

## BIODIVERSITY AND ECOLOGICAL APPRAISAL OF THE FRESHWATER ECOSYSTEM OF THE RIVER ARDA, BULGARIA

D. KIRIN<sup>1</sup>, V. HANZELOVÁ<sup>2</sup>, S. SHUKEROVA<sup>1</sup>, S. HRISTOV<sup>1</sup>, E. TURČEKOVÁ<sup>2</sup>, M. SPAKULOVA<sup>2</sup>, T. BARCIOVÁ<sup>2</sup>

<sup>1</sup>Agricultural University – Plovdiv, Mendeleev 12, 4000, Plovdiv, Bulgaria

<sup>2</sup>Parasitological Institute, Slovak Academy of Sciences, Hlinkova 3, 04001, Kosice, Slovakia

Corresponding author email: dianakirin@hotmail.com

### Abstract

*Study of biodiversity of freshwater fish and parasite communities from the River Arda, southern Bulgaria characterized by heavy metal pollution was carried out. Investigation of the actual state of the water pollution from an aspect of loading environment with heavy metals and their impact on fish, fish parasites and biodiversity of water organisms in general was accomplished. Assessment of fish parasites as potential bioindicators of water pollution was made. For examinations were used standard methods and techniques. The studies, belonging to 65 specimens of fishes representing 8 species during the three seasons were presented. The dominant structures of helminth communities are characterized. The model of fish species chosen for examination of the heavy metal content in this study were the European perch, *Perca fluviatilis* (L., 1758) and Macedonian vimba, *Vimba melanops* (Heckel, 1837) of the Arda River. The contents of heavy metals (Pb, Zn, Mn and Cu) (mean concentration, bioconcentration factors) in the tissues and organs of fish species and fish parasites (*Eustrongylides excisus* (Jägerskiöld, 1909) and *Caryophyllaeus* sp.) were discussed. The studies carried out could be used in various monitoring systems for screening the pollution on the water environment and the organisms inhabiting the anthropogenous ecosystems.*

**Key words:** biodiversity, freshwater fishes, helminth communities, heavy metal pollution, River Arda.

### INTRODUCTION

The Arda River is related to the Aegean water collecting region. The river forming picturesque defiles and gorges and giving exclusive attractiveness of the south-east parts of the Rhodopa Mountain, Southern Bulgaria. The valley of the river from the sources to the State border with Greece is indicated as a region with middle and high degree of importance in respect to the date about the species richness, the endemic and rare taxa. The variety of habitats – oak forests, Mediterranean bushes, rock massifs, at the upper parts - preserved beech forests, have determined the existence of an extraordinary biological variety. (Assyov, 2012; BirdLife International, 2013). Basic ecological problems in the region are caused from the negative impact and damages of the environmental components from the performed (Government decree No. 140, Official Newspaper 101/2000) mining-extractive, ore-dressing and metallurgy activity (mining of lead and zinc ores and flotation),

leading to a high pollution and acidifying of large surfaces with deposited mine masses (Kirin et al., 2004; Koev, 1998; Koev et al., 1998; Yorova et al., 1992).

Arda River is included in the National monitoring program (Regulation 1/2011).

Endoparasite species are particularly interesting as indicators of the ecological status of the freshwater ecosystems because the completion of their life cycle requires interactions with several host vertebrates and invertebrates, and the effects on each of the hosts differ according to the pollution level of the habitat in question (Baruš et al., 2007; Cone et al., 1993; Gelnar et al., 1997; Kennedy, 1997; MacKenzie et al., 1995; Marcogliese and Cone, 1997; Oros, Hanzelová, 2009; Overstreet, 1997; Sures, Siddall, 1999; Thielen et al., 2004; Tieri et al., 2006, etc.).

Fish parasite communities, heavy metal content and the state of freshwater ecosystem of the Arda River are studied from different authors (Kirin, 2002a,b; Kirin, 2003; Kirin, 2005;

Kirin, Turcekova, Shukerova, Pehlivanov, 2010; Marinova et al., 2007, etc.)

This paper presents the results from an examination of heavy metal content in sediments, fish tissues and organs, fish parasites and dominant structure of fish parasite communities from the Bulgarian border part of the Arda River (town of Majarovo).

## MATERIALS AND METHODS

During June, 2012 sediments, fish and fish parasites were collect and examined from the Arda River (town of Majarovo) (Fig. 1).



Figure 1. Aegean Water Basin and Arda River

Fig. 1. Aegean Water Basin and Arda River  
The town of Majarovo (41°38.25'N, 25°54.12'E) is situated on the riverside, about 30 km far away from the Studen kladenec Reservoir and about 10 km before the Ivaylovgrad Reservoir. It is distinguished with a less depth and slow running water, with rapids at some places. The waterside vegetation is represented mainly by *Salix* sp. and *Alnus glutinosa*, and the water vegetation – by the species *Fontinalis antipyretica* and *Drepanocladus aduncus*. The riverbed is considerably mote slanting with good formed sandy bottom. The region of the town and the riverside are distinguished with significant diversity of highly protected species and territories declared as protected with national and international nature protective status (Assyov, 2012; Kirin et al., 2004).

A total of 5 sediment samples and 65 fresh-water fish specimens belonging to 2 families and 8 species were collected and examined in June, 2012. The fish were caught by nets, by angling and electrofishing under a permit issued by the Ministry of Agriculture and food and Ministry of Environment and waters of Bulgaria. The scientific and common names of fish hosts were used according to the FishBase database (Fröse and Pauly, 2012).

Samples of 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 (Bireš et al., 1995).

The model of fish species chosen for examination of the heavy metal content in this study were the European perch, *Perca fluviatilis* (L., 1758) and Macedonian vimba, *Vimba melanops* (Heckel, 1837) of the Arda River.

Helminthological examinations were carried out following recommendations and procedures described by Bykhovskaya-Pavlovskaya (1985), Dubinina (1987), Georgiev et al. (1986), Gusev (1985), Hotenovskij (1985), Kulakowskaya (1961), Moravec (1994, 2001), Scholz and Hanzelova (1998), Scholz et al. (1998) etc.

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. (1997). Analyses of helminth community structure were carried out during the three seasons and in both levels: infracommunity and component community. The infracommunity data were used to calculate the total number of species, mean number of helminths, etc. (Kennedy, 1993, 1997; Magurran, 1988). Fish were weighed and measured. Samples of muscles, fat and liver were collected from all individuals. In order to determine the relative accumulation capability of the fish tissues in comparison to the sediments, bioconcentration factor (BCF=[Chost tissues]/[Csediments]))

were calculated (Sures et al., 1999). The bio-concentration factors were computed to establish the accumulation order and to examine fish for use as biomonitors of trace metal pollutants in freshwater environments. The differences in concentration factors were particularly discussed in respect to the bioavailability of trace metals from sediments. A linear correlation coefficient,  $r_s$  was used to test associations between the bottom sediments, fish tissues, organs and fish parasites.

## RESULTS AND DISCUSSIONS

### Fish communities

A total of 65 fish specimens from 8 species were collected and examined from the Arda River: *Alburnus alburnus* (Linnaeus, 1758), *Barbus plebejus* Bonaparte 1839, *Carassius gibelio* (Bloch, 1782), *Rutilus rutilus* (Linnaeus, 1758), *Scardinius erythrophthalmus* (Linnaeus, 1758), *Squalius orpheus* Kottelat & Economidis, 2006, *Vimba melanops* (Heckel, 1837) and *Perca fluviatilis* Linnaeus, 1758. From studied 8 species of fishes, 6 species were estimated as least concern species (LC=Least Concern; IUCN Red List Status) and for one species are not enough data (*V. melanops*, DD=Data Deficient; IUCN Red List Status). One fish species is included in Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011) (*V. melanops*, VU=Vulnerable). *A. alburnus*, *B. plebejus*, *R. rutilus* and *Sc. erythrophthalmus* are freshwater brackish, benthopelagic fish species. *V. melanops* is demersal fish species and *P. fluviatilis* is brackish, demersal introduced fish species. *Sq. cephalus* is pelagic fish species. Five species of fish (*A. alburnus*, *B. plebejus*, *R. rutilus*, *Sc. erythrophthalmus* and *Sq. cephalus*) are free of parasites.

### Helminth community structure

A total two taxa of helminths were fixed (*Eustrongylides excisus* (Jägerskiöld, 1909) and *Caryophyllaeus* sp.). They are belonging to classes Nematoda (1) and Acanthocephala (1). *Eustrongylides excisus* (Jägerskiöld, 1909), larvae is developed with participation of the first intermediate host oligochets (blackworm *Lumbricus variegatus* Linnaeus, 1758, sludge worm *Tubifex tubifex* (Müller, 1774), *Limnodrilus* sp.) and the second fish species,

amphibians (Marsh frog, *Pelophylax ridibundus* (Pallas, 1771) (= *Ranaridibunda* Pallas, 1771) and reptiles (Dice snake, *Natrix tessellata* (Laurenti, 1768)). The adult nematodes parasitic in the glandular stomach of cormorants (Great Black Cormorant *Phalacrocorax carbo* (Linnaeus, 1758) and Pygmy Cormorant *Microcarbo pygmeus* (Pallas, 1773) (= *Ph. pygmaeus* Pallas, 1773)) (Moravec, 1994). In Bulgaria, the species is presented of *Sander lucioperca* (Linnaeus, 1758) (= *Lucioperca lucioperca* Linnaeus, 1758) (as paratenic host) and of *Gobius* sp. (as intermediate host) of *Aspius aspius* (Linnaeus, 1758) from the Danube River (Kakacheva, Margaritov, Grupcheva, 1978; Margaritov, 1959); of *P. fluviatilis* from the Zhrebchevo Reservoir (Nedeva, Grupcheva, 1996) and from the Srebarna Lake (Hristov, 2010; Shukerova, Kirin, 2007; Shukerova et al., 2010); of *Silurus glanis* (Linnaeus, 1758); *Lotalota* (Linnaeus, 1758), *Neogobius melanostomus* (Pallas, 1814) (= *Neogobiuscephalarges* Pallas, 1814), *N. kessleri* (Günther, 1861), *P. fluviatilis* from the Danube River (Atanasov, 2012), etc. Caryophyllidean (Platyhelminthes: Eucestoda) parasites represent a widely distributed group of intestinal helminths of Cyprinidae and Siluridae fishes occurring in all zoogeographical regions except the Neotropics. Some caryophyllideans may be pathogenic for their fish hosts (Mackiewicz, 1994; Oros et al., 2010; Scholz and Hanzelová, 1998). *Caryophyllaeus brachycollis* Janiszewska, 1951 is developed with participation of the first intermediate host *Limnodrilus hoffmeisteri* Claparède, 1862 and *T. tubifex* and the second different fish species. *C. laticeps* (Pallas, 1781) is developed with first intermediate host *Limnodrilus claparedeanus* Ratzel, 1868, *T. tubifex* and *T. barbatus* (Grube, 1861). The supposition intermediate host of *Caryophyllaeides fennica* (Schneider, 1902) is the oligochaete *Stylaria lacustris* (Linnaeus, 1767) (Kakacheva-Avramova, 1983). In Bulgaria caryophyllidean tapeworms were presented from different fish species and freshwater ecosystems: as *C. laticeps* (Pallas, 1781) - of *Barbus barbatus* (Linnaeus, 1758) (Margaritov, 1959; 1966) from the Danube River; of *B. cyclolepis* Heckel, 1837 (Margaritov, 1959) from the Iskar River; of *A.*

*alburnus* (Margaritov, 1959) of the Tunja River; of *B. barbatus*, *Vimba carinata* (Pallas, 1814), *Abramis brama* (Linnaeus, 1758) and *Ballerus sapa* (Pallas, 1811) (= *A. sapa* Pallas, 1811) (Kakacheva, Margaritov, Grupcheva, 1978) from the Danube River; of *Leuciscus cephalus* (Linnaeus, 1758) (Cacic et al., 2004) from the Danube River; as *C. fennica* (Schneider, 1902) – of *B. barbatus* (Margaritov, 1959; 1966) from the rivers Iskar and Tunja; of *B. cyclolepis* and *L. cephalus* (Margaritov, 1959) from the Iskar River; of *L. cephalus*, *V. melanops* and *B. cyclolepis* (Margaritov, 1963/64) from the rivers Maritsa and Topolnitsa; of *B. cyclolepis*, *L. cephalus*, *V. melanops* (Kakacheva, 1965) from the rivers Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijska, Sushenitsa and Bedechka; of *B. barbatus* and *S. lucioperca* (Margaritov, 1966) from the Danube River; of *B. petenyi* (Kakacheva, 1969) from the rivers Nishava, Ogosta, Vodomerka, Buchinska, Vrabnishka, Barsiya, Chuprenska, Iskrecka, Botunya, Bebresh; of *L. cephalus* and *R. rutilus* (Margaritov, 1977) from the Shiposhnitsa River and Reservoir Iskar; of *V. carinata*, *A. brama*, *B. sapa*, *Ballerus ballerus* (Linnaeus, 1758) (= *Abramis ballerus*), *Blicca bjoerkna* (Linnaeus, 1758), *A. alburnus*, *B. barbatus*, *S. lucioperca*, *Sc. erythrophthalmus* and *Pelecus cultratus* (Linnaeus, 1758) (Kakacheva, Margaritov, Grupcheva, 1978) from the Danube River; of *L. cephalus* and *R. rutilus* (Kakacheva, Menkova, 1978) from the Palakariya River; of *B. barbatus* (Kakacheva, Menkova, 1981) from the Struma River; of *B. cyclolepis*, *A. alburnus*, *Sq. orpheus* (= *L. cephalus*) (Kirin, 2002b, 2003) from the Arda River; of *L. cephalus* (Cacic et al., 2004) from the Danube River; as *C. brachycollis* Janiszweska, 1953 - of *B. cyclolepis* and *L. cephalus* (Kakacheva, 1965) from the rivers Asenitsa, Sjujutlijska, Chepinska, Bedechka and Topolnitsa; of *L. cephalus*, *V. melanops*, *A. alburnus*, *B. cyclolepis*, *R. rutilus* (Margaritov, 63/64) from the rivers Maritsa, Vacha, Chepinska, Topolnitsa, Bistritsa; of *L. cephalus*, *Barbus petenyi* Heckel, 1852 and *B. barbatus* (Kakacheva, 1969) from the rivers Vrabnishka, Nishava, Mirkowska, Botunya, Ogosta, Malak iskar; of *L. cephalus* (Kakacheva, Menkova, 1978) from the

Palakariya River; of *B. petenyi*, *L. cephalus* (Kakacheva, Menkova, 1978) from the rivers Devinska, Sarneshka and Vacha; of *B. petenyi*, *B. barbatus*, *L. cephalus* (Kakacheva, Menkova, 1981) from the rivers Blagoevgradska Bistritsa, Struma, Zheleznitsa and Gradevska; of *P. fluviatilis* (Nedeva, Grupcheva, 1996) from the Zhebchevo reservoir; of *B. cyclolepis*, *A. alburnus*, *Sq. orpheus* (= *L. cephalus*) (Kirin, 2002b, 2003) from the Arda River; and of *L. cephalus* (Cacic et al., 2004) from the Danube River; as *Caryophyllaeus* sp. – of *L. cephalus* and *A. alburnus* (Kakacheva, 1965) from the rivers Maritsa, Syuyutlijska and Harmanlijska; of *Cyprinus carpio* Linnaeus, 1758 (Margaritov, 1975, 1976) from the Fish Farming–Yambol; of *C. carpio* (Kakacheva, Menkova, 1981) from the Fish Farming–Blagoevgrad; of *V. melanops* (Kakacheva, 1965) from the Harmanlijska River; of *Cobitis bulgarica* (Drensky, 1928) (Margaritov, 1966) from the Danube River; as *Caryophyllaeus* sp. juv. - of *Gobio gobio* (Linnaeus, 1758), *B. cyclolepis*, *V. melanops* (Kakacheva, 1965) from the river Maritsa, Chepinska and Harmanlijska; of *B. petenyi* (Kakacheva, Menkova, 1978) from the Palakariya River; of *C. carpio* (Margaritov, 1992) from the Fish farms–Yambol, Blagoevgrad, etc.

*Caryophyllaeus* sp., parasitic in *V. melanops* is generalist and *E. excisus*, which use fish as intermediate hosts represented the allogenic species for the helminth communities of the examined freshwater fish species of the Arda River ecosystem. *Caryophyllaeus* sp. of *V. melanops* and *E. excisus* of the parasite communities of *P. fluviatilis* of the Arda River were distinguished with high values of prevalence (P=52.17% and P=54.54%, respectively) but with lower value of mean intensity for *E. excisus* (MI=6±5.12, 1-14, SE Mean 1.48, C.V. 85.28; MI=1.6±1.52, 1-2, SE Mean 0.21, C.V. 30.98, respectively). The two helminth species are core species of the helminth communities of the perch and vimba, respectively.

#### **Content of heavy metals in sediments, fishes and parasites**

The result of the chemical analyzes (Pb, Cu and Zn) of 40 samples of muscle, liver, kidneys and bones of *Vimba melanops* and *Perca fluviatilis*

of the Arda River were presented (Table 1 and 2). The content of Pb, Cu and Zn in two parasite species: *Caryophyllaeus sp.* and *Eustrongilides excisus* were determined. The content of heavy metals in sediments from the two freshwater ecosystems was fixed. Based on the results of chemical analyzes, mean concentrations (mg/kg) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF=[C<sub>host/parasite tissues</sub>]/[C<sub>sediments</sub>]) were defined (Table 3 and 4).

Table 1. Content of heavy metals (Cmg/kg±SD) of *P. fluviatilis* and *E. excisus*

<i>Perca fluviatilis</i>	Arda River		
	Cu	Pb	Zn
$C_{E.excisus}$	12.94±0.02	51.65±0.29	321.22±0.11
$C_{Liver}$	11.58±0.33	1.95±0.02	71.48±0.25
$C_{Kidney}$	1.02±0.09	3.06±0.06	259.92±0.50
$C_{Bones}$	2.19±0.12	4.39±0.16	72.04±0.08
$C_{Muscles}$	0.55±0.03	0.18±0.04	25.89±0.16
Sediments mg/kg	25,51±1.02	19,98±0.45	682,56±1.45

Table 2. Content of heavy metals (Cmg/kg±SD) of *V. melanops* and *Caryophyllaeus sp.*

<i>Vimba melanops</i>	Arda River		
	Cu	Pb	Zn
$C_{Caryophyllaeus sp.}$	9.97±0.84	33.35±0.67	279.69±0.35
$C_{Liver}$	12.98±0.49	1.98±0.24	43.32±0.20
$C_{Kidney}$	1.55±0.04	4.51±0.26	148.44±0.22
$C_{Bones}$	2.49±0.24	2.18±0.14	79.35±0.67
$C_{Muscles}$	0.52±0.02	0.95±0.07	23.72±0.60
Sediments mg/kg	25,51±0.75	19,98±0.55	682,56±1.25

The highest mean content of Cu showed the sediment samples of river (25.5 mg/kg), followed by those of the parasite species *E.*

*excisus* (12.938 mg/kg). From fish tissues and organs, with the highest content of Cu are distinguished the liver (11.587 mg/kg).

Table 3. . Bioconcentration factor (BCF=[C<sub>host/parasite tissues</sub>]/[C<sub>sediments</sub>]) of *P. fluviatilis* and *E. Excisus*

<i>Perca fluviatilis</i>	Arda River		
	Cu	Pb	Zn
$C_{E.excisus}/C_{Sediments}$	0.507	2.595	0.471
$C_{Liver}/C_{Sediments}$	0.454	0.097	0.105
$C_{E.excisus}/C_{Liver}$	1.116	26.525	4.493
$C_{Kidney}/C_{Sediments}$	0.04	0.154	0.380
$C_{E.excisus}/C_{Kidney}$	12.684	16.872	1.235
$C_{Bones}/C_{Sediments}$	0.086	0.221	0.105
$C_{E.excisus}/C_{Bones}$	5.892	11.759	4.459
$C_{Muscles}/C_{Sediments}$	0.021	0.0009	0.037
$C_{E.excisus}/C_{Muscles}$	23.652	283.769	12.407
Sediments mg/kg	25,51	19,98	682,56

Table 4. Bioconcentration factor of *V. melanops* and *Caryophyllaeus sp.*

<i>Vimba melanops</i>	Arda River		
	Cu	Pb	Zn
$C_{Caryoph.sp.}/C_{Sediments}$	0.391	1.676	0.409
$C_{Liver}/C_{Sediments}$	0.508	0.099	0.063
$C_{Caryoph.sp.}/C_{Liver}$	0.768	16.819	6.456
$C_{Kidney}/C_{Sediments}$	0.060	0.226	0.217
$C_{Caryoph.sp.}/C_{Kidney}$	6.423	7.393	1.884
$C_{Bones}/C_{Sediments}$	0.097	0.109	0.116
$C_{Caryoph.sp.}/C_{Bones}$	3.996	15.313	3.525
$C_{Muscles}/C_{Sediments}$	0.020	0.047	0.035
$C_{Caryoph.sp.}/C_{Muscles}$	19.147	35.035	11.794
Sediments mg/kg	25,51	19,98	682,56

The highest mean content of Pb are defined in *E. excisus* (51.646 mg/kg), followed by those in the sediments (9.19 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of Pb in bones and kidneys (4.392 and 3.061 mg/kg, respectively). The mean content of Zn showed higher values in the sediments (682.5 mg/kg) than of *E. excisus* (321,221 mg/kg). Of tissues and organs, the highest concentrations were differed of Zn. The highest content of this trace heavy metal was detected for kidneys ( $C_{kidney}=259,918$  mg/kg), followed by those for bones and liver ( $C_{bones}=72,035$ ;  $C_{liver}=71,481$  mg/kg, respectively). The lowest values of Zn are detected in

the muscles of examined perch ( $C_{\text{muscles}}=25,889$  mg/kg) (Table 1).

BCF of *E. excisus*, parasite species of *P. fluviatilis* of the Arda River was the highest for Pb (BCF  $C_{E.excisus}/C_{\text{SedimentsPb}}=2.595$ ), followed by those for Cu (BCF  $C_{E.excisus}/C_{\text{SedimentsCu}}=0.504$ ) and Zn (BCF  $C_{E.excisus}/C_{\text{SedimentsZn}}=0.471$ ) (Table 3). With regard to the examined fish tissues and organs, BCF was the highest for Cu in liver (BCF $_{\text{liver/sedimentsCu}}=0.454$ ), followed by those for Zn in kidneys (BCF $_{\text{kidneys/sedimentsZn}}=0.380$ ) and for Pb in bones (BCF $_{\text{bones/sedimentsPb}}=0.221$ ). BCF was with the lowest values for the trace heavy metals for perch muscles. Accumulation of heavy metals in *E. excisus* to their content in the fish organs and tissues was the highest of Pb from the muscles (BCF $_{E.excisus/musclesPb}=283.769$ ), followed by those of Pb for liver (BCF $_{E.excisus/liverPb}=26.525$ ), of Pb for kidneys and of Pb for bones (BCF $_{E.excisus/kidneysPb}=16.872$ ; BCF $_{E.excisus/bonesPb}=11.759$ ). Generally, the accumulation of the trace heavy metals were the highest of fish parasite species *E. excisus*, compared to their contents in muscles. As a result of this study (Table 2), the content of Cu and Zn was the highest in the sediments of the Arda River ( $C_{\text{Cu}}=25.5$  and  $C_{\text{Zn}}=682.5$  mg/kg, respectively) and the content of Pb was the highest in *Caryophyllaeus* sp. (33.353 mg/kg). With regard to organs and tissues, the content was the highest for copper in the liver ( $C_{\text{liverCu}}=12.977$  mg/kg) and for lead and zinc it was the highest in the kidneys ( $C_{\text{kidneysPb}}=4.511$  and  $C_{\text{kidneysZn}}=148.441$  mg/kg, respectively). The highest values of bioconcentration factor for liver and copper and also for *Caryophyllaeus* sp., lead and zinc (BCF $_{\text{liver/Cu}}=0.508$ ; BCF $_{\text{Caryophyllaeussp./Pb}}=1.676$ ; BCF $_{\text{Caryophyllaeussp./Zn}}=0.409$ ) were established. The highest values of the bioaccumulation for lead were fixed (BCF $_{\text{musclesPb}}=35.035$ ; BCF $_{\text{liverPb}}=16.819$ ; BCF $_{\text{bonesPb}}=15.313$  and BCF $_{\text{kidneysPb}}=7.393$ ).

Generally, the accumulation of the trace heavy metals were the highest of fish parasite species *Caryophyllaeus* sp., compared to their contents in muscles (Table 4). A linear correlation coefficient, ( $r_s$ , Spearman correlation coefficient) were determined to test

associations between the bottom sediments, fish tissues, organs and fish parasites. Very significant correlation ( $p<0.001$ ) were fixed for relationship between Sediments $_{\text{Pb}}$ -*E. excisus* $_{\text{Pb}}$  and between Sediments $_{\text{Pb}}$ -*Caryophyllaeus* sp. $_{\text{Pb}}$  of the Arda River. The obtained values for the content of Pb, Zn and Cu in sediments, freshwater fish organs and tissues and their parasites from the Arda River are higher than those reported by other authors for the same ecosystem, but for another fish species (*Sq. cephalus*, *Sc. erythrophthalmus*) and fish parasites (*P. cuticola*, *Ac. anguillae* and *L. intestinalis*) but they are lower for *P. fluviatilis* (Kirin et al., 2010).

## CONCLUSIONS

As a result of this examination a total of 65 fish specimens from 8 species were collected and examined from the Arda River. *Caryophyllaeus* sp., parasitic in *V. melanops* is generalist and *E. excisus*, parasitic in *P. fluviatilis* is allogenic species.

The received data for heavy metal contents in sediments, fish tissues and organs and fish parasites from the Arda River were presented for the first time for *P. fluviatilis*, *V. melanops* and their parasites *E. excisus* and *Caryophyllaeus* sp., respectively. The highest mean content of Pb are defined in *E. excisus*

and *Caryophyllaeus* sp. (51.646 mg/kg, 33.353 mg/kg, respectively), followed by those in the sediments (19.98 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of lead in bones and kidneys of the perch and for the content of lead in liver of the vimba. Generally, the accumulation of the trace heavy metals were the highest of fish parasite species *E. excisus* and taxa *Caryophyllaeus* sp., compared to their contents in muscles of the two fish species, respectively.

The high values of the bioconcentration factors and of the significant correlations determined *E. excisus* and *Caryophyllaeus* sp. as sensitive bioindicators for lead.

## ACKNOWLEDGEMENTS

The authors would like to thank Ministry of Education, Youth and Science, financing

## REFERENCES

- Assyov B., 2012. Important Plant Areas in Bulgaria. Pensoft, Sofia-Moscow.
- Atanasov G., 2012. Fauna, morphology and biology on the endohelminths of fish from Bulgarian part of the Danube River. PhD these, Sofia.
- Baruš V., Jarkovský J., Prokeš M., 2007. *Philometra ovata* (Nematoda: Philometridae): a potential sentinel species of heavy metal accumulation. *Parasitol. Res.*, 100, 929-933.
- BirdLife International, 2013. Important Bird Areas factsheet: Majarovo. <http://www.birdlife.org>.
- Bíreš J., Dianovský J., Bartko P., Juhásová Z., 1995. Effects of enzymes and the genitv apparatus of sheep after administration of samples from industrial emissions. *BioMetals*, 8, 53-58.
- Bush A., Lafferty K., Lotz J., Shostak A., 1997. Parasitology meets ecology on its own terms. *Journal of Parasitology*, 83, 575-583.
- Bykhovskaya-Pavlovskaya I., 1985. Parasites of fish. Manual on study, Nauka, Leningrad, 121 (in Russian).
- Cakić P., Lenhardt M., Kolarević J., Nedeva I., Radev V., Karaivanova E., Atanassov G., 2004. The first data on chub (*Leuciscus cephalus* L.) parasites in the Serbian part of the Danube River. In: Proceedings of the 35th Conference of IAD, 49-55.
- Cone D., Marcogliese D., Watt W., 1993. Metazoan parasite communities of yellow eels (*Anguilla rostrata*) in acid and limed rivers of Nova Scotia. *Can. J. Zool.*, 71, 177-184.
- Dubinina M., 1987. Class tapeworms – Cestoda Rudolphi, 1808. In: O.N. Bauer (Ed.) Key to the Parasites of Freshwater Fishes of the USSR, Nauka, Leningrad (In Russian).
- Fröse R., Pauly D., 2012. FishBase. World Wide Web electronic publication, [www.fishbase.org](http://www.fishbase.org), version (07/2012).
- Gelnar M., Sebelova S., Dusek L., Kobkova B., Jurajda P., Zahradkova S., 1997. Biodiversity of parasites in freshwater environment in relation to pollution. *Parasitologia*, 39, 189-199.
- Georgiev B., Biserkov V., Genov T., 1986. In toto staining method for cestodes with iron acetocarmine. *Helminthologia*, 23, 279-281.
- Golemanski V. (Ed-in-Chief), 2011. Red Data Book of the Republic of Bulgaria. Joint edited of the Bulg. Acad. of Sci. and Ministry of Environment and Waters, Sofia, Vol. 2. – Animalia.
- Government decree No. 140. Official Newspaper 101/2000.
- Gusev A., Dubinina M., Pugachev O., Rajkova E., Hotenovskij I., Ergens R., 1985. Guide to Freshwater fish parasites Fauna SSSR. Nauka, Leningrad, 428 (In Russian).
- Hristov S., 2010. Circulation of some heavy metals in the freshwater ecosystem of the Srebarna Biosphere Reserve. *J. Ecology&Safety*, 4, 2:204-213.
- ISO 5667-6:1990. Guidance on sampling of rivers and watercourses. International Organization for Standardization.
- IUCN Red List Status (Ref. 90363) 02/2013.
- Kakacheva-Avramova D., 1965. Helminthological study of fishes from some water basins in Trakia. *Fauna of Trakia*, 2, 83-120 (In Bulgarian).
- Kakacheva-Avramova D., 1969. Helminth on fishes from rivers on western Balkan mountain. II. Trematoda, Cestoda, Acanthocephala, Nematoda. *Rep. Centr. Helm. Lab.*, 13, 61-75.
- Kakacheva-Avramova D., 1977. Studies on helminths of fishes in the Bulgarian section of the Danube River. *Helminthologia*, 3, 20-45 (In Bulgarian).
- Kakacheva-Avramova D., 1983. Helminths of freshwater fishes in Bulgaria. *Bul. Acad. Sci.*, Sofia (In Bulgarian).
- Kakacheva D., Menkova I., 1978. Helminthological study of fishes from the region of Rhodopa. *Helminthologia*, 6, 44-53.
- Kakacheva D., Menkova I., 1981. Contribution to helminthological study of fishes from Blagoevgrad region. *Helminthologia*, 11, 26-41.
- Kakacheva D., Margaritov N., Grupcheva G., 1978. Fish parasites of Bulgarian part of the Danube River. *Limnology of Bulgarian part of the Danube River*, *Bulg. Acad. Sci.*, 250-271 (In Bulgarian).
- Kennedy C., 1993. 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.
- Kennedy C., 1997. Freshwater fish parasites and environmental quality, an overview and caution. *Parasitologia*, 39, 249-254.
- Kirin D., 2002a. Biodiversity and ecological peculiarities of the helminth fauna in *Salmo trutta fario* from Arda river, Bulgaria. *Comptes rendus de l' Académie bulgare des Sciences*, 55, 7, 83-88.
- Kirin D., 2002b. Biodiversity and ecology of the helminth communities in *Leuciscus cephalus* from Arda river. *Comptes rendus de l' Académie bulgare des Sciences*, 55, 7, 89-94.
- Kirin D., 2003. Biodiversity and ecological evaluation of the helminth communities of *Barbus cyclolepis* and *Alburnus alburnus* from Arda river, Bulgaria. *Experimental pathology and parasitology*, 6, 11, 44-50.
- Kirin D., 2005. Ecological research of fishes and appraisal of the condition of the freshwater ecosystems from the Arda River, Bulgaria. *Journal of Environmental Protection and Ecology*, 6, 1, 91-96.
- Kirin D., Buchvarov G., Kuzmanov N., Koev K., 2003. Biological diversity and ecological evaluation of the fresh water ecosystems from the Arda River. *Journal of Environmental Protection and Ecology*, 4, 3, 550-556.
- Kirin D., Turčeková L., Shukerova S., Pehlivanov L., 2010. Biomonitoring of heavy metal pollution of the Arda River (south-eastern Rhodopa, Bulgaria). *Symposium IX. Slovak and Czech Parasitological days*, 24-28.05.2010, Liptovsky Jan, Slovenska republika, 40.

- Kirin D., Lecheva I., Stancheva A., 2004. Biodiversity and Evaluation of State of Terrestrial Ecosystems damaged by Ore-dressing. *Journal of Balkan Ecology*, 2, 4, 388-393.
- Koev K., 1998. Research on resistibility of *Sophora japonica* to antropogenic pollution. *Trav. Sci. Univ. Plovdiv, Plantarium*, 34, 6, 119-125.
- Koev K., et al., 1998. Morphological changes of tree species under industrial and transport pollution. *Trav. Sci. Univ. Plovdiv, Plantarium*, 34, 6, 126-136.
- Kulakovskaya O., 1961. Materials on the fauna of Caryophyllaeidae (Cestoda, Pseudophyllidae) of the Soviet Union. *Parazitol. Sb.*, 20, 339-355 (In Russian).
- MacKenzie K., Williams H., Williams B., McVicar A., Siddall R., 1995. Parasites as indicator of water quality and the potential use of helminth transmission in marine pollution studies. *Adv. Parasitol.*, 35, 85-144.
- Mackiewicz J., 1994. Order Caryophyllida van Beneden in Carus, 1863. In: L.F. Khalil, A. Jones and R.A. Bray (Eds.), *Keys to the Cestode Parasites of Vertebrates*. CAB International, Wallingford.
- Magurran A., 1988. *Ecological diversity and its measurement*. Cambridge University Press, London.
- Marcogliese D., Cone D., 1997. Parasite communities as indicators of ecosystem stress. *Parassitologia*, 39, 227-232.
- Margaritov N., 1959. Parasites of some freshwater fishes. Publishing House NIRR, Varna (In Bulgarian).
- Margaritov N., 1964. Notes for helminth fauna of our freshwater fishes. *Bulletin de L'institut de Zoologie et Musée*, 15, 199-202 (In Bulgarian).
- Margaritov N., 1966. Helminths of the digestive tract and the abdominal cavity of fishes of the Bulgarian section of Danube River. *Bulletin de L'institut de Zoologie et Musée*, 20, 157-173 (In Bulgarian).
- Margaritov N., 1975. Parasitological status of carp from state farms. *Fisheries*, 5, 1-3 (In Bulgarian).
- Margaritov N., 1976. Parasites and diseases of carp in state fish farms. *Rep. SSOFF*, 104, 40-44 (In Russian).
- Margaritov N., 1977. Effects of parasites and disease on reproduction of fish in the inland waters Bulgaria. *Fisheries*, 2, 4-6 (In Bulgarian).
- Margaritov N., 1992. Parasitic status of grown in fishponds cages in Bulgaria. *Yearbiik of Sofia University, Biological Faculty*, 81, 1, 60-73.
- Marinova S., Hristov H., Delchev D., Maslov O., 2007. Depositions Of Heavy Metals In The Region Of The Low Stream Of The Arda River. *AIP Conf. Proc.*, 899, 733.
- Moravec F., 1994. *Parasitic nematodes of freshwater Fishes of Europe*. Kluwer Academic Publishers, Dordrecht.
- Moravec F., 2001. Checklist of the metazoan parasites of fishes of the Czech Republic and the Slovak Republic (1873–2000). *Academia*, Prague.
- Nedeva I., Grupcheva G., Gabrashanska M., 1996. Analysis of the parasite fauna of predatory fishes in the contitions of the Zrebchevo Reservoir (1996) *Proceedings of International symposium Ecology, Burgas*, 68-70.
- Nedeva I., Grupcheva G., Gabrashanska M., 1996. Parasite fauna of *Carrasius auratus gibelio* (Blosch) in the Zhebchevo reservoir, Bulgaria. VII European Multicoquium of Parasitology, Italy.
- Oros M., Hanzelová V., 2009. Re-establishment of the fish parasite fauna in the Tisa River system (Slovakia) after a catastrophic pollution event. *Parasitol. Res.*, 104, 1497-1506.
- Oros M., Scholz T., Hanzelová V., Mackiewicz J., 2010. Scolex morphology of monozoic cestodes (Caryophyllidae) from the Palaearctic Region: a useful tool for species identification. *Folia Parasitologica*, 57, 37-46.
- Overstreet R., 1997. Parasitological data as monitors of environmental health. *Parassitologia*, 39, 169-175.
- Regulation 1/2011 for monitoring of the waters. Ministry of Environment and Water of Bulgaria.
- Scholz T., Drábek R., Hanselová V., 1998. Scolex morphology of *Proteocephalus* tapeworms (Cestoda: Proteocephalidae), parasites of freshwater fish in the Palaearctic Region. *Folia Parasitol.*, 45, 27-43.
- Scholz T., Hanselová V., 1998. Tapeworms of the Genus *Proteocephalus* Wienland, 1858 (Cestoda: Proteocephalidae), Parasites of Fish in Europe. *Studie AV ČR, N0.2/98*, Academia, Prague.
- Shukerova S., Kirin D., 2007. Helminth and helminth communities of *Percifluviatilis* of the Biosphere Reserve Srebarna. VI National Conference of zoology, Sofia, 21-22 May, 2007.
- Shukerova S., Kirin D., Hanzelova V., 2010. Endohelminth communities of the perch, *Percifluviatilis* (Perciformes, Percidae) from Srebarna Biosphere Reserve, Bulgaria. *Helminthologia*, 42, 2, 99-104.
- Sures S., Siddall R., 1999. *Pomphorhynchus laevis*: the intestinal acanthocephalan as a lead sink for its host, chub (*Leuciscus cephalus*). *Exp. Parasitol.*, 93, 66-72.
- Tieri E., Mariniello L., Ortis M., Berti M., Battistini M., 2006. Endoparasites of chub (*Leuciscus cephalus*) in two rivers of the Abruzzo region of Italy. *Vetrinaria Italiana*, 42, 3, 271-279.
- Thielen F., Zimmermann S., Baska F., Taraschewski H., Sures B., 2004. The intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) from barbel as a bioindicator for metal pollution in the Danube River near Budapest, Hungary. *Environmental Pollution*, 129, 421-429.
- Yorova K., et al., 1992. Influence of the increased concentrations on lead (Pb) in the soil on saplings from some tree species. *Trav. Sci. of HFTI, Planting*, 35, 109-111.