Bulletin of Environmental Contamination and Toxicology (2019) 103:261–266
https://doi.org/10.1007/s00128-019-02657-3

Metal Accumulation in Muscle and Liver of the Common Nase (*Chondrostoma nasus*) and Vimba Bream (*Vimba vimba*) from the Danube River, Serbia: Bioindicative Aspects

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Received: 13 February 2019 / Accepted: 15 June 2019 / Published online: 21 June 2019

Abstract
Accumulation of 17 elements in muscle and liver of common nase and vimba bream, caught between February and May 2016 in the Danube River (1173 river kilometer), were assessed by ICP-OES. The principal component analysis grouped muscle and liver samples based on element concentrations (muscle grouped by higher Ba and Sr values, and liver grouped by higher Al, B, Cd, Cr, Cu, Fe, Mn, and Zn values), but no grouping between the two species was observed. Concentrations of Ba, Cu, Fe, and Zn were significantly higher in muscle, and concentrations of Ba, Cd, Cu, and Mn in liver of common nase, while vimba bream had significantly higher concentrations of Cr and Fe in liver. Common nase has a higher affinity for bioaccumulation of Cu, Fe, and Zn in muscle, while vimba bream has a higher affinity for Al, Cd, and Cr in muscle.

Keywords Cyprinid fish · Metal bioaccumulation · Large river · ICP-OES · Pollution · Diet

The Danube River is the second longest river in Europe with rather low overall amount of metal contamination (Woitke et al. 2003). While most of the metal load in the Danube system is a result of natural processes, anthropogenic sources have a higher impact in its tributaries (Comero et al. 2014; Morina et al. 2016). Anthropogenic sources of metal pollution in the Danube River include municipal waters, industry, and agriculture (ICPDR 2005). Sediments in the Danube River near Belgrade have a low to moderate toxic metal contamination, with concentrations above the target values, but not exceeding the intervention values (Antonijević et al. 2014).

Pollution of waterbodies with metals presents a major threat to aquatic ecosystems and biota, including fish. Bottom sediments have a significant role in toxic metal pollution (Comero et al. 2014), as the majority of metal input can be stored in river sediments (Peng et al. 2009) and further transferred to the food chain (Havelkova 2008). Up to 62% of metals can be released back to the water column from sediments (Olivares-Rieumont et al. 2005), and from there, they follow the pattern water–phytoplankton–zooplankton–fishes–humans (Has-Schon et al. 2006). Colombo et al. (2000) noted that omnivorous/detritivorous fish can present a potential danger for human consumption.

Pollutants are not distributed uniformly through the fish body tissues (Terra et al. 2007). Even though fish muscle is commonly used for analyses, being the main fish part used for human consumption, some tissues accumulate significantly higher amounts of pollutants than the muscle (Henry et al. 2004; Uysal et al. 2009). Liver is a target organ for storage and bioaccumulation of metals due to presence of metallothioneins (De Smet et al. 2001; Atli and Canli 2003).

The common nase (*Chondrostoma nasus*) and vimba bream (*Vimba vimba*) are two benthic cyprinid fish species that are commercially exploited along the Serbian section

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00128-019-02657-3) contains supplementary material, which is available to authorized users.

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of the Danube River (Smederevac-Lalic 2013). The common nase is one of the most frequent fish species in Europe, living in medium-sized or large rivers, with moderately or fast-flowing water (Kottelat and Freyhof 2007). It feeds on benthic algae and detritus (Reckendorfer et al. 2001), but can supplement its diet with insect larvae, snails, and fish eggs (Sysa et al. 2006). It is considered as a good bioindicator of ecosystem health (Jirsa et al. 2008; Rakowitz et al. 2009). The vimba bream also inhabits medium-sized to large rivers (Kottelat and Freyhof 2007). It is an omnivorous fish (Okgerman 2013), with mollusks and insect larvae as main food items, followed by crustaceans and plants (Bubinas and Vaitonis 2003; Kottelat and Freyhof 2007). Both species show migratory behavior to a greater or lesser extent (Huber and Kirchhofer 1998; Kesminas et al. 1999; Kottelat and Freyhof 2007; Ovidio and Philippart 2008).

There is lack of data and knowledge on ecotoxicology of the common nase and vimba bream in the Danube River. While both species are not of a higher economic importance, they are nonetheless, commercially exploited and used for human consumption. The aim of this study was to investigate metal accumulation in tissues of these two cyprinid species in a moderately polluted part of the Danube River, to see if there is a difference in specific metal enrichment in different tissues and between species, as well as to check whether these fish are suitable for human consumption. Additionally, we wanted to find out if the common nase and vimba bream could be used alternatively as bioindicators of metal pollution in rivers.

Materials and Methods

Thirty common nase and 43 vimba bream individuals were caught by portable fish nets in the Danube River, near the city of Zemun, at the 1173 river kilometer, between February and May 2016. Total length (in cm) and total weight (in g) of all sampled individuals were measured, and scales were taken to estimate the age of the analyzed fish. After measuring, fish were dissected with a plastic laboratory set. Liver and muscle samples were removed, washed with distilled water and stored at − 18°C for the element analysis. Digestive tracts were placed in 96% ethanol for subsequent diet analysis.

Tissue portions of around 0.5 g (wet weight) were processed in a microwave digester (ETHOS EASY Advanced Microwave Digestion System 230 V/50 Hz, Milestone, Italy), using 9 mL of 65% HNO_3 (Suprapur®, Merck) and 1 mL of 30% H_2O_2 (Suprapur®, Merck), at the fresh fish temperature program. The potential presence of the analyzed elements in chemicals used for digestion was resolved by a number of blank samples. After cooling to room temperature, digested samples were diluted with distilled water to a total volume of 25 mL. Concentrations of 17 elements (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sr, and Zn) in tissue samples were assessed by ICP-OES (Spectro Genesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany). Concentrations of all elements were expressed as mg kg^{-1} wet weight (ww).

Bioaccumulation is the result of the combined chemical uptake from water through gills as well as from dietary sources (Arnot and Gobas 2006). The bioaccumulation factor (BAF) is measured as the ratio of the mean element content in fish tissue (C_{fish}), expressed as mg kg^{-1} ww, and the element concentration in water (C_{water}), expressed as mg L^{-1}.

Principal component analysis (PCA) was used as an unsupervised statistical method that summarizes the variation of a data set between samples to a set of uncorrelated components (each point is a particular linear combination of the original variables), in order to assess the differentiation among the analyzed fish tissue, based on the element content. We tried to present, in an approximate manner, clusters of individuals within smaller dimensional subspaces. The untreated data for element content in each tissue were used as input variables for Eigenvector Solo 7.0 software. The Varimax method was used for orthogonal factors rotation.

Concentrations of eleven elements (Al, B, Ba, Cd, Cr, Cu, Fe, Mn, Mo, Sr, and Zn) in common nase and vimba bream tissue samples of muscle and liver were used as input variables for data analysis. Exploratory, descriptive and data analyses were performed using the SPSS 13.0 software. Outliers have been removed on the basis of the following criterion: if Q1 and Q3 are the lower and upper quartiles, respectively, then an outlier is any observation outside the range: $[Q_1\text{-}1.5(Q_3–Q_1), Q_3\text{-}3(Q_3–Q_1)]$. Outliers have been removed in data cases for vimba bream (6/43 in liver and 3/38 in muscle samples) and common nase (1/30 in liver). Tests of homogeneity of variances based on Levene statistics were used. As ANOVA is robust to violation of the null hypothesis that the group variances are equal when the groups are of equal or approximately equal size, a two-way and one-way ANOVA were used to test the differences between concentrations of elements in common nase and vimba, in liver and muscle tissue, at the significance level of 0.05. We tested the null hypotheses that average concentrations of elements are equal between the common nase and the vimba bream, in each tissue separately. Differences in muscle and liver content between the species were also examined.

We compared the obtained results with the MAC values in fish meat intended for human consumption, as established by the European Union (Official Journal of the European Communities 2001), Food and Agriculture Organization of the United Nations (FAO 1983), and the national legislation of the Republic of Serbia (Official Gazette of FRY, No 28/2011).

Results and Discussion

The length and weight of sampled common nase individuals were, on average, smaller than vimba bream individuals. Four age classes were determined for nase, and three for vimba; for both species, most individuals were aged 6+ and 7+ (Supplementary Table 1). There were a certain similarities in dietary preferences of the analyzed species. The diet of common nase was composed of an almost equal percentage of fish fry, oligochaetes, other unidentified annelid species, and green algae, while vimba bream had a broader spectrum of food items, with domination of gammarids, followed by fish fry (Supplementary Fig. 1).

Levels of As, Co, Hg, Li, Ni, and Pb were below the detection limit, in both muscle and liver. The PCA analysis grouped Al, B, Cd, Cr, Cu, Fe, Mn, and Zn for higher values in liver, and Ba and Sr for higher values in muscle in both species (Supplementary Fig. 2). However, the PCA showed no differentiation between the common nase and vimba bream based on concentration of elements in muscle and liver.

Significant interaction between species and tissue was found in Cd, Cu and Fe. Concentrations of Ba, Cu, Fe, and Zn were significantly higher in muscle, as well as concentrations of Ba, Cd, Cu, and Mn in liver of common nase than in vimba bream. Concentrations of Cr and Fe were significantly higher in liver of vimba bream (Table 1). Statistically significant differences in element content, both in muscle and in liver, were observed for the majority of elements in both species (Supplementary Table 2). Liver is the tissue with a higher content of all analyzed elements, except for Ba and Sr. Standard errors are equal to or greater in liver than those in muscle, with the exception for Ba and Sr. There were no statistical differences regarding the element concentrations in tissues between individuals of different ages in both species.

Environmental problems related to metal pollution are the cause of increased ecological and global public health concern (Tchounwou et al. 2012). Non-degradability and potentially toxic nature of these elements are the reason for analyses of surface waters quality (Faciu et al. 2014), and fish represent major indicators for protection, conservation, restoration and management of water environments (Jungwirth et al. 2000).

Metal content in fish tissues is species-dependent (Meche et al. 2010). Several studies on metal accumulation in various fish species have been carried out along the Belgrade

Table 1 Elemental concentrations (mean value ± standard error) in liver and muscle of common nase and vimba bream

Elements		Common nase	Vimba bream	F statistics	*p* value
Al	Muscle	0.48 ± 0.10	0.79 ± 0.37	0.507	0.479
	Liver	2.99 ± 0.75	2.66 ± 0.59	0.125	0.724
B	Muscle	0.75 ± 0.04	0.76 ± 0.06	0.043	0.836
	Liver	0.97 ± 0.05	1.02 ± 0.04	0.768	0.384
Ba	Muscle*	0.46 ± 0.05	0.26 ± 0.03	14.571	0.000
	Liver*	0.23 ± 0.03	0.13 ± 0.02	7.938	0.006
Cd	Muscle	0.02 ± 0.01	0.03 ± 0.01	0.473	0.494
	Liver*	0.10 ± 0.01	0.06 ± 0.01	7.160	0.009
Cr	Muscle	0.04 ± 0.01	0.06 ± 0.01	2.045	0.158
	Liver*	0.04 ± 0.01	0.08 ± 0.01	5.395	0.023
Cu	Muscle*	0.87 ± 0.11	0.59 ± 0.08	4.118	0.047
	Liver*	19.93 ± 2.13	8.31 ± 0.55	34.353	0.000
Fe	Muscle*	7.34 ± 1.10	4.81 ± 0.69	4.170	0.046
	Liver*	83.06 ± 5.54	101.55 ± 5.78	4.585	0.036
Mn	Muscle	0.34 ± 0.05	0.30 ± 0.06	0.197	0.659
	Liver*	2.10 ± 0.08	1.84 ± 0.07	5.966	0.017
Mo	Muscle	0.05 ± 0.01	0.08 ± 0.02	2.017	0.161
	Liver	0.12 ± 0.02	0.14 ± 0.02	0.235	0.629
Sr	Muscle	1.82 ± 0.17	1.92 ± 0.25	0.087	0.769
	Liver	1.49 ± 0.10	1.42 ± 0.08	0.268	0.595
Zn	Muscle*	9.04 ± 0.72	6.22 ± 0.42	12.822	0.001
	Liver	22.85 ± 1.05	22.58 ± 0.90	0.039	0.844

Concentrations are expressed as μg g^{-1} wet weight. The values with * are significantly different elemental concentrations in tissues of common nase and vimba bream (for liver F(1, 64), for muscle F(1, 58), $p < 0.05$)

section of the Danube River (Jarić et al. 2011; Lenhardt et al. 2012; Sunjog et al. 2012; Subotić et al. 2013, 2015; Jovičić et al. 2015); however, none of the previous studies included neither common nase nor vimba bream.

There is a number of studies on metal accumulation in tissues of common nase in European rivers due to its commercial exploitation (Smederevac-Lalić 2013) and local endangerment (Kottelat and Freyhof 2007). In a study on the Nitra River (Slovakia), Andreji et al. (2012) detected higher levels of several elements in muscle tissue (Cu, Fe, Ni, Pb, and Zn) of common nase in comparison to our results. Higher accumulation levels in the Nitra River could be explained by water pollution, which was, until recently, affected by mining industry and absence of sewage treatment. Mn and Zn content in common nase muscle and liver from various Slovenian rivers (Bajc et al. 2005) is comparable to our findings. However, we detected higher concentration of Cu in both tissues, somewhat higher content of Fe in muscle, as well as notably lower content in liver. Milošković et al. (2016) found lower levels of several elements (Cd, Cu, Fe, and Zn), as well as higher levels of some highly toxic elements (As, Hg, and Pb) in muscle tissue of common nase from various rivers in Serbia. However, common nase from the Danube River was not included in their research. Ecotoxicological research on common nase was also conducted in Romania (Triebskorn et al. 2008) and Croatia (Zrnčić et al. 2013). Outside of Europe, Uysal et al. (2009) found lower concentration of Cu and Zn in muscle and liver of common nase from Turkey, and several times higher concentrations of Fe in these tissues.

In the study conducted in Međuvršje Reservoir, Đikanović et al. (2016) observed differences in levels of toxic metals in common nase of different age. Individuals aged 1^+ and 2^+ had higher concentrations of Mn, Sr, and Zn, and individuals aged 3^+ and 4^+ had higher values of Ni and Cu in muscle. However, we found no differences related to age, but only older specimens ($>5^+$) were included in our study.

In contrast to common nase, there are only a few studies on element concentrations in vimba bream, to the best of our knowledge. Among them are studies by Svecevičius (2007), who tested fish avoidance response to sublethal toxicity, Dušek et al. (2005), who tested muscle tissue of various fish species for mercury, but who pooled results obtained for vimba bream with other benthophagous species, and Strapáč et al. (2010), who focused on levels of Hg and Se in muscle tissue of vimba bream.

For elements such as Cu, Fe, Mn, and Zn, liver is the tissue that exhibits higher accumulation (Bajc et al. 2005; Uysal et al. 2009; Jarić et al. 2011; Sunjog et al. 2012; Subotić et al. 2013, 2015; Đikanović et al. 2016). On the other hand, muscle tissue has a higher accumulation of Ba and Sr (Jarić et al. 2011; Sunjog et al. 2012; Subotić et al. 2013, 2015; Đikanović et al. 2016).

Mean values of the metal load in the water column, used for calculation of bioaccumulation factor, were calculated from values reported by the Serbian Environmental Protection Agency for the sampling station Zemun (Supplementary Table 3). In muscle, common nase has a higher affinity for bioaccumulation of Cu, Fe, and Zn, while vimba bream has a higher affinity for bioaccumulation of Al, Cd, and Cr (Supplementary Table 4). There is a similar affinity for bioaccumulation of B and Mn in muscle of both species. In liver, common nase has a higher affinity for Al, Cd, and Zn, while vimba bream has a higher affinity for bioaccumulation of Cr and Fe.

Common nase individuals analyzed in this paper have a higher BAF in comparison to individuals from other rivers in Serbia (Milošković et al. 2016). Bioaccumulation of Cd, Cu, and Zn was higher in the Danube River than in any sampled location analyzed by this group of authors; however, the BAF for Al, Fe, and Mn was higher at all locations sampled by Milošković et al. (2016), except at one location for Al and Fe, and two locations for Mn. In addition, we compared the obtained BAF values, both for common nase and vimba bream, with the BAF for two other cyprinid species – carp *Cyprinus carpio* (Subotić et al. 2013) and sichel *Pelecus cultratus* (Subotić et al. 2015), also caught at the Belgrade section of the Danube River. Bioaccumulation of Cd, Cr, Cu, and Fe was higher in muscle tissue of common nase and vimba bream. Carp and sichel had higher BAF values for Al in muscle, while BAF value for Mn was equal in common nase, vimba bream, and sichel, but higher than in carp. Common nase and vimba bream have a higher BAF value for Zn in muscle than sichel, but lower than carp. Tao et al. (2012) investigated bioaccumulation of toxic and trace elements in several other cyprinid species (bighead carp *Hypophthalmichthys nobilis*, goldfish *Carassius auratus*, silver carp *Hypophthalmichthy smolitrix*, and common carp *Cyprinus carpio*) from China. Except for Cu bioaccumulation in bighead carp, they obtained higher BAF values for all analyzed elements in all four analyzed species than in common nase and vimba bream from the Danube River.

Metal pollution is currently a major environmental problem, because metal ions persist in the environment due to their non-degradable nature. The toxicity and bioaccumulation tendency of metals in the environment are a serious threat to the health of living organisms. Through bioaccumulation in fish tissues (especially muscle tissue), these toxicants reach human consumers. Detected metal concentrations were compared with MAC values in fish meat intended for human consumption. None of the analyzed elements were above the thresholds prescribed by national and European Union regulations: Cd (0.05 µg/g ww; Official Gazette of FRY, No 28/2011; Official Journal of the European Communities 2001), Cu (30 µg/g ww Official Gazette of FRY, No 28/2011), Fe (30 µg/g ww Official Gazette of FRY), and Zn

(100 µg/g ww Official Gazette of FRY, No 28/2011; 30 µg/g ww Official Journal of the European Communities 2001). Based on these results, there is no hazard for consummation of meat from these two species caught at the Belgrade section of the Danube River. A different situation is presented when considering BAF values. Generally, the bioaccumulation of elements from the environment is not significant unless BAF exceeds the value of 100 or more (United States Environmental Protection Agency 1991). Even though element levels in muscle tissue of common nase and vimba bream individuals did not exceed the MAC values, potential problems may emerge, especially regarding Cd, which had high BAF values in both tissues. Hofer and Lackner (1995) observed that Cd has an extremely slow excretion rate, and Jirsa et al. (2008) detected a correlation between Cd concentrations in nase liver and in river sediments. These findings indicate the need for a better legislation that would combine BAF and MAC values. Higher content of all elements in liver, except for Ba and Sr, emphasize the need for legislation to include other fish tissues, as some of them can also be used for human consumption (e.g. fish liver oil).

This study provide new information on ecotoxicology of common nase and vimba bream in the Danube River. This is especially significant for vimba bream for which data are lacking; this species is more abundant and has a higher biomass in the catch of both commercial and recreational fishers in waters around Belgrade (Jovičić et al. 2014), and thus more accessible to human consumers.

Currently, there is no data on population trends or endangerment levels of these two species in waters around Belgrade, as well as in Serbian waters in general. However, based on the fishery yield, it seems that vimba bream is more frequent in these waters. In this study, the two analyzed species have a similar length/weight, habitat, trophic position, and element bioaccumulation; for this reason, future monitoring studies could focus on either one of these two species as adequate bioindicators of metal pollution, with a potential shift to vimba bream, if future research show a decline and/or local endangerment of common nase. However, complementary analyses of element concentrations in water, sediments, and potential prey items are needed.

Acknowledgements We acknowledge the support provided by the Project No. 173045, funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia. The authors would like to thank Dr. Miroslav Nikolić and Dr. Ljiljana Kostić-Kravljanac for their help in analysis of metals.

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