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CONTENT OF COPPER, CADMIUM AND ARSENIC IN *Chondrostoma nasus* (Linnaeus, 1758) FROM THE DANUBE RIVER

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Abstract

This study presents the result of investigations of heavy metals (Cu, Cd) and metalloids (As) in the liver, skin and muscles of Chondrostoma nasus (Linnaeus, 1758), as well as in waters and sediments of the Danube River (Kudelin village, Vidin region), Bulgaria. The highest levels of Cu (23.05 ± 16.14 mg.kg^{-1}), Cd (0.08 ± 0.04 mg.kg^{-1}) and As (9.24 ± 4.79 mg.kg^{-1}) was found in samples of liver. The content of the studied elements decreased in the following order: liver>skin>muscle. The highest values for As content (0.07 ± 0.05 mg.l^{-1}) were found in surface waters, while the highest values for Cu content (204.09 ± 121.05 mg.kg^{-1}) were found in sediments. The circulation of the studied elements in the freshwater ecosystem was investigated. The excesses in the values of the obtained results were analyzed against regulated national and international standards.

Key words*: arsenic, cadmium, Chondrostoma nasus, copper, Danube River.*

INTRODUCTION

The Danube River is 2,857 km longest river in Europe. It flows into the Black Sea (Ilie et al., 2017a). Human activity has a substantial impact on the Danube and causes pollution of its waters with various pollutants, such as heavy metals (Ilie et al., 2017b). Contamination with heavy metals such as copper, cadmium, arsenic, lead, and others is significantly close to mining areas. Subsequently, many of the pollutants in these areas may enter the rivers (Cholakova et al., 2006). Fish cannot avoid the impact of all the contaminants found in the surrounding aquatic environment (Yarsan & Yipel, 2013). Pollutants, including heavy metals, accumulate in fish (Jovanović et al., 2017). In the tissues and organs of fish, heavy metals can be found in different concentrations. Metal concentrations also depend on the fish species (Lenhardt et al., 2012). Muscles and liver of fish are the most commonly tested for heavy metal content. Muscles - because they are an important part of the human diet and the liver – because it accumulates heavy metals in very high concentrations (Jovičić et al., 2018). Few authors have conducted studies on the content of heavy metals in tissues and organs of *C. nasus* from the Danube River (Milošković et al., 2016; Subotić et al., 2019) or from rivers and dams from the Danube River Basin (e.g. from the Nitra River (Stranai & Andreji, 2007; Andreji et al., 2012), from the rivers Pek, Tisa, South Morava, West Morava, Drina (Milošković et al., 2016), from rivers in Austria (Jirsa et al., 2008), from the dam Međuvršje from the West Morava River sub-basin (Đikanović et al., 2016a; Đikanović et al., 2016b). The content of heavy metals in waters and sediments from the Bulgarian section of the Danube River have also been carried out (Kirin et al., 2013; Kirin et al., 2014; Chunchukova et al., 2016; Chunchukova & Kirin, 2017; Chunchukova & Kuzmanova, 2017; Kirin & Chunchukova, 2017; Shukerova et al., 2017), to the Serbian section (Pajević et al., 2008; Antonijević et al., 2014; Ćirić et al., 2016; Milanov et al., 2016), to the Romanian section of the river (Woitke et al., 2003; Milenkovic et al., 2005; Teodorof et al., 2007; Vosniakos et al., 2008; Urdeş et al., 2010; Vuković et al., 2011; Gati et al., 2013; Ilie et al., 2014; Vuković et al., 2014; Ionescu et al., 2015a; Ionescu et al., 2015b; Morina et al., 2015; Burada et al., 2016; Gati et al., 2016; Morina et al., 2016; Radu et al., 2016; Teodorof et al., 2016; Tudor et al., 2016; Vasile et al., 2016; Ilie et al., 2017a; Ilie et al., 2017b; Radu et al., 2017; Rusu, 2017; Begy et al., 2018; Catianis et al., 2018).

This study presents the concentrations of heavy metals (Cu, Cd) and metalloids (As) in the liver, skin, and muscles of *Chondrostoma nasus* (Linnaeus, 1758), as well as in waters and sediments of the Danube River; to trace the circulation of the studied elements in the freshwater ecosystem.

MATERIALS AND METHODS

In 2019, fish, water and sediment were collected and examined from the Bulgarian section of the Danube River (Kudelin village, Vidin region, designated as Kudelin biotope). The village of Kudelin (44 ° 11 ′ 30 ″ N, 22 ° 40 ′ 5 ″ E) is located in northwestern Bulgaria, on the border with the Republic of Serbia and the Republic of Romania, the last settlement on the territory of the Republic of Bulgaria along the river course.

Thirty samples of common nase *Chondrostoma nasus* (L., 1758), five samples of water and four samples of sediment were collected. Species belonging to the caught fish specimens were determined by Karapetkova and Jivkov (2006), Kottelat and Freyhof (2007). The scientific name of the species is found in FishBase (Froese & Pauly, 2019). The fish are caught according to the requirements of the fishing permits for scientific research issued by the Executive Agency for Fisheries and Aquaculture, the Ministry of Agriculture, Food and Forests in Bulgaria. The metric data (weight (g) in grams, maximum length (L) and maximum body width (H) in centimetres) of all examined specimens *C. nasus* were determined (Table 1).

Table 1. Metric data (L, H and g) of the examined specimens *C. nasus* from the Danube River, Kudelin biotope

C. nasus	Min. - max.	Average ± SD
L	18-35.5	31.56 ± 3.44
H	4.3-8.5	6.64 ± 0.83
g	59-435	290.03 ± 76.12

From all specimens were collected samples of liver, skin and muscle using standard methods. The samples were analyzed in an accredited laboratory for atomic absorption spectrophotometry of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia by ISP "Optima 7000" Perkin-Elmer. Based on the results obtained from chemical analyzes, the concentrations of the investigated heavy metals in the tissues and organs of *Chondrostoma nasus* ($mg.kg^{-1}$ wet weight; $mg.kg^{-1}$ dry weight) as well as in water ($mg.l^{-1}$) and sediment ($mg.kg^{-1}$ dry weight) were determined. The bioconcentration factor (BCF) and the linear correlation coefficient of Spearman (r_s) were calculated. The software was used for statistical data processing, MS Excel (Microsoft, 2010), BioDiversity Pro (McAleece, 1997) and Statistica 10 (StatSoft Inc., 2011).

RESULTS AND DISCUSSIONS

The object of study is common nase (*Chondrostoma nasus* Linnaeus, 1758) from the Danube River, Kudelin biotope, northwestern Bulgaria. *C. nasus* is a freshwater fish of the Cyprinidae family. Occurs in rivers where the stream is moderate or fast. Prefers rocky or gravelly bottom. *C. nasus* is a herbivorous species (Kottelat and Freyhof, 2007).

The results of studies on the content of copper (Cu), cadmium (Cd) and arsenic (As) in the liver, skin and muscles in mean samples of 30 specimens of *C. nasus* are presented in Table 2 and Table 3. The content of the three elements in five water samples and four sediment samples from the Danube River, Kudelin biotope, was also determined.

From the tissues and organs of *C. nasus*, the highest levels of Cu, Cd, and As were found in liver samples (for wet weight, respectively C_{Cu} = 23.05 ± 16.14 $mg.kg^{-1}$; C_{Cd} = 0.08 ± 0.04 $mg.kg^{-1}$ and C_{As} = 9.24 ± 4.79 $mg.kg^{-1}$); followed by skin (wet weight respectively C_{Cu} = 1.64 ± 0.75 $mg.kg^{-1}$; C_{Cd} = 0.07 ± 0.04 $mg.kg^{-1}$ and C_{As} = 4.59 ± 2.33 $mg.kg^{-1}$) and muscle (wet weight respectively C_{Cu} = 0.47 ± 0.11 $mg.kg^{-1}$, C_{Cd} = 0.01 ± 0.01 $mg.kg^{-1}$ and C_{As} = 1.19 ± 0.59 $mg.kg^{-1}$). Concentrations of the studied heavy metals decrease in the order: liver>skin>muscles. In the water samples was found the highest content of As (C_{As} = 0.07 ± 0.05 $mg.l^{-1}$), followed by that of Cu (C_{Cu} = 0.04 ± 0.03 $mg.l^{-1}$) and Cd (C_{Cd} = 0.001 ± 0.001 $mg.l^{-1}$). In the sediment samples (dry weight) was found the highest content of Cu (C_{Cu} =

204.09 ± 121.05 mg.kg^{-1}), followed by the content of As (C_{As} = 19.52 ± 9.76 mg.kg^{-1}) and Cd (C_{Cd} = 1.54 ± 0.35 mg.kg^{-1}) (Table 2 and Table 3).

Table 2. Content of Cu, Cd and As (mg.kg^{-1} wet weight) in tissues and organs of *C. nasus* and water (mg.l^{-1}) from the Danube River, Kudelin biotope

Chondrostoma nasus		**Cu**	**Cd**	**As**
Liver	Min. - max.	11.08-45.31	0.03-0.13	2.82-13.95
	Mean ± SD	23.05 ± 16.14	0.08 ± 0.04	9.24 ± 4.79
Skin	Min. - max.	1.02-2.73	0.03-0.11	2.20-7.75
	Mean ± SD	1.64 ± 0.75	0.07 ± 0.04	4.59 ± 2.33
Muscle	Min. - max.	0.32-0.58	0.01-0.02	0.72-1.99
	Mean ± SD	0.47 ± 0.11	0.01 ± 0.01	1.19 ± 0.59
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. Content of Cu, Cd and As (mg.kg^{-1} dry weight) in tissues and organs of *C. nasus* and sediments (mg.kg^{-1} dry weight) from the Danube River, Kudelin biotope

Chondrostoma nasus		**Cu**	**Cd**	**As**
Liver	Min. - max.	28.18-111	0.09-0.34	12.61-36.81
	Mean ± SD	63.73 ± 38.01	0.23 ± 0.13	21.24 ± 11.03
Skin	Min. - max.	2.28-7.30	0.08-0.34	8.68-30.79
	Mean ± SD	4.46 ± 2.11	0.20 ± 0.12	16.64 ± 10.20
Muscle	Min. - max.	1.23 – 2.48	0.02-0.07	3.06-7.59
	Mean ± SD	1.97 ± 0.53	0.04 ± 0.02	4.75 ± 2.08
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 concentration of Cu, Cd and As in the tissues and organs of *C. nasus* is compared to the norms in Ordinance No. 31 of 2004 on the maximum levels of contaminants in foodstuffs (C_{Cu} = 10 mg/kg; C_{Cd} = 0.05 mg/kg; C_{As} = 1 mg/kg) by national law, as well as to the norms indicated by WHO (C_{Cu} = 20 mg/kg) and FAO (C_{Cu} = 30 mg/kg; C_{Cd} = 0.2 mg/kg). The concentration of Cu in the liver of *C. nasus* was established to exceed the norms specified in Ordinance No. 31 and WHO by 2.30 and 1.15 times, respectively. The concentration of Cd in the liver and skin of *C. nasus* exceeds the norms specified in Ordinance No. 31 by 1.56 and 1.32 times, respectively. The concentration of As in the liver, skin and muscles of *C. nasus* exceeds the norms specified in Ordinance No. 31 by 9.24, 4.59 and 1.19 times, respectively (Figure 1).

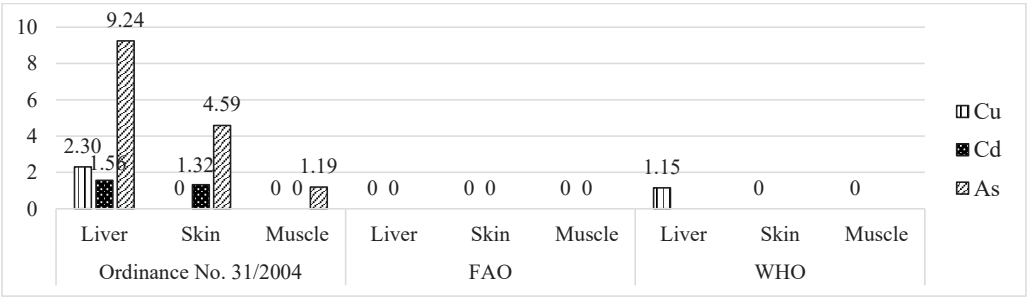

Figure 1. Exceedances of Cu, Cd and As content in liver, skin and muscle of *Chondrostoma nasus* from Danube River, Kudelin biotope according to regulatory documents

The concentration of Cu, Cd and As in the water samples is compared to the norms in Ordinance No. 18 of 2009 on the quality of water for irrigation of crops (C_{Cu} = 0.2 mg/dm^3; C_{Cd} = 0.01 mg/dm^3; C_{As} = 0.1 mg/dm^3), in Ordinance No. H-4 of 2012 on the characterization of surface water (C_{As} = 0.025 mg/l) and in the Ordinance on environmental quality standards for priority substances and certain other pollutants of 2010 (C_{Cd} = 0.0009 mg/l). The Cd concentration of the water samples was established to exceed 1.11 times the maximum permissible concentrations (MPC) specified in the Ordinance on environmental quality standards for priority substances and certain other pollutants. It was also found that the concentration of As in the water samples exceeds 2.96 times the MPC in Ordinance No. H-4 (Figure 2).

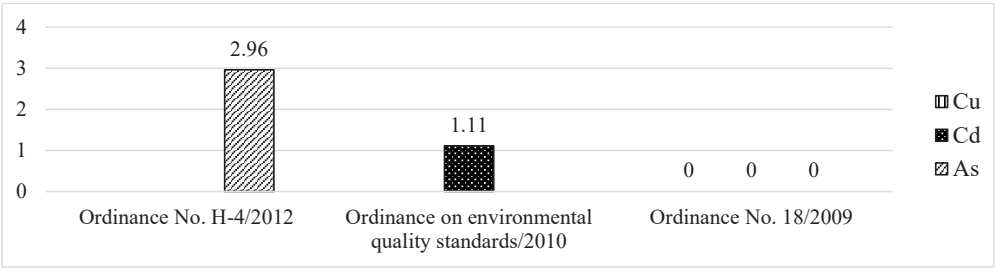

Figure 2. Exceedances of Cu, Cd and As content in surface waters of the Danube River, Kudelin biotope according to regulatory documents

The concentration of Cu, Cd and As in the sediment samples is compared to the norms in Ordinance No. 3 on the norms for permissible content of harmful substances in soils (C_{Cu} = 150 mg/kg and C_{Cd} = 2 mg/kg at pH = 7.4; C_{As} = 25 mg/kg) from national law and with the Dutch target values (C_{Cu} = 36 mg/kg; C_{Cd} = 0.8 mg/kg; C_{As} = 29 mg/kg). The concentration of Cu and Cd in the sediment samples was established to exceed 5.67 and 1.92 times the Dutch target values, respectively. Also, the concentration of Cu in sediments exceeds the MPC in Ordinance No. 3 by 1.36 times (Figure 3).

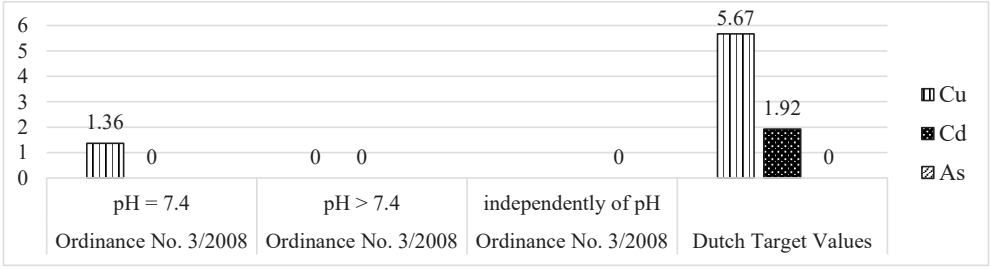

Figure 3. Exceedances of Cu, Cd and As content in sediments from the Danube River, Kudelin biotope relative to regulatory documents

The highest bioconcentration of both water (BCF = [$C_{host\ tissues}$]/[C_{water}]) and sediment (BCF = [$C_{host\ tissues}$]/[$C_{sediments}$]) was found regarding Cu, Cd and As in the samples from liver followed by those in the skin. Bioconcentration is the lowest in the muscles. The liver of the common nase bioaccumulates 4.99 times more Cu from the water compared to the accumulation of As and 7.98 times more compared to the degree of accumulation of Cd. The liver of *C. nasus* accumulates 1.60 times more As from the water, compared to that of Cd. The highest bioaccumulation of As from sediments in liver samples was established (3.48 times more than the accumulation of Cu and 7.2 times more than the accumulation of

Cd). The accumulation of Cd, both from water and from sediment in liver and skin samples, is close (statistically insignificant differences; $p>0.05$) and is 6-1 times higher than that in the muscle samples (Table 4, Table 5).

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

Chondrostoma nasus/Water	BCF_{Cu}	BCF_{Cd}	BCF_{As}
C_{liver}/C_{water}	622.86	78	124.82
C_{skin}/C_{water}	44.37	66	61.97
C_{muscle}/C_{water}	12.56	13	16.12

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

Chondrostoma nasus/Sediments	BCF_{Cu}	BCF_{Cd}	BCF_{As}
$C_{liver}/C_{sediments}$	0.31	0.15	1.08
$C_{skin}/C_{sediments}$	0.02	0.13	0.85
$C_{muscle}/C_{sediments}$	0.01	0.03	0.24

Positive linear correlations were found between the content of monitored elements in the liver, skin and muscles of the common nase and those in the waters and sediments of the Danube River ($r_s = 0.98 - 1.0$; $p<0.05$), proving the direct influence of the aquatic environment and the sediments on the content of the monitored elements in the tissues and organs of the common nase, except for the correlation between content of Cu in muscle and that in sediment samples ($r_s = 0.50$; $p>0.05$). A very high correlation and significance of the majority of the discussed results were found.

Studies on the content of heavy metals in tissues and organs of common nase from the Danube River are relatively small. To the present stage, such studies have not been carried out for a common nase from the Bulgarian section of the river. Jirsa et al. (2008) researched *C. nasus* from several rivers in Austria, including the Danube River, and established the highest content of cadmium and copper in liver samples. Subotic et al. (2019) studied muscle and liver of *C. nasus* from the Serbian section of the Danube River and found higher levels of Ba, Cu, Fe, and Zn in muscle samples and higher levels of Ba, Cd, Cu, and Mn in liver samples. Andreji et al. (2012) examined muscles of *C. nasus* from the Nitra River, Slovakia for heavy metal concentration and found that the metals decreased in the order: Zn>Cu>Fe>Mn>Pb>Ni>Cd. Đikanović et al. (2016a) analyzed the content of heavy metals in the liver, muscle and gills of nine fish species including *C. nasus* from the Međuvršje dam, Serbia. They established the highest concentrations of heavy metals in the liver and gills.

CONCLUSIONS

The first data for the content of Cu, Cd and As in the liver, skin and muscles of *C. nasus* is presented for the Bulgarian section of the Danube River (from the northwestern section of the river and the territory of the country). The highest content of the studied elements was found in liver samples and the lowest in the muscles. Exceedances of Cu, Cd and As content was detected in liver samples of *C. nasus*. Exceedances of Cd and As were found in skin samples, and only exceedance of As was found in muscle samples, according to national regulations. High BCF values and positive linear correlation dependencies are grounds *C. nasus* be proposed to be included as a bioindicator in biomonitoring systems regarding the content of the monitoring elements, based on their representation in liver samples. The most indicative and significant are the results for As, followed by those for Cu.

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