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The Accumulation and Distribution of Metals in Water, Sediment, Aquatic Macrophytes and Fishes of the Gruža Reservoir, Serbia

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Abstract The concentrations of iron, lead, cadmium, copper, manganese, mercury and arsenic were measured in water, sediment, five macrophytes (*Typha angustifolia*, *Iris pseudacorus*, *Polygonum amphybium*, *Myriophyllum spicatum* and *Lemna gibba*) and five fish species (*Sander lucioperca*, *Abramis brama*, *Carassius gibelio*, *Silurus glanis* and *Arystichtys nobilis*) in the Gruža Reservoir, used for water supply and recreational fishing. The concentrations of all examined elements were higher in sediment than in water. The values of the ratio between element concentrations in the sediment and those in the water were the highest for Fe and As. Among the five plant species, the highest concentrations of Pb and Mn were observed in *T. angustifolia*, while the highest concentrations of Fe, Cu and Hg were in *L. gibba*. *I. pseudacorus* and *P. amphybium* had the highest concentrations of Cd and As, respectively. Among the fish species, *C. gibelio* showed the highest tendency of element accumulation (Fe, Cd, Cu), followed by *S. lucioperca* (Pb, Hg), *A. brama* (Mn) and *A. nobilis* (As). The average concentrations of elements in fish muscle, except for As in *A. nobilis* (2.635 ± 0.241 mg kg^{-1} ww), were below the limits that are considered safe for human consumption in accordance with the European Commission Regulation and Official Gazette of Serbia.

Keywords Elements · Bioaccumulation · Macrophytes · Fishes

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Water contamination with metals is a very important problem in the contemporary world, and their presence in the aquatic environment is a serious issue that threatens not only the aquatic ecosystems but also human health (Jianguo et al. 2007). Metals from natural and anthropogenic sources are continually being released into aquatic ecosystems, and they are a serious threat because of their toxicity, long persistence and capacity for bioaccumulation (Papagiannis et al. 2003). The manufacture, traffic, utilization and disposal of many products cause the release of trace metals into the aquatic environment, and increased attention is being paid to possible effects upon humans (Yildirim et al. 2008).

Elements such as Fe, Cu and Mn are essential elements, since they play an important role in biological systems; whereas Hg, Pb, Cd and As are non-essential. Non-essential elements can be very harmful, even at low concentration, when ingested over a long period. Essential elements can also produce toxic effects when their intake is elevated excessively. The bioavailability of the various metals depends upon the nature of the aquatic ecosystem, including the pH, redox conditions and the presence of competing ligands that occur naturally (McGeer et al. 2004; Miretzky et al. 2004).

The concentrations of metals in sediment and in long-lived organisms reflect accumulation over a period of time, and are only weakly related to their content in water, which depends on a short-term factor (Mazej et al. 2010). Generally, species in relatively low trophic levels are exposed to comparatively lower concentration, although macrophytes can accumulate high levels of metals (Terra et al. 2008). On the other hand, McGeer et al. (2004) state that, with exception of methylmercury, biomagnification rarely occurs with metals.

The aim of the present study was to assess the concentration status, accumulation and distribution of seven elements (Fe, Pb, Cd, Cu, Mn, Hg and As) in water, sediment, native aquatic macrophytes and fishes of the Gruža Reservoir. This reservoir was very hyper-eutrophic in the nineties, and therefore was treated with copper sulfate over a 10-year period to prevent algal blooms. Another goal was to determine if elevated copper residues remained in the reservoir. Moreover, the element loading in fish muscle was determined to assess the risk to humans from the consumption of fish.

Materials and Methods

The Gruža Reservoir is situated in Central Serbia, near the city of Kragujevac, at an altitude of 269 m, with a surface area of 9.34 km^2 and a maximum depth of 31 m (Fig. 1). It was formed in 1985 to supply drinking water to the

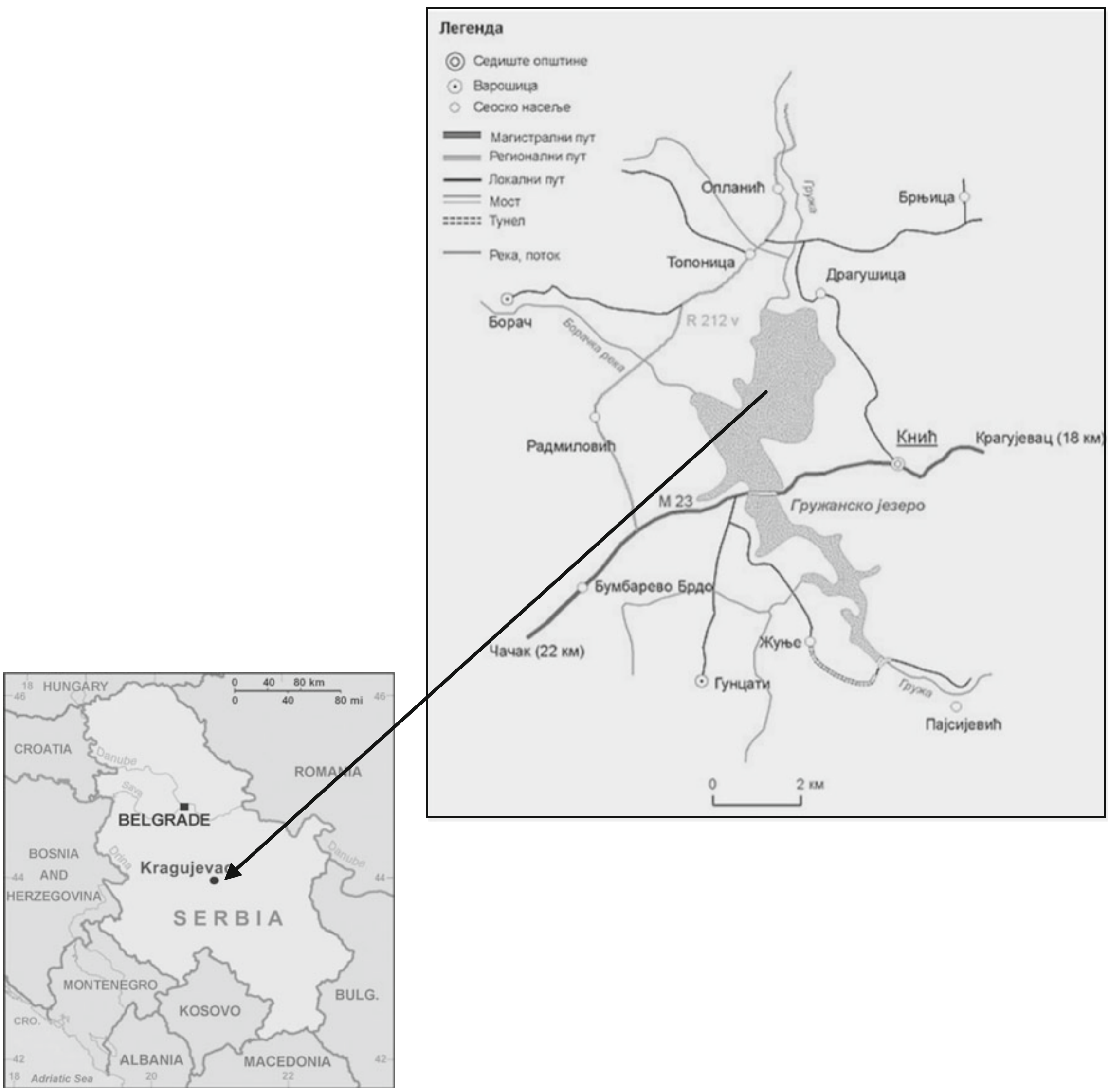

Fig. 1 Location of the Gruža Reservoir near the city of Kragujevac, Central Serbia

population of the region, and also for recreational purposes. Hence, it is essential to know the risk due to contaminants from the consumption of water and fish, and from skin contact during swimming.

This reservoir has been under a strong anthropogenic influence. A municipal transit road passes over the reservoir. The land around the reservoir is intensively processed with the use of invasive agrotechnical measures (excessive use of pesticides, herbicides and fungicides). Also, near the reservoir there are two factories for meat processing and mushroom cultivation. The field work was conducted during the summer of 2011. Samples of surface water and sediment were collected from various areas of the reservoir at a maximum depth of 1 m. Soon after collection, the water samples were filtered through 0.45 μm (pore size) Millipore filters. The sediment was taken with a grab, at a depth from 0 to 10 cm, after which it was preserved in bottles, labeled carefully and brought to the laboratory for further analysis.

Macrophytes *Typha angustifolia*, *Iris pseudacorus*, *Polygonum amphybium*, *Myriophyllum spicatum* and *Lemna gibba*, and fish species *Carassius gibelio*, *Abramis brama*, *Silurus glanis*, *Sander lucioperca* and *Arystichtys nobilis* were included in the study. The macrophytes were collected by hand, washed with lake water to remove loosely attached periphyton and sediment, and preserved with lake water in plastic bottles. Fish were collected with nets of different lengths, widths and mesh diameters. The total length (mm) and weight (g) of each fish was measured before dissection. Arithmetic mean values for total length and weight of fishes were: 580 mm and 595 g for *S. lucioperca*; 380 mm and 608 g for *A. brama*; 430 mm and 1,120 g for *C. gibelio*; 900 mm and 5,250 g for *S. glanis*; 865 mm and 12,350 g for *A. nobilis*, respectively. A muscle sample of approximately 5 g was collected from above the lateral line, washed with distilled water, packed into a polyethylene bag, and stored at −20°C prior to analysis.

In the laboratory, the sediment samples were dried in an oven and stone pieces were removed. Sediment samples were digested at 95°C for 1 h using a mixture of HCl–HNO_3–H_2O (6 mL of mixture of 1/1/1 for 1 g). The identified plant material was rinsed in distilled water, and dried at room temperature, followed by drying for 24 h at 105°C in a dryer (Binder/Ed 15053, Tuttlingen, Germany). Water and plants were prepared for chemical analysis according to standard procedures (APHA 1993). Fish samples (~1.5 g) were dried in a lyophilizer (Christ Alpha 2-4 LD, Harz, Germany), and then digested in an Advanced Microwave Digestion System (ETHOS 1, Milestone, Italy) using a mixture of 65 % nitric acid and 30 % hydrogen peroxide (Merck, Darmstadt, Germany, 10:2 v/v) at 220°C for 20 min. After cooling to room temperature and without filtration, the solution was diluted to a fixed volume (volumetric flask, 25 mL) with deionized water.

Concentrations of Fe, Pb, Cd, Cu, Mn, Hg and As were measured in water, sediment, plants and fish in triplicate using a Thermo Scientific iCAP 6500 Duo ICP instrument (Thermo Fisher Scientific, Cambridge, United Kingdom). Concentrations of As and Hg were measured by hydride technique (HG–ICP-OES) in order to increase the sensitivity and lower the threshold for detection limits. The potential presence of trace elements in chemicals used in sample preparation was resolved by using a number of blank samples. Two multi-elemental plasma standard solutions, Multi-Element Plasma Standard Solution 4, Specpure®, 1,000 μg mL^{-1} and Semiquantitative Standard 1, Specpure®, 10 μg mL^{-1} certified by Alfa Aesar GmbH & Co KG, Germany, were used to prepare calibration solutions for ICP-OES and HG–ICP-OES measurements, respectively. The detection limits for Fe, Mn, Cu, Pb, Cd, Hg and As in water were: 0.0100, 0.0070, 0.0030, 0.0100, 0.0100, 0.0010, 0.0010 mg L^{-1}, respectively. The detection limits for Fe, Mn, Cu, Pb, Cd, Hg and As in sediment were: 0.0056, 0.0089, 0.0030, 0.0076, 0.0065, 0.0010, 0.0010 mg kg^{-1}, respectively. The detection limits for Fe, Mn, Cu, Pb, Cd, Hg and As in plant material were: 0.0053, 0.0030, 0.0027, 0.0056, 0.0051, 0.000335, 0.000129 mg kg^{-1}, respectively. The detection limits for Fe, Pb, Cd, Cu, Mn, Hg and As in fish muscle were: 0.0018, 0.0093, 0.00047, 0.0028, 0.0025, 0.00027, 0.00022 mg kg^{-1}, respectively.

Concentrations of elements in water were expressed as mg L^{-1}, whereas in sediment, plant materials and fish tissue as mg kg^{-1} of dry weight (dw). Element concentrations in fish muscle tissue were also expressed as mg kg^{-1} wet weight (ww), for comparison with the maximum permitted concentrations for human consumption by the European Commission Regulation (1881/2006/EC) and Official Gazette of Serbia (Anonymous 2009a).

Mean values and standard deviations were calculated for each group. Differences in mean concentrations of elements in plants and fishes were analyzed by one-way ANOVA. The Pearson's correlation coefficient was used to test the correlation between the concentrations of elements in different fish species. Statistical analysis of data was carried out using SPSS 16.0 statistical package programs for Windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Of the different environmental matrices that were analyzed (water, sediment, macrophytes and fishes) the mean concentrations of Fe, Pb, Cd, Hg and As were the highest in the sediment (Table 1). Four of five macrophyte species had higher mean concentrations of Cu than the sediment,

Table 1 Concentrations of elements in water (mg L^{-1}), sediment, aquatic macrophytes and fish muscles (mg kg^{-1} dw) in the Gruža Reservoir

Subject	Number of samples	Element concentration						
		Fe	Pb	Cd	Cu	Mn	Hg	As
Water	3	0.097 ± 0.001	ND	ND	0.004 ± 0.001	0.025 ± 0.001	0.016 ± 0.001	0.259 ± 0.009
Sediment	3	5,320 ± 0.50	30.9 ± 0.007	2.53 ± 0.002	10.53 ± 0.006	623 ± 0.178	280 ± 0.24	11,120 ± 23.51
T. angustifolia[a]	5	2,610 ± 87.38	7.75 ± 0.28	1.5 ± 0.054	11.35 ± 0.17	1,240 ± 11.025	27.8 ± 1.00	4,273 ± 142.3
I. pseudacorus[a]	8	1,864 ± 60.23	2.2 ± 0.08	1.6 ± 0.053	26.9 ± 0.67	330.7 ± 5.10	182.6 ± 3.79	3,440 ± 110
P. amphybium[a]	6	1,490 ± 15.17	0.700 ± 0.015	0.200 ± 0.002	6.45 ± 0.01	223 ± 1.76	165 ± 1.03	4,280 ± 69.2
M. spicatum[a]	6	1,610 ± 41.75	2.65 ± 0.10	0.350 ± 0.014	11.6 ± 0.30	352 ± 14.0	124 ± 32.7	1,185 ± 53.75
L. gibba[a]	11	3,280 ± 27.31	3.4 ± 0.04	0.500 ± 0.000	55.5 ± 0.30	641 ± 3.19	279 ± 3.53	2,719 ± 14.71
S. lucioperca[b]	10	10.6 ± 6.19	0.935 ± 0.324	0.007 ± 0.004	0.478 ± 0.153	1.04 ± 1.06	0.147 ± 0.021	ND
		3.12 ± 1.82*	0.275 ± 0.095*	0.002 ± 0.0005*	0.140 ± 0.452*	0.306 ± 0.312*	0.043 ± 0.006*	ND*
A. brama[b]	10	6.8 ± 1.53	0.331 ± 0.157	0.005 ± 0.001	0.354 ± 0.060	3.08 ± 2.48	0.053 ± 0.003	ND
		2.8 ± 0.64*	0.138 ± 0.065*	0.002 ± 0.0005*	0.147 ± 0.250*	1.28 ± 1.03*	0.022 ± 0.001*	ND*
C. gibelio[b]	10	18.1 ± 9.65	0.437 ± 0.287	0.008 ± 0.001	0.877 ± 0.286	1.23 ± 0.59	0.084 ± 0.007	0.255 ± 0.150
		7.53 ± 4.02*	0.182 ± 0.119*	0.003 ± 0.0005*	0.365 ± 0.119*	0.512 ± 0.244*	0.035 ± 0.002*	0.106 ± 0.062*
S. glanis[b]	10	17.5 ± 7.77	0.150 ± 0.039	0.007 ± 0.001	0.704 ± 0.112	1.24 ± 1.22	0.078 ± 0.004	0.225 ± 0.119
		7.4 ± 3.29*	0.063 ± 0.016*	0.003 ± 0.0006*	0.296 ± 0.048*	0.524 ± 0.518*	0.033 ± 0.002*	0.095 ± 0.050*
A. nobilis[b]	10	2.28 ± 0.20	0.099 ± 0.010	0.003 ± 0.000	0.089 ± 0.011	0.028 ± 0.002	0.024 ± 0.001	3.45 ± 0.317
		1.74 ± 0.15*	0.076 ± 0.007*	0.002 ± 0.0004*	0.068 ± 0.008*	0.021 ± 0.001*	0.018 ± 0.0009*	2.635 ± 0.241*

Values are mean ± SD

ND not detected

[a] Macrophytes, [b] Fishes

* Concentrations of elements in fish muscles expressed as mg kg^{-1} ww

and two of five macrophytes had higher Mn concentrations than the sediment. The concentrations of elements in fish muscle tissue were almost always considerably lower than their concentrations in sediment or macrophytes. One exception to this was noted for Pb in *P. amphibium* and *S. lucioperca*. The concentration of As in water was by far the highest of all elements at 0.259 mg L^{-1}.

One way ANOVA revealed that the mean element concentrations were significantly different with different plant species at the 0.05 level ($p < 0.05$). In this study, a significant difference in Cu levels was found between fish species *S. glanis* and *A. nobilis* at the 0.05 level. Concentrations of As varied significantly between *A. nobilis* and all other species. Also, a significant difference in Hg was found between *A. nobilis* and all other fish species, *A. brama* and *C. gibelio*, and *A. brama* and *S. glanis* at the 0.05 level. Different fish species differed in their capabilities to take up and accumulate elements. A positive correlation was characteristic for Fe between *A. brama* and *C. gibelio* ($r = 0.99$; $p = 0.03$), Pb between *S. lucioperca* and *A. nobilis* ($r = 0.99$; $p = 0.02$), Cd between *S. lucioperca* and *C. gibelio* ($r = 0.99$; $p = 0.02$), Cu between *A. brama* and *S. glanis* ($r = 1.00$; $p = 0.005$), and Mn between *S. lucioperca* and *S. glanis* ($r = 0.99$; $p = 0.01$). On the other hand, negative correlations were characteristic for Fe between *C. gibelio* and *A. nobilis* ($r = -0.99$; $p = 0.04$) and Cu between *C. gibelio* and *A. nobilis* ($r = 0.99$; $p = 0.03$). In contrast, no significant correlations were obtained between fish species for Hg and As.

The ratios for element concentrations in sediment over water were the highest for Fe and As (Fig. 2a). Lead and cadmium were not detected in the water, due to the fact that their concentrations were lower than the detection limit of the method. The element concentrations in water followed the order: As > Fe > Mn > Hg > Cu. High concentration of As in water may be due to overuse of pesticides on agricultural areas around the reservoir. Low concentrations of soluble elements recorded in the lake water might be the result of their binding to particulate matter, which then settles onto sediment, and to uptake by plankton (Mazej et al. 2010). Generally, the

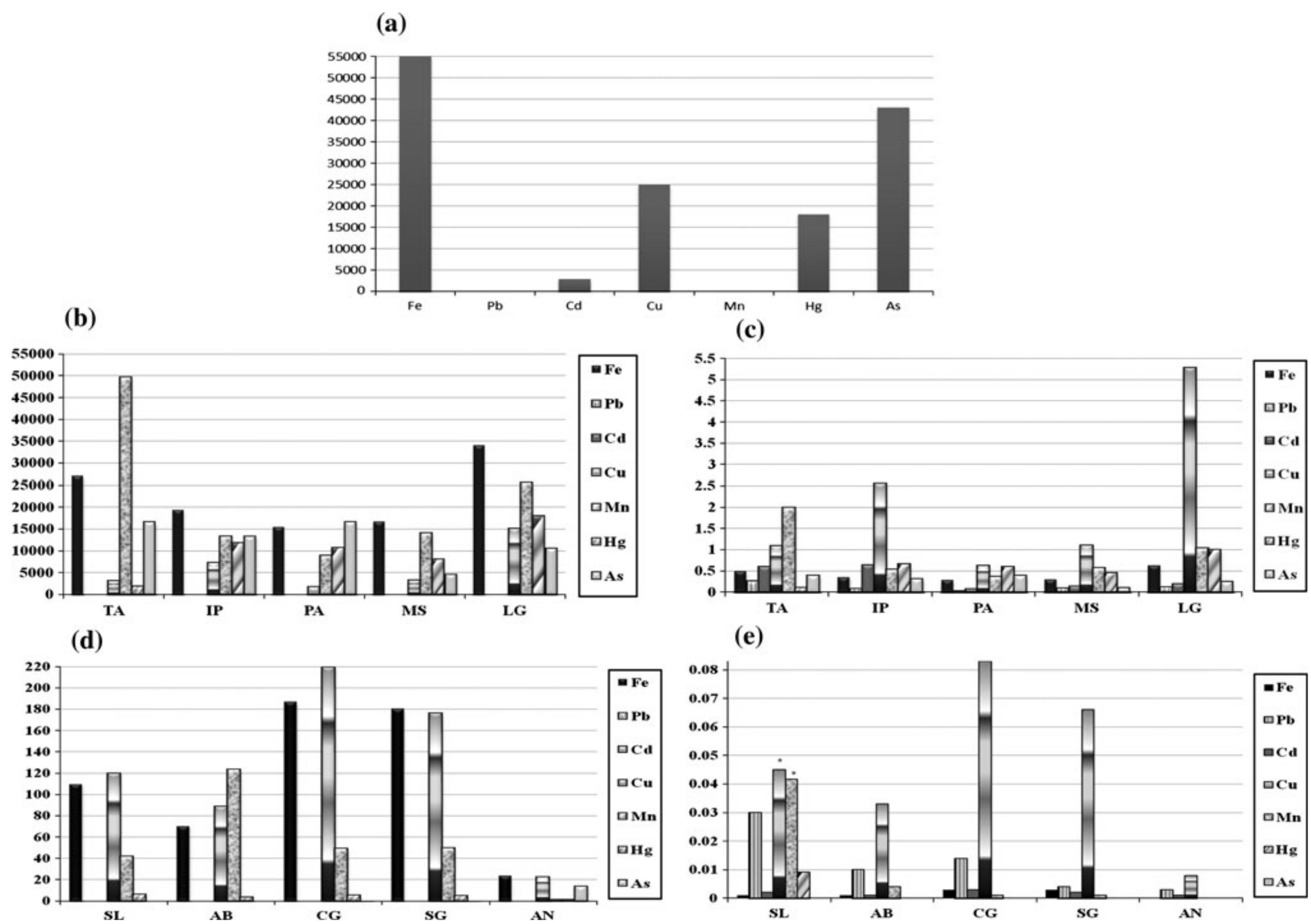

Fig. 2 The ratio of elements in the Gruža Reservoir: **a** sediment/water, **b** macrophytes/water, **c** macrophytes/sediment, **d** fishes/water, **e** fishes/sediment. TA, *T. angustifolia*; IP, *I. pseudacorus*; PA, *P. amphybium*; MS, *M. spicatum*; LG, *L. gibba*; SL, *S. lucioperca*; AB, *A. brama*; CG, *C. gibelio*; SG, *S. glanis*; AN, *A. nobilis*. *Because of very large differences between the element concentrations, the concentration was expressed as g kg^{-1}

results of our research showed the mean concentrations of investigated elements were far higher in the sediment than those calculated for the same elements in the lake water (Table 1), which is in accordance with previous findings by others (Samecka-Cymerman and Kempers 2001; Demirezen and Aksoy 2004; Pajević et al. 2008; Branković et al. 2010). In sediment, the order of concentration of elements was: As > Fe > Mn > Hg > Pb > Cu > Cd. The element accumulation in sediment is the result of long-term exposure, whereas element concentrations in water are mainly the result of recent contamination (Baldantoni et al. 2005).

Among the five plant species, the highest concentrations of Pb and Mn were observed in *T. angustifolia*, which has a highly developed root system by which the above mentioned elements accumulate from sediment. The highest concentrations of Cd were in *I. pseudacorus*, while the highest concentrations of Fe, Cu and Hg were in *L. gibba*. *P. amphybium* had the highest concentrations of As. The comparison of element content in macrophytes is often difficult because of the difference in the age of plants and presence of different pollution sources over time (Vardayan and Ingole 2006). Tolerant plants tend to restrict soil-root and root-shoot transfer, and therefore have much less accumulation in their biomass, while hyperaccumulators facilitate active uptake and translocation of these elements into their above-ground biomass (Ahmad et al. 2010). The ratio between concentration of investigated elements in plant and water was the highest for Mn for *T. angustifolia* and Fe for *L. gibba* (Fig. 2b). This might be the result of high accumulative capacity of these plants. The ratio between concentration of elements in plants and sediment was the highest for Cu for *L. gibba* (Fig. 2c).

Recently, considerable attention has been paid to determination of element concentrations in fish, due to their possible toxic effects in humans (Turan et al. 2009). In this study, concentrations of elements were measured in muscle tissue, since humans in this area consume only this part of the fish.

The concentrations of measured elements in fishes followed the order: *S. lucioperca* Fe > Mn > Pb > Cu > Hg > Cd > As; *A. brama* Fe > Mn > Cu > Pb > Hg > Cd > As; *C. gibelio* Fe > Mn > Cu > Pb > As > Hg > Cd; *S. glanis* Fe > Mn > Cu > As > Pb > Hg > Cd and *A. nobilis* As > Fe > Pb > Cu > Mn > Hg > Cd (Table 1). According to the present study, *C. gibelio* showed the highest tendency of element accumulation overall, followed by *S. glanis*, *S. lucioperca*, *A. brama* and *A. nobilis*. Mercury was accumulated most highly in the predatory *S. lucioperca*, which represented the highest trophic level amongst the five fish species. This result is in agreement with Dušek et al. (2005), who found that predators accumulated the most Hg, followed by benthophagus species, whereas omnivorous and planktivorous species contained the lowest concentrations of Hg. The species *S. lucioperca* also had the highest mean concentration of Pb. The values of the ratio between element concentrations in fishes and those in the sediment were the highest for Mn and Hg for this fish species (Fig. 2e). The omnivorous species, *C. gibelio* had the highest mean concentration of Cu. This is in agreement with Simić (2005), who also found that the highest concentration of Cu was accumulated in muscles of this species in the Gruža reservoir. This species also had the highest concentrations of Fe and Cd, which may result from the high concentrations of these elements in sediment and macrophytes. Also, the values of the ratio between element concentrations in fishes and those in the water were the highest for Cu and Fe for *C. gibelio*, which is in accordance with the nutrition of the species (Fig. 2d). The highest mean concentration of As was in *A. nobilis* which is in accordance with the statements of Storelle et al. (2005), that organisms feeding on algae and crustaceans appear to retain higher As concentrations than piscivorous species.

To compare observed concentrations of elements with the maximum permitted concentrations (MPC) in fish meat provided by the EU and the Official Gazette of Serbia, all concentrations were also expressed as mg kg^{-1} ww (Table 1). According to the European Commission Regulation (1881/2006/EC) the MPC concentrations for Pb, Cd and Hg in fish meat for human consumption are, respectively, 0.3, 0.05 and 0.5 mg kg^{-1} ww. There is no regulation in EU for maximum levels of most of the other elements. The maximum permitted concentration levels laid down in the Official Gazette of Serbia (Anonymus 2009a) for Pb, Cd, Hg, As, Fe (in tin containers) and Cu (in tin containers) are, respectively, 0.3, 0.05, 0.5, 2.0, 30.0, 30.0 mg kg^{-1} ww. Meat of *A. nobilis* had As values (2.635 ± 0.241 mg kg^{-1} ww) above the maximum permitted concentrations provided by the Official Gazette of Serbia. Meat of all other examined fishes can be safely utilized in human diet. Concentrations of As (0.259 ± 0.01 mg L^{-1}) and Hg (0.016 ± 0.001 mg L^{-1}) in water exceeded MPC for drinking water provided by the Official Gazette of Serbia (Anonymous 2009b) (As, 0.01 mg L^{-1}; Hg, 0.001 mg $L^{-1)}$. Since the mean concentrations for As and Hg in water and for As in *A. nobilis* exceeded the maximum permitted concentration levels laid down in the Official Gazette of Serbia, continued monitoring of elements in water and fish meat is needed.

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