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ECOLOGICAL STUDIES ON THE CIRCULATION OF NICKEL IN *ABRAMIS BRAMA* AND *POMPHORHYNCHUS TERETICOLLIS* FROM THE FRESHWATER ECOSYSTEM OF THE DANUBE RIVER, BULGARIA

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Abstract

During the ecological study of 45 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) by applying standard techniques for parasites, an infestation with the acantocephalan *Pomphorhynchus tereticollis* was found. The aim of the present study is to establish the content of nickelin the water, sediments, parasites, tissues and organs of *A. brama* and its parasite *Pomphorhynchus tereticollis*, and the possibilities of accumulation and circulation in the freshwater ecosystem of the Danube River. From the tissues and organs of the studied specimens *Abramis brama*, the content of nickel in the skin samples was higher than in the liver and muscles and ranged as follows: $Ni_{skin}>Ni_{liver}>Ni_{muscles}$ (in both infected and uninfected specimens).

The obtained values for nickel content in the liver and skin of the uninfected *A. brama* were found to be higher than the obtained values for nickel content in the liver and skin of *A. brama* infected with *P. tereticollis*. In contrast to that, the nickel content in the muscles of the infected *A. Brama* was higher than the nickel content in the muscles of the uninfected specimens of freshwater bream. The acanthocephalan *Pomphorhynchus tereticollis* showed significantly higher content of nickel than its host tissues and organs. Significant correlations ($p<0.05$) were fixed for relationships between $C_{P.\ tereticollis Ni} – C_{liverNi}$, $C_{P.\ tereticollis Ni}$- $C_{muscleNi}$ и $C_{P.\ tereticollis Ni} – C_{skinNi}$.

Keywords: nickel, *Abramis brama*, *Pomphorhynchus tereticollis*, River Danube.

INTRODUCTION

Heavy metal pollution of the aquatic environment has been a subject of consideration during the recent years. Typically, the content of heavy metals is substantially lower in water than in the sediments, which occur as a depot for them.

Pollution in aquatic ecosystems is usually observed by measuring the content of heavy metals in water, sediments, and living organisms. It is already established that some parasites accumulate significantly higher amounts of heavy metals than the tissues and organs of their hosts (Sures and Taraschewski, 1995; Sures et al., 1997; Sures and Siddal, 1999; Thielen et al., 2004; Turčeková and Hanzelová, 1996; Turčeková et al., 2002).

River Danube is under permanent negative anthropogenic impacts of industrial accidents and wastewaters.

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.).

Nickel is an essential element that is required for various biochemical and physiological functions in the living organisms (WHO, 1996). That essential element is found naturally in waters, but it may be increased by pollution.

This study aims to present the results of examinations of nickel contents in water, sediments, *Pomphorhynchus tereticollis*, skin, muscles, and liver of infected and uninfected with *P. tereticollis* freshwater breams from the Bulgarian part of the Lower Danube River (village of Vetren).

MATERIALS AND METHODS

During 2016, water, sediments, fish and fish parasites are collected and examined from the Lower Danube River (village of Vetren, Bulgarian part). The village of Vetren (440133'N, 270033'E) is situated on the riverside, in the northeastern part of the Danube Valley.

A total of 45 specimens of freshwater bream (*Abramis brama* (Linnaeus, 1758)) is collected and examined from the Danube River during 2016. *Abramis brama* is estimated as least concern species (LC = Least Concern; IUCN Red List

Status). Freshwater bream is brackish, benthopelagic, potamodromous fish species. Adults inhabit a wide variety of lakes and large to medium sized rivers.

Fish species is the most abundant in backwaters, lower parts of slow-flowing rivers, brackish estuaries and warm and shallow lakes. Adults usually occur in still and slow-running waters where they travel in large shoals. Larvae and juveniles live in still water bodies, feeding on plankton. Adults feed on insects, particularly chironomids, small crustaceans, mollusks, and plants. Larger specimens may feed on small fish (Fröse and Pauly, 2016).

The freshwater bream (*Abramis brama* (Linnaeus, 1758)) specimens chosen for examination of the heavy metal content in this study were weighed (total weigh from 24-291 g) and measured (total length from 13 – 26.5 cm).

Samples of muscles, skin, and liver are collected from all specimens of freshwater bream.

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 used and determined by criteria proposed by Bush et al. (1987).

Five specimens from the examined freshwater breams were infected with the acantocephalan *Pomphorhynchus tereticollis* (P% = 11.11, MI=2.6±1.85 (range 1-6); MA = 0.289±1.025). *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 water and sediments are collected according to the Guidance on sampling of rivers and watercourses – BSS ISO 5667-6:1990.

The samples of water, sediment, fish tissues, organs, and parasites are analyzed for content of nickel (Ni) 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 and parasites in comparison to water and sediments, bioconcentration factors (BCF = [Chost/parasites tissues]/[Cwater/sediments] are calculated (Sures et al., 1999).

The bioconcentration factors are used for estimation of trace metal pollution in a freshwater ecosystem by examined fish and their parasites.

The differences in concentration factors are discussed on the bioavailability of nickel from water and sediments. To determine the relative accumulation capability of parasites in comparison to host tissues, bioaccumulation factors (BAF = [Cparasite]/[C host tissues]) are calculated. A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) is determined to test the association between parasites and their hosts tissues and organs.

RESULTS AND DISCUSSION

The results of the content of nickel (Ni) in samples of water and sediments and samples of muscle, liver, and skin of *Abramis brama* 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 fish (infected and uninfected), parasites, water, and sediments, as well as the bioconcentration factor (BCF = [Chost/parasite tissues]/[Cwater/sediments]) are defined.

The content of nickel in samples of sediments ($C_{Sed/Danube}$ = 3.193 mg.kg^{-1}) is much higher than the content of nickel in samples of water ($C_{Water/Danube}$ = 0.017 mg.l^{-1}) from the examined freshwater ecosystem – Biotope Vetren on the Danube River.

From the tissues and organs of uninfected specimens fish the highest contents of nickel are determined in samples of skin (C = 1.446±0.329 mg.kg^{-1}), followed by these of liver (C = 1.061±0.509) and muscles (C = 0.458±0.092).

This purpose remains regarding the values of BCF, set against the levels of nickel in water and sediments of the Danube River (Biotope Vetren).

The highest bioconcentration factor (BCF) was for skin ($BCF_{Skin/Water}$ = 85.058; $BCF_{Skin/Sediments}$ = 0.453), followed by these for liver ($BCF_{Liver/Water}$ = 62.411; $BCF_{Liver/Sediments}$ = 0.332) and muscles ($BCF_{Muscles/Water}$ = 26.491; $BCF_{Muscles/Sediments}$ = 0.143) (Table 1).

Table 1. Content of nickel ($mg.kg^{-1}$) and bioconcentration factor (BCF) determined for the content of nickel in tissues and organs of uninfected *A. brama* and in water and sediment

A. brama	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	1.061±0.509	C_{Liver}/C_{water}	62.411	$C_{Liver}/C_{Sediments}$	0.332
Muscles	0.458±0.092	$C_{Muscles}/C_{water}$	26.491	$C_{Muscles}/C_{Sediments}$	0.143
Skin	1.446±0.329	C_{Skin}/C_{water}	85.058	$C_{Skin}/C_{Sediments}$	0.453
River Danube		Water ($mg.l^{-1}$)	0.017	Sediments ($mg.kg^{-1}$)	3.193

From the tissues and organs of infected with *P. tereticollis* freshwater breams the highest contents of nickel are determined in samples of skin (C_{Skin}=0.935±0.012 $mg.kg^{-1}$), followed by these of liver (C_{Liver}=0.856±0.141 $mg.kg^{-1}$) and muscles ($C_{Muscles}$=0.70±0.224 $mg.kg^{-1}$). The acanthocephalan *P. tereticollis* (C=60.00±6.00 $mg.kg^{-1}$) showed significantly higher content of nickel than its host *A. brama*. This purpose remains regarding the values of BCF, set against the levels of nickel in water and sediments of the Danube River (Biotope Vetren). The highest bioconcentration factor (BCF) was for *P. tereticollis* ($BCF_{P.tereticollis/Water}$= 3529.41; $BCF_{P.tereticollis/Sediments}$ = 18.791), followed by those for skin ($BCF_{Skin/Water}$ = 55.00; $BCF_{Skin/Sediments}$ = 0.293), for liver ($BCF_{Liver/Water}$ = 50.353; $BCF_{Liver/Sediments}$ = 0.268) and muscles ($BCF_{Muscles/Water}$ = 41.176; $BCF_{Muscles/Sediments}$ = 0.219) (Table 2).

Table 2. Content of nickel ($mg.kg^{-1}$) and bioconcentration factor (BCF) determined for the content of nickel in tissues, organs, and parasites of *A. brama* and in water and sediment

A. brama/ P. tereticollis	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	0.856±0.141	C_{Liver}/C_{water}	50.353	$C_{Liver}/C_{Sediments}$	0.268
Muscles	0.70±0.224	$C_{Muscles}/C_{water}$	41.176	$C_{Muscles}/C_{Sediments}$	0.219
Skin	0.935±0.012	C_{Skin}/C_{water}	55.00	$C_{Skin}/C_{Sediments}$	0.293
P. tereticollis	60.00±6.00	$C_{P. tereticollis}/C_{water}$	3529.41	$C_{P. tereticollis}/C_{Sediments}$	18.791
River Danube		Water ($mg.kg^{-1}$)	0.017	Sediments ($mg.kg^{-1}$)	3.193

The obtained values for nickel content in liver (C=1.061±0.509) and skin (C=1.446±0.329) of uninfected A. brama are found to be higher than the obtained values for nickel content in liver ($C_{Liver\ Ni}$=0.856±0.141) and skin ($C_{Skin\ Ni}$=0.935±0.012) of *A. brama* infected with *P.tereticollis*. In contrast of that, the nickel content in muscles ($C_{Muscles\ Ni}$=0.70±0.224) of infected A. brama was higher than the nickel content in muscles ($C_{Muscles\ Ni}$=0.458±0.092) of uninfected specimens freshwater bream. In general, the content of nickel in both infected and uninfected specimens A. brama in samples of skin are higher than in the liver and muscles and ranged as followed $Ni_{Skin}>Ni_{Liver}>Ni_{Muscles}$.

Regarding *P. tereticollis* the highest bioacumulation factors (BAF) was for muscles ($BAF_{P.tereticollis/Muscles}$ = 85.714), followed by those for liver ($BAF_{P.tereticollis/Liver}$ = 70.093) and skin ($BAF_{P.tereticollis/Skin}$ = 64.171) (Table 3).

A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) is determined to test the association between *P. tereticollis* and fish tissues and organs. Significant correlations ($p<0.05$) were fixed for relationships between *P. tereticollis*$_{Ni}$- liver$_{Ni}$, *P. tereticollis*$_{Ni}$- muscles$_{Ni}$ and *P. tereticollis*$_{Ni}$ - skin$_{Ni}$.

Table 3. Bioacumulation factors (BAF = [Cparasite]/[C host tissues]) of *P. tereticollis*

P. tereticollis/A. brama	BAF
$C_{P. tereticollis}/C_{Liver}$	70.093
$C_{P. tereticollis}/C_{Muscles}$	85.714
$C_{P. tereticollis}/C_{Skin}$	64.171

In the scientific papers, there are relatively small data for researches of the nickel content in fish from river Danube and their parasites. For example, Lenhardt et al. (2012) study the concentrations of 17 elements in tissues and organs of five economically important fish species from the Danube River (Serbia). In their study, the concentrations of nickel in muscle, gills, liver and gonads of *A. brama* were below the thresholds, and thus they were not subjected to statistical analysis.

CONCLUSIONS

1. New data for nickel contents in water, sediments, fish parasites, fish tissues and organs in both infected and uninfected specimens *A. brama* from the Danube River are presented. From the tissues and organs of the studied fish specimens freshwater bream, the lowest concentrations of nickel are found in the muscles.

2. The nickel content in muscles of infected *A. brama* was higher than the nickel content in muscles of uninfected specimens freshwater bream. The content of nickel in both infected and uninfected *A. brama* in the samples of skin are higher than in the liver and muscles and ranged as followed $C_{Skin\ Ni} > C_{Liver\ Ni} > C_{Muscles\ Ni}$. In general, the amendment of nickel content in a freshwater ecosystem in this study is in order: $C_{P.tereticollis\ Ni} > C_{Sediments\ Ni} > C_{Skin\ Ni} > C_{Liver\ Ni} > C_{Muscles\ Ni} > C_{Water\ Ni}$.

3. The highest bioconcentration was for $C_{P.\ tereticollis\ Ni}/C_{Water\ Ni}$ ($BCF_{P.tereticollis\ Ni/Water\ Ni}$=3529.41). Regarding *P. tereticollis* the highest bioaccumulation factor was for muscle, followed by those for liver and skin.

4. Significant correlations (p<0.05) were fixed for relationships between $C_{P.tereticollis\ Ni} – C_{Liver\ Ni}$, $C_{P.tereticollis\ Ni} – C_{Muscles\ Ni}$ and $C_{P.tereticollis\ Ni} - C_{Skin\ Ni}$.

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