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RESEARCH ARTICLE

Contents of Cu, Cd, and As in *Chondrostoma nasus*, *Pomphorhynchus laevis*, and *Contracaecum* sp. from an anthropogenically loaded segment of the Danube River in Bulgaria

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Abstract. The circulation of the contents of Cu, Cd, and As was traced in the system of *Chondrostoma nasus* – *Pomphorhynchus laevis* – *Contracaecum* sp. – water – sediments from the Danube River in the Kudelin biotope. New data on the content of the elements examined are presented. The concentrations of the three elements in the materials studied, which included tissues, organs, and parasites of common nase, and water and sediments from the Kudelin biotope, were higher than the limits set forth in Bulgarian and international legislative documents. The bioindicator role of the tissues, organs, and parasites examined was revealed, and the liver of common nase and *Contracaecum* sp. were good bioindicators for Cd content, while *P. laevis* was a good bioindicator for As content.

Keywords: As, bioindication, Cd, common nase, Cu, Kudelin

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Introduction

Once the Danube River enters Bulgarian territory, its low-lying areas are strongly polluted with heavy metals such as As and Cu (Stoyanova et al. 2018, 2019, Stoyanova and Kotsev 2020). Data are available on the contamination of the Timok River with As, Cu, Cd, Pb, Ni, Cr (Zhekova 2011), Cu (Stoyanova et al. 2019), Cu, As, Zn and others (Kotsev and Zhelezov 2014). The mines in the city of Bor have been identified as sources of pollution (Zhekova 2011, Kotsev and Zhelezov 2014). Urošević et al. (2018) examined the environmental impact of mining complexes (in Bor and Majdanpek) in Eastern Serbia and reported that mining and metallurgy were serious sources of air and water pollution. These authors reported concentrations of As in the air in the Bor area that were several times higher compared to those in other cities in Serbia. Winds can carry such pollutants to neighboring countries such as Bulgaria and Romania. Prelec (2021) reported that large amounts of As (600 times over the norm) were recorded in the air in Bor in December 2019. Fish tissues and organs can be used successfully as bioindicators of the presence of

Figure 1. Location of the Kudelin biotope along the Danube River, Bulgaria.

heavy metals/metalloids in aquatic environments (Authman et al. 2015), while various authors have demonstrated that fish parasites from the Danube River in Bulgaria can also be used as bioindicators (Chunchukova and Kirin 2017, Chunchukova and Kuzmanova 2017, Chunchukova et al. 2017a, 2017b, Kirin and Chunchukova 2017, Chunchukova et al. 2020). A few authors have studied the heavy metal contents in the tissues and organs of fishes from the Danube River (Chunchukova et al. 2017a, 2017b, Chunchukova et al. 2020 and others). Studies on concentrations of heavy metals/metalloids in fishes were also conducted, among others, in the Romanian segment of the Danube River (Mititelu et al. 2012, Pantelica et al. 2012, Ioniță et al. 2014) as well as in its Serbian (Kostić-Vuković et al. 2021, Subotić et al. 2021) and Croatian segments (Zrnčić et al. 2013). Studies on concentrations of heavy metals in fish parasites from the Danube River have also been conducted in Serbia (Despotović et al. 2007), Hungary (Thielen et al. 2004), Austria (Jirsa et al. 2008), and other countries. There is a lack of research on the concentrations of heavy metals in the tissues, organs, and parasites of common nase from the Bulgarian segment of the Danube River. Studies on the concentrations of heavy metals in the tissues and organs of common nase have been reported only from the Austrian (Jirsa et al. 2008), Serbian (Subotić et al. 2019), and Croatian (Zrnčić et al. 2013) segments of the river. Common nase is one of the fish species targeted in commercial and recreational fishing that is popular among consumers (Karapetkova and Zhivkov 2006, Subotić et al. 2019). These factors provided the impetus for the present study.

This study aims to trace the circulation of Cu, Cd, and As in the system of *Chondrostoma nasus* (L.) tissues and organs – *Contracaecum* sp. – *Pomphorhynchus laevis* – water – sediments from the Danube River in the Kudelin biotope.

Materials and Methods

In 2019–2021, a total of 270 specimens of common nase, *Ch. nasus* were caught from the vicinity of Kudelin village (designated as a biotope; 44°12’07.9" N 22°41’27.0" E). The village of Kudelin is located on the right bank of the Danube River (river km 844) about 2 km from the border with the Republic of Serbia in the Vidin District of northwestern Bulgaria (Fig. 1).

The fish were caught using various net gears permitted by the Executive Agency for Fisheries and Aquaculture, Ministry of Agriculture to catch fishes for scientific purposes. The fish were caught according to the standard BS EN 14757:2015 Water quality – Sampling of fishes with multi-mesh gillnets.

Catches of the specimens studied were conducted in three seasons: spring (22.03–22.06), summer (22.06–22.09), and fall (22.09-22.11) in each of the three years in which the study was conducted. The fish species was identified according to Karapetkova and Zhivkov (2006) and Fröse and Pauly (2022). The main metric data of total length (L) in cm, maximum height (H) in cm, and body weight (W) in g were calculated as follows: 14–39.6, 32.05 ± 3.77 for L; 3–9.5, 7.14 ± 1.03 for H; 17–792, 321.64 ± 104.71 for W.

In the present study, the contents of Cu, Cd, and As were determined in samples of liver, skin, and muscles of common nase, helminths (*P. laevis* and *Contracaecum* sp.), and water and sediments from the Danube River (Kudelin biotope) for the period of 2019–2021. The contents of the three elements examined in the fish and helminth samples are presented in mg kg^{-1} wet weight and mg kg^{-1} dry weight; in water samples – mg l^{-1}; in sediment samples – in mg kg^{-1} dry weight. Mean representative samples of liver, skin, and muscles, collected from a total of 270 specimens of common nase (30 specimens per season) were analyzed chemically.The common nase specimens collected were subjected to helminthological study (Zashev and Margaritov 1966). *P. laevis* and *Contracaecum* sp. were selected as model helminth species to study the content of heavy metals/metalloids.

A total of 27 water and 27 sediment samples were collected from the Danube River (Kudelin biotope) according to ISO 5667-6:2016 (ISO 2016) and ISO 5667-12:2017 (ISO 2017) standards. Samples of tissues and organs (liver, skin, and muscles), *Ch. nasus* parasites, and water and sediments from the Danube River were analyzed chemically to determine the content of Cu, Cd, and As. The chemical analyses were conducted in an accredited laboratory of the Institute of Biodiversity and Ecosystem Research (IBER) at the Bulgarian Academy of Sciences (BAS) with a PerkinElmer OPTIMA 7000 DV ICP-OES.

Bioconcentration (1) and bioaccumulation factors (2) and Spearman's rank correlation coefficients (r_s) (3) are presented (Sokal and Rohlf 1981, Sabljic and Protic 1982, Chok 2010, Yarsan and Yipel 2013):

(1) The bioconcentration factor (BCF) was calculated using the formula:

$$BCF = \frac{\left[C_{fishtissues\ and\ organs\,/\,parasites}\right]}{\left[C_{water\,/\,sediments}\right]}\ \ where:$$

$C_{\text{fish tissues and organs/parasites}}$ – concentration of the element studied in fish tissues and organs/parasites;

$C_{\text{water/sediments}}$ – concentration of the element studied in water/sediments;

The bioconcentration factor is the ratio of the concentration of the element studied in fish tissues and organs/parasites and the concentration of the element studied in water/sediments.

(2) The bioaccumulation factor (BAF) was calculated using the formula:

$$BAF = \frac{\left[C_{parasites}\right]}{\left[C_{fish\ tissues\ and\ organs}\right]}\ \ where:$$

$C_{\text{parasites}}$ – concentration of the element studied in parasites;

$C_{\text{fish tissues and organs}}$ – concentration of the element studied in fish tissues and organs.

The bioaccumulation factor indicates the ability of the parasites to accumulate the element studied compared to the tissues and organs of the fish hosts.

(3) Spearman's rank correlation coefficient (r_s) was calculated using the formula:

$$r_s = \frac{\sum_{i=1}^{n}((Rx_i) - \overline{R(x)})(R(y_1) - \overline{R(y)}))}{\sqrt{\sum_{i=1}^{n}((R(x_i) - \overline{R(x)}))^2 \sum_{i=1}^{n}((R(y_i) - \overline{R(y)}))^2}}$$

where:

rank(Rx_i) and *rank(Ry_i)* are the ranks of the samples.

Spearman's rank correlation coefficient (r_s) was used to examine the relationship among concentrations of heavy metals/metalloids in tissues and organs of fish, their parasites, water, and sediments. Coefficient values ranged from -1 to +1. The relation is weak when the value of r_s approaches 0. The differences were statistically significant at $P<0.05$.

The content of Cu, Cd, and As in tissues and organs and parasites of common nase from the Danube River were compared with the maximum permissible concentrations (MPC) in Ordinance No. 31 of 29 July 2004 on the maximum levels of contaminants in foodstuffs (10 mg kg^{-1} for Cu; 0.05 mg kg^{-1} for Cd; 1 mg kg^{-1} for As) and with the norms of the Food and Agriculture Organization (FAO 2022) (30 mg kg^{-1} for Cu; 0.2 mg kg^{-1} for Cd) and the World Health Organization (WHO) (20 mg kg^{-1} for Cu). The content of the elements studied in water was compared with the norms in Ordinance H-4 of 14 September 2012 on the characterization of surface water (average annual value, $CaCO_3$ 100-250 – 0.01 mg l^{-1} for Cu; MPC – 0.025 mg l^{-1} for As); the ordinance on environmental quality standards for priority substances and certain other pollutants (MPC, class 4 – 0.0009 mg l^{-1} for Cd); Ordinance No. 18 of 27 May 2009 on the quality of water for crop irrigation (MPC – 0.2 mg dm^{-3} for Cu; 0.01 mg dm^{-3} for Cd; 0.1 mg dm^{-3} for As). The content of the elements studied in sediments was compared with Dutch target values (36 mg kg^{-1} dry weight for Cu; 0.8 mg kg^{-1} dry weight for Cd; 29 mg kg^{-1} dry weight for As) and Ordinance No. 3 of 1 August 2008 on the norms for the permissible content of harmful substances in soils (MPC for agricultural land (pH 6.00–7.4) – 150 mg kg^{-1} for Cu;

Table 1
Content of Cu, Cd, and As in tissues, organs, and helminths of *Chondrostoma nasus*, water, and sediments

The Danube River/ Kudelin biotope		Cu		Cd		As	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Liver	mg kg^{-1} wet weight	3.03-55.65	26.23 ± 15.18	0.03-4.09	0.93 ± 0.86	2.43-48.70	10.71 ± 10.06
	mg kg^{-1} dry weight	7.39-426.60	85.61 ± 78.87	0.09-15.91	2.67 ± 3.11	5.09-146.25	30.17 ± 30.02
Skin	mg kg^{-1} wet weight	0.28-1.95	0.87 ± 0.37	0.03-0.28	0.12 ± 0.07	0.95-14.90	4.42 ± 3.24
	mg kg^{-1} dry weight	0.64-5.64	2.34 ± 1.07	0.08-2.26	0.50 ± 0.45	2.39-41.62	12.08 ± 9.41
Muscles	mg kg^{-1} wet weight	0.09-0.82	0.38 ± 0.19	0.01-0.23	0.07 ± 0.05	0.37-9.30	1.90 ± 1.92
	mg kg^{-1} dry weight	0.42-3.31	1.55 ± 0.73	0.04-0.69	0.24 ± 0.16	1.34-41.19	7.42 ± 8.10
Water	mg l^{-1}	0.01-0.32	0.07 ± 0.07	0.0004-0.10	0.01 ± 0.02	0.013-8.44	2.65 ± 2.21
Sediments	mg kg^{-1} dry weight	33.37-630.04	275.65 ± 184.80	0.15-88.91	6.08 ± 18.73	12.00-804.82	423.41 ± 270.48
Contracaecum sp.	mg kg^{-1} wet weight	0.25-51.56	15.07 ± 19.00	0.43-9.29	3.51 ± 3.36	27.96-111.54	79.10 ± 28.58
	mg kg^{-1} dry weight	45.00-77.00	65.67 ± 17.93	5.84-18.99	10.55 ± 7.33	123.00-308.33	190.46 ± 102.44
Pomphorhynchus laevis	mg kg^{-1} wet weight	3.27-14.69	9.25 ± 5.73	0.82-3.67	2.31 ± 1.43	130.61-587.76	370.07 ± 229.35

2 mg kg^{-1} for Cd; MPC for agricultural land, independently of pH – 25 mg kg^{-1} for As).

Statistical data processing was performed with MS Excel (Microsoft, 2010) and Statistica 10 (StatSoft Inc., 2011).

Results

Circulation of Cu, Cd, and As in the system of *Ch. nasus* tissues and organs – helminths – water – sediments from the Danube River, Kudelin biotope

The content of the heavy metals/metalloids studied was higher in common nase liver samples than in skin or muscle samples. A reliable functional (deterministic) dependence regarding Cu, Cd, and As, and their contents in corresponding samples of common nase liver, skin, and muscles (Spearman correlation coefficient $r_s = 1.0$, n = 3-9) was determined. The type of *Ch. nasus* tissue and organ samples were determined by the content of the elements analyzed (Friedman test F = 6.0; P = 0.049).

The content of Cu, Cd, and As in the common nase tissues and organs assayed decreased as follows: liver > skin > muscles. The concentrations of Cu in common nase liver samples were 69.03 times higher than those in muscle samples. The Cd content in liver samples was 13.29 times higher than that in muscle samples. As concentrations were 5.65 times higher in common nase liver samples than in muscle samples. The concentrations of heavy metals/metalloids in common nase liver samples decreased in the following order: Cu > As > Cd. The concentrations of the three elements in skin and muscle samples were as follows: As > Cu > Cd (Table 1).

The concentrations of the elements tested in the samples of water, sediments, and helminths were in the following order: As > Cu > Cd. Significant differences were noted between the concentrations of As ($t_{As} = 29.45$, P = 0.03) and Cu ($t_{Cu} = 21.37$, P = 0.04) in *P. laevis* and *Contracaecum* sp. The type of helminth in common nase was relevant to the content of both elements (Table 1).

The concentrations of Cu and Cd (mg kg^{-1} wet weight) in the samples examined of common nase tissues and organs were up to 374.71 and 93 times higher, respectively, than those in water. The content of As in samples of common nase liver and skin was 4.04 and 1.67 times higher, respectively, than that in water, while in the samples of muscles was 1.39 times lower. Only the concentrations of Cu in common nase liver samples exceeded concentrations of this element in its helminths, *P. laevis* and *Contracaecum* sp., which were 1.74 and 2.84 times higher, respectively. The content of Cu in muscle samples was 39.66 times lower than that in *Contracaecum* sp. and 24.34 times lower than that in *P. laevis*. The levels of Cd and As in *Contracaecum* sp. were up to 50.14 and 41.63 times higher, respectively, than those in the tissues and organs of its host. The concentrations of Cd and As in *P. laevis* were up to 33 and 194.77 times higher, respectively, than those in common nase tissues and organs. The content of Cu, Cd, and As in *Contracaecum* sp. was 215.29, 351, and 29.85 times higher, respectively, than that in water. The concentrations of Cu, Cd, and As in *P. laevis* were 132.14, 231, and 139.65 times higher, respectively than those in water.

The concentrations of Cu and As in the nematode *Contracaecum* sp. were 4.20 and 2.22 times lower, respectively, than those in sediments, while that of Cd was 1.74 times higher.

The values of the bioconcentration factor for Cu, Cd, and As in common nase tissues and organs compared to water were the highest for Cu in the liver. The values of the bioconcentration factor in *P. laevis* in comparison to water were the highest for Cd and the lowest for Cu. The values of the bioconcentration factor in *Contracaecum* sp. compared to water were the highest for Cd and the lowest for As. The value of the bioconcentration factor in common nase liver compared to sediments was the highest for Cd and the lowest for As. The value of the bioconcentration factor in *Contracaecum* sp. in comparison to sediments was the highest for Cd and the lowest for Cu (Table 2).

Table 2
Bioconcentration and bioaccumulation factors

Chondrostoma nasus/ Water	BCF_{Cu}	BCF_{Cd}	BCF_{As}
C liver / C water	374.71	93.00	4.04
C skin / C water	12.43	12.00	1.67
C muscles / C water	5.43	7.00	0.72
C *P. laevis* / C water	132.14	231.00	139.65
C *Contracaecum* sp. / C water	215.29	351.00	29.85
Chondrostoma nasus/ Sediments	BCF_{Cu}	BCF_{Cd}	BCF_{As}
C liver / C sediments	0.31	0.44	0.07
C skin / C sediments	0.01	0.08	0.03
C muscles / C sediments	0.01	0.04	0.02
C *Contracaecum* sp. / C sediments	0.24	1.74	0.45
Helminths/ *Chondrostoma nasus*	BAF_{Cu}	BAF_{Cd}	BAF_{As}
C *P. laevis*/ C liver	0.35	2.48	34.55
C *P. laevis*/ C skin	10.63	19.25	83.73
C *P. laevis*/ C muscled	24.34	33.00	194.77
C *Contracaecum* sp./ C liver	0.57	3.77	7.39
C *Contracaecum* sp./ C skin	17.32	29.25	17.90
C *Contracaecum* sp./ C muscles	39.66	50.14	41.63

A very strong correlation was found in the content of Cu and Cd between the tissues/organs of common nase and its parasites *P. laevis* (r_s = 0.98–0.99, P < 0.001 for Cu; r_s = 0.94-0.99, P < 0.001 for Cd) and *Contracaecum* sp. (r_s = 0.98–0.99, P < 0.001 for Cu; r_s = 0.99, P < 0.001 for Cd); between tissues/organs of common nase and water (r_s = 0.96–0.98; P < 0.001 for Cu; r_s = 0.96–0.99, P < 0.001 for Cd); between tissues/organs of common nase and sediments (r_s = 0.97–0.99; P < 0.001 for Cu; rs = 0.97–0.99, P < 0.001 for Cd); between *Contracaecum* sp. and water (r_s = 0.98; P < 0.001 for Cu; r_s = 0.96; P < 0.001 for Cd); and between *P. laevis* and water (r_s = 0.93; P < 0.001 for Cu). A very strong correlation was noted in the content of As between *P. laevis* and tissues/organs of common nase (r_s = 0.92–0.95, P < 0.001); between *Contracaecum* sp. and skin of common nase (r_s = 0.91, P < 0.001); and between tissues/organs of common nase and sediments (r_s = 0.91–0.95, P < 0.001).

Excesses of Cu, Cd, and As in tissues and organs of Ch. nasus, helminths, water, and sediments from the Danube River, Kudelin biotope

The highest excess of the three elements investigated was reported in the liver samples – 2.62 times (for Cu), 18.6 times (for Cd), and 10.71 times (for As) compared to MPC in Ordinance No. 31. The concentrations of Cu in liver exceeded the WHO norm by 1.31 times. Concentrations of Cd in the liver exceeded FAO norm by 4.65 times. In samples of common nase liver and muscles, excesses of Cd (2.4 times in skin samples and 1.4 times in muscle samples) and As (4.42 times (in skin samples and 1.9 times in muscle samples) were noted in comparison to values in Ordinance No. 31. No excess Cu was detected.

The highest excess As was noted in *P. laevis* and *Contracaecum* sp. and was 370.07 and 79.1 times higher, respectively, compared to the MPC in Ordinance No. 31. The concentration of Cu in two helminths exceeded the norms in Ordinance No. 31 (by 46.2 times in *P. laevis* and 70.2 times in *Contracaecum* sp.) and the FAO (11.55 times in *P. laevis* and 17.55 times in *Contracaecum* sp.). Only in *Contracaecum* sp. was excess Cu noted at 1.51 times compared to the MPC in Ordinance No. 31.

The highest As excess in water samples was 106 and 26.5 times higher than the norms in Ordinance H-4 and Ordinance No. 18, respectively. The highest excess of Cd content in water was 11.11 times higher than the MPC in the Ordinance on environmental quality standards. Cu content exceeded the norm in Ordinance H-4 by 7 times, but it did not exceed that in Ordinance No. 18.

Excesses of all three elements investigated were found in sediment samples, with the highest As excess at 16.94 times that in Ordinance No. 3. As also exceeded Dutch target values by 14.6 times. Cu and Cd concentrations in sediments exceeded Dutch target values by 7.66 and 7.6 times, respectively and Ordinance No. 3 by 1.84 and 3.04 times, respectively.

The results obtained for the excesses of the heavy metals/metalloids investigated in tissues, organs, and parasites of common nase indicated that the liver of *Ch. nasus* and the nematode *Contracaecum* sp. were good bioindicators for Cd, and the acanthocephalan *P. laevis* was a good bioindicator for As content.

Discussion

Studies on the concentrations of heavy metals/metalloids in tissues and organs of common nase from the Danube River and its basin in other countries have been conducted by a few authors. Jirsa et al. (2008) investigated heavy metals (including Cu and Cd) in the liver, intestine, muscle, and gills of common nase from the Austrian segment of the Danube River. These authors reported lower concentrations of Cu and Cd in the liver of *Ch. nasus* (Cu 25–333 μ g^{-1} dry weight; Cd 1.57 μ g^{-1} dry weight in uncontaminated places, and 5.58 μ g^{-1} dry weight in contaminated places) in comparison to the values noted in the present study. Zrnčić et al. (2013) studied the concentrations of some heavy metals/metalloids (including Cd and As) in muscles of *Ch. nasus* from the Croatian segment of the Danube River. These authors reported lower concentrations of Cd and As (Cd – 0.005-0.018 μ g^{-1} dry weight and 0.008 ± 0.005 μ g^{-1} dry weight; As – 0.011-0.045 μ g^{-1} dry weight and 0.022 ± 0.098 μ g^{-1} dry weight) than those reported in the present study. Studies on the content of heavy metals/metalloids in tissues, organs, and parasites of common nase from the Danube River and its basin in Bulgaria are lacking.

Various authors have investigated concentrations of Cu, Cd, and As in the water and sediments from the Bulgarian segment of the Danube River. Nachev (2010) and Nachev and Sures (2016) examined the content of these three elements in the water from the Danube River in the vicinity of the village of Novo Selo (in 2005 Cu – 17.5 μg L^{-1}, Cd – below detection limits, As – 2.2 μg L^{-1}; in 2006 Cu – 23.5 μg L^{-1}, Cd – below detection limits, As – 2.275 μg L^{-1}) and at the confluence of the Iskar River into the Danube River (in 2005 Cu – 5.15 μg L^{-1}, Cd – below detection limits, As – 2.692 μg L^{-1}; in 2006 Cu – 6.1 μg L^{-1}, Cd – below detection limits, As – 2.382 μg L^{-1}). Nachev et al. (2022) investigated the levels of Cu, Cd, and As in water from the Danube River in the vicinity of the town of Vidin (in 2010 Cu – 11.6 ± 4.6 μg L^{-1}, Cd – below detection limits, As – 1.8 ± 0.3 μg L^{-1}; in 2011 Cu – 8.0 ± 8.3 μg L^{-1}, Cd – below detection limits, As – 1.9 ± 0.3 μg L^{-1}). The concentrations of Cu, Cd, and As in water reported in previous studies were several times lower than those noted in the present study. Chunchukova and Kuzmanova (2017) and Chunchukova et al. (2020) examined the content of As in water and sediments from the lower segment of the Danube River in Bulgaria (Vetren biotope). The authors reported the following concentrations: $C_{AsWater}$ = 0.013 mg l^{-1} and $C_{AsSediments}$ = 5.74 mg kg^{-1}, which were 203.85 and 73.77 times lower, respectively, than those noted in the Kudelin biotope. Kirin et al. (2013) reported a concentration of Cu in sediments from the Danube River, Vetren biotope of

$C_{CuSediments}$ = 84.332 mg kg^{-1}, which was 3.27 times lower than that in the present study. Shukerova et al. (2017) examined Cd levels in water and sediments from the Danube River (Vetren biotope) and reported the following concentrations:$C_{CdWater}$ = 0.020 mg l^{-1} and $C_{CdSediments}$ = 4.307 mg kg^{-1}. The concentrations of Cd in water reported by these authors were two times higher than those in the water from the Kudelin biotope, while those in sediments were 1.41 times lower.

Based on the observed excesses in the muscles of *Ch. nasus*, it is recommended to restrict the consumption of this fish species from the study area. Because of the excesses of the investigated elements in common nase tissues and organs, it is recommended to conduct systematic studies on the content of heavy metals/metalloids in common nase tissues, organs, and helminths and in the water and sediments of the Danube River and from other freshwater ecosystems in Bulgaria.

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Author contributions. P.G.Z., D.A.K. designed the work, investigation, wrote the original draft, reviewed, edited. All authors approved the manuscript for publication.

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