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ACCUMULATION OF LEAD IN *BARBUS BARBUS*, *ALBURNUS ALBURNUS* AND IN THEIR COMMON PARASITE *POMPHORHYNCHUS TERETICOLLIS* FROM RIVER DANUBE (VETREN AREA), BULGARIA

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Abstract

During 2016, 45 specimens of barbel (Barbus barbus (Linnaeus, 1758)) and 45 specimens of bleak (Alburnus alburnus (Linnaeus, 1758)) are collected and examined from the Danube River. The aim of the study is to analyse the lead content in sediments, tissues and organs of two fishes that inhabit different water levels and in their common parasite - P. tereticollis from the Bulgarian section of River Danube. New data for the lead contents in sediments, parasites, tissues and organs of barbel and bleak from the Danube River are presented. From the tissues and organs of the studied fish specimen B. barbus, the lowest concentrations of lead were found in skin, while in A. alburnus the lowest concentrations of lead were found in muscles. The acanthocephalan Pomphorhynchus tereticollis showed significantly higher content of lead than its hosts tissues and organs. Bioconcentration factor for lead (Pb) in the tissues and organs of barbel and bleak and their common parasite Pomphorhynchus tereticollis were presented and discussed with respect to their content in sediments. Highly significant correlation ($p<0.01$) was fixed for relationship between P. tereticollis $_{Pb}$-Skin $_{Pb}$ for Barbus barbus. Highly significant correlation ($p<0.01$) was also fixed for relationship between P. tereticollis $_{Pb}$-Sediments $_{Pb}$ for Alburnus alburnus.

Key words*: Alburnus alburnus, Barbus barbus, Danube River, lead, Pomphorhynchus tereticollis.*

INTRODUCTION

The content of heavy metals in different tissues and organs of fishes and in their parasites and the state of freshwater ecosystem of the Danube River has been studied from different authors (Atanasov, 2012; Gabrashanska et al., 2004; Subotić et al., 2015; Sures et al., 1994; Sures and Siddall, 1999; Schludermann et al., 2003; Thielen et al., 2004; Kirin et al., 2013; Kirin et al., 2014; Nachev and Sures, 2009; Nachev et al., 2013; Nachev, 2010; Nedeva et al., 2003; Ricking and Terytze, 1999; Woitke et al., 2003, etc.).

This paper presents the results of examinations of heavy metal contents in sediments, fish tissues and organs of two fishes that inhabit different water levels and their common parasite- *P. tereticollis* from the Bulgarian part of Danube River.

MATERIALS AND METHODS

During 2016, sediments, fish and fish parasites are collected and examined from the Lower Danube River (village of Vetren, Bulgarian part). The village of Vetren (44^0133'N, 27^0033'E) is situated on the riverside, in the northeastern part of the Danube Valley.

A total of 3 samples of sediment, 45 samples of barbel *(Barbus barbus* Linnaeus, 1758) and 45 samples of bleak *(Alburnus alburnus* Linnaeus, 1758) from the Danube River are collected and examined in 2016. The scientific and common names of fish hosts are used according to the Fish Base database (Fröse and Pauly, 2016). The barbel *(Barbus barbus* Linnaeus, 1758) and bleak *(Alburnus alburnus* Linnaeus, 1758) species chosen for examination of the heavy metal content in this study were weighed (total weigh: from 285-788 g for barbel; from 10-22 g for bleak) and measured (total length: from 29-45 cm for barbel; from 10 - 14 cm for bleak).

Helminthological examinations are carried out following recommendations and procedures described by Petrochenko (1956), Bauer et al. (1981), Bykhovskaya-Pavlovskaya (1985), Gusev (1985), etc. Identification of *P. tereticollis* was based on resurrection of the species (Špakulová et al., 2011).

Samples of sediments were collected during the spring, summer and autumn season, according

to the Guidance on sampling of rivers and watercourses – BSS ISO 5667-6:1990. Samples of muscles, skin and liver are collected from all samples of fish. The content of lead (Pb) in samples of sediment, fish tissues, organs and parasites was established by ICP Spectrometry (ISO 8288:1986; BDS EN ISO 17294-2:2016; Bíreš et al., 1995). In order to determine the relative accumulation capability of the fish tissues and parasites in comparison to the sediments, bioconcentration factor (BCF= [Chost/parasite tissues]/[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. The differences in concentration factors are discussed in respect to the bioavailability of lead from sediments. A Spearman's rank correlation coefficient, r_s and levels of significance were determined to test the relationships between bottom sediments, fish tissues, organs and parasites.

RESULTS AND DISCUSSIONS

A total of 45 specimens of barbel (*Barbus barbus* Linnaeus, 1758) and 45 specimens of bleak (*Alburnus alburnus* Linnaeus, 1758) are collected and examined from the Danube River. *Barbus barbus* and *Alburnus alburnus* are estimated as least concern species (LC=Least Concern; IUCN Red List Status).

Barbel is freshwater, benthopelagic, potamodromous fish species. Inhabits from premontane to lowland reaches of clear, warm, medium sized to large rivers with fast current and gravel bottom. Occasionally found in lakes. Frequently overwinters in large group, inactive or active in slow-flowing river habitats. Adults often form shoal, hiding under overhanging trees or bridges during the day. Adults are encountered most active during dusk and dawn while larvae and juveniles are active during both day and night. Larvae and juvenile stay on the bottom in very shallow shoreline habitats and leave the shores for faster-flowing waters as they grow. Lives in the deeper, faster-flowing upper reaches of rivers with stony or gravel bottom (barbel zones). Feeds chiefly on benthic invertebrates, such as small crustaceans, insect larvae, mollusks, mayfly and midge larvae and also on small fish and sometimes algae (Fröse and Pauly, 2016).

Bleak is freshwater, brackish, benthopelagic, potamodromous fish species. It inhabits open waters of lakes and medium to large rivers. Bleak forms large aggregations in backwaters and other still waters during winter. Adults occur in shoals near the surface. Larvae live in littoral zone of rivers and lakes while juveniles leave shores and occupy a pelagic habitat, feeding on plankton, drifting insects or invertebrates fallen on the water surface. This fish species feeds mainly on plankton, including crustaceans and insects. Bleak spawns in shallow riffles or along stony shores of lakes, occasionally above submerged vegetation (Fröse and Pauly, 2016).

The result of the content of lead (Pb) in samples of sediments and samples of muscle, liver and skin of *Barbus barbus* and *Alburnus alburnus* and their common 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 fish, parasites and sediments, as well as the bioconcentration factor (BCF= [Chost/parasitetissues]/ [Csediments]) are defined.

From the fish tissues and organs of barbel the highest contents of lead was determined in samples from liver (2.592±2.30 $mg.kg^{-1}$), followed by those from muscles (1.727±1.317 $mg.kg^{-1}$) and skin (1.53±0.718 $mg.kg^{-1}$)(Table 1).

Table 1. Lead concentration ($mg.kg^{-1}$) in sediments, different organs of *Barbus barbus* and its parasites *P. tereticollis*

Barbus barbus	Mean±SD	Range
Sediments	45.256±15.958	33.940-67.825
Liver	2.592±2.30	0.52-6.715
Muscles	1.727±1.317	0.461-4.756
Skin	1.53±0.718	0.781-3.271
P. tereticollis	135.713±26.28	94.231-180.833

From the fish tissues and organs of bleak the highest contents of lead was determined in samples from liver (4.30±3.627 $mg.kg^{-1}$), followed by those from skin (1.642±0.8 $mg.kg^{-1}$) and muscles (1.449±0.410 $mg.kg^{-1}$) (Table

2). In general the content of lead in liver is higher in bleak than barbel.

Table 2. Lead concentration ($mg.kg^{-1}$) in sediments, different organs of *Alburnus alburnus* and its parasites *P. tereticollis*

Alburnus alburnus	Mean±SD	Range
Sediments	45.256±15.958	33.940-67.825
Liver	4.30±3.627	0.631-9.154
Muscles	1.449±0.410	0.46-1.815
Skin	1.642±0.8	0.943-3.779
P. tereticollis	172.770±18.37	154.40-191.139

The acanthocephalan *P. tereticollis* showed significantly higher content of lead (135.713 $mg.kg^{-1}$ for *Barbus barbus*; 172.770 $mg.kg^{-1}$ for *Alburnus alburnus*), than its hosts tissues and organs. This purpose remains regarding the values of BCF, set against the levels of lead in sediments of the Danube River (Biotope Vetren). Regarding *Barbus barbus* the highest BCF $_{P.\ tereticollis}$ was for skin (88.70) followed by those for muscle (78.583), liver (52.358) and sediments (2.999) (Table 3).

Table 3. Bioconcentration factor (BCF=[Chost/parasite tissues]/[C Sediments]) of *B. barbus* and *P. tereticollis*

Sediments/ *B.barbus*/ *P. tereticollis*	BCF
$C_{P.\ tereticollis}/C_{Sediments}$	2.999
$C_{Liver}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{Liver}$	0.057 52.358
$C_{muscle}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{muscle}$	0.038 78.583
$C_{Skin}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{Skin}$	0.033 88.70

Regarding *Alburnus alburnus* the highest BCF $_{P.\ tereticollis}$ was for muscle (119.23) followed by those for skin (105.22), liver (40.18) and sediments (3.818) (Table 4).

A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) is determined to test the association between the sediments, fish tissues, organs and sediments. Highly significant correlation ($p<0.01$) was fixed for relationship between *P. tereticollis* $_{Pb}$-Skin $_{Pb}$ for *Barbus barbus*. Highly significant correlation ($p<0.01$) was fixed also for relationship between *P. tereticollis* $_{Pb}$-Sediments $_{Pb}$ for *Alburnus alburnus*.

Table 4. Bioconcentration factor (BCF=[Chost/parasite tissues]/[C Sediments]) of *A. brama* and *P. tereticollis*

Sediments / *A. brama* / *P. tereticollis*	BCF
$C_{P.\ tereticollis}/C_{Sediments}$	3.818
$C_{Liver}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{Liver}$	0.095 40.18
$C_{muscle}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{muscle}$	0.032 119.23
$C_{Skin}/C_{Sediments}$ $C_{P.\ tereticollis}/C_{Skin}$	0.036 105.22

The maximum lead level permitted for fish is 0.2 $mg.kg^{-1}$ according the EU and Bulgarian food codex (Anonymus, 2004); 2.0 $mg.kg^{-1}$ for WHO and 0.5 $mg.kg^{-1}$ for FAO. Lead content in analyzed organs and tissues of *B. barbus and A. alburnus* are found to be higher than limits. These results showed human health risk with respect to the concentrations of lead in analyzed samples of freshwater bream from the Bulgarian part of the Danube River.

CONCLUSIONS

As a result of this study is presented new data for lead content in liver, muscle and skin of *B. barbus* and *A. alburnus* and their common parasite - *P. tereticollis* from Danube River (Biotope Vetren). The acanthocephalan *P. tereticollis* showed significantly higher content of lead than its hosts tissues and organs. In general the highest content of lead was found in liver and it was higher than content of lead in muscle and skin for both studied fish species. From the tissues and organs of the studied fish specimen *B. barbus*, the lowest concentrations of lead were found in skin, while in *A. alburnus* the lowest concentrations of lead were found in muscles. These results showed human health risk with respect to the concentrations of lead in analyzed samples of barbel and bleak from the Bulgarian part of the Danube River.

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