Environ Sci Pollut Res (2016) 23:17105–17112
DOI 10.1007/s11356-016-6875-y

RESEARCH ARTICLE

Metal concentrations in water, sediment and three fish species from the Danube River, Serbia: a cause for environmental concern

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Received: 11 December 2015 / Accepted: 9 May 2016 / Published online: 23 May 2016

Abstract The aim of this study was to investigate the presence of metal contamination in water, sediments and three different fish species. All samples were taken from the Danube River in Belgrade Region, a location upstream from Grocka. Concentrations of Cd, Hg and Pb in water samples were not detected, while concentrations of Zn, Fe, Cu and As were in the range of 0.004–0.41 mg L^{-1}. Iron was the most deposited metal in sediment samples (17,530.00 mg kg^{-1}). For the purpose of heavy metal determination in fish tissue, silver carp, common carp and wels catfish were collected. Concentrations of Pb, Cd and As were determined in muscle, digestive tract and liver by inductively coupled plasma-optical emission spectrophotometry (IPC-OES). The highest concentration of Pb was in the digestive tract of all three fish species, while Cd was mostly deposited in the liver. The highest concentration of Hg was in the muscle tissue of wels catfish, and these values are above the maximum residual levels prescribed by the European Union and the maximum allowed concentrations (MACs) for Serbia. Concentration of As was mostly deposited in the liver, but under the MAC.

Responsible editor: Céline Guéguen

Highlights • Cd, Hg and Pb concentrations in water were not detected.
• Danube sediments had high levels of Fe, Zn and Pb.
• High levels of Hg were found in the muscle tissue of wels catfish from the Danube River.

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Keywords Danube · Common carp · Silver carp · Wels catfish · Metal concentration · Sediment

Introduction

Heavy metals are among the most common environmental pollutants and are equally undesirable in the air, water and soil, when these values are above threshold levels. The presence of heavy metals in the environment is due to anthropogenic and biogenic sources, but regardless of the sources of pollution, the consequences are undeniable (Ajeagah et al. 2013), except natural sources. In aquatic systems, heavy metals are deposited into the sediments and are non-biodegradable (Milenković et al. 2005; Vuković et al. 2012, 2014). Heavy metals can accumulate in aquatic organisms (Milanov et al. 2016; Mendil et al. 2010; Squadrone et al. 2013; Subotić et al. 2013). Some metals like copper (Cu), zinc (Zn) or iron (Fe) are important for many biochemical processes in living organisms. They are essential elements for aquatic plants and animals (Wei et al. 2014). However, in cases when concentrations of those metals are too high in the environment than in the biota, they become hazardous. Much research has shown that metal pollutants from water can bioaccumulate in different tissues of aquatic organisms (Janjić et al. 2015; Noël et al. 2013; Verep et al. 2012). Various species of fish can be used as bioindicators of contamination with heavy metals and other pollutants (Dural et al. 2007). Heavy metals like Cu, Zn and Fe are essential for fish metabolism, while the roles of others, such as Hg, Cd and Pb, are not known. Field studies and laboratory experiments have shown that accumulation of heavy metals in tissues depends mainly on the metal concentrations in water and the exposure period, although some other environmental factors including salinity, pH value, hardness

and temperature also play significant roles in metal accumulation (Alloway 2013).

Sediments are the main reservoirs of metals in lakes. Sediment transport along the upstream–downstream river gradient, especially in high flow periods, is one of the main pathways of metal input into these ecosystems (Alloway 2013). Because metal concentration in sediments is less variable than that in water, sediments are suitable for monitoring long-term metal deposition in ecosystems (MacDonald et al. 2000). However, measuring metal concentrations in either water or sediment does not provide information on the risk posed by metal bioaccumulation or biomagnification (Maceda-Veiga et al. 2013). These processes are firstly driven by metal availability to the biota (i.e. bioavailability), which, in turn, is related to water and sediment variables, such as pH value, oxygen concentration, water hardness and temperature, and sediment characteristics, including organic carbon content (Adhikari et al. 2009). However, species traits like trophic position, age, body size or home range also modify metal bioaccumulation patterns (Gammons et al. 2006), illustrating that a combination of sentinel species with different ecological attributes will provide the best picture of the risk posed to the biota by metal pollution (Jorgensen 2011). Feeding habits have a great influence on pollutant accumulation (Milanov et al. 2016). Additionally, in different fish species, heavy metals can be deposited in various amounts in their tissue (muscle, liver or digestive tract). There are five potential routes for pollutant to enter fish via food, non-food particles, gills, oral consumption of water and the skin (Milanov et al. 2016).

Fish are suitable bioindicators for metal pollution because they occupy a range of trophic levels, and they have a known ability to concentrate pollutants (e.g. pesticides, biphenyls, heavy metals) (Agarwal et al. 2007; Bervoets and Blust 2003). In addition, since many species, including humans, consume fish as part of their diet, fish best reflect the consequences of metal pollution in lakes on wildlife and humans (Jorgensen 2011).

The Danube River is an international river and the second largest river in Europe and is significant for its commercial fishing. The presence of pollutants in the Danube River is the topic of previous research (Milenković et al. 2005; Subotić et al. 2013; Vuković et al. 2012, 2014) and international treaties and agreements such as the Danube Convention by the International Commission for the Protection of the Danube River (Subotić et al. 2013). Since August 2003, Serbia has been a full member of this association, which stipulates monitoring of water quality as well as river ecosystem (Pajević et al. 2008).

The aims of this study were (1) to measure concentrations of heavy metals in water and sediment, (2) to assess the bioaccumulation of heavy metals in three species of fish from the Danube River, (3) to determine the distribution of heavy metals among different tissues of the Danube fish, and (4) to determine the distribution ratio of heavy metals in the liver, muscle and digestive tract content of the Danube fish.

Materials and methods

Sample collection

Water, sediment and fish samples were taken from the Belgrade Region of the Danube River, near Vinča during 2013. This sampling site is located downstream from Belgrade, in Grocka, a former village, which is now in the urban Belgrade conglomerate (N 44° 40′, E 20° 43′) (Fig. 1).

Water samples ($n = 74$) were collected 20–30 cm under the water surface with a 5-L Friedinger bottle (SCHOTT DURAN®, Elmsford, North America) and mixed. Subsamples of 500 mL were bottled in pre-cleaned plastic flasks (Qorpak, USA).

Sediments ($n = 10$) were obtained using an Ekman grab sampler from a depth of 1 m on the margin of the river bank. The sediment was immediately mixed, and about 200 g was stored at 4 °C in metal-free plastic bags.

In order to determine heavy metals in fish tissue, 15 samples of each fish species, Silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*) and wels catfish (*Silurus glanis*), were collected from professional fishermen during 2013. Each fish from each catch was identified to species level, and a random subsample of 10 individuals per species was used for metal analysis. As the juveniles of these species (<90 mm total length, TL) could not be reliably identified, they were excluded from the analysis.

The fish used for metal analyses were euthanised with an overdose of MS-222 (3-aminobenzoic acid ethyl ether, Sigma-Aldrich®). Fish were then transported in a refrigerator to the laboratory. There, fish were measured with accuracy of 1 mm (TL) and weighed with accuracy of 0.01 g (total body wet weight) (OHAUS Pioneer, Parsippany, UK). Fish were then dissected. Portions of muscle (approx. 500 mg) from below the dorsal fin, digestive tract content and entire liver were stored in polypropylene vials previously pre-cleaned with nitric acid (10 %) and rinsed three times in deionised water. These were immediately frozen and stored at −20 °C. Fish muscle was selected in order to determine the risk posed by metal pollution to humans consuming fish meat, while liver was selected because it is a key organ in detoxification processes and is the target organ for accumulation of heavy metals (Miller et al. 1992).

Heavy metal analysis

Water subsamples were stored in the dark at 4 °C. All solutions were filtered using a Whatman GF/C fibreglass filter

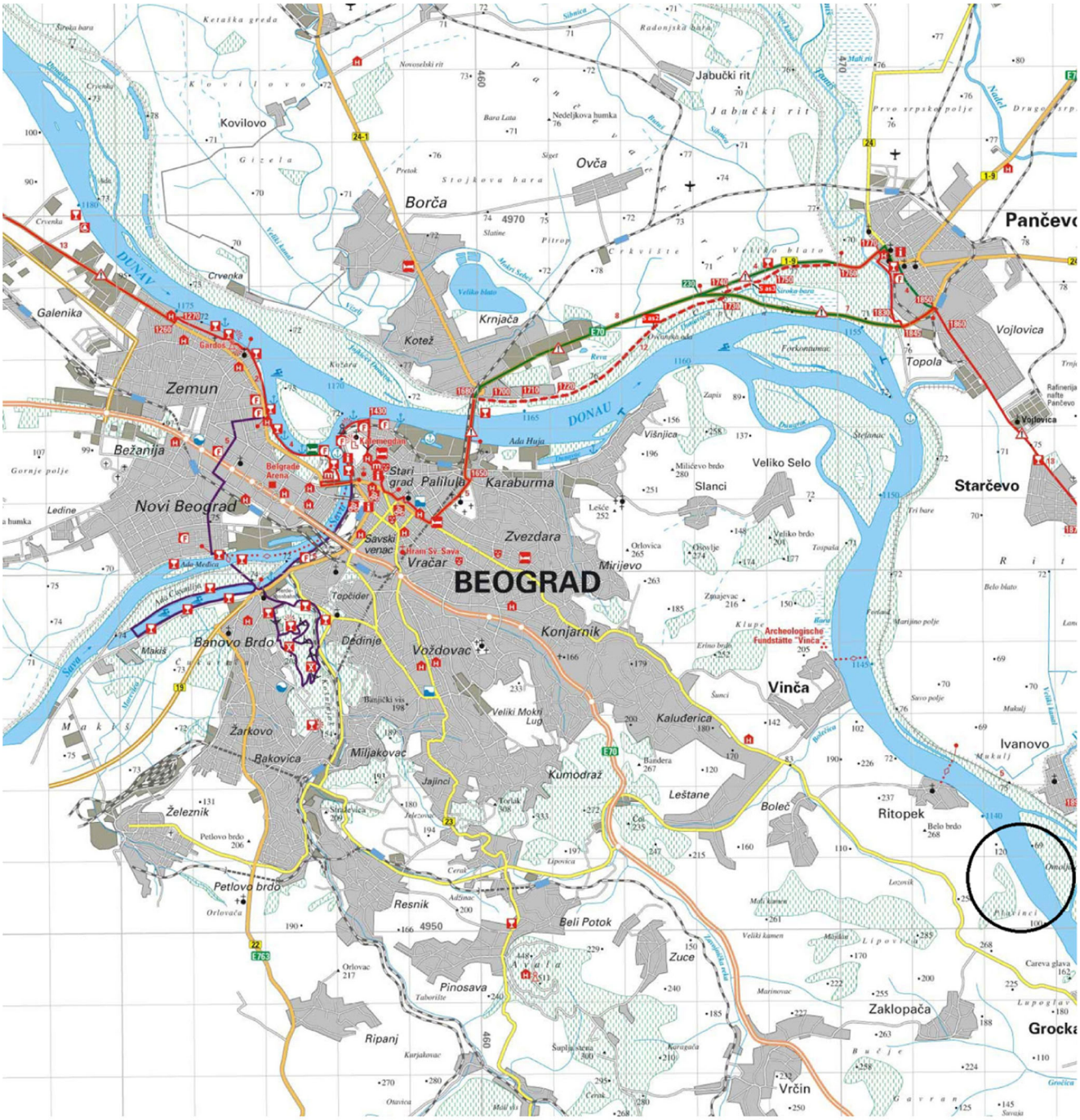

Fig. 1 Map of the sampling area. Encircled part of the map shows the place where the samples were taken. Circle line is black, and other colours are part of the map and different colored lines are not significance

paper (Sigma-Aldrich Co, UK) to remove any fine suspended particulates. Water samples were acidified to pH <2 by the addition of concentrated *Suprapur* HNO_3 (Merck, Germany) (1 mL of acid to 0.5 L of the sample). Standard As solutions were prepared in the range of 10–60 μg L^{-1}. All solutions were filtered using a Whatman GF/C fibreglass filter paper and then analysed in triplicate.

The samples of sediment were first dried at 110 °C for 24 h and then mechanically homogenised to obtain a powder. Approximately 0.5 g of each dry sediment sample was wet digested with nitric acid and hydrogen peroxide in a microwave closed system using the temperature program 180–240 °C during 35 min.

Concentrations of the following heavy metals were determined in the waters, sediments, fish muscle, fish digestive tracts and fish livers: Zn, Fe, Cu, As, Cd, Hg and Pb.

Analysis of the elements was carried out by inductively coupled plasma-optical emission spectrophotometry (ICP-

OES). Details of the instrumental operating conditions are presented in Table 1. The mercury analysis was performed by a PerkinElmer 4100 atomic absorption spectrophotometer (Norwalk, CT, USA) equipped with the MHS 15 CVAAS system according to the method of our previous study (Milanov et al. 2016). The quality of the analytical process was controlled by the analysis of BCR-185R reference materials of bovine liver as well as IAEA-336 lichen reference material. The concentrations found were within 90–115 % of the certified values for all measured elements.

Limits of detection of heavy metals in water samples were 0.009, 0.008, 0.005 and 0.5 mg kg^{-1} for Pb, Cd, Hg and As, respectively, while detection limits in fish tissue samples were 0.04. 0.05, 0.02 and 0.05 mg kg^{-1} for Pb, Cd, As and Hg, respectively (Milanov et al. 2016).

Heavy metal concentrations for waters are reported as milligrams per litre, those for sediments are reported as milligrams per kilogramme of wet weight (ww) while those for fish tissues are reported as micrograms per gramme of wet weight. Also, heavy metal concentrations for fish tissues were compared with the maximum allowed concentrations (MACs) in fish meat for human consumption. According to EU legislation (European Commission Regulation 2006; Official Journal of the European Communities 2001), MAC for Cd, Hg and Pb is 0.05, 0.05 and 0.30 μg g^{-1} ww, respectively. Serbian legislation prescribed MAC for As, Cd, Hg, Pb, Cu, Fe and Zn in fish meat at 2.0, 0.1, 0.5, 1.0, 30.00, 30.00 and 100.00 μg g^{-1} ww, respectively (Official Gazette of RS 2011) (Official Gazette RS, No. 28/2011).

Statistical analysis

All samples were collected and analysed in duplicate, and the results are expressed as mean ± standard deviation. Statistical analysis of the results was elaborated using software GraphPad Prism version 5.00 for Windows, GraphPad software, San Diego, CA, USA, www.graphpad.com. Statistical analysis was performed using Student's *t* test and analysis of variance (ANOVA) with multiple comparison Tukey's test to determine the significance of differences between means. A level of 0.01 and of 0.05 was considered significant.

Results

The limit of detection for each sample type was verified based on the instrumental detection limit, sample mass (g) and volume to which it had been diluted. The average detection limits for each of the assessed elements were as follows (mg kg^{-1}): 0.223 (As), 0.010 (Cd), 0.019 (Co), 0.046 (Cu), 0.044 (Fe), 0.437 (total Hg), 0.271 (Pb) and 0.031 (Zn).

The highest concentration of Fe was found in the sediments from the Danube River compared with Zn, Pb, Cu, As, Cd and Hg. Heavy metal concentrations in sediments ranged from 0.80 mg kg^{-1} (Pb) to 17,530 mg kg^{-1} (Fe). The concentration of heavy metal in water ranged from 0.004 ± 0.001 mg L^{-1} (Cu) to 0.063 ± 0.007 mg L^{-1} (Zn) (Table 2). The concentrations Cd, Hg and Pb were not detected in water samples.

The average body weights of silver carp, common carp and wels catfish were 6.60 ± 1.72, 4.47 ± 1.51 and 19.73 ± 7.93 kg, respectively.

There were significant differences between fish species with regard to levels of Pb, Cd, Hg and As in muscle, liver and digestive tract content (Table 3) ($P < 0.01$; $P < 0.05$).

Silver carp generally had the highest metal concentrations among the three fish species. Silver carp had the highest concentrations of Pb (1.30 ± 0.07 μg g^{-1} ww) and As (0.07 ± 0.01 μg g^{-1} ww) in their digestive tract contents compared to the other fish species. Silver carp also contained the highest concentrations of Cd and As in the liver. However, wels catfish had the highest mean values of Pb (0.06 ± 0.01 μg g^{-1} ww) and Hg (0.53 ± 0.10 μg g^{-1} ww) in muscle.

Nonetheless, digestive tract content was the main metal depository for the heavy metals, with the exception of Cd (Table 2). Concentrations of As differed significantly ($P < 0.01$) between the same tissues of the three fish species (Table 2). The concentrations of As ranged from 0.04 to 0.08 μg g^{-1} in tissues of silver carp, from 0.01 to 0.02 μg g^{-1} in tissues of common carp and from 0.003 to 0.006 μg g^{-1} in tissues of wels catfish. These levels of As did not exceed 2 mg kg^{-1}, the MAC prescribed by the National Regulation of the Republic Serbia. The average Hg concentration in wels catfish muscle was 0.53 mg kg^{-1} ww, which exceeded the 0.5 mg kg^{-1} ww threshold (Official Gazette of RS, No. 28/2011, Official Gazette of RS 2011; Official Journal of the European Communities 2001).

The comparison of heavy metal distribution ratios among the tissues of the three fish species is presented in Table 4.

The most abundant metal in the digestive tract content of silver carp was Pb, which was 24.42 times higher than that in muscle, while in wels catfish, this ratio was only 1.45. The concentration of Cd in the digestive tract content of wels catfish was 9.50 times higher than that in the muscle. Analysis of the correlation between accumulated heavy metals in muscle and liver showed that higher concentrations of Cd were related to the distribution of other metals. These ratios were 13.66, 8.00 and 8.32 in silver carp, common carp and wels catfish, respectively. Distribution of Hg followed a similar pattern, but Hg ratios between muscle and liver were lower, ranging from 0.44 to 1.28. The ratio of As in muscle and liver was between 1.27 and 1.69. The distribution ratio between liver and digestive tract content for Pb was in the range of 0.11 to 1.07.

Table 1 ICP-OES instrumental operating conditions

Parameter	Value
RF generator power (W)	1200
Frequency of RF generator (MHz)	40.68
Plasma gas flow rate (L min^{-1})	12
Auxiliary gas flow rate (L min^{-1})	0.2
Nebulisation gas flow rate (L min^{-1})	0.85
Sample uptake rate (L min^{-1})	1
Type of detector	Solid state
Type of spray chamber	Cyclonic
Injection tube diameter (mm)	0.3
Measurement replicates	3
Elements	As 193.695; Cd 228.802; Cu 324.754; Fe 259.939; Pb 220.353; Zn 213.856

Discussion

Knowledge of toxic heavy metal concentrations in fish is important, from the aspect of environmental management as well as human health (Gu et al. 2016a). In humans, heavy metal toxicity most commonly involves brain and kidney damage, but other manifestations can also occur, and some metals (such as arsenic) clearly show carcinogenic properties. An individual with heavy metal intoxication, if they are ingested in high doses, typically shows uncharacteristic symptoms, such as weakness or headache (Dural et al. 2007).

Concentrations of Zn and Fe were higher in Danube River sediments than in waters, which indicates slow precipitation of these metals. Concentrations of heavy metals in sediment samples from the Danube River are often higher than concentrations of heavy metals in water samples (Gundacker 2000; Pajević et al. 2008; Vuković et al. 2012).

The presence of heavy metals in water depends on many environmental factors like influence of industry and other forms of pollution. Analysis of water samples has shown the presence of copper and arsenic in traces, while zinc was present in an amount lower than 0.050 mg L^{-1} as reported for Danube water (Pantelica et al. 2012). The concentration of iron was up to 10 times higher than of other metals in water, while the presence of As in traces makes the water polluted. These results are in accordance with previous research of water quality in the Danube River (Pajević et al. 2008). The mean concentration of arsenic was 0.006 mg L^{-1} which is lower than the limit for the content of this heavy metal in natural mineral water by FAO and WHO (2012). The metal concentrations in the examined water samples in comparison to the limit values of Serbian regulations indicate the second class of water quality of Danube River. The metal content in the tested water samples does not indicate the pollution of aquatic ecosystems, nor risk to human health. The increased concentration of heavy metals in sediments is the result of higher concentrations of these metals in the water, which is easily adsorbed on the surface of the sediment and poses as an ecological threat to wildlife watercourses (Gu et al. 2016b). The analysis of the sediments from the Danube River indicates the presence of all tested metals in much higher concentrations than in water, but these results are below the acceptable level of Hg or the target and limit value recommended by Serbian Regulations. The presence of metals in sediments is an indicator of the deposition in the riverbed and represents a potential risk to the ecosystem, especially the observed mercury concentration. In subsequent investigations of health risk assessment, concentrations of As, Cd, Pb and Hg were determined in freshwater fish species.

Table 2 Heavy metal concentrations in water samples (mg L^{-1}) and sediment (mg kg^{-1}) from the Danube River, expressed as mean ± standard deviation

Heavy metal	Water (mg L^{-1})	Sediment (mg kg^{-1})
Zn	0.063 ± 0.007	270.40 ± 17.98
Fe	0.41 ± 0.01	17,530.00 ± 971.7
Cu	0.004 ± 0.001	50.93 ± 3.34
As	0.006 ± 0.001	13.89 ± 1.05
Cd	ND	1.69 ± 0.13
Hg	ND	0.80 ± 0.09
Pb	ND	64.92 ± 2.39

ND not detected

Metal concentrations in the muscle, digestive tract content and liver of each fish species showed great variations. In fact, metal concentrations were sometimes significantly different within each tissue from an individual fish species ($P < 0.01$; $P < 0.05$). A previous study also showed that different fish species contained different levels of heavy metals in their tissues (Monroy et al. 2014). This may be related to the differences in ecological needs and metabolic activities among

Table 3 Heavy metal concentrations (μg g^{-1} wet weight) in tissues of the three examined fish species, expressed as mean ± standard deviation

Species	Matrices	Heavy metal concentrations			
		Pb	Cd	Hg	As
Silver carp	Muscle	$0.056^{A} \pm 0.01$	0.01 ± 0.002	$0.16^{A} \pm 0.02$	$0.04^{A} \pm 0.007$
	Digestive tract content	$1.30^{A} \pm 0.07$	$0.07^{AB} \pm 0.009$	$0.26^{a} \pm 0.04$	$0.07^{A} \pm 0.01$
	Liver	$0.14^{A} \pm 0.02$	$0.21^{AB} \pm 0.01$	0.20 ± 0.02	$0.08^{A} \pm 0.01$
Common carp	Muscle	$0.048^{A} \pm 0.02$	0.01 ± 0.002	$0.24^{A} \pm 0.05$	$0.01^{A} \pm 0.003$
	Digestive tract content	$0.21^{A} \pm 0.02$	$0.10^{AC} \pm 0.02$	$0.22^{aA} \pm 0.03$	$0.02^{A} \pm 0.004$
	Liver	$0.06^{A} \pm 0.01$	$0.13^{A} \pm 0.02$	0.22 ± 0.04	$0.02^{A} \pm 0.003$
Wels catfish	Muscle	$0.06^{A} \pm 0.01$	0.01 ± 0.003	$0.53^{A} \pm 0.10$	$0.003^{A} \pm 0.0008$
	Digestive tract content	$0.10^{A} \pm 0.01$	$0.14^{ABC} \pm 0.02$	$0.30^{A} \pm 0.05$	$0.006^{A} \pm 0.001$
	Liver	$0.10^{A} \pm 0.01$	$0.12^{B} \pm 0.01$	0.23 ± 0.02	$0.005^{A} \pm 0.001$

Different superscript uppercase and lowercase letters indicate significant differences ($P < 0.01$ and $P < 0.05$) between the same tissues of the different fish species

different fish species. The differences in metal concentrations in the tissues might be a result of the capacity of individual tissues to induce metal-binding proteins such metallothioneins (Canli and Guluzar 2003).

The present data show that concentrations of Pb, Cd and As in the digestive tract content and liver were higher than those in the muscle of all three fish species. The concentration of Pb in the digestive tract content of silver carp was significantly higher than that in the other tissues. The distribution was similar to that of the other two fish species and in the following order: digestive tract content > liver > muscle. The concentration of Pb in the muscle of wels catfish was low (0.06 ± 0.01 μg g^{-1}), and this result is in accordance with the results reported previously (Zrnčić et al. 2013). Two fish species, silver carp and common carp, feed on plankton and algae which may be one of the reasons for their higher bioaccumulation of Pb.

Table 4 Ratio distribution of heavy metals between different tissues among three fish species

Species	Tissue ratio	Heavy metal distribution			
		Pb	Cd	Hg	As
Silver carp	M:DT	1:24.42	1:4.29	1:1.65	1:1.71
Common carp		1:4.46	1:6.66	1:0.89	1:1.41
Wels catfish		1:1.45	1:9.50	1:0.58	1:1.86
Silver carp	M:L	1:2.56	1:13.66	1:1.28	1:1.69
Common carp		1:1.32	1:8.00	1:0.89	1:1.27
Wels catfish		1:1.55	1:8.30	1:0.44	1:1.46
Silver carp	L:DT	1:0.11	1:3.18	1:0.78	1:1.10
Common carp		1:0.99	1:1.29	1:0.99	1:1.05
Wels catfish		1:1.07	1:0.87	1:0.76	1:0.78

M muscle, *DT* digestive tract content, *L* liver

Significantly higher concentrations of Cd were present in the liver of plankton-feeding fishes (0.01–0.121 μg g^{-1}), while in the liver of wels catfish, the Cd concentration was 0.012 μg g^{-1}. The bioaccumulation of Cd in liver has been reported in other studies (Jarić et al. 2012; Poleksić et al. 2010). Also, higher concentrations of Cd in the digestive tract content of wels catfish are in accordance with its feeding habits.

Hg concentrations were higher than concentrations of the other heavy metals in all investigated tissues of all three fish species. Hg exhibits high toxicity, with tendency to bind the sulfur-containing group of proteins and accumulate in muscle (Has-Schön et al. 2006), which could explain the highest concentration we detected (0.53 ± 0.10 μg g^{-1}) in the muscle of wels catfish. Hg concentrations were similar in all three tissues of common carp, but this heavy metal was found at significantly lower concentrations in the muscle of silver carp than in other tissues. These results are not in accordance with the results of previously published research (Zrnčić et al. 2013), but this might be due to different sampling sites as well as fish species.

The As levels in fish tissues in this study were lower (0.003 to 0.08 μg g^{-1}) than those reported in other studies, which reported levels ranging from 0.021 to 0.048 μg g^{-1} in common carp (Zrnčić et al. 2013). Also, in a previous study of Lenhardt et al. (2011), the highest concentrations of As were found in tissues of silver carp. However, these higher concentrations of As reported in other studies could be explained by the presence of As in water, which indicates its presence in plankton and algae and, therefore, a higher concentration would be expected in tissues of plankton-feeding fishes.

Pb concentrations were in the following order in all three fish species: digestive tract content > liver > muscle; this order also applies for Cd and As in silver carp. However, the highest concentrations of Cd and As were deposited in the liver tissue

of silver carp, while the muscle of wels catfish had the highest average concentration of Hg. These results indicate a significant capability of these fish species to intake these metals. Nonetheless, the levels of heavy metals detected in edible fish muscle were also generally below the maximum residue limits of these pollutants as proposed by the Official Journal of the European Communities (2001). The meat of Danube fish species should be utilised in the human diet only in limited quantities, and it is necessary to establish permanent monitoring of heavy metal concentrations in this location.

Conclusions

This study provides information on the heavy metal concentrations in water, sediment and three fish species from the Danube River, Serbia. Some elements (Pb, Cd, Hg, As) were found to be significantly different ($P < 0.01$; $P < 0.05$) between the tissue of fish species. Regular monitoring of heavy metal concentration should be conducted in the future since the levels of Hg exceeded the legislated limits in some of the examined samples. Other examined heavy metals in fish tissue were at acceptable levels for human composition.

Acknowledgments This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, via the project "Selected biological hazards to the safety/quality of food of animal origin and control measures from farm to consumer" (31034).

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