ECOLOGIA BALKANICA

International Scientific Research Journal of Ecology

Volume 4, Issue 2
December 2012

UNION OF SCIENTISTS IN BULGARIA – PLOVDIV

UNIVERSITY OF PLOVDIV PUBLISHING HOUSE

International Standard Serial Number
Print ISSN 1314-0213; Online ISSN 1313-9940

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The current issue of Ecologia Balkanica is published with the financial support of Bulgarian Science Fund, Ministry of Education, Youth and Science, project №НП03 014.

"Ecologia Balkanica" is published with the kind cooperation and support of Student Council at the University of Plovdiv "Paisii Hilendarski"- Plovdiv, Bulgaria.
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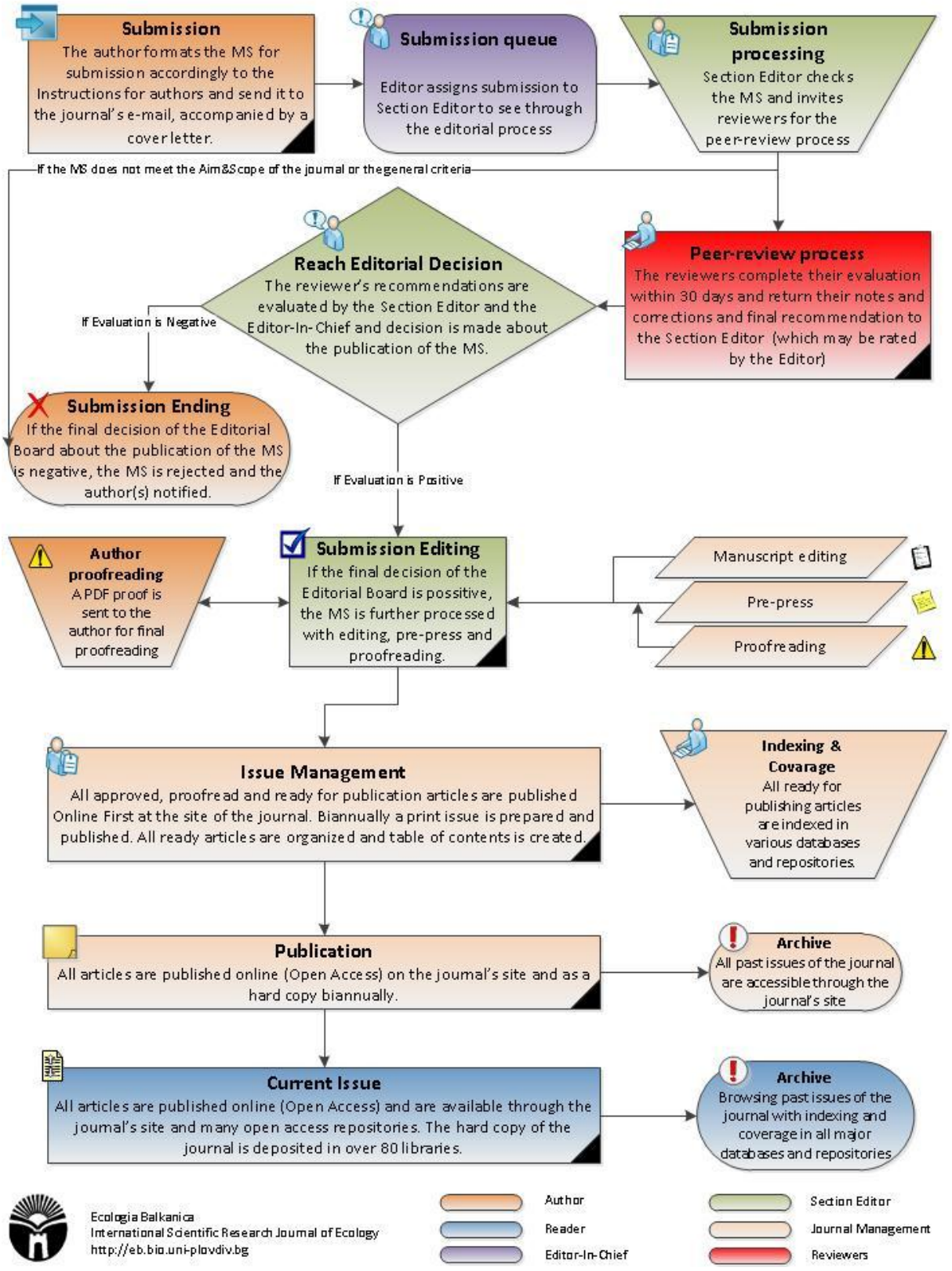

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The Otter (Lutra lutra L.) Signs and the Banks Tree Cover: A Survey in Central and Eastern Bulgaria

Radostina Dimitrova, Dilian Georgiev*

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Abstract. The study was carried out during 2011 – 2012 at different water basins (rivers and micro dams) situated in Central and Eastern Bulgaria. Transects with 600 m length were walked along the banks in otter signs search. The search was stopped when the first otter signs were registered. As a whole we found domination of the very good or excellent bank tree cover. A total of 61% of transects were with tree cover between 50% and 100%. Almost equal were the stretches without or with little tree vegetation. At all transects surveyed the spraints dominated with 61%. All other otter signs were with low percentage. The otter tracks (foot prints) were on second place with 20% of occurrence, followed by the food remains with 7%. At 12% of transects we did not find any otter signs. As a whole the otter was registered in 88% of transects, a sign for a good population. Highest preference we registered for walking or feeding of the otter at the open banks with no or with weak tree cover.

Key words: otter, Lutra lutra, activity, vegetation.

Introduction

The European otter (*Lutra lutra* L.) is a semi-aquatic carnivore having home ranges mainly situated at the littoral zone of the water basins, at the stripe about 20 meters away from the water edge. The marking of these home ranges is made by excrements (so called "spraints") and anal gland secretions. The tree bank vegetation favors the species making good shelter and hunting sites under water (KRUUK, 1992; 2006).

According to different statistical analyses, some authors register positive correlation between the tree cover and the otter activity (BAS *et al*., 1984; MACDONALD & MASON, 1985) or lack of such correlation (KRUUK, 2006). Using infrared cameras GUTER *et al*. (2008) found that the presence of more otter signs is strongly related with often otter presence at a particular area.

The otter signs are widely used for the species population monitoring (FOSTER-TURLEY *et al*., 1982; MACDONALD & MASON, 2004) which is now in progress in Bulgaria (GEORGIEV, 2008).

The aim of our study is to (i) investigate the habitat condition according to the bank tree cover in different water basins in Bulgaria, and (ii) to study if some otter activity is related with the different percentage of the tree cover on the banks.

Material and Methods

The study was carried out during 2011 – 2012 at different water basins (rivers and micro dams) situated in Central and Eastern Bulgaria. Transects with 600 m length were

walked along the banks in otter signs search. The search was stopped when the first otter signs were registered (FOSTER-TURLEY *et al*., 1982). The banks tree cover was noted as 0% (lack of tree vegetation), up to 25%, up to 50%, up to 70% and 100% (dense flood forest wood). A total of 80 such stretches were investigated at the rivers Mochuritsa, Tulovo, Topolyane and Gradina forests, Sredetska, Merichlerska, Sazlyika, Stryama, Omurovska, Sokolitsa, Blatnitsa, Pyasachnik, Maritsa, small stretch at the Danube near village of Vardim, rivers and micro dams in Dervent Heights and Stara Planina.

The preference index was calculated $P = P_{i1}/P_{i2}$, where P_{i1} is the proportion of a particular otter sign (tracks, spraints or food remains), and P_{i2} – the proportion of a particular bank stretch according to the percentage of the tree cover (mentioned above).

Results and Discussion

Habitat condition. As a whole we found domination of the very good or excellent bank tree cover. A total of 61% of transects were with tree cover between 50% and 100%. Almost equal were the stretches without or with little tree vegetation (Fig. 1).

Otter signs and population condition. At all transects surveyed the spraints dominated with 61%. All other otter signs were with low percentage. The otter tracks (foot prints) were on second place with 20% of occurrence, followed by the food remains with 7%. At 12% of transects we did not find any otter signs.

As a whole, the otter was registered in 88% of transects, a sign for a good population (Foster-Turley *et al*., 1982).

The preference. Among all the otter signs the spraints strongly dominated but mostly at the stretches with 100% bank tree cover (Fig. 3). At the other tree cover types the otter signs were more evenly distributed. The highest percentage without any otter signs had these transects without tree cover.

Highest preference we registered for walking or feeding of the otter at the open banks with no or weak tree cover (Table 1).

Conclusion

1. As a whole we found domination of the very good or excellent bank tree cover and good otter population at the study area.

2. Among all the otter signs the spraints strongly dominated but mostly at the stretches with 100% bank tree cover so the species monitoring surveys could be focused on such stretches.

3. Highest preference we registered for walking or feeding of the otter at the open banks with no or weak tree cover so a priority of such banks can be given in food remains or footprint measure studies.

Table. 1. Number of different otter signs found in various banks according to the tree cover and preference index: TC – type of the tree cover, N – number of transects with such cover, P – preference index.

C	N	spraints	tracks	food remains	no signs	P-sprainting	P-walking	P-feeding
0%	15	7	4	1	4	0,73	1,71	1,21
10%	7	5	0	1	1	1,12	0,00	1,70
25%	9	6	3	0	1	1,05	1,50	0,00
50%	12	8	2	0	2	1,05	0,75	0,00
100%	37	25	8	4	2	1,06	0,96	1,36
Total	80	51	17	6	10	-	-	-

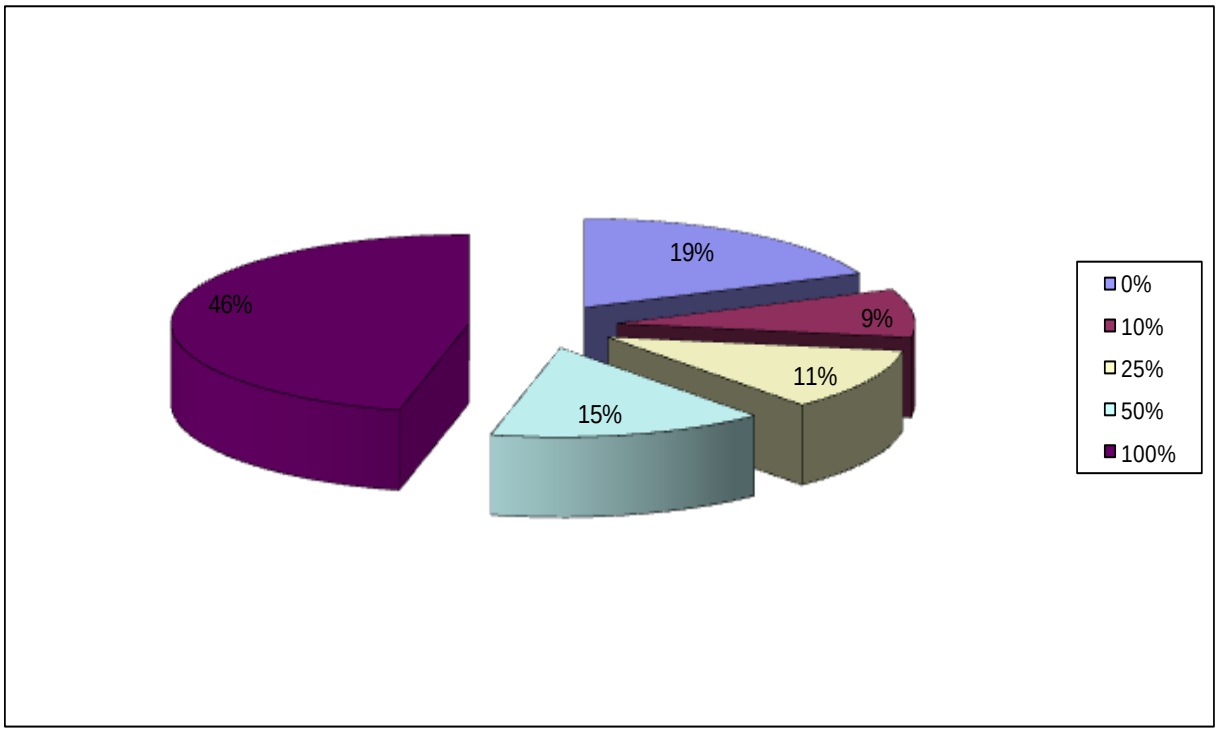

Fig.1. Percentage of transects with different bank tree cover at the study area.

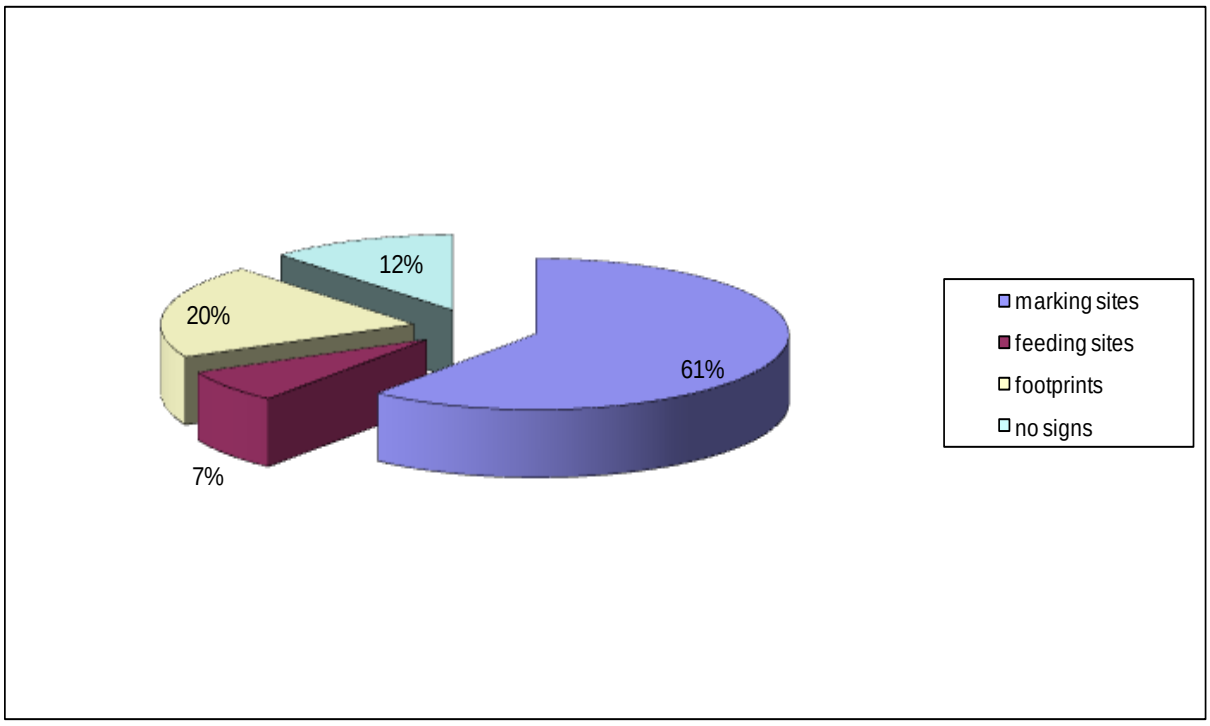

Fig. 2. Percentage of the different otter signs at transects studied.

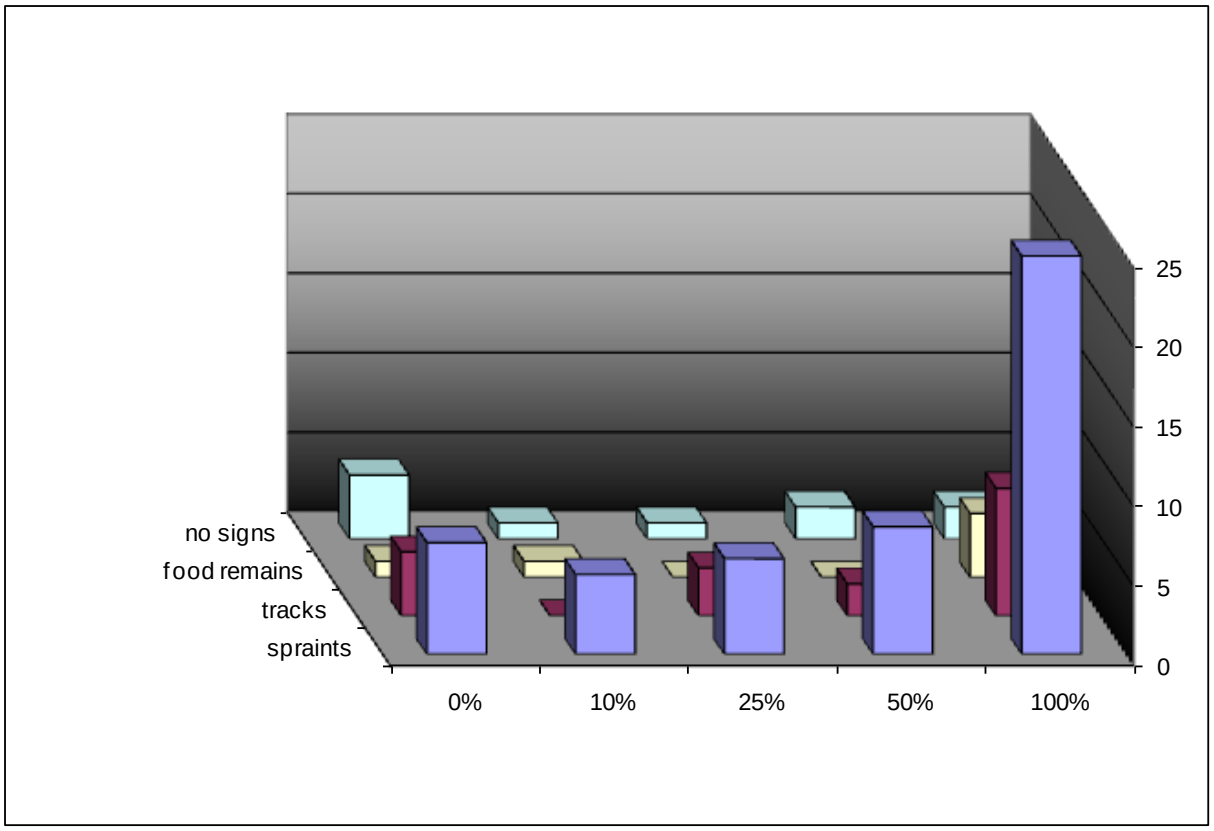

Fig. 3. Number of different otter signs found in various banks according to the tree cover.

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Received: 22.05.2012
Accepted: 20.08.2012

Union of Scientists in Bulgaria – Plovdiv
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ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 5-14

Eco-Biological Characteristics of Medicinal Plants in the Protected Area "Nahodishte Na Blatno Kokiche", Gradina Village, Parvomay (Bulgaria)

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Abstract. In the present work we investigated medicinal plants of the flora of the protected area "Nahodishte na blatno kokiche" the village of Gradina, Parvomay Municipality. Eco-biological characterization of the plants was done, and the species were grouped according to biological groups, life forms, floral elements and flowering time. Medicinal plants are also classified according to their attitude towards water, light and heat.

Key words: medicinal plants, herbs, eco-biological groups, protected area, Gradina Village

Introduction

The purpose of this study is, on the basis of fieldwork and literature data analysis, to make eco-biological characteristics of the medicinal plants found within the protected area "Nahodishte na blatno kokiche" at the village of Gradina.

The results of this study supplement the results of the studies of PAVLOV *et al*. (1990), TASHEV (1991) and GEORGIEV & KOEV (2012) in the protected area "Nahodishte na blatno kokiche" the village of Gradina, giving an idea about the genetic resources of medicinal plants in it as well.

The protected area is located in Plovdiv field of the Upper Thracian Valley. Floristic regions of Bulgaria are first published in Flora of NR Bulgaria (vol. 3, 1966). According this the study, the examined area falls into Thracian Lowland floristic region.

Climatic regionalization of the country (STANEV *et al*., 1991) refers the protected area "Nahodishte na blatno kokiche", Gradina Village, to the Eastern Thracian sub-region of Middle Eastern climatic region of Bulgaria, Transient-continental climatic subregion of the European-continental climatic region. It is characterized with relatively mild winters and hot summers, with well pronounced drought, especially during the months August – September, which are the driest months of the year. The annual rainfall is presented in Table 1. The average annual rainfall is 551 mm, which defines the area as places with little annual rainfall (GALABOV, 1982). The average annual temperature is about 12.1°C, which coincides with that of the country between 11 and 12°C (GALABOV, 1982). The average humidity is 72% and coincides with the average of the country (GALABOV, 1982).

Soils are mainly alluvial meadow, as at some places they are mixed with clay and fertile black earth vertisoils.

Material and methods

We studied the medicinal plants in protected area "Nahodishte na blatno kokiche" Gradina, Parvomay Municipality (234.4417 ha), located in the middle reaches of the Maritza River. The protected area was declared with a major motive of protecting the habitat of summer snowflake (*Leucojum aestivum* L.) and natural lowland riparian forest of clear polish (*Fraxinus oxycarpa* Bieb. Ex Willd.), field elm (*Ulmus minor* Mill.) and summer oak (*Quercus robur* L.).

Table 1. The average monthly amount of precipitations, measured in station Sadovo

Months	mm
January	42
February	35
March	38
April	45
May	61
June	68
July	48
August	31
September	36
October	43
November	52
December	52
Per annum	551

The diversity of medicinal plants in the protected area "Nahodishte na blatno kokiche", Gradina Village, was investigated through the systematic collection of materials during the growing seasons of 2011-2012. The frequency of attendance was complied with the climatic conditions in the region and the phytorhythms conditioned by them. The earliest collecting started from the beginning of February, and the latest – by the end of September, which allowed us to range the taxonomic diversity and its seasonal dynamics. The identification of species was done by using Handbooks "Plants in Bulgaria" (DELIPAVLOV & CHESHMEDZHIEV 2003), "Flora of People Republic of Bulgaria" (YORDANOV, 1963-1979; VELCHEV, 1982-1989; KOZHUHAROV, 1995); "Flora of Bulgaria" (STOYANOV, 1966, 1967); "Qualifier of trees and bushes in Bulgaria" (GRAMATIKOV, 1992).

Processing of the factual material and floristic characteristics were made by standard methods (STANEV 1976; VASSILEV & ANDREEV 1992; GUSEV *et al*., 2004). Characteristic of the healing plants was done according to: eco-biological structure (analysis of biological types) spectrum of life forms by RAUNKIER (1934), distribution of the floral elements by Walter (ASYOV, 2006), ecologic groups in terms of the water factor, time flowering and conservation significance of the flora. Conservation status of the species at national level was determined by the "Red Book of Bulgaria" (PEEV, 2012), "Biological Diversity Act" (2002, 2007) and internationally by Lucas (1983), ECE (1991) list of IUCN: "Red List of Threatened Plants" (1998), Appendix № 1 of the Convention on the Conservation of European wild flora and fauna and natural habitats (Bern Convention) and the Annexes of the Convention on International Trade with Endangered Species of Wild Fauna and Flora (CITES).

As a result of floristic research and literature reviews (PAVLOV *et al*. 1990; TASHEV 1991; GEORGIEV & KOEV 2012) in the period 2011-2012 on the territory of the protected area "Nahodishte na blatno kokiche", Gradina Village, 175 higher plant species from 119 genera and 50 families were found. Phytofund consists only of angiosperms from Division Magnoliophyta. 30 species of these belong to Class Liliopsida, distributed in 22 genera and 11 families, and 145 species belong to Class Magnoliopsida, distributed in 97 genera and 39 families.

Results

Basing on the analysis of literary publications on medicinal plants of the flora of Bulgaria (DELIPAVLOV & CHESHMEDZHIEV 2003; LAW ON MEDICINAL PLANTS IN BULGARIA, 2006; NIKOLOV, 2007; LANDZHEV, 2010), in the protected area "Nahodishte na blatno kokiche", Gradina Village, 95 species medicinal plants (Appendix 1**)** from 83 gen-

era and 43 families were found, representing 54.3% of the species, 69.7% of the genera and 86.0% of the families in the protected area. These plants represent 12.7% of the species included in Appendix № 1 of the Law on Medicinal Plants (2006).

The distribution of taxa, regarding biological types (Table 2), shows that perennial herbaceous plants have the most significant presence (49 species), representing 51.6% of the whole establishment listed, followed by trees with 10 species – 10.% and 9 species of shrubs – 9.5%.

Table 2. Distribution of medicinal plants regarding biological types.

Biological type	Number of taxa	% of medicinal plants in protected area
tree	10	10.5
tree - shrub	2	2.1
shrub - tree	3	3.1
shrub	9	9.5
shrub - perennial	0	0
perennial	49	51.6
biennial - perennial	2	2.1
biennial	6	6.3
annual - biennial	7	7.4
annual	7	7.4

Analysis of medicinal plants, regarding floral elements (Walter) (Table 3), shows dominant presence of the Euro-Asian species (21), forming 22.1% of the sample group, followed by the Euro-Mediterranean – with 18 species or 18.9%, and Sub-Mediterranean – 10 species or 10.5%.

Table 3. Distribution of medicinal plants according to floral elements.

Floral elements by Walter	Number of taxa	% of medicinal plants in protected area
Adv	1	1.0
Boreal	8	8.4
Eur	7	7.4
Eur - As	21	22.1
Eur - Med	18	18.9
Eur - OT	2	2.1
Eur - Sib	6	6.3
Eur - subMed	1	1.0
Kos	7	7.4
Med	2	2.1
Med - CAs	1	1.0
Pont - Med	3	3.1
subBoreal	5	5.3
subMed	10	10.5
sPont	3	3.1

Hemicryptophytes, regarding the biological spectrum of the studied species (Table 4), are dominant – 42 species representing 44.2% of the total number of medicinal taxa in the reserve, followed by phanerophytes – 23 species or 24.2%, and hemicryptophytes to therophytes – 8 species and 8.4%, respectively.

Table 4. Distribution of medicinal plants according to their life forms.

Life form	Number of taxa	% of medicinal plants in protected area
Phanerophytes (Ph)	23	24.2
Chamephytes (Ch)	1	1.0
Hemicryptophytes (H)	42	44.2
Cryptophytes (Cr)	7	7.4
Therophytes (T)	7	7.4
Therophytes to hemicryptophytes (T-H)	7	7.4
Hemicryptophytes to therophytes (H-T)	8	8.4

The distribution of the sample group of plants, regarding time of flowering (Table 5), shows the maximum density in months V-IX (81 taxa). Among them the flowering species are most during the months V-VII and V-VIII, with 11 species for each period; V-VIII – 11 species, and V-IX – 8 species. Despite clearly less expressed flowering, in so-called months of relative peace: November, December, January and February – only 3 taxa, it is noteworthy that the vegetation of medicinal species is found within the area throughout the year.

Table 5. Distribution of medicinal plants according to the flowering period.

Flowering time, months of the year	Number of taxa	% of medicinal plants in protected area
I-XII	1	1.0
II-III	1	1.0
II-IV	1	1.0
III-IV	1	1.0
III-V	2	2.1
III-VI	1	1.0
III-VIII	1	1.0
III-IX	2	2.1
IV-V	9	9.5
IV-VI	5	5.3
IV-VII	5	5.3
IV-VIII	2	2.1
IV-X	1	1.0
V	2	2.1
V-VI	11	11.6
V-VII	4	4.2
V-VIII	11	11.6
V-IX	8	8.4
V-X	3	3.1
V-XI	1	1.0
VI-VII	4	4.2
VI-VIII	7	7.4
VI-IX	5	5.3
VI-X	3	3.1
VII-VIII	3	3.1
VII-X	1	1.0

Referring the territory of the protected area "Nahodishte na blatno kokiche", Gradina Village, to the category of floodplain or dense forests, we also determine a high percentage: total 32.6% of hygrophytes, hygromezophytes and mezohygrophytes (Table 6). It should be noted, however, the dominance of mezophytes, represented by 56 species (59.0 %) that exceeds almost twice the previous groups.

Table 6. Distribution of medicinal plants by ecological groups regarding the water as a factor

Ecological group	Number of taxa	% of medicinal plants in protected area
Hygrophytes	20	21.0
Hygromezophytes	9	9.5
Mezohygrophytes	2	2.1
Mezophytes	56	59.0
Xeromezophytes	7	7.4
Xerophytes	1	1.0

Analysis of the distribution of medicinal plants, regarding ecological groups toward light as a factor (Table 7), shows the prevalence of helophytes – 52 or 54.7 %, followed by the groups of hemischiophytes with 26 species or 27.4%, and schiophytes represented by 17 species and 17.9%, respectively.

Table 7. Distribution of medicinal plants by ecological groups regarding the light as a factor

Ecological group	Number of taxa	% of medicinal plants in protected area
Helophytes	52	54.7
Hemisciophytes	26	27.4
Sciophytes	17	17.9

Regarding the edaphic factor, the sample species are divided into 9 groups (Table 8). Indifferents are dominant – 69 species or 72.6%, followed by lithophytes 11 species – 11.6% and psamophytes 6 – 6.3%, respectively. All other groups are poorly represented - by 1 or 2 species.

Table 8. Distribution of medicinal plants to bedrock (substrate).

Groups	Number of taxa	% of medicinal plants in protected area
Indifferents	69	72.6
Calciophytes	2	2.1
Lithophytes	11	11.6
Lithophytes and Calciophytes	1	1.0
Lithophytes and Psamophytes	1	1.0
Halophytes	1	1.0
Psamophytes	6	6.3
Nitrophytes	1	1.0
Hydrophytes	3	3.1

Analysis of medicinal plants, regarding the heat as a factor, divides them into two groups. The group of thermophytes, that are dominant – 69 species or 72.6 %, while the

remaining 26 species – 27.4% belong to the group mezotherms.

The distribution of medicinal plants in phytogeographic centers (Fig. 1) (STEFANOV, 1943) shows that with the largest percentage – 30.5% are the species from Southern Continental Center, followed by those coming from the Mountainous one – 25.3%, Northern Continental Center and Sylvan boreal – 21.0%; and lastly are the types of Mediterranean Center – 2.1%.

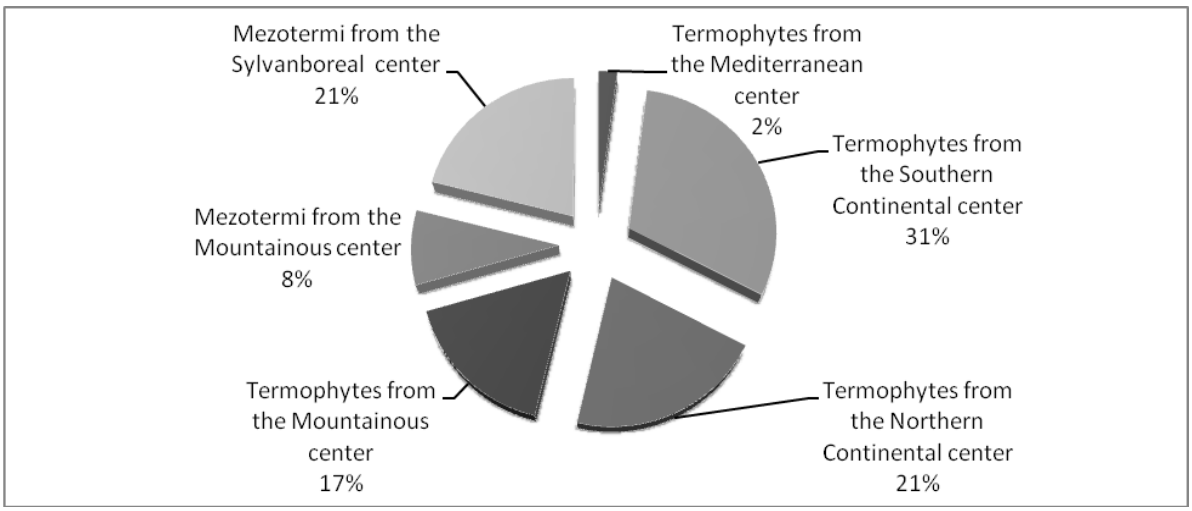

Fig. 1. Distribution of medicinal plants in phytogeographic centers (after STEFANOV, 1943)

On the basis of their structural-physiological characteristics and the vegetation activity, the plants fall into three groups (STEFANOV, 1943): fixed – 32 species, variable with secondary extended habitats – 27 species and those penetrated by secondary displacement – 36 species.

In the protected area we found four taxa of conservation importance, representing 4.2% of medicinal plants occurring in it: bear's breech (*Acanthus spinosus*) is an endangered species (*Endangered*) (DIMITROVA, 2009, following PEEV, 2011) and is included in Appendix 3 of Biodiversity Act of Bulgaria; summer snowflake (*Leucojum aestivum*) is vulnerable (*Vulnerable*) (EVSTATIEVA, 2009) and is included in Appendix 4 of Biodiversity Act of Bulgaria; and two other species are included in Appendix No 2 of the Convention on International Trade with Endangered Species of Wild Fauna and Flora: Loose-Flowered Orchid (*Orchis laxiflora* subsp. *elegans*) and Lesser Butterfly-orchid (*Platanthera bifolia*).

Discussion

The number of medicinal plants, found in the examined protected area (95 species), represents 12.7% of the total number of herbal plants, listed in LAW ON MEDICINAL PLANTS IN BULGARIA – 744 herb species; *Specialized encyclopedia of medicinal plants in Bulgaria* – 380 species (NIKOLOV, 2007); *Encyclopedia of medicinal plants in Bulgaria* – 224 species (LANDZHEV, 2010). This can be said as a very high concentration of these species in the research object, having in mind that the territorial correlation of 23.4 km² of protected area to the total area of the country (GALABOV, 1982) ranged of 0.021%

The reported dominant presence of hemicryptophytes gives us reason to assume relatively rapid negative change of the species establishment in acute changes in hydrostatic mode of the protected area "Nahodishte na blatno kokiche", Gradina Village, as there were significant decreases in rainfall and river level, observed in recent years. Moreover, about one third (32.6%) of those species, established during our study,

are highly dependent on moisture reserves of the edaphic factor.

The presence of nine different categories of plants, associated with the substrate type, suggests a mosaic distribution in horizontal and vertical direction of the typical for the protected area "alluvial-black soils" (GALABOV, 1982). The dominance of indifferent species is in direct correlation with these characteristics of the soil factor – 72.6%. The relatively poor performance of lithophytes and psamophytes is based on limited areas of sandy sediments and ballast (gravel) deposits, present locally in the territory and occupied by the species discussed.

The dominance of thermophile species is in direct correlation with the climatic characteristics of the region, typical with influence of Mediterranean warm currents along the Maritsa River (GALABOV, 1982).

The wide range of the vegetation season, covering the twelve months of the year, allows the available resources of medicinal plants to be fully exploited as a gene bank for the production of propagating material for cultivation experiments of wild species.

Conclusions

1. The protected area "Nahodishte na blatno kokiche", Gradina Village, is a significant depot of medicinal plants, including 12.7% of all wild plants in Bulgaria used for medical purposes;

2. The hemicryptophytes have a dominant position in the total amount of the herbal species reported;

3. Despite the well-known Mediterranean climatic influence in the region, the overwhelming amount from the analyzed group of plants remains the typical European and Euro-Asian forms;

4. Relatively high percentage of hydro- and hygrophytes shows a significant degree of vulnerability and change in species establishment in increasingly distinct tendencies of drought in the last decade;

5. Reporting the medicinal plants in the study area is a prerequisite for changing the status of the protected area in the reserve according to Bulgarian Low for Environmental Protection and improving the care and conservation of plant diversity.

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Received: 21.05.2012
Accepted: 13.11.2012

Appendix 1: *Systematic list of medicinal plants in protected area:*

Class	Family	Species
Liliopsida	Alismataceae	*Alisma plantago-aquatica* L.
	Alliaceae	*Allium scorodoprasum* L.
	Amaryllidaceae	*Leucojum aestivum* L.
	Iridaceae	*Iris graminea* L.
		Iris pseudacorus L.
	Liliaceae	*Polygonatum latifolium* Desf.
		Polygonatum odoratum (Mill.) Druce
	Orchidaceae	*Orchis laxiflora* Lam. *ssp.elegans* (Heuff.) Soo
		Platantera bifolia (L.) Rich.
	Poaceae	*Cynodon dactylon* (L.) Pers.
	Thyphaceae	*Thypha latifolia* L.
Number of species		**11**
Magnoliopsida	Acanthaceae	*Acanthus spinosus* L.
	Aceraceae	*Acer campestre* L.

		Acer tataricum L.
	Apiaceae	*Bifora radians* Bieb.
		Chaerophyllum bulbosum L. *ssp. bulbosum* L.
		Chaerophyllum temulentum L.
		Daucus carota L.
		Heracleum sibiricum L.
		Oenanthe angulosa Griseb.
	Aristolochiaceae	*Aristolochia clematitis* L.
	Asteraceae	*Artium lappa* L.
		Bellis perennis L.
		Carlina vulgaris L.
		Cichorium intybus L.
		Cirsium arvense (L.) Scop. *var. incanum* Fisch.
		Cirsium arvense (L.) Scop. *var. vestitum* Wimm et Grab.
		Lactuca serriola L.
		Taraxacum officinale Weber
		Tragopogon pratensis L. *ssp. orientalis* (L.) Celak.
	Boraginaceae	*Buglossoides purpurocaerulea* (L.) I. M. Johnston
		Cynoglossum officinale L.
		Symphytum officinale L.
	Brassicaceae	*Capsella bursa-pastoris* (L.) Medik.
	Cannabaceae	*Humulus lupulus* L.
	Capryfoliaceae	*Sambucus ebulus* L.
		Sambucus nigra L.
		Viburnum opulus L.
	Caryophyllaceae:	*Stellaria graminea* L.
		Stellaria media (L.) Cirillo.
	Celastraceae	*Euonymus europaeus* L.
	Convolvulaceae	*Convolvulus arvensis* L.
	Cornaceae	*Cornus mas* L.
		Cornus sanguinea L. *ssp. sanguinea* L.
	Corylaceae	*Carpinus betulus* L.
		Corylus avellana L.
	Dipsacaceae	*Dipsacus fullonum* L.
	Euphorbiaceae	*Euphorbia plathyphylus* L.
		Euphorbia velenovskyi Bornm. *var. trichocarpa* Bornm.
		Euphorbia villosa Waldst. & Kit. ex Willd.
	Fabaceae	*Coronilla scorpioides* (L.) W. D. J. Koch
		Galega officinalis L.
		Lotus corniculatus L.
		Melilotus alba Medik
		Ononis arvensis L.
		Trifolium pratense L.
	Fagaceae	*Quercus pedunculiflora* K. Koch
		Quercus robur L.
	Lamiaceae	*Ajuga reptans* L.
		Ballota nigra L. *ssp. nigra* L.
		Glechoma hederacea L.

		Lamium maculatum L.
		Lamium purpureum L.
		Mentha aquatica L.
		Mentha pulegium L.
		Prunella vulgaris L.
		Teucrium scordium L.
	Malvaceae	*Althaea officinalis* L.
	Moraceae	*Morus alba* L.
	Oleaceae	*Fraxinus ornus* L.
		Fraxinus oxycarpa Willd.
		Ligustrum vulgare L.
	Plantaginaceae	*Plantago lanceolata* L.
		Plantago major L.
	Polygonaceae	*Persicaria hydropiper* (L.) Opiz
		Polygonum patulum Bieb.
	Ranunculaceae	*Clematis vitalba* L.
		Ficaria verna Hudson
	Rosaceae	*Agrimonia eupatoria* L.
		Crataegus monodyna Jacq.
		Geum urbanum L.
		Malus sylvestris (L.) Mill.
		Potentilla argentea L.
		Prunus spinosa L.
		Rosa canina L.
		Rubus caesius L.
	Rubiaceae	*Galium aparine* L.
	Salicaceae	*Populus alba* L.
		Salix fragilis L.
	Scrophulariaceae	*Pseudolysimachium orchideum* (Crantz) T. Wraber
		Veronica anagallis-aquatica L.
	Solanaceae	*Solanum dulcamara* L.
	Ulmaceae	*Ulmus minor* Mill.
	Urticaceae	*Urtica dioica* L.
	Violaceae	*Viola odorata* L.
Number of species		**84**
Total number of medicinal species		**95**

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 15-23

Study on the Total Coliforms Count and Coli Titter in the Waters of Kardzhali Reservoir, Bulgaria

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Abstract. The aim of this study was to examine TC (total coliforms) and coli titter from two sampling stations in the aquatory of Kardzhali Dam Lake and one station in River Arda, in August, 2011. The values of the TC index in the reservoir vary from 1900±674 cfu/100ml in station I, to 1293±194 cfu/100ml in station II, while the TC value of River Arda reaches 1698±134 cfu/100ml. In Reservoir Kardzhali, the smallest volume of water in which *Escherichia coli* cells were found, varies between 5 and 15 ml, while for the River Arda the value of coli titter is equal to 1. With highest percentage, regarding the presence of microbiological species in the reservoir waters, is the genus *Klebsiella* (70%), followed by *Citrobacter* (15%), *Enterobacter* (10%) and *Serratia* (5%), respectively represented by the species *Klebsiella oxytoca, Citrobacter freundii*, *Enterobacter cloacae* and *Serattia marcescens*. In the river Arda two genuses were found - *Serratia* (50%) and *Salmonella* (50%).

Key words: coli titter, total coliforms, Kardzhali Reservoir.

Introduction

The permanent control of the sanitary parameters, determining water quality, is used worldwide to monitor and control the quality and safety of various types of water reservoirs, and for prevention of illnesses caused by the polluted water.

These infections can arise from the bacteria naturally present in the aquatic environment or microorganisms present in the water as a result of contamination with human or animal faeces (ABRAHAM, 2011). According to a series of authors (ZMYSTLOWSKA *et al.*, 2000a,b; ZMYSTLOWSKA *et al.*, 2003; NIEWOLAK & TUCHOLSKI, 2000a,b), the presence of *Escherichia coli* in the water is evidence for the possible presence of variety of microorganisms, that can cause gastrointestinal diseases (DHSS, 1982; BLACKWOOD, 1978; FAPOHUNDA *et al.*, 1994).

Many potential pathogens could be associated with water, so it is thus impractical to screen samples for all possible disease causing agents. Instead various, indicator organisms have been used as surrogate markers of risk. (BARRELL *et al.*, 2000).

Most waterborne infectious diseases are related to faecal pollution of water sources therefore aquatic microbiology is mainly based on the need to identify indicators of faecal pollution such as coliforms and *E. coli*. (HUNTER, 1997).

The desirable qualities for a useful water quality indicator are (WHO, 1996):

- Presence in large numbers in human and warm blooded animal faeces.
- Easy to identify by simple methods.
- Does not grow as part from the natural microflora of various types of water reservoirs.
- Their presence and elimination after treatment of the water is similar to the pathogens.

The coliforms are part of the *Enterobacteriaceae* family, which includes Gram-negative, non-spore-forming, oxidize-negative, straight rods, facultative anaerobic bacteria that ferment lactose with the formation of acid and gas within 24-48h at 35- 37^{o}C. They appear as non-specific indicators of faecal pollution.

During the years different schemes are developed for the classification of coliforms. The earliest are the one of MCCONKY (1909) and BERGEY & DEAN (1908). Enough biochemical reactions for the identification of coliforms are established during the 20th years of the 20th century (HENDRIKS, 1978). Large numbers of those reactions are still in use today. The main disadvantage is their long duration.

In the modern microbiology the efforts are directed in developing reliable, fast methods for detection and determining presence of indicator microorganisms. This directs the efforts towards developing tests for fast identification of coliforms, including basically the genus *Escherichia, Klebsiella, Citrobacter, Enterobacter* and *Serratia.*

This became possible after the introduction of chromogenic substances, which may be added to the conventional nutrient media. The chromogenic components are modified by the bacterial enzymes or by specific bacterial metabolites; as a result the chromogenic substance changes its colour or fluorescent after the modification. This makes it possible to establish which colonies, possess this specific metabolic activity. It also makes it possible to determine the number of microorganisms from a specific indicator group in a mixed sample. The time required for the determination of different indicator bacteria can be cut down to 16-18h (ASHBOLT, 2001).

These methods, although fairly easy, give information primary about the TC represented in the water, but they don't give a clear picture about species affiliation of microorganisms. Often, other species from the family *Enterobacteriaceae*, which are part of the natural micro flora of the water body, give positive reaction. Therefore further confirmative identification tests are needed.

The aim of this study was to determine the total coli forms (TC), coli titter and coli index, by the usage of chromogenic medium, in two sampling stations in the aquatory of Kardzhali dam-lake and in Arda River and to identify the isolated strains.

Materials and Methods

The research was carried out in August 2011, in the aquatory of Reservoir Kardzhali and Arda River, in the part where the river infuses in the reservoir. The water samples were collected from two sampling stations, located in the different parts of the reservoir: station I - located in close range of the settlement Glavatarci and station II- in the transitional zone of the reservoir (Halach Dere) and one station (III) on the Arda River, in the region where the river flows into the reservoir (Fig. 1). The precise location of the sampling stations is determined with the help of GPS receiver Garmin 76CSx.

Fig. 1. Sampling stations located on Kardzhali Dam Lake and Arda River.

The water samples were collected by direct immersion of sterile glass jars with a volume of 500 ml, 50 cm below the water surface and stored in cold conditions until their processing, not later than 6 h after sampling.

The coli titter is determined as the smallest volume of water in which the presence of *Escherichia coli* is established.

The coli index is determined as the number of *Escherichia coli* cells in 1l water sample, and it is calculated based on the coli titter value with the equation:

$$\text{COLI INDEX} = \frac{1000}{\text{COLI TITTER}}$$

Total coliforms (TC) and *E. coli* number were determined after filtration of 5 ml water sample through membrane filters (Membrane Solutions) with pore size 0.45 μm, and cultivation on chromogenic medium SLS Coliform Agar (HiChrome) for 48 h on 37°C.

Strains for identification were isolated based on their specific colouring during cultivation on SLS Coliform Agar. From every station 10 strains were isolated.

For verification of their purity, strike seeding on Levin medium was used. Biochemical reactions, based on the system for fast identification Enterotube II (ENCISE), were used for identification of the isolated strains.

T-test was applied for statistical evaluation of the results (MS Excel).

Results and discussion

The values for total coliforms, coli titter and coli index from the waters of Kardzhali Reservoir and Arda River are shown in Table 1.

Table1. Total coliforms, coli titter and coli index in the waters of Kardzhali Reservoir and Arda River for August, 2011.

Parameter	Station		
	I	II	III
TC (cfu/ 100 ml)	1900±674	1293±194	1698±134
coli titter (ml/ 1cfu)	5	15	1
coli index (cfu/ 1l)	200	67	1000

The values of TC, established in station II, which is located at the transitional zone of the reservoir between the lake like zone of the water

body and riverine zone, are within the range of 1293±194 cfu/100ml. They are significantly lower in comparison with the values from station III - 1698±134 cfu/100ml ($p<0.05$), and station I - 1900±674 cfu/100ml ($p<0.05$). At the same time, no reliable differences were found between the average values of total coliforms between station I and station III.

The long retention time of Kardzhali dam-lake, determines it as a lake like water reservoir, where a continuous process of self-purification takes place (TRAYKOV, 2003). The self-purification of the water is due to the action of physicochemical (continuous water exchange, settling of suspended particles along the longitudinal axis of the reservoir, where microorganisms are adsorbed; bactericidal effect of UV- rays, etc.) and biological (zooplankton; antagonism between the species, etc.) environmental factors. In this kind of water bodies the trophic diversity, and in consequence the quantity of microorganisms, reduces form the tail to the dam of the reservoir (STRASKRABA, 1998). The data deviates from this model. Increased values of the total coliforms in the region of station I, may be due to the influence of village Glavatarci, which is situated in close range of the station, and also from the fact that the study is conducted in a period of intensive tourist season. The settlement does not have two way sewage systems, and this gives the assumption that the sewage waters are filtrated in the reservoir. This assumption is supported by the high values of coli titter, which is an indicator of faecal contamination, from 5 ml water in this station and coli index 200 cfu *E. coli*/1l. Similar data is shown in the work of other authors, studying the processes of intensive urbanisation in the zones surrounding the large water reservoirs (MALLIN *et al.*, 2000).

The biochemical characteristic of the strains isolated for identification and determining the coliform ratio in Kardzhali Reservoir, shows that all strains are Gram-negative, catalase–positive, oxidise–negative, facultative anaerobic, straight rod-shaped bacteria. When cultivated on Levin medium, they form colonies with purple colouring and metallic shine, which is typical for lactose–positive representatives of family *Enterobacteriaceae* (Bergey`s manual, 2005). Detailed results regarding the biochemical identification of the isolated strains are shown in Tables 2, 3 and 4.

The strains isolated from station I are characterized with the highest diversity of species and fall into four genera, based on their biochemical characteristics (Fig. 2a). The *Klebsiella* genus represents the main share. The strains assimilate glucose with release of acid and gas (CO_2), they produce acid from lactose, arabinose and sorbitol, utilize citrate, they have lysine and ornithine decarboxylase activity, tryptophan activity and decompose urea to ammonia. Based on the obtained results all strains of the genus were defined as *K. oxytoca* (strains 1, 6 and 8) and *K. ozaenae* (strain 4) (Bergey`s manual 2005, Enterotube II Code Book 2009).

Thirty percent (30%) of the studied strains fall into genus *Citrobacter*. Into this genus fall species that degrade glucose and other carbohydrate sources with the release of acid and gas. The genus is represented by *C. freundii* (strains 3, 9 and 10), which is normally present in the human microflora. Often it is isolated from clinical patients.

Table 2. Biochemical characteristic of the strains isolated from station I of Kardzhali Reservoir

Test	STRAIN №									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Gram	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+
Indol	+	-	+	+	-	+	+	+	-	-
MR	+	+	+	+	+	+	+	+	+	+
VP	-	+	-	-	+	-	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	+
H_2S	-	-	+	-	-	-	-	-	+	+
Urea	+	+	+	-	+	+	+	+	-	-
PA	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	+	-	-	+	-	+	+	+	-	-
Ornithine decarboxylase	-	+	+	+	+	-	-	-	-	-
Glucose, acid	+	+	+	+	+	+	+	+	+	+
Glucose, gas	+	+	-	-	+	-	-	+	-	-
Lactose	+	+	-	-	+	+	-	+	-	-
L-Arabionse	-	-	+	+	-	-	-	-	+	+
D-Adonitol	+	+	+	+	+	+	+	+	+	+
D-Sorbitol	+	+	+	-	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-
O/F	F	F	F	F	F	F	F	F	F	F
Enterotube II code	34723	32323	33173	26240	32323	24723	24223	34733	21061	21061

In the third place of occurrence is genus *Enterobacter*, represented by *E. cloacae* (strains 2 and 5). The species is widely spread in nature as part of the normal microflora of water and soil (Bergey`s manual 2005). They fall into the conditionally pathogenic bacteria in the colon of humans and animals. If they enter elsewhere they can cause urinary tract infections, wound, sepsis etc. (HAIDUSHKA *et al.,* 2011).

Strain №7 assimilates all tested carbohydrate sources, with the exception of dulcitol, with the release of acid, without forming gas. It decomposes citrate and decarboxylates amino acids lysine and ornithine in anaerobic conditions, doesn't hydrolyse urea and is indol negative. Based on its biochemical characteristic the strain falls into genus *Serratia*.

All isolated strains from station I fall into the so called faecal coliforms and are used as indicator for faecal pollution (WHO, 1999). Their presence in combination with the high values of coli titre, indicate recent contamination with sewage water of the studied region.

Table 3. Biochemical characteristic of the strains isolated from station II of Kardzhali Reservoir

Test	STRAIN №									
	1	2	3	4	5	6	7	8	9	10
Gram	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+
Indol	+	+	+	+	+	+	+	+	+	+
MR	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-	-	-
Urea	+	+	+	+	+	+	+	+	+	+
PA	-	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+	+	+	+	+
Glucose, acid	+	+	+	+	+	+	+	+	+	+
Glucose, gas	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
L-Arabionse	+	+	+	+	+	+	+	+	+	+
D-Adonitol	+	+	+	+	+	+	+	+	+	+
D-Sorbitol	+	+	+	+	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-
O/F	F	F	F	F	F	F	F	F	F	F
Enterotube II code	36763	36763	36763	36763	36763	36763	36763	36763	36763	36763

All isolated strains from station II fall into genus *Klebsiella* and are represented by the species *K.oxytoca* (Fig. 2b). Representatives of the species are found in human faeces, but more often are part of the normal microflora of water and soil.

Isolated species from station III fall into genus *Serratia* (strains 1, 2, 3 and 6) and genus *Salmonella*, respectively represented with the strains 4, 5, 7 and 8 (Fig. 2c). The bacteria of the genus Salmonella are among the main causes of food borne infectious diseases in humans (MOLBAK, 2006). They retain viability up to 4 months in still waters and 5 to 6 days in flowing waters (HAIDUSHKA, 2011). Their high titter in the waters of Arda River is an evidence of contamination with sewage water. This along with the high values of coli titter (1ml) and coli index (200 cfu *E. coli*/ 1l), creates the risk for occurrence of infectious outbreaks.

Conclusions

Highest levels of contamination with sewage water, based on the study of coli titter (1ml) and coli index (200 cfu *E. coli*/ 1l) in the three studied stations, are established in the waters of the Arda River.

Table 4. Biochemical characteristic of the strains isolated from station III of Kardzhali Reservoir

Test	STRAIN №								
	1	2	3	4	5	6	7	8	9
Gram	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+
Indol	-	-	-	-	-	-	-	-	+
MR	+	-	-	+	-	-	-	+	+
VP	+	+	+	-	+	+	+	-	-
Citrate	+	+	+	+	+	+	+	+	-
H_2S	-	-	-	+	+	-	-	+	-
Urea	-	+	-	+	+	-	-	+	-
PA	-	-	-	-	-	-	-	-	-
Lysine decarboxylase	+	+	+	+	+	+	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+	+	+	+
Glucose, acid	+	+	+	+	+	+	+	+	+
Glucose, gas	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+
L-Arabionose	+	-	-	+	+	+	-	+	+
D-Adonitol	-	-	+	+	-	-	-	-	-
D-Sorbitol	+	+	+	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-	-
O/F	F	F	F	F	F	F	F	F	F
Enterotube II code	36161	36123	36321	37161	37161	36063	35161	37161	36560

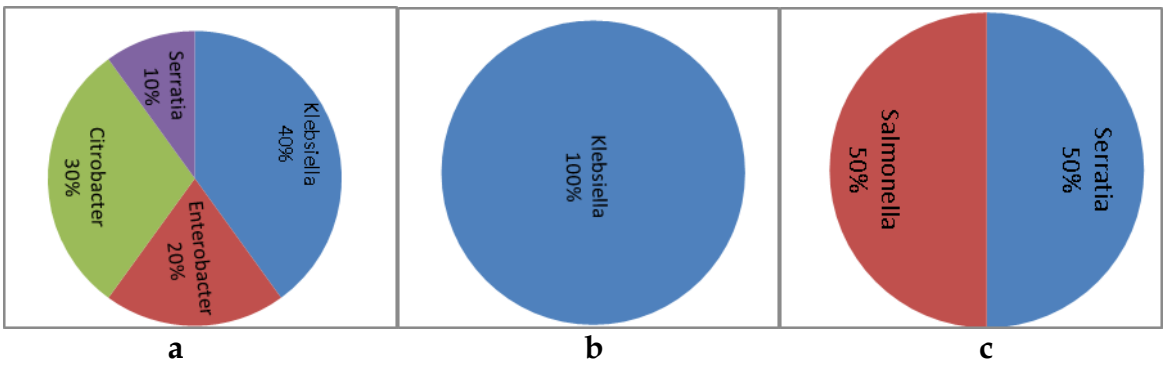

Fig 2. Percentage ratio of identified genus form family *Enterobacteriaceace* by stations (a\station I; b\ station II; c\station III)

The high number of coliforms and the species composition is indicative for contamination and loading of Arda River with sewage waters in the section

before it flows in the Kardzhali Dam-lake.

The absence of pathogenic species, combined with a decrease in the number of TC, coli titter and coli index in station II is evidence of the self-purifying capability of the studied reservoir.

The new rise in the number of TC in station I in proximity to Glavatarci village, as well as the presence of faecal coliforms, indicates secondary site of contamination with sewage waters in the region.

Acknowledgements

The study is funded by the Scientific Research Fund to Ministry of Education, Youth and Science - contract DO 02-307.

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Received: 16.07.2012
Accepted: 16.10.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 25-31

Influence of Vegetation on the Avifauna in Two Urban Parks in Plovdiv, Bulgaria

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Abstract. This study is related to the investigation of the impact that vegetation caused on the avifauna of the parks "Lauta" and "Tsar Simeon Garden" located in the city of Plovdiv, Bulgaria. As a result of the study, we found that the biodiversity of the vegetation in park "Tsar Simeon Garden" is highly significant in comparison with the same in park "Lauta". In the park "Tsar Simeon Garden" introduced plant species dominate over indigenous species, as compared with the park "Lauta". The differences in the vegetation are caused by different management approaches carried out in the both parks. The degree of similarity between trees and bushes in both parks is low. The vegetation also had a significant influence on the avifauna living within the parks. The avian biodiversity in park "Lauta" is two times higher in comparison with the avian biodiversity in park "Tsar Simeon Garden". The degree of similarity in the avifauna between both parks is significant, due the fact that all of the species, except one documented in "Tsar Simeon Garden" being documented in park "Lauta" as well. Eighteen new species of birds were recorded and described for the first time in the city of Plovdiv. The conservation status of the avifauna in park "Lauta" is quite significant in comparison with the same in park "Tsar Simeon Garden".

Key words: avifauna; birds; vegetation; native; introduced; indigenous; urban parks

Introduction

Most forecasts indicate that after four decades two thirds of the Earth's population will live in big cities. The concentration of the population and the compression of the infrastructure will increase the need of green areas. The urban parks are of great importance not only for human population, but also for many animals which find appropriate living conditions within the parks. So far, most of the bird species which live in cities are observed namely in the urban parks.

At this moment, there is no published systematic data about the vegetation in the parks "Lauta" and "Tsar Simeon Garden" in the city of Plovdiv, Bulgaria. There is a lack of information about the complex of avifauna in the both parks. Until this moment the influence of the vegetation on the avifauna in urban parks has not been studied in Bulgaria.

The aim of this study was to determine the impact of the vegetation on the avifauna in the both urban parks - "Lauta" and "Tsar Simeon Garden", located in the city of Plovdiv, Bulgaria.

Materials and Methods

Study area. The study was conducted within the park "Lauta" and park "Tsar Simeon Garden" in the city of Plovdiv(42° 9' N, 24° 45' E), one of the most populated

cities in Bulgaria (over 365 000 inhabitants on 102 km^2) (PETROVA, 2011).

Data collection and analyses. The fieldwork was carried out in the years 2010 and 2011. The plant species were determined according to the Qualifier of the plants in Bulgaria (DELIPAVLOV *et al*., 1992), Botanical Atlas (CHESHMEDZHIEV & STOYCHEV, 2005), Decorative dendrology (VAKARELOV & GENCHEVA, 2005), Qualifier of the bushes and trees in Bulgaria (GRAMATIKOV, 1992), Flora of Bulgaria (STOYANOV & STEFANOV, 1948), and Dendrology (STEFANOV & GANCHEV, 1953). Unpublished data concerning the species composition and distribution of plants in park "Tsar Simeon Garden" also was used (HADZHIISKI, 2003). Latin and Bulgarian names are written according to the Qualifier of the plants in Bulgaria (DELIPAVLOV *et al*., 1992). The data concerning the general characteristics of the plant species in the essay are according VAKARELOV & GENCHEVA (2005). Herbaceous species are presented in the floristic characteristic of species composition because in city parks they are of minor importance for species richness and abundance of the avifauna.

The classification of the plant species in terms of extent of occurrence was performed following BRAUN-BLANQUET *et al*. (1939). For the purposes of this study the species were arranged in three larger groups in terms of percentage of occurrence:

- *Rare (fewest) species*, representing 20% of the vegetation in the park, corresponding to I degree of abundance according to BRAUN- BLANQUET *et al*. (1939);
- *Common species* that represent 21% to 60% of the vegetation in the park, corresponding to II degree of abundance according to BRAUN- BLANQUET *et al*. (1939);
- *Very frequently common species* that represent 61% to 100% of the vegetation in the park, corresponding to IV and V degree of abundance according to BRAUN-BLANQUET *et al*. (1939).

The vertical structure of the vegetation was estimated according to the model of DAJO (1975).

The similarity in the vegetation and the avifauna of both parks were estimated by the Index of Similarity (S) of Sørensen by the next formula (DAJO, 1975):

$$S = \frac{2C}{A+B} \times 100$$

where: ***A*** - number of species that occur only in park "Tsar Simeon Garden"

B - number of species that occur only in park "Lauta"

C - number of species that are found within the both parks

The studies concerning avifauna characteristics were performed and the data was provided for usage from Atanas Irikov, Ph.D at University of Plovdiv, Faculty of Biology, department of Ecology. They include the observations of the bird species in park "Lauta" from 2000 to 2010 in all seasons and in park "Tsar Simeon Garden" from 2008 to 2011. Different bird species were recorded using transects and absolute statement by visual and acoustic observations and registrations. Binoculars with a magnification of 30x50 were used for observations.

The field qualifier of MICHEV & SIMEONOV (1991) was used to determine bird species and the Latin and Bulgarian names of the birds were given according it.

The conservation status reflects the basic legal measure provided for the Protection of Birds in Bulgaria - Biodiversity Act (State Gazette, 2002). The conservation status of the birds has been prepared according to the Atlas of Breeding Birds in Bulgaria (BSPB, 2007). The categories of threats are based on the criteria of IUCN (BirdLife International 2000; 2004).

Results and Discussion

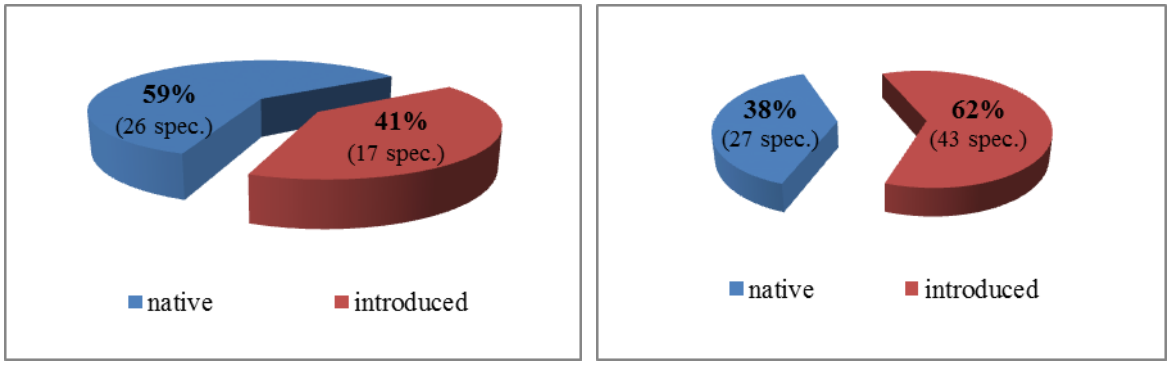
Estimation of the vegetation in the parks "Lauta" and "Tsar Simeon Garden" situated in city of Plovdiv.

In park "Tsar Simeon Garden," 70 species of trees and bushes were identified.

In the park "Lauta" were identified 41 species of trees and bushes. The trees and bushes were described in the following order: family; genus; species; short characteristic (from ecological point of view) of the species; form of the crown; height in meters and under what type of form were recorded in the park area.

Origin of the vegetation.

In park "Tsar Simeon Garden" introduced plant species dominate (62%) over native (38%) in comparison with park "Lauta" where more native plant species were observed (59%) in comparison to the introduced plant species (41%) (Fig.1).

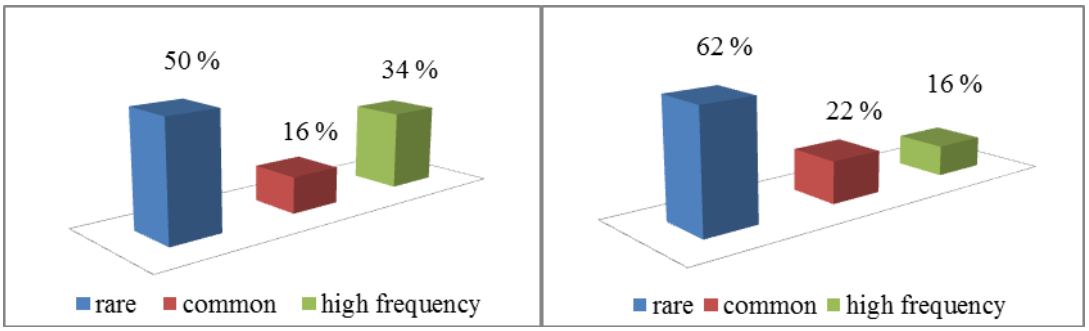


a) *b)*

Fig.1. Percetage of native to introduced plant species in park "Lauta"(*a*) and park "Tsar Simeon Garden" (*b*)

Frequency of occurrence of the plant species

The frequency of occurrence of the vegetation showed the typical appearance of the park systems, namely the existence of many rare species with low numbers. In both parks the species with an average frequency of occurrence were approximately equally represented. There was a serious difference in the common species, which was reflected by the fact that in park "Lauta" there were few plants with a much higher frequency in comparison to the park "Tsar Simeon Garden" (Fig.2).



a) **b)**

Fig. 2. Frequency of occurrence of the plant species in park "Lauta" (a) and in park "Tsar Simeon Garden" (b)

Spatial structure - vertical and horizontal (level) structure and types of the crowns

Vegetation in park "Lauta" was characterized by a complex spatial structure and the presence of five clear distinct levels in the vertical direction, while in park "Tsar Simeon Garden" there was no clear distinct level of bushes and low wood.

The horizontal spatial distribution of the vegetation in park "Lauta" was observed to be much more homogeneous in comparison to the mosaic character of the

vegetation in park "Tsar Simeon Garden", probably as a result of the presence of many paths and roads within.

In park "Lauta" there was a wide variety of types of tree crowns in comparison to park "Tsar Simeon Garden", suggesting that "Lauta" is more preferable for avifauna.

Comparison of the vegetation in parks "Lauta" and "Tsar Simeon Garden"

The species diversity of the vegetation in park "Tsar Simeon Garden" was significantly greater (70 species) in comparison with the species diversity of the vegetation in park "Lauta" (43 species). The differences in the vegetation between the two examined parks may be explained by the fact that "Tsar Simeon Garden" was managed and in all seasons recreational activities were maintained, directed and regulated, whereas natural regeneration and succession processes dominated in "Lauta" due to the almost complete absence of such events. The similarities between the both parks were mostly in the species biodiversity (Fig.3).

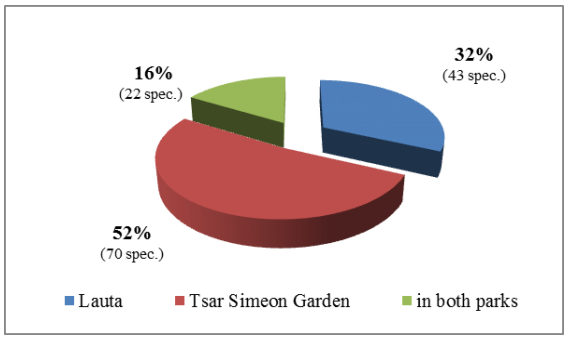


Fig. 3. Degree of similarity between the plant species from trees and bushes-levels in park "Lauta" and park "Tsar Simeon Garden".

Estimation of the avifauna in the parks "Lauta" and "Tsar Simeon Garden" in Plovdiv

The species diversity of the avifauna in park "Lauta" was twice as high (42 species) than the avifauna in park "Tsar Simeon Garden" (22 species). Twenty-one species occurred only in "Lauta", twenty one species were found in both parks and one species was recorded only for park "Tsar Simeon Garden".

The bird species in both parks were described in the following order: Order; Family; Genus; Species; under what type of form the species were recorded within the parks; category of threat and conservation status.

The complex of avifauna in park "Lauta" was determined to be two times higher in comparison with the complex of avifauna in park "Tsar Simeon Garden". Most of the species in both parks occurred in the high tree level, then the average, low and at last, in the level of the bushes.

Fewer bird species were observed in park "Tsar Simeon Garden" (Fig. 4), probably due to the under-developed level of the bushes and low trees; the predominance of the introduced plant species; and because of the management practices in the park. Hence the living conditions were significantly different from the conditions in the natural forest ecosystems.

In park "Lauta" the opposite tendency was observed: there were more bird species and better developed vertical structure of the vegetation; predominantly native plant species; the park is unmanaged, and hence closer to natural conditions.

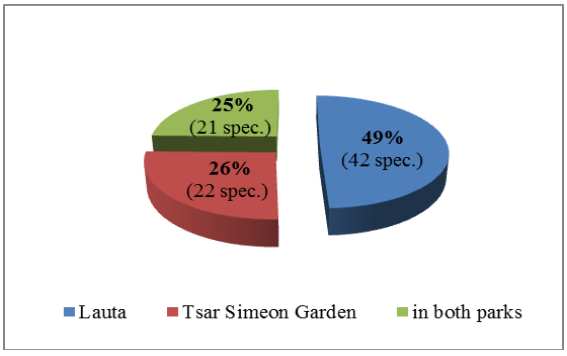


Fig. 4. Percentage of bird species in park "Lauta" and park "Tsar Simeon Garden

Purpose and way of usage of the parks area from the bird species.

The results are presented in Table 1.

Conservation status of the avifauna in the examined urban parks

In park "Lauta" were observed *28 species* strictly protected throughout the cou-

Table 1. Purpose and way of usage of the parks area by the bird species

Species in "Lauta"	Species in "Tsar Simeon Garden"	Usage:
26	26	propagation
16	9	wintering
13	6	feeding
3	2	night
5	3	assylum
3	0	temporary shelter and or shelter
3	2	other purposes (hunting and finding food, temporary wandering, searching for wintering)
0	2	trend to synantropization and adoptation to managed park areas

ntry and in park "Tsar Simeon Garden" - *14 species*.

The conservation status of the avifauna in park "Lauta" is more significant in comparison to the conservation status of the avifauna in park "Tsar Simeon Garden". In "Lauta" there is a higher number of species in threatened categories.

The conservation status of the avifauna in park "Tsar Simeon Garden" includes: *14 species* protected throughout the country; *6 species* under the protection of regulated use; *1 species* under protection and control (BA, Appendix 4); *1 species* with high priority for protection (BA, Appendix 2), protected throughout the country (BA, Appendix 3).

Bird species which fell into categories of endangerment are as follows: *18 species* without falling into category of endangered; *1 species* Spec exhausted in Europe; *1 species* Spec 2 exhausted in Europe; *2 species* Spec 3 decreasing in Europe.

The conservation status of bird species in park "Lauta" includes: *28 species* protected throughout the country; *9 species* under the protection of regulated use BDA application 4;

1 species under the protection and control of art 45 BA; *4 species* priority for protection and endangered species (BA, Appendix 2) protected throughout the country (BA, Appendix 3).

Bird species which fell into endangered categories were as follows: *27 species* without falling into category of endangerment; *1 undetermined species*; *1 species* endangered in Bulgaria; *2 species* Spec declining in Europe; *2 species* Spec 2 exhausted in Europe; *3 species* Spec 3 decreasing in Europe; *1 species* Spec 3 vulnerable in Europe; *2 species* vulnerable in Bulgaria, SPEC 3 exhausted in Europe; *3 species* Spec 3 exhausted in Europe.

Bird species, recorded for the first time in the city of Plovdiv

18 species of birds were recorded for the first in Plovdiv: *15 species* in park "Lauta" and *3 species* in park "Tsar Simeon Garden" (Table 2).

Conclusions

The frequency of occurrence of plant species in both parks met the typical appearance of the park systems, namely the existence of many rare species with low numbers. In both parks, where the study was conducted, the species with an average frequency of occurrence were approximately equally represented. Park "Tsar Simeon Garden" had a higher diversity of species but most species had a low frequency of occurrence. In park "Lauta" the species diversity was lower but most of the species, were with high frequency of occurrence.

Park "Lauta" was characterized by a more complicated vertical structure of the vegetation. There were five very well developed levels in the vertical direction, while in park "Tsar Simeon Garden" was a distinct lack of low bush and low wood

levels. In the horizontal distribution of the vegetation, park "Lauta" was much more homogeneous in comparison with the mosaic character of park "Tsar Simeon Garden". A wide variety of tree crowns were observed in park "Lauta" in comparison with park "Tsar Simeon Garden".

Table 2. Bird species recorded for the first time in Plovdiv

Park	Latin name	Common name
"Tsar Simeon Garden"	*Erithacus rubecula* (L.)	European robin
	Sitta europaea (L.)	Wood Nuthatch
	Carduelis chloris (L.)	European Greenfinch
Number of new species 3		
"Lauta"	*Accipiter nisus* (L.)	Eurasian Sparrowhawk
	Accipiter brevipes (Sev.)	Levant Sparrowhawk
	Falco tinnunculus (L.)	Common Kestrel
	Picus canus (Gmel)	Grey–faced Woodpecker
	Dendrocopos minor (L.)	Lesser Spotted Woodpecker
	Lanius colurio (L.)	Shrike
	Troglodytes troglodytes (L.)	Winter wren
	Erithacus rubecula (L.)	Wood Nuthatch
	Turdus pilaris (L.)	Fieldfare
	Hippolais pallida (Hemp., Ehronb.)	Eastern Olivaceous Warbler
	Aegithalos caudatus (L.)	Long–tailed Tit
	Sitta europaea (L.)	Wood Nuthatch
	Certhia familiaris (L.)	Eurasian Treecreeper
	Coccotrhaustes coccothraustes (L.)	Hawfinch
	Montifringilla nivalis (L.)	White–winget Snowfinch
Number of new species 15		
Total number of new species 18		

The similarity between trees and bushes in both parks was low (39%). More than half of the plants (52%) appeared only in park "Tsar Simeon Garden", another part (32%) were typical only for park "Lauta" and a few plant species (16%) occurred within both parks.

According to the results of the conducted study the vegetation in the parks "Lauta" and "Tsar Simeon Garden" has a significant influence on the complex of birds living within the parks.

In both parks most of the bird species were nesting in the high wood level, then at the average level, low level and at the least level of the bushes. Less birds were observed in park "Tsar Simeon Garden" due to under-developed low level, which includes bushes and low trees.

The degree of similarity of the complex of birds in both parks was essential (66%). Half of the species (49%) of birds were found only in park "Lauta", while only 26% of species were specific to "Tsar Simeon Garden". Twenty-five percent of registered bird species occurred within the two parks.

The conservation status of the avifauna in park "Lauta" is much more significant than the same in park "Tsar Simeon Garden".

Acknowledgements

I would like to thank professor Ivan Kiryakov for his help concerning the botanical part of this study,

to the colleagues from the Municipality of Regions "Centralen" and "Trakia" in city of Plovdiv for their cooperation. Thanks to engineer Stamo Stamov and engineer Vasil Hadjiiski for providing me some important data concerning the plant species in both parks and thank you also to Vladimir Dobrev and Georgi Gerdjikov (from BSPB) for providing me data concerning the bird species.

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Received: 20.07.2012
Accepted: 29.09.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 33-41

Eco-physiological Study on the Influence of Contaminated Waters from the Topolnitza River Catchment Area on Some Crops

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Abstract. The present study is a small part of a program for an investigation of the water conditions in the Topolnitza Dam Lake, Topolnitza River and its catchment area. The sensitivity of seeds and young wheat, sunflower and mustard plants to heavy metal stress was examined at laboratory conditions. Our results showed that seedling growth was more sensitive to heavy metals in comparison to seed germination. The length of shoot and root has been adversely affected due to water contamination when compared to the control. A certain negative effect on the photosynthetic pigments content was registered.

Key words: crops, pollution, heavy metals, Topolnitza River, Bulgaria

Introduction

Mining of minerals and metals is important for the economic or industrial development of society all over the world. In the past century, much of the mining has concentrated on the extraction of such metals as gold, silver, iron, copper, lead, zinc, chromium, mercury, then some others, for example, As, Cd and Se are often enriched in mineral deposits and recovered as by-products. Disposal of mines resulting in waste tailings posed a significant risk to the surrounding environment. Their improper management resulted in the migration of heavy metals to the surrounding environment, contributing to the contamination of soil substrates, destruction of ecological landscape, groundwater pollution and decrease in biological diversity. Metals are currently of much environmental concern due to the fact that they tend to bio-accumulate in food chain and are harmful to humans and animals. The mechanisms of metal toxicity induction are not fully understood for crop plants. Metals may directly or indirectly interfere with the metabolic activities by altering the conformation of proteins for example enzymes, transporters or regulatory proteins by their strong affinities as ligands to sulfhydryl and carboxylic groups (SHARMA & SHARMA, 1996).

The photosynthetic surface area and leaf chlorophyll contents are the major factors determining the total biomass production in plants. Cadmium and lead are reported to have inhibitory effect on photosynthesis, transpiration, carbohydrate metabolism and other metabolic activities (KUPPER *et al*., 1996; VASSILEV *et al*., 1998; XU *et al*., 1998). STOBART *et al*. (1985) have reported that Cd particularly inhibits the chlorophyll biosynthesis and decreases total chlorophyll content and chlorophyll a/b ratio, while Pb toxicity emerges with the disturbance of mitosis, toxicity of nucleoli, inhibition of root elongation due to mitotic effects on the root tip cells of *Brassica juncea*, appearance of chlorosis, inhibition of enzymatic activities and reduction in photosynthesis.

The Topolnitza River catchment area is rich of mining activities and many metals are persistent in the water basins. These waters are being used by farmers to irrigate crop plants without considering its adverse effects - the metals, presented in them, may accumulate in plants in excessive quantities quite enough to cause the clinical problems in animals and human being consuming these metal rich crops. Hence, monitoring of accumulation and distribution of metals in crop plants cultivated in agricultural land irrigated with water from Topolnitza Dam Lake, Topolnitza River and its catchment area is necessary.

Experiments were planned and conducted to study the influence of contaminated waters from the Topolnitza River catchment area on some crop plants cultivated in laboratory conditions.

Materials and methods

Collection of water samples

Sampling was made in the beginning of October 2011 when is the period of the autumn low flow. Water samples were collected in clean containers (5 l) from selected points of Topolnitza River catchment area, as follows: Site 1 (control) – Micro dam near Borimechkovo village; Site 2 – Topolnitza River, near Lesichevo village; Site 3 – Topolnitza Dam Lake, near the wall; Site 4 – Topolnitza River, near Poibrene village; Site 5 – Topolnitza River, near Petrich village; Site 6 – Topolnitza River, near Chavdar village; Site 7 – Topolnitza River, near the town of Zlatitza; Site 8 – Medetska River, before Topolnitza River (Fig.1).

Water analysis

In situ were measured temperature (BDS 17.1.4.01), pH (ISO 10523:2008) and the conductivity (EN 27888) of the water. Chemical analyses, including Pb (ISO 17294-2), Fe (ISO 6332), Mn (BDS 17.1.4.15), Ni (ISO 17294-2), Cu (ISO 17294-2) and As (ISO 17294-2) content in the collected water samples, were made in the Regional laboratory – Plovdiv.

Bioassays

The experimental bioassays were performed in the laboratory of Ecology, Faculty of Biology, University of Plovdiv. Three crop plants were tested – *Sinapis alba*, *Triticum aestivum* and *Helianthus annuus*. Fifty seeds of each species for each treatment were placed into experimental containers (petri dishes with filter paper for mustard and pots with perlite for wheat and sunflower) and irrigated with 50 ml of the collected water samples. Each treatment was made in triplicate. Irrigation was repeated periodically when needed. The seedlings were harvested after period of one week for *Sinapis alba* and two weeks for *Triticum aestivum* and *Helianthus annuus*.

Fig.1. Location of the sampling sites

Estimation of morphometric and physiologic parameters of plants

The following parameters were measured immediately after harvesting: germination of seeds (in %), shoot and root length (in cm), shoot-to-root ratio, chlorophyll content (mg g^{-1} fresh weight) and chlorophyll *a/b* ratio.

Pigment analysis was performed according SCHLYK (1965) after extraction with 90% acetone. Wavelength absorbance was measured at 662 nm for chl *a* and 644 nm for chl *b* in a SPECOL 11 absorption spectrophotometer (Faculty of Biology, University of Plovdiv). Concentrations of chl *a*, chl *b* and chl *a*+*b* were calculated for each sample and expressed on a fresh weight basis (PETROVA, 2011). We reported our

results as mean ± SD, where standard deviations were calculated on the base of triplicate analysis of chlorophyll content.

Statistical analysis

To confirm the variability of data obtained and validity of results, all the data were subjected for the statistical analyses using STATISTICA 7.0 package (STATSOFT INC., 2004).

Results and Discussion

Physico-chemical properties of the water

The results from the physic-chemical analysis of the collected water samples showed that the water quality is worst in Site 8 (Medetska River), where the pH was quite acidic (Table 1). The highest conductivity (374.0 µS) correlated with the increased heavy metal content in the same sample.

Table 1. Physico-chemical parameters of water samples

Water sample	Water temperature, °C	pH	EC, µS	Fe, mg l^{-1}	Mn, µg l^{-1}	Pb, µg l^{-1}	Ni, µg l^{-1}	Cu, µg l^{-1}	As, µg l^{-1}
Site 1	19.0	7.5	97.7	-	-	-	-	-	-
Site 2	12.2	6.7	106.3	-	-	-	-	-	-
Site 3	12.6	7.4	111.5	-	-	0.001	0.005	0.005	0.005
Site 4	11.8	6.5	158.2	0.240	0.064	0.006	0.011	0.018	0.090
Site 5	12.2	7.1	137.3	0.540	0.005	-	-	0.005	-
Site 6	12.5	7.3	290.0	0.380	0.171	0.006	0.005	0.048	0.012
Site 7	13.0	7.3	60.5	0.050	0.047	0.025	0.004	0.033	0.008
Site 8	11.1	4.8	374.0	0.131	0.027	0.025	0.040	0.092	0.010

This contamination was significantly lower in the water sample from Site 7, probably due to the dilution effect. There was registered an increment of the pH, conductivity, As and Cu concentrations in Site 6 and Site 5, located hereafter. The results of the As content were highest in water sample from Poibrene Village (Site 4), the conductivity was also quite high. We suppose that in this sampling site some organic pollution has been presented due to an illegal discharging of household waste waters, disposal of livestock manure, etc. The effect of that kind of anthropogenic impact on water quality had been well known in many rivers (VALCHEVA & POPOV, 2007; VALCHEVA & POPOV, 2008). In Site 3 (Topolnitza Dam Lake near the wall) the EC value was lower, that could be explained with some processes like dilution and sedimentation, and close to this was the water sample from the next sampling site - Site 2.

The values of measured characteristics of the water, obtained in this study, were higher in comparison with data from previous research in this region (PETROVA *et al*., 2011). This could be due both to the different sampling periods, the variances in water debit, and also to the autumn senescence and consequent eutrophication of the rivers.

Germination

Seed germination is a critical phase of the life-cycle in most plant species, particularly in cultivar plants. Stressful conditions exert a strong effect on the dynamics of seed germination and seedlings establishment. There was not observed considerable effect of contaminated waters on the seed germination capacity for wheat and sunflower in comparison with the mustard (Fig. 2). This issue may relate to permeability of seed coat to metal ions. Different degree of permeability led to a different degree of germination inhibition.

No negative effect was observed in the biotest with *Helianthus annuus* seeds. All cases resulted in a stimulation of the germination process, most pronounced in water sample from Site 4 (123%). The lowest values in the biotest with *Triticum aestivum*

(88% in comparison with the control) were found in pots irrigated with water from Site 7, followed by the Site 8 (94%). The mustard seeds showed higher sensitivity and the germination varied from 55% in pots irrigated with water from Site 6, followed by Site 8 (65%), through Site 4 (89%) to Site 5 (91%), Site 7 (93%) and Site 3 (94%).

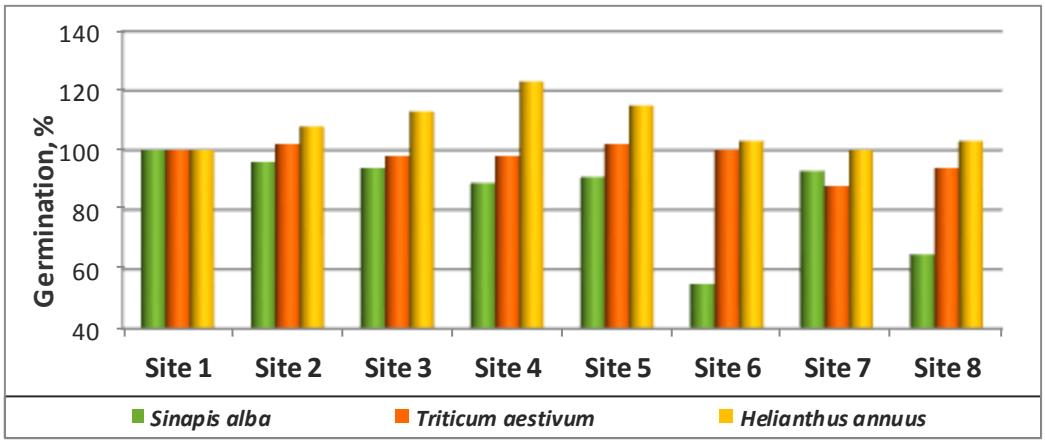

Fig. 2. Germination results for the studied plant species, % in comparison with control sample

Our results can support to the observations described by other authors (FARGASOVA, 1994; WIERZBICKA & OBIDZINSKA, 1998; PANDEY, 2006, 2008; URUÇ & YILMAZ, 2008) of the effect of contaminated with some heavy metals (Ni, Cu, Pb) waters on seed germination of different crop plants.

Growth

The root and shoot length and elongation rate are essential for plants exploring for water and mineral nutrients. Our results showed that seedling growth was more sensitive to heavy metals in comparison to seed germination. The length of shoot and root has been adversely affected due to water contamination when compared to the control (Fig. 3).

As a result from all bioassays conducted, we found the stronger inhibition on the plant growth, both for the aboveground and underground plant parts, in pots irrigated with water from Site 8 (Medetska River). The effect of this water sample was most intense at the root length – we observed a reduction of 95% to wheat seedlings, 93% to mustard seedlings and 87% to sunflower seedlings in comparison with the control plants. Suppression of the shoot growth was also well expressed - 75% (*Sinapis alba*), 70% (*Triticum aestivum*) and 69% (*Helianthus annuus*) shorter seedlings in comparison with the control.

The effect of the tested water samples on sunflower plants growth and development was negative. An exception was the stimulated root elongation in experimental plants irrigated with water from Site 4, which assumed that have been rich in organic matter.

Mustard seedlings had similar shoot length in all cases (except water sample 8). We registered an inhibition of the root growth in pots treated with water from Site 6 (39%) and a positive impact of water samples from Site 2 (169%), Site 4 (132%), Site 5 (129%) and Site 7 (122%) when compared to the control.

We found some stimulation of the root elongation of the wheat seedlings in all bioassays except the Site 8, and an increment of the wheat shoot growth in experimental pots irrigated with water from Site 5 and Site 6. AYDINALP & MARINOVA (2009) studied the effects of heavy metals (Cd, Cr, Cu and Ni) on *Medicago sativa* plants. Their results, similarly to ours, showed that the low concentration of these chemical elements had positive effect on shoot and root growth, but in large quantities they became toxic.

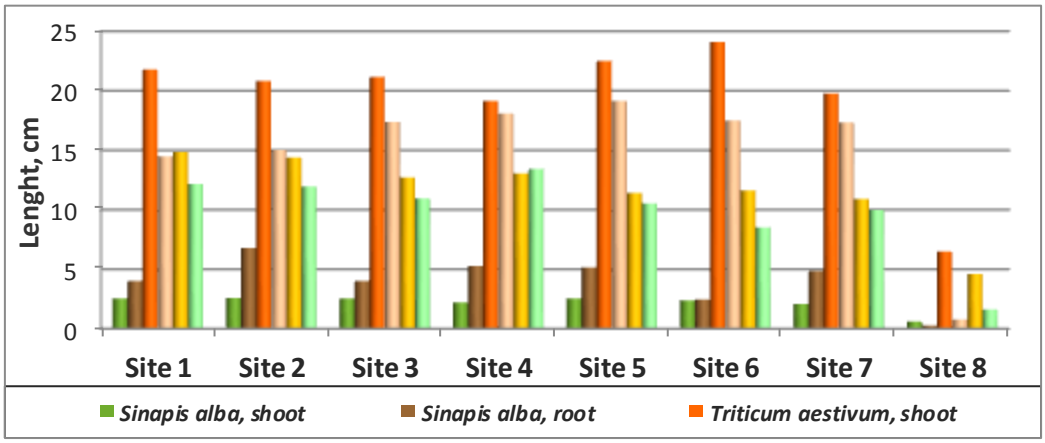

Fig. 3. Shoot and root length of the seedlings (in cm)

The above results were in agreement with the findings of CHEN *et al.* (2003) in soybean, RAI *et al.* (2005) in *Phyllanthus amarus* and XU *et al.* (2008) in garlic. The inhibitory action in root and shoot length might be due to reduction in cell division, toxic effect of heavy metals on photosynthesis, respiration and protein synthesis. These obviously contributed to the retardation of normal growth (KUPPER *et al.*, 1996).

HAGEMEYER *et al.* (2002) and MARCHANO *et al.* (2002) also suggested that the morphological and structural effects caused by metal toxicity in plants was due to decrease in root elongation, root tip damage, decrease in root formation, suppression of elongation growth rate of cells, affecting the ultra-cellular structure of meristematic cells and inhibition of the size of plant cells and inter cellular spaces.

Shoot-to-root ratio is a useful parameter for evaluation of the quality of the environment in which plants grow. In our study it was 2.5 (sunflower), 3.5 (mustard) and 5.5 (wheat) times higher in seedlings irrigated with water from Site 8 when compared to the control. These results could be explained with the toxic effect of the water contaminants (mainly heavy metals) and were in agreement with MAMI *et al.* (2011) findings.

The lowest values of this parameter for all three studied plant species we observed under influence of the water samples from Site 4, followed by Site 7 and Site 5 (Fig. 4). An explanation for these results could be found in the mentioned favorable effect of heavy metals in low concentrations on the root extension.

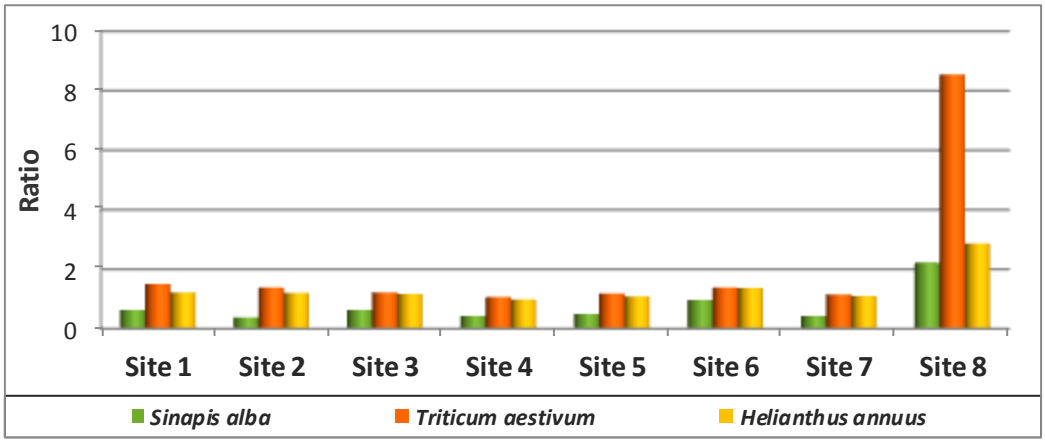

Fig. 4. Shoot-to-root ratio of the seedlings

Pigment content

It is considered that the growth parameters do not always provide enough objective information to phytotoxicity of the environment and therefore in plant bioassays, it is advisable to include functional indicators. Leaf chlorophyll content is an important parameter for testing plant status. For example, it can be used as an index of the photosynthetic potential as well as of the plant productivity (CARTER, 1998). In addition, chlorophyll gives an indirect estimation of the nutrient status (FILELLA *et al.*, 1995) and is closely related to various types of plant stresses (GITELSON & MERZLYAK, 1994).

The pigment analysis indicated that the changes in the levels of photosynthetic pigments in cotyledons varied in wide range (Fig. 5). The strongest inhibitory effect in wheat bioassays was observed at Site 8 - 57% to chlorophyll *a* and 48% to chlorophyll *b* in comparison with the control sample, followed by Site 4 and Site 5. In the rest of water samples an increased concentation was found. Stimulation of the synthesis of chlorophyll in the presence of low doses of zinc and nickel in the middle, accompanied by an increased ratio have been found by other authors (MACIFE & TAYLOR,1992; SARITA & ABHA, 2007) and could explain our results.

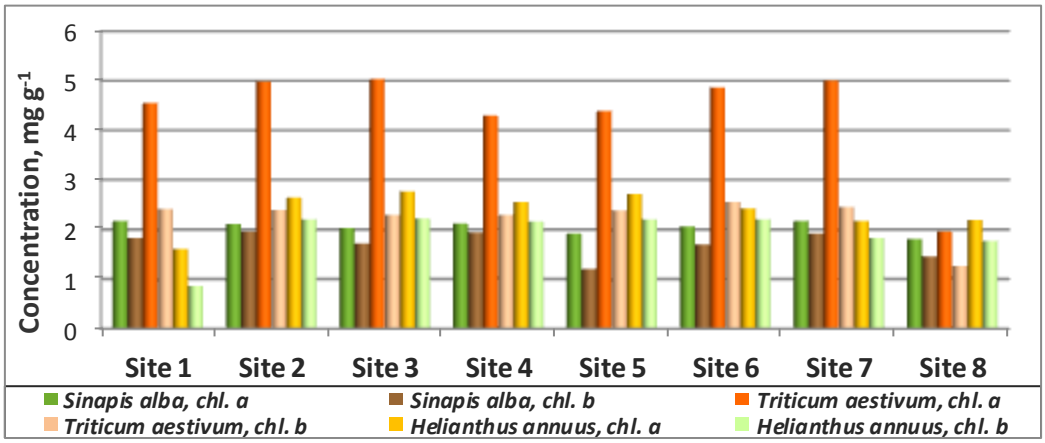

Fig. 5. Chlorophyll content in the leaves of the investigated plants (mg g^{-1} fresh weight)

The bioassay with sunflower plants revealed that the lowest chlorophyll content was determined in the control and in all other cases there was an increment of its concentration – up to 171% to chlorophyll *a* and to 259% to chlorophyll *b* both in water sample from Site 3. Similar to our findings, raise of the level of photosynthetic pigments in sunflower plants were observed by SINGH *et al.* (2004). In the course of their experiment with industrial wastewaters, after the 30th day of exposure the chlorophyll content was higher, however, extend the time the effect became unfavorable and led to a reduction of its amount in comparison with the control.

The mustard seedlings of the water sample 8 were most affected - 17% inhibition of chlorophyll *a* and 21% of chlorophyll *b*, followed by water from Site 5. The change in the amount of both chlorophylls was not clear and there was no statistical reliability.

The change in the chl *a/b* ratio (which had the advantage to be a dimensionless parameter) could be used as more informative indicator in ecological investigations. In all bioassays conducted, the highest ratio values were observed in seedlings with lowest total chlorophyll content – *Sinapis alba* at Site 5, *Triticum aestivum* at Site 3 and *Helianthus annuus* at Site 1 (Fig. 6). The decrease of chlorophyll content may be due to an increase of chlorophyll degradation or to a decrease of chlorophyll synthesis. During the process of chlorophyll degradation, chl *a* is converted in chl *b* (FANG *et al.*, 1998) and this may

explain the depression of chlorophyll content together with the increase of the ratio chl *a/b*.

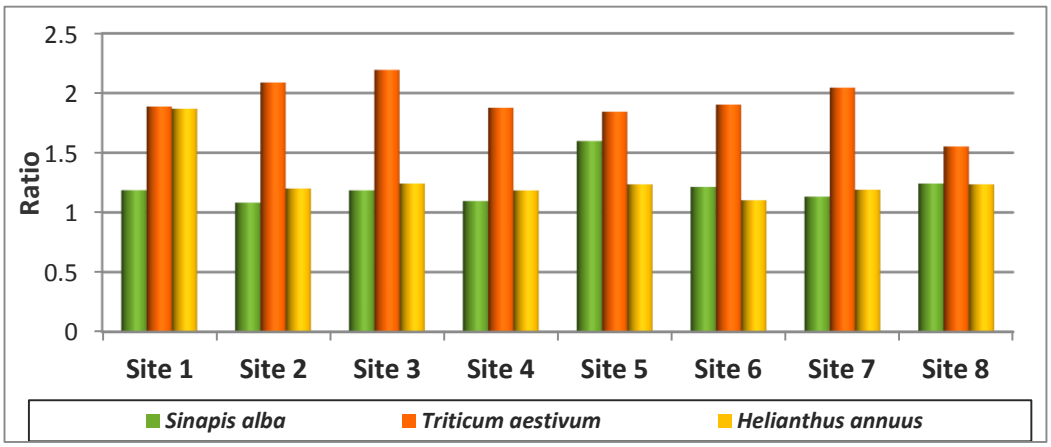

Fig. 6. Ratio chlorophyll *a/b* in the leaves of the investigated plants

Conclusion

The physico-chemical analyses of water samples from Topolnitza River catchment area have revealed that these effluents carry very high toxic metal content and are one of the major sources of soil and water pollution in this area. It indicated that the explored water basins (used for irrigation) and the surrounding agricultural land are not fit for agricultural practices. As a result, it is possible to reduce the biomass production without any visible symptoms of metal toxicity (chlorosis, necrosis, dryness). Further, the study revealed that these crop plants are not fit for consumption by human beings and livestock.

Acknowledgements

This study was carried out with the financial assistance of University of Plovdiv, Faculty of Biology, by Fund BF 006.

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Received: 25.07.2012
Accepted: 13.11.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 43-49

Data on the Hawthorn Psyllid Cacopsylla melanoneura (Förster) Populations in Southeast Romania

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Abstract. In 2009, the hawthorn psyllid, *Cacopsylla melanoneura* (Förster) (Hemiptera: Psyllidae) was investigated in Băneasa area within the framework of a pests monitoring program initiated at the Research Development Institute for Plant Protection Bucharest. This psyllid is an important pest for apple orchards in Europe, as it is a vector of the apple proliferation phytoplasma. The overwintered adults colonized the hawthorn and apple as well as the sweet cherry plants in the neighboring of the apple orchards starting with the beginning of March. Egg laying period began in late March (on hawthorn) and early April (on apple). The larvae completed their development on hawthorn and on apple between middle April and June. No eggs or larvae were found on sweet cherry. The adults of the new generation were observed since the beginning of May until June, after that they left the hawthorn and apple plants. Our data indicated that the hawthorn, compared with apple, is mostly preferred as host plant for *C. melanoneura*, thus the most abundant populations of this insect have been observed on this.

Key words: *Cacopsylla melanoneura*, population dynamics, biology, hawthorn, apple

Introduction

The hawthorn psyllid *Cacopsylla melanoneura* (Förster) (Sternorryncha: Psyllidae) is an important pest in apple orchards in Europe, being involved in the spread of the phytoplasma pathogen which causes the apple proliferation disease (TEDESCHI *et al.*, 2002; TEDESCHI & ALMA, 2004; DELIC *et al.*, 2007; PEDRAZZOLI *et al.*, 2007; FIALOVA *et al.*, 2008; MALAGNINI *et al.*, 2010). The psyllid *Cacopsylla picta* (Förster) (CARRARO *et al.*, 2001b; FRISINGHELLI *et al.*, 2000; JARAUSCH *et al.*, 2003) and the leafhopper *Fieberiela florii* (Stal) (KRCZAL *et al.*, 1989; TEDESCHI & ALMA, 2006) are also reported as vectors of this pathogen.

C. melanoneura psyllid is described in the European literature as a species that develops one generation per year and overwinters as adult on coniferous plants (TEDESCHI *et al.*, 2002; ČERMÁK & LAUTERER, 2008). The studies on psyllid in different regions and under different climatic conditions revealed that, the overwintered adults named remigrants (MAYER *et al.*, 2009) migrate earlier in spring to plant species of the genus *Crataegus*, *Malus* and *Pyrus* where the copulation, oviposition and larval development take place. The adults of the new generation named emigrants (MAYER *et al.*, 2009) leave these host plants and come back to their coniferous overwintering hosts.

In Romania, the researches with respect to *C. melanoneura* psyllid refer only to descriptions and illustrations of morphological characters and genital apparatus for the adults collected from species of *Crataegus* (hawthorn), *Pinus montana mughus* (mountain pine) and *Abies alba* (European silver fir) in different locations in the South and South-West of the

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country (DOBREANU & MANOLACHE, 1962). The data are included in Psylloidea volume in *Fauna of Romania* helping in identifying the psyllid species. The winter adults of psyllid were also reported in pear orchards (CHIRECEANU & HONDRU, 1993). Studies concerning the role of *C. melanoneura* as vector in the epidemiology of apple proliferation disease in Romania are not available.

In the present work, we report the results of the investigations on the presence and abundance of the hawthorn psyllid *Cacopsylla melanoneura* in Băneasa area (South-East Romania) to obtain information on the biology and the population dynamics, as well as its preferences for different plant hosts. Based on data provided by the psyllid collections, we could outline the life cycle, structure and size of populations in relation to host plants.

Materials and Methods

The study on the population dynamics of *C. melanoneura* psyllid was carried out during spring 2009 (March-June) on apple, spontaneous hawthorn (*Crataegus monogyna* Jacquin) and sweet cherry trees located in the fruit trees research platform of Băneasa. Six collection points were used: an abandoned old apple orchard (1,2ha), an apple plot without pesticide (20 trees disposed in two rows), two times insecticide treated apple plot (11 trees disposed in two rows), two hawthorn trees inside of small brushwood, two hawthorn and one sweet cherry trees in the vicinity of abandoned apple orchard. The brushwood included species of woody plants such as pine and oak. Before beginning these investigations, a preliminary visual inspection had been made around the area to detect the presence of first *C. melanoneura* overwintered adults and to select the collection points. The adult population densities of psyllid were monitored weekly using the yellow sticky traps and the shoots beating methods from March until June. Two yellow sticky traps (15x30 cm, atraCERAS-type of Romanian origin) per hawthorn collecting points (one trap per tree), four traps per apple and one trap on sweet cherry were set in trees canopy on 1st March. Five shoots per tree at each collection point of hawthorn and sweet cherry trees and twenty-five shoots per apple were beaten upon an entomological net of 47 cm diameter in opening. The eggs and larvae density was estimated only on the hawthorn inside the brushwood and on abandoned apple orchard by examination of six floral formations and counting them under a stereomicroscope. The sampling was weekly. To precise the moment of eggs and nymphs emergence, the leaves and shoots of the plants were carefully examined daily. The psyllid adults were examined under a stereomicroscope and identified to species level according to adult's description in *Fauna of Romania* (DOBREANU & MANOLACHE, 1962) and the identification key for the Central European *Cacopsylla* species (BURCKHARDT, 2010). Taking into account the striking morphological resemblance between the adults of *C. melanoneura* and *C. affinis* species which live permanently together in mixed population on *Crataegus* spp. (DOBREANU & MANOLACHE, 1962; TEDESCHI *et al.*, 2009), and that this fact made difficult their separation, we appreciated this species complex as being *C. melanoneura* ones.

Daily air temperatures (average, minimum and maximum) and precipitations recorded throughout the collecting period were acquired using the Metrilog's system placed on Research Development Institute for Plant Protection Bucharest area. The averages of three days temperatures were calculated and used on graphic.

Results

Throughout the monitoring period a total of 6718 adult psyllids have been collected using the yellow sticky traps combined with shoots beating method on spontaneous hawthorn, apple and sweet cherry plants. Out of these, *C. melanoneura* was the predominant species, 6700 adults were separated, representing 99.73% of all psyllids. Data on the simultaneous weekly collections made possible to outline the population dynamics of *C. melanoneura* adults in the investigated area, as they are presented in Fig. 1 (A-F). The results

indicated that the overwintered adults populated the hawthorn and apple and also the sweet cherry trees since the beginning of March to the end of May. They were collected beginning with 2nd of March on hawthorn as well as apple trees, and one week later (10th of March) on sweet cherry tree using the shoots beating. In this time, the average daily temperatures were above 4°C (Fig. 2). In figure 2 we illustrated the minimum, average and maximum values of three days temperatures and precipitation fallen in Băneasa area during the monitoring period. The cumulative number of overwintered adults that colonized the hawthorn trees reached values 3.6 times higher than those on apple and 9.9 times higher than those on cherry tree. The dynamic curves of overwintered adults, designed by yellow sticky traps captures, marked two peaks in their migratory movement to spring host plants, except for the insecticidal treated apple which showed only one peak. The two peaks of captures were registered in the first and second week of May, when the average temperatures reached 15°C and 20°C, respectively. Measured by the beating method, the overwintered adults dynamic showed only one peak, one week later than the first peak of yellow sticky traps captures.

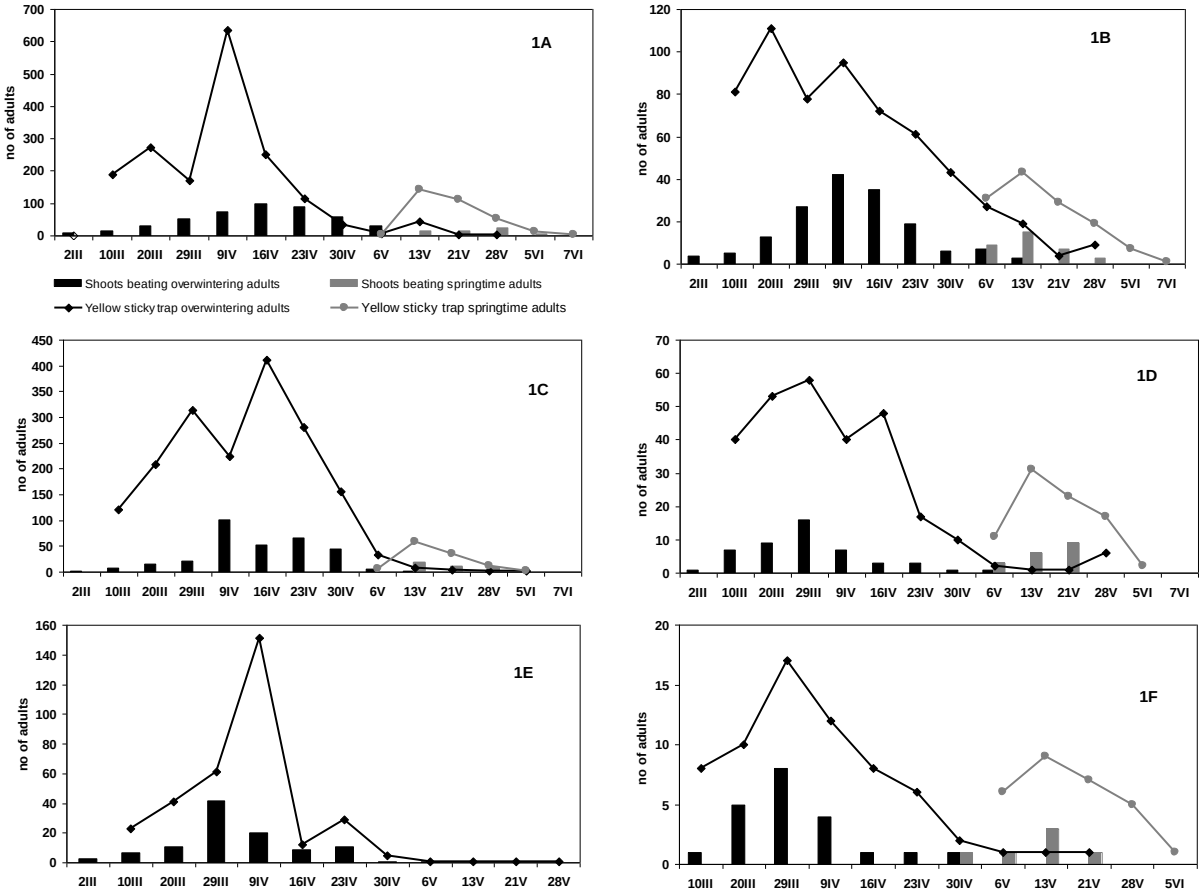

Fig. 1. Population dynamics of *Cacopsylla melanoneura* psyllid (overwintering and spring adults) on hawthorn plants inside of the brushwood (1A), on hawthorn plants in the neighborhood of abandoned apple orchard (1C), on abandoned apple orchard (1B), on apple without pesticide (1D), on two times insecticide treated apple (1F) and sweet cherry trees (1E) in the year 2009.

The new adults of spring generation, noted and registered on hawthorn and apple but not on cherry trees, emerged in first week of May, when the average temperatures were over 14°C. They were collected to the first week of June; they spent approximately a period time of five weeks on both hosts. The population of spring

adults on hawthorn was about two times higher than on apple.

Following the comparative results related to the number of adults recorded on each plant species by using the two collecting methods, we could observe that the yellow sticky traps indicated a longer time of adults' activity, with 2-3 weeks for the overwintered and with 1-2 weeks for the spring adults, than the beating sampling method.

Taking into account the three apple points that were investigated, a greater number of adults were recorded in abandoned apple, twice higher than in pesticide untreated and seven times higher than in pesticide treated apple. The adults density also varied in relation to the place where the hawthorn trees were located, namely it was 1.2 times higher on hawthorn placed inside the bushes than on the individual trees near the apple. Fluctuations of the psyllid adults dynamic generally followed a similar pattern on all surveyed plants.

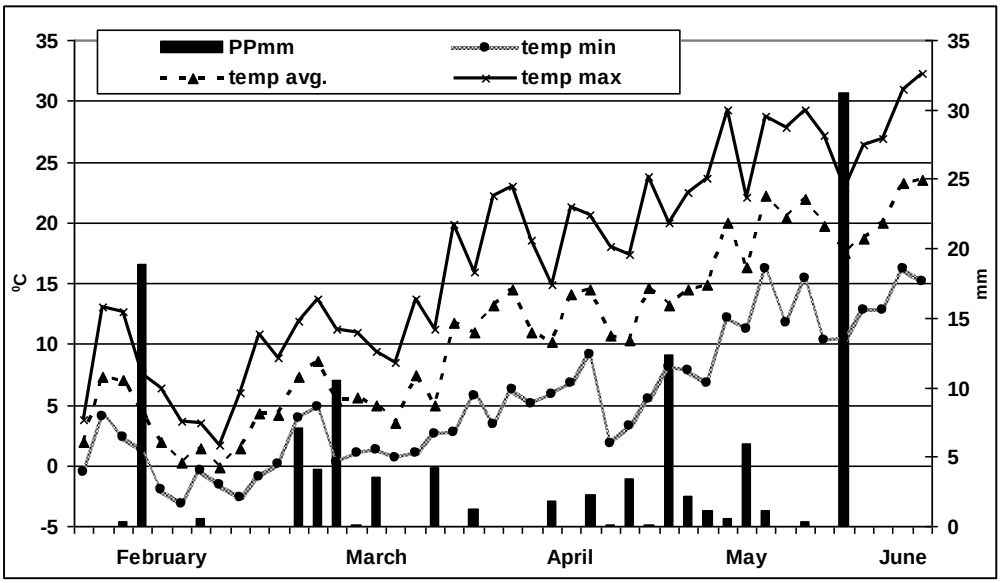

Fig. 2. The average, minimum and maximum air temperatures and precipitations recorded throughout the collecting period in Băneasa area

In figure 3, we illustrated the dynamics of the eggs and succeeding larvae populations, sampled in two collecting points, the abandoned apple and the hawthorn inside of brushwood.

With the end of March (on hawthorn) and the beginning of April (on apple), the overwintered females started to lay their eggs on leaves and floriferous organs by the end of May. Therefore the first eggs were observed on 29th March on hawthorn, and about one week later on apple (9th April). The first psyllid larvae began to emerge on hawthorn on 16th April, one week earlier than on apple (23rd April). The abundance levels of *C. melanoneura* eggs and larvae on hawthorn were of three and four times higher to those on the abandoned apple. During the spring period, when both eggs and larvae populations were increasing, the climatic conditions in collecting zone were favorable, characterized by the average and maximum daytime temperatures more than 12°C, and 15°C, respectively, and by very low precipitation. Our results indicated the psyllid eggs and larvae populations marked one peak. The maximum number of laying eggs was recorded in middle of April on hawthorn trees, and two weeks later on apple trees. For the populations of larvae, the maximum number in their dynamics was marked at the end of May on reproductive host plants, hawthorn and apple trees.

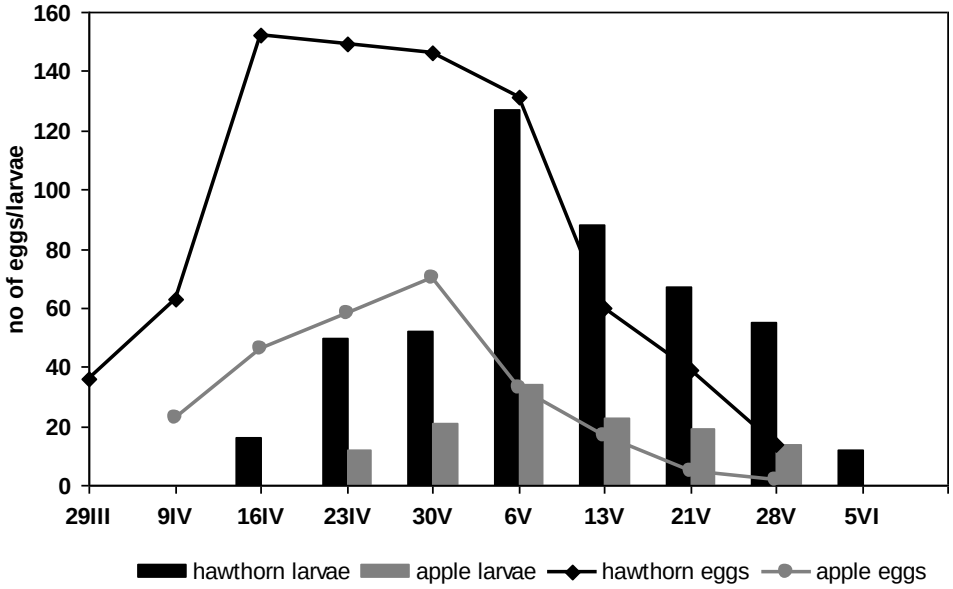

Fig. 3. Population dynamics of eggs and larvae of *Cacopsylla melanoneura* on hawthorn and on apple in the year 2009

Discussion

This entomological study is the first contribution to the knowledge of the presence, abundance and the biology of *C. melanoneura* and their comparatively population dynamics in relation to the host plants in Romania. By monitoring and capturing a large number of *C. melanoneura* specimens, this study allows us to establish the population dynamics course and the life cycle of the psyllid, from the overwintered adults to the new adults of spring generation, on hawthorn and apple plants under climatic conditions of Băneasa area, which could be valid for the climate conditions in South East of the county. The results in this work paper are in accordance with the literature findings (TEDESCHI *et al.*, 2002; ČERMÁK & LAUTERER, 2008; MAYER *et al.*, 2009) which indicate that the *C. melanoneura* species completes one generation per year on hawthorn and apple plants from the end of winter to the early summer. According to our data, *C. melanoneura* spent a little more than three months time on their spring reproductive host, hawthorn and apple plants. The overwintered adults arrived on spring hosts starting with the beginning of March, and the adults of new generation left them in the first seven days of June. This information could provide a good indication to forecast the adults' migration to apple orchards and also to warn over the optimal moment of the insecticide treatments against them. The eggs and larvae dynamics showed a maximum population between the end of April to beginning of May. For this period time the warning over the second chemical treatment date, corresponding to a prior-blossoming time, could be possible.

As our data specify, beside the hawthorn and apple plants, a high number of *C. melanoneura* overwintered adults populated the sweet cherry plants located near the apple orchards. This result may indicate that this species could play the role of an intermediate host plant where insects may cut off their migration from conifers to their spring reproductive host plants. No eggs, larvae or new adults were observed on sweet cherry, suggesting that it seems to be not an adequate host plant for the psyllid development. The overwintered adults from the sweet cherry needed to migrate to the hawthorn or apple plants to develop the new generation. Comparative observations under laboratory conditions showed that the psyllid is attracted by the odors of hawthorn and apple to the cherry detriment (GROSS & MEKONEN, 2004).

The substantially more abundant adult's population captured by us on

hawthorn, representing their main host plants, strongly confirmed the greater preference for this one than for apple. A similar situation was found in case of the psyllid eggs and larvae. Results are in compliance with those found in ovipositions bioassays conducted in Germany (MAYER *et al.* 2009) where the *C. melanoneura* preferred the hawthorn as host plant for reproduction and development, rather than the apple plants.

The presence of the high populations of the psyllid on hawthorns placed in the neighborhood of the apple and also on the abandoned apple orchard, could represent a great threat for the productive apple orchards in Romania, both being important components in the epidemiology and risk of apple phytoplasma spreading by psyllid (TEDESCHI *et al.* 2009).

Based on our results a permanent survey of the hawthorn psyllid in apple orchards shall be carried on in order to detect its possible role as vector in apple proliferation phytoplasma spreading in Romania.

Acknowledgments

The authors would like to thank Dr. Rosemarie Tedeschi from University of Turin, Italy, for the scientific literature and support offered. Also, we would like to offer our thanks to Mrs. Carmen Ghinea, ESP Assistant Professor in "Spiru Haret" University, for having kindly reviewed the English version of the present paper. This research was integrated in the research project PN-09-ICDPP-01-02/2009 financed by the Romanian ANCS.

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Received: 25.07.2012
Accepted: 13.11.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 51-60

Ecotoxicology Assessment of Waste Water Emitted From Radomir Metal Industries (Bulgaria)

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Abstract. The purpose of this article is to evaluate the toxicological effect of wastewater emitted from „Radomir Metal Industries". It has been registered that the range of 50% mortality (LC_{50}) of great water flea (*Daphnia magna* St.) is limited between 75% and 80% effluent. The data mortality rate-effluent dilution for *Pseudorasbora parva* well correlated with linear regression, R^2=0.86. LC_{50} is reported from exposure to raw sewage (100%). Results indicate that even when individual concentrations of toxic metals are within the permissible limits the effluent remains toxic for the hydrobiota perhaps due to the combine effect of the contaminants.

Keywords: ecotoxicology, heavy metals, effluent, *D. magna* St., *P. parva* Temminck & Schlegels.

Introduction

Metals are perceived as pollutants but they are also naturally existing substances in the environment. Elements like Cd, Hg, Pb, Ni are accepted as priority pollutants for aquatic environment. Most metals do not undergo microbial or chemical degradation and are toxic and their total concentration in soils persists for a long time after their introduction (ADRIANO, 2003; KIRPICHTCHIKOVA *et al*., 2006). Their content in aquatic environment is very divers, and it was well known that neither total nor dissolved total metal concentrations are good predictors for their bioavailability and toxicity (NEWMAN, 2008). Many chemical mixtures, with concentrations of individual chemicals considered as nontoxic, are often presented in aquatic systems. However, it is reckoned that such chemical mixtures where individual constituents are present at low, non-toxic concentrations may trigger toxicity due to additive or synergistic effects among the constituents (RAJAPAKSE *et al*., 2002; MONTVYDIENE & MARCIULIONIENE, 2004).

The surface water environment is under the influence of continuous industrial pressure. It has long been recognized that the formation of organic and inorganic metal complexes and metal sorption to particulate material reduces metal bioavailability and toxicity in the water compartment (LUCK *et al*., 2008; SAEEDI *et al*., 2004). Bio-assessment can be used as a tool to detect the presence of hazardous chemicals in the environment evaluating the effects of mixtures with the

combined effects that can be expressed as synergism, additivity and antagonism demonstrating bioavailability of contaminants to different species (LOUREIRO *et al*., 2006; LANDIS *et al*., 2011; LYUBENOVA & KALCHEV, 2011; LYUBENOVA *et al*., 2012). However assessment of water quality in the presence of mixture of pollutants by using single–species biotest may be insufficient for a more biologically complex system; therefore, organisms of different phylogenetic levels and ontogenesis have to be involved in these investigations (MONTVYDIENE & MARCIULIONIENE, 2004).

The purpose of article is to evaluate the toxicological effect of wastewater emanated from Radomir Metal Industries".

Material and Methods

Study area. Bulgarian metal casting plant Radomir Metals is based the southwestern town of Radomir, Pernik district. The plant has been constructing on 1 800 000 m² area and situated at about 50 км south-west of the capital of the Republic of Bulgaria - Sofia, on the road E79 (Coordinates: 42°31'10"N 22°59'12"E). The purification facilities fully meet the requirements of the European Standards for the quality of the air and water (http://radomirmetal industries.com/en/application/index).

„Radomir Metal Industries" has a complex permit № 145-NO/2008 on within the scope of paragraph 2.2. Annex 4 of the Law on Environmental Protection. Under the complex permit "Radomir Metal Industries has established annual emission standards presented in Table 1.

It was reported (PASKALEV, 2001), that there are some strongly polluted sections in Struma River – after the towns of Pernik, Kjustendil, and Dupnitza. Sources of pollution are the industrial wastewater of the towns of Radomir and Pernik, as well as domestic sewage of some of the bigger settlements (the town of Blagoevgrad and others). In the area of enterprise "Radomir Metal Industries" soil and water monitoring at several different points are conducted. According to the data of Basin Directorate - West Region for wastewater, even those emitted from the production are in the emission rate.

Table 1. Direct annual pollutant emission standards

Pollutants	**Mixed stream wastewater (industrial and domestic-faecal) kg.year^{-1}**
Total nitrogen	709.40
Total phosphorous	77.45
Arsenic and its compounds	1.54
Cadmium and its compounds	0.15
Chromium and its compounds	1.54
Copper and its compounds	8.52
Mercury and its compounds	0.15
Nickel and its compounds	0.42
Lead and its compounds	1.54
Zinc and its compounds	15.02
Phenols	4.64
Total organic carbon	6954.5
Chlorides	-
Cyanides	0.57

"Radomir Metal Industries" has owns water treatment plant. Purification is a mechanical, biological, as is done drying and stabilization of sludge. Receiver of purified water was the Struma River (II - Second category receiving water). Along the outlet wastewater to the water treatment point, canal water is intensively used for

irrigation. Industrial wastewater is discharged to the water purification plant through a channel running through arable land. In this regard, research in this work is done to establish whether there is compliance with established standards.

Characteristics of the test – objects. The biological tests evaluated the toxicity of wastewater, polluted air, soil, sediment, etc. or a particular pollutant using standard test organisms. The latter are exposed to different concentrations of the substance and report mortality or change in morphology and physiology of organisms. In determining the toxicity we followed standard protocols in order to have comparability of results.

For the purpose of study are conducted tests with test-objects: *Daphnia magna* (ISO 6341/1996, 10); *Pseudorasbora parva* (ISO 7346/1:1996, 10). The aims of bioassays are to determine the substance concentration or dilution of water waste, in which occurred 50% mortality (LC_{50}) or change in the appropriate indicator in the test-organisms for determined time.

In assessing the toxicity of the effluent of "Radomir Metal Industries" are using change in mortality *of D. magna* (ISO 6341/1996) and *P. parva* (ISO 7346/ 1:1996) respectively, at a constant temperature 20±2°C.

By pretesting a series of concentrations (in %) for the final testing are defined: 100, 95, 90, 85, 75 и 70 where the *D. magna* mortality percentage is rendered in account. Used for testing fish of species *P. parva* are from one and the same generation and with approximately the same dimensions (length ± 5 mm). The sample has been acclimatized in aquaria (1 l aerated water for a fish) and normally fed two weeks until the test beginning. The fish is not fed and the water is not aerated during the test. For the final testing the following dilutions are used: 20%, 40%, 60%, 80%, 100% included smallest lethal and highest not lethal dilution, defined by the pre-testing, for 48 h in a two repetitions. The tests are conducted in aquariums with an individual volume of 10 l (with the appropriate dilution of the effluent and the control with a boiled tap water) and with 10 fishes in every aquaria. The dead fishes have been removed two times a day. In 24 h during the test the wastewaters at different dilution have been renewed and the alive fishes have been moved to the new aquariums. LD_{50} is calculated by graphical interpolation (ISO 6341/1996, ISO 7346/1:1996).

The toxicity of Zn ($Zn_2SO_4.7H_2O$) on *P. parva* is also been tested using concentrations: 0, 0.001, 0.010, 0.030, 0.040, 0.050 μg.l^{-1}.

Sample analysis

Reagents. All solutions were prepared with analytical reagent grade chemicals and ultra-pure water (18 MΩ cm) generated by purifying distilled water with the Milli-QTM PLUS system

Nitric acid: Suprapur HNO_3 (67% v/v) was purchased from Fluka.

The stock standard solutions of Cd, Cu, Cr, Fe, Hg, Ni, Pb and Zn 1000 mg.l^{-1} were Titrisol, Merck in 2% v/v HNO_3 and were used to prepare calibration standards.

Sampling. Fish samples (whole fish body) are thoroughly washed with MQ water. The fish specimens were dissected and samples of fish gills are quickly removed and washed again with MQ water. Fish gills were analyzed as obtained without further homogenization. Fish gills (sample amount between 0.1 and 0.3 g) were digested with nitric acid in MW oven (step 1 - 250 W for 3 min; step 2 - 400 W for 3 min, step 3 - 600 W for 3 min), solutions cooled, transferred in 5 ml volumetric flask and diluted up to the mark with Milli-Q water.

Instrumentation. Determination of Fe and Zn: Flame atomic absorption spectrometric measurements were carried out on a Perkin Elmer Zeeman 1100 B spectrometer with an air/acetylene flame. The instrumental parameters were optimized in order to obtain maximum signal-to-noise ratio.

Determination of Cd and Pb in fish gills: Electrothermal atomic absorption spectrometric measurements were carried out on a Perkin Elmer Zeeman 3030 spectrometer with an HGA-600 atomizer. Pyrolytic graphite-coated graphite tubes with integrated platforms were used as atomizers. Pd as $(NH_4)_2PdCl_4$ was used as

modifier for ETAAS measurements of Cd. Pretreatment temperatures used were 500 °C for Cd and Pb and atomization temperatures 1300 °C for Cd and 1900 °C for Pb.

Determination of Cd, Cu, Cr, Ni, Pb in water samples: Samples measured by ETAAS under optimized instrumental parameters.

Determination of Hg in water samples: Water samples were previously digested with 1 ml HNO_3, Mercury content was measured by cold vapour AAS (Varian AA 240 atomic absorption spectrometer equipped with a continuous flow VGA-77 Vapor Generation Accessory) under optimal instrumental parameters.

Assessment of water contamination. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) have been determined for the sewage (ISO 6060:1989). In environmental chemistry, the chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers), making COD a useful measure of water quality. It is expressed in milligrams per litre ($mg.l^{-1}$), which indicates the mass of oxygen consumed per litre of solution. Older references may express the units as parts per million (ppm).

Statistics. All obtained results were statistically processed. It has been shown that the resulting averages are representative of performance using t-test. The statistical significance level in this study was defined at $p < 0.05$.

Results

Results of samples from wastewater

The table below presents the results of some additional analysis of samples of wastewater and limit concentrations of metals under Regulation №6 (Table 2). The marginal limit rate are defined in the particular circumstances of production taken into account for the manufacture of iron and steel, production of iron and steel castings, cast cars and other non-ferrous metals.

The results show that the resulting concentrations are within the limits of regulation.

Table 2. Average values with standard deviation of the analyzed parameters of samples effluent compared to the Marginal limit concentrations

Elements	**Marginal concentrations*, µg.l^{-1}**	**Acidified sample, unfiltered, µg.l^{-1}**	**Not acidified sample, unfiltered, µg.l^{-1}**
Cd	500	3.2±0.2	2.3±0.2
Cu	500	12.0±2.0	8±1
Cr	500	2.8±0.2	2.1±0.1
Fe	5000	189±14	156±12
Ni	500	4±1	3±1
Pb	200	3.8±0.1	2.1±0.1
Zn	2000	230±15	190±12
Hg	10	<1	<1
Other indicators	pH – 7.92 Chemical oxygen demand (COD) (BBM 0208:2001; t = 20^0±3) - 17.2±1.63 COD (oximetric) 20.48 $mg.l^{-1}$ – filtered sample; 73.10 $mg.l^{-1}$ – unfiltered sample	NH_4^+ - 0.2 $mg.l^{-1}$ NO_3^- - 5.7 $mg.l^{-1}$	SO_4^{2-} - 19.5 $mg.l^{-1}$ Cl – 10 $mg.l^{-1}$ PO_4^{3-} - < 0.1 $mg.l^{-1}$l

Results from the test with D. magna and P. parva

Daphnia magna

The experiments were carried out with *D. magna*, the rate of mortality (LC_{50}) have been determined. The dose-response curve plotted as a cumulative number of dead organisms by each dose using is represented (Fig. 1). The dose-effect of the effluent on the mortality rate of *D. magna* correlated well as a linear regression (R^2=0.956).

With the increasing of dilution the toxicity is reduced. Test results showed that the range of 50% mortality appears approximately at 86% effluent concentration (undiluted effluent is 100% in the scale, Fig. 2).

Pseudorasbora parva (Temminck & Schlegel, 1846) Stone moroko

The dependence of mortality of *P. parva* from effluent with different dilutions is showed on Fig. 3.

The dependence of mortality of P. *parva* from different concentration of Zn in the water is showed on Fig. 4.

Conducting the tests with selected dilutions showed that surviving is superior to that of *Daphnia magna*, LC_{50} is reported from exposure to raw sewage (100%).

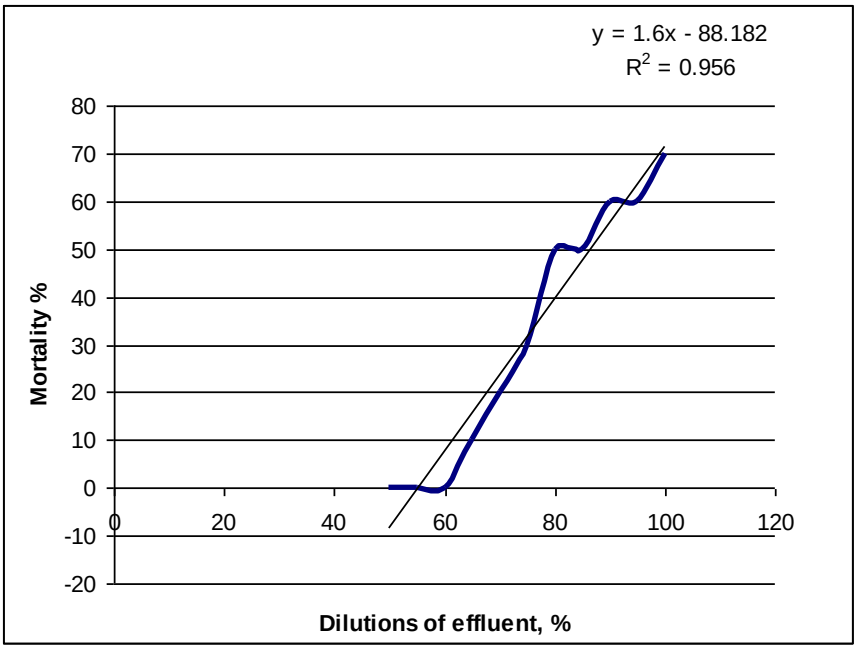

Fig. 1. Dependence of mortality of *D. magna* by diluting the effluent

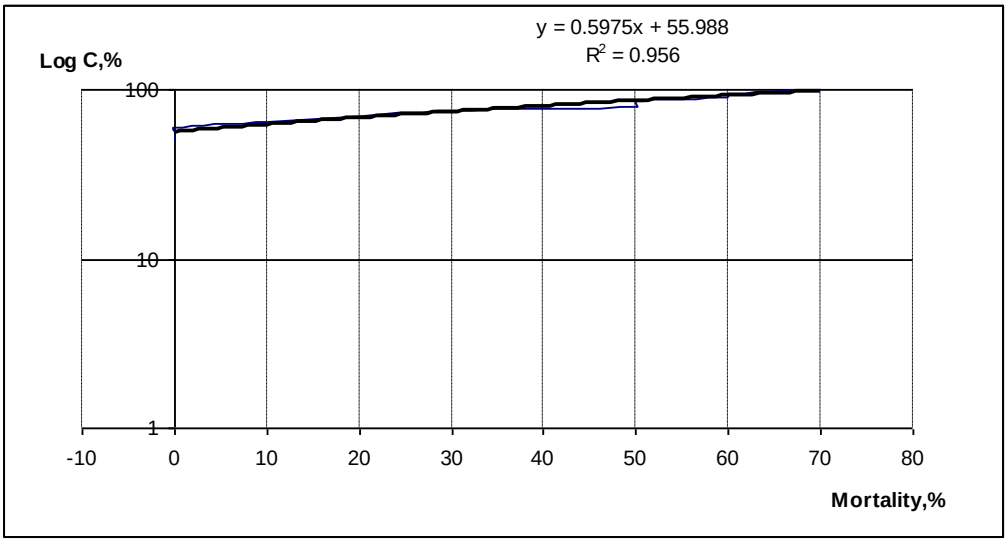

Fig. 2. LC_{50} for *D. magna* under different dilutions of effluent

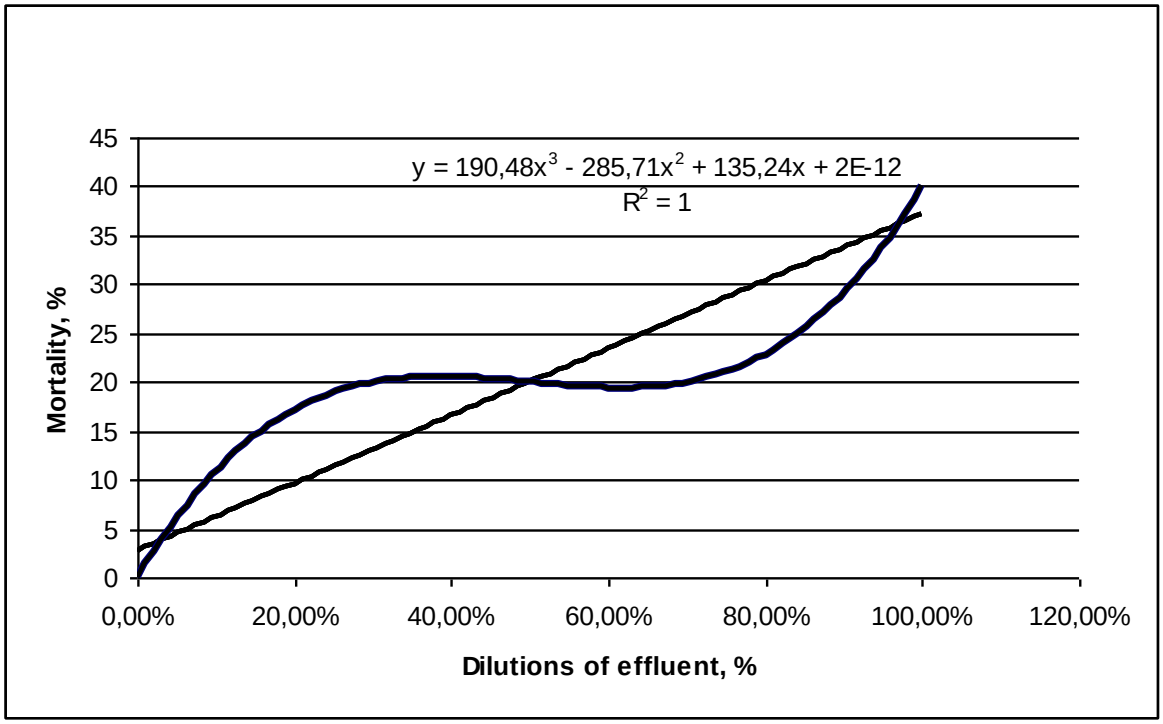

Fig. 3. Dependence of mortality of *P. parva* from effluent with different dilutions

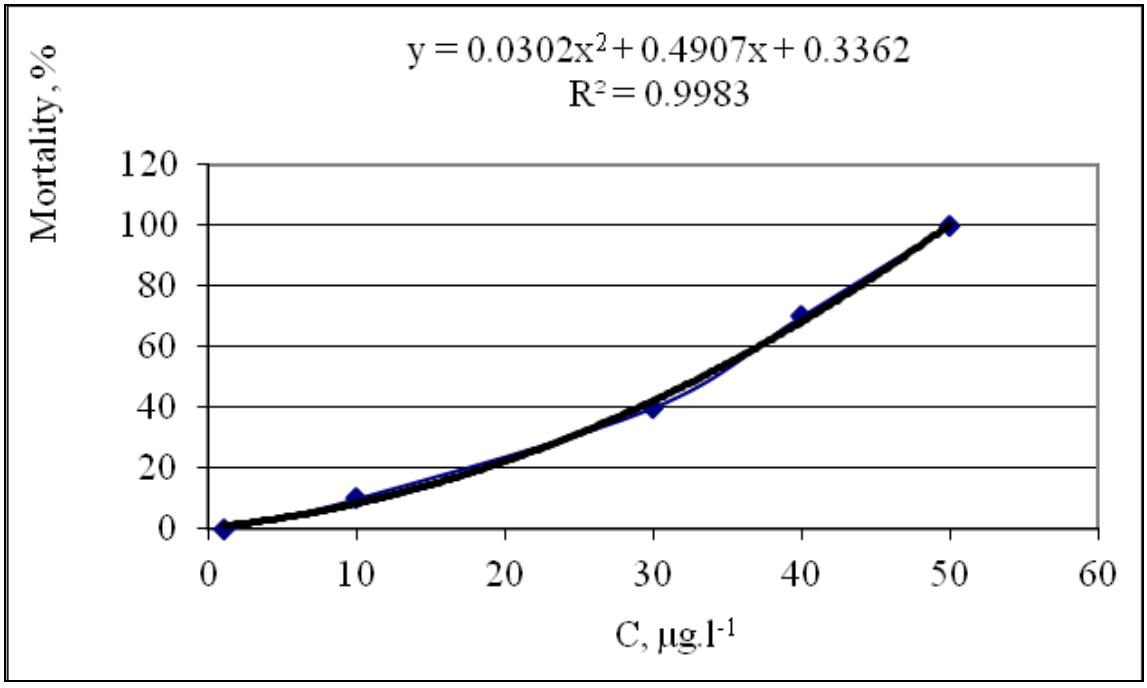

Fig. 4. LC_{50} for *P. parva* under different concentration of Ni in the water ($mg.l^{-1}$)

In all samples of gill Cd is <0.01 ppm, and Pb - <0.1 ppm. For untreated fish, found in the gills content of Fe and Zn is respectively 2.8 and 3.9 ppm. The contents of only two of the studied heavy metals in the gills of *P. parva* increases - low for Zn (1.12 times) and almost double for Fe (1.7 times) compared to untreated specimens.

Discussion

Daphnia magna

D. magna is a Crustacean in the order of *Cladocera*. This aquatic animal extensively used as a test organism in aquatic toxicology due to their small size, short life cycle and amenability to lab culture. *D. magna* is the most sensitive (PETERS & BERNARDI, 1987). *D. magna* are used as a model organism to simulate response of wide ranges of aquatic invertebrates to toxicants (LANDIS *at al*., 2011; PETERS & BERNARDI, 1987; VEDLEGG, 2004; VILLAVICENCIO, 2005). The great water flea (*D. magna*) has been reported as the most sensitive test-object in relation of different pollutants (organophosphates, organochlorines, heavy metals, pyrethroids etc.) among all known biological objects including experimental animals (PETERS & BERNARDI, 1987; Vedlegg for Cyprodinil Kilde: Svensk undersøkelse, 2004). The test of mortality has been also reported as the most effective and reliable (HERMENS *et al*., 1984) with high degree of predictive performance (VILLAVICENCIO, 2005).

The dose-effect of the effluent on the mortality rate of *D. magna* correlated well as a linear regression with correlation coefficient R^2=0.956 (fig.1). The observed tendency is a typical pattern for the dependence of cumulative mortality versus environmental concentration or dose of the toxicant (LANDIS *et al*., 2011). The comparable dose-response curve has been reported when the effect of different concentrations of four compounds on *D. magna* mortality was investigated (KUNGOLOS *et al*., 2001).

Relationship between the concentration of dissolved copper in the overlying water and the mortality of exposed D. *magna*, yielding an estimated LC_{50} of 26 $mg.l^{-1}$ dissolved copper (GILLIS *at al*., 2006). Water analyses of effluent from the plant of heavy industry "Radomir Metal Industries", given in table 2, showed that the amount of cooper in the waste water is approximately 12±2 $mg.l^{-1}$. The acute toxicity has been studied of heavy metal chromium as well as tannery effluent (chromium containing) water has been evaluated by laboratory bioassay experiments for 48 h duration in water flea, *D. magna* (TALAPATRA & BANERJEE, 2005). The median lethal concentration (LC_{50}) value of chromium metal for 48 h duration was determined to be 0.4027 ppm. Whole effluent toxicity (WET) test confirmed median lethal concentration (LC_{50}) values of tannery effluent water for 48 h duration was 6.540% dilutions respectively. Chromium content in tannery effluent water was found 3.47 ppm or it is approximately 0.225 ppm (TALAPATRA & BANERJEE, 2005). The data in our experiments is showed that the content of Zn and of Cr in wastewater is relatively 0.00189± 0.0014 and 0.0028 ± 0.0002 ppm (table 2). The survival rate of great water flea (*D. magna*) in our study showed that the effluent from the plant of heavy industry "Radomir Metal Industries" is toxic to the test hydro biota, although the concentrations of individual heavy metals are within the allowed limits, accepted from the Regulation № 6. The effluent remains toxic perhaps the combine affect that also occur. It has been reported that the joint toxicity of the mixtures of pollutants remains much higher than that of the individual chemicals (HERMENS *et.al*., 1984).

Pseudorasbora parva (Temminck & Schlegel, 1846) Stone moroko

Stone moroko (also known as topmouth gudgeon), *P. parva*, is a fish belonging to the Cyprinid family, native to Asia, but introduced and now considered an invasive species in Europe (WITKOWSKI, 2006). A review of literature on the ichthyofauna of lowland rivers of Central Europe (WITKOWSKI *et al.* 2000, 2004) indicates that the species is now a constant component of the Central European fauna, and is often a dominant. *Pseudorasbora parva* is a small planktivore and feeds mainly on aquatic insects, algae, and zooplankton (ZHANG *et*

al., 2000; XIE *et al*., 2000; YANG *et al*., 2004). In both its original distribution range and in secondarily invaded areas the stone moroko inhabits shallow lakes, carp ponds, irrigation canals, ditches, slow sections of lowland rivers and their oxbows. It prefers much-vegetated places (BERG, 1949; KOZLOV, 1974; MUCHACEVA, 1950; WITKOWSKI, 2000).

The data mortality rate-effluent dilution well correlated with polynomial equation $190{,}48x^3 - 285{,}71x^2 + 135{,}24x + 2E\text{-}12$, $R^2=1$ (Fig.3). Conducting the tests with selected dilutions showed that surviving is superior compare to that of daphnia. LC_{50} is reported from exposure to raw sewage (100%). *P. parva* is generally regarded as a pest due to its very high reproductive rate, which gives rise to dense populations of fish (WELCOMME, 1988). This species is more resistant than many European fish species to a moderate degree of pollution, elevated temperatures, and low water levels. There is evidence that it also can move a limited distance through polluted water (BANARESCU, 1999). High success in population growth and the colonization rate of P. *parva* could be explained by its wider ecological physiological tolerance (ROSECCHI *et al*., 1993; 2001). Our results confirm that this species has high tolerance to the water pollution and that wastewater has slight toxic effect on populations of hydro biota. Nevertheless the tested toxicity of Zn is expressed by ascending polynomial curve (fig. 4), where $LC_{50\%}$ for 48 h treatment $_{is}$ 33, 44 $mg.l^{-1}$. Perhaps the combine effect between the toxicants in effluent that occurs is expressed by inverse antagonism (NEWMAN, 2008).

Conclusions

The concentrations of the toxicants in the waste waters are under limits. Nevertheless the toxic effect of metal plant sewage on the tested hydro biota has been registered. For the great water flea (*D. magna*) 48 h LC_{50} appears approximately at 86% effluent concentration. The combined toxic effect of the effluent on the two tested species is different. The surviving of *P. parva* is superior to that of *D. magna*. The 48 h LC_{50} is reported from exposure to raw sewage (100%). Even the concentrations of individual heavy metals are within the allowed limits the effluent remains toxic due to the combine effect of toxicants in waste waters that occurs. For the *P. parva* the inverse antagonism is observed. Also the gills content of Fe and Zn increases in treated fishes.

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Received: 15.06.2012
Accepted: 26.08.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 61-67

Comparison of Terrestrial Isopod (Isopoda, Oniscidea) Assemblages from Two Types of Forests from North Western Romania

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Abstract. In 2008 we compared the terrestrial isopod assemblages from two different habitats, a beech forest and a mixed beech and spruce forest, from north western Romania (Huta Certeze locality). The samples were taken from April to September using pitfall traps. We identified a total of 7 species: *Ligidium germanicum, Trichoniscus sp., Hyloniscus transsilvanicus, Protracheoniscus politus, Porcellium collicola*, *Trachelipus difficilis* and *Porcellio scaber*. A greater diversity and species richness were noticed in the beech forest. The poverty of species in the mixed forest was a consequence of the forest type, the anthropogenic impact and the dry environment. High surface activity of individuals was noticed in the summer months. Even if the species compositions of the two compared isopod assemblages were not identical, there weren't statistically significant differences between them.

Keywords: Huta Certeze, Isopoda, Oniscidea, woodlice, beech forest, mixed forest, microhabitat, surface activity.

Introduction

The diversity of terrestrial isopod species is in direct connection with the number of microhabitats, which allow the coexistence of several species (LOPES *et al.*, 2005). Small scale differences of the terrestrial isopod fauna are extremely relevant, their composition depending on the characteristics of the existing microhabitats (VILISICS *et al.*, 2011). In Romania, studies of terrestrial isopods ecology were usually made from a large scale group of habitats, showing how the general characteristics of the area influences the terrestrial isopods (e.g. MURESAN *et al.*, 2003; HOTEA *et al.*, 2003; TOMESCU *et al.*, 2002, 2005, 2008, 2011). In this context we intended to analyse and compare two assemblages of terrestrial isopods from two neighbouring habitats located in Oas Mountains, Huta Certeze (north western Romania). Some studies on terrestrial isopods were carried out in the volcanic mountains from the same region of Romania (HOTEA *et al.*, 2003; HOTEA & HOTEA, 2009; VILISICS, 2008). One of these analyzed the isopod fauna of the Maramures Depression, including Huta Certeze locality, where from only one species was reported (VILISICS, 2008). Our objective was to analyze the

ecology of the terrestrial isopod assemblages from Huta Certeze, based on the populated habitat type and sampling season.

Material and Methods

The samplings were carried out near Huta Certeze locality, in Huta Pass region (Satu Mare County, north western Romania). The investigated habitats are situated in the eastern part of Oas Mountains, one of the volcanic mountains in the Eastern Carpathians (POSEA & BADEA, 1984). The samples were taken using pitfall traps from April to September 2008. Five traps were settled in a row in each habitat, put at the distance of 5 m from each other, collected monthly from april to september. The content of 49 pitfall traps were analyzed. The individuals were identified in the laboratory using the speciality literature (RADU, 1983, 1985) and the currently accepted nomenclature (SCHMALFUSS, 2003).

Samples were taken from two habitat types, located at a distance of approximately 300 m between them. The first habitat (47°57'30,48"N/23°30'22,56"E) was represented by a typical beech forest, situated at an altitude of 579 m a.s.l. This habitat was devoid of herbaceous vegetation, with a thick layer of litter. The second habitat (47°57'26,16"N/23°30'38,63"E) was represented by a mixed spruce and beech forest, located at 584 m a.s.l. This habitat is located near a quarry, being affected by human activities. The forest's substrate presents only a thick carpet of dead leaves.

The results were analyzed comparatively to seasons and habitats by the next parameters: number of species, relative abundance and species frequency in traps. Diversity of species was calculated by the Shannon-Wiever diversity index (SHANNON & WIEVER, 1949). Kruskal-Wallis test was used to estimate the significance of the differences between the sampling periods (ZAR, 1999) and Mann-Whitney test (ZAR, 1999) to compare the habitats.

Results

A total of 751 individuals of the next seven species were identified in the two habitats: *Ligidium germanicum*, *Trichoniscus sp.*, *Hyloniscus transsilvanicus*, *Protracheoniscus politus*, *Trachelipus difficilis*, *Porcellium collicola* and *Porcellio scaber*. The traps captured only two female specimen of *Trichoniscus sp.*, which were insufficient to determine exactly this species. Six species (401 specimen) were identified in the beech forest, and four species (350 specimen) in the mixed forest. Some of them were common for both of the habitats.

The greater abundance of individuals was recorded in the second half of the sampling period in the beech forest (Table 1). The high value of abundance was due to the large number of *P. politus* and *T. difficilis* individuals. *L. germanicum* appeared only in spring. The presence of the other species didn't influence the relative abundance. The highest diversity was observed in May. For this, an important role is that of the species represented by few individuals that only appear in spring. According to the Kruskal-Wallis test, in the beech forests the differences between the species composition in different time periods are not significant ($H(5, N=36)=1.008475$, $p=0.96$).

The relative abundance and diversity values in the mixed forest are presented in Table 1. The abundance depended on the parameters of *P. politus* population, the highest represented species. The highest abundance and diversity values occurred in July and August (Table 1). As in the case of the beech forest, the seasonal differences of the species composition in terrestrial isopods were not significant ($H(5, N=24)=2.370010$, $p=0,79$).

The species abundance was higher in the beech forest due to *T. difficilis*, while in the mixed forest *P. politus* has the higher abundance. The differences between the diversity of the terrestrial isopod assemblages from the two habitats are really high (Table 2). However the difference between the species composition of the two habitats is not significant (Mann–Whitney test: $p=0.66$).

The composition and species' ratio within the assemblage of terrestrial isopods from the two forests differs depending on the period of the year. In the beech forest in the first two months the predominant species were *L. germanicum* and *P. politus*.

Table 1. Relative abundance and species diversity of terrestrial isopods depending on the period of the year in the beech forest (L g – *L. germanicum*, H t – *H. transsilvanicus,* Tr – *Trichoniscus* sp., T d – *T. difficilis*, P p – *P. politus*, P c – *P. collicola*, P s – *P. scaber,* H – diversity)

	Beech forest								**Mixed forest**					
	Relative abundance							H	Relative abundance					H
	Total	*L g*	*Tr*	*P p*	*P c*	*T d*	*P s*		Total	*L g*	*H t*	*Tr*	*P p*	
April	5.48	29.17	-	4.90	66.67	0.96	100	1.44	9.42	-	-	-	9.70	0
May	6.98	58.33	-	3.68	33.33	3.38	-	1.14	8.57	100	12.50	-	8.23	0.29
June	18.70	8.33	100	22.7	-	16.91	-	0.86	10	-	12.50	-	10.00	0.13
July	26.43	4.16	-	33.13	-	24.64	-	0.74	28.29	-	50	-	27.94	0.17
August	18.70	-	-	24.54	-	16.91	-	0.69	38.86	-	25	100	39.12	0.12
September	23.69	-	-	11.04	-	37.2	-	0.49	4.85	-	-	-	5.00	0

Table 2. Abundance, frequency and diversity of species in both habitats (L g – *L. germanicum*, Tr – *Trichoniscus* sp., H t – *H. transsilvanicus*, P p – *P. politus*, T d – *T. difficilis*, P c – *P. collicola*, P s – *P. scaber*, H – diversity, A – relative abundance, F - frequency)

Habitat		***L g***	***Tr***	***H t***	***P p***	***P c***	***T d***	***P s***	**H**
Beech forest	A	5.98	0.24	-	40.65	0.74	51.62	0.74	0.96
	F	21.43	3.57	-	85.71	7.14	89.29	3.57	
Mixed forest	A	0.28	0.28	2.28	97.14	-	-	-	0.15
	F	4.76	4.76	23.81	100	-	-	-	

Table 3. Numerical abundance (N) and relative abundance (A%) of species in the terrestrial isopod assemblages in the two forests (Sept. - September)

Habitat			**April**	**May**	**June**	**July**	**August**	**Sept.**
Beech forest	*L. germanicum*	N	7	14	2	1	-	-
		A%	31.82	50	2.667	0.943	-	-
	Trichoniscus sp.	N	-	-	1	-	-	-
		A%	-	-	1.333	-	-	-
	P. politus	N	8	6	37	54	40	18
		A%	36.36	21.43	49.33	50.94	53.33	18.95
	P. collicola	N	2	1	-	-	-	-
		A%	9.091	3.571	-	-	-	-
	T. difficilis	N	2	7	35	51	35	77
		A%	9.091	25	46.67	48.11	46.67	81.05
	P. scaber	N	3	-	-	-	-	-
		A%	13.64	-	-	-	-	-
Mixed forest	*L. germanicum*	N	-	1	-	-	-	-
		A%	-	3.333	-	-	-	-
	H. transsilvanicus	N	-	1	1	4	2	-
		A%	-	3.333	2.857	4.04	1.471	-
	Trichoniscus sp.	N	-	-	-	-	1	-
		A%	-	-	-	-	0.735	-
	P. politus	N	33	28	34	95	133	17
		A%	100	93.33	97.14	95.96	97.79	100

This ratio changes from June, when along with *P. politus*, *T. difficilis* prevail, *L. germanicum* presenting only a small percentage (Table 3). *P. politus* dominated all sample in the mixed forest (Table 3).

Discussion

The composition of isopod assemblages differed in the two forests, despite their relatively small spatial distance. There are species present in both habitats (*P. politus, L. germanicum, Trichoniscus sp.*) and characteristic species for each habitat. Two of these are typical for forest habitats (*T. difficilis* and *P. collicola*) and one is euritopic (*P. scaber*) (RADU, 1985). Deciduous forests are generally more favorable than coniferous forests for terrestrial isopods, the leaves of conifers being inefficient for the isopods' development (SOUSA *et al*., 1998). Previously TOMESCU *et al*. (2002) noticed a lower number of species in mixed forests. This is the case of Huta-Certeze too, where the species richness and diversity were higher in the beech forest than in the other habitat. The mixed forests were more exposed to anthropogenic impact, due to the quarry and also to the dryness. ALMERAO *et al*., (2006) pointed out similarly the negative effect of the anthropogenic impact on invertebrate assemblages, especially terrestrial isopods. Only species with wide ecological valence appear in affected areas (VILISICS *et al*., 2011). A higher richness and a diversity of species have been reported in the natural areas and in the wetlands (HORNUNG *et al*., 2008). This poverty of species in mixed forests doesn't appear to be caused exclusively to one reason (mixed forest with conifers, degree of degradation, dryness), but appears to be a result of their aggregation. Also, the method of collecting with pitfall traps led to the capture of few species with more activity at the surface (ARNDT & MATTERN, 2005; TOMESCU *et al*., 2008; MAGRINI *et al*., 2011). Species with low mobility lack from the traps (MESSINA *et al*., 2011). Also, in other cases the obtained results using pitfall traps were considered to be influenced by the isopods` mobility (IVANOV, 2011).

Species common to the two habitats are represented by a different number of individuals depending on habitat and period. *L. germanicum* prefers wetland forests (RADU, 1983). Thus its presence in the forest from Huta Certeze was expectable, since it was previously reported in the volcanic mountains from north western Romania (RADU, 1983; HOTEA *et al*., 2003; VILISICS, 2008). *L. germanicum*`s high frequency in the beech forest suggests its preference for natural areas. Some authors consider this species as relict, specific to areas with low anthropogenic impact (TUF & TUFOVA, 2008), being common to natural areas from higher altitudes (HORNUNG *et al*., 2009). *P. politus* is the most common isopod species from Huta Certeze, being a forest species (MURESAN *et al*., 2003; HOTEA *et al*., 2003; TOMESCU *et al*., 2008, 2011). Its presence in large number of individuals in both forest types suggests that this species is able to use a variety of habitats, as it was previously reported (TUF & TUFOVA, 2008). *P. politus* is also considered a common species for natural and semi-natural areas (HORNUNG *et al*., 2009; VILISICS & LAPANJE, 2005).

Besides the common species in both habitats, in the mixed forest *H. transsilvanicus* also occurs. The presence of this species exclusively in this dry habitat is unusual, because it is considered to prefer forest wetlands (RADU, 1983). In other beech forests it was recorded along with *P. politus* and *T. wachtleri* (TOMESCU *et al*., 2002) (now synonimized with *T. difficilis*, see Schmalfuss 2003). For smaller species with soft bodies the preferences towards the microhabitat are satisfied by the existence of small places of shelter, regardless of general characteristics of the habitat (VILISICS *et al*., 2005, 2011). Thus, the small number of captured individuals indicates the scarcity of favorable microhabitats, althought the species recently was identified even in the plains from north-western Romania (FERENTI *et al*., 2012). *H. transsilvanicus* was identified in several

periods that indicates that this species is constantly present in the region.

The exclusive presence and high abundance of *T. difficilis* in the beech forest suggests that the species is linked to natural forests. In the surrounding areas in Maramures, this is the most common terrestrial isopod species, being found even in coniferous forests (VILISICS, 2008). The other two species, *P. collicola* and *P. scaber*, also appear in the beech forest. The presence of *P. scaber* in the beech forest can be explained by the adjacent motel and other buildings which are favorable habitats to a synanthropic species (TOMESCU *et al*., 2005). Perhaps it may entered into the forest from this habitat, being easily accommodated due to its euritopic nature (TUF & TUFOVÁ, 2008). Its continuous migration between wet and dry habitats has been previously observed (DEN BOER, 1962). *P. scaber*`s absence from the mixed forest, is probably a consequence of the fact that this forest is surrounded by relatively natural beech forests. So, the species is present in the areas near the buildings, but it can not spread inside the forest. The human impact from the mixed forest only reduced the abundance of the native species, linked to natural areas, because synantropic species can not pass the beech forest.

The high relative abundance of representative species occured in summer, fact recorded in other areas, too (SFENTHOURAKIS, 1992; VADKERTI & FARKAS, 2002). This can be a consequence of the increased surface activity of the gravid females, which are looking for shelters for their descendants in this period (DANGERFIELD & HASSALL, 1994).

The diversity of terrestrial isopod assemblages increases up to 500-600 m, after which it decreases (LOPES *et al*., 2005). The values were high in the beech forest of Huta Certeze compared to other areas from Romania located in similar or higher altitudes (TOMESCU *et al*., 2002). The differences of diversity values between the two habitats are a consequence of the uniformity of the terrestrial isopods assemblage from the mixed forest. Furthermore, *P. politus* prevailing in both habitats determined the differences between them to be not significant.

Acknowledgements: In this way we want to thank to anonymous referees for their constructive comments, which highly improved the quality of the early version of the manuscript.

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Received: 20.08.2012
Accepted: 11.10.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 69-76

Evaluation of Soil Physical and Chemical Properties in Poplar Plantations in North of Iran

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Abstract. Soil physical and chemical properties and some quantitative characteristics of *Populus deltoides* Marsh (clone 79.51) plantations and relationships between them were evaluated in Guilan plain of north of Iran. Two same aged poplar plantations with low and high qualities were selected. In each poplar plantation, fifteen sample plots with systematic sampling method were selected. In each sample plot diameter at breast height (DBH) as well as height of all trees within them was determined. Soil samples were taken from 0-20cm in each plot and soil texture, water holding capacity (WHC), bulk density (B.D) and particle density (P.D) as well as soil porosity, O.C, N, available P and exchangeable K were determined for each soil sample in laboratory. Tree data and soil properties between two plantations were analysed using independent samples t-test (Student's t test at $p < 0.05$). The results showed that among soil physical properties, percentage of clay, sand, B.D and WHC and amongst soil chemical properties O.C, N, available P and exchangeable K were significantly different between two plantations. Heavy textured soils with high B.D are undesirable for growing of *populus deltoides* in study area. The results also indicated that poor quality plantations has negative effect on soil nutrient and reduces its fertility. Reduction of nutrient availability had negative effects on quantity and quality of poplar trees.

Key words: Soil Properties, *Populus deltoides*, Soil Texture, Iran.

Introduction

The majority of northern Iran is covered by the Caspian forest, a deciduous temperate commercial forest, of about 1.8 million hectares located on the northern slopes of the Alborz Mountains overlooking the Caspian Sea (HOSSEINI, 2006). Between mountain region and Caspian Sea, there are plain areas that during last decades have been involved in agriculture, reforestation or deforestation activities.

Guilan province, as one of the provinces located in north of Iran, has vast plain areas (with about 3600 km^2) covered by natural forests, crops and poplar plantations. Because of population growth, increasing demand for wood and declining of forest harvesting, development in plantation of fast growing species especially poplars has occurred in the plain of Guilan province in recent years. Poplar has been planted by villagers and big companies at various level in north of Iran and the planted areas in Guilan province is about 30000 ha (KIADALIRI, 2003; ZIABARI, 1993). Although the majority of poplar plantations in this area have suitable quantitative and qualitative growth, some of these plantations don't show appropriate conditions. The efficiency of plantations can

depend highly on site properties especially soil characteristics and water availability. DICKMANN & STUART (1983) declared that poplars could grow almost everywhere, but perform up to their full potential only on the productive sites. Soil physical properties which play major roles in water holding capacity, aeration and root penetration, have a strong influence on the growth of poplars. Poplars in general require light textured, permeable, deep and moist, and well aerated soils (BIRLER, 1983). DICKMANN & STUART (1983) showed that poplars will grow well on both upland and bottomland sites, where the soil is well drained and has good water holding capacity. The surface water table of Guilan plain usually is high and on the other hand the soil texture of this area often is classified as heavy soil. Therefore, it seems that the conditions of soils and water table in some parts of this region can be considered undesirable for poplar stands.

On the other hand as SINGH & SHARMAN (2007) and AUGUSTO *et al.* (2002) stated tree plantations influence soil physical, chemical and biological properties negatively or positively through litter fall, accretion and decomposition of organic matter. It seems that different poplar plantations with various quality and quantity have been able to change some soil properties in recent years in Guilan plain.

Although in Iran and the other neighbouring countries, such as Turkey, there are some studies about soil characteristics for poplar planting, most of them are related to soil nutrition and classification (KIADALIRI, 2003; SAYYAD *et al.*, 2005), and a few studies have focused on the physical and chemical soil properties of poplar plantations lands. In this study, two adjacent, same age and same clone of poplar plantations (*Populus deltoides*, clone 79.51) with different growth performance were selected in west of Guilan plain/Iran. The topographic conditions and climatic factors are the same; therefore it seems that after about 30 years soil properties have a strong impact on growth of poplar plantations. On the other hand previous reports indicate that before plantation of poplar in these areas, both of these places covered by not-usable native covers, and after the cutting down of them, the poplar plantations were established. So it is predictable that through these years the quality and quantity of poplar plantations can impact on soil properties especially on surface layers of the soil. On the base of above mentioned matters, it seems that there are complex relationships between soil properties and poplar plantations. This study tries to assess the favourable soil properties for poplar stands and also explain influence of poplar plantations on soil attributes.

Materials and Methods

The study was carried out in Guilan province, on the northern parts of Iran (45°25′N, 36°45′E). Experimental plots were located at an altitude of 80 m above sea level. Average annual rainfall is 1542.2 mm without dry season. Annual mean temperature is 19.7°C and average minimum and maximum temperature vary from 2.5°C in January to 30.3°C in July. The area is placed on flat and uniform terrain with poor drainage and has low water permeability. The soils predominantly have clay and clay loams texture, and the soils of the study site are classified as Inceptisols on the base of soil taxonomy (Shafarood Company, 2004).

In this area two adjacent poplar plantations apparently with different quantitative and qualitative performance were selected, (in this article they are referring as good and poor plantations (GP and PP)). The area of each plantation is about 70 hectare and the distance between them is about 3 km. Both of these plantations were on flat and uniform terrain and *Populus deltoides* Marsh. (Clone 79.51) were planted by 3 × 4 m distances in each plantation in 1982 (Shafarood Company, 2004). Approximately 30 years ago, these areas were dominated by natural forests containing native tree species such as *Carpinus betulus, Alnus glutinosa, Parrotia persica and Pterocarya fraxinifolia.* This natural stands were cleared cut and were planted by poplar.

In each plantation, fifteen sample plots (100×100m) by systematic sampling method were selected. Height and diameter at breast height (DBH) were measured for all of trees within sample plots. In each sample plot, three soil samples were taken from 0-20cm and after mixing, composed one soil sample from one plot (MARANON *et al.*, 1999). In this way, thirty soil samples were taken from both of stands and transferred to soil laboratory for analyzing. All of soil samples were air-dried and passed through a 2mm mesh. Soil texture by hydrometric method (BOUYOUCOS, 1962), soil pH in water suspension of 1:2.5 (soil: liquid ratio), bulk density (B.D) by clod method, water holding capacity (WHC) (GHAZANSHAHI, 1999), total nitrogen (N) by Kjeldal method (BREMNER, 1996), organic carbon (O.C) by Walkely and Black method (WALKLEY & BLACK, 1934) were determined. Available P (P) was analyzed according to the standard methods (OLSEN *et al.*, 1954), and exchangeable K (K) was analyzed after extraction using 1M ammonium acetate at pH7.0 was determined by flame-photometer (BLACK *et al.*, 1965). The differences of quantitative characteristics of trees and soil properties between two plantations were analyzed based on independent samples t-test (Student's t test at $p < 0.05$), and correlation between variables were determined by Pearson correlation coefficient. In order to find the most effective factors on the separation of two plantations, PCA (Principal Component Analysis) was used. For statistical analysis, SPSS (version 15.0) and "PC–ORD"program version 4.17 were used.

Results

Height and DBH (Diameter of Breast Height), as two main quantitative features of tree differed significantly between two poplar stands (Table 1). Means of height and DBH in GP were higher than the value of these variables in PP. It has been specified that after about 30 years although the clone of poplar plantations has been the same, the trees in GP are able to produce further biomass.

As Table 2 shows, soil physical properties between GP and PP are significantly different. The percentage of clay as well as B.D is higher in PP, while the amount of sand is lower. WHC as a factor showed maximum capacity of moisture holding in each soil was higher in GP compare to PP. As it can be seen in Table 3, the majority of the chemical soil properties differ significantly between two plantations. The amount of the main nutrient elements (N, P, and K) is higher in GP compared to PP. No significant difference was found between C/N ratios of two plantations. The amounts of N, P and K, as the most important nutrient elements and also C are higher in GP. Higher amounts of these elements indicate superior conditions of soil fertility which have been provided by poplar trees of GP.

Table 1. Means ± SD of DBH and height of trees in GP and PP and probability values

	Height (m)	DBH*** (cm)
GP*	24.2 ± 1.45	32.65 ± 3.95
PP**	18.08 ± 1.28	21.73 ± 1.99
P value	0.000	0.000

*GP: Good Plantation, **PP: Poor Plantation, ***DBH: Diameter of Breast Height

Table 2. Means ± SD of physical soil properties in GP and PP, and probability values.

Soil Parameter / Site	P.D (g/cm3)	B.D (g/cm3)	WHC (%)	Sand (%)	Silt (%)	Clay (%)
GP	2.27 ± 0.14	1.55 ± 0.07	40.86 ± 2.52	24.22 ± 7.78	38.01± 6.12	37.73 ± 7.52
PP	2.32 ± 0.15	1.65 ± 0.09	38.39 ± 2.27	18.91 ± 3.11	37.08 ± 3.39	44.01 ± 3.41
P value	0.412	0.003	0.009	0.021	0.610	0.006

Table 3. Means ± SD of chemical soil properties in GP and PP, and probability values

Soil Parameter / Site	O.C (%)	N (%)	P (mg/kg)	K (mg/kg)	C/N	pH (1:2.5)
GP	3.4 ± 0.66	0.32 ± 0.09	10.02 ± 8.37	164.93 ± 71.98	10.78 ± 1.06	5.42 ± 0.28
PP	1.92 ± 0.62	0.17 ± 0.05	2.20 ± 1.33	104.67 ± 26.25	10.88 ± 1.13	5.38 ± 0.30
P.value	0.000	0.000	0.002	0.005	0.814	0.677

Table 4. Pearson Correlation coefficient between D.B.H and height of trees and soil properties in PP

	Sand (%)	Silt (%)	Clay (%)	WHC (%)	P.D (g/cm³)	B.D (g/cm³)	pH (1:2.5)	O.C (%)	N (%)	P (mg/kg)	K (mg/kg)	C/N
D.B.H* Pearson Correlation	-0.16	0.01	0.13	-0.25	0.25	0.27	-0.03	-0.22	-0.04	-0.26	0.04	-0.47
Sig.(2-tailed)	(0.579)	(0.969)	(0.640)	(0.377)	(0.365)	(0.332)	(0.914)	(0.436)	(0.889)	(0.346)	(0.089)	(0.078)
Height Pearson Correlation	0.50	0.26	-0.72**	-0.50	-0.04	0.35	0.27	0.27	0.40	0.33	0.01	-0.14
Sig. (2-tailed)	(0.057)	(0.346)	(0.003)	(0.057)	(0.890)	(0.208)	(0.338)	(0.328)	(0.136)	(0.235)	(0.964)	(0.623)

Table 5. Pearson Correlation coefficient between D.B.H and height of trees and soil properties in GP

	Sand (%)	Silt (%)	Clay (%)	WHC (%)	P.D (g/cm³)	B.D (g/cm³)	pH (1:2.5)	O.C (%)	N (%)	P (mg/kg)	K (mg/kg)	C/N
D.B.H Pearson Correlation	0.34	0.15	-0.47	-0.55*	0.17	-0.12	0.40	0.54*	0.62*	0.67**	0.42	-0.49
Sig.(2-tailed)	(0.209)	(0.594)	(0.077)	(0.035)	(0.557)	(0.671)	(0.144)	(0.039)	(0.014)	(0.007)	(0.118)	(0.064)
Height Pearson Correlation	0.39	0.26	-0.62*	-0.05	-0.14	-0.31	-0.10	0.22	0.23	0.52*	0.16	-0.21
Sig. (2-tailed)	(0.146)	(0.344)	(0.014)	(0.872)	(0.633)	(0.268)	(0.716)	(0.427)	(0.408)	(0.048)	(0.563)	(0.444)

The results of Pearson correlation coefficient in PP showed that among soil properties, clay content has considerable negative correlation with height of trees (Table 4). Among soil properties in GP, percentage of C, total N and P correlated positively with D.B.H and soil saturated moisture content showed negative correlation to it. Height of trees in GP and clay content showed negative correlation, but P is correlated positively with height of trees (Table 5).

Figure 1 and Table 6, resulted from PCA analysis, show that DBH, height of poplar as well as O.C, N, P and K negatively correlated to the first axis of PCA. While clay content shows significant correlation with positive direction of the first axis of PCA.

Table 6. The Pearson and Kendall correlation coefficient between D.B.H, height of trees, soil properties and the axes of PCA

	r (Axis 1)	r-sq (Axis 1)	r (Axis 2)	r-sq (Axis 2)
D.B.H	-.854*	.730	.172	.030
Height	-.847*	.718	.054	.003
PD	.284	.080	.740*	.548
BD	.568*	.322	-.262	.069
WHC	-.310	.096	-.250	.063
Soil Porosity	-.259	.067	.881*	.776
pH	-.205	.042	-.633*	.400
Clay	.777*	.603	.089	.008
Sand	-.708*	.502	-.163	.027
O.C	-.924*	.855	-.059	.004
N	-.949*	.900	.026	.001
P	-.881*	.777	-.030	.001
K	-.750*	.562	.010	.000
C/N	.237	.056	-.339	.115

* Significant Pearson and Kendall correlation coefficient ($p<0.05$)

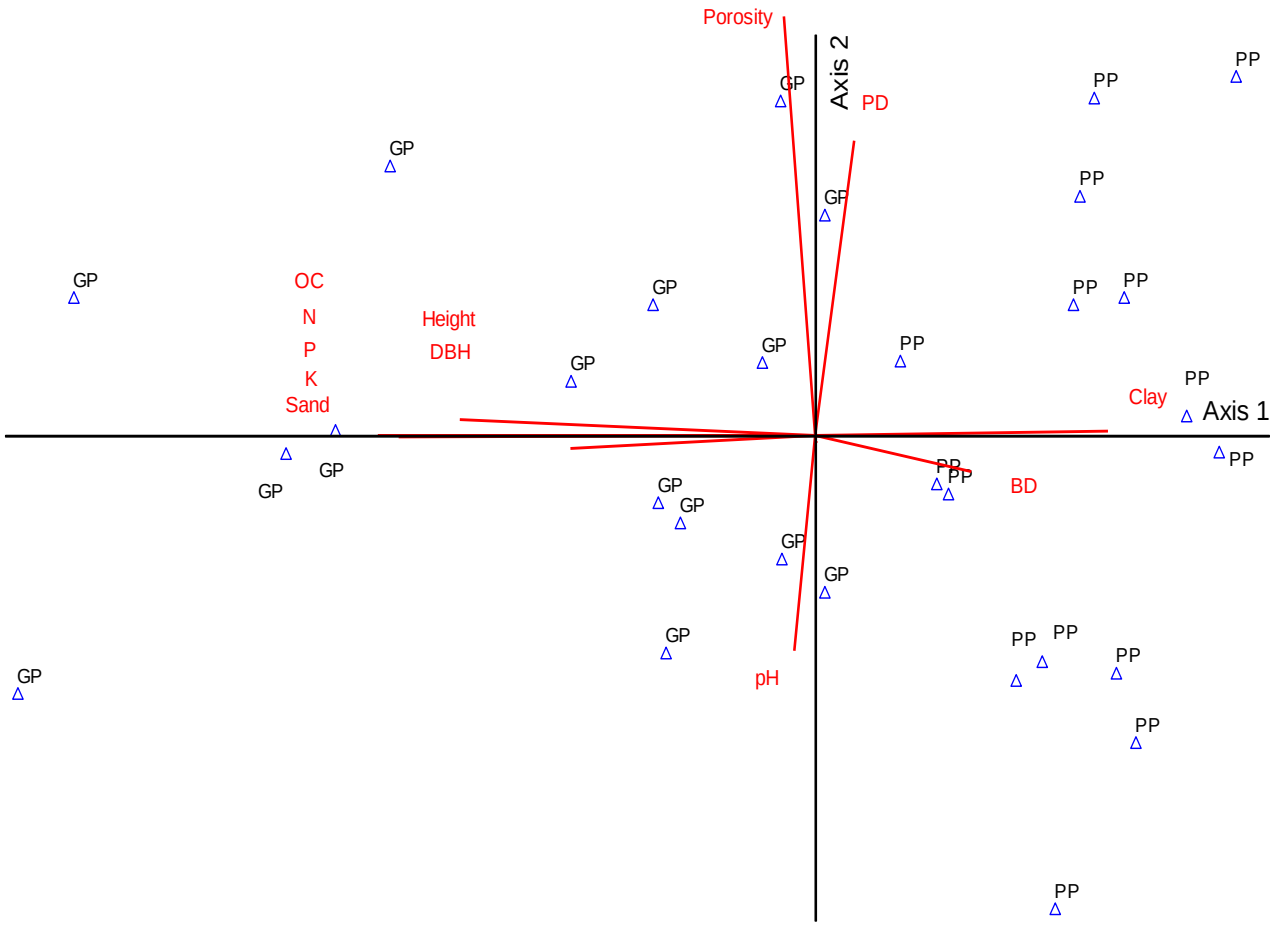

Fig 1. Distribution of soil factors, DBH and height of trees in relation to axes of PCA.

Discussion

The results showed that the main quantitative indicators of tree stands included DBH and height differ significantly between two poplar plantations. Growth of poplar depends upon various factors such as clone, quality of planting stock, spacing of trees, intercrops, site quality, climate and management practices (TEWARI, 1995). In this study, as two poplar plantations are the same age, the clones of them and the spacing of trees is also the same, it seems that environmental conditions effect the growth of the trees in two plantations. As these two plantations are almost close to each other, the environmental conditions such as climatic and topographic factors are similar to each other; however there are some soils properties are significantly affected on growing parameters.

According to the results, soil texture differs between two plantations and it can be one of the most important factors that influenced poplar trees. The content of clay is higher in PP compare to GP and in both stands height of trees showed negative correlation to clay content. TUFEKCIGLU *et al.* (2003) and ISEBERANDS (2007) declared that soil texture is one of the most important factors for poplar plantations. TUFEKCIGLU *et al.* (2003) also showed that the amount of sand in the soils had a positive correlation with growth of hybrid poplars and also they found a negative correlation between clay content and mean annual height growth. Light texture, high soil porosity and high soil water content in GP create suitable conditions related to aeration and moisture content in this poplar plantation. BIRLER (1983) stated poplars require soils of light textured, permeable, deep and moist and good aerated. As STANTURFT *et al.* (2002) reported heavy soils are considered less favourable for poplar growth than coarser textured soils, it seems that in our experimental sites also increasing of B.D and clay content in soil resulted lower growth of poplar trees. In contrary, increasing sand content has improved soil aeration and porosity and provides a better soil condition for poplars growth. According to above mentioned matters, soil texture have very important role in differences between two plantations. The important issue is that although theses plantations are almost close to each other, soil texture is different. Variability of soil chemical, physical and biological properties in small scale declared in several studies. WANG *et al*. (2008) stated that local spatial variability is the variation of the property within allocation at a finer scale, related to spatial configuration of the data locations within the neighbourhood. Heterogeneity of soil properties may be occurred at large scale (region) or at small scale (community), even in the same type of soil or in the same community (DU FENG *et al.*, 2008). WEINDORF & ZHU (2010) and KAVIANPOOR *et al*. (2012) in their studies mentioned the variations of soil texture in small scale. Since the soil texture can not change in a short time (SHAHOEI, 2006), it seems that soil texture was differed between two plantations before deforestation. The important note that it is not subject to forest managers in that time.

According to the results, GP contains higher main nutrient contents compare to PP. On the base of related reports (preliminary studies before plantation) for this region, the majority of soil chemical properties did not have significant differences in about 30 years ago, when these poplar plantations were established. According to quantitative growth of trees in two plantations, it seems that over the time, appropriate quantitative and qualitative growth of trees in GP has been able to create positive conditions to improve soil chemical and nutrient properties. BINKLEY & SOLLINS (1990), AUGUSTO *et al.* (2002), SINGH & SHARMA (2007) noted the effect of poor quality and quantity of litter on inappropriate decomposition of them and weak nutrient release to the soil. Due to appropriate quantitative growth of trees in GP, it seems that the amount of litter falls in this plantation is more than PP. The rates of forest litter falls and decomposes of them contribute to the regulation of nutrient cycling, primary productivity, and the

maintenance of soil fertility in forest ecosystems (FIORETTO *et al.*, 2003, ONYEKWELU *et al.*, 2006). As WANG *et al.* (2008) demonstrated that litter decomposition provides organic and inorganic elements for the nutrient cycling processes and controls nutrient return to the forest ecosystem. On the other hand in GP, DBH shows positive correlation with C, N and P and also height of trees with P. CHANG-BING *et al.* (2004) showed the effect of N on increase of DBH and also TUFEKCIGLU *et al.* (2003) stated positive correlation between height of hybrid poplar and P availability of soil.

Above mentioned matters declare multifaceted relationships between trees and nutrient availability in the soil of these plantations. The better quality and quantity of trees in GP improve soil nutrient availability and higher contents of these elements have caused better quantitative conditions of poplar trees. High availability of nutrient was related to high productivity of poplar trees as evidenced by their higher height and greater diameter. Poor stand reduces soil fertility and reduction in availability of nutrient elements has had negative effects on growth and quality of stand. The results showed that after about 30 years, not only biomass and wood amount in the poor quality stand have been reduced, but soil nutrient availability was also diminished. It seems that in the early years after plantations soil physical properties, especially soil texture had an important role in trees growth of two plantations. These results demonstrate the essential role of soil properties and detail studies for careful selection of the sites for poplar plantation.

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Received: 10.09.2012
Accepted: 24.11.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 77-83

Primary Production Dynamics of Two Dominant Macrophytes in Wular Lake, a Ramsar Site in Kashmir Himalaya

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Abstract. Growing season changes in the organic matter, organic carbon and chlorophyll content of the two dominant macrophytes, *Nymphoides peltatum* and *Ceratophyllum demersum* of Wular Lake, a Ramsar Site in Kashmir Himalaya were analysed during March- November 2011. The content of organic matter and organic carbon for *Nymphoides peltatum* were 114.1 g m^{-2} and 53.1 g C m^{-2} and *Ceratophyllum demersum* were 57.0 g m^{-2} and 26.4 g C m^{-2}. Chlorophyll A (Chl a) and chlorophyll A+B (Chl a+b) pigments ranged from 1.75 mg g^{-1} (Chl a) and 2.1mg g^{-1} (Chl a+b) in *Nymphoides peltatum to* 4.41 mg g^{-1} (Chl a) and 5.69 mg g^{-1} (Chl a+b) in *Ceratophyllum demersum*. In full leaf out, the latter aquatic plants exceeded 15-20% coverage of the open water surface.*Ceratophyllum demersum* and *Nymphoides peltatum* achieved maximum growth in June and August respectively, but significant differences in their growth dynamics was observed. At the end of the vegetation period, these plants sink to the bottom and decompose.

Keywords: aquatic macrophytes, organic matter, organic carbon, chlorophyll.

Introduction

Macrophytes, as a component of freshwater ecosystems have diverse roles to play in the structure and functioning of these ecosystems (PANDIT, 1984; WETZEL, 2001). Water plants, including macrophytes are universally recognized as important participants in the natural processes of water self-purification (GAYEVSKAYA, 1966; DEMBITSKY *et al*., 1992). The occurrence and distribution of aquatic vascular macrophytes depends on water depth, transparency, regime, chemical composition, pH and salinity (MADSEN *et al*., 2006; VIS *et al*., 2007). The presence of certain macrophytic species in aquatic ecosystems also depends on composition and properties of sediments (DAWSON & KRYSZTOF, 1999; HEEGARD *et al*., 2001; BARENDREGT BIO, 2003; MAKELA *et al*., 2004). Owing to their high rate of biomass production, macrophytes have primarily been characterized as an important food resource for aquatic organisms, providing both living (grazing food webs) and dead organic matter (detritivorous food webs). Macrophytes being excellent indicators of lake condition also act as "nutrient source" and "nutrient pumps" (PANDIT, 1984; STRIVASTAVA *et al*., 2008). On the other hand, the process of eutrophication may get accelerated due to high productivity of aquatic macrophytes creating large

problems, particularly at the time of decomposition of their biomass (NIKOLIC *et al.*, 2007). It is therefore important to monitor aquatic ecosystems for growth and development of macrophytes with high biomass production, thus protecting the ecological balance in these ecosystems.

Shallow freshwater ecosystems are characterized by high productivity. Phytoplankton and emergent, floating and submerged macrophytes are principal primary producers, which differ in productivity, utilization of different sources of carbon dioxide, nutrients and light energy (HORNE & GOLDMAN, 1994). In general, it can be stated that macrophytes are dominant and principal primary producers in shallow freshwater aquatic ecosystems (WETZEL, 2001). The aim of the present study was to analyze the primary production dynamics of the two dominant macrophytes by monitoring their levels of organic matter and organic carbon and estimating their photosynthetic potential *via* the total chlorophyll content.

Materials and methods

Study area. The work underlying this paper was carried out in the largest freshwater lake of Indian Sub-continent, Wular Lake, a Ramsar site in Kashmir Himalaya in the course of the 2011 vegetation period. The lake, is a shallow one (open water area 24 Km^2), located 34 Km north-west of Srinagar city on the Kashmir valley between 34^0 16′ -34^0 20′ N latitude and 74^0 33′ – 74^0 44′ E longitude. Its altitude is 1580 m and its depth, on average, 3.6 m throughout length, reaching 5.8 m at its deepest point (Watlab). The major inflows to Lake Wular are Jhelum, Madumati and Erin. The location of Lake Wular has a mean annual temperature of 10.7^{o}C and 17.5^{o}C for the vegetation period. The amplitude difference of the mean monthly temperatures is 21.9^{o}C, the temperatures ranging from 0.8^{o}C in January to 21.1^{o}C in July. The survey and analysis of the present lake was carried out at monthly intervals from March to November, 2011. For detailed study and investigation, the lake was divided into nine sampling sites (Fig. 1).

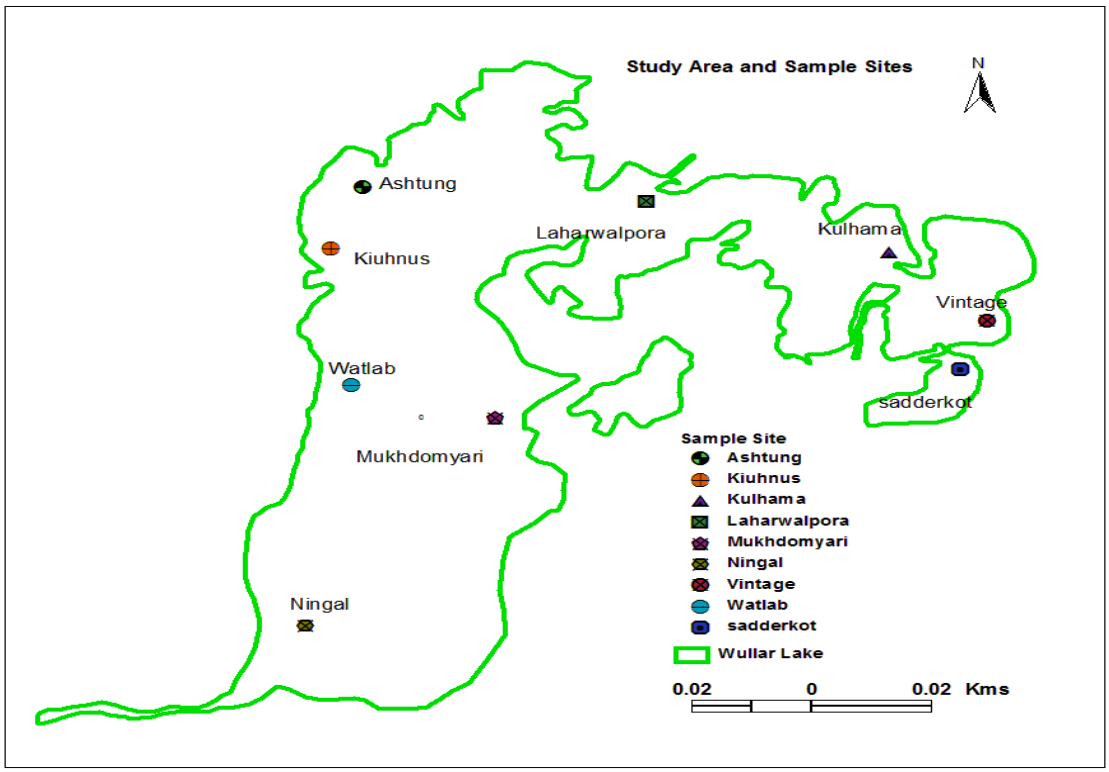

Fig. 1. Study area and sampling sites.

Methods.Organic matter, organic carbon and chlorophyll contents were measured according to standard methods (APHA, 1998). Vascular aquatic macrophytes were collected from a boat, using a 0.5 m^2 wooden frame and a 0.25 m^2 Ekman dredge (WELCH, 1948). Macrophytes were sampled in three replications, from nine spots (sampling sites), throughout the growing season in one year. Whole plants were taken from sample areas of 0.5 m^2 or 0.25 m^2. Plant material was labeled and taken to laboratory where it was rinsed, wrung out and weighed. Samples were dried at 105^oC for 30 hours *i.e*., until a constant weight was reached. Plant material was ground and burnt for as long as it emitted gases. The residue was incinerated at 550 ^oC for 6 hours. After cooling in a desiccator, the residue was weighed to calculate the content of organic matter, which was expressed in g m^{-2}. As most of the higher plants contain 46-48% of carbon in dry weight, organic carbon content was calculated using the factor 46.5% and expressed in g C m^{-2}. Chlorophyll content (Chlorophyll A, Chl a and chlorophyll A+B, Chl a+b) was determined spectrophotometrically, in acetone extracts from fresh samples (LICHTENTALER & WELLBURN, 1983). Descriptive statistics were calculated for content of organic matter and organic carbon (Table 1) using StatSoft Statistica 7.0 statistics software.

Results

During the investigated period, the macrophytes *Nymphoides peltatum*, and *Ceratophyllum demersum* dominated Lake Wular waters along with other 10 macrophytes. In full vegetation, the two dominant aquatic plants flourished and overgrew 15-20 % of the open water surface, while the surface of the deepest part of the lake remains free of aquatic vegetation.

Nymphoides peltatum and *Ceratophyllum demersum* were analyzed for organic matter, organic carbon and chlorophyll pigment contents, *i.e*., for indicators of biomass production in the investigated aquatic ecosystem.

Nymphoides peltatum occurred in the course of March. They reached maximum growth in summer and they persisted until late fall, when some plants were still in flower. *Nymphoides peltatum* plants inhabited the shallower parts of the lake, growing at the depths between 1.5 and 5 m. The average annual contents of organic matter and organic carbon in *Nymphoides peltatum* plants were 114.1 g m^{-2} and 53.1 g C m^{-2}, respectively (Table 1).

In the investigation period, *Ceratophyllum demersum* occurred in late March. They occupied limited areas at the depths between 2 and 5.6 m of the lake. The average annual contents of organic matter and organic carbon in *Ceratophyllum demersum* plants were 63.75 g m^{-2} and 29.75 g C m^{-2}, respectively (Table 1). The analyzed parameters (content of organic matter and content of organic Carbon) showed relatively high variation coefficients and variation (Table 1). The average values of chlorophyll pigment content ranged from 1.75 mg g^{-1} (Chl a) and 2.1 mg g^{-1} (Chl a+b) in *Nymphoides peltatum in* the month of November to 4.41 mg g^{-1} (Chl a) and 5.69 mg g^{-1} (Chl a+b) in *Ceratophyllum demersum* in June (Table 2). Our results show species specificity in the seasonal dynamics of pigment content (Table 2). *Nymphoides peltatum* and *Ceratophyllum demersum* exhibited significant differences in chlorophyll content between the seasons in a single vegetation period. Seasonal variation in chlorophyll content may provide indirect indication of the dynamics of bioproduction by the analyzed plant species. The species *Nymphoides peltatum* has the longest vegetation period and is distinguished for largest and longest photosynthetic activity, which results in the largest organic production and thus impact on the eutrophication process. We also show that the two species differed significantly in chlorophyll content.

Discussion

It has been emphasized that there is a direct relationship between the primary production dynamics of macrophytes and light regime, temperature, water depth, sediment composition and the amount of

available nutrients (SHILLA & DATIVA, 2008; ZHU *et al.,* 2008). In our study we also show that these ecological factors affected the primary production in Wular Lake.

Different macrophyte species may exhibit seasonally variable growth patterns (WETZEL, 2001). In our study, the floating, rooted, species *Nymphoides peltatum*, characterized by a long flowering period and high biomass productivity achieved maximum growth in summer and persisted till late fall, when individual plants could still be found in flower. The contents of organic matter (around 71% in relation to dry matter) and organic carbon (33% in relation to dry matter) in *Nymphoides peltatum* were somewhat lower than those found in literature (RICH *et al*., 1971).

Table 1. Growing season changes in organic matter• and organic carbon content• for the two aquatic plant species in Wular Lake.

Plant species	Bio-mass*	March	April	May	June	July	August	Sep-tember	Octo-ber	Novem-ber	Mean	SD±	V	I
Ceratophyllum demersum	**OM**	18	45	95	110	105	70	30	25	15	57	38.6	1488.5	67.7
	OC	8	21	44	51	49	32	14	12	7	26.4	17.9	320.3	67.7
Nymphoides peltatum	**OM**	66	85	135	210	190	230	45	36	30	114.1	79	6241.9	69.2
	OC	31	39	63	98	88	107	21	17	14	53.1	36.7	1348.4	69.1

*Legend: OM-organic matter (g m^{-2}); OC-organic carbon (g C m^{-2}); SD-standard deviation; V-variation coefficient; I-variation.
•Average results based on independent analyses from nine sites.

Table 2. Growing season changes in chlorophyll A• and A+B• (mg g^{-1} dry matter) for the two aquatic plant species in Wular Lake**.**

Plant species	Chloro-phyll	March	April	May	June	July	August	Sep-tember	Octo-ber	Novem-ber	Mean	SD ±	V	I
Ceratophyllum demersum	**A**	3.7	4	4.3	4.41	4.12	3.8	3.3	3.2	2.6	3.7	0.6	0.3	15.8
	A+B	4.83	5.28	5.5	5.69	5.34	4.94	4.22	4.36	3.38	4.8	0.7	0.5	15.3
Nymphoides peltatum	**A**	1.86	1.92	2.1	2.51	3.21	3.56	2.42	2.11	1.75	2.4	0.6	0.4	26.3
	A+B	2.1	2.22	2.52	3.11	4.01	4.68	2.93	2.41	2.15	2.9	0.9	0.8	31.0

Legend: SD-standard deviation; V-variation coefficient; I-variation.
• Average results based on independent analyses from nine sites.

Still lower biomass values were registered for *Nymphoides* (KELLY, 1989; NIKOLIC *et al*., 2009), they pointed out that water chemistry had an exceptionally high effect on macrophytic species and their bioproduction. On the other side, KAUL (1971) registered higher biomass values for *Nymphoides peltatum* at the peak of the vegetation period. In case of species with large leaf area such as *Nymphoides peltatum*, the biomass production dynamics is significantly affected by the rate of colonization by epiphytic organisms (HOPSON & ZIMBA, 1993). The submerged, unrooted species *Ceratophyllum demersum,* which occurs at all depths and in all regions and seasons (KUNII & MAEDA, 1982), was found in Wular Lake on a limited area, near the bottom of this aquatic ecosystem, where it did not form large biomass. This might be due to *Ceratophyllum* and its high requirements for nitrogen and nitrogen containing substan-

ces, which are moderately abundant in Wular Lake. NIKOLIC *et al*. (2009) reported this observation in case of *Ceratophyllum demersum* in Lake Provala (Serbia). Compared with the N. *peltatum, Ceratophyllum demersum* had lower values of the organic matter and organic carbon, which were somewhat lower than those found in literature (VIS *et al*., 2007). The obtained data are a good illustration of the primary production dynamics of *Ceratophyllum demersum* in Wular Lake, and they also showed lowest variations. Overall *Ceratophyllum demersum* grew and produced biomass fairly uniformly through the vegetation period, with an exception in June when it reached a peak of the vegetation growth. The average values of organic matter were about 75 %, in agreement with the available literature (WESTLAKE, 1965, 1971, 1975; NIKOLIC *et al*., 2009). The values of organic carbon we obtained for the analyzed species were slightly higher than those reported by WESTLAKE, (1975)), while other authors (RICH *et al*., 1971; VIS *et al*., 2007) recorded considerably higher average values.

The analysis of chlorophyll A and B in the two macrophytes indicated the presence of species specificity as well as of seasonal dynamics of these pigments. The content of chlorophyll A has a wide range of variation in aquatic plants, which speaks in favour of plant adaptation to different ecological conditions, in the first place light and temperature (SCHAGERL & PICHLER, 2000). In Wular Lake, the floating species *Nymphoides peltatum* and the submerged species *Ceratophyllum demersum* exhibited significant differences in chlorophyll content among the dates of measurement throughout the vegetation period. The results of pigment content for the analyzed macrophytic species were in agreement with the available literature.

The seasonal variation in pigment content was an indication of bioproduction dynamics with *Nymphoides peltatum* having the longest period of bioproduction. Consequently, due to the decay and decomposition of its biomass after the end of the vegetation period, which significantly bolster the eutrophication process, this species had the highest effect on the process of secondary pollution of the lake (NIKOLIC & BILJNI, 2005).

Conclusions

It may be concluded from the above that the investigated aquatic ecosystem is dominated by macrophytes, one floating (*Nymphoides peltatum*) and another *submerged* (*Ceratophyllum demersum*). These plant species are characterized by uneven biomass during the vegetation period, which is brought about by the ambient climatic conditions and the trophic state of the investigated aquatic ecosystem. The enormous biomass which they form by the end of the vegetation period causes secondary pollution of the lake, which directly affects the trophic level of the ecosystem by accelerating the eutrophication process in this lake. Therefore it is necessary to monitor and control their growth and development. The results of the present study have implications for efficient eco-restoration of the lake ecosystem through scientific management of macrophytic vegetation**.**

Acknowledgements

The authors are highly thankful to Head, Department of Environmental science, University of Kashmir for providing necessary facilities for carrying out the research work.

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Received: 13.09.2012
Accepted: 27.11.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 2 December 2012 pp. 85-92

Control of Three Stored−Product Beetles with Artemisia haussknechtii (Boiss) (Asteraceae) Essential Oil

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Abstract. Fumigant toxicity of the essential oil of aerial parts from *Artemisia haussknechtii* (Boiss) (Asteraceae) was investigated against the cowpea weevil *Callosobruchus maculatus* (Fab.), the rice weevil *Sitophilus oryzae* (L.), and the red flour beetle *Tribolium castaneum* (Herbst). Dry ground plants were subjected to hydro−distillation using a Clevenger−type apparatus and the chemical composition of the volatile oil was studied by gas chromatography−mass spectrometry (GC−MS). The major components of the oil were camphor (29.24%), 1, 8−cineol (27.62%), yomogi alcohol (5.23%), and camphene (4.80%). The essential oil in same concentrations was assayed against (1−7 days old) adults of insect species and percentage mortality was recorded after 24, 48, and 72 h exposure times. LC_{50} values were varied between 19.84 and 103.59 µL L^{-1} air, depending on insect species and exposure time. *Callosobruchus maculatus* was more susceptible than other species. These results suggested that *A. haussknechtii* oil might have potential as a control agent against *C. maculatus*, *S. oryzae* and *T. castaneum*.

Key words: *Artemisia haussknechtii*, essential oil, stored−product beetles.

Introduction

Insects are one of the major causes of grain losses during storage (SCOTTI, 1978). Crop losses due to insect pests changed between 10 and 30% for major crops (FERRY *et al.*, 2004). The major insect pests of stored grains are members of Coleoptera that are the largest order in the animal kingdom, with over one−third of a million described species (BOOTH *et al.*, 1990). Insect pests of stored grain feed directly on commodities and can be conveniently divided into primary pests (such as *Callosobruchus sp.*, *Sitophilus sp.* and *Rhyzopertha sp.*) those that attack intact commodities, and secondary pests (such as *Oryzaephilus sp.*, *Cryptolestes sp.*, *Cadra sp.* and *Tribolium sp.*) which require the commodity to be damaged before they can attack it (REES, 2007).

Fumigation plays a very important role in insect pest elimination in stored products (ZETTLER & ARTHUR, 2000). Few chemicals are suitable for use as fumigants. They should be biologically active and control pest insects without damage to stored grain, sufficiently volatile to be removed by aeration, unabsorbed by the grain, inflammable and inert with metals. The use of phosphine (PH_3) is increasing due to the convenience of formulations, the relatively short−term hazard, and low retention of residues. However, PH_3 fumigation may become increasingly limited in use because resistance of stored grain insects to phosphine has now been discovered in more than 45 countries (BELL & WILSON, 1995; CHAUDHRY, 1995). The use of methyl bromide is being restricted because of its

potential to damage the ozone layer (BUTLER & RODRIGUEZ, 1996). Plant essential oils and their components have been shown to possess potential for development as new fumigants and they may have advantages over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability (ISMAN, 2008).

The genus *Artemisia* (Asteraceae family) with the common Persian name of 'dermane' and also with common English name of 'wormwood' includes 34 species that are found wild all over Iran of which two are endemic (MOZAFFARIAN, 1996). *Artemisia* species are popular plants that are used for the treatment of diseases such as hepatitis, cancer, inflammation and infections by fungi, bacteria, and viruses (KIM *et al.*, 2002). Local people in the western part of Iran use *A. haussknechtii* in dyspepsia and other gastrointestinal disorders (KHANAHMADI *et al.*, 2009).

The present work was carried out to determine the possible fumigant toxicity of the essential oil of *A. haussknechtii* against the cowpea weevil *Callosobruchus maculatus* (Fab.), the rice weevil *Sitophilus oryzae* (L.), and the rust−red flour beetle *Tribolium castaneum* (Herbst).

Material and methods

Insect culture.

Callosobruchus maculatus, *S. oryzae* and *T. castaneum* were reared on bean grains, whole rice and wheat flour mixed with yeast (10:1, w/w), respectively. Adult insects, 1–7 days old, were used for fumigant toxicity tests. The cultures were maintained in the dark in a growth chamber set at 27±2°C and 60±5% r.h. All experiments were carried out under the same environmental conditions.

Plant material.

Aerial parts of *A. haussknechtii* were collected at full−flowering stage in June 2010 in the region of karand in the west of Kermanshah (latitude: 37° 32', longitude: 45° 05'; altitude: 1313 m). Plant taxonomists in the Department of Biology at Urmia University, confirmed the taxonomic identification of plant species. The voucher specimens have been deposited at the herbarium of the Department of Plant Protection at Urmia University. The plant material was dried naturally on laboratory benches at room temperature (23−24°C) for 5 days until crisp. The dried material was stored at −24 °C until needed and then hydro distilled to extract its essential oil (NEGAHBAN *et al.*, 2007).

Extraction and analysis of essential oil.

Essential oil was extracted from the plant samples using a Clevenger−type collector where the plant material is subjected to hydro distillation. Conditions of extraction were: 50 g of air−dried sample; 1:10 plant material/water volume ratio, 2 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (0.9% w/w) was calculated on a dry weight basis. Extracted oil was stored in a refrigerator at 4 °C.

The constituents of essential oil were analyzed by gas chromatography mass spectrometry (GC−MS) (Thermo−UFM). The GS conditions were as follows: capillary column 1−ph (30 m x 0.25 mm, film thickness 0.25 µm); helium as a carrier gas (0.5 ml/min); oven temperature program, initially 40°C rising to 250°C (80°C/min, 3 min); injector and detector temperature of 250°C. The identification of individual compounds were based on comparison of their relative retention times with those of authentic samples on a capillary column, and by matching their mass spectra of peaks with those obtained from authentic samples and published data (DAVIES, 1990).

Fumigant toxicity.

In order to test the toxicity of essential oil, concentrations 30, 38, 49, 63 and 80 $\mu l.l^{-1}$ air of the oil were dissolved in 100 µl acetone, dried in air for 2 min and applied on a filter−paper (Whatman No.1) strip measuring 4 × 5 cm that was attached to the lower side of the jars lid. Twenty adults (1–7 days old) of insects were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical center of 1 L glass jars, which were then sealed with air−tight lids. Thus, there was no direct contact between the oil and the insects. In the control jars, only acetone applied on the filter papers.

Mortality was determined after 24, 48 and 72 h from commencement of the exposure. Each experiment was replicated four times for each concentration. When no leg or antennal movements were observed, insect was considered dead.

Data analysis.

The mortality data were corrected using Abbott's formula (ABBOT, 1925) for the mortalities in the controls, and then subjected to probit analyses to estimate LC_{50} and LC_{95} values. The percentage mortality was determined for analysis of variance (ANOVA) according to the general linear model (GLM). Significant differences were identified by honest significant difference (HSD) tests at the 5% level and entered in the fig. Data processing was conducted by the SPSS 16.0 for Windows.

Results

Fumigant toxicity.

In all cases, considerable differences in mortality of insects to essential oil vapor were observed with different concentrations and times. Probit analysis showed that *C. maculatus* (LC_{50}=59.29 µl.l^{-1} air) was more susceptible to *A. haussknechtii* oil than *S. oryzae* (LC_{50}=84.49 µl.l^{-1} air) and *T. castaneum* (LC_{50}=103.59 µl.l^{-1} air). The corresponding LC_{95} values were 263.67, 303.8 and 286.75 µl.l^{-1} air, respectively. The 95% fiducial limits of the LC_{50} and LC_{95} values for three species are shown in Table 1.

Table 1. Result of probit analysis to calculate LC_{50} and LC_{95} values.

Insects	Time [h]	LC_{50}[a]	LC_{95}[a]	χ^2 [df = 3]	*p*	Inter-cept	Slope
C. maculatus	24	59.29 (46.72−101.42)	263.67 (131.09−9766.62)	0.79[b]	0.85	0.50	2.53
	48	37.53 (30.62−42.80)	79.72 (64.78−126.90)	2.06[b]	0.55	-2.91	5.02
	72	19.84 (0.07−27.35)	42.31 (34.18−236.29)	2.04[b]	0.56	−1.48	5.00
S. oryzae	24	84.49 (65.50−214.66)	303.80 (149.79−7822.18)	1.12[b]	0.77	−0.70	2.96
	48	53.02 (42.79−70.55)	188.80 (112.51−1253.75)	1.58[b]	0.66	−0.14	2.98
	72	29.19 (18.50−35.00)	68.01 (54.91−122.86)	1.77[b]	0.62	−1.56	4.47
T. castaneum	24	103.59 (78.11−328.17)	286.75 (149.26−5963.63)	0.33[b]	0.95	−2.49	3.72
	48	65.75 (54.84−95.75)	199.12 (121.65−971.56)	0.55[b]	0.90	−1.21	3.41
	72	31.54 (16.17−39.42)	109.37 (75.64−448.05)	1.00[b]	0.80	0.34	3.04

[a] Ninety−five percent lower and upper fiducial limits are shown in parenthesis.
[b] Since the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits.

From the graph in Fig. 1 it can be seen that, *A. haussknechtii* oil was relatively more toxic to *C. maculatus* than to *S. oryzae* and *T. castaneum*. The least concentration (30 µl.l^{-1} air) of the oil yielded about 27% mortality of *C. maculatus* after a 24 h exposure but the mortalities of *S. oryzae* and *T. castaneum* at the same concentration and time were about 5% and 1%, respectively. At the 72 h exposure time, kills of *C. maculatus* reached 100% with a 49 µl.l^{-1} air concentration. By contrast, 80% mortality was achieved for *S. oryzae* and about 63% for *T. castaneum* at the same exposure time and concentration.

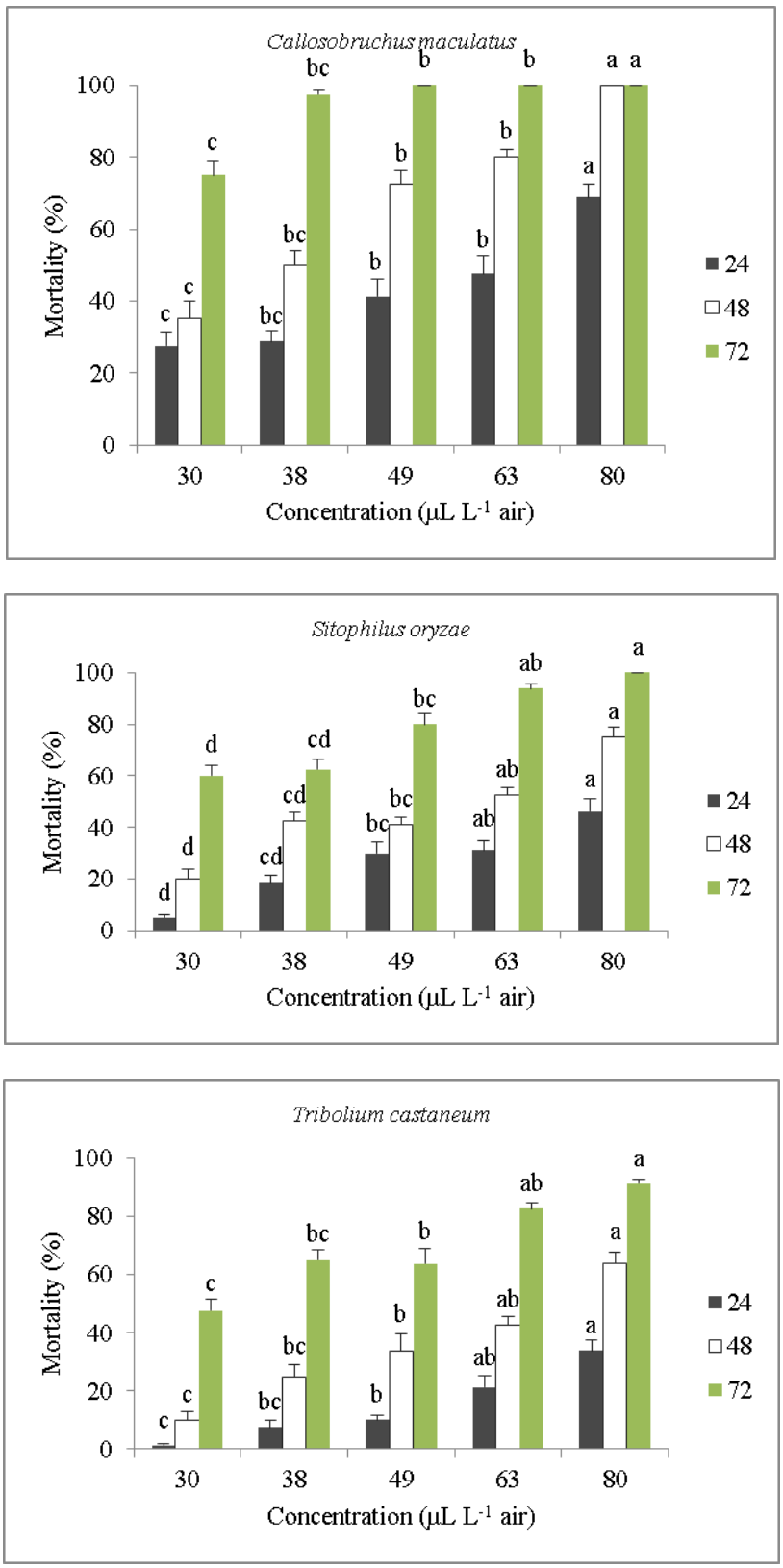

Fig 1. Mean mortality (%) of *Callosobruchus maculatus*, *Sitophilus oryzae*, and *Tribolium castaneum* exposed to different concentrations of *Artemisia haussknechtii* essential oil. Different letters over columns indicate significant differences according to Tukey test at α=0.05. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±).

The essential oil of plant tested was toxic against insects. According to the results of ANOVA, the differences among all treated doses and exposure times of the essential oil were significant at $p<0.05$ (Fig. 1).

Chemical constituents of A. haussknechtii.

The oil from *A. haussknechtii* contained camphor (29.24%), 1, 8−cineole (27.62%), yomogi alcohol (5.23%), camphene (4.80%), p−cymene (3.35%) and α−pinene (2.36%) (Table 2).

Table 2. Main chemical components of *A. haussknechtii* essential oil.

No.	Constituents	Percentage
1	α−Pinene	2.36
2	Camphene	4.80
3	β−Pinene	1.68
4	1,8−Cineole	27.62
5	Camphor	29.24
6	Yomogi alcohol	5.23
7	p−Cymene	3.35
8	*Artemisia* alcohol	1.96
9	Linalool	1.73
10	Borneol	2.18
11	Caryophylene oxide	0.98
	Total	**98.36**

Discussion

The essential oil of *A. haussknechtii* was analyzed with different researchers (JALALI HERAVI & SERESHTI, 2007; SERESHTI & SAMADI, 2007; KHANAHMADI *et al.*, 2009). Extract and essential oil of *A. haussknechtii* have been shown to possess antioxidant and anti-microbial activities (KHANAHMADI *et al.*, 2009). In our best knowledge, studies have not been reported previously concerning the activity of *A. haussknechtii* as a fumigant on insect pests. The fumigant activity of essential oils from other *Artemisia* species has been evaluated against a number of stored product insects including oils from *A. annua* (L.) against *T. castaneum* and *C. maculatus* (TRIPATHI *et al.*, 2000), and from *A. tridentata* Nutt. ssp. *vaseyana* (Rydb.) against some stored−grain insects (DUNKEL & SEARS, 1998). *Artemisia sieberi* (Besser) had fumigant activity against some insect pests (NEGAHBAN *et al.*, 2006; 2007), and oils from three *Artemisia* species against *Sitophilus granarius* (L.) (KORDALI *et al.*, 2006).

One of the most valued properties of essential oils is their fumigant activity against insects, since it may involve their successful use to control pests in storage without having to apply the compound directly to the insects. Based on the results from fumigant bioassays the essential oil tested showed high toxicity when that was applied against insects with insecticidal activity dependent on oil concentration and exposure time. When *C. maculatus* were fumigated for 48 h, a concentration 38 $\mu l.l^{-1}$ air oil was necessary to cause 50% mortality (LC_{50}), while a concentration of 63 $\mu l.l^{-1}$ air for *S. oryzae* and for *T. castaneum* concentration near to 80 $\mu l.l^{-1}$ air was enough to cause equal mortality when were used (Fig. 1). Moreover, slopes of probit lines estimated that any increase in essential oil concentration, was imposed the highest mortality to *C. maculatus* when compared to other tested insects at 72 h exposure time. Furthermore, intercept of probit line for this pest at 24 h exposure time was higher than *S. oryzae* and *T. castaneum*, showing the higher response threshold (Table 1, Fig. 1).

Experiment showed that *C. maculatus* is more susceptible than *S. oryzae* and *T. castaneum* (Table 1 and Fig. 1). HASHEMI & SAFAVI (2012) studied fumigant toxicity of *Platycladus orientalis* (L.) Franco (Cupressaceae) against *C. maculatus, S. oryzae*, and *T. castaneum*. LC_{50} values of the leaf and the fruit oils at 24 h were estimated 6.06 and 9.24 $\mu l.l^{-1}$ air for *C. maculatus*, 18.22 and 21.56 $\mu l.l^{-1}$ air for *S. oryzae*, and 32.07 and 36.58 $\mu l.l^{-1}$ air for *T. castaneum,* respectively. NEGAHBAN & MOHARRAMIPOUR (2007) reported fumigant toxicity of essential oils from *Eucalyptus intertexta* R.T. Baker, *Eucalyptus sargentii* Maiden and *Eucalyptus camaldulensis* Dehnh (Myrtaceae) against *C. maculatus, S. oryzae*, and *T. castaneum*. The LC_{50} values to the selected essential oils were 2.55, 3.87, and 3.97 $\mu l.l^{-1}$ air for *C. maculatus*, 6.93, 12.91, and 12.06 $\mu l.l^{-1}$ air for *S. oryzae*, and 11.59, 18.38, and 33.50 $\mu l.l^{-1}$ air for *T. castaneum*, respectively. In another study by NEGAHBAN *et al* (2007), fumigant toxicity of *A. sieberi* essential oil

was reported against *C. maculatus*, *S. oryzae*, and *T. castaneum*. *Callosobruchus maculatus* was significantly more susceptible than *S. oryzae* and *T. castaneum*; the LC_{50} values were 1.45 µl.l^{-1} air for *C. maculatus*, 3.86 µl.l^{-1} air for *S. oryzae*, and 16.76 µl.l^{-1} air for *T. castaneum*. These findings are consistent with the results of this study as *C. maculatus* was more susceptible to the essential oils, and *T. castaneum* was more tolerant than *S. oryzae* and *C. maculatus*.

GC−MS analyses of the oil revealed that the percentage of oxygenated monoterpenoids is higher than other compounds (Table 2). The loss of insecticidal activity of essential oil in the course of time may be attributed to rapid evaporation and degradation of the chemicals (OBENG−OFORI *et al.*, 1997). It was demonstrated that, oils with high content of hydrogenated compounds loss their activity quicker than those containing mainly oxygenated compounds (REGNAULT−ROGER *et al.*, 2002). Structure–activity relationships of plant compounds against stored product insects have been well studied. REGNAULT−ROGER & HAMRAOUI (1995) studied the structure–activity relationship between monoterpenoids and fumigant activity against *Acanthoscelides obtectus* (Say): the oxygenated structures prove to be the most active compounds. It could be demonstrated that toxicity of the essential oil of *A. haussknechtii* related to the high percentage of oxygenated compounds such as camphor (Table 2). In addition, the insecticidal activity of an essential oil could be attributed either to the major compound of the oil, or to the synergic and/or antagonistic effects of all the components of the oil. For example, KRISHNARJAH *et al.* (1985) demonstrated that the association of ρ−cymene and β−pinene resulted in a higher toxicity in *Sitotroga cerealella* (Olivier) than that of the components used separately.

The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility, they have fumigant activity that might be of importance for controlling stored−product insects (REGNAULT−ROGER & HAMRAOUI, 1995). The toxic effects of *A. haussknechtii* could be attributed to major constituents such as camphor (29.24%), 1, 8−cineole (27.62%), p−cymene (3.35%) and α−pinene (2.36%). The monoterpene camphor might have broad insecticidal activity against stored−product insects and act as the fumigant in *A. haussknechtii* oil. Camphor from several *Artemisia* species reported that is toxic against stored−product beetles (DUNKEL & SEARS, 1998; KORDALI *et al.*, 2006; NEGAHBAN *et al.*, 2007; TANI *et al.*, 2008). 1, 8 cineole isolated from *A. annua* is a potential insecticidal allelochemical that could reduce the growth rate, food consumption and food utilization in some post-harvest pests and house hold insects (JACOBSON & HALBER, 1947; KLOCKE *et al.*, 1989; OBENG−OFORI & REICHMUTH, 1997; TRIPATHI *et al.*, 2001). ρ−cymene had fumigant toxicity on *A. obtectus* (REGNAULT−ROGER & HAMRAOUI, 1995). OJIMELUKWE & ADLER (1999) found α−pinene was toxic to *Tribolium confusum* du Val.

In conclusion, our research revealed that the essential oil of *A. haussknechtii* possesses a potential for use in the management of *S. oryzae*, *T. castaneum*, and especially *C. maculatus*. In the light of recent interest by agrochemical companies in developing plant−based pesticides, this product would be an environmentally friendly chemical and safe for application. However, further studies also need to be conducted to evaluate the cost, efficacy and safety of this essential oil on wide range of pests in commercial store.

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Received: 07.10.2012
Accepted: 09.12.2012

ECOLOGIA BALKANICA - INSTRUCTIONS TO AUTHORS - 2013

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. Studies conducted on the Balkans are a priority, but studies conducted in Europe or anywhere else in the World is accepted as well, after a consideration of the Editorial Board. *The Editorial Board of "Ecologia Balkanica" reserves its right to deny 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. All manuscripts must be accompanied with a cover

letter, which can be downloaded from http://eb.bio.uni-plovdiv.bg/about/instructions-to-authors/. All fields from the cover letter form must be filled out and the cover letter 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 in principle, it will be forwarded to a Section Editor, who will assign 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

Submission of a manuscript implies: that the work described has not been published before (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. The manuscript text should be **MS-Word** processed (all versions are acceptable, including 2007 and 2010),

justified, font size 11, font "Book Antiqua", without footnotes, column or page breaks, single spaced (about 60 lines per page), on A4 (210 x 297 mm) paper, with margins of exactly 2.5 cm on each side. Pages should not be numbered.

The manuscripts should conform to the following format:
Title: Provide a title that is concise but also an informative synthesis of the study. Where appropriate, include mention of the family or higher taxon.
Author(s): Full first name(s), middle initials and surname(s) in bold italic. The corresponding author should be marked with the *-symbol.
Address(es): As complete as possible. E-mail address is given only for the corresponding author.
Abstract: Maximum of 300 words and should summarize the essential results and conclusions with no description of methods, discussions, references and abbreviations.
Key words: Normally 3–10 words suitable for information-retrieval system.

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

The *Introduction* has to explain the actuality of the researched problem and give the aim of the study.
Materials and Methods have to provide sufficient information to permit repetition of the experiment and/or fieldwork. The technical description of study methods should be given only if such methods are new; otherwise a short presentation is enough.
The *Results* section must be a concise presentation of the finding of the study. Avoid the presentation of same information as text and/or figure and/or table.
Discussion section should be separate from the results section at full-length papers and should deal with the significance of the results and their relationship to the aims of the paper. Also include how the findings of the paper will change, influence the state of our knowledge about model organism. In separate cases a joint section "Results and Discussion" is allowed but not preferable.

Conclusions should shortly describe the main contributions and recommendations of the study without including citations and statistics.
In the *Acknowledgements* section all persons and organizations that helped during the study in various ways, as well as the organization that financed the study must be listed.

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

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

Example:

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

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

Example:

Fig. 1. Indicative map of the study area.

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

Citations and references

Literature citations in the text should indicate the author's surname in SMALL CAPITALS (for more information on SMALL CAPITALS - *http://en.wikipedia.org/wiki/Small_caps* and how to make them in Word - *http://office.microsoft.com/en-us/word-help/change-the-capitalization-of-text-HA010210665.aspx*) 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.

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. 220 p.

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. [http://www.statsoft.com].

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

Website:

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

In case of papers written in other than Latin letters, if there is an English (or German, or French) title in the summary, it may be used. If there is not such a summary, the author's 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. 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.)".

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%).

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 authors of taxa and for publications listed in reference 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 of the manuscript, 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. Since 2011, hardcopy reprints are no longer sent to the authors.

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"We are looking forward to welcoming you in Plovdiv!"

The Second Anniversary Scientific Conference on Ecology will be held in the second largest city in Bulgaria – Plovdiv. Plovdiv's history spans 6,000 years and is among the world's-oldest cities. The city has historically developed on seven syenite hills and because of that Plovdiv is often referred to in Bulgaria as "The City of the Seven Hills".

Plovdiv is a picturesque city with many parks and gardens, museums and archaeological monuments. Its oldest part, called the Old town, is a historic preservation site with houses from the period of the national revival in the XVII and XIX centuries. One of the most impressive views to the city is right there – at the Ancient Theater (an excellently preserved Roman theater).

""...this is the most beautiful city which you can imagine. Its beauty shines from the distance."

Lucian

Important dates:

Abstract Submission Deadline - ***30 May 2013***

Registration Fee Payment Deadline - ***30 May 2013***

Full Text Submissions Deadline – ***31 October 2013***

Conference Date - ***01 November 2013***

Student Conference Date - ***02 November 2013***

The Second Anniversary Scientific Conference on Ecology will be held in Plovdiv (Bulgaria) on November 1st 2013. On November 2nd 2013 will follow the Fifth Student's Scientific Conference "Ecology – A Way of Thinking" 5 (for Bulgarian students and PhD students only). More information about the conference will be available soon.

The conference aims to provide an ideal platform for people to share research ideas and experiences in the fields of Ecology, Environmental Conservation and related areas. The official languages of the conference are Bulgarian and English. We welcome abstract submissions.

The program and abstracts will be available as printed proceedings at the conference. The full text articles will undergo a peer-review process and if they receive a positive evaluation they will be published in the international scientific journals ***"Ecologia Balkanica"*** (http://eb.bio.uni-plovdiv.bg) or ***"Education and Science without Borders"*** (http://esjournal.cz/), depending on the area of the submitted article.

Publishing partners:

Ecologia Balkanica
INTERNATIONAL SCIENTIFIC RESEARCH JOURNAL OF ECOLOGY

EDUCATION *and* **SCIENCE** *without borders*

Second Anniversary Scientific Conference on Ecology

on the occasion of the 25th anniversary
of the Department of Ecology and Environmental Conservation,
Faculty of Biology, University of Plovdiv 'Paisii Hilendarski"

01-02 November 2013

Plovdiv, Bulgaria

The staff of the Department of Ecology and Environmental Conservation at the Faculty of Biology, University of Plovdiv "Paisii Hilendaarski". (2007)

Allow Us to Introduce Ourselves

The Department of Ecology and Environmental Conservation is a specialized structural link at the Faculty of Biology, University of Plovdiv "Paisii Hilendarski". Its main task is to organize and carry out educational, scientific and applicable activities in the field of ecology and environmental conservation.

The department carries out teaching courses for obtaining the educational-qualification degree "bachelor of science" of "Ecology and Environmental Conservation", "Ecological biotechnologies", "Biology", "Medicinal Biology", 'Biology & Chemistry", 'Biology & Physics" and 'Biology & English", "master of science" of "Ecology and ecosystems conservation" , "Ecology of Aquatic Ecosystems and Aquaculture Production" and "Ecology, Management and Control of the Environment" and also PhD on "Ecology and conservation of the natural ecosystems". The scientific production of the department is rich, various and purposeful by its content.

Anniversary Scientific Conference on Ecology, 2008

Group photo of the participants of the First Anniversary Scientific Conference on Ecology in Plovdiv in 2008, at the House of the Scientists in the Old Town.

Second Anniversary Scientific Conference on Ecology

on the occasion of the 25th anniversary of the Department of Ecology and Environmental Conservation, Faculty of Biology, University of Plovdiv 'Paisii Hilendarski"

The conference >>>

Traditionally the conference will be held in three thematic sections:

- Ecology, biodiversity and conservation
- Ecological monitoring and Applied ecology
- Ecological education

Topics of interest include, but are not limited to: 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, ecotoxicology, marine biology, animal evolution, ecological monitoring and bioindication, landscape and urban ecology, conservation ecology, new methodical contributions in ecology.

Organizing Committee >>>

Assoc. Prof. Iliana Velcheva, PhD
Assoc. Prof. Angel Tsekov, PhD
Ch. Assist. Prof. Atanas Irikov, PhD
Ch. Assist. Prof. Gana Gecheva, PhD
Ch. Assist. Prof. Dilian Georgiev, PhD
Assist. Prof. Slaveya Petrova, PhD
Assist. Prof. Ivelin Mollov, PhD
Vesela Yancheva, PhD Student
Stela Stoyanova, PhD Student
Borislava Todorova
Bogdan Nikolov

Abstracts & Posters >>>

The Organizing Committee invites the submission of abstracts for oral presentation and poster display. The abstracts topic should be related to conference main highlights.

The abstract should be no more than 2000 characters, including spaces. Please indicate in the registration on-line form whether you wish an oral or a poster contribution. Abstracts are submitted via Registration form, which can be downloaded from the link given below, filled out offline and then sent to the corresponding e-mail address.

All accepted abstracts will be published in the conference abstract book.

Submission deadline – 30 May 2013

Posters for the Poster session must be printed on a single sheet, format A1 (594mm x 841mm) portrait orientation.

Participants may present maximum of two abstracts or posters at the conference with one registration fee.

Registration Fee >>>

Standard Academic fee – 30 Euro

PhD Student fee – 15 Euro (PhD student status proof is needed).

Accompanying person fee – 15 Euro (another set of conference materials).

Contact Information and Registration >>>

The Registration Form can be downloaded from http://web.uni-plovdiv.bg/ecology/files/SACE2013_registration_form.doc and should be filled in and sent to **ecologybf@abv.bg**, by 30 May 2013, with subject "SACE2013 registration". A confirmation e-mail for registration will be sent back to you by the organizing committee. If you have any questions about the conference, use the e-mail given above.

Information about the payment methods will be given in our second announcement in early 2013, as well as more information about the conference. For more information also visit the following link: http://web.uni-plovdiv.bg/ecology/