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Scientific paper
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Biodiversity and heavy metal pollutions in freshwater ecosistems in border areas from Tunja river

The purpose of the carried out examinations is to investigate heavy metal pollutions and the biological variety of the freshwater ecosystems of the Tunja River, Bulgaria, and to make an ecological evaluation of their condition. The Tunja River is related to the Aegean water collecting region. Basic abiotic (temperature, pH, conductivity μS/sm, COD, BOD_5, ionic groups – PO_4 mg/l, N-NO_3 mg/l, N-NO_2 mg/l and heavy metals – Pb, Zn, Cd and Cu, structure of the river bed, dominant tree vegetation, etc.) and biotic characteristics (total number of specimens, prevalence, mean intensity, etc.) were determined. The bioindicative macrozoobenthos was characterized for assessment of the ecological status of the examined freshwater ecosystem. Helminths communities of Squalius orpheus and heavy metal contaminations in fish tissues, organs and chub parasite Acanthocephalus anquillae were determined. The present studies are connected with applying of the new approach for integrated environmental assessment of the freshwater ecosystem based on the living communities and fish parasite communities.

Key words: *bioindication, heavy metals, macrozoobenthos, chub parasite communities, Tunja River*

INTRODUCTION

Riparian habitats are characterized by a wide variety of abiotic parameters of the restricted area, which is a prerequisite for high species diversity of the elements of flora and fauna. Middle and lower part of the river Tunja have created conditions for the development of largest longoza forests. These unique ecosystems are constantly subjected to negative anthropogenic, climatic and hydrological impacts. This makes it necessary to seek, a reasonable compromise between human activity and sustainability of natural systems. The lack of flooding and low Tunja in recent years have triggered a process leading to change and increase floristic diversity. The trend of kserofitization is expected to change the appearance of dense habitats and the disappearance of a number of characteristic species. Studies of the biodiversity and the state of freshwater ecosystem of the Tunja River are comparatively small [17; 22-25; 31; 33-34; 37].

The major negative anthropogenic impact on the Tunja River ecosystem associated with the changes of the studied freshwater communities are farm activities (using of fertilizers, pesticides; wastewater from livestock, etc.; 50% of the region are agricultural lands), constructions, tourism, etc. [5,6,32]. Tunja River is included in the National monitoring program (Water Body Type BGTR12 – Medium sized rivers) [28].

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These researches would allow making recommendations for preservation of the biodiversity, to plan possibilities for preservation of the natural water resources and negative aspects of transborder impact, etc.

MATERIALS AND METHODS

The Tunja River (Fig. 1) is one of the biggest rivers in central part of South Bulgaria (East Aegean Water Basin). The river springs from the central part of Stara Planina.

Mountain, Peak Yurushka Gramada, eastern of the Botev Peak. The Tunja River (350 km in Bulgaria from total 390 km) is the biggest tributary of Maritsa River, influent to Maritsa River on the territory of Turkey, before town of Odrin. The river has a watershed area of 7884 km^2, its main tributaries being Mochuritsa, Popovska and Sinapovska river.

The basic abiotic and biotic characteristics of the freshwater ecosystem biotopes were determined (temperature, pH, conductivity μS/sm, COD, BOD_5, ionic groups – PO_4 mg/l, N-NO_3 mg/l, N-NO_2 mg/l and heavy metals – Pb, Zn, Cd and Cu, structure of the river bed, dominant tree vegetation, etc.). Samples of water and sediments were collected according to the Guidance on sampling of rivers and watercourses - ISO 5667-6:1990, introduced as a Bulgarian standard in 2002. Heavy metal concentration of the water and sediment samples, fish tissues, organs and parasites were carried out according to standard techniques. The samples were analyzed for content of Cd, Cu, Pb and Zn by ICP Spectrometry [2].

Figure 1 - Tunja River

During the three seasons (spring, summer and autumn) of 2011-2012, 18 samples of water and sediments, 19 taxa and 103 specimens of macrozoobenthos in the region between village of Konevec and town of Elhovo from the Tunja River (last part of the river, before the border area with Turkey), were examined. Samples of macrozoobenthos were collected by the EN 27828:1994/ISO 7828:1985; EN ISO 9391:1995/ISO 9391:1993; EN ISO 5667-1:2006/AC:2007; EN ISO 5667-3:2003/AC:2007, etc. Method for presentation on biological data (EN ISO 8689-2:2000) are enjambment.

Methods have been developed at European level projects AQEM and STAR (AQEM consortium, 2004). All samples were fixed in 4% formaldehyde. They are laboratory sorted by systematic groups and then are kept in 70% ethyl alcohol. Analyses of the biological diversity of bioindicative groups of organisms (bioindicative macroinvertebrate fauna - macrozoobenthos) were determined according to quantitative data. The criteria and methods have been applied [4,13,14, 26,29,30,36]. Modified Irish Biotic Index (MIBI), Ecological Quality Ratios Index for Ecological Quality Assessment (EQR), Index based on presence of Ephemeroptera, Plecoptera, Trichoptera Index (EPT), Rhithron Feeding Type Index (RETI) and Saprobic index of Pantle and Buck (SPB) were used for biomonitoring analysis. For an ecological evaluation of the situation of the analyzed freshwater ecosystems, principal biotic indexes have been fixed: Tn/spe – total number of taxa; Tn/sps – total number of specimens, HB (index of Brillouin, diversity), Dmg (index of Margalef, diversity); H' (index of Shannon, diversity), etc [13,18-21,30,37].

The model fish species chosen for this study are from Cyprinidae (chub, *Squalius orpheus* Kottelat & Economidis, 2006). The fish specimens were caught by nets in the river with the Official permission from the Ministry of Agriculture and Food, Republic of Bulgaria. Fish samples (33 specimens) were collected in the period between May and September, 2012. The scientific and common names of the fish hosts were used according to the FishBase database [12]. The analysis of the dominant structure of the found fish parasite taxa were presented to the level of the component communities. The ecological terms prevalence, mean intensity are used, based on the terminology of Bush et al. [3]. Analyses of helminth community structure were carried out during the three seasons and in both levels: infracommunity and component community [3]. The infracommunity data were used to calculate the total number of species, mean number of helminths, the Brillouns diversity index (HB), etc. [16,20].

RESULTS AND DISCUSSION

I. General characterization of the studied biotopes. Physicochemical monitoring.

Studies have been performed of freshwater ecosystems from the Tunja River between the village of Konevec (Biotop 1, after town of Yambol and near to Gorna Topchiya Reserve) and the town of Elhovo (Biotop 2, after town of Elhovo and before Balabana Reserve). Biotope 1 is 264.4 km from the springs of the river and 75.9 km from the border area with R. Turkey. The length of the area is 200-250 m Watercourse is slow and easy. In places woody debris are formed. The bottom substrate of the river is silt and mud. Biotope 2 distances of 251.8 km from the springs of the river and 70.5 km from the border. The length of the section is 300 m. Flow is slow and easy. Rare places in the riverbed sand sediments are formed by vegetation, as well as wood and concrete waste parts. The substrate of the river bed is sand, mud and silt. The section under research in the biotopes is 14-16 m wide and about 0.5-1.5 m deeps. The banks are stable, overgrown with vegetation and deeply shaded on the right bank of solid wood finish. The waterside wood vegetation is represented mainly by *Populus alba*, *Salix alba*, *Ulmus minor*, *Fraxinus oxicarpa*, *Fraxinus excelsior*, *Quercus peduncoliflora*, *Quercus robur*, *Acer campestre*, *Tamarix tetrandra*. Left bank is found in places overgrown with tall natural and semi-natural grassland. Macrophytes have secondary coverage and are from the taxa *Typha* sp., *Potamogeton* sp. and *Phragmites austriacus*, mainly on the left bank. *Typha* sp. and *Potamogeton* sp. are bioindicators for β-mezosaprobic ecological status.

The water of the river in this region of study (Biotope 1, 2) is alkali with acidity from 8.58 pH to 8.87 pH, measured by temperature of the water from 18.5 ^{o}C – 31.4 ^{o}C during the three seasons of the study. Values of the COD and BOD_5 are for second and third category of surface flowing waters, respectively. The waters of the Tunja River in the examined region are distinguished by a low content of ionic groups, compared to Limit Admissible Concentration (LAC) for second category of surface flowing waters (in accordance with the BG State Standards – Regulation 7) with the exception of PO_4, $N\text{-}NO_2$ and $N\text{-}NO_3$. In the two biotopes their content was compared for IIIrd and above IIIrd category of waters (2.3-2.9 mg/l; 0.05-0.06 mg/l, and 21.4-25.3 mg/l, respectively). Conductivity for all samples was compared for the first category of surface following waters (Table 1). There was not any increased content of heavy metals detected in water samples of Tunja River (Pb, Cd, Cu, Zn) for the two biotopes of the period of this research (in accordance with the BG State Standards – Regulation 7 of the Ministry of Environment and Waters of Bulgaria for LAC for second category of surface flowing waters).

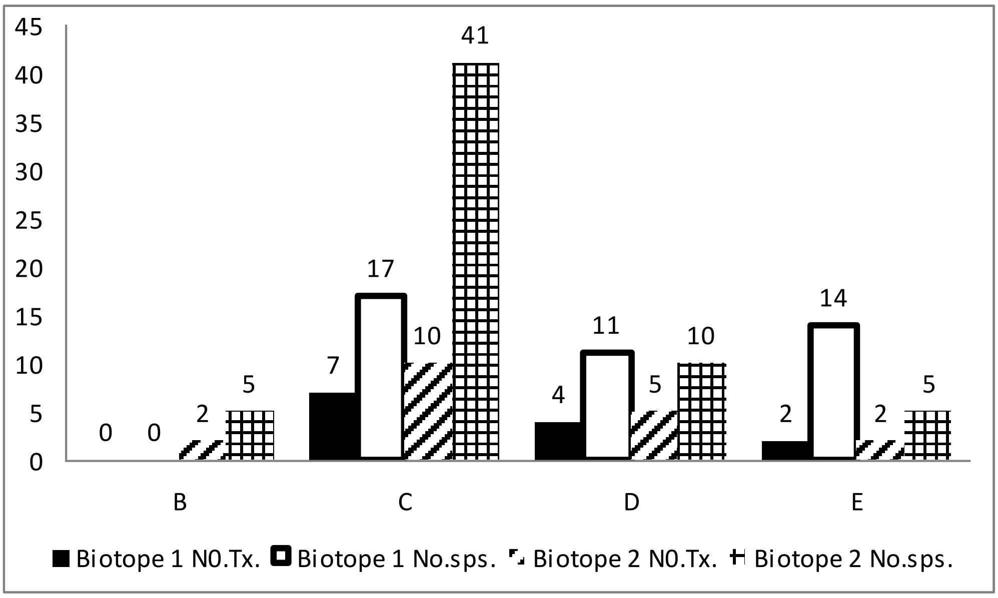
Table 1 - Basic abiotic indices of the studied freshwater ecosystem from the Tunja River

Abiotic indices	Min-Max	Mean±SD	SE mean	C.V.	Category
Temperature (^{o}C)	18.5 - 31.4	24.95±9.122	6.45	36.56	
Acidity (pH)	8.58 - 8.87	8.725±0.205	0.145	2.35	III
Conductivity µS/cm	378 - 384	381±4.243	3.0	1.114	I
COD mg/l	43 - 45	44±1.414	1.0	3.214	II
BOD_5 mg/l	17 - 21	19±2.828	2.0	14.886	III
PO_4 mg/l	2.3-2.9	2.6±0.424	0.3	16.318	>III
$N\text{-}NO_3$ mg/l	21.4-25.3	23.35±2.758	1.95	11.810	>III
$N\text{-}NO_2$ mg/l	0.05-0.06	0.055±0.007	0.003	12.856	III

II. Biodiversity of the macrozoobenthos and bioindication.

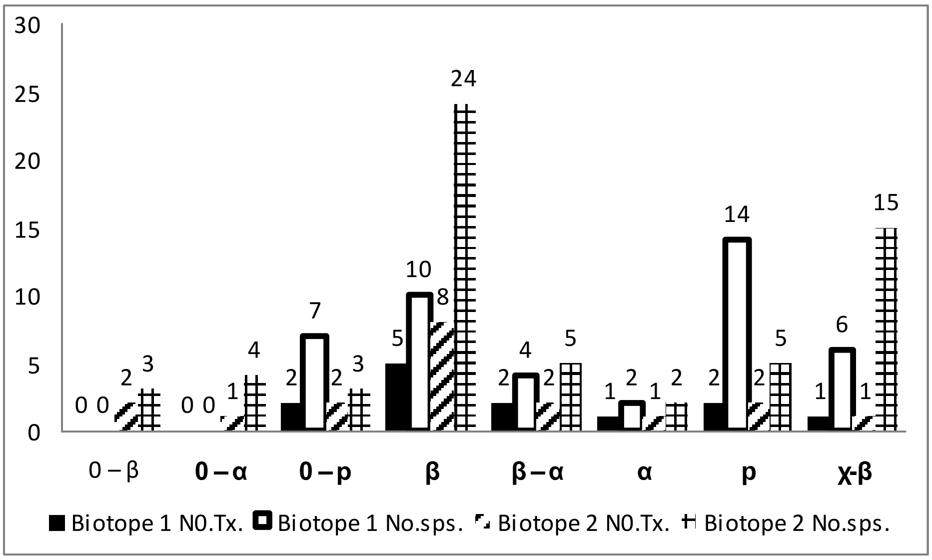
The biomonitoring study of the hydrobiontic macroinvertebrate fauna (macrozoobenthos) in the research region during the three seasons of the research period show the presence of 19 bioindicating taxa and 103 specimens from 13 orders.

With higher number of taxa and specimens is distinguished Biotope2 (13 taxa and 49 specimens; 19 taxa and 79 specimens, respectively). In Biotope 1, *Chironomus plumosus* L. showed the highest number of specimens (9), followed by *Baetis vernus* Curt. and *Gammarus pulex* (L.) (on 6 specimens, respectively), *Nematoda* sp. and *Tubifex tubifex* Müller, 1774 (on 5 specimens, respectively). In Biotope 2 with the highest number of specimens were fixed *G. pulex* (15 specimens), followed by *Ch. plumosus* (4 specimens). The determined bioindicating taxa were from four taxa of sensitivity (B, C, D, E) (Fig. 2). The dominant were these from group C (relative tolerant forms) – on 7 taxa and 17 specimens in Biotope 1; 10 taxa and 41 specimens in Biotope 2, followed by group D (tolerant forms) – on 4 taxa and 11 specimens; 5 taxa and 10 specimens, respectively for the two biotopes. On the third position is group E (more tolerant forms). It was presented with two taxa and on 14 and 5 specimens, respectively in biotopes 1, 2. More weakly were presented groups B (less sensitive forms) – only in Biotope 1 with 2 taxa and 5 specimens. The most sensitive group (A) was not presented. Totally for the two biotopes for sensitive group C was fixed 10 species and 58 specimens, followed by group D – 5 taxa and 21 specimens; group E – 2 taxa and 19 specimens; group B – 2 taxa and 5 specimens. Only *Dytiscus* sp. bioindicating 0-α-mesosaprobity (presented only in Biotope 1) and only *G. pulex* bioindicating χ-β-mesosaprobity (presented in the two biotopes, but with higher number of specimens in Biotope 2) (Fig. 3). Two species of determined macrozoobenthos bioindicating p-saprobity (*T. tubifex* and *Ch. plumosus*). They are with higher number of specimens in Biotope 1. *Asellus aquaticus* (L.) bioindicating α-mesosaprobity (on 2 specimens in the two biotopes). Two taxa showed 0-p saprobity (Nematoda sp. and *Tipula* sp.) and another two showed α-β-mesosaprobity (*Tabanus* sp.; *Culex* sp.). *Hyphydrus* sp. and *Stenophylax* sp. bioindicating o-β-mesosaprobity. With the highest number were taxa bioindicating o-β-mesosaprobity – 7 taxa (*Radix peregra* Drap., *B. vernus*, *Leptophlebia* sp., *Aechna* sp., *Notonecta* sp., *Haemopsis sanguisuga* L., *Chaoborus flavicans* Meigen).



Note: No.taxa – Number of taxa; No.sps. – Number of specimens.

Figure 2 - Number of bioindicative taxa (specimens) and sensitive groups



Note: No.taxa – Number of taxa; No.sps. – Number of specimens.

Figure 3 - Number of bioindicative taxa (specimens) and saprobic groups

Totally, with the highest number of taxa and specimens were presented β-mesosaprobity macro-zoobenthos (8 taxa and 34 specimens), followed by χ-β-mesosaprobity (2 taxa and 21 specimens), by p-saprobity (2 taxa and 19 specimens) and 0-p-saprobity (2 taxa, 10 specimens). All other saprobity groups were presented with less number of specimens than 10.

III. Helminth communities of Squalius orpheus Kottelat & Economidis, 2006 and bioindication.

As a result of the helminthological study of 33 specimens *Squalius orpheus* Kottelat & Economidis, 2006 from the Tunja River, a total of 8 species of helminths was recovered. This are the species: *Ichthyocotylurus pileatus* (Rud., 1802) (metacercaria); *Clinostomum complanatum* (Rud., 1819) (metacercaria); *Caryophyllaeides fennica* (Schneider, 1902); *Caryophyllaeus brachycollis* Janiszewska, 1951; *Bothriocephalus achielognatii* Jamaguti, 1934; *Ligula intestinalis* (L., 1758) (plerocercoid); *Acanthocephalus anguillae* (Müller, 1780); *Rhabdocona denudata* (Dujardin, 1945). All helminth species were reported for the first time in the helminths communities in Sq. *orpheus* from the Tunja River. Fish were catched between two biotopes and before town of Elhovo. Highest mean intensity (MA) showed *I. pileatus* (MA=7.00), followed by mean intensity of *C. brachycollis* (MA=5.00). Dominant structute of the determinated helminth communities were definited according criterias of Bush et al. According these criterias, only *Acanthocephalus anguillae* (P%=51.50) are a core species for the helminth communities of the chub. *C. brachycollis* (P%=15.15) are component species of this communities. All the rest species of helminthes were accidental species for the helminth communities of *Sq. orpheus* from the Tunja River.

IV. Heavy metal (Cu, Pb, Zn and Cd) contaminations in fish and fish parasites.

The contamination of Cu, Pb, Zn and Cd in tissues and organs of *Sq. orpheus* and chub acanthocephalans *Ac. anguillae* were analyzed (Table 2). Bioconcentration factor (BCF= [Chost(parasite)tissues] / [Csediments] and bioaccumulation factor (BAF= [Cparasite] / [host tissues]) were calculated [35]. The bioconcentration factor allowed to establish the accumulation order and to examine fish used as biomonitors of trace of metal pollutants in freshwater ecosystems.

Table 2 - Heavy metal contamination in Sq. orpheus and fish parasites Ac. anguillae

Heavy metals mg/kg	Cu	Pb	Zn	Cd
Ac. anguillae	**7.3**	**152.93**	**71.16**	**<0,01**
BCF	0.52	2.82	1.09	-
Skin	4.8(4.2-5.2)	16.12(13,0-18,2)	40.27	0,4(<0,1-0,4)
BAF	1.52	9.49	1.77	
Bone	2.93(2,25-4,2)	16.03(12,5-20,1)	18.29(12.5-28.3)	0,5(0,2-0,9)
BAF	2.49	9.54	3.9	
Muscle	2.38(2,2-4,2)	4.65(1,4-5,2)	5.84(2.5-14.92)	<0,01
BAF	3.07	32.89	12.18	
Fet	6.22(6,2-6,5)	13.38(0,4-12,8)	42.2(25.39-63.14)	<0,01
BAF	1.17	11.43	1.7	
Sediments	13.95	54.12	65.28	2.05

The contamination of lead in tissues of *Ac. anguillae* is 2.82 more than it's in sediments from the river and 1.09 more for zinc. Bioaccumulation factors are higher in *Ac. anguillae* for Cu, Pb and Zn than in examined fish tissues and organs. In all cases they are the highest in the muscles of the tested fishes against the contaminations in the acanthocephalans (BAF_{Cu}= 3.7; BAF_{Pb}=32.98; BAF_{Zn}=12.18). Parasite bioindication is essential by determination of the ecological status of ecosystems.

Studies of authors in recent years have focused mainly on tracking changes in different groups physicochemical indicators of freshwater ecosystem of the Tunja River [22-24]. Publications describing the results of hydrobiological monitoring based on bioindicating matcrozoobenthos date back about 30 years ago [17,31,37].

In this study, an integrated new approach is applied for an ecological evaluation of the situation of the examined ecosystem. Established in *Sq. orpheus* helminth communities are poor of species diversity, but they are presented with a higher total number of helminths. The results for helminth communities of *Sq. orpheus* showed improvement in saprobity in research area and the presence of species dominating in helmith communities (prevalence and mean intensity), indicating α–mezosaprobity (*A. anguillae*, *P. laevis*) and β–α-mezosaprobity (*I. pileatus*, followed by that of *C . brachycollis*). They are closely linked and dependent on the biology and ecology of specific, identified in the survey helminth species and the role of intermediate host as bioindicators of natural ecosystems studied (Table 3).

Table 3 - Spearman correlation coefficient (r_s) and levels of significance determined for relatioships between the content of heavy metals in the bottom sediments, Sq. orpheus and A. anguillae

x-y	**Heavy metals**	**r_s**	**p**
Sediment-skin	Cu	0.402	<0.05
	Pb	0.775**	<0.01
	Zn	0.745**	<0.01
Sediment-bone	Cu	0.415*	<0.05
	Pb	0.759**	<0.01
	Zn	0.748**	<0.01
Sediment-muscle	Cu	0.057^{ns}	>0.05
	Pb	0.882***	<0.001
	Zn	0.715**	<0.01
Sediment-fet	Cu	0.421*	<0.05
	Pb	0.738**	<0.01
	Zn	0.732	<0.01
Sediment- *A. anguillae*	Cu	0.982***	<0.001
	Pb	0.999***	<0.001
	Zn	0.996***	<0.001

Note: *significant correlation p<0.5;
**highly significant correlation p<0.01;
***very significant correlation p<0.001;
^{ns} non-sygnificant correlation p>0.05.

Determined indices of diversity, evennes and dominance significantly showed better environmental conditions in Biotope 2 (after town of Elhovo) compared with these in the Biotope 1. Especially convincingly this was confirmed by the indices of the helminth communities of the chub caught before town of Elhovo (before Biotope 2).

The waters of the Tunja River in the region under research for the three seasons were distinguished as a whole by a low content of ionic groups and heavy metals for second category of surface flowing waters. According these examinations values of saprobity showed β-mezosaprobity in lower part of the Tunja River (Biotope 1, after town of Elhovo) and α□ □-mezosaprobity in Biotope 1 (after town of Yambol and village of Konevec) as well as before town of Elhovo, according to the data of fish parasite communities. Studied ecosystem is with biotic index 3 and EQR 0.6 in Biotope 2 and is distinguished with biotic index 2 and EQR 0.4 in Biotope 1. Anthropogenic impacts are from farm activity near the region of these biotopes. The river ecosystem as a whole has good condition during the spring period of the study and bad, during the summer and autumn (Table 4).

Table 4 - Basic biotic indicators for ecological appraisal

Basic biotic indicators	**Macrozoobenthos communities**		**Helminth communities**
	Biotope 1	**Biotope 2**	***Sq. Orpheus***
Total N0. of taxa	14	20	8
Total N0. of specimens	49	79	111
EPT	2	4	-
RETI	0.25	0.45	-
Dmg	3.34	4.35	1.49
H’	2.35	2.59	1.95
HB	2.0	2.25	1.40
Saprobity	α	β	α
BI (EQR)	2(0.4)	3(0.6)	-

Note: Tn/tx – Total number of taxa; Tn/sps – Total number of specimens; EPT–Ephemeroptera/Plecopera/Trichotera Index; RETI–Rhithron Feeding Type Index; Dmg - index of Margalef, diversity; H’ – index of Shannon, diversity; HB - index of Brillouin, diversity; BI(EQR)-Biotic index (Ecological Quality Ratios for Ecological Quality Assessment).

The present studies were made with applying the new approach for integrated environmental assessment in two biotopes of the river, giving rise to changes in the definition of ecological status and trophic structure of river ecosystem with free living communities and fish parasite communities.

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REFERENCES

[1] AQEM consortium, 2004. AQEMdip: AQEM data input program.Downloadable from http://www.eu-star.at.

[2] Bíreš, J., J. Dianovský, P. Bartko, Z. Juhásová. Effects of enzymes and the genetiv apparatus of sheep after administration of samples from industrial emissions. *BioMetals* 8: 53-58 (1995).

[3] Bush, A., K. Lafferty, J. Lotz, A. Shostak, 1997. Parasitology meets ecology on its own terms: Margolis et al. revised. - Journal of Parasitology 83: 575-583.

[4] Cheshmedjiev, S., Soufi, R., Vidinova, Y., Tyufekchieva, V., Yaneva, I., Uzunov, Y., E. Varadinova Multi-habitat sampling method for benthic macroinvertebrate communities in different river types in Bulgaria. *Water Research and Management*, Vol. 1, No. 3 55-58 (2011).

[5] Dimitrov, Y. *Proceedings of Jubilee Scientific Conference*, Agricutural University, Plovdiv, 2009, v. LIV.

[6] Dimitrov, Y., N., Palagacheva. Risky periods of pesticide (insecticidae and fungicidae) pollution of vegetable grown in greenhouses. *Agro-knowledge Journal „Agroznanje"*, 13, 1, 153-158 (2012).

[7] EN 27828:1994/ISO 7828:1985. Water Quality – Methods of Biological Sampling. Guidance of Handnet Sampling of Aquatic Benthic Macroinvertebrates. International Organization for Standartization.

[8] EN ISO 9391:1995/ISO 9391:1993. Water Quality – Sampling in deep waters for macroinvertebrates – Guidance on the use of colonization, qualitative and quantitative samplers. International Organization for Standartization.

[9] EN ISO 8689-1:2000. Method for presentation on biological data (EN ISO 8689-2:2000). International Organization for Standartization.

[10] EN ISO 5667-1:2006/AC:2007. Water quality - Sampling - Part 1: Guidance on the design of sampling programmes and sampling techniques (ISO 5667-1:2006). International Organization for Standartization.

[11] EN ISO 5667-3:2003/AC:2007. Water quality - Sampling - Part 3: Guidance on the preservation and handling of water samples. International Organization for Standartization.

[12] Fröse, R., D. Pauly. FishBase. World Wide Web electronic publication, www.fishbase.org, version (07/2012).

[13] Furse, M., D. Hering, K. Brabec, A. Buffagni, L. Sandin, P. Verdonschot, 2006. The Ecologycal Status of European Rivers: Evaluation and Inter-calibration of Assessment Methods. *Hydrobiologia* 566:311-324 (2006).

[14] Haase, P., J. Murraj-Blight, S. Lohse, St. Pauls, A. Sundermann, R. Gunn, R. Clarke. Assessing the impact of errors in sorting and identifying macroinvertebrate samples. *Hydrobiologia* 566:505-521 (2006).

[15] ISO 5667-6:1990. Guidance on sampling of rivers and watercourses. International Organization for Standartization.

[16] Kennedy C. The dynamics of intestinal helminth communities in eels *Anguilla anguilla* in a small stream: long-term changes in richness and structure. *Parasitology* 107, 71-78 (1993).

[17] Kovachev, S., Y. Uzunov. The Mutual Effects of the Tundja River and Zhrebchevo Dam Lake on the Development of the Macrozoobenthic community. *Hydrobiology* 15: 43-48 (1981).

[18] Lenat, D. Water Quality Assessment Using a Qualitative Collection Method for Benthic Macroinvertebrates. *Journal of North American Benthological Society* 7: 222-223 (1988).

[19] Lenat, D. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. *Journal of North American Benthological Society* 12(3): 279-290 (1993).

[20] Magurran, A. *Ecological diversity and its measurement*. Princeton University Press, Princeton, 1988.

[21] McGarrigle, M.L., Lucey, J., Clabby, K.J., 1992. Biological assessment of river water quality in Ireland. - In: Newman, P.J., Piavaux, M.A., Sweeting, R.A. (Eds.), River Water Quality: Ecological Assessment and Control. Commission of the European Communities, Luxembourg, 371–393.

[22] Mihaylova, G. Evaluation of nutrient loads to surface waters in the lower part of the catchment area of the river Tunja. *Ecology and Future,* 8(4), 43-49 (2009).

[23] Mihaylova, G., G. Kostadinova. Macrozoobenthos Communities as Indicator for Ecological Status Assessment of Surface Water Bodies in Tunja River, *Bulgaria. Journal of Balkan Ecology*, 15(2), 173-189 (2012).

[24] Mihaylova, G., G. Kostadinova, G. Petkov. Assessment of physic-chemical status of surface water in lower part of Tunja River, Bulgaria. *Agricultural Science and Technology* 4(3), 277, 284 (2012).

[25] Naydenova, L., G. Gecheva, M. Marinov. Integrated assessment of the ecological status of rivers in the sub-basin of the river Tunja. Anniversary Scientific Conference of Ecology (Proceedings of), Plovdiv, 543-550 (2008).

[26] Pielou, E. *Ecological diversity*. Wiley, New York, 1975.

[27] Regulation 7/1986. Parameters and Norms for Assesment of the Surface Water Quality. Ministry of Environment and Water of Bulgaria.

[28] Regulation 1/2011 for monitoring of the waters. Ministry of Environment and Water of Bulgaria.

[29] Rosenberg, D. M., V. H. Resh. Introduction to freshwater biomonitoring and benthic macroinvertebrates, pp. 1-9, 1997.

[30] Russev, B. *Fundamentals of Saprobiology*, University Publisher "Kliment Ohridski", Sofia, 1993 [In Bulgarian].

[31] Russev, B., M. Nikolova, M. Dimitrova. Hydrobiological and Saprobiological Alterations in the Tundja R. I. 1955-1967. *Hydrobiology* 22: 59-73 (1984).

[32] Stefanov, St., Y., Dimitrov. Effective Chemicals for Cotton Thrips Thrips-Tabaci and Aphis-Gossypii Protection. *Rasteniev'd nauki* 23: 72-75 (1986).

[33] Stoichev, S. The zoobenthos of the Koprinka Reservoir, Central Bulgaria. *Lautrbornia* 40: 39-41 (2001).

[34] Stoichev, S. Contribution to the study of the Nematode fauna (Nemathelminthes: Nematoda) in Tunja River, Central and South-East Bulgaria. *Acta zool. bulg*. 58(1): 73-82 (2006).

[35] Sures, S., R. Siddall. *Pomphorhynchus laevis*: the intestinal acanthocephalan as a lead sink for its host, chub (*Leuciscus cephalus*). *Exp. Parasitol.* 93: 66-72 (1999).

[36] Yaneva, I., S. Cheshmedzhiev. Hydrobiological Monitoring. Surface Running Waters. Irish Biotic Index, in: *National Program for Biomonitoring of Bulgaria*, Peev, D., S. Gerasimov (Eds.), Gea-Libris Publisher, 1999, pp. 138-195.

[37] Yaneva, I., B. Russev. Trends in Changes of the Hydrobiological and Saprobiological State of the Tundja River. II. May – November, 1981. *Hydrobiology* 26: 15-29 (1985).

IZVOD

BIODIVERZITET I ZAGAĐENJA TEŠKIM METALIMA U SLATKOVODNOM EKOSISTEMU U POGRANIČNIM ZONAMA REKE TUNJA

Cilj ispitivanja je da se ispita sadržaj teških metala i zagađenja na biološku raznovrsnost slatkovodnih ekosistema reke Tunja, Bugarska, i da se da ekološka procena njihovog stanja. Reka Tunja sakuplja vode Egejskog regiona. Izvršeno je određivanje osnovnih abiotičkih (temperatura, pH, provodljivost i μs/sm, HPK, BPK5, jonske grupe – PO_4 mg/l, N-NO_3 mg / l, N-NO_2 mg / l, i teški metali - Pb, Zn, Cd i Cu, struktura rečnog korita, dominantno drvo vegetacija, itd) i biotičkih karakteristika (ukupan broj uzoraka, učestalost, intenzitet, itd. Bioindikativna makrozobentoza je karakteristična za procenu ekološkog statusa ispitivanog slatkovodnog sistema. Određivani su mikroorganizmi Helminths of Squalius orpheus, paraziti Acanthocephalus anquillae i teški metali u tkivima i organima riba. Sadanje studije su povezani sa primenom novog pristupa za intregisanu ocenu životne sredine slatkovodnih ekosistema na osnovu životnih zajednica i zajednica parazita riba.

Ključne reči: *bioindikacija, teški metali, makrozoobentosa, parazitske zajednice, reka Tunja*

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