Scientific Papers. Series D. Animal Science. Vol. LXIII, No. 2, 2020
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

A CONTRIBUTION TO THE STUDIES ON THE CONTENT OF CU, CD AND AS IN *ALBURNUS ALBURNUS* (LINNAEUS, 1758) FROM THE DANUBE RIVER

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Abstract

The study aimed to present the results of investigations on the content of heavy metals/metalloids (Cu, Cd and As) in tissues and organs (liver, skin and muscles) of Alburnus alburnus (Linnaeus, 1758), and in waters and sediments of the Danube River (Kudelin, Vidin), Bulgaria. The highest Cd contents were found in liver samples (0.80 ± 0.56 mg.kg^{-1}), followed by contents in skin samples (0.21 ± 0.15 mg.kg^{-1}) and muscle (0.08 ± 0.07 mg.kg^{-1}). The highest As contents were found in liver (28.14 ± 10.98 mg.kg^{-1}) and skin (14.36 ± 11.45 mg.kg^{-1}), and the lowest in muscle (2.14 ± 0.64 mg.kg^{-1}). The highest content of Cu was established in liver samples (7.32 ± 2.39 mg.kg^{-1}), followed by this in the skin (3.78 ± 2.24 mg.kg^{-1}) and muscles (1.29 ± 1.03 mg.kg^{-1}). The highest values for As contents (0.07 ± 0.05 mg.l^{-1}) were determined in surface waters, and the highest values for Cu contents were determined in sediments (204.09 ± 121.05 mg.kg^{-1}). The results of studies on the circulation of the studied elements in the water-sediment-tissue and organs of A. alburnus system were analysed.

Key words*: Alburnus alburnus, arsenic, Bulgaria, cadmium, copper, Danube River.*

INTRODUCTION

The Danube River is the only river in Europe which course from west to east. In this way, the river is a connection between the countries of Western, Central and Eastern Europe (Schiemer et al., 2004). The Danube River Basin occupies ten per cent of Europe's surface. It covers the territory of 19 countries. The Danube River is adversely affected by the presence of numerous sources of pollution throughout its course. The river collects pollutants of various nature and takes them to its delta into the Black Sea. The upper reaches of the rivers are less polluted than the lower ones (Gasparotti, 2014). The Bulgarian territory of the Danube River falls into the lower reaches of the river (Polačik et al., 2008). Northwestern Bulgaria is a region with a long history of ore mining. The larger mines are situated near Chiprovtsi town and Martinovo village. They are a source of contamination with heavy metals (arsenic, copper, lead, zinc, etc.) (Dimitrova et al., 2016). Various authors have conducted studies on the heavy metals pollution in the mining area near Chiprovtsi and the Ogosta and Chiprovska rivers (Cholakova et al., 2006; Dimitrova et al., 2007; Mladenova et al., 2010; Mladenova et al., 2011; Kotsev and Zhelezov, 2014; Dimitrova et al., 2016; Benderev et al., 2017; Gerginov et al., 2017). A significant proportion of heavy metals that enter the aquatic environment pass into the sediments. They remain there for an extended period and continue to be a source of pollution and a threat to living organisms. Heavy metals have different toxicity. Some of them are dangerous to organisms even in small quantities (e.g. cadmium, lead, mercury), while others (e.g. copper, zinc, iron) are required in small amounts and dangerous in large numbers (Ionescu et al., 2014; Ionescu et al., 2015). Heavy metals and metalloids can be established in various tissues and organs of fish. At significantly higher concentrations, they can be found in the liver, kidneys and gills of fish (Javed and Usmani, 2017). Different authors have investigated the concentrations of heavy metals in tissues and organs of fish from the Danube River (Diaconescu et al., 2008; Nachev et al., 2010; Urdeş et al., 2010; Lenhardt et al., 2012; Kirin et al., 2014; Subotić et al., 2014; Milanovet al., 2016; Milošković et al., 2016; Burada et al., 2017; Chunchukova and Kirin, 2017; Chunchukova and Kuzmanova, 2017;

Chunchukova, Kirin, Shukerova, et al., 2017; Chunchukova, Kirin, Kuzmanova, et al., 2017; Jovanović et al., 2017; Kirin and Chunchukova, 2017; Shukerova et al., 2017, etc.). Studies are also carried out on the content of heavy metals in waters and sediments from the Bulgarian territory (Kirin et al., 2014; Chunchukova and Kirin, 2017; Chunchukova and Kuzmanova, 2017; Kirin and Chunchukova, 2017; Shukerova et al., 2017, etc.); from the Serbian territory (Antonijević et al., 2014; Ćirić et al., 2016; Milanov et al., 2016, etc.), from the Romanian area (Urdeş et al., 2010; Radu et al., 2017; Begy et al., 2018, etc.) of the Danube River.

This study aimed to determine the concentrations of heavy metals and metalloids (Cu, Cd, and As) in the tissues and organs (liver, skin, and muscles) of *Alburnus alburnus* (Linnaeus, 1758) as well as in waters and sediments of the Danube River; to analyze the circulation of the studied elements in the water-sediment-tissue and organs of *A. alburnus* system.

MATERIALS AND METHODS

During 2019, fish, water and sediments samples were collected from the Bulgarian territory of the Danube River (Kudelin village, designated as Kudelin biotope). The Kudelin village (44 ° 11 ′ 30 ″ N, 22 ° 40 ′ 5 ″ E) is located along the Danube River in northwestern Bulgaria.

Thirty specimens of *Alburnus alburnus* (L., 1758) were captured and investigated. Five water samples and four sediment samples were also collected. The fish are collected in accordance with the scientific fishing requirements of the Executive Agency for Fisheries and Aquaculture in Bulgaria. The species of fish were determined by Karapetkova and Jivkov (2006); Kottelat and Freyhof (2007). The scientific name of fish is written according to FishBase (Froese and Pauly, 2019). Maximum body length (L) in centimeters, maximum body width (H) in centimeters, and weight (g) in grams were recorded for all specimens of *A. alburnus* (Table 1). According to standard methods, mean representative samples of organs (liver) and tissues (skin and muscle) of the collected specimens of bleak were prepared. The chemical analysis of the samples was carried out by ICP “Optima 7000” Perkin-Elmerin an accredited laboratory for atomic absorption spectrophotometry of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia.

Table 1. L, H and g of the specimens *A. alburnus* from the Danube River (Kudelin biotope)

Alburnus alburnus	Min – max	Average ± SD
L	8.5 – 12.8	10.59 ± 1.05
H	1.8 – 3	2.21 ± 0.27
g	4 – 13	6.60 ± 2.14

The concentrations of the examined heavy metals (Cu, Cd) and metalloids (As) are presented in $mg.kg^{-1}$ wet weight and $mg.kg^{-1}$ dry weight (for samples of liver, skin and muscle of *A. alburnus*), in $mg.l^{-1}$ (for samples of water) and $mg.kg^{-1}$ dry weight (for samples of sediment). The bioconcentration factor for water/sediments ($BCF = [C_{host\ tissues}]/[C_{water/sediments}]$) was determined in this study. The linear correlation coefficient of Spearman (r_s) also was determined. Data processing was performed with MS Excel (Microsoft, 2010), BioDiversity Pro (McAleece, 1997) and Statistica 10 (StatSoft Inc., 2011).

RESULTS AND DISCUSSIONS

Thirty specimens of bleak (*Alburnus alburnus* Linnaeus, 1758) were collected and investigated for the content of copper, cadmium and arsenic from the Danube River (Kudelin biotope). *Alburnus alburnus* is a freshwater fish of the Cyprinidae family. Bleak is a brackish fish that inhabits lakes and larger rivers, is found in the upper water layers, and feed on insects and invertebrates (Kottelat and Freyhof, 2007).

The study provides information on the concentrations of heavy metals and metalloids (copper, cadmium and arsenic) in tissues and organs of 30 specimens *A. alburnus*, as well as in five samples of water and four samples of sediment from the Danube River (Kudelin biotope).

The highest concentrations of the three elements (Cu, Cd and As) were found in the liver of *A. alburnus* ($C_{Cu} = 7.32 \pm 2.39$ $mg.kg^{-1}$ wet weight; $C_{Cd} = 0.80 \pm 0.56$ $mg.kg^{-1}$ wet weight and $C_{As} = 28.14 \pm 10.98$ $mg.kg^{-1}$ wet weight); followed by

those in the skin (C_{Cu} = 3.78 ± 2.24 mg.kg^{-1} wet weight; C_{Cd} = 0.21 ± 0.15 mg.kg^{-1} wet weight and C_{As} = 14.36 ± 11.45 mg.kg^{-1} wet weight). The lowest concentrations of Cu, Cd and As were reported in the muscle of *A. alburnus* (C_{Cu} = 1.29 ± 1.03 mg.kg^{-1} wet weight; C_{Cd} = 0.08 ± 0.07 mg.kg^{-1} wet weight and C_{As} = 2.14 ± 0.64 mg.kg^{-1} wet weight). The content of the three elements in tissues and organs of bleak reduced as follows: liver > skin > muscles. The concentrations of the studied elements in the water samples were established to decrease in the order: As>Cu>Cd (respectively C_{As} = 0.07 ± 0.05 mg.l^{-1}; C_{Cu} = 0.04 ± 0.03 mg.l^{-1} and C_{Cd} = 0.001 ± 0.001 mg.l^{-1}). While the concentrations of the investigated elements in the sediment samples decreased in the order: Cu>As>Cd (respectively C_{Cu} = 204.09 ± 121.05 mg.kg^{-1} dry weight, C_{As} = 19.52 ± 9.76 mg.kg^{-1} dry weight and C_{Cd} = 1.54 ± 0.35 mg.kg^{-1} dry weight) (Table 2 and Table 3).

Table 2. Cu, Cd and As (mg.kg^{-1} wet weight) in liver, skin and muscle of *A. alburnus* and water (mg.l^{-1}) from the Danube River (Kudelin biotope)

Alburnus alburnus		Cu	Cd	As
Liver	Min – max	5.25 – 10.26	0.11 – 1.49	17.03 – 39.66
	Mean ± SD	7.32 ± 2.39	0.80 ± 0.56	28.14 ± 10.98
Skin	Min – max	1.26 – 5.68	0.04 – 0.39	3.73 – 30.46
	Mean ± SD	3.78 ± 2.24	0.21 ± 0.15	14.36 ± 11.45
Muscle	Min – max	0.41 – 2.47	0.01 – 0.16	1.63 – 3.04
	Mean ± SD	1.29 ± 1.03	0.08 ± 0.07	2.14 ± 0.64
Water	Min – max	0.01 – 0.08	0.001 – 0.003	0.01 – 0.13
	Mean ± SD	0.04 ± 0.03	0.001 ± 0.001	0.07 ± 0.05

Table 3. Cu, Cd and As (mg.kg^{-1} dry weight) in liver, skin and muscle of *A. alburnus* and sediments (mg.kg^{-1} dry weight) from the Danube River (Kudelin biotope)

Alburnus alburnus		Cu	Cd	As
Liver	Min – max	10.42 – 27.24	0.33 – 2.20	41.95 – 93.45
	Mean ± SD	19.25 ± 7.31	1.46 ± 0.91	63.48 ± 21.76
Skin	Min – max	3.33 – 9.41	0.11 – 1.34	12.23 – 45.10
	Mean ± SD	6.36 ± 2.81	0.67 ± 0.61	25.56 ± 14.21
Muscle	Min – max	1.65 – 6.48	0.05 – 0.60	6.07 – 7.96
	Mean ± SD	3.77 ± 2.43	0.23 ± 0.26	6.90 ± 0.80
Sediments	Min – max	94.66 – 362.5	1.11 – 1.88	12.32 – 33.02
	Mean ± SD	204.09 ± 121.05	1.54 ± 0.35	19.52 ± 9.76

The reported concentrations of copper, cadmium and arsenic in liver, skin and muscle samples of *A. alburnus* were compared to norms in national (Ordinance No. 31 of 2004 on the maximum levels of contaminants in foodstuffs) and international (WHO and FAO) documents. The norms for Cu, Cd and As determined in Ordinance No. 31 of 2004 are 10 mg/kg; 0.05 mg/kg and 1 mg/kg, respectively. The norm for Cu indicated by WHO is 20 mg/kg. The norms for Cu and Cd reported by FAO are 30 mg/kg and 0.2 mg/kg, respectively. It was found that the Cu content in the liver, skin and muscles of *A. alburnus* did not exceed the norms specified in Ordinance No. 31, WHO and FAO. It was found that the Cd content in the liver, skin and muscles of *A. alburnus* exceeded the norms specified in Ordinance No. 31, respectively by 16.08, 4.24 and 1.52 times. The Cd content in liver and skin also exceeded the standards defined by FAO by 4.02 and 1.06 times, respectively. It was established that the As

content in liver, skin and muscles of *Alburnus alburnus* exceeded the norms specified in Ordinance No. 31, respectively by 28.14, 14.36 and 2.14 times (Figure 1).

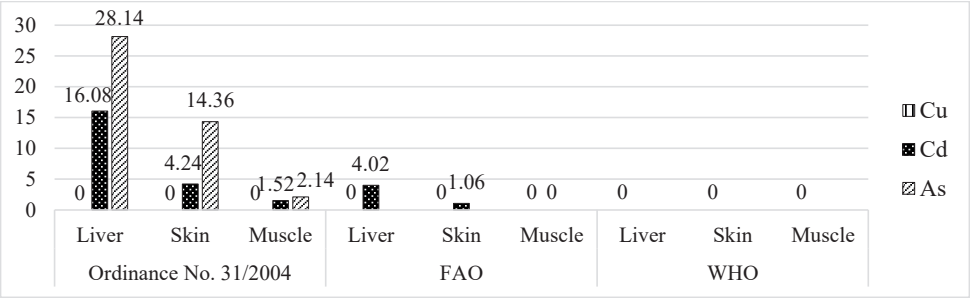

Figure 1. Exceedances of Cu, Cd and As in tissues and organs of *Alburnus alburnus* from Danube River (Kudelin biotope) according to national and international documents

The concentrations of copper, cadmium and arsenic in water samples were compared to norms in national documents (Ordinance No. 18 of 2009 on the quality of water for irrigation of crops; Ordinance No. H-4 of 2012 on the characterization of surface water and Ordinance on environmental quality standards for priority substances and certain other pollutants of 2010). The norms for Cu, Cd and As determined in Ordinance No. 18 of 2009 are 0.2 mg/dm^3; 0.01 mg/dm^3 and 0.1 mg/dm^3, respectively. The norm for As recorded in Ordinance No. H-4 of 2012 is 0.025 mg/l. The norm for Cd recorded in Ordinance on environmental quality standards is 0.0009 mg/l. Exceedances of Cd and As were found. The cadmium content exceeded the norms shown in Ordinance on environmental quality standards by 1.11 times, while the arsenic content exceeded the norms shown in Ordinance No. H-4 by 2.96 times (Figure 2).

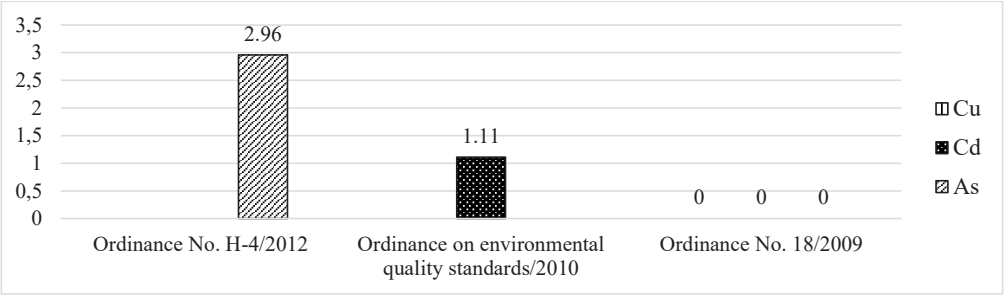

Figure 2. Exceedances of Cu, Cd and As in surface waters of the Danube River (Kudelin biotope) according to national documents

The reported concentrations of copper, cadmium and arsenic in sediment samples were compared to the norms in national (Ordinance No. 3 on the norms for permissible content of harmful substances in soils) and international (Dutch target values) documents. The maximum permissible concentrations (MPC) for Cu, Cd and As determined in Ordinance No. 3 are 150 mg/kg; 2 mg/kg at pH = 7.4 and 25 mg/kg, respectively. The Dutch target values for Cu, Cd and As are 36 mg/kg; 0.8 mg/kg and 29 mg/kg, respectively. Exceedances of Cu and Cd were found. The copper content exceeded 1.36 times the MPC in Ordinance No. 3. The copper and cadmium content exceeded the Dutch target values by 5.67 and 1.92 times, respectively (Figure 3).

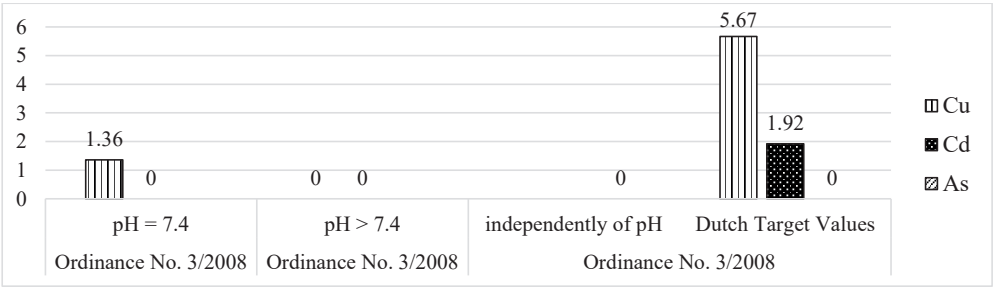

Figure 3. Exceedances of Cu, Cd and As in sediments from the Danube River (Kudelin biotope) relative to national and international documents

High bioconcentration of water/sediments was found for all three studied elements in the liver of bleak. In the muscles of bleak, the bioconcentration was the lowest. The study showed that the liver of *A. alburnus* bioaccumulated 4.09 times more Cd from the water compared to the accumulation of Cu and 2.11 times more Cd from the water compared to the accumulation of As. The liver of *A. alburnus* accumulated 1.92 times more As from the water, compared to that of Cu. The liver of *A. alburnus* bioaccumulated 36.12 times more As from the sediments compared to the accumulation of Cu and 10.56 times more Cd from sediments compared to that of Cu (Table 4–5).

Table 4. Bioconcentration factor BCF = [$C_{host\ tissues}$]/[C_{water}]

Alburnus alburnus/Water	BCF_{Cu}	BCF_{Cd}	BCF_{As}
C_{liver}/C_{water}	197.84	804	380.20
C_{skin}/C_{water}	102.27	212	193.98
C_{muscle}/C_{water}	34.81	76	28.85

Table 5. Bioconcentration factor BCF = [$C_{host\ tissues}$]/[$C_{sediments}$]

Alburnus alburnus/Sediments	BCF_{Cu}	BCF_{Cd}	BCF_{As}
$C_{liver}/C_{sediments}$	0.09	0.95	3.25
$C_{skin}/C_{sediments}$	0.03	0.44	1.31
$C_{muscle}/C_{sediments}$	0.02	0.15	0.35

Positive linear correlations were established between the Cu, Cd and As content in the investigated tissues and organs of the bleak and those in the water and sediments of the Danube River (r_s = 0.86 – 0.99 relative to water content; $p<0.05$ and r_s = 0.96 – 0.99; $p<0.05$). The positive linear correlations showed the direct impact of the water and the sediments on the content of the three studied elements in the liver, skin and muscles of the bleak. In this study, it was established a very high correlation and significance of correlation dependencies.

There are studies for heavy metals in bleak, but from the lower sections of the Danube River in Bulgaria. These are the first studies of the border sections of Danube River in North-Western Bulgaria. Few such studies, however, exist in other countries. Chunchukova and Kuzmanova (2017) established higher As concentration in the liver of bleak than in the muscles and skin. Shukerova et al. (2017) investigated *Alburnus alburnus* from the Bulgarian section of the Danube River for cadmium content and found higher Cd content in liver than in muscle and skin. Chunchukova et al. (2017a) analyzed the lead content in liver, skin and muscles of bleak from the Bulgarian section of the Danube River, in the area of the Vetren village, and found the highest lead content in the liver. Burada et al. (2017) measured the concentrations of heavy metals and metalloids (chromium, copper, mercury, nickel, lead and arsenic) in muscles of fifteen fish species, and they found the highest level of lead in *A. alburnus*. Duck et al. (2008) investigated the concentrations of Cd and Zn in tissues and organs (gills, bones, muscles, digestive system, liver and kidneys) of *A. alburnus* from the Topolnitsa dam, in Bulgaria. They found the highest concentration of Cd and Zn in kidneys and liver of *A. alburnus*.

CONCLUSIONS

This study provides new data on the content of heavy metals/metalloids (Cu, Cd and As) in *A. alburnus* for the upstream Danube River from the Bulgarian section. The concentration of the

investigated elements in tissues and organs of bleak decrease in the order: liver>skin>muscles. The study showed exceedances of Cd and As in the liver, skin and muscles of the examined specimens of *A. alburnus*. The obtained results do not indicate exceedances of Cu in the examined liver, skin and muscle samples. Exceedances of Cd and As, and of Cu and Cd were found in water samples and sediment samples, respectively. Due to the high values of bioconcentration factor and the positive linear correlations, *A. alburnus* can be used as a bioindicator for Cu, Cd and As content. The results for Cd, as well as those for As, are the most significant.

ACKNOWLEDGEMENTS

We thank the Agricultural University – Plovdiv and Centre of research, technology transfer and protection of intellectual property rights at the Agricultural University for the funds received in connection with PhD thesis. We also thank Ms Hristova for the chemical analyzes performed at the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia.

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