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RELATIONSHIPS IN THE SYSTEM *BARBUS BARBUS – POMPHORHYNCHUS TERETICOLLIS – UNIO TUMIDUS* IN CONNECTION WITH THE CIRCULATION OF LEAD IN THE FRESHWATER ECOSYSTEM OF THE DANUBE RIVER, BULGARIA

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Abstract

During the summer of 2015, 13 specimens of barbel (*Barbus barbus* (Linnaeus, 1758)) were examined by standard techniques for parasites. All the examined barbels (100%) were infected with the acantocephalan *Pomphorhynchus tereticollis.*The study of the intestinal content and fishfood fixed the presence mainly of *Unionidae* molluscs.The swollen river mussel *(Unio tumidus* Philipsson, 1788*; Mollusca, Unionidae)* is one of the most common *Unionidae* in the studied habitat *(Biotope Vetren, the DanubeRiver)*. The aim of the research wass to study the lead content in samples of sediments, *Unio tumidus*, *Pomphorhynchus tereticollis,* skin, muscles and liver of *B. barbus* from the Danube River.

From the tissues and organs of the studied fish specimens *Barbus barbus*, the lowest concentrations of lead were found in the muscles. The content of lead in the samples of liver is higher than in the skin and muscles. The alteration was in the following order: $Pb_{liver}>Pb_{skin}>Pb_{muscles}$. *P. tereticolliss* howed significantly higher content of lead than its host tissues and organs. The content of lead in *Unio tumidus* was higher than the content of lead in the tissues and organs of *B. barbus*. A highly significant correlation ($p<0.01$) was fixed for the relationships between the Pb content in the acanthocephalan and mollusks ($C_{P.\ tereticollis Pb}$-$C_{U.tumidus Pb}$) and between that in the acanthocephalan and the skin of the fish ($C_{P.\ tereticollis Pb}$-C_{skinPb}).

Keywords: lead, *Unio tumidus*, *Barbus barbus, Pomphorhynchus tereticollis*, River Danube.

INTRODUCTION

River Danube is under permanent negative anthropogenic impacts of industrial accidents and wastewaters. As a result, pollutions of the water ecosystems killed a lot of fish and other freshwater organisms (Literathy and Laszlo, 1995, 1999). Lead is well known toxic contaminant in an aquatic environment with major source anthropogenic activities. In recent years there had been various studies reporting concentrations of lead in different fish species.

These concentrations may vary among different fish species, different tissues and organs and different locations. In aquatic environment bottom sediments occur as a depot for different pollutants including lead and its content typically is higher in benthic organisms.

Heavy metal content in fish and parasites and the state of freshwater ecosystem of the Danube River are studied from different authors (Atanasov, 2012; Gabrashanska et al., 2004; Kirin et al., 2013; Kirin et al., 2014; Nachev, 2010; Ricking and Terytze, 1999; Woitke et al., 2003, etc.). Aim of this research is to study the lead content in samples of sediments, *Unio tumidus*, skin, muscles, and liver of *B. barbus* and its acanthocephalan *Pomphorhynchus tereticollis* from Danube River (village of Vetren).

MATERIALS AND METHODS

During the summer of 2015, sediments, mussels, fish and fish parasites are collected and examined from the Lower Danube River (village of Vetren, Bulgarian part) (Fig. 1). The village of Vetren (44^{0}133'N, 27^{0}033'E) is situated on the riverside, in the northeastern part of the Danube Valley.

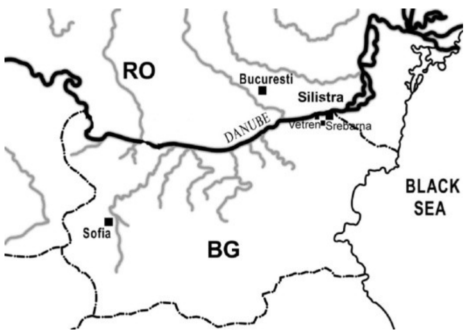

Fig. 1. *River Danube*

A total of 13 specimens of barbel (*Barbus barbus* (Linnaeus, 1758)) is collected and examined from the Danube River during the summer of 2015. *Barbus barbus* is estimated as least concern species (LC=Least Concern; IUCN Red List Status). Barbel is freshwater, benthopelagic, potamodromous fish species. This fish species inhabits from premontane to lowland reaches of clear, warm, medium-sized to large rivers with fast current and gravel bottom and occasionally can be found in lakes.

Barbel frequently overwinters in a large group, inactive or active in slow-flowing river habitats. Adults of this fish species are encountered most active during dusk and dawn while larvae and juveniles are active during both day and night. Larvae and juvenile of barbels stay on the bottom in very shallow shoreline habitats and leave the shores for faster-flowing waters as they grow. This fish species lives in the deeper, faster-flowing upper reaches of rivers with stony or gravel bottom (barbel zones) (Fröse and Pauly, 2016). *Barbus barbus* feeds chiefly on benthic invertebrates, such as small crustaceans, insect larvae, mollusks, mayfly and midge larvae and also on small fish and sometimes algae in the sediments and organism associated with the sediments (Fröse and Pauly, 2016; Lammens and Hoogenboezem, 1991). Barbel's diet is influenced by the available local invertebrate fauna and the age and the body size of fish (Moravec et al., 1997).

According to Aves et al. (2006) lead (Pb) does accumulate internally from the diet when present at levels representative of those in naturally contaminated diets. During the Helminthological examinations of *B. barbus* one of the most frequently identified food in the intestine was the Unionidae.

Unio tumidus (Philipsson, 1788) is one of the most common molluscs in Biotope Vetren, River Danube. *U. tumidus* has been assessed as a Least Concern as this species has the wide distribution throughout Europe (LC=Least Concern; IUCN Red List Status). Swollen river mussel is a freshwater species which can be found inhabiting both rivers and lakes. This mollusk species is listed as Critically Endangered in Austria, Endangered in Germany and as Vulnerable in Switzerland (IUCN Red List Status).

The swollen river mussel is not under special protection measures in Bulgaria.

The barbel (*Barbus barbus* (Linnaeus, 1758)) speciments chosen for examination were weighed (total weigh from 307-568 g) and measured (total length from 30.5 – 40cm). Samples of muscles, skin, and liver are collected from all individuals. Helminthological examinations are carried out following recommendations and procedures described by Bauer et al. (1981), Bykhovskaya-Pavlovskaya (1985), Georgiev et al. (1986), Gusev (1985), Moravec (1994, 2001), etc. The identification of *Pomphorhynchus tereticollis* was based on the resurrection of the species (Špakulová et al., 2011). Main parameters of infection (prevalence %, mean abundance, mean intensity) are determined (Bush et al., 1987).

As a model parasite species to determine the lead content is selected *Pomphorhynchus tereticollis*. This acanthocephalan is a dominant species of helminth communities of barbel during the sampling period (P% = 100; MA = MI = 50.54 ± 40.56). All examined barbels were infected with the acantocephalan *Pomphorhynchus tereticollis. Pomphorhynchus tereticollis* is autogenic species, matured in fish. Intermediate hosts of *P. tereticollis* are *Gammarus* sp (Westram et al., 2011). *Pomphorhynchus tereticollis* was found in *A. brama, Ballerus sapa, B. barbus, Gymnocephalus schraetser* and *Neogobius fluviatilis* from Bulgarian section of river Danube (Kirin et al. 2013, Kirin et al. 2014). *P. tereticollis* was found in *S. cephalus*, *C. carpio carpio* and *A. brama* from Latorica River (Oros and Hanzelová, 2009). *P. tereticollis* was also found in *Sq. cephalus*, *P. fluviatilis* and *B. carpathicus* from Hornad River (Kohut et al. 2015).

Samples of sediments are collected according to the Guidance on sampling of rivers and watercourses – BSS ISO 5667-6:1990. The samples of sediment, mussels, fish tissues, organs, and parasites are analyzed for content of lead (Pb) by ICP Spectrometry (Bíreš et al., 1995; ISO 8288:1986; BDS EN ISO 17294-2:2016).

To determine the relative accumulation capability of the fish tissues, parasites and molusks in comparison to the sediments, bioconcentration factors (BCF = [Chost/parasites tissues/ mollusks]/ [Csediments] are calculated (Sures et al., 1999). The bioconcentration factors are used for estimation of trace metal pollution in freshwater ecosystem by examined fish and their parasites. In order to determine the relative accumulation capability of parasites in comparison to host tissues and mollusks, bioaccumulation factor (BAF= [Cparasite]/ [C host tissues/mollusks]) and to determine the relative accumulation capability along the food chain with one trophic level difference, biomagnification factor (BMF=[C barbel tissues]/[C mollusks]) are calculated. The differences in concentration factors are discussed on the bioavailability of lead from sediments and mussels. A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) is determined to test the association between parasites and their host's tissues and organs and between parasites and mussels.

RESULTS AND DISCUSSION

The result of the content of lead (Pb) in samples of sediments, mollusk swollen river mussel (*U. tumidus* Philipsson, 1788*; Mollusca, Unionidae)*, samples of muscle, liver, and skin of *Barbus barbus* and its parasite *P. tereticollis* from the Danube River are presented.

Based on the results of chemical analyzes, mean concentrations ($mg.kg^{-1}$) in tissues, organs of the mollusks, fish, parasites and sediments, as well as the bioconcentration factor (BCF=[C host/parasite tissues/mollusks]/[C sediments]), bioaccumulation factor (BAF=[C parasite]/[C host tissues/mollusks]) and biomagnification factor (BMF=[C host tissues]/[C mollusks]) were defined.

The contents of lead are the highest in samples of sediments from the examined freshwater ecosystem – Biotope Vetren on the Danube River ($C_{Sediments/Danube}$=43.251 $mg.kg^{-1}$). From the fish tissues and organs, the highest contents of lead are determined in samples of liver (C_{Liver}=4.453±1.423 $mg.kg^{-1}$), followed by those of skin (C_{Skin}=3.123±2.767) and muscles (C_{Muscle}=2.188±0.966) (Table 1).

The mean content of lead in swollen river mussel ($C_{U.\ tumidus}$ =8.064±2.771) was higher than in barbels organs and tissues, but more than 5 times less than the content of lead in sediments.

The acanthocephalan *P. tereticollis* ($C_{P.\ tereticollis}$=91.519±10.061 $mg.kg^{-1}$) showed a significantly higher content of lead than its host *Barbus barbus* and than mollusks and sediments. In general, the amendment of lead content in studied system from the freshwater ecosystem is in order: $Pb_{P.\ tereticollis}$>$Pb_{Sediments}$>$Pb_{U.\ tumidus}$>Pb_{Liver}>Pb_{Skin}>$Pb_{Muscles}$. This purpose remains regarding the values of bioconcentration factor (BCF), set against the levels of lead in sediments of the Danube River (Biotope Vetren).

The highest BCF was for *P. tereticollis/*sediments ($BCF_{P.\ tereticollis/Sediments}$=2.116), followed by *U. tumidus*/sediments ($BCF_{U.\ tumidus/Sediments}$=0.186), liver/sediments ($BCF_{Liver/Sediments}$=0.103), skin/sediments ($BCF_{Skin/Sediments}$=0.072) and muscles/sediments ($BCF_{Muscles/Sediments}$=0.051).

Table 1. Content of lead ($mg.kg^{-1}$) and bioconcentration factor (BCF) determined for the content of lead in *U. tumidus*, tissues, organs and parasites of *B. barbus* and in the bottom sediments

B.barbus/ U. tumidus / P. tereticollis	Mean±SD	Relationships	BCF
Liver	4.453±1.423	$C_{Liver}/C_{Sediments}$	0.103
Muscles	2.188±0.966	$C_{Muscles}/C_{Sediments}$	0.051
Skin	3.123±2.767	$C_{Skin}/C_{Sediments}$	0.072
Unio tumidus	8.064±2.771	$C_{U.\ tumidus}/C_{Sediments}$	0.186
P. tereticollis	91.519±10.061	$C_{P.\ tereticollis}/C_{Sediments}$	2.116
River Danube		Sediments ($mg.kg^{-1}$)	43.251

The obtained values of bioconcentration factors for liver and muscles of barbel in this study are higher than the obtained values in previous researches for the same host and parasite species from the same Biotope (Kirin et al., 2013).

The obtained value of bioconcentration factor for *P. tereticollis* in this study are lower than the obtained values from Kirin et al. (2013).

Regarding *P. tereticollis* the highest bioacumulation factors (BAF) was for muscle (BAF_{Muscle} = 41.827), followed by those for skin (BAF_{Skin} = 29.304), liver (BAF_{Liver} = 20.547) and *U.tumidus* ($BAF_{U.\ tumidus}$ = 11.349) (Table 2).

Table 2. Bioacumulation factors (BAF = [[Cparasite]/[C host tissues/mollusks]) of *P. tereticollis*

P. tereticollis / U. tumidus / B. barbus	BAF
$C_{P.\ tereticollis}/C_{U.\ tumidus}$	11.349
$C_{P.\ tereticollis}/C_{Liver}$	20.547
$C_{P.\ tereticollis}/C_{Muscle}$	41.827
$C_{P.\ tereticollis}/C_{Skin}$	29.304

The values of Biomagnification factor were the highest for liver (BMF_{Liver} = 0.552), followed by those for skin (BMF_{Skin} = 0.387) and muscle (BMF_{Muscle} = 0.271) (Table 3).

Table 3. Biomagnification factor BMF = [C host tissues]/[C mollusks]) of Pb in of *B. barbus* towards Pb in *U. tumidus*

River Danube	Tissues and organs of *B. barbus*	BMF Pb
U. tumidus	Liver	0.552
	Muscle	0.271
	Skin	0.387

A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) is determined to test the association between *P. tereticollis* and fish tissues, organs and *U. tumidus*. Highly significant correlations ($p<0.01$) were fixed for relationships between *P. tereticollis* $_{Pb}$ - *U. tumidus* $_{Pb}$ and *P. tereticollis* $_{Pb}$ - skin $_{Pb}$. Very significant correlations were established for *P. tereticollis* in previous researches of this acanthocephalan (Kirin et al., 2013).

The obtained values in this study for lead content in muscles (2.188±0.966 mg.kg^{-1}) and liver (4.453±1.423 mg.kg^{-1}) of barbel are a lot higher than the obtained values for lead content in muscles (0.11±0.11 µg/g) and liver (0.12±0.12 µg/g) of barbel from Serbian part of River Danube (Morina, 2016). According to Bulgarian food codex, the maximum lead level permitted for fish muscles is 0.3 mg.kg^{-1}. Lead content in analyzed fish organs and tissues of *B. barbus* are found to be much higher than limits.

CONCLUSIONS

1. New data for heavy metal contents in sediments, mussels, fish parasites, fish tissues and organs from the Danube River are presented. Lead content in analyzed fish organs and tissues of *B. barbus* are found to be much higher than the maximum lead levels in Bulgarian food codex. From the tissues and organs of the studied fish specimens *B. barbus*, the lowest concentrations of lead are found in the muscles.

2. The content of lead in the samples of the liver are higher than in the muscles and skin and ranged as followed $Pb_{Liver}>Pb_{Skin}>Pb_{Muscles}$. In general, the amendment of lead content in a studied system from the freshwater ecosystem is in order: $Pb_{P.\ tereticollis}>Pb_{Sediments}>Pb_{U.\ tumidus}>Pb_{Liver}>Pb_{Skin}>Pb_{Muscles}$. The highest bioconcentration was for *P. tereticollis/*sediments, followed by *U. tumidus*/sediments, liver/sediments, skin/sediments and muscles/sediments.

3. Regarding *P. tereticollis* the highest bioacumulation factor was for muscle, followed by those for skin, liver and *U. tumidus*. The values of Biomagnification factor were the highest for liver and ranged as follows $BMF_{Liver}>BMF_{Skin}>BMF_{Muscle}$. Highly significant correlations ($p<0.01$) were fixed for relationships between *P. tereticollis*$_{Pb}$ - *U. tumidus*$_{Pb}$ and *P. tereticollis*$_{Pb}$ - skin$_{Pb}$. These highly significant correlations determine *P. tereticollis* as a sensitive indicator for a lead.

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