responses in snails. - *Ecological Indicators*, 48: 636-648. [DOI].

CHAFFAI R., H. KOYAMA, 2011. Heavy metal tolerance in *Arabidopsis thaliana*. - *Advances in Botanical Research*, 60: 1-49. [DOI].

CHARY N., C. KAMALA, D. RAJ. 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. - *Ecotoxicology and Environmental Safety*, 69(3): 513-524. [DOI].

CONTI E., S. DATTILO, G. COSTA, C. PUGLISI. 2017. The ground beetle *Parallelomorphus laevigatus* is a potential indicator of trace metal contamination on the Eastern Coast of Sicily. - *Ecotoxicology and Environmental Safety*, 135: 183-190. [DOI].

CUI S., Q. ZHOU, L. CHAO. 2007. Potential hyperaccumulation of pb, zn, cu and cd in endurant plants distributed in an old smeltery, northeast China. - *Environmental Geology*, 51(6): 1043-1048. [DOI].

DAR M., I. GREEN, M. NAIKOO, F. KHAN, A. ANSARI, M. LONE. 2017. Assessment of biotransfer and bioaccumulation of cadmium, lead and zinc from fly ash amended soil in mustard–aphid–beetle food chain. - *Science of the Total Environment*. [DOI].

DEVKOTA B., G. SCHMIDT. 2000. Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. - *Agriculture Ecosystems & Environment*, 78(1): 85-91. [DOI].

DUDKA S., M. PIOTROWSKA, H. TERELAK. 1996. Transfer of cadmium, lead, and zinc from industrially contaminated soil to crop plants: A field study. - *Environmental Pollution*, 94(2): 181-188. [DOI].

ELSOKKARY I., J. LAG. 1980. Status of some trace elements in Egyptian soils and in wheat grains. - *Beitrage zur Tropischen Landwirtschaft und Veterinarmedizin (Germany DR)*. 18(1): 35-47.

ELSOKKARY I. 1996. Synopsis on contamination of the agricultural ecosystem by trace elements: An emerging environmental problem. - *Egyptian Journal of Soil Science (Egypt)*, 36(1-4): 1-22.

GALL J., R. BOYD, N. RAJAKARUNA. 2015. Transfer of heavy metals through terrestrial food webs: A review. - *Environmental Monitoring and Assessment*, 187(4): 1-21. [DOI].

HECKEL P., T. KEENER. 2007. Sex differences noted in mercury bioaccumulation in *Magicicada cassini*. - *Chemosphere*, 69(1): 79-81. [DOI].

HELIÖVAARA K., R. VÄISÄNEN. 1990. Concentrations of heavy metals in the food, faeces, adults, and empty cocoons of *Neodiprion sertifer* (Hymenoptera, Diprionidae). - *Bulletin of Environmental

Contamination and Toxicology, 45(1): 13-18. [DOI].

HOPKIN S. 1989. *Ecophysiology of metals in terrestrial invertebrates*. Elsevier Applied Science Publishers.

HSU M., K. SELVARAJ, G. AGORAMOORTHY. 2006. Taiwan's industrial heavy metal pollution threatens terrestrial biota. - *Environmental Pollution*, 143(2): 327-334. [DOI].

HUNTER B., L. HUNTER, M. JOHNSON, D. THOMPSON. 1987. Dynamics of metal accumulation in the grasshopper *Chorthippus brunneus* in contaminated grasslands. - *Archives of Environmental Contamination and Toxicology*, 16(6): 711-716. [DOI].

HURD L. 1999. Ecology of praying mantids, - In: Prete F. R., Wells H., Wells P. H. and Hurd L. E. (Eds.): *The praying mantids.* Johns Hopkins University Press, pp. 43–49.

JUNG M., J. LEE. 2012. Bioaccumulation of heavy metals in the wolf spider, *Pardosa astrigera* L. Koch (Araneae: Lycosidae). - *Environmental Monitoring and Assessment*, 184(3): 1773-1779. [DOI].

KABATA A., H. PENDIAS. 2001. Trace elements in soils and plants. - *New York. CRC.*

KARADJOVA I., E. MARKOVA. 2009. Metal accumulation in insects (Orthoptera, Acrididae) near a copper smelter and copper-flotation factory (Pirdop, Bulgaria). - *Biotechnology & Biotechnological Equipment*, 23(sup1): 204-207. [DOI].

LASKOWSKI R. 1991. Are the top carnivores endangered by heavy metal biomagnification? - *Oikos*: 387-390. [DOI].

MALIZIA D., A. GIULIANO, G. ORTAGGI, A. MASOTTI. 2012. Common plants as alternative analytical tools to monitor heavy metals in soil. - *Chemistry Central Journal*, 6(S2): S6. [DOI].

MARSCHNER H. 2012. *Marschner's mineral nutrition of higher plants*. London. Academic press.

MOHAMED R., K. ALLAM, A. FARAG, S. EL HAMAMY, N. MAHMOUD. 2011. Assessment of environmental impact of some industries in Helwan. - *Journal of Nuclear and Radiation Physics*, 6(1&2): 13-29.

NATH S., H. ANAND, P. HALDAR. 2011. Study of short horned grasshoppers in relation to heavy metals accumulation. - *Journal of Entomological Research*, 35(3): 291-293.

NEWMAN M., M. UNGER. 2003. *Fundamentals of ecotoxicology*, 2nd ed. London. Taylor & Francis Ltd.

NFON E., I. COUSINS, O. JÄRVINEN, A. MUKHERJEE, M. VERTA, D. BROMAN. 2009. Trophodynamics of mercury and other trace elements in a pelagic food chain from the Baltic Sea. - *Science of the Total Environment*, 407(24): 6267-6274. [DOI].

NICA D., M. BURA, I. GERGEN, M. HARMANESCU, D. BORDEAN. 2012. Bioaccumulative and conchological assessment of heavy metal transfer in a soil-plant-snail food chain. - *Chemistry Central Journal*, 6(1): 55. [DOI].

NORDBERG G., B. FOWLER, M. NORDBERG. 2014. *Handbook on the toxicology of metals*. Academic Press.

NOTTEN M., A. OOSTHOEK, J. ROZEMA, R. AERTS. 2005. Heavy metal concentrations in a soil-plant-snail food chain along a terrestrial soil pollution gradient. - *Environmental Pollution*, 138(1): 178-190. [DOI].

NUMMELIN M., M. LODENIUS, E. TULISALO, H. HIRVONEN, T. ALANKO. 2007. Predatory insects as bioindicators of heavy metal pollution. - *Environmental Pollution*, 145(1): 339-347. [DOI].

OPALUWA O., M. AREMU, L. OGBO, K. ABIOLA, I. ODIBA, M. ABUBAKAR, N. NWEZE. 2012. Heavy metal concentrations in soils, plant leaves and crops grown around dump sites in Lafia Metropolis, Nasarawa State, Nigeria. - *Advances in Applied Science Research*, 3(2): 780-784.

PHIPPS T., S. TANK, J. WIRTZ, L. BREWER, A. COYNER, L. ORTEGO, A. FAIRBROTHER. 2002. Essentiality of nickel and homeostatic mechanisms for its regulation in terrestrial organisms. - *Environmental Reviews*, 10(4): 209-261. [DOI].

RAKIB M., M. ALI, M. AKTER, M. BHUIYAN. 2014. Assessment of heavy metal (Pb, Zn, Cr and Cu) content in roadside dust of Dhaka Metropolitan City, Bangladesh. – *International Research Journal of Environment Sciences*, 3: 1-5.

ROSS H. 1987. Trace metals in precipitation in Sweden. - *Water, Air, and Soil Pollution*, 36(3-4): 349-363. [DOI].

ROSS S. 1994. *Toxic metals in soil-plant systems*. John Wiley & Sons Ltd

ROTH-HOLZAPFEL M. 1990. Multi-element analysis of invertebrate animals in a forest ecosystem (Picea abies. L.). - In: Lieth H., Markert B. (Eds.): *Element concentration cadasters in ecosystems: Methods and assessment and evaluation*. VCH verlagsgesellschaft. Weinheim.

SAEKI K., M. OKAZAKI, S. MATSUMOTO. 1993. The chemical phase changes in heavy metals with drying and oxidation of the lake sediments. - *Water Research*, 27(7): 1243-1251. [DOI].

SAWABY R., H. EL-HAMOULY, M. NASSER. 2010. Pilot study of population density and biodiversity index of Mantodea fauna in El-Fayoum governorate-Egypt. - *Egyptian Academic Journal of Biological Sciences*, 3(2): 19 - 26.

SIMON E., S. HARANGI, E. BARANYAI, M. BRAUN, I. FÁBIÁN, S. MIZSER, L. NAGY, B. TÓTHMÉRÉSZ. 2016. Distribution of toxic elements between biotic and abiotic components of terrestrial ecosystem along an urbanization gradient: Soil, leaf litter and ground beetles. - *Ecological Indicators*, 60: 258-264. [DOI].

SOFIANSKA E., K. MICHAILIDIS, V. MLADENOVA, A. FILIPPIDIS. 2013. Multivariate statistical and GIS-based approach to identify heavy metal sources in soils of the Drama plain, Northern Greece. *Proceedings of the Bulgarian Geological Society National Conference with International Participation Geosciences*, pp. 131-132.

SOLIMAN M., A. HAGGAG, M. EL-SHAZLY. 2017. Assessment of grasshopper diversity along a pollution gradient in the Al-Tebbin region, South Cairo, Egypt. - *Journal of Entomology and Zoology Studies*, 5: 298-306. [DOI].

SPSS STATISTICS. 2008. SPSS statistics software for windows, release 17.0. SPSS Statistics, Chicago, IL.

ZHANG C., N. SONG, G. ZENG, M. JIANG, J. ZHANG, X. HU, A. CHEN, J. ZHEN. 2014. Bioaccumulation of Zinc, Lead, Copper, and Cadmium from contaminated sediments by native plant species and Acrida cinerea in South China. - *Environmental Monitoring and Assessment*, 186(3): 1735-1745. [DOI].

ZHANG Z., X. SONG, Q. WANG, X. LU. 2012. Cd and Pb contents in soil, plants, and grasshoppers along a pollution gradient in Huludao City, Northeast China. - *Biological Trace Element Research*, 145(3): 403-410.

ZHANG Z., Q. WANG, D. ZHENG, N. ZHENG, X. LU. 2010. Mercury distribution and bioaccumulation up the soil-plant-grasshopper-spider food chain in Huludao City, China. - *Journal of Environmental Sciences*, 22(8): 1179-1183. [DOI].

ZHANG Z., X. LU, Q. WANG, D. ZHENG. 2009. Mercury, cadmium and lead biogeochemistry in the soil–plant–insect system in Huludao City. - *Bulletin of Environmental Contamination and Toxicology*, 83(2): 255-259. [DOI].

ZHENG D., Q. WANG, Z. ZHANG, N. ZHENG, X. ZHANG. 2008. Bioaccumulation of total and methyl mercury by arthropods. - *Bulletin of Environmental Contamination and Toxicology*, 81(1): 95-100. [DOI].

ZHUANG P., Z. HUILING, S. WENSHENG. 2009. Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: Field study. - *Journal of Environmental Sciences*, 21(6): 849-853. [DOI].

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New Highest Altitudes for Some Ground Beetles (Coleoptera: Carabidae) From the Western Rhodopes Mts. (Bulgaria)

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Abstract. As result of a monitoring survey in the Western Rhodopes Mts. were established nine representatives of the Bulgarian carabid fauna, for which there are no currently published data demonstrating their presence at such a high altitude. These are *Abax carinatus*, *Asaphidion flavipes*, *Bembidion stephensii*, *Harpalus attenuatus*, *Harpalus pumilus*, *Microlestes apterus*, *Paradromius linearis*, *Poecilus cupreus* and *Pterostichus leonisi*. Five of them were collected on the territory of Grashtitsa Village, another three – near Krastava Village, and one species was found in both localities (both representing new highest altitude records). The establishment of these new altitudes warns about the movement of the species in height. Given the role of the ground beetles as bioindicators, and on the background of the worldwide environmental trends, we might conclude that this fact is a result of the global climate changes, combined with the presence of some anthropogenic load in the studied region.

Key words: Carabidae, anthropogenic load, bioindication, areal changes.

Introduction

Global or local climatic changes are frequently mentioned as one of the reasons for shifts in geographic ranges of the species. However, anthropogenic factors (i.g. pollution, intensification of agriculture, changes in land use, etc.) also took place. There is a general problem of separating climatic effects from human effects in interpreting biological patterns (ALEKSANDROWICZ, 2011).

The droughting, as result of climatic and anthropogenic changes, has led to serious detrimental changes in the faunistic complexes with possible unforeseen alterations and trends in the future. A key characteristic is the expansion of the northern limits of distribution of some historically southern species (ALEKSANDROWICZ, 2011; OHLEMÜLLER *et al.*, 2006).

The effects of the global climate changes, along with the pollution of the environment, are registered on the territory of Bulgaria too. The country falls within the area of droughting. The total amount of the precipitation and river runoff are decreasing with characteristic minima (droughts) in 4 – 5 years. The areas occupied by more xerophytic plant species are increasing. The state of the ecosystems consisting of cold resistant and hygrophilous forest species is deteriorating. Further warming and droughting of the climate would have an extremely adverse impact on the species and habitat diversity in Bulgaria (MOEW, 2005; RAEV & TINCEV, 2015).

Drought and even desertification processes are most intensively occurring in the regions where the effect of the anthropogenic impacts supplements the natural arid conditions (BRAGINA, 2004). Such impacts are the massive plowing of virgin lands (especially around the middle of the 20th century), the regulation of watercourses and the distortion

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of water balance in the territory, and the surge of the anthropogenic press with the influx of population and unregulated tourism.

Ground beetles (Coleoptera: Carabidae) are one of the best studied groups of invertebrates with regards to their taxonomy and phylogeny, geographical distribution, habitat preferences, ecological requirements and adaptations. Epigean and hypogean carabid beetles are proved to be excellent and „multitask" indicators of climate change. Moreover, their response time to climate changes seems to be shorter than for plants (BRANDMAYR & PIZZOLOTTO, 2016). Given the fact that the water in the soil is the ecological factor, which has the greatest influence on the ground beetle fauna (EYRE & LUFF, 1990), it could be expected that the drought processes will affect especially adversely the distribution of the carabids.

Western Rhodopes Mts. are very interesting from a research point of view. Thanks to the specific climate many typical habitats have being preserved, as well as numerous glacial relicts and endemic species. The region is well studied in terms of the carabid fauna, although the last purposive and thorough study was conducted before more than 10 years (GUÉORGUIEV & LOBO, 2006). The establishment of new records about the altitudinal ranges of the species may be resulting from insufficient exploration, but given the long-term research interest to the mountain and the availability of relatively rich information about the ecology of these species, it can be expected that these new findings derived from the expansion of their range in altitudinal direction.

Materials and Methods

The material was collected during a monitoring survey in the Western Rhodopes Mts.

Almost entire territory, with the exception of the most southern and southwestern parts, fall within the Transitional climate zone. The climate is mountainous version of the transitional one, with average annual temperatures between 10°C and 5°C, which decrease with increasing of the altitude. The Rhodopes Mts. are one of the faunistically richest areas in Europe.

The ground beetles were collected in 2015 and 2016 – 2017, respectively, in the vicinity of the village of Krastava (1209 m; 41°56'25"N, 23°51'49"E), and in the Grashtitsa hamlet, in the land of the village of Stoykite (1340 m a.s.l.; 41°39'05"N, 24°37'04"E). Pitfall traps were used, respectively, with salt-vinegar saturated solution and with formaldehyde.

The material is deposited in the IBER – Bulgarian Academy of Sciences, Sofia.

Results

During the whole study a total of 97 ground beetle species was found, of which 58 in the area of Krastava and 61 in Grashtitsa. As result of the investigation were established nine representatives of the Bulgarian carabid fauna, for which there are no currently published data demonstrating their presence at such a high altitude. Five of them were collected on the territory of Grashtitsa, another three – near Krastava, and one species was found in both localities (both representing new highest altitude records). These new findings represent almost 10% of all established during the study species.

The establishment of these species for the first time on such a height is possible to be due to inadequate studies in the area, but given the long-standing interest of many researchers to the Rhodopes Mts., it could be concluded that these species have changed their area. These were:

Asaphidion flavipes (Linnaeus, 1761)

The highest known so far in Bulgaria locality is at about 1200 m a.s.l. in the region of the town of Trigrad (also in the Western Rhodopes Mts.).

Range type: Western Palearctic.

Biotopic preferences: Mesohygrophilous. Inhabits the clayey banks of slowly flowing or small standing water reservoirs, swamps and marshes. Also in humid meadows and swampy forests.

Material examined: Grashtitsa (1♀, 1♂).

Bembidion (Peryphanes) stephensii stephensii Crotch, 1866

The highest known so far in Bulgaria locality is at about 1150 m a.s.l. The highest known so far locality in the Western Rhodopes

Mts. is at 1050 m a.s.l. in the region of the town of Batak.

Range type: European.

Biotopic preferences: Mesohygrophilous. Mostly found on river and lake shores, often on cliffs.

Material examined: Krastava (1♀, 1♂); Grashtitsa (3♀♀).

Poecilus (Poecilus) cupreus cupreus (Linnaeus, 1758)

The highest known so far in Bulgaria locality is at about 1300 m a.s.l. The highest known so far locality in the Western Rhodopes Mts. is in the regions of the towns of Trigrad and Sarnitsa, both at about 1200 m a.s.l.

Range type: Euroasiatic (steppe).

Biotopic preferences: Mesophilous. Inhabits open areas with dense grass vegetation, agrocoenoses and highly anthropogenic areas. Rarely in forests. Common.

Material examined: Grashtitsa (29♀♀, 30♂♂).

Pterostichus (Argutor) leonisi Apfelbeck, 1904

So far known in Bulgaria up to approximately 300 m a.s.l. For the Rhodopes Mts. there are only unconfirmed sources (see Guéorguiev & Lobo, 2006).

Range type: Central and Eastern European.

Biotopic preferences: Hygrophilous. Found in very moist, seasonally flooded and swampy forests, as well as on the banks of rivers and lakes.

Material examined: Krastava (1♀).

Abax (Abacopercus) carinatus carinatus Duftschmid, 1812

The highest known so far in Bulgaria locality is at about 1300 m a.s.l. The highest known so far locality in the Western Rhodopes Mts. is at 1185 m a.s.l. in the region of Barutin.

Range type: Central and Eastern European.

Biotopic preferences: Typical mesophilous species. Inhabits a variety of forest ecosystems. Common.

Material examined: Grashtitsa (1♀).

Harpalus (Harpalus) attenuatus Stephens, 1828

The highest known so far in Bulgaria locality is at 1180 m a.s.l. at Magareshki Dol River near Borino Village (also in the Western Rhodopes Mts.).

Range type: European-Neareastern-Mediterranean.

Biotopic preferences: Mesophilous. Inhabits mainly mesoxerophytic forest communities on sandy soils.

Material examined: Krastava (1♂).

Harpalus (Harpalus) pumilus Sturm, 1818

The highest known so far in Bulgaria locality is at about 1000 m a.s.l. The highest known so far locality in the Western Rhodopes Mts. is at 540 m a.s.l. in the region of Ognyanovo Village.

Range type: European and Central Asian.

Biotopic preferences: Xerophilous. Inhabits open biotopes with sandy soils, including agrocoenoses.

Material examined: Krastava (1♀, 1♂).

Paradromius (Manodromius) linearis (Olivier, 1795)

The highest known so far in Bulgaria locality is at about 1000 m a.s.l. The highest known so far locality in the Western Rhodopes Mts. is at about 300 m a.s.l. in the region of Asenovgrad Town.

Range type: Western Palearctic.

Biotopic preferences: Mesohygrophilous. Found in moist meadows, swamps, marshes, as well as on the banks of rivers and lakes.

Material examined: Grashtitsa (1♂).

Microlestes apterus Holdhaus, 1912

So far known in Bulgaria up to approximately 300 m a.s.l. This species has never been established in the Rhodopes Mts. so far.

Range type: Balkan subendemic.

Biotopic preferences: Mesoxerophilous. Inhabits open biotopes, mostly dry meadows.

Material examined: Grashtitsa (1♀, 2♂).

Discussion

A wide variety of vertebrate and invertebrate species has moved northwards and uphill in response to the global warming. These changes have already been documented across Eurasia (Dudko & Ivanov, 2006; Ohlemüller *et al.*, 2006; Bespalov *et al.*, 2010; Aleksandrowicz, 2011; Teofilova *et al.*, 2015).

Hygrophilous carabid beetles with smaller body sizes, as *Asaphidion flavipes*, *Bembidion stephensii*, *Paradromius linearis* and *Pterostichus leonisi* are proved to be more vulnerable (NIEMELÄ *et al.*, 2002). Therefore, the change in their habitats towards lower humidity levels could cause alterations in their geographical ranges. Probably the change in the areal namely of these species should be considered as the most emphatic signal for a change in the environmental conditions in their habitats. It is likely that the new environmental settings have proved to be too unfavourable for these sensitive and generally stenotopic species, and they migrated following their primary mesohygrophily.

The typical forest species like *Abax carinatus* are highly vulnerable to the change and degradation of the forest areas. Many studies show the displacement of the autochthonous European nemoral complex by the more plastic species of steppe origin (DESENDER & TURIN, 1989; KODZHABASHEV & PENEV, 2006; ALEKSANDROWICZ, 2011; TEOFILOVA *et al.*, 2015). Of particular importance is that the distortions among the species associated with mature and old forests is possible to detect correctly almost five years after the intervention in the forests, as the changes in populations occur in about 2 – 4 years (JACOBS *et al.*, 2008). Therefore, the recommendations for forest management, aimed at the conservation of biodiversity, but based on short-term studies, in the best case would be incomplete, and at worst would have been catastrophic for the maintenance of the species in the managed landscapes.

Usually the studied region falls into forest zone E, according to De Martonne aridity index (RAEV & TINCEV, 2015), characterised by low vulnerability level with optimal moisture conditions. The unsustainable use of the forest resources, however, is a fact in many places in the Western Rhodopes Mts. There has been a change in the age structure, canopy, hidrotermic conditions and, in some places, also in the species composition of the forests, as a result of conducted clear-cuttings and subsequent successional changes. It is possible that these processes have influenced the distribution of the typical forest mesophile *Abax carinatus*. Forest specialists do not develop well in areas affected even by moderate levels of anthropogenization. Usually in urbanized areas generalists or species of open habitats displace them (VENN *et al.*, 2003; MAGURA *et al.*, 2008; BARANOVÁ *et al.*, 2013).

Species from the European-Asian (steppe) complex, as *Poecilus cupreus*, often indicate for the presence of natural and semi-natural steppes and very often constitute an essential element of the fauna of the arable lands, as well as lands recently deprived of the autochthonous ligneous vegetation. At these newly created habitats and at the early successional stages dominate species able to fly and to adapt to a wide range of environmental conditions (BRANDLE *et al.*, 2000). These are mostly eurytopic species. In this case as eurybiont of the open areas can also be noted *Harpalus pumilus*.

Rare species require more time for colonization than common species, because the number of dispersing individuals is smaller (VERHAGEN *et al.*, 2008). With the raise of the anthropogenic load the proportion of open living forms and ecologically plastic eurybionts increases (NIEMELÄ *et al.*, 2002; GORGIEVSKA *et al.*, 2009, etc.). To some extent, the same is true of some representatives of the Mediterranean complex, as is the *Harpalus attenuatus*.

The most vulnerable species are those with small geographical ranges (HUGHES *et al.*, 1997). Thus, the change in the habitats leads to further pressure of the rare, priority species, at the expense of those with wide distribution. Species with limited distribution in this case appear to be *Pterostichus leonisi*, *Abax carinatus* and the Balkan subendemic *Microlestes apterus*.

The nine mentioned ground beetle species differ in terms of zoogeography and habitat preferences, which emphasizes the complexity of the problem with the shifting of geographical ranges.

Over the past decades intensified processes of secondary xerophytization have been seen, a possible consequence of global climate changes and destruction of the natural ligneous vegetation with changes in its species and age structure. This successional degradation is strongly reflecting on the contemporary state of the fauna, which manifests through species impoverishment, severe dystrophy of the zoocoenoses and substitution of natural communities with ecologically plastic, invasive elements.

In such conditions, species try to escape these new severe environmental situations, and in many cases, they leave or change their present areals moving northwards or higher in the mountains. It is proved that populations shift elevation ranges to track suitable climate rather than adapting to novel conditions (HUA & WIENS, 2013). Although scarce, there are examples for this in some studies from Bulgaria concerning the carabid fauna (KODZHABASHEV & PENEV, 2006; TEOFILOVA *et al*., 2015; JOCQUE *et al*., 2016) or other groups of animals (e.g. Lyubomirova, 2012 – pers. com.). There are also evidences for the "Mediterranization" of the Heteroptera fauna of Austria (RABITSCH, 2008). In most cases, however, similar signs for areal changes remain unnoticed by the authors.

The establishment of new altitudes for these nine carabid species warns about the movement of the species in height. Knowing that carabids signal effects caused by climate change (ASHWORTH, 1996; BRANDMAYR & PIZZOLOTTO, 2016), and on the background of the worldwide environmental trends, it can be concluded that this fact is a result of the global climate changes. It also proves the presence of some anthropogenic load in the studied region.

Forest ecosystems in Bulgaria currently are subjected to increasing negative climatic influence (RAEV & TINCEV, 2015). If the observed tendency for the moving of the species in altitude is a short-term phenomenon or it comes to the general trend linked to the multiannual climate change? Future detailed studies in the area could bring more clarity and perhaps hope.

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References

ALEKSANDROWICZ O. 2011. Recent records of steppe species in Belarus, first indications of a steppe species invasion? - In: Kotze D.J., T. Assmann, J. Noordijk, H. Turin, R. Vermeulen (Eds.): *Carabid Beetles as Bioindicators: Biogeographical, Ecological and Environmental Studies*. Pensoft. *ZooKeys*, 100: 475-485. [DOI]

ASHWORTH A.C. 1996. The response of arctic Carabidae (Coleoptera) to climate change based on the fossil record of the Quaternary Period. - *Annales Zoologici Fennici*, 33: 125-131.

BARANOVÁ B., D. FAZEKAŠOVÁ, T. JÁSZAY, P. MANKO. 2013. Ground beetle (Coleoptera: Carabidae) community of arable land with different crops. - *Folia Faunistica Slovaca*, 18(1): 21-29.

BESPALOV A.N., R.YU. DUDKO, I.I. LYUBECHANSKII. 2010. Additions to the ground beetle fauna (Coleoptera, Carabidae) of the Novosibirsk Oblast: do the southern species spread to the north? - *Euroasian Entomological Journal*, 9(4): 625-628.

BRAGINA T.M. 2004. [*Regularities of Soil Animal Population Changes in Desertification: The Case of Arid Steppe Zone in Central Asia*]. Dissertation, Moscow, 290 p. (In Russian)

BRÄNDLE M., W. DURKA, M. ATLMOOS. 2000. Diversity of surface dwelling beetle assemblages in open-cast lignite mines in Central Germany. - *Biodiversity and Conservation*, 9: 1297-1311.

BRANDMAYR P., R. PIZZOLOTTO. 2016. Climate change and its impact on epigean and hypogean carabid beetles. - *Periodicum Biologorum*, 118(3): 147-162. [DOI]

DESENDER K., H. TURIN. 1989. Loss of habitats and changes in the composition of the ground and tiger beetle fauna in four West European countries since 1950 (Coleoptera: Carabidae, Cicindelidae). - *Biological Conservation*, 48(4): 277-294. [DOI]

DUDKO R.YU., E.A. IVANOV. 2006. [New findings of carabid beetles (Coleoptera, Carabidae) in Novosibirsk Oblast]. - In: *Entomological Research in Western Siberia*. Proceedings of the Kemerovo branch of the Russian Entomological Society, vol. 4, Kemerovo, pp. 15-18. (In Russian)

EYRE M.D., M.L. LUFF, 1990. A preliminary classification of European grassland habitats using carabid beetles. - In: Stork N.E. (Ed.): *The Role of Ground Beetles in Ecological and environmental Studies*. Andover, UK. Intercept, pp. 227-236.

GORGIEVSKA C.A., D. PRELIK, S. HRISTOVSKI, B. GEORGIEV. 2009. Comparative analysis of structural characteristics of ground beetles community (Carabidae: Coleoptera) along an urban-rural gradient in Skopje city and its surrounding. - *Ekologija i Zaštita na Životnata Sredina*, 12(1/2): 31-44.

GUÉORGUIEV B.V., J.M. LOBO. 2006. Adephagous beetles (Insecta: Coleoptera: Adephaga) in the Western Rhodopes (Bulgaria and Greece). - In: Beron P. (Ed.): *Biodiversity of Bulgaria. 3. Biodiversity of Western Rhodopes (Bulgaria and Greece) I*. Sofia. Pensoft & National Museum of Natural History, pp. 283-346.

HUA X., J.J. WIENS. 2013. How Does Climate Influence Speciation? - *The American Naturalist*, 182(1): 1-12. [DOI]

HUGHES J.B., G.C. DAILY, P.R. EHRLICH. 1997. Population diversity: Its extent and extinction. - *Science*, 278: 689-692. [DOI]

JACOBS J.M., T.T. WORK, J.R. SPENCE. 2008. Influences of succession and harvest intensity on ground beetle (Coleoptera, Carabidae) populations in the boreal mixed-wood forests of Alberta, Canada: species matter. - In: Penev L., T. Erwin, T. Assmann (Eds.): *Back to the Roots and Back to the Future. Towards a New Synthesis amongst Taxonomic, Ecological and Biogeographical Approaches in Carabidology*. Proceedings of the XIII European Carabidologists Meeting, Blagoevgrad, 20–24 August 2007. Sofia-Moscow. Pensoft, pp. 425-450.

JOCQUE M., T. M. TEOFILOVA, N. D. KODZHABASHEV. 2016. Light trapping as a valuable rapid assessment method for ground beetles (Carabidae) in a Bulgarian wetland. - *Acta Zoologica Bulgarica*, 68(4): 529-534.

MOEW. 2005. [*National Plan for the Conservation of Biological Diversity, 2005–2010*]. Sofia. Ministry of Environment and Water, 27 p. (In Bulgarian)

KODZHABASHEV N.D., L.D. PENEV. 2006. The ground beetles (Coleoptera: Carabidae) of South Dobrudzha, Bulgaria. - *Acta Zoologica Bulgarica*, 58(2): 147-180.

MAGURA T., B. TÓTHMÉRÉSZ, T. MOLNÁR. 2008. A species-level comparison of occurrence patterns in carabids along an urbanisation gradient. - *Landscape and Urban Planning*, 86(2): 134-140. [DOI]

NIEMELÄ J., D.J. KOTZE, S. VENN, L. PENEV, I. STOYANOV, J. SPENCE, D. HARTLEY, E. MONTES DE OCA. 2002. Carabid beetle assemblages (Coleoptera, Carabidae) across urban-rural gradients: an international comparison. - *Landscape Ecology*, 17: 387-401. [DOI]

OHLEMÜLLER R., E.S. GRITTI, M.T. SYKES, C.D. THOMAS. 2006. Towards European climate risk surfaces: the extent and distribution of analogous and non-analogous climates 1931–2100. - *Global Ecology and Biogeography*, 15(4): 395-405. [DOI]

RABITSCH 2008. The times they are a-changin': driving forces of recent additions to the Heteroptera fauna of Austria. - In: Grozeva S., N. Simov (Eds.): *Advances in Heteroptera Research. Festschrift in Honour of 80th Anniversary of Michail Josifov*, pp. 309-326.

RAEV I., G. TINCEV. 2015. Assessment of drought related climate change impacts on forests in Bulgaria. - *Silva Balcanica*, 16(1): 5-24.

TEOFILOVA T.M., E.P. MARKOVA, N.D. KODZHABASHEV. 2015. Ground beetles (Coleoptera: Carabidae) from the region of Cape Emine (Central Bulgarian Black Sea coast). Part I. Taxonomic and zoogeographic structure, life forms, habitat and humidity preferences. - *ZooNotes*, 68: 1-18.

VENN S.J., D.J. KOTZE, J. NIEMELÄ. 2003. Urbanization effects on carabid diversity in boreal forests. - *European Journal of Entomology*, 100: 73-80. [DOI]

VERHAGEN R., R. VAN DIGGELEN, R. VERMEULEN. 2008. Community assemblage of the Carabidae fauna in newly created habitats. - *Baltic Journal of Coleopterology*, 8(2): 135-148.

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Comparative Anatomical Leaf Analyses of Carduus nutans and Carduus thoermeri from Bulgaria

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Abstract: The present study provides a comparative anatomical leaf analysis of *Carduus nutans* L. and *C. thoermeri* Wienm., which belong to the Asteraceae family. Bifacial leaf structure, anomocytic and anisocytic types of stomatal apparatus and two types of trichomes (multicellular covering and glandular) were found on the leaves of both species. The quantitative anatomical characteristics varied considerably, however; basing on the statistical analyses, the most significant characteristics for distinguishing the two taxa are: the number of stomata in the abaxial epidermis, the number of capitated glandular trichomes and the number of multicellular covering trichomes in the upper (adaxial) and lower (abaxial) epidermis.

Key words: anatomical study, *Carduus nutans*, *Carduus thoermeri*, leaf, thistle.

Introduction

The Asteraceae (Compositae) family is the largest among the flowering plants, including about 1100 genera and 25000 species (Heywood *et al.,* 1977), and according to Stevens (2001) – about 1620 genera and over 23600 species. Genus *Carduus* (Thistle) belongs to this family and it is globally represented with about 90 species, naturally spread in Northern Europe, Asia and Africa (Kazmi, 1964; Bohm & Stuessy, 2001; Keil, 2006; Mabberley, 2008). *Carduus nutans* L. and *Carduus thoermeri* Wienm. are widely distributed representatives of the genus in the Bulgarian flora (Delipavlov & Cheshmedzhiev, 2003).

The basic anatomical characteristics of the representatives of the Asteraceae family were described by Metcalfe & Chalk (1950, 1979, 1989), the description includes: types of trichomes, stomatal apparatus, type of mesophyll, and the sheath cells of the vescular bundles. In accordance with those findings, Zarinkamar (2007) confirmed the anomocytic and anisocytic type of the stomatal apparatus of the species *C. thoermeri*, the stomata of the studied species found on the two leaf surfaces.

The taxonomic significance of the characteristics of the leaf epidermis in the representatives of the Asteraceae family was described by a number of authors (PALMER & GERBETH-JONES, 1986; JAYEOLA *et al.*, 2001; ADEDEJI & ILLOH, 2004; ADEDEJI, 2004). The secretory structures and their location also have an important diagnostic value in taxonomic studies (METCALFE & CHALK, 1950, 1979, 1989; FAHN, 1979).

The number, size, density and position of the stomata in the leaf epidermis are influenced by the environmental conditions and have an adaptive significance for plants (PARKHURST, 1978; ZARINKAMAR, 2006; NINOVA & DUSHKOVA, 1977, 1978a, 1978b; NINOVA *et al.*, 1984; CAIAZZA & QUINN, 1980; SALGARE & ACHARECAR, 1990; CASE, 1994). Changes in stomatal density might be a result of changing the amount of water in the environment (EDWARDS & MEIDNER, 1978), the light intensity (RETALLACK, 2001; LU *et al.*, 1993), the temperature (CIHA & BRUN, 1978), the geographical location (RETALLACK, 2001), the concentration of CO_2 in the atmosphere (BRISTOW & LOOI, 1968; ILKUN, 1978; WOODWARD, 1987; WOODWARD & BAZZAZ, 1988). VENDRAMINI *et al.* (2002) noted a thick leaf bladefor *C. thoermeri* species.

There are a number of studies on the anatomical and morphological characteristics of the achenes of the representatives of tribe Cardueae (WAGENITZ *et al.*, 1982; PEREZ-GARCIA & DURAN, 1987; CHERNIK, 1984; HÄFFNER, 2000). OLIVIERI *et al.* (1983) investigated the type and location of the achenes in the head of the species *Carduus pycnocephalus* and *Carduus tenuiflorus*. Studying the morphology of the achenes of the species of the genus *Carduus*, KÖSTEKCI & ARABACI (2015) found that their characte-ristics could be used as diagnostic taxonomic traits for the representatives of the genus.

The aim of the present study was to differentiate the taxonomically significant anatomical features of *C. nutans* and *C. thoermeri* leaves, taking into account the scarce studies of those characteristics of the two species, belonging to the *C. nutans* group.

Material and Methods

The plant material (leaves) was collected in 2014-2015 from 4 floristic regions of Bulgaria (Table 1). The species were identified at the Department of Botany and Methods of Teaching Biology, Faculty of Biology, Plovdiv University "Paisii Hilendarski" according to DELIPAVLOV & CHESHMEDZHIEV (2003). The voucher materials was deposited in the Herbarium of Agricultural University - Plovdiv (SOA).

An anatomical analysis of the leaf epidermis and leaf lamina of the studied species was made following classical methods (METCALFE & CHALK, 1950). For this purpose, the leaves were fixed in 70% ethanol, followed by making histological preparations of epidermis and cross section of the lamina. The following qualitative and quantitative anatomical characteristics were investigated: type and number of trichomes, type and number of stomata in the adaxial and abaxial epidermis, lamina thickness, cuticle thickness and palisade tissue thickness. Quantitative data about the characteristics number of trichomes and number of stomata were based on the observations of 50 visible adaxial and abaxial epidermis areas for each species. The results obtained for the anatomical features lamina thickness, adaxial and abaxial cuticle thickness, as well as thickness of the palisade tissue, were based on measurements of 10 leaf blades for each species from each site. Light microscopic images were taken with a Magnum T Trinocular microscope, equipped with a photo-documentation system Si5000 (zoom x100 up to x400) at the Department of Botany and Methods of Teaching Biology, Fa culty of Biology, Plovdiv University "Paisii Hilendarski". The electron microscopic analysis was done with a Scanning electrone microscope JEOL JSM-5510 (Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski" (zoom x50 to x10000). Statistical data processing was performed with the IBM SPSS, ver. 20.

Results and Discussion

The leaf epidermis of the studied species is represented by 4 types of cells: basic epidermal cells, stomatal guard cells, subsidiary cells and hair cells. The stomata are disorderly scattered in both epidermis–amphistoma leaves.

Table 1. List of studied taxa and localities of their collection.

Species	Locality (latitude/longitude and altitude)	Name of locality, Floristic region, year of sampling	Voucher specimen
Carduus nutans L.	41°:40′N/24°:44′E/1431 m a.s.l.	Rozhen, Rhodopes Mts (*Central*), 2015	062053
	42°:19′N/27°:43′E/30 m a.s.l.	Arkutino, Black Sea Coast(*South*), 2015	062047
	41°:76′N/25°:35′E/451 m a.s.l.	Tchernootchene, Rhodopes Mts (*East*) 2015	062049
Carduus thoermeri Wienm. [syn. *C. nutans* subsp. *lejophyllus* (Petrovič) Stoj. &Stef.]	42°:12′N/25°:21′E/212 m a.s.l.	Chirpan, Thracian Plain,2015	060240
	41°:95′N/24°:86′E/367m a.s.l.	Bachkovo Rhodopes Mts (*Central*), 2014	062040
	41°:51′N/24°:49′E/663 m a.s.l.	Laki Rhodopes Mts (*Central*), 2015	062051

Metcalfe & Chalk (1979, 1989) indicated the same type of leaves in the Asteraceae family. The type of trichomes (syn. hairs) is the same in both species – multicellular covering trichomes and sparsely located capitate glandular ones, on both leaf surfaces. Covering trichomes are uniseriate, consisting of 4 to 6 cells (Fig. 1). Glandular trichomes have one secretory cell – the head of the trichome, which is above the epidermal surface, on a short one-celled stalk (Fig. 2).

In our study, we did not find a flagellum type of trichomes, as Freire *et al.* (2005) reported in another species of the genus *Carduus (C. acanthoides)*. As a result of the study, a mixed type of stomatal apparatus was found for both species – anisocytic (predominant) and anomocytic types (Fig. 3).

The cross section of the lamina showed a dorsiventral structure (Fig. 4), which is typical of the Asteraceae family according to Metcalfe & Chalk (1950, 1979, 1989): presence of adaxial and abaxialleaf epidermis, covered with a cuticle; a mesophyll (differentiated into palisade and spongy parenchyma tissues). The palisade tissue is located under the adaxial epidermis and it is represented by 2 to 3 layers of tightly packed cells. The spongy parenchyma is located on the lower leaf surface.

The veins were clearly outlined with vascular bundles in them (Fig. 5). The vascular bundles are closed collaterally, covered with sclerenchyma bow. Only in the midrib they may be opened (Fig. 5). Metcalfe & Chalk (1950, 1979, 1989) announced that the vascular bundles of the Asteraceae are covered by a parenchyma sheath structured by large cells. Their statement was also confirmed in our study, where the vascular bundles are covered with parenchyma sheath.

The comparative statistical analysis of the data from the three localities about *C. nutans* (Fishers' criterion) distinguishes the characteristics with significant differences within the species (Table 2).

The statistical analysis of the quantitative anatomical features of *C. nutans* leaves from the three studied localities showed that there are intraspecific differences in the following features: number of stomata per unit area, number of glandular trichomes and number of coveringtrichomes in the adaxialand abaxial epidermis (Table 2). The number of stomata in the adaxial epidermis between Arkutino and Tchernoochene localities, as well as between Tchernoochene and Rozhen was significantly different ($p \leq 0.001$). As far as their number in the abaxial epidermis is concerned, there were differences between all the studied localities: Arkutino and Rozhen ($p \leq 0.001$), Tchernoochene and Rozhen ($p \leq 0.001$), Arkutino and Tchernoochene ($p \leq 0.05$), respectively.

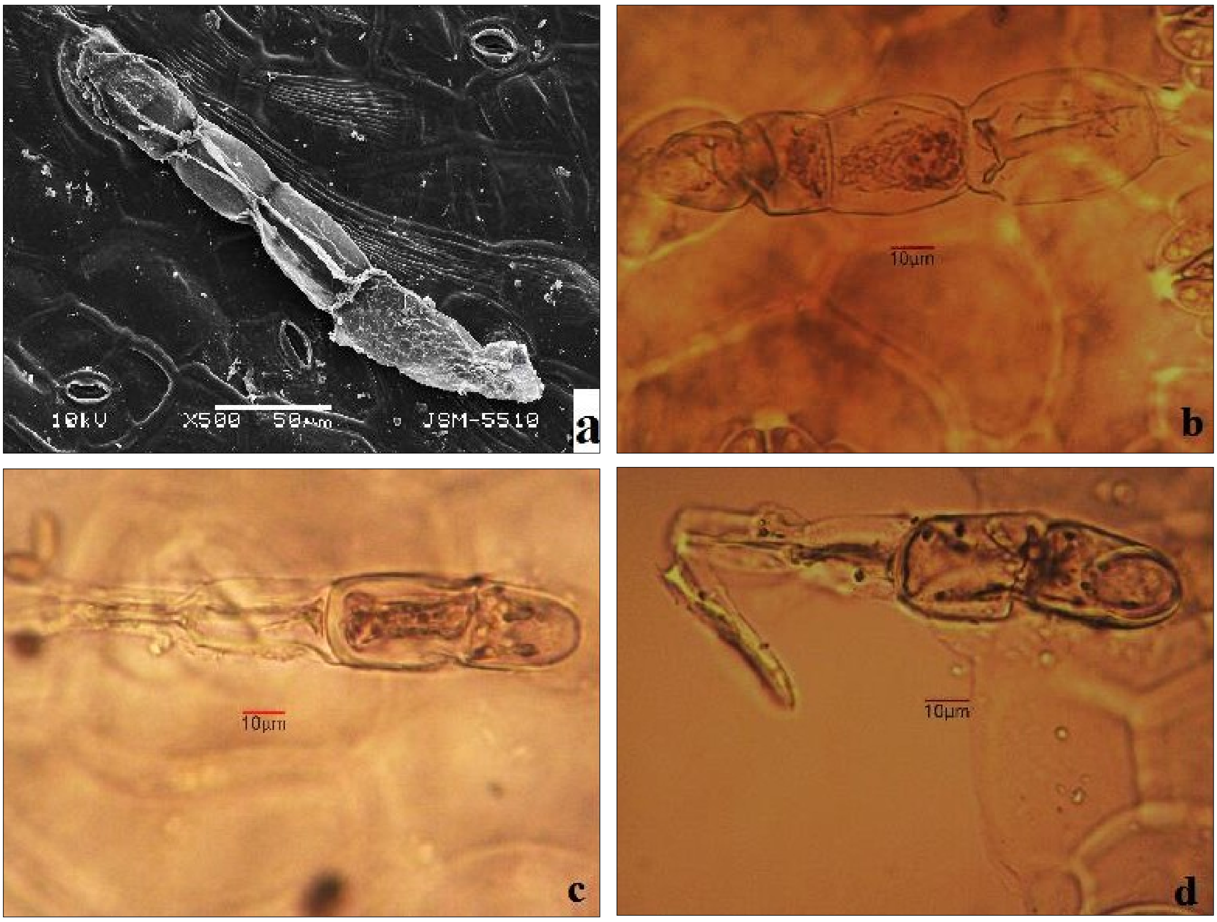

Fig. 1. Scanning electron microscopy (a) and light microscopy (b, c, d) of *Carduus nutans* (a, b) and *Carduus thoermeri* (c, d) trichomes.

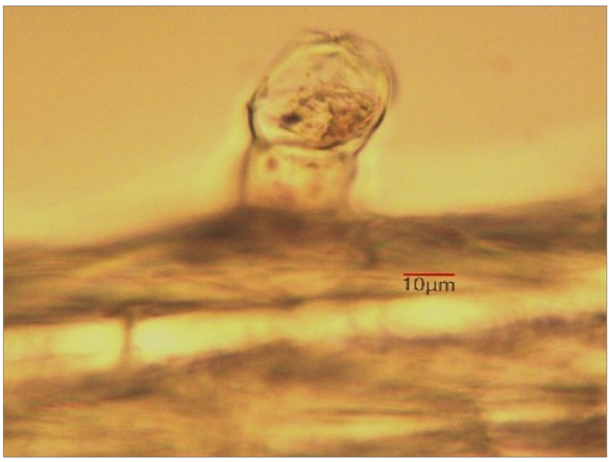

Fig. 2. Glandular trichome of leaf epidermis.

Fig. 3. Anomocytic and anisocytic stomatal type of *Carduus nutans* (a) and *Carduus thoermeri* (b).

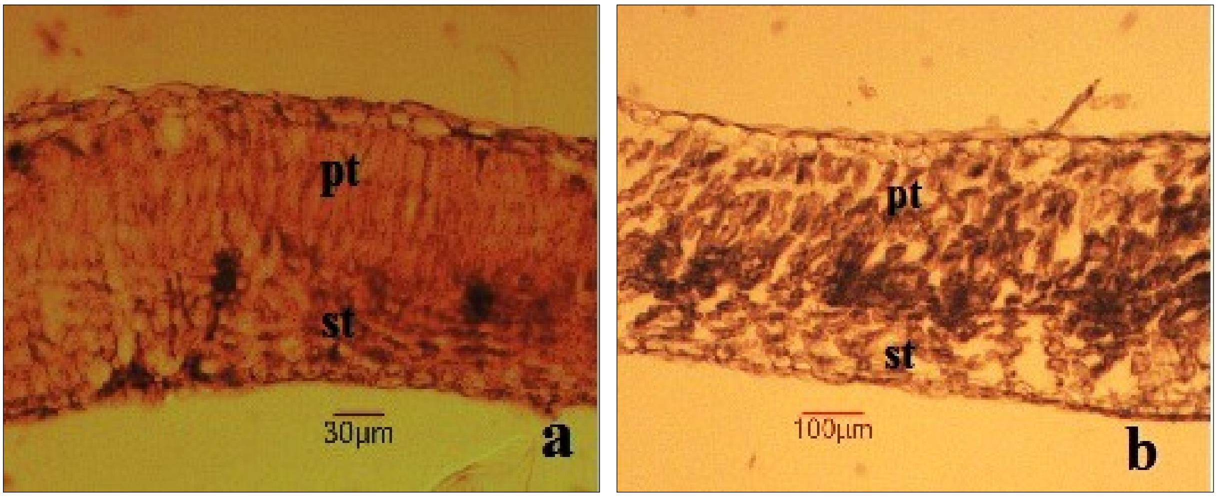

Fig. 4. Cross section of *Carduus nutans* (a) and *Carduus thoermeri* (b) leaf lamina. Legend: pt – Palisade tissue; st – Spongy tissue.

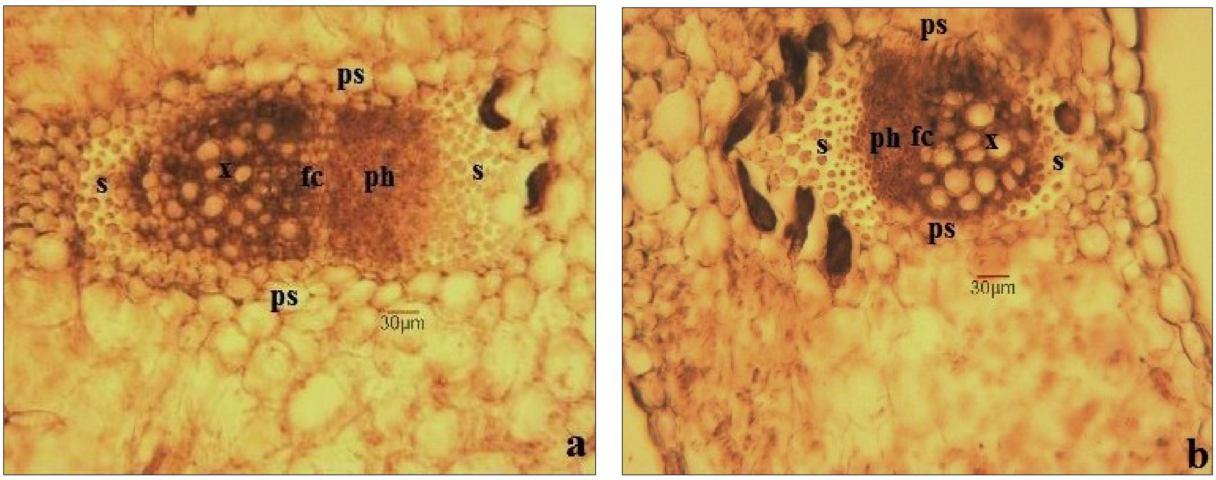

Fig. 5. Middle rib with opened collateral vascular bundles at *Carduus nutans* (a) and *Carduus thoermeri* (b). Legend: x – Xylem; ph – Phloem; fc – Fascicular cambium; s – Sclerenchyma bow; ps – Parenchyma sheath.

The amount of the two types of trichomes varied greatly between the different localities. Referring to the number of capitated glandular trichomes in the adaxial epidermis, there were differences between the studied specimens from Arkutino and Tchernoochene ($p \leq 0.001$) and those from Tchernoochene and Rozhen ($p \leq 0.05$). In the abaxial epidermis statistically significant difference ($p \leq 0.05$) was found only between Arkutino and Rozhen. The difference in the amount of the covering trichomes in the adaxial epidermis was statistically significant between Arkutino and Tchernoochene localities ($p \leq 0.001$), whereas in the abaxial epidermis differences were established between Arkutino and Rozhen and Tchernoochene and Rozhen ($p \leq 0.001$).

The results of the statistical processing of the measured quantitative indicators for the three localities of *C. thoermeri* are presented in Table 3.

The results of the statistical processing of the measured quantitative indicators for the three localities of *C. thoermeri* show that, similar to *C. nutans*, there was a great intraspecific variation of the quantitative anatomical characteristics (Table 3). Statistically significant differences between the localities were established in terms of the following characteristics: the number of stomata per unit area of the adaxial and abaxial epidermis, the number of multicellular covering trichomes in the adaxial and abaxial epidermis, the leaf blade thickness and the thickness of the palisade tissue. The difference in the number of stomata in the adaxial epidermis was statistically significant only between Bachkovo and Chirpan ($p \leq 0.01$), while significant differences in their number in the abaxial epidermis were found between Laki and Bachkovo ($p \leq 0.01$) and between Bachkovo and Chirpan ($p \leq 0.001$). The number of multicellular covering trichomes in the adaxial leaf surface varied greatly between the different localities and was statistically significant, as follows: Laki and Chirpan ($p \leq 0.001$), Laki and Bachkovo ($p \leq 0.01$), Bachkovo and Chirpan ($p \leq 0.05$), respectively. In the abaxial epidermis, the difference in the number of those trichomes was not found only among the specimens collected from Laki and Bachkovo, while between Laki and Chirpan and Bachkovo and Chirpan the differences were significant ($p \leq 0.001$). The lamina thickness was statistically different between Laki and Bachkovolocalities ($p \leq 0.05$). In terms of the thickness of the palisade tissue there were differences between Laki and Bachkovo ($p \leq 0.001$) and Bachkovo and Chirpan ($p \leq 0.05$).

Both species were collected from the Central Rhodopes Mts., which necessitated the comparison of the mean values of the anatomical indicators in the frames of the floristic region (Students' T-Test). The results obtained are presented in Table 4.

In the floristic region of the Central Rhodopes Mts., the largest statistically significant difference was found in the characteristics: the number of capitated glandular trichomes in the abaxial epidermis and the number of multicellular covering trichomes in the adaxial epidermis ($p \leq 0.001$), followed by the number of multicellular covering trichomes in the abaxial epidermis ($p \leq 0.01$) and the number of capitated glandular trichomes in adaxial epidermis ($p \leq 0.05$). The aggregated mean values of the quantitative anatomical characteristics in the two studied species were compared applying the Student's T-test (Table 5).

The statistical processing of the data obtained for *C. nutans* and *C. thoermeri* showed a difference in the number of stomata in the abaxial epidermis, the number of capitated glandular trichomes in both epidermis, the number of multicellular covering trichomes in the abaxial epidermis ($p \leq 0.001$) and the number of multicellular covering trichomes in the adaxial leaf surface ($p \leq 0.05$). The established differences in the above-mentioned quantitative indicators supplement the characteristic of the two taxa and support the results of the molecular genetic analysis by Denev *et al.* (2017) to distinguish them at the subspecies level.

Conclusions

The comparative anatomical leaf analysis of *Carduus nutans* L. and *Carduus thoermeri* Wienm was carried out for the first time in Bulgaria.

Table 2. Comparison of the mean values of anatomical features by localities of *Carduus nutans*. Legend: X_1, X_2, X_3 – Mean value; SE – Standard Error of Mean value; F –Fishers' criterion; *p* - Accuracy indicator; *p*≤0.05 - *; *p*≤0.01 - **; *p*≤0.001 - ***

Feature	1. Arkutino X_1±SE	2. Tcherno-otchene X_2±SE	3. Rozhen X_3±SE	F	*p*	Multiple comparisons (Scheffe)		
						X_1 –X_2	X_1 –X_3	X_2 –X_3
Stomata number (Adaxial epidermis)	51.06±2.40	78.20±2.28	54.28±2.69	36.32	0.000	-27.14***	-3.22	23.92***
Stomata number (Abaxial epidermis)	114.54±3.80	129.26±3.83	92.00±2.94	28.00	0.000	-14.72*	22.54***	37.26***
Glandular hair number (Adaxial epidermis)	0.30±0.09	0.93±0.13	0.51±0.12	7.82	0.001	-0.63***	-0.21	0.42*
Glandular hair number (Abaxial epidermis)	0.27±0.08	0.60±0.11	0.69±0.13	4.23	0.016	-0.33	-0.42*	-0.09
Non glandular hair number (Adaxial epidermis)	2.40±0.15	1.41±0.20	1.86±0.21	6.92	0.001	0.99***	0.54	-0.45
Non glandular hair number (Abaxial epidermis)	1.14±0.16	1.11±0.15	2.19±0.21	12.22	0.000	0.03	-1.05***	-1.08***
Leaf lamina (Width) µm	385.10±19.27	352.70±24.37	404.10±18.06	1.57	0.227	32.40	-19.00	-51.40
Upper cuticle (Width) µm	6.20±0.25	6.60±0.43	6.40±0.54	0.22	0.801	-0.40	-0.20	0.20
Lower cuticle (Width) µm	6.80±0.25	6.10±0.38	6.30±0.30	1.32	0.284	0.70	0.50	-0.20
Palisade tissue (Width) µm	190.60±3.85	177.90±10.47	166.80±9.44	1.99	0.156	12.70	23.80	11.10

Table 3. Comparison of the mean values of anatomical features by localities of *Carduus thoermeri*. Legend: X_1, X_2, X_3 – Mean value; SE – Standard Error of Mean value; F –Fishers' criterion; *p* - Accuracy indicator; *p*≤0.05 - *; *p*≤0.01 - **; *p*≤0.001 - ***

Feature	1. Laki X_1±SE	2. Bachkovo X_2±SE	3. Chirpan X_3±SE	F	*p*	Multiple comparisons (Scheffe)		
						X_1 -X_2	X_1 –X_3	X_2 –X_3
Stomata number (Adaxial epidermis)	56.58±2.20	63.02±2.35	51.98±2.26	5.96	0.003	-6.44	4.60	11.04**
Stomata number (Abaxial epidermis)	103.04±3.43	87.40±3.01	107.64±3.44	10.33	0.000	15.64**	-4.60	-20.24***
Glandular hair number (Adaxial epidermis)	0.30±0.10	0.09±0.05	0.12±0.06	2.55	0.081	0.21	0.18	-0.03
Glandular hair number (Abaxial epidermis)	0.06±0.04	0.12±0.06	0.03±0.03	1.04	0.36	-0.06	0.03	0.09
Non glandular hair number (Adaxial epidermis)	3.12±0.18	2.28±0.21	1.50±0.18	18.46	0.000	0.84**	1.62***	0.78*
Non glandular hair number (Abaxial epidermis)	3.33±0.17	2.70±0.23	1.50±0.18	21.96	0.000	0.63	1.83***	1.20***
Leaf lamina (Width)µm	364.40±24.82	431.50±17.95	396.00±9.47	3.29	0.053	-67.10*	-31.60	35.50
Upper cuticle (Width)µm	5.60±0.27	6.10±0.18	5.80±0.42	0.69	0.512	-0.50	-0.20	0.30
Lower cuticle (Width)µm	5.50±0.31	6.70±0.42	6.30±0.50	2.16	0.135	-1.20	-0.80	0.40
Palisade tissue (Width)µm	147.70±5.06	197.80±4.28	162.40±10.66	12.63	0.000	-50.10***	-14.70	35.40*

Table 4. Comparison of the mean values of the anatomical indicators of *Carduus nutans* and *Carduus thoermeri* from the floristic region of the Rhodopes Mts (Central). Legend: X – Mean value of *C. nutans*; Y – Mean value of *C. thoermeri*; SE – Standard Error of Mean value; T – Students' T-Test;*p* - Accuracy indicator; *p*≤0.05 - *; *p*≤0.01 - **; *p*≤0.001 - ***

Feature	*C. nutans* x±SE	*C. thoermeri* y±SE	T	*p*	(x-y)±SE
Stomata number (Adaxial epidermis)	54.28±2.69	59.80±1.63	-1.85	0.067	-5.52±2.99
Stomata number (Abaxial epidermis)	92.00±2.94	95.22±2.40	-0.81	0.420	-3.22±3.98
Glandular hair number (Adaxial epidermis)	0.51±0.12	0.20±0.06	2.42	0.018	0.32±0.13*
Glandular hair number (Abaxial epidermis)	0.69±0.13	0.09±0.04	4.45	0.000	0.60±0.13***
Non glandular hair number (Adaxial epidermis)	1.86±0.21	2.70±0.14	-3.38	0.001	-0.84±0.25***
Non glandular hair number (Abaxial epidermis)	2.19±0.21	3.02±0.15	-3.23	0.002	-0.83±0.26**
Lamina (Width) µm	404.10±18.06	397.95±16.78	0.23	0.822	6.15±27.03
Upper cuticle (Width)µm	6.40±0.54	5.85±0.17	0.98	0.353	0.55±0.57
Lower cuticle (Width)µm	6.30±0.30	6.10±0.29	0.43	0.669	0.20±0.46
Palisade tissue (Width)µm	166.80±9.44	172.75±6.59	-0.52	0.608	-5.95±11.46

Table 5. Comparison of the mean values of anatomical characteristics in *Carduus nutans* and *Carduus thoermeri*. Legend: X – Mean value of *C. nutans*; Y – Mean value of *C. thoermeri*; SE – Standard Error of Mean value; T – Students' T-Test;*p*– Accuracy indicator; *p*≤0.05 - *; *p*≤0.01 - **; *p*≤0.001 - ***

Feature	*C. nutans* x±SE	*C. thoermeri* y±SE	T	*p*	(x-y)±SE
Stomata number (Adaxial epidermis)	61.18±1.72	57.19±1.35	1.82	0.070	3.99±2.19
Stomata number (Abaxial epidermis)	111.93±2.39	99.36±2.02	4.02	0.000	12.57±3.13***
Glandular hair number (Adaxial epidermis)	0.58±0.07	0.17±0.04	5.08	0.000	0.41±0.08***
Glandular hair number (Abaxial epidermis)	0.52±0.06	0.07±0.03	5.08	0.000	0.41±0.08***
Non glandular hair number (Adaxial epidermis)	1.89±0.11	2.30±0.12	-2.48	0.014	-0.41±0.17*
Non glandular hair number (Abaxial epidermis)	1.48±0.11	2.51±0.13	-6.08	0.000	-1.03±0.17***
Lamina (Width) µm	380.63±12.21	397.30±11.50	-0.99	0.325	-16.67±16.78
Upper cuticle (Width) µm	6.40±0.24	5.83±0.17	1.93	0.059	0.57±0.29
Lower cuticle (Width) µm	6.40±0.18	6.17±0.25	0.75	0.45	0.23±0.31
Palisade tissue (Width) µm	178.43±5.04	169.30±5.62	1.21	0.23	9.13±7.54

There were no differences found between the two species, regarding the following qualitative anatomical characteristics – leaf lamina structure (bifacial structure), stomatal type (anisocytic and anomocytic) and hair type (capitated glandular hairs and multicellular uniseriate covering hairs), while intraspecific variations in the quantitative leaf characteristics were found in the different localities, probably, related to ecological adaptation. Statistically significant differences between the two taxa were observed in the following characteristics: the number of stomata in the abaxial epidermis, the number of glandular hairs and the number of covering hairs in the adaxial and abaxial epidermis.

References

ADEDEJI O. 2004. Leaf epidermal studies of the species of *Emilia* Cass. (Senecioneae, Asteraceae) in Nigeria. – *Botanica Lithuanica*, 10(2): 121-133.

ADEDEJI O., H.C. ILLOH. 2004. Comparative foliar anatomy of ten species in the genus *Hibiscus* Linn. in Nigeria. - *New Botanist*, 31: 147-180. [DOI]

BOHM B.A., T.F. STUESSY. 2001. *Flavonoids of the Sunflower family (Asteraceae)*. Springer-Verlag, Wien, New York.

BRISTOW J.M., A.S. LOOI. 1968. Effect of carbon dioxide on the growth and morphogenesis of *Marsilea*. - *American Journal of Botany*, 55: 884-889. [DOI]

CAIAZZA N.A., I.I. QUINN. 1980. Leaf morphology in *Arenaria patula* and *Lonicera japonica* along a pollution gradient. - *Bulletin of the Torrey Botanical Club*, 107: 9-18. [DOI]

CASE S. 1994. *Leaf stomata as bio indicators of environmental change.* Access Excellence Activities Exchange: Fellows' Collection.

CHERNIK V. 1984. [The development and structural features of achenes in *Rhaponticum carthamoides* and *R. scariosum* (Asteraceae)]. – *Botanicheskiy Zhurnal*, 69(11): 1491-1497 (in Russian).

CIHA A.J., W.A. BRUN. 1978. Stomatal size and frequency in soybeans. - *Crop Science*, 15(3): 309-313. [DOI]

DELIPAVLOV D., I. CHESHMEDZHIEV. 2003. [*Key to the plants of Bulgaria*]. Plovdiv. Academic Publishing House of Agricultural University, pp. 405-407 (in Bulgarian).

DENEV I., K. TODOROV, I. KIRILOVA, R. MLADENOV, P. STOYANOV, I. DIMITROVA-DYULGEROVA. 2017. Genetic diversity of Bulgarian representatives of genus *Carduus* L. (Asteraceae) as revealed by variability in sequences of internal transcribed spacers region. – *Biotechnology & Biotechnological Equipment*, 32(2): 387-396. [DOI].

EDWARDS M., H. MEIDNER. 1978. Stomatal responses to humidity and the water potentials of epidermal and mesophyll tissue. - *Journal of Experimental Botany*, 29: 771-780. [DOI]

FAHN A. 1979. *Secretory Tissues in Plants.* London: Academic Press.

FREIRE S.E., A. MARAMBARRI, N. DBAYÓN, G. SANCHO, E. URTUBEY, C. MONTI, M.C. NOVOA, M.N. COLARES. 2005. Epidermal characteristics of toxic plants for cattle from the Salado river basin (Buenos Aires, Argentina). - *Boletin de la Sociedad Argentina de Botanica*, 40(3-4): 241-281.

HÄFFNER E. 2000. On the phylogeny of subtribe Carduinae (tribe Cardueae, Compositae). - *Englera*, 21: 3-208. [DOI]

HEYWOOD V.H., J.B. HARBORNE, B.L. TURNER. 1977. An overture to the Compositae. - In: Heywood V.H., J.B. Harborne, B.L. Turner (Eds.). *The Biology and Chemistry of the Compositae*. Academic Press, London, pp. 1-20.

ILKUN G.M. 1978. [Pollutants of the atmosphere and plants]. Scientific Thought, Kiev, 247 p. (In Russian).

JAYEOLA A.A., J.R. THORPE, J.A. ADENEGAN. 2001. Macromorphological and micromorphological studies of the West African *Rhizophora* L. – *Feddes Repertorium*, 112: 349-356. [DOI]

KAZMI S.M.A. 1964. Revision der Gattung *Carduus* (Compositae). Teil II. - *Mitteilungen der Botanischen Staatssammlung München*, 5: 279-550.

KEIL D.J. 2006. *Carduus*. - In: Flora of North America Editorial Committee (Eds.), *Flora of North America*, Oxford University Press., 19, pp. 91-94, New York/Oxford.

KÖSTEKCI S., T. ARABACI. 2015. Cypselar morphology of *Carduus* L. (Asteraceae) taxa in Turkey and its taxonomic implications. - *Acta Botanica Gallica*, 162(1): 11-26. [DOI]

LU Z., M.A. QUINONES, E. ZEIGER. 1993. Abaxial and adaxial stomata from Pimacott (*Gossypium barbadense* L.). Differ in their pigment content and sensitivity to light quality. - *Plant, Cell and Environment*, 16: 851-885.

MABBERLEY D.J. 2008. *Mabberley's Plant-Book* 3rd ed., Cambridge University Press.

METCALFE C.R., L. CHALK. 1989. *Anatomy of the Dicotyledons.* 2nd ed. v. 2. Clarendon Press, Oxford.

METCALFE C.R., L. CHALK. 1950. *Anatomy of the Dicotyledons: Leaves, Stem, and Wood in Relation to Taxonomy with Notes on Economic Uses.* v.2. Claredon Press, Oxford.

METCALFE C.R., L. CHALK. 1979. *Anatomy of the Dicotyledons: Systematic Anatomy of Leaf and Stem, with a Brief History of the Subject.* v.1. 2nd ed. Claredon Press, Oxford.

NINOVA D., P. DUSHKOVA, K. TODOROVA, T. MILCHEVA. 1984. [Comparative analysis of morphological, natomical and physiological changes of experimentally grown tree and shrub species in the region of KCM "D. Blagoev"].- *Contemporary Theoretical and Applied Aspects of Plant Ecology*, pp. 503-515. (In Bulgarian).

NINOVA D., P. DUSHKOVA. 1977. [Structural and functional research on plants from industrial areas. I. Epidermal-stomatographic analysis of shrub plants in the area of KCM "D. Blagoev"]. - *Forestry Science*, 14(6): 32-40. (In Bulgarian).

NINOVA D., P. DUSHKOVA. 1978a. [Structural and functional research on plants from industrial areas. II. Epidermal-stomatographic analysis of tree plants in the area of KCM "D. Blagoev"]. - *Forestry Science*, 15 (1): 3-16. (In Bulgarian).

NINOVA D., P. DUSHKOVA. 1978b. [Structural and functional research on plants from industrial areas. III. Comparative analysis of leaf lamina in shrub plants in the area of KCM "D. Blagoev"]. - *Forestry Science*, 15 (3): 3-8. (In Bulgarian).

OLIVIERI I., M. SWAN, P.H. GOUYON. 1983. Reproductive system and colonizing strategy of two species of *Carduus* (Compositae). - *Oecologia*, 60: 114-117. [DOI]

PALMER P.G., S. GERBETH-JONES. 1986. A scanning electron microscope survey of the epidermis of East African grasses. IV. – *Smithsonian Contributions to Botany*, 62: 1-120.

PARKHURST D.F. 1978. The adaptive significance of stomatal occurrence on one or both surfaces of leaves. - *Journal of Ecology*, 66: 367-383. [DOI]

PEREZ-GARCIA F., J.M. DURAN. 1987. Fruit anatomy of *Onopordum nervosum* Boiss. (Asteraceae). - *Phytomorphology*, 37 (1): 35-38.

RETALLACK G.J. 2001. 300 million year record of atmospheric carbon dioxide from fossil plant cuticles. - *Nature*, 411:287-290. [DOI]

SALGARE S., C. ACHARECAR. 1990. Effect of industrial air pollution from chembur India on the micromorphology of some wild plant. - *Advances in Plant Science*, 3(1): 1-7.

STEVENS P.F. 2001. *Angiosperm phylogeny website*,v. 9. Available at: [mobot.org].

VENDRAMINI F., S. DÍAZ, D.E. GURVICH, P.J. WILSON, K. THOMPSON, J.G. HODGSON. 2002. Leaf traits as indicators of resource-use strategy in floras with succulent species. - *New Phytologist*, 154:147-157. [DOI]

WAGENITZ G., M. DITTRICH, J. DAMBOLDT. 1982. *Centaurothamnus*, eine neue Gattung der Compositae-Cardueae aus Arabien. - *Candollea*, 37: 101-115.

WOODWARD F.I. 1987. Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. - *Nature*, 327: 617-618. [DOI]

WOODWARD F.I., F. BAZZAZ. 1988. The responses of stomata al density to CO_2 partial pressure. - *Journal of Experimental Botany*, 39: 1771-1781.

ZARINKAMAR F. 2006. Density, Size and distribution of stomata in different monocotyledons.-*Pakistan Journal of Biological Sciences*, 9(9): 1650-1659. [DOI]

ZARINKAMAR F. 2007. Stomatal Observation in Dicotyledons. - *Pakistan Journal of Biological Sciences*, 10(2): 199-219. [DOI]

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Natural Habitats in the Forest-Free Zone of Malusha Peak (Protected Area "Bulgarka" BG0000399 and Protected Area "Central Balkan – Buffer" BG0001493)

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Abstract. The object of the study was to investigate the natural habitats in the forest-free zone of Malusha Peak, located on the main watershed ridge of the Shipka part of the Central Balkan Mountain range, part of the protected area (PA) "Bulgarka" BG0000399 and PA "Central Balkan – Buffer" BG0001493. According to the EUNIS classification used for making the analyses, seven types of habitats were described in the studied area, including 5 grasslands, 1 shrubland and 1 rocky. According to Annex 1 of the Bulgarian Biological Diversity Act, Annex 1 of the Directive 92/43/EEC and the Red Book of Bulgaria, Volume 3, five of the habitats have a conservation status, among them 6110* Rupicolous calcareous or basophilic grasslands of *Alysso-Sedion albi*, being a priority for habitat conservation. The largest part of the studied area (about 95%) is occupied by the habitats E2.33 Balkan mountain hay meadows and E 4.438 Oro-Moesian calciphile stripped grasslands. The habitats Oro-Moesian calciphile stripped grasslands, as well as the H3.2A13 Balkan Range of the calcicolous chasmophyte communities, are characterized by a high degree of naturalness, relic origin and a large number of endemic plant species, which makes them particularly valuable for the studied area.

Key words: Central Balkan, EUNIS, habitats, Malusha, Bulgarka Natural park, protected areas.

Introduction

Malusha Peak is situated on the main watershed ridge of the Balkan mountain range in the central part of Shipka Mt. of the Central Balkan mountain range. Kazanlak Valley and the village of Sheynovo are located to the south side, the Uzana Area is to the west of the peak and the historical peak Shipka is to the east. The area is part of PA Bulgarka, code BG0000399, and the PA

"Central Balkan – Buffer", code BG0001493, according to the Pan-European Ecological Network NATURA 2000. The total area of the studied territory including the forest-free area of the peak itself covers 37.73 ha.

The vertical amplitude of the studied area is about 82 m (between 1259 and 1341 m a.s.l.). The northern and southern exposures are predominant. The relief is quite broken with rock revelations. In most cases the slope varies between 25° and 40° and only at the tops it is moderate. The studied area is mainly composed of Medium-Triassic limestone. The soils are mainly brown, only in some places they are humus-carbonate, often quite shallow and stony. In many places there are rock revelations. The highest part of the territory is Malusha Peak (1341.1 m).

According to forest vegetation zoning of Bulgaria, the studied area belongs to the Balkan Mountain south slope forest vegetation province of the Temperate Continental Forest Zone. According to the geobotanical zoning of Bulgaria, Malusha Peak refers to the Illyrian (Balkan) province of the European broadleaved forest zone and the Central Balkan geobotanical area, Troyan-Kalofer region (Bondev, 2002), where the typical vegetation is represented by *Acer heldreichii* Orph., *Festuca balcanica* (Acht.) Markgr.-Dann. subsp. *balcanica*, *F. balcanica* subsp. *neicevii* (Acht.) Markgr.-Dann., *Micromeria frivaldszkyana* (Degen) Velen., *Betonica bulgarica* Degen & Nejceff, *Veronica austriaca* L. subsp. *neiceffii* (Degen) Peev, *Cynoglossum germanicum* Jacq.

The ridges of Malusha Peak are in the zone of mesophilous beech and eel forest belt (900-1500 m a.s.l.), (Bondev, 1991). Paleobotanical studies have proved that the vast beech belt gained its present appearance approximately 2000 years BC, preceded by hornbeam and coniferous forests (Filipovich, 1981; Filipovich *et al.*, 1998). Fir, which formed monodominant forests in some places during the mixed deciduous forest stage, is now found in the beech forests, mostly on the northern slopes (Neychev, 1909). The separate stages in the development of the forest vegetation throughout Holocene in the Balkan mountain range can be easily followed on the northern macroslope of the Balkan Mountain range, which is almost forest-free (Filipovich *et al.*, 1997). Those are: the mixed deciduous forest stage dominated by *Tilia, Ulmus, Fraxinus* and *Quercus*; the stage of hornbeam and coniferous forests and beech expansion. The southern steep and sunlit slopes are dominated by grassland and meadow vegetation and the natural coniferous species, found only mixed in beech forests, have a limited distribution. The active anthropogenic activity over the past few centuries has contributed to lower artificially the upper border of the forest in order to free territories for the feeding needs of pastoral livestock. Historical data show that in the past there were a large number of livestock animals grazing on the mountain ridge, mainly sheep. Vegetation was quite different from the present. In general, the forests occupied less area in that territory, but forest communities were almost entirely dominated by natural species. Mainly beech forests prevailed. Livestock farming and agriculture have been largely extensive. The meadows were mown. The mountain has been intensively used for grazing domestic animals, which has been related to periodic fires, burning on the slopes. The bare ridges, the meadows and small pieces of arable land occupied much larger areas on the mountain slopes. Over the last few decades, the grazing regime has been severely reduced and, in many areas, – practically suspended, which is a prerequisite for the occurrence of successions in the composition and structure of the flora in the studied territory (Meshinev & Popov,2000). Studies carried out so far on the species composition of the area show relatively high floristic diversity and the presence of new species for that floristic region (Marinov *et al.*, 2015). The great amount of collected information with regard to the flora of the area, provoked the aim of the present study, namely describing the characteristics of the natural habitats in the forest-free zone of Malusha Peak.

Material and Methods

The localization and characterization of the natural habitats was carried out during the period 2010-2017 by the transect route method. The selection of transects was carried out with the aim of covering the maximum area of the region and achieving representativeness for the habitat diversity.

The species composition of the established habitats is described on the basis of the floristic analysis of the studied area (Marinov *et al.*, 2015).

Nomenclature of species composition was accomplished acording to Delipavlov & Cheshmedjiev (2011) and Assyov & Petrova (2012).

The study of the habitats of the investigated territory was carried out according to EUNIS classification (European Environment Agency, 2017). It is the most complete and constantly updated EU classification with detailed definitions of natural habitats. The relevant category of threats for natural habitats is indicated according to the criteria adopted in the Red Book of the Republic of Bulgaria, Volume 3 (RDB) (Biserkov *et al.*, 2015) and the corresponding classification codes in the Habitats Directive 92/43/EEC (1992) and the Biological Diversity Act of Bulgaria – BDA (Biological Diversity Act, 2002-2007). Habitats according to Directive 92/43/EEC are compared to Kavrakova *et al.* (2009). The established habitats in the forest-free part of the ridge of Malusha Peak are presented in Fig. 1.

Information on the following major characteristics of the natural habitats was collected:

- Location of the habitat in the studied area;
- Dominant and typical plant species;
- Major ecological characteristics of the communities – altitude range, soil types, prevailing exposure and slope of the terrain in degrees.

Results and Discussion

The grasslands of Malusha Peak are predominantly occupied by grassy species and shrubs are less represented, spreading mainly around the upper border of the forest. Mosses and lichens are most often subordinate species in the plant communities. Mesophytic species are most widely spread. Most of the plant species are indifferent to the rock-based response and soil fertility. From a plant-geographic point of view, the vegetation cover is almost entirely of the Central-European type (Bondev, 2002).

For hundreds of years, the grasslands on the mountain ridges, maintained through grazing by stock, have developed as specific semi-natural habitats that preserve rich biodiversity.

As a result of the research studies, the following habitats were identified:

Meadows, pastures, steppes and outskirts of forests

E1.111 Middle European stone crop swards

The habitat is a dry land of limestone rocks and rock revelations from the plains to the mountains. The main location is in the plains and the hilly belt up to 900-1000 m a.s.l., but the habitats can be found even higher. Most often they occupy small areas and form complexes with perennial grasslands. The soils are underdeveloped, poor, mainly stony, alkaline or neutral. The vegetation comprises pioneer species, open, thermophilic, consisting predominantly of mosses, lichens, terrophytes and succulents. *Sedum album* L., *S. annuum* L., *S. anopetalum* DC. (syn. *S. ochroleucum* Chaix), *S. acre* L., *S. hispanicum* L., *S. maximum* L., are prevalent, found with other species of *Alysso alyssoidis-Sedion albi*. The habitat is characterized by the rich diversity of mosses (*Syntrichia* spp., *Bryum* spp., *Grimmia* spp.) and lichens (*Xanthoria* spp., *Cladonia* spp., *Collema* spp.), which use the better hydrological conditions in the autumn and especially in the winter for vegetation and development (Gusev *et al.*, 2015).

That is a rare habitat in the studied area. It is found in several places on limestone revelations and stone chippings on the southern slope of Malusha Peak. The dominating species are: *Sedum album* L. and *S. anopetalum* (syn. *S. ochroleucum* Chaix). Other species found are: *Acinos arvensis* (Lam.) Dandy, *Alyssum turkestanicum* Regel & Schmalh. (syn. *S. desertorum* Stapf), *Arenaria serpyllifolia* L., *Asplenium ruta-muraria* L., *Bromus tectorum* L., *Chondrilla juncea* L., *Convolvulus arvensis* L., *Erodium cicutarium* (L.) L'Her., *Festuca dalmatica* (Hackel) K. Richter, *Melica cilliata* L., *Potentilla obscura* Willd., *Teucrium chamaedrys* L.

The habitat is included in the RDB – Habitats under code 01E1, category 'Nearly Threatened' [NT], (Gusev *et al.*, 2015), in the Biological Diversity Act and the Bern Convention (1979). In Annex 1 of Directive 92/43/EEC it is identified by code *6110 'Rupicolous calcareous or basophilic grasslands of Alysso-Sedion albi'*.

E2.33 Balkan mountain hay meadows

Mountain hay meadows are secondary, continuous succession plant communities that originated in the place of destroyed mesophilous oak and beech forests in the mountains. The soils are brown forest (*Eutric* and *Dystric Cambisols*) and dark-colored *Mollic Cambisols*, fresh or wet. Mountain hay meadows are maintained and preserved by forest restoration through grazing or systematic mowing. When those activities ceased, processes of restoration of forest vegetation are observed. Under the influence of grazing, the mountain meadows are quickly covered with turf and they become mountain pastures. Their optimum development is between 1000 and 1600 m a.s.l. (Rusakova & Dimitrov, 2015).

This is one of the most common habitats in the ridges of Malusha Peak. It is found on relatively moist and rich soils, usually above 800-900 m a.s.l. They are mostly used for hay production or grazing. Due to the depopulation of the small villages in the area, the habitat is also characterized by the spread of shrubs and the restoration of the forest vegetation. There is a rich diversity of cereal grasses: *Agrostis capillaris* L., *Avenula pubescens* (Hudson) Dumort., *Cynosurus cristatus* L., *Dactylis glomerata* L., *Festuca nigrescens* Lam., *F. pratensis* Hudson, *Holcus mollis* L., *Poa pratensis* L. The species composition is quite varied. The following species are also found: *Agrimonia eupatoria* L., *Antoxanthum odoratum* L., *Filipendula vulgaris* Moench, *Galium album* Miller, *G. verum* L., *Hypochoeris radicata* L., *Luzula campestris* (L.) DC., *Leucanthemum vulgare* Lam., *Lotus corniculatus* L., *Peucedanum carvifolia* Vill., *Potentilla argentea* L., *P. reptans* L., *Plantago lanceolata* L., *Ranunculus acris* L., *Stachys germanica* L., *Stellaria graminea* L., *Thymus pulegiodes* L., *Trifolium alpestre* L., *T. repens* L., *Veronica chamaedrys* L. as well as the species of conservation importance: *Lilium albanicum* Griseb., *Betonica bulgarica* Degen & Nejceff and *Orchis militaris* L.

The habitat is included in the RDB – Habitat under code 16E2, category "vulnerable" [VU], (Rusakova & Dimitrov, 2015), in the BDA and the Bern Convention (1979). In Annex 1 of Directive 92/43/EEC it is identified by code *6520 Mountain hay meadows.*

E4.438 Oro-Moesian calciphile stripped grasslands

That habitat has a limited distribution in the mountains of Bulgaria. Among the main characteristics of the habitat are the high altitude – from about (1600) 2000 to 2900 m a.s.l. and the alkaline soil-forming rocks – limestone shales and marble. Soils are predominantly skeletal and quite dry during the vegetation season. The habitat is located on the ridges and on the slopes of the mountains in Bulgaria. Plant communities formed in those environmental conditions are most often open, the projective cover in some places being very low – about 20-30%, rarely reaching up to 60%. In habitats of this type there are dry, rocky and stony terrains with northern to southern exposure, some of them very steep, with shallow soils (rendzinas) on limestone or dolomites, on the ridges and on the slopes around them. The phytocoenoses developing in the Balkan mountain range are dominated by: *Sesleria latifolia* (Adamovic) Degen, *S. rigida* Heuffel ex Reichenb. subsp. a*chtarovii* (Deyl) Deyl, etc. Their distribution is limited and the area of the separate plots is small. The communities are open and their floristic composition is quite rich (RUSAKOVA, 2015).

For the area of Malusha Peak the most typical communities are dominated by: the two species of *Sesleria* – *Sesleria latifolia* (Adamovic) Degen and *Sesleria rigida* Heuffel ex Reichenb. subsp. A*chtarovii* (Deyl) Deyl, which form monodominant spots of different sizes – from several square meters to several hundred square meters.

The species composition is diverse. The following species are found: *Allium paniculatum* L., *Anthyllis vulneraria* L., *Asyneuma canescens* (Waldst. & Kit.) Griseb. et Schenk, *Carex humillis* Leyss., *Cerastium banaticum* (Rochel) Heuff., *Euphrasia salisburgensis* Funck, *Festuca dalmatica* (Hackel) K. Richter, *Filipendula vulgaris* Moench, *Gentiana cruciata* L., *Hieracium hoppeanum* Schult., *Linum catharticum* L., *Luzula multiflora* (Retz.) Lej., *Potentilla cinerea* Chaix ex Vill., *Primula veris* L., *Scabiosa columbaria* L., *Seseli libanotis* (L.) Koch, *Teucrium chamaedrys* L., *Thymus vandasii* Velen., *Trifolium alpestre* L., as well as the species of conservation importance: *Veronica austriaca* L. ssp. *Neiceffii* (Degen) Peev, *Draba lasyocarpa* Rochel, *Carum graecum* Boiss. & Heldr., *Festuca balcanica* (Acht.) Markgr.-Dann. subsp. *neicevii* (Acht.) Markgr.-Dann., *Laserpitium siler* L., *Sempervivum erythraeum* Velen., *Trinia glauca* (L.) Dumort.

The habitat is included in the RDB – Habitat under code 25E4, category 'endangered' [EN], (RUSAKOVA, 2015), in the BDA. In Annex 1 of Directive 92/43/EEC it is identified by code *6170 Alpine and subalpine calcareous grasslands; subtype 36.43. - Calciphilous stepped and garland grasslands.*

E 5.21 Xero-thermophile fringes

The habitat is often found in the studied territory; however, it occupies small areas everywhere, most often located at forest fringes. In most places it has a secondary origin, because it is located along the roads and at woodland fringes, most often in dry and sunlit areas. The species composition is quite varied and includes both forest species and ruderals, and, species typical of xerophytic grassland cenoses.

The following species are found in the habitat: *Achillea millefolium* L., *Agrostis capillaris* L., *Anthemis tinctoria* L., *Avenula pubescens* (Hudson) Dumort., *Carlina vulgaris* L., *Centaurea stenolepis* A. Kern., *Clematis vitalba* L., *Clinopodium vulgare* L., *Coronilla varia* L., *Dactylis glmerata* L., *Daucus carota* L., *Digitalis grandiflora* Mill., *Dorycnium herbaceum* Vill., *Galium album* Mill., *Gymnadenia conopsea* (L.) R. Br., *Leucanthemum vulgare* Lam., *Mentha spicata* L., *Origanum vulgare* L., *Picris echioides* L., *Plantago lanceolata* L., *Poa pratensis* L., *Potentilla pilosa* Willd., *Silene italica* (L.) Pers., *Stachys germanica* L., *Trifolium alpestre* L. The species *Atropa bella – donna* L. and *Dactylorhiza saccifera* (Brongn.) Soó are also quite widely spread.

The habitat has no conservation status.

E5.43 Shady woodland edge fringes

Plant communities, called 'tall herbs', are widely distributed throughout the country in the river valleys. They represent a variety of mixed phytocoenoses with grasses of 1 m to 1,5 and even 2 m height. They usually occupy narrow strips (up to 2-3 m, often narrower) along the running waters and at the wet riverbanks. Most of them can grow both in the water (in shallow water of 0.10-0.20 m) and in over-wet soils. The species composition of the cenoses is quite diverse and depends both on the altitude and the sunlight, as well as on the surrounding plant communities. According to that, they can be divided into three main sub-types corresponding to different codes of EUNIS habitat classification. The following subtype is spread on the studied territory: *Nitrophilous tall-herb in the river valleys in shady areas of forests.* Those communities belong to the semi-ruderal syntaxons of the *Galio-Urticetea* class, union *Aegopodion podagrariae* (order *Lamio albi-Chenopodietalia boni-henrici*) and order *Convolvuletalia sepium*. They form strips near streams and small rivers in shaded places, in humid valleys and slopes with high air and soil humidity. Most often the species found are: *Aconitum lamarckii* Rchb., *Aegopodium podagraria* L., *Alliaria petiolata* (M. Bieb.) Cavara & Grande, *Anthriscus sylvestris* (L.) Hoffm., *Carex pendula* Hudson, *Chaerophyllum hirsutum* L., *Chelidonium majus* L., *Circaea lutetiana* L., *Galeopsis speciosa* Mill., *Galium aparine* L., *Geranium phaeum* L., *G. robertianum* L., *Geum urbanum* L., *Glechoma hederacea* L., *Heracleum sibiricum* L. (Valchev *et al.*, 2015).

The habitat is included in the RDB – Habitat under code 28E5, category 'endangered' [EN], (Valchev *et al.*, 2015) and in the BDA. In Annex 1 of Directive 92/43/EEC it is identified by code *6430 Hydrophylious tall herb fringe communities of plains and of mountain to alpine levels.*

Bushes and Small Shrubs

F2.333 Subalpine bramble brush

A relatively rare habitat, with a secondary origin, spread at woodland edge fringes in the eastern part of the studied area. Those are usually communities formed in the succession processes associated with the reestablishment of forest ecosystems.

The habitat has no conservation status.

Cave and Rocky Habitats

H3.2A13 Balkan Range calcicolous chasmophyte communities

This habitat occupies vertical or steep limestone rock walls, as well as sharp rock ridges. There is no soil or it is very poor (most often Rendzic Leptosols). Separate plant individuals or small groups of plants are usually scattered at great distances from one another, the biotic links between them are poorly expressed and in most cases the organisms live in an environment created by them. Total projective cover is scarce. Limestone rock revelations: walls, garlands, peaks, etc. in the Balkan mountain range are localized mainly in the higher parts, especially on ridges. There are quite a few endemic, rare and endangered species in them (Gusev & Rusakova, 2015).

In some places there are large limestone rock revelations in the studied territory. Depending on the sunlight and slope, plant communities of different species composition are formed, alternating with bare rocks. Communities of the Balkan endemic *Haberlea rhodopensis* Friv. are formed on darker and wet limestone rocks. The sunlit rock blocks, where there is a thicker soil layer, communities of the endemites typical of the Balkan mountain develop, such as *Festuca balcanica* (Acht.) Markgr.-Dann. subsp. *neicevii* (Acht.) Markgr.-Dann. and *Micromeria frivaldszkyana* (Degen) Velen. Other rare species are also found in those communities, for example *Seseli bulgaricum* P. W. Ball.

The habitat is included in the RDB – Habitat under code 08H3, category 'vulnerable' [VU], (Gusev & Rusakova, 2015) and in the BDA. In Annex 1 of Directive 92/43/EEC it is identified by code *8210 Calcareous rocky slopes with chasmophytic vegetation.*

Data analysis shows that grasslands are the most widely spread on the studied territory. Out of the total area of the forest-free zone of the peak itself, which is approximately 37.73 ha, the largest share (95%) is occupied by *E2.33 Balkan mountain hay meadows (18.68 ha)* and *E4.438 Oro-Moesian calciphile stripped grasslands (17.069 ha)*. The area occupied by the habitats E5.21 The Xero-thermophile fringes and F2.333 Subalpine bramble brush is a small share of the studied area, most often at the forest fringes. Due to the small area occupied by the two habitats, they are not indicated on the map (Fig. 1).

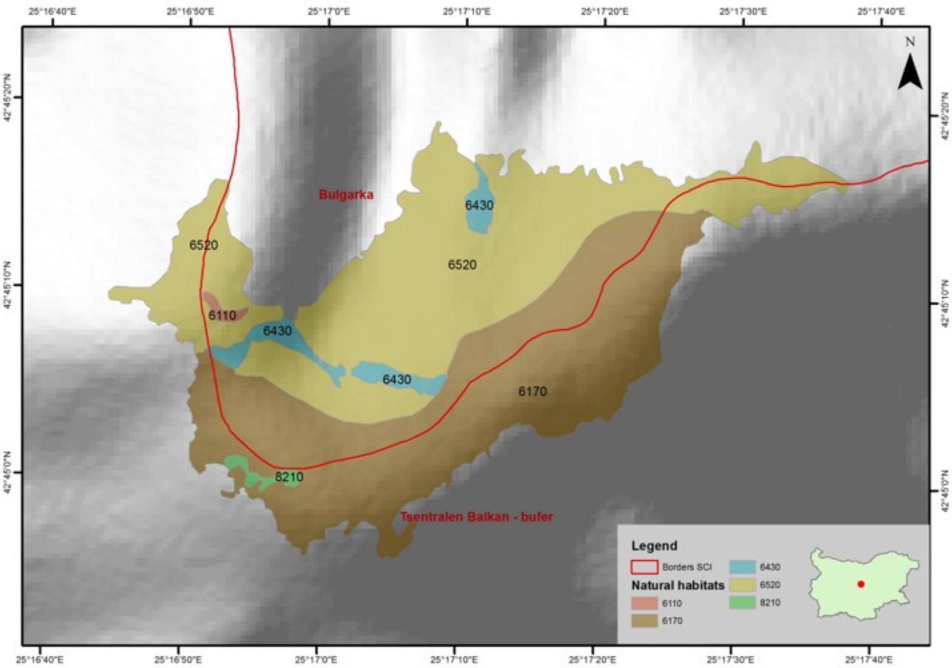

Fig. 1. Map of the studied territory by the identified habitat types, according to the EUNIS classification.

Seven major habitat types were identified according to EUNIS classification, five of them having a conservation status and included in Directive 92/43/EEC under codes: E1.111/6110; E4.438/6170; E5.43/6430; E2.33/6520 and H3.2A13/8210, respectively, as well as in Annex 1 of the Biological Diversity Act (Table 1). According to the two normative documents, the priority conservation type of habitat is *6110 Rupicolous calcareous or basophilic grasslands of Alysso-Sedion albi*.

According to the Red Book of the Republic of Bulgaria, Volume 3, two of the five habitats of conservation importance have the status of endangered (EN), two are vulnerable (VU) and one nearly threatened (NT), (Table 1). The habitats E1.111 Middle European stone crop swards and Balkan mountain hay meadows are also protected by Bern Convention.

Conclusions

Five grasslands, one shrubland and one rocky habitats found in the forest-free zone of Malusha Peak, are subject to protection in the protected areas BG0000399 "Bulgarka" and BG0001493 "Central Balkan – Buffer". All the habitats with the exception of F2.333 Subalpine bramble bush and E 5.21 Xero-thermophile fringes have a conservation status determined by the Bulgarian legislation.

The study of natural habitats shows a typical habitat diversity in Malusha Peak area. Along with the priority habitat *6110 Rupicolous calcareous or basophilic grasslands of Alysso-Sedion albi*, the habitats *E4.438 Oro-Moesian calciphile stripped grasslands* and *H3.2A13 Balkan Range of calcicolous chasmophyte communities*, which are characterized by a high degree of naturalness, relic origin and a large number of endemic plant species, are also especially valuable for that concrete area.

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Table 1. The identified habitat types according EUNIS classification, conservation status in Red Book of Bulgaria and Directive 92/43/EEC under codes and areas.

Habitat code	Habitat name	Habitat code Red Book	Threat category	Habitat HD 92/43	Area ha
E1.111	Middle European stone crop sward	**01E1**	**NT**	**6110 -** *Rupicolous calcareous or basophilic grasslands of *Alysso-Sedion albi*	0.148028
E2.33	Balkan mountain hay meadow	**16E2**	**VU**	**6520 -** Mountain hay meadows	18.687587
E4.438	Oro-Moesian calciphile stripped grasslands	**25E4**	**EN**	**6170** - Alpine and subalpine calcareous grasslands	17.269172
E5.21	Xero-thermophile fringes	-	-		
E5.43	Shady woodland edge fringe	**28E5**	**EN**	**6430 –** Hydrophylious tall herb fringe communities of plains and of mountaine to alpine levels	1.375677
F2.333	Subalpine bramble brush	-	-		
H3.2A13	Balkan Range calcicolous chasmophyte communities	**08H3**	**VU**	**8210 –** Calcareous rocky slopes with chasmophytic vegetation	0.24861

References

ASSYOV B., A. PETROVA (Eds.). 2012. *Conspectus of the Bulgarian Vascular Flora. Distribution Maps and Florestic Elements.* Ed. 4, BBF, Sofia.

Bern Convention. 1979. *Convention on the conservation of European wildlife and natural Habitats.* Available at: [conventions.coe.int].

Biological Diversity Act. 2002-2007. *State Gazette* 77/09.09.2002: 9-42, 94/16.11.2007: 2-44. Available at: [chm.moew.government.bg].

BISERKOV V., CH. GUSEV, V. POPOV, G. HIEBAUM, V. RUSAKOVA, I. PANDURSKI, Y. USUNOV, M. DIMITROV, R. TSONEV, S. TSONEVA (Eds.). 2015. *Red Data Book of the Republic of Bulgaria. Vol. 3. Natural Habitats.* Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences & Ministry of Environment and Water, Sofia.

BONDEV I. 1991. [*The Vegetation of Bulgaria. Map in scale 1:600000 with explanatory text.*] Publ. House of Sofia University "St. Kliment Ochridsky", Sofia. (In Bulgarian).

BONDEV I. 2002. [Geobotanical division.] – In: Kopralev, I. (Ed.*): Geography of Bulgaria. Physical Geography. Socio-economic Geography*, ForKom Publishers, Sofia, pp. 336–352. (In Bulgaria).

DELIPAVLOV D., I. CHESHMEDZHIEV. (Eds.). 2011. [*Key to the Plants of Bulgaria.]* Agrarian University Academic Press, Plovdiv. (In Bulgarian).

Directive 92/43/EEC**.** 1992. *Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of Wild Fauna and Flora*. – Official Journal L 206, 22.07.1992: 0007 - 0050. Available at: [eur-lex.europa.eu].

European Environment Agency. 2017. EUNIS habitat types. Available at: [eunis.eea.europa.eu].

FILIPOVICH L. 1981. Late Post-Glacial period development of forest vegetation in the upper parts of the Balkan mountain range. – *Phytologia*, 18: 3-20.

FILIPOVICH L., I. STEFANOVA, M. LAZAROVA, M. PETROVA. 1997. Holocene vegetation in Stara Planina (the Balkan range). I. – *Phytologia Balcanica*, 3(2–3): 15–39.

FILIPOVICH L., I. STEFANOVA, M. LAZAROVA, M. PETROVA. 1998. Holocene vegetation in Stara Planina (the Balkan range). II. – *Phytologia Balcanica*, 4(1–2): 13–25.

GUSEV CH., V. RUSAKOVA. 2015. Calcareous rocks with hasmophytic vegetation. - In: Biserkov V., Ch. Gusev, V. Popov, G. Hiebaum, V. Rusakova, I. Pandurski, Y. Uzunov, M. Dimitrov, R. Tsonev, S. Tsoneva (Eds.): *Red Book of the Republic of Bulgaria. Vol. 3. Natural Habitats*. Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences & Ministry of Environment and Water, Sofia. pp. 381-384

GUSEV CH., V. RUSAKOVA, D. DIMITROV. 2015. Pioneer thermophilic grass communities on calcareous rocks and rocky areas. - In: Biserkov V., Ch. Gusev, V. Popov, G. Hiebaum, V. Rusakova, I. Pandurski, Y. Uzunov, M. Dimitrov, R. Tsonev, S. Tsoneva (Eds.): *Red Book of the Republic of Bulgaria. Vol. 3. Natural Habitats.* Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences & Ministry of Environment and Water, Sofia. pp. 129-131

KAVRAKOVA V., D. DIMOVA, M. DIMITROV, R. TSONEV, T. BELEV, K. RAKOVSKA (Eds.). 2009. *Guide for identifying habitats of European significance in Bulgaria. Second Ed.* The World Wildlife Fund, Danube-Carpathian Programme and the Green Balkans Federation, Sofia.

MARINOV Y., I. CHESHMEDZHIEV, R. MLADENOV, I. DIMITROVA-DYULGEROVA, D. BELKINOVA, I. TENEVA-DZHAMBAZOVA, P. STOYANOV. 2015. Floristic analysis of the central part of Mt Shipka (Central Balkan, Bulgaria). - *Phytologia Balcanica,* 21(3): 297–308.

MESHINEV T., A. POPOV (Eds.). 2000. Mountain forest-free zone of the National Part Central Balkan. Biological Diversity and problems of its conservation. Sofia, BSBCP.

NEYCHEV I. 1909. [Materials on the flora of Gabrovo region and the Balkan mountain range (from Kademliya to Bedek) – Collection of folk sayings.] - *Nauka i Knizhnina*, 24: 1-83. (In Bulgarian).

RUSAKOVA V. 2015. Alpine and Subalpine open calcareous grassland habitats. - In: Biserkov V., Ch. Gusev, V. Popov, G. Hiebaum, V. Rusakova, I. Pandurski, Y. Uzunov, M. Dimitrov, R. Tsonev, S. Tsoneva (Eds.): *Red Book of the Republic of Bulgaria.Vol. 3. Natural Habitats.* Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences & Ministry of Environment and Water, Sofia. pp. 188-190

RUSAKOVA V., M. DIMITROV. 2015. Mountain hay meadows. - In: Biserkov V., Ch. Gusev, V. Popov, G. Hiebaum, V. Rusakova, I. Pandurski, Y. Uzunov, M. Dimitrov, R. Tsonev, S. Tsoneva (Eds.): Red *Book of the Republic of Bulgaria. Vol. 3. Natural Habitats.* Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences & Ministry of Environment and Water, Sofia. pp. 168-170.

VALCHEV V., V. GEORGIEV, R. TSONEV. 2015. Tall-herb communities along the rivers in plain areas. - In: Biserkov V., Ch. Gusev, V. Popov, G. Hiebaum, V. Rusakova, I. Pandurski, Y. Uzunov, M. Dimitrov, R. Tsonev, S. Tsoneva (Eds.): *Red Book of the Republic of Bulgaria. Vol. 3. Natural Habitats*. Institute of Biodiversity and Ecosystem Research – Bulgarian Academy of Sciences & Ministry of Environment and Water, Sofia. pp. 196-198.

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The Trophic Spectrum of the Northern White-Breasted Hedgehog (Erinaceus roumanicus Barrett-Hamilton, 1900) in Plovdiv City, Bulgaria

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Abstract. The food spectrum of the Northern white-breasted hedgehog (*Erinaceus roumanicus* Barrett-Hamilton, 1900) was studied based on the number of specimens/fruits in its excrements. A total of 181 scats (19 food items) from hedgehog were collected in park "Otdih i Kultura" in Plovdiv City, Bulgaria and were analyzed during the spring-summer period of 2017. In the study area, the hedgehog consumed mainly beetles (Coleoptera undet.) - 49.19%, followed by the ants (Hymenoptera, Formicidae undet.) - 10.69%. The value of Berger-Parker dominance index was 0.49, which determines the hedgehog as a polyphagous rather than an oligophagous. The food niche of the species in Plovdiv City during the spring-summer season was with a value of 0.19.

Key words: *food spectrum, Northern white-breasted hedgehog, Erinaceus roumanicus, Plovdiv City.*

Introduction

The food spectrum of the Northern white-breasted hedgehog (*Erinaceus roumanicus* Barrett-Hamilton, 1900) in Plovdiv City has not been investigated so far.

Hedgehogs consume a variety of food, but mainly of animal origin – different invertebrates, small vertebrates (rodents, amphibians, reptiles, eggs of birds and their young). Of secondary importance are fruits, mushrooms and others (PESHEV *et al.*, 2004).

A study of the diet of hedgehogs, mainly *Erinaceus concolor* but possibly also some *Erinaceus europaeus,* in an urban environment by OBRTEL & HOLISOVA (1981), found that the diet was consisted with mainly arthropods from the litter on the surface of the soil. Millipedes, earwigs and beetles were important year round, while chafers, weevils, ants, aquatic insects attracted to street lights and comatose bees were taken opportunistically.

Vegetable matter including fruits, acorns and berries, same as fungi constitute a relatively small proportion of the natural diet (REEVE, 1994).

The diet of hedgehogs is affected by food availability, as well as the diet of urban hedgehogs may vary from that of rural hedgehogs. A study of the contents of the gastrointestinal tract of 87 hedgehogs showed that suburban hedgehogs ate more fruit, isopods and tipulid larvae than did rural individuals, while the rural animals ate more lepidopteran larvae (caterpillars) (DICKMAN, 1988).

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In New Zealand, a study examining stomach contents of 10 hedgehogs plus 90 hedgehog droppings found that slugs and millipedes were the main items in the diet of suburban hedgehogs (snails were also eaten frequently) (BROCKIE, 1959).

The aim of this study is to study the food spectrum of the Northern white-breasted hedgehog (*Erinaceus roumanicus* Barrett-Hamilton, 1900) in Plovdiv City, Bulgaria.

Material and Methods

The food spectrum of the Northern white-breasted hedgehog (*Erinaceus roumanicus*) in the city of Plovdiv was studied based on the number of specimens/fruits in its excrements.

A total of 181 scats from hedgehog were collected in park "Otdih i Kultura" in Plovdiv City, Bulgaria and were analyzed during the spring-summer period of 2017.

The excrement collection method does not allow complete and accurate yield and determination of the animal's food spectrum and there is a significant error. Moreover, this method does not make it possible to specify the number of animals leaving the excrement, their sex and age. However, such studies have made a significant contribution to the research of mammalian feeding ecology.

The excrements were placed in 75% ethanol and then dried for 14 days. The food components were defined in the Department of Ecology and Environmental Conservation, Faculty of Biology, University of Plovdiv "Paisii Hilendarski", by morphological criteria, by means of a binocular magnifying glass at a 10x magnification lens.

Sampling adequacy was determined using Lehner`s formula (LEHNER, 1996):

$$Q = 1 - \frac{N_1}{I},$$

rising from 0 to 1, where N_1 is the number of the food components occurring only once, and *I* is the total number of the food components.

The quantitative analysis of the hedgehog's food spectrum was done by taking into account the percentage of the individual food components in the scat contains (DAAN, 1973).

Food specialization was calculated using the Berger-Parker (*d*) dominance index (MAGURRAN, 1988):

$$d = \frac{n_{i\,max}}{N},$$

where: *d* is the index of dominance; *N* is the number of individuals from all food components; $n_{i\ max}$ is the number of the most numerous food component. The Berger-Parker index (d) varies between 1/N and 1. A value closer to 1 means a higher specialization in the choice of food; a value closer to 1/N is typical for a species that is a general feeder (polyphage).

The food niche breadth (*B*) of the hedgehog was calculated by the Levin index (HURLBERT, 1978):

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

where: *B* is the food niche breadth; *R* is the number of individual categories of food components; P_i is the relative rate of occurrence (proportion) of each category of food component.

The proportion (P_i) was calculated using the formula (MAGURRAN, 1988):

$$P_i = \frac{n_i}{N},$$

where: n_i is the number of taxon *i*; *N* is the number of all taxa from the food spectrum.

Results and Discussion

A total of 19 food items were determined in the diet of hedgehog from Plovdiv city - 15 insect species, 3 plant species and 1 mammal species. The sampling adequacy is considered sufficient - 0.68.

The percentage of the individual food components mentioned above was calculated and presented in Table 1.

The main components of hedgehog's trophic spectrum in the study area were beetles (Coleoptera undet.) - 49.19%, followed by ants (Formicidae undet.) - 10.69%. These insects are widespread in the city of Plovdiv and are relatively easy to capture and rich in protein, spending the least foraging costs. The other food components had a significantly lower percentage, more important of which are: Hemiptera undet. - 6.88%, Asian ladybeetle (*Harmonia axyridis*) - 6.44%, firebug (*Pyrrhocoris apterus*) - 5.86%, earwigs (*Forficula* sp.) - 5.42 %, Coccinellidae undet. - 3.95%, seven-spot ladybird (*Coccinella septempunctata*) - 3.81%, 24-spot ladybird (*Subcoccinella vigintiquatuorpunctata*) - 3.51%.

For comparison in the spring-summer period of 2014-2016, in the region of Sashtinska Sredna Gora (Mikov & Georgiev, 2016) hedgehogs consumed mainly ants (Formicidae undet.) – 77.04%, followed by beetles (Coleoptera undet.) – 14.97%, indicating that these two food components were essential to the hedgehog.

In Europe, *H. axyridis* is considered to be an invasive alien species because of its potential to disrupt native ladybird communities (Adriaens *et al.*, 2008). Feeding on this ladybug, the hedgehog assists the populations of native species of ladybirds.

Although alfalfa ladybird *Subcoccinella vigintiquatuorpunctata* attacks mainly alfalfa (*Medicago sativa* L.) it was recorded as a pest of more than 70 plant species belonging to the families Leguminosae and Caryophyllaceae (Ali, 1976). Feeding on this ladybird, the hedgehog has a positive effect on agronomy, reducing the population of this species.

The value of the Berger-Parker dominance index for the hedgehog's diet in Plovdiv was 0.49 considering the species as a polyphagous rather than an oligophagous. In contrast, Mikov & Georgiev (2016) determined the hedgehog from Sashtinska Sredna Gora Mts. as oligophagus predator using Berger-Parker dominance index – 0.77 (Table 1). It is possible that the reason for the difference in the two study areas is that the ants (Formicidae undet.) and the beetles (Coleoptera undet.) in Plovdiv City were not as widespread and abundant as in the region of Sashtinska Sredna Gora Mts., and hence the hedgehog has supplemented its food spectrum with true bugs (Hemiptera undet.), asian ladybeetle (*Harmonia axyridis*) and firebug (*Pyrrhocoris apterus*).

The food niche of the hedgehog in Plovdiv City during the spring-summer period was 0.19, while during the period of 2014-2016, in the region of Sashtinska Sredna Gora Mts. it was 0.05 (Mikov & Georgiev, 2016) (Table 1). These values indicated that the hedgehog niche in Plovdiv City was nearly 4 times wider than in the region of Sashtinska Sredna Gora Mts. This is probably due to lesser distribution and lower abundance of true bugs (Hemiptera undet.), asian ladybeetle (*Harmonia axyridis*) and firebug (*Pyrrhocoris apterus*), in the region of Sashtinska Sredna Gora Mts., which in Plovdiv City were relatively often consumed.

According to a study of the food spectrum of the similar European hedgehog (*Erinaceus europaeus*) in the upper Waitaki Basin, New Zealand, the most commonly eaten prey were Coleoptera (present in 81% of 192 guts), Lepidoptera (52%; n = 192), Dermaptera (49%; n = 192), Hymenoptera (42%; n = 192) and Orthoptera (31%; n = 319). Weta remains occurred in 22% of guts, with the gut of one adult male containing 283 *Hemiandrus* legs. Eggshell was recorded in 4% of 615 guts. Native lizard remains were found in 6% of 615 guts (Jones *et al.*, 2005).

Another study of European hedgehog (*Erinaceus europaeus*) diets revealed that although a wide variety of invertebrates were consumed, the bulk of energy was provided by only four prey types (carabid beetles, earthworms, Lepidoptera larvae, and tipulid larvae). Within these four types, hedgehogs showed a clear tendency to concentrate on only one type at a time and to switch from one

Table 1. Number and percentage of individual food components in the composition of the food spectrum, Berger-Parker dominance index and food niche breadth of the Northern white-breasted hedgehog (*Erinaceus roumanicus*) in Plovdiv City (current study) and the region of Sashtinska Sredna Gora Mts. (after MIKOV & GEORGIEV, 2016).

Taxa	Plovdiv City		Sashtinska Sredna Gora Mts.	
	N	%	N	%
Scolopendra cingulata	-	-	1	0.07
Total Chilopoda	**-**	**-**	**1**	**0.07**
Adalia bipunctata	6	0.88	-	-
Anisodactylus sp.	-	-	2	0.13
Carabidae undet.	-	-	5	0.33
Carabus sp.	-	-	1	0.07
Cerambycidae undet.	-	-	3	0.20
Cetoniinae undet.	-	-	4	0.26
Coccinella septempunctata	26	3.81	-	-
Coccinellidae undet.	27	3.95	1	0.07
Coleoptera undet.	336	49.19	227	14.97
Harmonia axyridis	44	6.44	-	-
Harpalus sp.	-	-	1	0.07
Neodorcadion sp.	-	-	4	0.26
Oodes sp.	-	-	1	0.07
Otiorhynchus sp.	1	0.15	32	2.11
Scarabaeinae undet.	1	0.15	1	0.07
Subcoccinella vigintiquatuorpunctata	24	3.51	-	-
larva of Coccinellidae undet.	1	0.15	-	-
Total Coleoptera	**466**	**68.23**	**282**	**18.60**
Forficula sp.	37	5.42	26	1.72
Total Dermaptera	**37**	**5.42**	**26**	**1.72**
Coreus sp.	-	-	3	0.20
Hemiptera undet.	47	6.88	2	0.13
Nezara viridula	-	-	1	0.07
Pyrrhocoris apterus	40	5.86	-	-
Total Hemiptera	**87**	**12.74**	**6**	**0.40**
Formicidae undet.	73	10.69	1168	77.04
Vespula sp.	1	0.15	-	-
Total Hymenoptera	**74**	**10.83**	**1168**	**77.04**
Insecta undet.	-	-	2	0.13
larva/pupa of Insecta undet.	-	-	1	0.07
Total Insecta	**-**	**-**	**3**	**0.20**
Mammalia undet.	2	0.29	1	0.07
Total Mammalia	**2**	**0.29**	**1**	**0.07**
♀ Acrididae undet.	-	-	1	0.07
♀ Caelifera undet.	-	-	3	0.20
Acrididae undet.	-	-	2	0.13
Caelifera undet.	2	0.29	2	0.13
Total Orthoptera	**2**	**0.29**	**8**	**0.53**
Plantae undet.	-	-	3	0.20
Total Plantae	**-**	**-**	**3**	**0.20**
Rodentia undet.	-	-	3	0.20
Total Rodentia	**-**	**-**	**3**	**0.20**
Ficus carica	-	-	1	0.07
Morus sp.	13	1.90	-	-
Rosa canina	-	-	2	0.13
Rubus sp.	-	-	2	0.13
undefined fruit	1	0.15	5	0.33
Total fruits	**14**	**2.05**	**10**	**0.66**
undefined herbaceous plant	1	0.15	5	0.33
Total herbaceous plants	**1**	**0.15**	**5**	**0.33**
Total specimens/fruits:	**683**	**100.00**	**1516**	**100.00**
Berger-Parker dominance index:	**0.49**		**0.77**	
Food niche breadth:	**0.19**		**0.05**	

group to another on a seasonal basis. Preference indices (which relate diet and availability) suggested that these four prey types plus the gastropods and dermapterans were the preferred prey (WROOT, 1984).

In studying the food spectrum of introduced European hedgehog (*Erinaceus europaeus*) in a dryland habitat of the South Island, New Zealand, it was found that the most commonly eaten foods were beetles, including rare native species (in 94% of droppings), earwigs (92%), spiders (25%) and native skinks (14%). Remains of at least three skinks were found in one dropping. Earwigs and darkling beetles (Tenebrionidae) were the most preferred food types, and Hymenoptera and cylindrical bark beetles (Colydiidae) were least preferred (JONES & NORBURY, 2010).

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References

ADRIAENS T., G. GOMEZ, D. MAES. 2008. Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium. - *BioControl*, 53(1): 69-88. [DOI]

ALI M. 1976. Studies on the induction of food preference in alfalfa ladybird, *Subcoccinella vigintiquatuorpunctata* L. (Coleoptera: Coccinellidae). - *Symp. Biol. Hung.*, 16: 23-28. [DOI]

BROCKIE R. 1959. Observations on the food of the hedgehog (*Erinaceus europaeus* L.) in New Zealand. - *New Zealand Journal of Science*, 2: 121-136.

DAAN N. 1973. A quantitative analysis of the food intake of North Sea cod, *Gadus morhua*. - *Netherlands Journal of Sea Research*, 6(4): 479-517. [DOI]

DICKMAN C. 1988. Age-related dietary change in the European hedgehog (*Erinaceus europaeus*). - *Journal of Zoology*, 215(1): 1–14. [DOI]

HURLBERT S. 1978. The measurement of niche overlap and some relatives. - *Ecology*, 59(1): 67-77. [DOI]

JONES C., G. NORBURY. 2010. Feeding selectivity of introduced hedgehogs *Erinaceus europaeus* in a dryland habitat, South Island, New Zealand. - *Acta Theriologica*, 56(1): 45–51. [DOI]

JONES C., K. MOSS, M. SANDERS. 2005. Diet of hedgehogs (*Erinaceus europaeus*) in the upper Waitaki Basin, New Zealand: Implications for conservation. - *New Zealand Journal of Ecology*, 29(1): 29-35.

LEHNER P. 1996. *Handbook of ethological methods*. Cambridge. Cambridge University Press, 672 p.

MAGURRAN A. 1988. *Ecological Diversity and its Measurement*. Princeton. Princeton University Press, 179 p.

MIKOV A., D. GEORGIEV. 2016. [The food spectrum of the Northern white-breasted hedgehog (*Erinaceus roumanicus*) in the region of Sashtinska Sredna Gora]. - In: *Eighth students scientific conference "Ecology - A way of thinking" 8*. Plovdiv. Published by Department of Ecology and Environmental conservation, University of Plovdiv, pp. 7-14. (In Bulgarian).

OBRTEL R., V. HOLISOVA. 1981. The diet of hedgehogs in an urban environment. - *Folia Zoologica*, 30: 193-201.

PESHEV C., D. PESHEV, V. POPOV. 2004. *Fauna of Bulgaria*. Vol. 27 Mammalia. Sofia, BAN, Akademichno Izdatelstvo "Marin Drinov": 96-97. (In Bulgarian).

REEVE N. 1994. Hedgehogs: Diet and Feeding. - In: Reeve N. (Ed.): *Hedgehogs*. London, UK. T & AD Poyser Ltd., pp. 53-95.

WROOT A. 1984. *Feeding ecology of the European hedgehog Erinaceus europaeus*. University of London. Royal Holloway.

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Dynamics of Macroalgae at Two Different Ecological Sites in Alexandria Coastal Waters

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Abstract. A year cycle investigation of the macroalgae dynamics in Alexandria coastal waters was conducted at two selected stations that maximize possible differences in seawater characteristics. Eleven genera, including seventeen species were identified at the Eastern Harbour (EH) and Abou Talat (AT). The total species richness was higher at EH, but the algal species composition was highly similar at the two sites. The floristic ratio at EH indicates a mixed flora of warm temperate nature. The prevalence of red and brown algae was detected at relatively high salinities, and green opportunistic algae at lower salinities, and with nutrient pulses indicate its importance as ecological factors regulate the structure of the macroalgae communities in Alexandria waters. The changes in algal proportion reflected the anthropogenic influence and/or improvement in environmental quality at times. Incidents of massive green macroalgae proliferation occurred at a wide range of environmental variations, and with the sharing of other red species. *Ulva fasciata* represented a perennial species and the spring warming and nutrient enrichment seem to interact with its massive growth. The study is yet the first attempt to measure the algal biomass of different species under different physical and chemical ecological stresses.

Key words: macroalgae, abundance, production, mass proliferation, environmental factors.

Introduction

Macroalgae represent one of the major primary producers in coastal marine systems (VALIELA *et al.*, 1997). Its diversity maintains ecosystem's stability (PARKER *et al.*, 2001), and are widely utilized to characterize and monitor coastal systems (LELIAERT *et al.*, 2000). Macroalgae have great ecological and biological importance as providing nutrition, protection, and accommodation to other living organisms (BERNECKER & WEHRTMANN, 2009; PEDERSEN *et al.*, 2012; GUIDONE & THORNBER, 2013). They act as biological indicators for detecting an anthropogenic impact on the coastal ecosystems (VASQUEZ & GUERRA, 1996). The broad fluctuations of ecological processes are strong stress inducers in macroalgae diversity, production and geographical distribution (BORGES *et al.*, 2006). Database about marine macroalgae along the coast of Alexandria is very preliminary; the previous work based on collections at specific locations (KHALIL, 1987; ALEEM, 1993). Hence, the present study is an attempt to detect influences of abiotic factors in fostering temporal and regional dynamics of macroalgal abundance, assemblage structure and production during a year cycle.

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Materials and Methods

The Eastern Harbour (N31˚12′-31˚13′/E29˚53′-29˚54′) is a sheltered semi-enclosed area (about 2.53 km^2) in the central urban area of Alexandria City. It has an intense long-term process of eutrophication. The harbour is a site of different man activities as fishing, Yachts sports, boat building, and recreation, particularly at its northwestern part. Abou Talat at the west of Alexandria is fully exposed to wave action and not heavily inhabited, but situated in the neighbor of highly polluted regions (El-Mex Bay and El-Noubaria Canal (Fig. 1). The study extends for eight months between March 2014 to February 2015, when each site was visited twice per season.

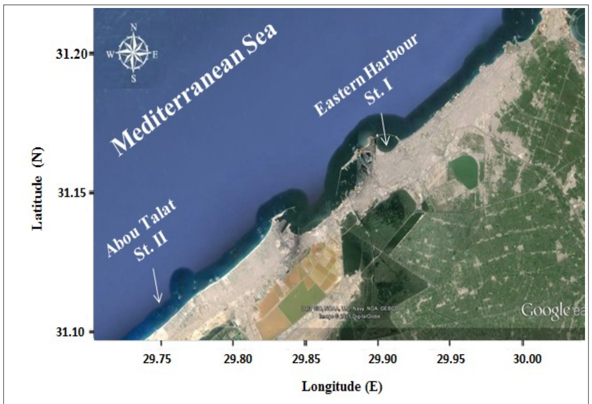

Fig. 1. Location of the sampling stations.

The environmental factors; water temperature (ºC), salinity, dissolved oxygen (mg l^{-1}), and pH values were measured in the field using the hydro lab. (HANA, Model HI 9828). Nutrient concentrations of the dissolved NH_4, NO_2, NO_3, SiO_4 and PO_4 were determined following STRICKLAND & PARSONS (1972). Each time at each site, living macroalgae samples were taken from a depth of about 20 cm at two marked areas of different dimensions following the seaweeds field manual (DHARGALKAR & KAVLEKAR, 2004), carefully separated from the substrate under the hold fast using a knife and/or handpicked. The seaweed samples of about 100 g fresh weights were kept in a labeled plastic bag filled with local seawater and vigorously shaken to dislodge any extraneous matters as stones, sand particles, animals and epiphytic microalgae, and brought back to the laboratory in icebox containing frozen gel cold packs. The specimens of each species were gently scrubbed under running tap water, rinsed with distilled water. The taxonomic guides consulted for identification include ALEEM (1993), and ABBOTT & HUISMAN (2004) and JHA *et al*. (2009). The abundance of macroalgae community at the two sites was estimated by following the scale of JAMES (2007), and TREVOR (2007), which is a broad estimate of frequency.

Chlorophyll content based on certain weights was extracted in 90% acetone, and measured spectrophotometrically and calculated applying the equation of WELLBURN (1994).

The floristic similarity between the number and percentage of taxa shared by the different sites was calculated as percent similarity (C) (MATHIESON & PENNIMAN, 1986), and the floristic ratio according to CHENEY (1977).

Multiple regression analysis was done using Excel software version 4.

Results

The physical and chemical characteristics of the surface water at the two sites are given in Table 1.

Temperature oscillated normally between a minimum in winter (February) and a maximum in summer (August), showed clear seasonality. The surface water started to be warmer by April. The changes in salinity were irregular, and not as obviously seasonal, comparatively higher during June. The abrupt drop in salinity at AT in October might reflect clear signs of discharged water influence. The water was well oxygenated, and the levels never fell down below 5.5 mg O_2 l^{-1}, Abou Talat waters appeared richer, with highest between August and October. The pH values differ slightly between the two stations, but, all in the alkaline side (mean 8.06±0.12 and 8.15±0.09, respectively), its lowest in September and October.

Table 1. Physical and chemical parameters of the surface water.

	Temp. (°C)	Salinity	pH	D. O. (mg l^{-1})	NO_2 (µM)	NO_3 (µM)	NH_4 (µM)	TN (µM)	SiO_4 (µM)	PO_4 (µM)
Eastern Harbour										
March	17.70	36.36	8.17	10.55	0.40	5.72	2.97	9.09	2.22	0.23
April	24.09	35.59	8.73	7.50	0.20	1.57	0.59	2.35	0.46	0.32
June	28.60	38.85	8.03	10.71	0.83	7.24	3.29	11.35	1.08	0.54
August	30.30	35.23	7.89	7.11	0.78	5.35	7.61	13.73	1.48	0.41
September	27.70	34.06	7.68	5.52	2.10	7.75	2.75	12.59	2.68	1.85
October	24.10	35.02	7.64	6.33	1.48	11.99	3.38	16.84	1.31	1.13
December	21.00	35.77	8.09	6.17	1.08	11.93	1.98	14.99	0.63	0.59
February	17.30	36.51	8.23	10.65	0.23	3.11	1.08	4.41	0.63	0.23
Mean	23.85±6.5	35.92±2.9	8.06±0.12	8.07±2.5	0.88±0.6	6.38±3.7	2.95±1.4	10.67±6.2	1.31±1.3	0.66±0.41
Abou-Talat										
March	18.2	35.99	8.32	10.10	0.23	3.75	0.18	4.16	0.68	0.09
April	24.51	37.26	8.57	6.80	0.13	2.57	0.90	3.59	0.63	0.18
June	28.90	38.46	8.09	7.46	0.25	4.43	0.23	4.90	0.97	0.50
August	29.80	33.38	8.16	10.31	4.28	17.31	2.16	23.74	1.77	0.54
September	25.00	34.28	7.78	10.60	0.28	7.53	1.53	9.34	0.74	0.36
October	23.3	32.22	7.81	8.11	1.88	23.22	5.72	30.81	2.79	1.88
December	20.2	34.95	8.23	9.41	0.68	19.73	3.42	23.83	6.21	0.23
February	16.4	37.41	8.25	10.55	0.10	5.72	0.27	6.09	0.63	0.05
Mean	23.29±6.5	35.5±2.9	8.15±0.09	9.17±1.4	0.975±0.6	10.5±9.68	1.8±1.6	13.3±7.5	1.8±1.2	0.48±0.4

The seawater was characterized generally by high levels of the measured nutrients, indicating influences of discharged water at times arrival. No particular seasonal trend was detected. The total nitrogen (NO_2 + NO_3 + NH_4 concentrations) showed distinct changes in terms of the relative proportion of the dominant nitrogen forms; nitrate at EH contributed 64.38% of the total nitrogen and 82.21% at the latter station. It's concentrations at the harbour (mean 6.83±1.33 µM) fall down at a level < 2 µM in April, and it was around 12 µM in October and December, while, higher levels were measured at AT with its extreme in October. The seasonal variations of the nitrite concentrations followed the patterns of NO_3 and NH_4. Ammonium values gained two major peaks in August and October at the two stations, respectively. Silicate exhibited erratic value changes with a minimum in April, and relatively higher levels in September (EH), and highest in October (AT). Phosphate levels were relatively higher at EH than AT, the lowest levels were measured in the cold periods, and the highest in autumn and with no adhere to a clear seasonal pattern. The calculated nutrient ratio in EH indicated high N:P ratio most of the year with two major peaks. Such values signal Si and P limitation at times (Fig. 2).

Macroalgae community structure and composition

Eleven genera, including 17 species were identified at EH and AT, as 3 genera with 7 species of Chlorophyta, 3 genera with 3 species of Phaeophyta and 5 genera with 7 species of Rhodophyta. The regional distribution indicated Chlorophyta and Rhodophyta of permanent existence at the two stations, with the highest species richness at EH in early spring and lowest in mid-autumn and early winter (Table 2).

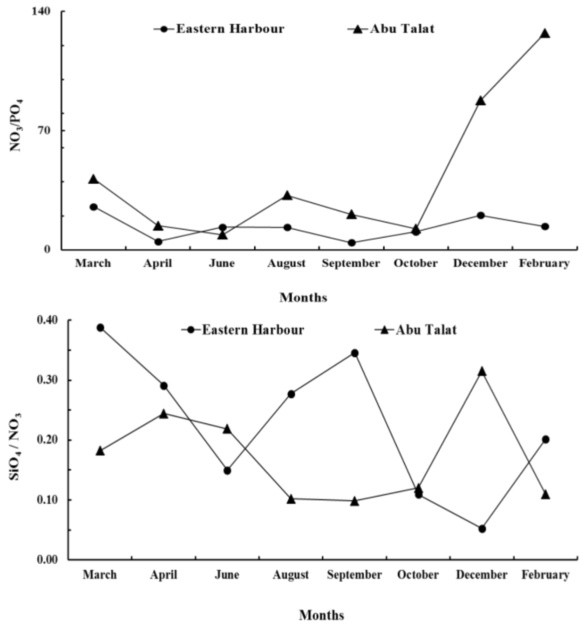

Fig. 2. Nutrient ratios at the Eastern Harbour and Abou Talat.

The succession showed *U. fasciata* Delile contributes a perennial form with different degrees of dominance, *U. compressa* Linnaeus with exclusive occurrence at AT, and *U. lactuca* Linnaeus and *U. linza* Linnaeus at EH during February and April as well. Other Chlorophyta species; *Cladophora pelluceda* (Hudson) Kützing contributed the major at AT in August and September, and the Rhodophyta *Corallina officinalis* Linnaeus at EH between June and December. The Phaeophyta species, *Petalonia fascia* (O.F. Müller) Kuntze, *Padina pavonica* (Linnaeus) Thivy and *Sargassum vulgare* C. Agardh detected at EH in February and March with relatively lower sharing.

Mass proliferation of macroalgae species

Four incidents of massive macroalgae blooms occurred at EH in February, April, August, and September, and other three events at AT during the first two months and in October as well. The blooms covered > 40% of the marked area, maintaining a wide range of environmental variations. The bloom at EH occurred at the lowest water temperature (February) compared with others in August and September. However, all the bloom periods, particularly in August characterized by relatively low salinity and high NO_3 and NH_4 concentrations. Moreover, SiO_4 and PO_4 reached the highest accompanying the algal bloom in September. The causative bloom species and the accompanied ones were different during the bloom periods. In February, *U. fasciata* dominated, followed by *U. lactuca*, while, the Phaeophyta "*Padina pavonia* and *Sargassum vulgare*" were of much lower existence. During August, *U. fasciata* remained the major; yet, *C. officinalis* shared actively the degree of dominance. Other accompanied Rhodophyta were *Cladophora pelluceda* and *Antithamnion nageli*. During September, *U. fasciata* was still the leader, and *C. lehmanniana* ranked the second significant constituent. Other existed species of rare occurrence were *C. pelluceda* and *C. elongata*. At Abou Talaat, the algal blooms attributed mainly to the proliferation of *U. compressa* and *U. fasciata*, particularly in February under almost equal contributions. However, in October *Cl. pelluceda* considerably shared the bloom.

Biomass of the recorded macroalgae species

Chlorophyll *a* content of the recorded species (Table 3) indicates the minimum value registered for *C. elongata* at EH in September and the maximum for *Cl. pellucida* in August. The latter species gained its highest value at AT in September, and the minimum for *U. compressa* in June. The common green *U. fasciata* exhibited higher chlorophyll *a* content most of the period at EH compared with AT, particularly during winter and with varied nutrient concentrations.

Discussion

The measured physico-chemical parameters conformed to previously reported ranges (EL-ZAYAT, 2012), although large inter-annual variability has been recognized, particularly for NO_3 and PO_4. The exceeded values of N/P ratio above the Redfield ratio in March and December at EH attribute to human activity (LAPOINTE *et al.*, 2004), might indicate the overall growth is often N limited.

Table 2. Temporal and regionalabundances of recorded macroalgae.

Months / Spp.	March	April	June	August	September	October	December	February
	2014							2015
Eastern Harbour								
Chlorophyta								
Chaetomorpha linum								+
Cladophora lehmanniana					+++			
Cladophora pellucida				++	+			
Ulva fasciata	++	+++	++	+++	+++	+++	++	+++++
Ulva linza		+++						
Ulva lactuca								++
Phaeophyta								
Petalonia fascia	+							
Padina pavonia								+
Sargassum vulgare								+
Rhodophyta								
Amphiroa fragilissima	+++							
Antithamnion nägeli				+				
Corallina elongata	++				+			
Corallina officinalis		++	+++	+++	+	+++	+++	
Gigertina teedi	+							
Griffithsia equisetifolia			+					
Griffithsia flosculosa								
Abou Talat								
Months / Spp.	**March**	**April**	**June**	**August**	**September**	**October**	**December**	**February**
	2014							2015
Chlorophyta								
Cladophora pellucida				+++	+++	++		
Ulva compressa	++	+++	++	+	+	+++	+++	+++
Ulva fasciata	++	+++	+	++	+++	+++	+++	++
Rhodophyta								
Corallina officinalis		+						

+, 1–5% cover; ++, 5–25% cover; +++, 25–50% cover; ++++, 50–75% cover; +++++, >75% cover.

Seaweeds community structures were analyzed by functional form groups, coverage, species composition, and biomass. Such items are the main focusing of worldwide investigations for seasonal distribution of macroalgae communities and their ecological patterns (PRATHEP, 2005; WELLS *et al*., 2007; CHOI, 2008). Surprisingly, little is known about these patterns in Alexandria waters. Referring the taxonomical works of KHALIL (1987) and ALEEM (1993), the present checklist of the marine algae at the two coastal areas of Alexandria is yet a part of their checklist.

A very strong similarity within 70 % of the algal species composition was found between the two sites.

The calculated floristic ratio at EH (4.33) indicated a mixed flora of warm temperate, yet, the limited community structure at AT hindered such definition. KHALIL (1987) defined the nature of vegetation along the coastal area of Alexandria as rather boreal as subtropical.

Table 3. Chlorophyll *a* content (mg g^{-1} DW) in the collected macroalgae species.

Eastern Harbour	Chl a	Abou Talat	Chl a
March			
Corallina elongata	0.08	*Ulva compressa*	0.71
Griffithsia flosculosa	0.17	*Ulva fasciata*	0.17
Ulva fasciata	0.40		
April			
Corallina officinalis	0.07	*Corallina officinalis*	0.19
Corallina elongata	0.07	*Ulva compressa*	0.10
Ulva fasciata	0.34	*Ulva fasciata*	0.67
Ulva linza	0.11		
Ulva lactuca	0.85		
June			
Corallina officinalis	0.06	*Ulva compressa*	0.04
Ulva fasciata	0.24	*Ulva fasciata*	0.35
August			
Cladophora pellucida	1.35	*Cladophora pellucida*	0.19
Corallina officinalis	0.07	*Ulva fasciata*	0.32
Ulva fasciata	0.40		
September			
Cladophora lehmanniana	0.04	*Cladophora pellucida*	1.32
Cladophora pellucida	1.32	*Ulva fasciata*	0.18
Corallina elongata	0.02		
Ulva fasciata	0.65		
October			
Ulva fasciata	0.56	*Cladophora pellucida*	0.51
Corallina officinalis	0.07	*Ulva compressa*	0.46
		Ulva fasciata	0.28
December			
Ulva fasciata	0.70	*Ulva compressa*	0.61
Corallina officinalis	0.03	*Ulva fasciata*	0.40
February			
Ulva lactuca	0.95	*Ulva compressa*	0.80
Chaetomorpha linum	0.44	*Ulva fasciata*	0.07
Ulva fasciata	0.74		

The present study showed that seaweeds in Alexandria coastal waters exposed to a variety of environmental stresses, offering some evidences about the relative influence of the measured factors on the spatiotemporal macroalgae structure and production. Such changes are regarded a consequence of habitat modification mediated by a combination of physical and chemical disturbances (WORM *et al.*, 2006; WERNBERG & CONNELL, 2008).

The current data might confirm the role of temperature as driving regulating factor that mainly recognized during spring and summer, affecting negatively the species diversity and positively algal production

(SZÉCHY & NASSAR, 2005). This role became more important when nutrients were in excess regardless the geographical locality. The observed extremely high pH values during the macroalgae bloom periods might represent an ecological factor affecting the species diversity. Recent studies in coastal areas documented pH can reach levels up to 10 during microalgae and macroalgae dense blooms (HANSEN, 2002; BJÖRK *et al.*, 2004; MIKHAIL & LABIB, 2013).

The present prevalence of the red and brown algae at relatively high salinities and the green opportunistic algae at relatively low salinities and under nutrient pulses particularly with the massive algal growth supports others (NIELSEN *et al.*, 1995; VALIELA *et al.*, 1997; BENEDETTI-CECCHI, 2001) for their importance as ecological factors regulate the structure of the macroalgae communities in Alexandria waters.

Since the changes of macroalgae biomass at Alexandria near-coastal areas received a very rather limited attention, and according to the available literatures and knowledge, the present study throughout a year cycle is yet the first attempt to measure algal biomass of different macroalgae species under different physical and chemical ecological stressors. Despite the relatively limited data, the calculated regression analyses indicate the variability in temperature, salinity, pH, NO_3, and NH_4 explains about 53% and 92% of the algal abundance at EH and AT stations, respectively, and 85% and 71% of chlorophyll *a* changes.

The present data confirm the occurrence of incidents of the massive green macroalgae proliferation at a wide range of environmental variations, and with the sharing of other Rhodophyta species. Although the distinct inter-annual variations and the different response of the different species to variable environmental conditions hinder definition and prediction of principle factor/factors control *Ulva* abundance and growth, the study stresses the importance of the spring-increased temperature that interacts with the growth and abundance of opportunistic macroalgae at AT when nutrient enrichment occurs. Slight seasonal variations of seawater temperature with the algal growth were reported (HURTADO & RAGAZA, 1999; LEE, 1999). The study also revealed the strong contribution of salinity on the proliferation of *U. fasciata* and *U. compressa* in accordance with others (ERIKSSON & BERGSTROM, 2005; LARSEN & SAND-JENSEN, 2006).

The present regional and temporal distribution of algal community structure explains relative reduction in the number of Phaeophyta and increased Chlorophyta, indicating changes in the environment status. Brown algae were scarcely recorded at EH, while, both brown and red algae were almost absent at AT. The changes in proportion have been considered to be indicative of anthropogenic influences and shifts in quality status (GIACCONE & CATRA, 2004; WELLS *et al.*, 2007; CORZO *et al.*, 2009). The brown algae are not as tolerant as the green algae, they are very sensible to pollution. The significant difference between the two sites confirms the previously discussed geographical sensitive characteristics of seaweed communities (KAREZ *et al.*, 2004). The highest species number of red algae in March at EH attributed to lack of competition with green algae, and might indicate some improvement in environment quality and stability at times. The presence of *C. elongata* in March as indicator for intermediate quality (SOLTAN *et al.*, 2001; BENEDETTI-CECCHI, 2001) supports the above-mentioned result. The dominance of green species explains their ability to adapt more readily to changes in the environment whereby proportions increase with decreasing quality status. Cormaci et al. (2003) pointed out that increased/decreased red/green/brown species proportions indicate degradation and/or environmental stability.

Macroalgae dynamics and the factors influencing their variability are important aspects of the ecological, environmental, aesthetic, and socio-economic value of Alexandria ecosystems. The results can increase the overall success of management strategies, helping decision makers.

References

ABBOTT I.A., J.M. HUISMAN. 2004. *Marine green and brown algae of the Hawaiian Islands*. Bishop Museum Press, Honolulu, 259 p.

ALEEM A.A. 1993. *The marine algae of Alexandria,* Egypt, University Alexandria, 139 p.

BENEDETTI-CECCHI L. 2001. Variability in abundance of algae and invertebrates at different spatial scales on rocky sea shores. - *Marine Ecology Progress Series*, 215: 79-92. [DOI]

BERNECKER A., I. WEHRTMANN. 2009. New records of benthic marine algae and cyanobacteria for Costa Rica, and a comparison with other Central American countries. - *Journal Helgoland Marine Research*, 63: 219-229. [DOI]

BJÖRK M., L. AXELSSON, S. BEER. 2004. Why is *Ulva intestinalis* the only macroalga inhabiting isolated rock pools along Swedish Atlantic coast. - *Marine Ecology Progress Series*, 284: 109-116. [DOI]

BORGES A., L. SCHIETTECATTE, G. ABRIL, B. DELILLE, F. GAZEAU. 2006. Carbon dioxide in European coastal waters. - *Estuarine, Coastal and Shelf Science Journal,* 70(3): 375-387.

CHENEY D.P. 1977. R/P and C/P – A new and improved ratio for comparing seaweed floras. - *Journal of Phycology*, 13: 129.

CHOI C.G. 2008. Algal flora in Hallyeo–haesang national park, Southern coast of Korea. - *Journal of Korean Fish*, 41: 371-380. [DOI]

CORMACI M., G. FURNARI, G. GIACCONE. 2003. Macrofitobenthos. - In: Gambi M. C., M. Dapiano (Eds.), *Manuale di metodologie di campionamento e studio del benthos marino mediterraneo*. - *Biologia Marina Mediterranea*, 10: 233-262.

CORZO A., S. VAN BERGEIJK, E. GARCÍA-ROBLEDO. 2009. Effects of green macroalgal blooms on intertidal sediments: net metabolism and carbon and nitrogen contents. - *Marine Ecology Progress Series,* 380: 81-93. [DOI]

DHARGALKAR V., D. KAVLEKAR. 2004. *Seaweeds - a field manual*. 1st Ed. National Institute of Oceanography, Goa.1-2.

EL-ZAYAT F. 2012. *Macroalgae and associated harmful microalgae in different habitats along Alexandria coast*. Alexandria: Alexandria University, 129 p.

ERIKSSON B., L. BERGSTROM. 2005. Local distribution patterns of macroalgae in relation to environmental variables in the northern Baltic Proper. - *Estuarine, Coastal and Shelf Science Journal,* 62: 109-117. [DOI]

GIACCONE G., M. CATRA. 2004. Rassegna sugli indici di valutazione ambientale con macroalghe per definire lo stato ecologico delle acque costiere del Mediterraneo (Direttiva 2000/60/CE). - *Biologia Marina Mediterranea*, 11(1): 57-67.

GRASSHOFF K. 1976. *Methods of seawater analysis*. Verlag Chemie, Weinheim/New York, 317 p.

GUIDONE M., C. THORNBER. 2013. Examination of *Ulva* bloom species richness and relative abundance reveals two cryptically co-occurring bloom species in Narragansett Bay, Rhode Island. - *Harmful Algae*, 24: 1-9. [DOI]

HANSEN P.J. 2002. Effect of pH on the growth and survival of marine phytoplankton: implications for species succession. - *Marine Ecology Progress Series,* 28: 279-288. [DOI]

HURTADO A.Q., A.R. RAGAZA. 1999. *Saragssum* studies in Currimao, Ilocos Norte, Northern Philippines. I. Seasonal Variations in the biomass of *Saragssum carpophyllum* J. Agadh, *Saragssum ilicifolium* (Turner) C. Agardh and *Saragssum siliquoseum* J. Agardh (Phaeophyta, Saragssaceae). - *Botanica Marina*, 42: 321-325.

JAMES T. 2007. *Running a biological recording scheme or survey*. A handbook to help scheme or society administrators.

Report, National Biodiversity Network, 61 p.

JHA B., C.R.K. REDDY, M.C. THAKUR, M.U. RAO. 2009. *Seaweeds of India, the diversity and distribution of seaweeds of the Gujarat Coast.* Springer, Dordrecht, Netherlands, 215 p.

KAREZ R., S. ENGELBERT, P. KRAUFVELIN, M.F. PEDERSEN, U. SOMMER. 2004. Biomass response and changes in composition of ephemeral macroalgal assemblages along an experimental gradient of nutrient enrichment. - *Aquatic Botany*, 78: 103-117.

KHALIL A.N. 1987. A list of the marine algae from the Alexandria Coast, Egypt. - *Bulletin of National Institute of Oceanography, ARE*, 13: 229-24.

LAPOINTE B.E., P.J. BARILE, W.R. MATZIE. 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. - *Journal of Experimental Marine Biology and Ecology*, 308: 23-58. [DOI]

LARSEN A., K. SAND-JENSEN. 2006. Salt tolerance and distribution of estuarine benthic macroalgae in the Kattegat-Baltic Sea area. - *Phycologia,* 45: 13-23. [DOI]

LAVERY P. S., R. J. LUKATELICH, A. J. MCCOMB. 1991. Changes in the biomass and species composition of macroalgae in a eutrophic estuary. - *Estuarine, Coastal and Shelf Science Journal*, 33: 1-22. [DOI]

LEE R.E. 1999. *Psychology*. Cambridge University Press, New York, 614 p.

LELIAERT F.R.J., J.J. ANDERSON, BOLTON, E. COPPEJANS. 2000. Sub tidal under story algal community in kelp beds around the Cape Peninsula (Western Cape, South Africa). - *Botanica Marina*; 43: 359-366.

MATHIESON A.C., C.A. PENNIMAN. 1986. A phytogeographic interpretation of the marine flora from the Isle of Shoals, USA. - *Botanica Marina*, 29: 413-434.

MIKHAIL S. K., WAGDY LABIB. 2013. Red tide outbreaks in Alexandria waters: Invasive harmful species. - *Proceeding of The 15th Conference on Harmful Algal Blooms*. Korea Republic, pp. 41-44.

NIELSEN R., A. KRISTIANSEN, L. MATHIESEN, H. MATHIESEN. 1995. Distributional index of the benthic macroalgae of the Baltic Sea area; - *Acta Botanica Fennica*, 155: 1-51.

PARKER J.D., J.E. DUFFY, R.J. ORTH. 2001. Plant species diversity and composition: experimental effects on marine epifaunal assemblages, - *Journal of Marine Ecology Progress*, 224: 55-67. [DOI]

PEDERSEN M.F., L.B. NEJRUP, S. FREDRIKSEN, H. CHRISTIE, K.M. NORDERHAUG. 2012. Effects of wave exposure on population structure, demography, biomass and productivity of the kelp *Laminaria hyperborea*. - *Marine Ecology Progress Series,* 451: 45-60. [DOI]

PRATHEP A. 2005. Spatial and temporal variations in diversity and percentage cover of macroalgae at Sirinat Marine National Park, Phuket province, Thailand. - *Science Asia,* 31: 225-233. [DOI]

SOLTAN D., M. VERLAQUE, C.F. BOUDOURESQUE, P. FRANCOUR. 2001. Changes in macroalgal communities in the vicinity of a Mediterranean sewage outfall after the setting up of a treatment plant. - *Marine Pollution Bulletin*, 42: 59-70. [DOI]

STRICKLAND J.D.H., T.R. PARSONS. 1972. A practical handbook of seawater analysis, 2nd edn., - *Journal of the Fisheries Research Board of Canada,* 167(2): 1-310.

SZÉCHY M. T. M., C. A. G. NASSAR. 2005. Flora ficológica bentônica da Baía da Ribeira, sul do estado do Rio de Janeiro: Avaliação após duas décadas de operação da Central Nuclear Almirante Álvaro Alberto. - In: Pereira R.C. *et al*. (Orgs). - *Formação de Ficólogos: Um compromisso com a sustentabilidade dos recursos aquáticos.* Rio de Janeiro: Museu Nacional, pp. 373-397.

TREVOR J. 2007. *Running a biological recording scheme or survey*. A handbook to help scheme or society administrators. NBN Development Officer for National Societies & Recording Schemes.

VALIELA I., J. MCCLELLAND, J. HAUXWELL, P. J. BEHR, D. HERSH, K. FOREMAN. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. - *Journal of Limnology and Oceanography*, 42: 1105-1118. [DOI]

VASQUEZ J., N. GUERRA. 1996. The use of seaweeds as bioindicators of natural and anthropogenic contaminants in northern Chile. - *Hydrobiologia*, 326-327(1): 327-333. [DOI]

WELLBURN A.R. 1994. The spectral determinations of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. - *Journal of Plant Physiology*, 144: 307-313. [DOI]

WELLS E., M. WILKINSON, P. WOOD, C. SCANLAN. 2007. The use of macroalgal species richness and composition on intertidal rocky seashores in the assessment of ecological quality under the European Water Framework Directive. - *Journal Marine Pollution;* 55(1-6): 151-161. [DOI]

WERNBERG T., S. CONNELL. 2008. Physical disturbance and subtidal habitat structure on open coasts: effects of wave exposure, extent and intensity. - *Journal of Sea Research*, 59: 237-248. [DOI]

WORM B., B. EDWARD, N. BEAUMONT, J. DUFFY, F. CARL, S. BENJAMIN, B. JEREMY. 2006. Impacts of biodiversity loss on ocean ecosystem services. - *Science* 314: 787-790. [DOI]

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Synopsis

Food – A Resource and Waste, from Ecological and Social Aspects

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Abstract. The purpose of this synopsis is to trace the important issue of food as a resource and waste from an ecology point of view and their interconnection with social ecology, to present important contemporary aspects in understanding this issue and to identify approaches in creating ecological and social attitude toward food. This research focuses on the processes of food waste reduction. Apart from the purely quantitative (material) aspects, it draws our attention to the immaterial side of waste, which has to do with our value system.

Key words: Ecology, food, waste, values, attitudes, sociology.

Introduction

Mankind has rarely managed to achieve balance among adequate food production and food distribution, because of the fact, noted by Stuart (2009) that in some countries there are 200 % more food than the country needs. It is necessary to create safe financial mechanisms, market access and equal opportunities for the agricultural production, to meet world food needs (Quinn & Bencko, 2013). Nowadays, there is an ever-growing private sector looking for an impact on the public sphere.

Food has always been essential to the human population throughout the ages, and procuring it is a socially determined process. Although it is one of the most important ecological factors, food reproduction, processing, consumption and transformation are socially mediated (Dimitrov, 1983). In his work, Vernadskii (1967) expresses his view on food having a tendency of transforming people from socially heterotrophic beings to socially autotrophic ones.

In 1927, Elton (1927) states that: "Food is the main factor in an animal community and its complete structure and the activities of this community depend mainly on the available food". In the course of various life processes, food performs a complex flexing, structuring and shaping role.

According to the classical ecology, life depends on the physical environment and the continuous exchange of substances and energy in the natural ecosystems. Odum (1971) defines the ecosystem as "any unity between living organisms in a particular level and their interactions with the physical environment, causing energy flow to create a

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precisely defined trophic structure, species diversity, and recycling of minerals"; the ecosystem is the main functional unit in nature. The author explains that in the processes of the transformation of food resources, a large number of complex chemical, physical and biochemical transformations are performed, causing potential energy to be released and converted into thermal, mechanical and partially into electric energy, later used by the body for its motor and labor activities, to maintain body temperature and for the normal functioning of cells, tissues, organs and systems, in all living organisms.

Various interactions perform biochemical transformations and energy transfer from one organism to another precisely through the processes of feeding (BOGOEV & KENAROVA, 2009). From a functional point of view, the natural ecosystem consists of: energy flow, food webs, the structure of its components, recycling of minerals, development and evolution, as well as their management. Nature has developed a number of strategies to get rid of the unnecessary quantities, and it does not produce "waste" but ensures their conversion into new inert material, as noted by LEIMGRUBER (2015).

Throughout human history and its accompanying civilization processes, different types of waste have been created. Parallel to the increase in volume, their variety, complexity, toxicity and the associated difficulties and costs of disposal are increasing. Traditional methods and technologies, such as landfilling or incineration, are expensive, inefficient, unsustainable, and quite often dangerous to human health. From the anthropocentric point of view, waste is a material (solid, liquid or gaseous) that is no longer wanted or cannot be used (UNSD, 1997). People develop behavior in the "not-in-my-yard" style, because of which they perform uncontrolled polluting activities.

In the 20th century, with the advancement of the economy, surpluses of food products treated as waste were emerging. Food waste was found to form and to be a major component of solid waste in municipal landfills (STEPTOE, 1995; SCHEIBEHENNE *et al.*, 2007). This is not just an ethical issue, it is clear that the loss of edible food leads to negative economic, environmental and social consequences (TOBLER *et al.*, 2011).

Material and Methods

The available research on the problem under consideration and the legislation in force in Europe and Bulgaria are the basis on which this article was prepared. The relationship between two independent sciences - ecology and sociology is considered in order to enhance any preventive activity, environmental culture and education for food waste reduction. A worldwide legal framework has been established to regulate the production, consumption, packaging, transport, classification, and grading of food and food products. Legislative acts with a number of rules and regulations have been developed and adopted for each of the Member States of the European Union. However, the literature does not clarify the general definition of the term "Food waste" and does not provide consistent data on the amount of waste generated during the different phases of the so-called "Food Supply Chain" (GARRONE *et al.*, 2014; MOLLER *et al.*, 2014; FALASCONI *et al.*, 2015). There is a general consensus that in the developed countries most of the food waste is produced down the chain (FAO, 2011; LIPINSKI *et al.*, 2013; ÖSTERGREN *et al.*, 2014; GARRONE *et al.*, 2014) and that on the territory of Europe thousands of tons of usable food is thrown away.

The article uses the terms "waste" and "food" whose interpretation is given according to current regulations.

In the Ordinance № 2 of 23.07.2014 on the classification of waste, promulgated, SG, no. 66 of 08.08.2014 specifies the conditions and the order of classification according to types and properties that can ensure ecological management in compliance with the Waste Management Act – WMA (Ordinance № 2, 2014). It applies to substances, objects or parts of objects corresponding to the definition of waste within the meaning of § 1 (17) of the Additional WMA Provisions, according to which "Waste" is any substance or object which the holder has released or intends to release, or is required to release.

In the European Union Regulation, the term "Food" and the embedded meaning in it are described in Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28th January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (EC, 2002). An analogue interpretation has been carried out in our current Food Law in Bulgaria, with the latest amendment in State Gazette, issue 92 from 17th November 2017; it set out the general principles and requirements of food law, as well as the establishment of a European Safety Authority and the introduction of procedures relating to them (Foods Law, 1999-2017). Article 2 and the two legislative acts state the following:

- For the purposes of this Regulation, *"Food"* (or *"Food Product"*) means any substance or product, whether processed, partially processed or raw, which is intended for or reasonably expected to be suitable for human consumption. The term "Food" includes beverages, chewing gums, and any substances, including water, which are intentionally incorporated into food in the process of manufacturing, preparation or treatment. It also includes water after the point of compliance, in accordance to Article 6 of Council Directive 98/83/EC and without prejudice to the requirements of Directive 98/83 and EC Directive 80/778/EEC (EC, 1998).
- The term '*Food*' does not include:

  * fodder;

  * livestock, unless prepared to be placed on the market for human consumption;

  * plants before harvesting;

  * medicinal products within the meaning of Council Directives 65/65/EEC (EC, 1965) and 92/73/EEC (EC, 1992);

  * cosmetics within the meaning of Council Directive 76/768 /EEC (EC, 1976);

  * tobacco and tobacco products within the meaning of Council Directive 89/622/EEC (EC, 1989);

  * narcotic or psychotropic substances, within the meaning of the "Single convention on narcotic drugs of 1961", amended by the 1972 protocol amending the "Single convention on drugs of 1961" (UNTC, 1972) and the 1971 "United nations convention on psychotropic substances" (UN, 1971);

  * residues and pollutants.

Social Ecology – Present and future

The human population, as a complex of the global macro-system and biosphere, as well as of the regional and local ecosystems, and through food and metabolism, the flows of energy, is in constant interdependence and relationship to its structure and functioning, which was examined by Dazho (1975). By consuming food produced by the producers and by a number of consumers of I, II and III level, the human appears as a consumer who can occupy a different trophic level (Owen, 1981).

Modern society, characterised by increasing globalisation and high-tech industrialisation, is moving further away from nature and from nature's naturally occurring processes (Mantarova, 2010). The environmental problems of the present are not only severe but also multilateral (Vladimirov, 2009). Climate change is real and already happening. Consumers' behaviour of a part of the mankind generates significant amounts of waste, including food waste. Food waste decompositions lead to the release of methane, as a strong "greenhouse effect causing" gas is 21 times stronger than carbon dioxide and its accumulation leads to the loss of many non-renewable resources.

Overproduction and consumer policy lead to over-accumulation, which has increasingly strong and negative effect and becomes a real problem relayed to food supply to mankind. Food wasted by some, while others die of hunger reported by FAO (2014) is common. Worldwide, between 30 and 50% of all food intended for human consumption is lost (LUNDQVIST *et al.*, 2008), creating the astonishing 1.3 billion tons of waste per year (GUSTAVSSON *et al.*, 2011). In fact, almost a billion people could be saved from malnutrition with less than a quarter of the food that is lost (FUSIONS, 2015).

This situation requires a new environmental policy and culture, as well as a different attitude and behaviour of consumers in their environment (DIMOV & MANTAROVA, 2010).

The person is a social being and is actively involved in the Food Safety (FAO, 2002). DIMITROV (1983) points out the issue of emerging of new environments; these are in fact the first artificial ecosystems or the so-called "agro-ecosystems", in which necessary conditions are created to meet the food and food needs of the human society. The agro-ecosystems are manageable and the interference in them is continuous and aims to maximise production and achieve population satisfaction with food. As a result of this, the fine balance in the distribution of a number of important elements such as C, N, P, S is disturbed, which affects and changes the natural recycling of minerals and the basic functions of natural ecosystems.

According to DIMITROV (1983), the high intensity of the substance exchange processes in the agro-ecosystems is associated with a significant accumulation of organic production as biomass that could be used for different purposes by us, human beings.

The success in the field of agriculture and forestry are largely related to the huge energy flows that are imported artificially, in comparison to the natural ecosystems where the processes are self-regulating (ODUM, 1971). At harvesting, 15% of the biomass remains in the form of plant residues that are included in the biotic cycle (DIMITROV, 1983).

Recently, there has been an increasingly close relationship between ecology and the sciences related to the development of human society. As a result of the mutual intertwining of issues from two independent sciences, today we can talk about the emerging of a new science, so-called *Socioecology*; its main subject is the relationship between society and nature, the demographic processes and their projection on macro and micro levels, the density of populated areas and the pressure they put on ecosystems. Food is a relevant issue in socio-economics only in the context of questions that refer to the different behaviours in choosing food and food products and their sensible consumption, and with solutions for their management, effective practices for their use, and specific proposals for reducing food waste.

According to VLACHOV (2011), ecologists are increasingly aware of the need to convince the society that human abuse on the environment is pushing the planet and humanity toward degradation; as a result, a separate science has been developed, called Environmental Sociology. This science is a specific paradigm in sociology that emphasize the issues of nature and society, and it studies the principles, relations, norms, methods of optimization and harmonization of the noosphere interactions (BONEVA, 2011). It also could be described as a science that focuses on the system of society and nature, based on the methodological and theoretical sociological reflection on them. It is important to emphasise that socio-ecology is not limited to empirical studies of ecological problems but also to their theoretical understanding as phenomena of social importance (SMOLNIKOV, 2011).

As SAHLINS (1964) insists, unquestionably the most important ecological object is the human being, and as such, they need a favourable surrounding environment, cared for adequately, and maintained through a dialogue between cultures and the environment. That is why, most of the ecological problems are also social (Boneva, 2011).

In the context of these issues, different adaptive strategies are established, drawing attention to the relations among people, human societies and their natural social and artificially created environments (Dimov & Mantareva, 2010), and placing emphasis on the welfare of individuals, families, communities. It is assumed that the welfare could be achieved through well-developed models in education for the different age groups (Panayotova & Vakleva, 2011), and by imposing strict preventive measures and by adopting a person's right to choose and manage their own fate. Serafimova (2013) pointed out that measures and norms for "a good living" have been developed as a result of people's attempts to rethink qualitatively the importance of these interactions. It is of great importance to develop further knowledge and introduce our students to the environmental protection cause, by including wider environmental issues into the curriculum content, by organising and stimulating participation in pro-environmental campaigns, by conveying and disseminating pro-environmental models of thinking and behaviour, related to food and food consumption.

All that shows explicitly that it is necessary for ecology to be perceived as a socio-cultural practice of the future.

Factors influencing decision-making on the use of food

Consumer's perception of food is a phenomenon influenced by a wide range of characteristics. It could give us better knowledge of how to deal with wasteful behaviour and allow us to design effective prevention of food waste.

In order to understand how the behaviour, mindset, and culture of the individual affect the choice of usable food products to be disposed of, several different factors are considered, and according to Contento (2010), they could be divided into 4 main categories:

- biological predispositions – taste, hunger, satiety mechanisms, sensory-specific satiety;
- sensory-affective factors – past experience associations with food, on physiological and social level, related to determinants - beliefs, norms, mindset, and skills that we have developed throughout our lives;
- social determinants - building a relationship through the cultural, economic and informational environment in which we currently live (e.g. availability of food, public policy, time, cost and media);
- economic factors - food price, time, resource.

Generating waste is a natural consequence of our human lives and day-to-day activities. Food Sale Services are in a very convenient position to focus on the research of the causes of food waste generation, but there is not much literature about them. Their research is mainly on food waste. It focuses on measuring the ratio between served and unconsumed food, as well as overproduction of prepared and unused food (Van Birgelen *et al.*, 2009).

The reasons behind food being turned into waste have not been studied well enough yet. As Hernandez-Carrion (2014) points out that the role of the consumer for the market significance of a particular product is very important. Research in this field shows that sensory factors, food attractiveness, consumer health, convenience and price are the most important factors for choosing a product, as well as the factors related to the product itself (i.e. its internal and external properties).

The motivation behind the choice of food can be influenced by consumer's interest in general health, their awareness in personal health care, the appearance and pleasure the food product delivers, the ideological reasons, the price of the product and the eating habits of an individual (JOHANSON *et al.*, 2011).

Undoubtedly, it influences the consumer's purchasing decisions (YADAVALI & JONES, 2014). Consumers rely on the mass media for a lot of the information about the various products; the media is an endless source for creating incentives for the purchase of new quantities and types of goods. They often present controversial information about food, nutrition, and health, as well as the introduction of different beliefs and mindset in a healthy lifestyle and eating (JOHANSON *et al.*, 2011). Advertising is also an important factor influencing the reallocating of costs and the decision of making certain purchases, but it is not always in the consumer's interest (YADAVALI & JONES, 2014).

The growing economic parameters is decisive and improves the standard of living for one part of the population for the expense of another part of it; that is reflected in the increase in consumption and the growth of waste generated by industries and households (GRAZHDANI, 2016). On the other hand, the income of the consumer has a significant connection to their purchase choices and is an important factor in shaping their consumer's behaviour, related to the generation of food waste. It is noticed to impact not only the food choices but also affect their subsequent behaviour in the generating of household food waste (COX & DOWNING, 2007).

Income dynamics predetermine also the impact on decision-making related to the quantity, quality, and safety of food products choice, which changes in the face of economic crises. The relationship between the personal income of an individual consumer and the household waste from food products they generate is not yet well studied.

Many studies observe that improved living conditions and rise in the income lead to a reduction in the negative effects of human consumption on the environment, but ANDREONI & LEVINSON (2001) and PLASSMANN & KHANNA (2006) do not share this theory and point out a significant number of conflicting situations. According to LEVINSON (2002) and STERN (2004), the "Poverty impacting the environment" hypothesis is not based on easily summarised assumptions. The obtained model "income - natural recourses" often reveals a very simple "cause-and – effect" relationship (GALEOTTI, 2007; CHOUMERT *et al.*, 2013). That leads to the important research question that needs to be addressed: how the environmental food waste policies interfere with people's lives and how they influence the forming of specific consumer attitude to food waste.

Factors influencing the sustainable choice

Geopolitical instability, human conflicts, economy and markets manipulations can cause significant food security problems as pointed out by (QUINN & BENCO, 2013). The ecologic integrity reflects the sustainability of the environment. The sustainable lifestyle adopted by man is related to the use of natural and personal resources, with the aim to improve their relations and actions with the surrounding environment as well as the cultural processes and their driving factors.

By eliminating the social gap between rich and poor and embracing food management as a resource rather than waste, the negative impact on the environment will be reduced. This requires active efforts to change. For billions of people on Earth, poverty is inversely proportional to food security; in unstable and poor countries – members of the EU, the institutions derive wealth and health instead of providing it (QUINN & BENCKO, 2013). Excessive consumption in both developed and developing countries is a leading factor.

The World Summit on Environment and Development in Rio de Janeiro in 1992 provides a great opportunity to define the conditions for sustainable development and food safety. "Food safety is guaranteed when all people have the physical and average economic opportunity to obtain food at all times, in safe and sufficient quantities, to meet their dietary needs and to allow them to lead an active, complete and healthy lifestyle" (GOLLIN *et al.*, 2005).

Food safety is a fundamental human right that is achieved through availability, accessibility and use of a sufficient quantity of healthy, suitable and nutritious food. Food extended life means preserving to a certain degree the prosperity of all, which should be constant in time.

The Concept for Sustainable Development helps to integrate specific limitations in the balanced management (TILMAN *et al.*, 2002). The safety objectives are numerous. The most important ones, according to VEREIJKEN (1992) are:

1. Support of organizations and communities in their development, as well as of organizations dedicated to education and raising people's awareness, related to improving the living conditions of the human population;

2. Implementation and management of food production programmes, food distribution, and food access;

3. Development of tools and methods for food safety management and environmental protection.

Food is considered as an integral part of the human right to life. Considering the interrelations with the socio-cultural and management processes, the process of balancing economic growth, social development and nature conservation must be regarded. The most recent sustainability studies emphasise mainly on the concentrated efforts in the areas related to sustainable energy and water consumption or the resources consumption (BRYNJARSDÓTTIR *et al.*, 2012). The United Nations Environment Programme (UNEP) aims to raise people's awareness about food value and its impact on the environment, as well as to redirect food consumption models to foods requiring fewer resources and as a consequence to trigger a change in the behaviour of their use.

Unfortunately, good intentions are not always reflected in everyday practices (VERMEIR & VERBEKE, 2008). It is generally known that the individual mindset and behaviour are the most difficult to change. In order to achieve a change of behaviour it is important to turn to important factors as personality traits, eating habits and the effectiveness of consumer practices (VERMEIR & VERBEKE, 2008), rating the knowledge, the level of education, the general beliefs and the environmental concerns (MILFONT *et al.*, 2006; LAIDLY, 2011).

That is how we face the problems of the individual attitude and behaviours, the way the change of common values would affect the public attitude toward protecting nature and food as a resource.

In order to make the relation "Values-Context-Behaviour" work, two types of behaviour could be differentiated:

- Enforcing those values that can be achieved and underestimating those that could be blocked;
- Prioritising values that are blocked could leave to an increase in their subjective significance, due to the inability to achieve them.

People often have different values. Very often, mass culture and media create wrong models of personal growth and prosperity. Communities with lower social standing copy those models, but it is more likely their behaviour to have modified values, and according to PAUNOV (2010), the value priorities influence behaviour. They must be activated by accessible and meaningful goals for the person, which should be manifested in a particular motivated behaviour in everyday life. Turning value into a motif under the influence of various factors, beliefs, expectations, mindset will affect the consumer's specific day-to-day actions and behaviour. Knowledge, beliefs, mindset and relationship to food are the results of cultural origin and eating habits established from childhood, but also of the constant flow of information related to food that surrounds us every day (JOHANSON *et al.*, 2011).

Undoubtedly, variables such as household income, number of children, gender, and age structure affect not only the individual risk perception and their attitude toward risk (VARELA & FISZMAN, 2013), but also individual behaviour and choice, related to food and food waste generation.

There is a growing interest in science, focusing on the research of technologies that could improve the awareness and efficiency in obtaining foods resources and could prevent food loss by consumers in everyday life. Researchers report that as the development of technology and technological processes advance increased consumer interest has been noticed toward those associated with safe food production (ERGONUL, 2013), followed by tendencies in increasing consumer requirements (CARDELLO *et al.*, 2007).

A number of studies reveal that women achieve a higher degree of concern and openness in sustainable behaviour (LUCHS & MOORADIAN, 2011). That could also be observed in families that share a positive attitude toward sustainable behaviour (GANGLBAUER *et al.*, 2013), which also depends on the social class (LAIDLEY, 2011).

In this respect, we maintain that the social inequalities in the society determine the differences in the distribution of the environmental risks benefits and damages, in relation to food waste in their identification, assessment, and response; these risks create new inequalities which top the already existing ones and reinforce them.

Factors influencing our behaviour on forming food waste

There are studies on the relevant factors that influence the generation of food waste and the development of various preventive measures and strategies against it. Knowing them well and turning them into a specifically motivated behaviour would have a significant impact on the amount of waste generated. Causes for food waste are found at every stage on the way of the food resources and ready-made food.

COHEN (2008) describes that smell and observation of delicious food activate the neurons that release dopamine, a neurotransmitter that promotes appetite. Hunger can lead a person to over-replenishing, by consuming large amounts of food. The food excess automatically becomes food waste. In this sense, inaccurate perception is the reason for food waste generation. In the world, twice as much food is still produced than it is required for individual needs (FOX & FIMECHE, 2013). Approximately half of the food waste in developed countries is generated by consumers, and in particular by households. Menawhile 60% of the food losses could be avoided and that 20% of the discarded foods are due to date errors on the labels of the products.

The physical and mental development in early childhood is remarkable. "Likes and Dislikes" are formed as part of the developing sense of self-love and taste (SAKAMOTO, 2001). The earliest childhood is the most important time to establish eating habits. YAMAMOTO (2008) suggests that tastes are acquired through child experience and learning, although they are given by nature. OGAWA (2003) describe how taste appraisals are generally not present in children but can be learned over time by being introduced to dishes designed to enhance their taste. ANDO (2003) describes how childhood tastes evolve very similar to the way speech does, in other words, the greater the variety of foods experienced during their childhood, the wider the tastes of the child.

Some researchers show interdependence between the opinions and food preferences of parents and their children (BIRCH, 1980; ROZIN, 1984). The hypothesis is that the food preferences of mothers are related to the liking and dislike of certain foods of their children; in its support, PLINER & PELCHAT (1986) report that children inherit the preferences of their parents. Younger people seem to be less aware and concerned about their health than older people are, according to a study by JOHANSON *et al.* (2011).

Food losses occur at all stages of the food supply chain and the food losses generated are around 40% of the total food production (GUSTAVSSON *et al.*, 2011; BERETTA *et al.*, 2013). Calculations accumulated since 1974 to date show a 50% increase in edible food losses across all food supply chain units. The poor logistics cannot be justified ethically and ecologically when it comes to delivering food to the different food serving establishments: restaurants, canteens, schools and nurseries, hospitals, nursing homes, etc.

In each of the sectors, there is consumer behaviour leading to the disposal of entirely edible food. EU data show that 30% of fruit and vegetables are not reaching the shelves of hypermarkets because of their unattractive appearance and because of their "unsellable with regard to the consumer preferences" due to their shape and colour.

There is also a similar practice seen in harvesting and food processing, as losses for Europe alone are estimated at 4 billion Euros per year. Food losses are generated in the agricultural and commercial sector because of improper storage, labelling errors, expired shelf life, damaged packaging, non-marketable appearance, and poor consumer habits when purchasing, storing and using the food products. Store and consumer food losses are estimated at 179 kg per inhabitant annually in Europe.

A recent FAO (2011) report on food losses and food waste on a global level shows that North Americans waste 95 to 115 kg food per person annually; Africans, south of the Sahara, - 6 to 11 kg; that is in a relation to 925 million people in the world living with a risk of malnutrition. The US Department of Agriculture claims that 27% of the food produced or imported in the country is disposed in landfills, directly or as waste.

The European Commission estimates the annual food waste in the 27 EU Member States altogether to be at 89 million tones or 179 kg per single person; that is without taking into consideration the losses at the agricultural and fisheries activities level, as per the report on "How to avoid food waste: strategies for a more efficient food chain in the EU" (2011/2175 (INI), Committee on Agriculture and Rural Regions Development (EP, 2011). In their study, GANGLBAUER *et al.* (2013) trace how the economic factor could help the attempts to reduce food waste, resulting in the daily cost reduction. With the help of such publications and the increase of information provided, it is expected that the percentage of households, influenced to recycle, will increase; and it is essential, in the process of reducing food losses and achieving better recycling and less landfilling. The knowledge and skills related to the use of appropriate refrigeration equipment and the proper storage of food and food products, as well as meal planning and the preparation of shopping lists can greatly reduce the amount of food discharged.

Suggested and current solutions for reducing food waste

Preventing generation of waste means avoiding waste by not manufacturing products which then must be recycled or disposed of safely in accordance to the Waste Prevention Policy laid down by the European legislation. A significant impact on the efficiency of specific coordinated strategies to reduce food waste could be achieved by carefully researching the food waste generation process at each stage and sector, which participate in a different way in the food supply chain, and by using shorter logistics routes between the different stages of food production management. In the EU acts a Retail Market Forum, in direct relation to sustainability, which includes the European Round Table on Sustainable Food Production and Consumption, the High-Level Forum for a better functioning food supply chain in Europe, an informal network of Member States "Friends of Sustainable Food", Consumer Goods Forum and many others. The exchange of good practices in the European Union and the use of global experience could have an impact on the efficiency.

As set out in EU legislation, it is necessary to reduce and minimize the waste resulting from the use of inappropriate and problematic methods of post-harvest agricultural production and management; to provide high-tech and infrastructure improvements in processing and packaging. By reducing the price of fresh food below its cost before its expiry, it will enable lower-income consumers to buy higher-quality goods at lower prices.

In order to avoid unnecessary waste, it is essential to update the already established rules for waste control, as well as the classifications and the standards for their appearance, shape, and size of fresh fruit and vegetables, because in many cases they are the cause of food disposal and food waste; it is also important to optimize the most suitable storage temperature.

It is important to emphasize the importance of including traders in food redistribution programs, targeting low-income citizens and food sector manufacturers, related to the supply of different pack sizes, and even to rethink the benefits of single item sales for single - person households; it would be extremely useful to tolerate public procurement that would help and guarantee free reallocation of unsold food products in the shops to those malnourished people in the society who cannot afford them.

The National Waste legislation of all Member States should be tuned to the EU Waste Management legislation, in order to define the responsibilities of all beneficial countries. All EU Member States have to achieve the same objectives, although the differences in their industry indicators vary in different countries.

The state and the municipalities with the relevant municipal councils, the economic and scientific structures, the non-governmental organizations and households, all participate in the waste generation. In many eastern European countries, waste accumulation control and levels of recycling are still insufficient. Ecological modernization is slowed down by the lack of resources (social, administrative, etc.), and the environmental culture can be identified as a post-modern phenomenon, which is an important part of the socio-culture and play a different role, depending on the social development, as DULOV (2010) notes.

Introducing households from different social groups and different cultures in the waste distribution requires individual efforts (KARIM GHANI *et al.*, 2013).

Four types of measures should be used when motivating citizens to pursue pro-environmental behaviour:

- administrative measures (legal obligations);
- economic measures (fees and taxes);
- physical measures (e.g. recycling bins and frequent waste collection);
- information (campaigns and guidelines).

The different instruments mentioned above should be applied combined, however, a number of studies show that they could be inefficient in waste management practice at national, regional and local levels. It is noted that different peculiarities at individual levels may prevent participation in the recycling collection – that will be the purpose of our forthcoming research in particular.

By improving consumers' perceptions (i.e. perceived efficiency) and widening their knowledge (sales dates, use of refrigerators, meal planning, shopping lists and visibility of food costs), the individual consumer can be influenced. According to GRISKEVICIUS et al. (2012), in order for the impact strategies to have an optimal effect, they have to work *with* and not *against* developed trends. They should trigger unconscious and impulsive attraction associations and to influence quickly. KINASZ *et al.* (2015) sets the task of developing a list of good management practices that have a positive impact on the elimination or minimisation of food waste.

The most commonly used approaches are awareness raising at schools or public campaigns where consumers are informed about topics such as food purchase, storage, preparation or actual shelf life. Higher education has a positive correlation with healthy patterns (Bus & Worsley, 2003). Women are those who have a higher awareness of possible nutritional threats, similar to those with higher education and high incomes (Stobelaar *et al.*, 2006; Shafie & Rennie, 2012). Groups with a higher economic status are more motivated to adopt certain food and nutrition ideologies, while those with a lower status focus on convenience, price and product knowledge.

Conclusions

Food waste is not the main cause of hunger in the world, but its generation is a global problem and is becoming the source of a number of environmental, economic, moral and ethical issues. Human society and its accompanying civilization processes face a real challenge, whose solution could be found through the methods and programmes of education. The development of pro-ecologic attitude and behaviour should begin with examples in homes and families that will find their natural continuation in the areas of further education and work. It is behind the necessity for the development of specialized programs designed for the various stages of school-age education, which aim to create a sustainable attitude towards the environment and shape healthy pro-environmental behaviour that will become a priority value in their everyday activities, related to environmental protection. It is necessary and imperative to promote scientific and civic culture and awareness of the causes of waste and the consequences it brings. It is desirable and useful to exchange good practices at European and international level by the principles of sustainable development and solidarity, to improve the preservation, and the "non-marketable" goods to be put into food banks, public kitchens and charitable aid.

References

Ando S. 2003. What Is a Balanced Diet? - In: Mebaesya Food Culture Desk, Ed., *Unbalanced Diets and Disliking Vege - Tables in Children*, Mebaesya, Tokyo, pp. 17-28.

Andreoni J., A. Levinson. 2001. The simple analytics of the environmental Kuznets curve. - *Journal of Public Economics,* 80(2): 269-286 [DOI]

Beretta C., F. Stoessel, U. Baier, S. Hellweg. 2013. Quantifying food losses and the potential for reduction in Switzerland. - *Waste Manage*, 33(3): 764-773. [DOI]

Birch L.L. 1980. The Relationship between Children's Food Preferences and Those of Their Parents. - *Journal of Nutrition Education*, 12:14-18. [DOI]

Bogoev V., A. Kenarova. 2009. [*Fundamentals of Ecology*], Pensoft - Sofia-Moscow, 575 p. (In Bulgarian).

Boneva M. 2011. [*Social ecology and globalization*.], Faber 2011. Veliko Tarnovo. (In Bulgarian).

Brynjarsdóttir H., M. Håkansson, J. Pierce, J., E. Baumer, C. DiSablo, P. Senders. 2012. Sustainably unpersuaded: how persuasion narrows our vision of sustainability. - In: *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems,* Session: Critical Perspectives on Design, CHI 2012, May 5–10 2012, Austin, Texas, USA, pp. 947-956. Available at: [stackpathdns.com].

Bus A., A. Worsley. 2003. Consumers' Health Perceptions of Three Types of Milk: A Survey in Australia. - *Appetite*, 40(2): 93-100. [DOI]

Cardello A.V., H.G. Schutz, L.L. Lesher. 2007. Consumer Perceptions of Foods Processed by Innovative and Emerging Technologies: A Conjoint Analytic Study. - *Innovative Food Science and Emerging Technologies*, 8: 73-83. [DOI].

CHOUMERT J., P. COMBES MOTEL, H.K. DAKPO. 2013. Is the environmental Kuznets curve for deforestation a threatened theory? A meta – analysis of the literature. - *Ecological Economics*, 90: 19-28. [DOI].

COHEN D. A. 2008. Neurophysiological pathways to obesity: below awareness and beyond individual control. – *Diabetes*, 57(7): 176-873. [DOI].

CONTENTO I. R. 2010. Overview of Determinants of Food Choice and Dietary Change: Implications for Nutrition Education. - In: *Nutrition Education: Linking Research, Theory, and Practice*. Chapter 2. Jones & Bartlett Publishers, pp. 26-42.

COX J., P. DOWNING. 2007. Food behavior Consumer Research - findings from the quantitative survey, Final report, Brook Lyndhurst, WRAP, Banbury, June, Project code: RWM005-001, 43 p. Available at: [warp.org].

DAZHO B. 1975. [*Fundamentals of Ecology*], Moscow, Publ. "Progress" (In Russian).

DIMITROV T. 1983. [*The man in the biosphere.]* Series Man and Nature - 12. State Publishing House "Science and Art" Sofia, 212 p. (In Bulgarian).

DIMOV N., A. MANTAROVA. 2010. [*The development of ecological culture - part of the modernization of the Bulgarian society*.] Sofia. (In Bulgarian).

DULOV VL. 2010. Ecological values and attitudes. Ecological culture in the modernizing society. Sofia, pp. 336.

EC. 1965. Council Directive 65/65/EEC of 26 January 1965 on the approximation of provisions laid down by Law, Regulation or Administrative Action relating to proprietary medicinal products. - *Official Journal of the European Union*, 022: 369-373. Available at: [eur-lex.europa.eu].

EC. 1976. Council Directive (76/768/EEC) of 27 July 1976 on the approximation of the laws of the Member States Member States relating to cosmetic products. - *Official Journal of the European Union*, L 262: 169-200. Available at: [eur-lex.europa.eu].

EC. 1989. Council Directive 89/622/EEC of 13 November 1989 on the approximation of the laws, regulations and administrative provisions of the Member States concerning the labelling of tobacco products. - *Official Journal of the European Union*, L 359: 0001-0004. Available at: [eur-lex.europa.eu].

EC. 1992. Council Directive 92/73/EEC of 22 September 1992 widening the scope of Directives 65/65/EEC and 75/319/EEC on the approximation of provisions laid down by Law, Regulation or Administrative Action relating to medicinal products and laying down additional provisions on homeopathic medicinal products). - *Official Journal of the European Union*, L 297: 0008-0011. Available at: [eur-lex.europa.eu].

EC. 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption). - *Official Journal of the European Union*, L 330: 0032-0054. Available at: [eur-lex.europa.eu].

EC. 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. - *Official Journal of the European Union*, L 31: 1–24. Available at: [eur-lex.europa.eu].

EP. 2011. Report on "How to avoid food waste: strategies for a more efficient food chain in the EU" (2011/2175 (INI)), Committee on Agriculture and Rural Development. Available at: [europarl.europa.eu]

ELTON C. 1927. *Animal Ecology*, Macmillan, New York.

EPL. 2002-2015. [Environmental protection law am. and supplements]. – *State Gazette*, 91/2002-101/2015, Available at: [moew.government.bg]. (In Bulgarian)

ERGONUL B. 2013. Consumer Awareness and Perception to Food Safety: A Consumer Analysis. - *Food Control*, 32: 461-471. [DOI].

FALASCONI L., M. VITTUARI, A. POLITANO, A. SEGRE. 2015. Food waste in school catering: An Italian case study. - *Sustainability*, 7(11): 14745-14760. [DOI].

FAO. 2002. *The state of Food Insecurity in the World 2001*. Food and Agricultural Organization, Rome. ISBN 92-5-104628-X. Available at: [fao.org].

FAO. 2011. *Global food losses and food waste - extent, causes and prevention*, FAO, Rome. ISBN 978-92-5-107205-9. Available at: [fao.org].

FAO. 2014. The State of Food Insecurity in the World 2014. Strengthening the enabling environment for food security and nutrition. Rome, FAO. E-ISBN 978-92-5-108543-1. Available at: [fao.org].

FOODS LAW. 1999-2017. [Foods law]. - *State Gazette*, 90 of 15 October 1999, last amended. SG. no. 92 from 17.11. 2017. Available at: [batel.bg]. (In Bulgarian).

FOX T., C. FIMECHE. 2013. *Global food: Waste not, want not.* The institution of Mechanical Engineers, Tech. Rep. Available at: [imache.org].

FUSIONS. 2015. *FUSIONS Definitional Framework for Food Waste*. World Resources Institute (WRI). Food Loss and Waste Accounting and Reporting Standard. Web page. Available at: [eu-fusions.org].

GARRONE P., M. MEACINI, A. PEREGO. 2014. Surplus food recovery and donation in Italy: the upstream process. - *British Food Journal*, 116(9): 1460-1477. [DOI].

GANGLBAUER E., G. FITZPATRICK, R. COMBER. 2013. Negotiating food waste: using a practice lens to inform design. *ACM Transactions on Computer-Human Interaction*, 20(2): Article No. 11. [DOI].

GALEOTTI M. 2007. Economic growth and the quality of the environment: taking stock. - *Environment, Development and Sustainability*, 9(4): 427-454. [DOI]

GOLLIN D., M. MORRIS, D. BYERLEE. 2005. Technology Adoption in Intensive Post Green Revolution Systems. - *American Journal of Agricultural Economics*, 87(5): 1310-1316. [DOI].

GRAZHDANI D. 2016. Assessing the variables affecting on the rate of solid waste generation and recycling: An empirical analysis in Prespa Park. - *Waste Management*. 48: 3-13. [DOI].

GRISKEVICIUS V., S.M. CANTU, M.V. VUGT. 2012. The evolutionary bases for sustainable behavior: implications for marketing, policy, and social entrepreneurship. - *Journal of Public Policy and Marketing*, 31(1): 115-128. [DOI].

GUSTAVSSON J., C. CEDERBERG, U. SONESSON, R. VAN OTTERDIJK, A. MAYBECK. 2011. *Global food losses and food waste: Extent, causes and prevention.* Food and agriculture organization of the United Nations, Tech. Rep. 4 p.

HERNANDEZ-CARRION M., P. VARELA, I. HERNANDO, S. FISZMAN, A. QUILES. 2014. Persimmon Milkshakes with Enhanced Functionality: Understanding Consumers' Perception of the Concept and Sensory Experience of a Functional Food. *LWT – Food Science and Technology*, 62: 384-392. [DOI].

JOHANSON S., T. NAES, M. HERSLETH. 2011. Motivation for Choice and Healthiness Perception of Calorie - Reduced Dairy Products. A Cross-Cultural Study. - *Appetite*, 56: 15-24. [DOI].

KARIM GHANI W., I. RUSLI, D. BIAK, A. IDRIS. 2013. An application of the theory of planned behaviour to study the influencing factors of participation in source separation of food waste. - *Waste Management*, 33(5): 1276–1281. [DOI].

KINASZ T.R., R.B. REIS, T.B. MORAIS. 2015. Presentation of a Validated Checklist as a Tool for Assessing, Preventing and Managing Food Waste in Food Services. - *Food and Nutrition Sciences*, 6(11): 985-991. [DOI].

LAIDLEY T.M. 2011. The Influence of social class and cultural variables on environmental behaviors: municipal - level evidence from Massachusetts. - *Environment and Behavior*, 45(2): 170-197. [DOI].

LEIMGRUBER W. 2015. The food we Waste - Material and Ethical Issues in

Geography. - *Journal of Agricultural Science and Technology*, B5(2015): 1-14. [DOI].

LEVINSON A. 2002. The ups and downs of the environmental Kuznets curve. - In: List J., A. de Zeeuw (Eds.), *Recent Advances in Environmental Economics*. Edward Elgar Publishing, London, pp. 119-141.

LIPINSKI B., C. HANSON, J. LOMAX, L. KITINOJA, R. WAITE, T. SEARCHINGER. 2013. Reducing Food Loss and Waste. Working Paper. Installment 2 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute. Available at: [worldresourcesreport.org].

LUCHS M.G., T.A. MOORADIAN. 2011. Sex, personality, and sustainable consumer behaviour: elucidating the gender effect. - *Journal of Consumer Policy*, 35(1): 127-144. [DOI].

LUNDQVIST J., C. DE FRAITURE, D. MOLDEN. 2008. *Saving Water: From Field to Fork – Curbing Losses and Wastage in the Food Chain*. SIWI Policy Brief. SIWI. Available at: [indiana.edu].

MANTAROVA A. 2010. *[The Environmental Culture in the Modernizing Society]*. Institute of Sociology at the Bulgarian Academy of Sciences, Sofia. (In Bulgarian).

MILFONT T.L., J. DUCKITT, L.D. CAMERON. 2006. A cross-cultural study of environmental motive concerns and their implications for proenvironmental behavior. - *Environment and Behaviour*, 38(6): 745-767. [DOI].

MOEW. 2010. *[National Strategic Plan for Gradual Reduction of Quantities of Biodegradable Waste for Deposits for the Period 2010-2020 in Bulgaria]*. Ministry of Environment and Waters, Sofia. Available at: [moew.government.bg]. (In Bulgarian).

MOEW. 2014. *[National waste management program 2014-2020 in Bulgaria]*. Ministry of Environment and Waters, Sofia. Available at: [moew.government.bg]. (In Bulgarian).

MOLLER H., O.J. HANSSEN, J. GUSTAVSSON, K. ÖSTERGREN, A. STENMARCK, P. DEKHTYAR. 2014. Report on review of (food) waste reporting methodology and practice, FUSIONS project, Wageningen. Available at: [eu-fusions.org].

ODUM E. 1971. *Fundamentals of ecology*, 3rd ed. Philadelphia. London.

OGAWA Y. 2003. Development of Palate, Taste and Appetite. - In: *Mebaesya Food Culture Desk, Ed., - Unbalanced Diets and Disliking Vegetables in Children*, Mebaesya, Tokyo, 2003, pp. 29-46.

Ordinance № 2 from 23.07.2014 on the classification of wastes, prom. - *State Gazette*, no. 66 of 08.08.2014.

ÖSTERGREN K., J. GUSTAVSSON, H. BOS-BROUWERS, T. TIMMERMANS, O.-J. HANSEN, H. MOLLER, G. ANDERSON, C. O'CONNOR, H. SOETHOUDT, T. QUESTED, S. EASTEAL, A. POLITANO, C. BELLETTATO, M. CANALI, L. FALASCONI, S. GAIANI, M. VITTUARI, F. SCHNEIDER, G. MOATES, K. WALDRON, B. REDLINGSHOLER. 2014. FUSIONS definitional framework for food waste, FUSIONS project, Wageningen. Available at: [eu-fusions.org].

OWEN O. 1981. [*Conservation of natural resources*.], Sofia, Zemizdat. (In Bulgarian).

PANAYOTOVA M., ZL. VAKLEVA. 2011. To the issue of adolescents' awareness of risk factors for food contamination and their impact on human health. - In: *Collection of Reports from the Jubilee National Scientific Conference with International Participation "The Ovew and the Universe"*. 6-8 October, Smolyan, Bulgaria. pp. 197-205.

PAUNOV M. 2010. [*Values and values priorities of the Bulgarians; peculiarities, similarities and differences with other European countries*.] Sofia. (In Bulgarian).

PLASSMANN F., N. KHANNA. 2006. Preferences, technology and the environment: understanding the environmental Kuznets curve hypothesis. - *American Journal of Agricultural Economics*, 88(6): 632-643. [DOI].

PLINER P., M. PELCHAT. 1986. Similarities in Food Preferences between Children and Their Siblings and Parents. - *Appetite*, 7(4): 333-342. [DOI].

QUINN J., V. BENCKO. 2013. Food insecurity: How to orchestrate a global health crisis. - Health, 5(6): 1055-1061. [DOI].

ROZIN P. 1984. The Acquisition of Food Habits and Preferences. - In: Matarazzo J. D., M. S. Weiss, J. A. Herd, N. E. Miller, S. M. Weiss (Eds.), *Behavioral Health: A Handbook of Health Enhancement and Disease Prevention*, Wiley, New York, 1984, pp. 590- 607.

SAHLINS M. 1964. *Culture and environment: The study of cultural ecology.* Horizons of anthropology. Chicago: Aldine. Shah, H., pp. 132-147.

SAKAMOTO M. 2001. *A Guide to Children's Nutrition and Food Education.* Ishiyaku Publishing Inc., Tokyo, pp. 102-103.

SERAFIMOVA M. 2013. [Religion or Ecology]. Neofit Rilski University, Blagoevgrad. (In Bulgarian).

SHAFIE F.A., D. RENNIE. 2012. Consumer Percepions towards Organic Food. - *Procedia – Social and Behavioural Sciences*, 49: 360-367. [DOI].

SMOLNIKOV N.S. 2012. *About social ecology.* PUNIPU. Social and economic sciences. No. 13 (37). (In Russian).

SCHEIBEHENNE B., L. MIESLER, P. TODD. 2007. Fast and Frugal Food Choices: Uncovering Individual Decision Heuristics. - *Appetite*, 49: 578-589. [DOI].

STEPTOE A., T. POLLARD, J. WARDLE. 1995. Development of a Measure of the Motives Underlying the Selection of Food: The Food Choice Questionnaire. - *Appetite*, 25: 267-284. [DOI].

STERN D.I. 2004. The rise and fall of the environmental Kuznets curve. - *World Development*, 32(8): 1419-1439. [DOI].

STOBELAAR D.J., G. CASIMIR, J. BORGHUIS, I. MARKS, L. MEIJER, S. ZEBEDA. 2006. Adolescents' Attitudes towards Organic Food: A Survey of 15- to 16-Year Old School Children. - *International Journal of Consumer Studies*, 31: 349-356. [DOI].

STUART T. 2009. *Waste: Uncovering the Global Food Scandal.* London: Penguin Books.

TILMAN D., K.G. CASSMAN, P.A. MATSON, R. NAYLOR, S. POLASKY. 2002. Agricultural Sustainability and Intensive Production Practices. - *Nature*, 418: 671-677. [DOI].

TOBLER C., V. VISSCHERS, M. SIEGRIST. 2011. Eating Green. Consumers' Willingness to Adopt Ecological Food Consumption Behaviors. - *Appetite*, 57: 674-682. [DOI].

UN. 1971. Convention on Psychotropic Substances, 1971. Available at: [unodc.org].

UNSD. 1997. Glossary of Environment Statistics. - United Nations Statistics Division, Glossary of Environment Statistics, Series: F, No. 67. Available at: [unstats.un.org].

UNTC. 1972. "Single convention on narcotic drugs of 1961", amended by the 1972 protocol amending the "Single convention on drugs of 1961". Geneva, 25 March 1972. - *United Nations, Treaty Series*, vol. 976, p. 3. Available at: [treaties.un.org].

VARELA P., S. FISZMAN. 2013. Exploring Consumers' Knowledge and Perceptions of Hydrocolloids Used as Food Additives and Ingredients. - *Food Hydrocolloids*, 30: 477-484. [DOI].

VAN BIRGELEN M., J. SEMEIJN, M. KEICHER. 2009. Packaging and Proenvironmental Consumption Behavior: Investigating Purchase and Disposal Decisions for Beverages. - *Environment and Behavior*, 41: 125-146. [DOI].

VEREIJKEN P. 1992. A Methodic Way to More Sustainable Farming Systems. - *Netherland Journal of Agricultural Science*, 40: 209-224.

VERMEIR I., W. VERBEKE. 2008. Sustainable food consumption among young adults in Belgium: theory of planned behaviour and the role of confidence and values. - *Ecological Economics*, 64(3): 542-553. [DOI].

VERNADSKII V.I. 1967. *Biosphere.* Moscow. (In Russian).

VLADIMIROV L. 2009. [*Risks to environmental security.*] VFU "Chernorizets Hrabar". Varna. (In Bulgarian).

VLACHOV S. 2011. [*Ecology.*] Acad. Prof. "Prof. Marin Drinov", Sofia. (In Bulgarian).

WOLSINK M. 1994. Entanglement of Interests and Motives: Assumptions behind the NIMBY-Theory on Facility Siting. - *Urban Studies,* 31(6): 851-66. [DOI].
WMA. 2012. Waste Management Act. - *State Gazette.,* No. 44 of 12 June 2012, rev. SG. No. 53 of 13 July 2012.
YADAVALI A., K. JONES. 2014. Does Media Influence Consumer Demand? The Case of Lean Finely Textured Beef in the United States. - *Food Policy*, 49: 219-227. [DOI].
YAMAMOTO T. 2008. *Children's Plate and Food Likes/Dislikes.* 2nd Edition, Health Education, pp. 10-14.

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ECOLOGIA BALKANICA - INSTRUCTIONS FOR AUTHORS - 2017

General information

Submissions to "*Ecologia Balkanica*" can be original studies dealing with all fields of ecology, including ecology and conservation of microorganisms, plants, aquatic and terrestrial animals, physiological ecology, behavioral ecology, population ecology, population genetics, community ecology, plant-animal interactions, ecosystem ecology, parasitology, animal evolution, ecological monitoring and bioindication, landscape and urban ecology, conservation ecology, as well as new methodical contributions in ecology. **The journal is dedicated to publish studies conducted on the Balkans and Europe.** Studies conducted anywhere else in the World may be accepted only as an exception after decision of the Editorial Board and the Editor-In-Chief. ***The Editorial Board of "Ecologia Balkanica" reserves its right to reject publication of any manuscript that does not fit the aim and scope or does not comply with these instructions.***

Manuscript submission

The following types of manuscripts are accepted: *short research notes* (up to 4 pages), *research articles* (4 to 10 pages) and *review papers* (10 to 20 pages). *Short research notes* are shorter submissions of a preliminary nature or those including new records or observed phenomenon, etc. *Research articles* should present significant original research in the various fields of ecology, mentioned above. *Review papers* should deal with topics of general interest or of contemporary importance, being synthetic rather than comprehensive in emphasis. Authors of Review papers should consult with the Editor before submission. The Editor may also invite review articles concerning recent developments in particular areas of interest. The Editor reserves the right to decide if a manuscript should be treated as a Short note or Research article. In general, studies that are purely descriptive, mathematical, documentary, and/or natural history will not be considered.

Manuscripts must conform strictly with the instructions to authors and sent to the Editor. **<u>All manuscripts must be accompanied with a cover letter, signed by ALL authors,</u>** 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.

Legal requirements

Ecologia Balkanica follows the standards for Ethics and Publication Malpractice set by the Committee on Publication Ethics (COPE). Conformance to standards of ethical behavior is therefore expected of all parties involved: Authors, Reviewers, Editors, and the Publisher. Submission of a manuscript implies: that the work described has not been published previously (except in the form of an abstract, or as part of a published lecture, or thesis); that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities - tacitly or explicitly - at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Manuscript preparation

Language

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.
Affiliation(s) with postal address: 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. **<u>Avoid presentation of the same information as text and/or figure and/or table.</u>**
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 ($\mathbf{H}_{\text{burned}}$) and control ($\mathbf{H}_{\text{control}}$) 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. *When the figures are presenting maps of the studied area, we recommend using some kind of GIS software for the preparation of the maps, or use of other indicative or topographical maps.*

Satellite or aerial photos (especially from Google Earth) of the studied area will no longer be acceptable! **<u>All figures must be placed within the text at the desired position by the author(s).</u>**

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. **<u>Please provide DOI numbers for all electronic resources (journal articles, online articles, books), if they have DOI, when cited in the references list!</u>**

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].

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.)".

Unpublished theses (BSc, MSc, PhD, DSc) are not considered officially published scientific literary sources, therefore from January 2015, "*Ecologia Balkanica*" no longer allows citations of such references.

Citing references that are still "in press" is also considered frown upon, but not forbidden. If possible, please avoid using such references.

For your effortlessness, from January 2015, "*Ecologia Balkanica*" provides an EndNote™ style template based on the Harvard reference style (with modifications) available at our site.

Additional requirements

For special symbols (Greek letters, symbols for male and female etc.) use the Symbol list on the Insert menu in Microsoft Word with the following preferable fonts: Symbol, Webdings, Wingdings, Wingdings 2 and Wingdings 3. Degree symbols (°) must be used (from the Symbol list) and not superscript letter "o" or number "0". Multiplication symbols must be used (×) and not small "x" letters. Spaces must be inserted between numbers and units (e.g., 3 kg) and between numbers and mathematical symbols (+, –, ×, =, <, >), but not between numbers and percent symbols (e.g., 45%).

Nomenclature and units. Follow internationally accepted rules and conventions: use the International system of units (SI). If other quantities are mentioned, give their equivalent in SI. Please use a standard format for the units and be uniform in the text, tables and figures. Please prefer one of two possible styles: "m/s" or "m s-1". When using "m s-1" leave a space between symbols.

Small capitals and italic letters. The Latin genus and species names must be cited completely once in the text and should be typed in *italic*. Family names of the authors in the citations and the publications listed in the reference list must be in SMALL CAPITALS, but never for collectors, preparators, acknowledgements, etc.

Statistics

Mean values should always be accompanied by some measure of variation. If the goal is to describe variation among individuals that contribute to the mean standard deviation (SD) must be used. When the aim is to illustrate the precision of the mean standard errors (SE) should be given. The last paragraph of Materials and Methods section should briefly present the significance test used. Quote when possible the used software. Real p values must be quoted both at significance or non-significance. The use of the sign is acceptable only at low values of p (e.g. $p<0.0001$).

Ethics

The authors of articles that are based on experiments that caused injuries or death of animals should explain and justify the grounds of the study and state that the scientific results of the study is at least in trade-off with the sufferings caused. In the Materials and Methods section of the manuscript, the authors should detail as precisely the conditions of maintenance, transport, anaesthesia, and marking of animals. When available, references should be added to justify that the techniques used were not invasive. When alternative non-harming techniques exist, but were not used, the manuscripts may not be considered for publication.

Proofs and Reprints:

Proof will be sent to the **first (or corresponding) author** for checking (a PDF file) only once and it should be returned without delay. Corrections should be limited to typographical errors. No additional changes of the manuscript are allowed. Following publication, the first (or corresponding) author will be provided with electronic copy (PDF) of the article. Hardcopy reprints are no longer sent to the authors, since 2011.

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