DOI: 10.22620/agrisci.2018.24.005

DIFFERENCES IN ACCUMULATION OF NICKEL IN INFECTED AND UNINFECTED WITH *POMPHORHYNCHUS LAEVIS* SPECIMENS OF *ALBURNUS ALBURNUS* FROM THE FRESHWATER ECOSYSTEM OF DANUBE RIVER, BULGARIA

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Abstract

During the ecological study of 28 specimens bleak (*Alburnus alburnus* (Linnaeus, 1758)) by applying standard techniques for parasites was found infestation with the acantocephalan *Pomphorhynchus laevis* (Müller, 1776). Aim of the present study is to establish the content of nickel in water, sediments, tissues and organs of *A. alburnus* and its parasite *Pomphorhynchus laevis*, and the possibilities of accumulation and circulation in the freshwater ecosystem of Danube River.

From the tissues and organs of the studied specimens *Alburnus alburnus*, the content of nickel in samples of liver are higher than in the skin and muscles (in both infected and uninfected specimens). Differences were observed not only in nickel content, but also in the amendment of nickel content of infected ($Ni_{Liver} > Ni_{Muscles} > Ni_{Skin}$) and uninfected ($Ni_{Liver} > Ni_{Skin} > Ni_{Muscles}$) specimens of bleak. The obtained values for nickel content in liver, muscles and skin of *A. alburnus* infected with *P.laevis* were found to be lower than the obtained values for nickel content in liver, muscles and skin of uninfected specimens of bleak. The acanthocephalan *Pomphorhynchus laevis* showed significantly higher content of nickel than its host tissues and organs. Significant correlation ($p<0.05$) was fixed for relationships between $C_{P.\ laevis\ Ni} – C_{Skin\ Ni}$.

Keywords: nickel, *Alburnus alburnus*, *Pomphorhynchus laevis*, River Danube.

INTRODUCTION

Heavy metal pollution in the aquatic environment is usually observed by measuring the content of heavy metals in water, sediments and living organisms. The content of heavy metals in fish tissues can be used as indicator for the state of the freshwater ecosystem (Canbek et al., 2007; Jovičić et al., 2014).

The content of heavy metals in fish depends not only on the contamination levels of the environment, but also to their trophic position. It has been also established that some parasites accumulate significantly higher amounts of heavy metals than their hosts (Sures and Taraschewski, 1995; Sures et al., 1999; Sures and Siddal, 1999; Thielen et al., 2004; Turčeková and Hanzelová, 1996).

River Danube is under permanent negative anthropogenic impacts of industrial accidents and wastewaters. Heavy metal contamination of freshwater ecosystem of the Danube River was studied by different authors (Gabrashanska et al., 2004; Kirin et al., 2013; Kirin et al., 2014; Nachev, 2010; Ricking and Terytze, 1999; Woitke et al., 2003, etc.). Parasite fauna of *A. alburnus* from the Bulgarian section of the Danube River was studied from few authors (Kakacheva-Avramova, 1977; Atanasov, 2012; Kirin et al., 2013; Chunchukova et al., 2018).

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

MATERIALS AND METHODS

During 2016, water, sediments, fish and fish parasites were 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 (Fig. 1).

Polačik et al. (2008) established that *Alburnus alburnus* is one of the most abundant fish species in the shoreline zone of the Danube River in Bulgaria.

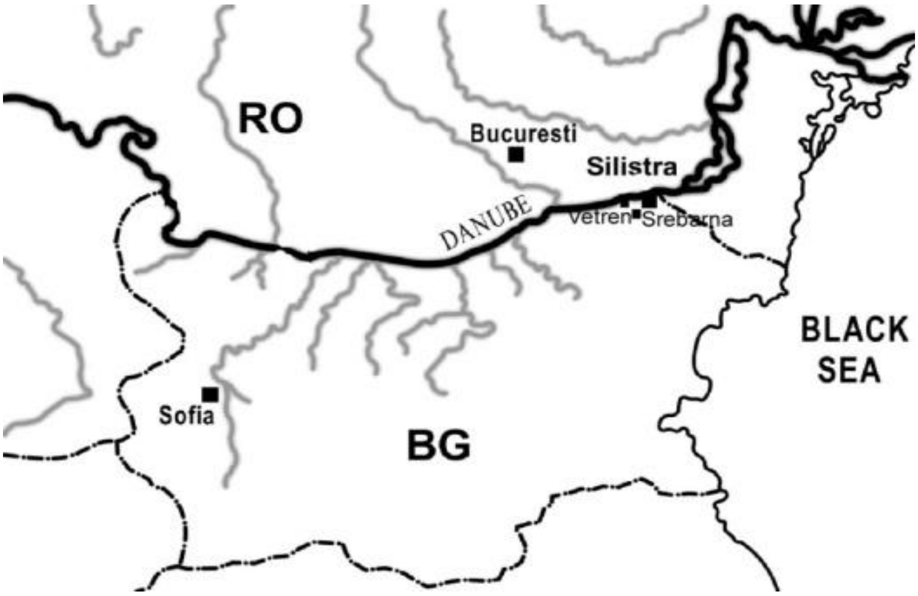

Fig.1. *River Danube*

*Alburnus alburnus*is estimated as least concern species (LC = Least Concern; IUCN Red List Status). Bleak is freshwater, brackish, benthopelagic, potamodromous fish species. This fish species inhabit open waters of lakes and medium to large rivers. Adults of *A. alburnus* occur in shoals near the surface. The diet of bleak includes mainly plankton, as well as crustaceans and insects (Fröse and Pauly, 2018).

The bleak (*Alburnus alburnus* (Linnaeus, 1758)) specimens chosen for examination of the heavy metal content in this study were weighed (total weigh from 10–23 g) and measured (total length from 10–14 cm). Samples of muscles, skin and liver were collected from all specimens of bleak. Helminthological examinations are carried out following recommendations and procedures described by Bauer et al. (1981), Bykhovskaya-Pavlovskaya (1985), Georgiev et al. (1986), Gusev (1985), etc. Main parameters of infection (prevalence %, mean abundance, mean intensity) are used and determined by criteria proposed from Bush et al. (1987).

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

In order 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 freshwater ecosystem by examined fish and their parasites. The differences in concentration factors are discussed in respect to the bioavailability of nickelfrom water and sediments. In order 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

A total of 28 specimens of bleak (*Alburnus alburnus* (Linnaeus, 1758)) were collected and examined from the Danube River during 2016. Ten specimens from the examined bleaks were infected with the acantocephalan *Pomphorhynchus laevis* (P% = 35.71, MI = 2.0 ± 1.095 (range 1-4); MA = 0.71±1.16). *Pomphorhynchus laevis*is an intestinal parasite of many freshwater fish, most often by a family Cyprinidae and less frequently by families Salmonidae, Percidae, Siluridae and others (Kakacheva-Avramova, 1983). *P. laevis* was found in *A. alburnus* from Danube River (Margaritov, 1966; Kakacheva-Avramova, 1977; Atanasov, 2012; Chunchukova et al., 2018).

This acanthocephalan develops with the participation of an intermediate host – *Gamaruspulex* (Amphipoda) (Petrochenko, 1956).

The results of the content of nickel (Ni) in samples of water and sediments and samples of muscle, liver and skin of *Alburnus alburnus* and its parasite *P. laevis* 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/parasitetissues]/[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 liver (C=12.85±14.17$mg.kg^{-1}$), followed by these of skin (C=0.610±0.019) and muscles (C=0.565±0.096). 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 forliver ($BCF_{Liver/Water}$=755.88; $BCF_{Liver/Sediments}$=4.024) followed by these for skin ($BCF_{Skin/Water}$ =35.882; $BCF_{Skin/Sediments}$=0.191), and muscles ($BCF_{Muscles/Water}$=33.235; $BCF_{Muscles/Sediments}$=0.177) (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. alburnus* and in water and sediment

A. alburnus	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	12.85±14.17	C_{Liver}/C_{water}	755.88	$C_{Liver}/C_{Sediments}$	4.024
Muscles	0.565±0.096	$C_{Muscles}/C_{water}$	33.235	$C_{Muscles}/C_{Sediments}$	0.177
Skin	0.610±0.019	C_{Skin}/C_{water}	35.882	$C_{Skin}/C_{Sediments}$	0.191
River Danube		Water ($mg.l^{-1}$)	0.017	Sediments ($mg.kg^{-1}$)	3.193

From the tissues and organs of infected with *P. laevis* bleaks the highest contents of nickel were determined in samples ofliver (C_{Liver}=6.960±6.290 $mg.kg^{-1}$), followed by these of muscles ($C_{Muscles}$=0.441±0.173 $mg.kg^{-1}$) and skin (C_{Skin}=0.242±0.013 $mg.kg^{-1}$). The acanthocephalan *P. laevis* (C=58.93±27.07 $mg.kg^{-1}$) showed significantly higher content of nickel than its host *A. alburnus.* 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. laevis* ($BCF_{P.laevis/Water}$=3466.47; $BCF_{P.laevis/Sediments}$=18.456), followed by those for liver ($BCF_{Liver/Water}$=409.412; $BCF_{Liver/Sediments}$=2.180), for muscles ($BCF_{Muscles/Water}$=25.941; $BCF_{Muscles/Sediments}$=0.138) and for skin ($BCF_{Skin/Water}$ =14.235; $BCF_{Skin/Sediments}$=0.076),(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. alburnus* and in water and sediments

A. alburnus / P. laevis	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	6.960±6.290	C_{Liver}/C_{water}	409.412	$C_{Liver}/C_{Sediments}$	2.180
Muscles	0.441±0.173	$C_{Muscles}/C_{water}$	25.941	$C_{Muscles}/C_{Sediments}$	0.138
Skin	0.242±0.013	C_{Skin}/C_{water}	14.235	$C_{Skin}/C_{Sediments}$	0.076
P. laevis	58.93±27.07	$C_{P. laevis}/C_{water}$	3466.47	$C_{P. laevis}/C_{Sediments}$	18.456
River Danube		Water ($mg.kg^{-1}$)	0.017	Sediments ($mg.kg^{-1}$)	3.193

The obtained values for nickel content in liver ($C_{Liver\ Ni}$=12.85±14.17), skin ($C_{Skin\ Ni}$ =0.610±0.019) andmuscles ($C_{Muscles\ Ni}$=0.565±0.096) of uninfected *A. alburnus* were found to be higher than the obtained values for nickel content in liver ($C_{Liver\ Ni}$=6.960±6.290), skin (C_{SkinNi}=0.242±0.013) and muscles ($C_{Muscles\ Ni}$ =0.441±0.173) of *A. alburnus* infected with *P. laevis*. In general, the content of nickel in both infected and uninfected specimens of *A. alburnus* in samples of liver are higher than in the muscles and skin. For the uninfected specimens of bleak nickel content ranged as followed:$Ni_{Liver}>Ni_{Skin}>Ni_{Muscles}$, while for the infected with *P. laevis* specimens it ranged: $Ni_{Liver}>Ni_{Muscles}>Ni_{Skin}$. In general, thenickel content in liver, muscles and skin of uninfected specimens of bleak are higher than in liver, muscles and skin of *A. alburnus* infected with *P. laevis.* Similar results were observed for arsenic from Chunchukova and Kuzmanova (2017). Regarding *P. laevis* the highest bioacumulation factor (BAF) was for skin ($BAF_{P.laevis/Skin}$ =243.51), followed by those for muscles ($BAF_{P.laevis/Muscles}$ =133.63) and liver ($BAF_{P.laevis/Liver}$= 8.467) (Table 3).

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

P. laevis/*A. alburnus*	BAF
$C_{P.\ laevis}/C_{Liver}$	8.467
$C_{P.\ laevis}/C_{Muscles}$	133.63
$C_{P.\ laevis}/C_{Skin}$	243.51

A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) is determined to test the association between *P. laevis* and fish tissues and organs. Significant correlation ($p<0.05$) was fixed for the relationship between *P.laevis* $_{Ni}$– skin $_{Ni}$.

In the scientific papers there are relatively small data for researches of the nickel content in water, sediments, bleak and its parasites from Danube River. For example, Subotić, et al. (2015) study the concentrations of 11 elements in muscle tissues of bleak (*A. alburnus*) and pike (*E. lucius*) from the Danube River (Serbia). In their study the concentrations of nickel were below the detection threshold in the bleak muscle.

Data for heavy metal concentrations in *Alburnus alburnus* - parasite system are scarce.For example, Gabrashanska and Nedeva (1996) studied the concentrations of Cu, Cr and Zn inparasite-host system *Alburnus alburnus – Ligulaintestinalis.* Chunchukova and Kuzmanova (2017) studied the content of arsenic inparasite-host system: *A. alburnus – P. laevis* and the impact of the acanthocephalan on his host.

CONCLUSIONS

New data for nickel content in fish parasites, fish tissues and organs in both infected and uninfected specimens *A. alburnus*from the Danube River are presented. From the tissues and organs of the studied fish specimens the lowest concentrations of nickel were found in the skin of infected with *P. laevis* bleaks. The nickel content in liver, muscles and skin of infected with *P. laevis* specimens of *A. alburnus* was lower than the nickel content in liver, muscles and skin of uninfected specimens of bleak.

The content of nickel in both infected and uninfected *A. alburnus* in the samples of liver are higher than in the samples ofskin and muscles. Differences were observed not only in nickelcontent, but also in the amendment of nickel content of infected ($Ni_{Liver}>Ni_{Muscles}>Ni_{Skin}$) and uninfected ($Ni_{Liver}>Ni_{Skin}>Ni_{Muscles}$) specimens of bleak. In general, the amendment of nickel content in freshwater ecosystem in this study is in order: $C_{P.laevisNi}> C_{SedimentsNi}> C_{LiverNi}> C_{Muscles\ and\ SkinNi}>C_{WaterNi}$. The highest bioconcentration was for $C_{P.\ laevisNi}/C_{Water\ Ni}$ ($BCF_{P.laevisNi/Water\ Ni}$=3466.47). Regarding *P. laevis*the highest bioacumulation factor was for skin ($BAF_{P.tereticollis/Skin}$ =243.51). Significant correlation ($p<0.05$) was fixed for relationships between $C_{P.laevisNi}$–$C_{Skin\ Ni}$.

ACKNOWLEDGEMENT

This research work was carried out with the support of Agricultural University - Plovdiv, financing PhD thesis NoD31. The author would like to thank to Ms R. Hristova (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences) for her technical help with the chemical analyses.

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