DOI: 10.22620/agrisci.2017.01.006

ARSENIC CONTENT IN PARASITE-HOST SYSTEM: *ALBURNUS ALBURNUS -POMPHORHYNCHUS LAEVIS* AND THE IMPACT OF THE ACANTHOCEPHALAN ON HIS HOST

Mariya Chunchukova*, Dimitrinka Kuzmanova

Agricultural University – Plovdiv

***E-mail: m.chunchukova@abv.bg**

Abstract

During the ecological study of 45 specimens of bleak (*Alburnus alburnus* (Linnaeus, 1758)) by applying standard techniques for parasites in ten specimens of fish an infestation was found with the acantocephalan *Pomphorhynchus laevis*. Aim of the present study is to establish the content of arsenic in water, sediments, tissues and organs of *A. alburnus* and its parasite *Pomphorhynchus laevis*, and the impact that the acanthocephalan has on the content of arsenic in his host tissues and organs. From the tissues and organs of the studied specimens of *Alburnus alburnus*, the content of arsenic in samples of liver were higher than in the samples of muscles and skin, and ranged as followed: $C_{As/Liver}>C_{As/Muscles}>C_{As/Skin}$ (in both infected and uninfected specimens). The obtained values for the content of arsenic in skin of uninfected specimens of *A. alburnus* were found to be more than 3 times higher than the obtained values for content of arsenic in skin of *A. alburnus* infected with *P. laevis*. In general the content of arsenic in tissues and organs of uninfected *A. alburnus* was higher than the content of arsenic in tissues and organs of infected with *P. laevis* specimens of bleak. The acanthocephalan *Pomphorhynchus laevis* showed 115-3042 times higher content of arsenic than its host tissues and organs. Significant negative correlation ($p<0.05$) was fixed for the relationship between $C_{As/P.\ laevis} - C_{As/Sediments}$.

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

INTRODUCTION

The fish community in the shoreline zone of the Danube River in Bulgaria is presented by 44 fish species and *Alburnus alburnus* is one of the most abundant (Polačik et al., 2008). Due to the potential risk for human, the levels of contaminants in fish tissues are of great interest. Heavy metal content in fish and their parasites, and the state of freshwater ecosystem of the Danube River are studied by different authors (Atanasov, 2012; Gabrashanska et al., 2004; Kirin et al., 2013; Kirin et al., 2014; Nachev, 2010; Ricking and Terytze, 1999; Subotić et al., 2015; Woitke et al., 2003; Zrnčić et al., 2013; etc.). Arsenic is a trace metaloid that can occur in different oxidation states and inorganic and organic forms. Organic arsenic compounds may occur where waters are significantly impacted by industrial pollution (Smedley, 2002).

This study aims to present the results of examinations of arsenic contents in water, sediments, *Pomphorhynchus laevis*, skin, muscles and liver of infected and uninfected with *P. laevis* bleaks and the impact that the acanthocephalan has on the arsenic content of his host tissues and organs.

MATERIALS AND METHODS

In 2016, water, sediments, fish and fish parasites were collected and examined from the Lower Danube River (village of Vetren, Bulgarian part).

A total of 45 specimens of bleak (*Alburnus alburnus* (Linnaeus, 1758)) were collected and examined from the Danube River. *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 inhabits open waters of lakes and medium to large rivers. Adults of bleak occur in shoals near the surface. Juveniles fish leave shores and occupy a pelagic habitat, feeding on plankton, drifting insects or invertebrates fallen on the water surface. Bleak feeds mainly on plankton, including crustaceans and insects. This fish species spawns in shallow riffles or along stony shores of lakes, occasionally above submerged vegetation (Fröse and Pauly, 2017).

The bleak (*Alburnus alburnus* (Linnaeus, 1758)) specimens chosen for examination of the heavy metal content in this study were weighed (total weigh from 10-22 g) and measured (total

length from 10 – 14 cm). Samples of muscles, skin and liver were collected from all specimens of bleak. Helminthological examinations were carried out following recommendations and procedures described by Petrochenko (1956) Bauer et al. (1981), Bykhovskaya-Pavlovskaya (1985), Gusev (1985), etc. Main parameters of infection (prevalence %, mean intensity) were used and determined by criteria proposed from Bush et al. (1987).

Ten specimens from the examined bleaks were infected with the acantocephalan *Pomphorhynchus laevis* (P%=22.22, MI=2.44±1.81 (range 1-6)). *Pomphorhynchus laevis* is autogenic species, matured in fish. Intermediate host of *P. laevis* is *Gammarus pulex* and definitive hosts are fish most often from family Cyprinidae, and less often from families Salminidae, Percidae, Siluridae,etc. (Kakacheva-Avramova, 1983). *Pomphorhynchus laevis* was found in *Abramis brama*, *Abramis sapa, Acipenser ruthenus, Alburnus alburnus, Alosa pontica, Barbus barbus, Carassius auratus gibelio, Esox lucius*, *Gymnocephalus schraetser, Leuciscus cephalus, Pelecus cultratus, Pomatoschistus minutus, Sander lucioperca, Scardinius erytrophtalmus, Silurus glanis* and *Zingel zingel* from Bulgarian section of river Danube (Atanasov 2012). *P. laevis* was found in *A. alburnus* from Danube River and from other authors (Kakacheva-Avramova, 1977; Margaritov, 1966).

The samples of water, sediment, fish tissues, organs and parasites were analyzed for content of arsenic (As) by ICP Spectrometry. 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] were calculated (Sures et al., 1999). The bioconcentration factors were used for estimation of trace metal pollution in freshwater ecosystem by examined fish and their parasites. The differences in concentration factors were discussed in respect to the bioavailability of arsenic from 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]) were calculated. A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) was determined to test the association between parasites and water, sediments and their hosts tissues and organs.

RESULTS AND DISCUSSION

The results of the content of arsenic (As) 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/parasite tissues]/[Cwater/sediments]) were defined.

The content of arsenic in samples of sediments ($C_{Sed/Danube}$=5.74 $mg.kg^{-1}$) was much higher than the content of arsenic in samples of water ($C_{Water/Danube}$=0.013 $mg.l^{-1}$) from the examined freshwater ecosystem – Biotope Vetren on the Danube River. From the tissues and organs of uninfected specimens of fish the highest content of arsenic was determined in samples of liver (C=0.600±0.690), followed by those of muscles (C=0.073±0.029) and skin (C=0.066±0.037). This purpose remains regarding the values of BCF, set against the levels of arsenic in water and sediments of the Danube River (Biotope Vetren). The highest bioconcentration factor (BCF) was for liver ($BCF_{Liver/Water}$=46.154; $BCF_{Liver/Sediments}$=0.105), followed by those for muscles ($BCF_{Muscles/Water}$=5.615; $BCF_{Muscles/Sediments}$=0.013) and skin ($BCF_{Skin/Water}$=5.077; $BCF_{Skin/Sediments}$=0.011) (Table 1).

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

A. alburnus	Mean ± SD	Relationships	BCF	Relationships	BCF
Liver	0.600±0.690	C_{Liver}/C_{water}	46.154	$C_{Liver}/C_{Sediments}$	0.105
Muscles	0.073±0.029	$C_{Muscles}/C_{water}$	5.615	$C_{Muscles}/C_{Sediments}$	0.013
Skin	0.066±0.037	C_{Skin}/C_{water}	5.077	$C_{Skin}/C_{Sediments}$	0.011
River Danube		Water ($mg.l^{-1}$)	0.013	Sediments ($mg.kg^{-1}$)	5.74

From the tissues and organs of infected with *P. laevis* specimens of bleak the highest content of arsenic was determined in samples of liver (C=0.554±0.710), followed by those for muscles (C=0.050±0.025) and skin (C=0.021±0.008). The acanthocephalan *P. laevis* (C=63.9±12.84) showed significantly higher content of arsenic than its host *A. alburnus*. This purpose remains regarding the values of BCF, set against the levels of arsenic in water and sediments of the Danube River (Biotope Vetren). The highest bioconcentration factor (BCF) was for *P. laevis* ($BCF_{P.laevis/Water}$=4915.38; $BCF_{P.laevis/Sediments}$=11.132), followed by those for liver ($BCF_{Liver/Water}$=42.615; $BCF_{Liver/Sediments}$=0.097), muscles ($BCF_{Muscles/Water}$=3.846; $BCF_{Muscles/Sediments}$=0.009) and skin ($BCF_{Skin/Water}$=1.615; $BCF_{Skin/Sediments}$=0.004) (Table 2).

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

A.alburnus / P. laevis	Mean±SD	Relationships	BCF	Relationships	BCF
Liver	0.554±0.710	C_{Liver}/C_{water}	42.615	$C_{Liver}/C_{Sediments}$	0.097
Muscles	0.050±0.025	$C_{Muscles}/C_{water}$	3.846	$C_{Muscles}/C_{Sediments}$	0.009
Skin	0.021±0.008	C_{Skin}/C_{water}	1.615	$C_{Skin}/C_{Sediments}$	0.004
P. laevis	63.9±12.84	$C_{P.\ laevis}/C_{water}$	4915.38	$C_{P.\ laevis}/C_{Sediments}$	11.132
River Danube		Water ($mg.kg^{-1}$)	0.013	Sediments ($mg.kg^{-1}$)	5.74

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

P. laevis / A. alburnus	BAF
$C_{P.\ laevis}/C_{Liver}$	115.34
$C_{P.\ laevis}/C_{Muscles}$	1278
$C_{P.\ laevis}/C_{Skin}$	3042.86

The obtained values for the content of arsenic in liver, muscles and skin of uninfected specimens of *A. alburnus* were found to be higher than the obtained values for the content of arsenic in liver, muscles and skin of *A. alburnus* infected with *P. laevis*. The greatest differences were observed for the content of arsenic in skin. The content of arsenic in skin of uninfected specimens of *A. alburnus* (C=0.066±0.037) was found to be more than 3 times higher than the content of arsenic in skin of *A. alburnus* infected with *P. laevis* (C=0.021±0.008). In general, the content of arsenic in both infected and uninfected specimens of *A. alburnus* in samples of liver were higher than in samples of muscles and skin, and ranged as followed: $C_{As/Liver}>C_{As/Muscles}>C_{As/Skin}$.

Regarding *P. laevis* the highest bioacumulation factor (BAF) was for skin ($BAF_{P.laevis/Skin}$=3042.86), followed by those for muscles ($BAF_{P.laevis/Muscles}$=1278) and liver ($BAF_{P.laevis/Liver}$=115.34) (Table 3).

A linear correlation coefficient (Spearman's rank correlation coefficient, r_s) was determined to test the association between *P. laevis* and water, sediments and fish tissues and organs. Significant negative correlation ($p<0.05$) was fixed for the relationship between $C_{As/P.laevis} - C_{As/Sediments}$.

Atanasov (2012), Kakacheva-Avramova (1977) and Margaritov (1966) established for *A. alburnus* from the Danube River infestation with the acanthocephalan *Pomphorhynchus laevis.* Other reported acanthocephalan with host *A. alburnus*

from the Danube Basin is *Acanthocephalus anguillae* (Gelnar et al., 1994). For rivers in Bulgaria were reported the acanthocephalans *Neoechinorhynchus rutili* and *Telosentis exiguous* with host *A. alburnus* (Kirin, 2001).

In the scientific papers there are relatively small data for researches of the arsenic content in *A. alburnus* from Danube River. Zrnčić et al. (2013) studied the heavy metal contamination of fish in the Croatian part of the Danube. In their study the concentration of arsenic in muscles of *A. alburnus* was 0.042±0.016 microgram per dry weight. Subotić et al. (2015) studied the element concentrations of muscle tissue of bleak from the Danube River near Belgrade. In their study the concentration of arsenic in muscles of *A. alburnus* was 1.06±0.21 μg/g dry weight.

The ability of *P. laevis* to accumulate heavy metals was studied from many authors mostly as parasite of *Barbus barbus* (Nachev et al., 2010; Nachev et al., 2013; Schludermann et al., 2003; Sunjog et al., 2012; Sures et al., 2005; Thielen et al., 2004; etc.)

Data for heavy metal concentrations in *Alburnus alburnus*-parasite system and the impact of parasites on this host are scarce. For example, Gabrashanska and Nedeva (1996) studied the concentrations of Cu, Cr and Zn in *A. alburnus* and its parasite *Ligula intestinalis*. They observed higher concentrations of Cu, Cr and Zn in plerocercoid of *L. intestinalis* than in muscles of fish.

CONCLUSIONS

New data for arsenic contents in fish parasites, fish tissues and organs in both infected and uninfected specimens of *A. alburnus* from the Danube River is presented. From the tissues and organs of the studied specimens of bleak the lowest concentrations of arsenic were found in the skin. The content of arsenic in skin of infected with *P. laevis* specimens of *A. alburnus* was more than 3 times lower than the content of arsenic in skin of uninfected specimens of bleak. The content of arsenic in both infected and uninfected *A. alburnus* in the samples of liver are higher than in the samples of muscles and skin and ranged as followed: $C_{As/Liver}>C_{As/Muscles}>C_{As/Skin}$. In general, the amendment of arsenic content in freshwater ecosystem in this study is in order: $C_{As/P.laevis}>C_{As/Sediments}>C_{As\ /Liver}>C_{As\ /Muscles}>C_{As/Skin}>C_{As/Water}$. The highest bioconcentration factor was for $C_{As/P.laevis}/C_{As/Water}$ ($BCF_{As/P.laevis}/_{As/Water}$=4915.38). Regarding *P. laevis* the highest bioacumulation factor was for skin, followed by the one for muscles and liver.

REFERENCES

Atanasov, *G*., 2012. Fauna, morphology and biology on the endohelminths of fish from Bulgarian part of the Danube River. PhD these, Sofia (In Bulgarian).

Bauer, *O.N., V.A. Musselius, Yu. A. Strelkov*, 1981. Diseases of pond fish. 320 pp. Moscow, Legkaya Pishchevaya Promishlenost' Publishers (In Russian).

Bush, *A., K. Lafferty, J. Lotz, A. Shostak*, 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).

Fröse, *R., D. Pauly*, 2017. Fish Base. World Wide Web electronic publication, www.fishbase.org

Gabrashanska, M, Nedeva I., 1996. Content of heavy metals in the system fish-cestodes. Parassitologia, 38, p. 58.

Gabrashanska, *M., I. Nedeva, P. Cakic, M.Galves-Morros, E. Karaivanova, G. Atanasov, M. Lenhardt,* 2004. Heavy metals in fish parasite system from the Danube river (Bulgarian and Serbian parts). Macro and Trace elements, 22 Workshop, Jena Germany, 613-618.

Gelnar, *M., B. Koubkova, H. Plankova, P. Jurajda,* 1994. Report on metazoan parasites of fishes of the river Morava with remarks on the effects of water pollution. Helminthologia, 31, 47-56.

Gusev, *A., M. Dubinina, O. Pugachev, E. Rajkova, I. Hotenovskij, R. Ergens*, 1985. Guidae to Freshwater fish parasites Fauna SSSR.Nauka, Leningrad, 428 (In Russian).

IUCN Red List Status www.iucnredlist.org

Kakacheva-Avramova D., 1983. Helminths of freshwater fishes in Bulgaria. Bulgarian Academy of Sciences, Sofia, 261. (In Bulgarian).

Kakacheva-Avramova, D., 1977. Studies on helminths of fishes in the Bulgarian section of the Danube River. *Helminthologia*, 3, 20-45 (In Bulgarian).

Kirin, *D.*, 2001. Biodiversity and ecology of helminthes communities of *Alburnus alburnus* frpm Veleka and Resovska Rivers, Bulgaria. Comptes rendus de l'Académie bulgare des Sciences 54, 99-102.

Kirin, D., *V. Hanzelova, S. Shukerova, D. Kuzmanova*, 2014. Biodiversity, bioindication and helminth communities of *Abramis brama* (Linnaeus, 1758) from the Danube River and Srebarna Lake, Bulgaria. Turkish Journal of Agricultural and Natural Sciences, Special Issue: 1, 727-733.

Kirin, *D., V. Hanzelova, S. Shukerova, S. Hristov, L. Turcekova, M. Spakulova*, 2013. Helminth communities of fishes from the River Danube and Lake Srebarna, Bulgaria Scientific Papers. Series D. Animal Science. Vol. LVI ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN-L 2285-5750

Lenhardt, *M., I. Jaric´, Ž. Višnjic-Jeftic´, S. Skoric´, Z. Gacic, M. Pucar, A. Hegediš*, 2012. Concentrations of 17 elements in muscle, gills, liver and gonads of five economically important fish species from the Danube River. Knowledge and Management of Aquatic Ecosystems (2012) 407, 02. DOI: 10.1051/kmae/2012028

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 Zoolog**ieetMusée*, 20, 157-173 (In Bulgarian).

Nachev, M. S. Zimmerman, T. Rigaud, B. Sures, 2010. Is metal accumulation in *Pomphorhynchus laevis* dependent on parasite sex or infrapopulation size? Parasitology, 137, 1239–1248.

Nachev, *M*., 2010. Bioindication capacity of fish parasites for the assessment of water quality in the Danube River. PhD these, Universität Karlsruhe.

Nachev, M., G. Schertzinger, B. Sures, 2013. Comparison of the metal accumulation capacity between the acanthocephalan Pomphorhynchus laevis and larval nematodes of the genus Eustrongylides sp. infecting barbel (Barbus barbus). Parasite Vector 6, 21.

Petrochenko V.I., 1956. Acanthocephala of Domestic and Wild Animals. NAS of SSSR,

Polačik, *M., T. Trichkova, M. Janáč, M. Vassilev & P. Jurajda,* 2008. The Ichthyofauna of the Shoreline Zone in the Longitudinal Profile of the Danube River, Bulgaria. *Acta Zoologica Bulgarica*, 60 (1), 77-88.

Ricking, *M., K. Terytze*, 1999. Trace metals and organic compouds in sediment samples from the River Danube in Russe and Lake Srebarna (Bulgaria). Environ. Geol., 37, 40-46.

Schludermann, C., R. Konecny, S. Laimgruber, J. W. Lewis, F. Schiemer, A. Chovanec, B. Sures, 2003. Fish macroparasites as indicators of heavy metal pollution. Parasitology, 126, S61–S69.

Smedley, P. L., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Applied Geochemistry. 17 (5), 517–568.

Subotić, S., *Ž. Višnjić-Jeftić, S. Spasić, A. Hegediš, J. Krpo-Ćetković, M. Lenhardt*, 2015. Element concentrations in muscle tissue of two fish species from different trophic levels (bleak and pike) in the Danube near Belgrade. Proceeding in: VII International Conference "Water & Fish", 500-505.

Sunjog, K., Z. Gačić, S. Kolarević, Ž. Višnjić-Jeftić, I. Jarić, J. Knežević-Vukčević, B. Vuković-Gačić, M. Lenhardt, 2012. Heavy metal accumulation and the genotoxicity in barbel (Barbus barbus) as indicators of the Danube River pollution. Sci. World J. (Article ID 345774), 1–6.

Sures, B., F. Thielen, F. Baska, J. Messerschmidt, A. von Bohlen, 2005. The intestinal parasite *Pomphorhynchus laevis* as a sensitive accumulation indicator for the platinum group metals Pt, Pd, and Rh. Environmental research, 98, 83-88.

Sures, *B., R. Siddal, H. Taraschewski*, 1999. Parasites as accumulation indicators of heavy metal pollution. Parasitol Today 15(1), 16–21.

Thielen, F., S. Zimmermann, F. Baska, H. Taraschewski, B. Sures, 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.

Woitke, *P., J. Wellmitz, D. Helm, P. Kube, P. Lepom, P. Litheraty,* 2003. Analysis and assessment of heavy metal pollution in suspended solids and sediments of the river Danube. Chemosphere, 51, 633-642.

Zrnčić, S*, D. Oraić, M. Ćaleta, Ž. Mihaljević, D. Zanella, N. Bilandžić*, 2013. Biomonitoring of heavy metals in fish from the Danube River. Environ Monit Assess, 185, 1189–1198.