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**Original Scientific Article**

# ELEMENTAL CONCENTRATIONS IN DIFFERENT TISSUES OF EUROPEAN PERCH AND BLACK BULLHEAD FROM SAVA LAKE (SERBIA)

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**Summary:** Distribution of 17 chemical elements in gills, muscle, and liver of the European perch (*Perca fluviatilis*) and black bullhead (*Ameiurus melas*) from the Sava Lake (Serbia) was studied to detect bioaccumulation patterns in relation to the species' diet and trophic level. Concentrations of Ba, Cd, Co, Cr, Li, Ni, Pb, B, and Se were below the detection limits. Concentrations of Al, Fe, Mn, Sr, and Zn were higher in gills of the black bullhead; As, Mo, and Sr were higher in liver of the European perch; Fe and Zn were higher in liver of the black bullhead. In muscle, a significant difference between species was found only for Sr. Copper was detected only in liver of the black bullhead. Similarity in elemental concentrations in both species could be explained by a relatively similar diet of these two species, while the differences, especially in gills, could be explained by different habitat preferences.

**Key words:** heavy metal; pollution; Percidae; Ictaluridae; Danube River

## Introduction

In recent years, one of the environmental problems of increasing concern is the contamination of freshwater fish with metals, which enter the water bodies through atmosphere, drainage, surface runoff, and soil erosion (1). While some metals are required as essential nutrients for fish, including copper, selenium, iron and zinc, their over-accumulation can pose a food safety concern. For this reason, Food and Agriculture Organization (2) declared that metals or chemicals of particular concern include arsenic, cadmium, chromium, lead, methyl mercury, nickel and selenium. Iron is not toxic to fish, but long-term exposure to high levels can be harmful (3). Iron is present in proteins such as haemoglobin and myoglobin and stored in proteins such as ferritin and hemosiderin, which are found in high concentrations in the fish liver (4). According to (5), molybdenum is relatively non-toxic to fish as compared to other metals,

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and exposure to sub-lethal concentrations of molybdenum activates neither physiological nor cellular stress response in fish.

Metals enter the fish body through the body surface, gills, or digestive tract (6) causing numerous physiological disorders. The toxic effects of metals may cause inner organ alterations, immune system disturbances, changes in blood parameters, and the reduction of organism's overall vitality and resistance to diseases, influencing individual growth rates, reproduction and mortality (7). Metals can also lead to increased mortality of fish fry and loss of genetic variability (1). Heavy metal contamination of freshwater fish represents a problem not only for piscivorous fish, birds, and mammals that consume contaminated prey (8), but also a potential human health associated with fish consumption (9).

According to (8), metal uptake and accumulation in fish is a complex process that depends on metal concentration, time of exposure, source of metal uptake, environmental conditions (water temperature, pH, hardness, salinity), and intrinsic factors (fish age, feeding habits). Various metals also differ in their affinity for different fish tissues (10). As their concentrations tend to increase along the food chain through biomagnification, piscivorous fish are often able to accumulate higher metal concentrations (10).

Feeding habits, behaviour, and regulatory ability can influence the accumulation patterns of a species (11). European perch (*Perca fluviatilis* L.) and black bullhead (*Ameiurus melas* Rafinesque) have the capacity to tolerate general disturbances in the environment. Both species are opportunistic and piscivorous when they reach adult age, with the difference that European perch is a diurnal feeder, while black bullhead is restricted to nocturnal feeding, as all ictalurids (12). Both species exhibit ontogenetic diet shifts: juvenile European perch feeds on zooplankton, subadults feed on benthic macroinvertebrates, and adults feed on fish (13); juvenile black bullhead feeds mainly on larvae/nymphs of aquatic insects, leeches, and crustaceans, while adults also feed on clams, snails, plant material, and fishes (14).

Gills, muscle, and liver are considered to be three key tissues in the monitoring of heavy metal accumulation in fish (15). Gills represent the primary site of metal uptake from the water. Although muscle tissue has a low metal accumulating ability, it is essential to include it in monitoring programs due to its role in the human diet (16). Liver is the organ with detoxificating and accumulating role (3), and appears to be the main heavy metal storage tissue (17).

In the present study we investigate the distribution and accumulation of 17 metals and trace elements in gills, muscle, and liver of the European perch and black bullhead in the Sava Lake, Serbia. The aim of the study was to detect the bioaccumulation patterns in the selected species in relation to their diet and trophic level. The study is also important from the human health perspective, because the Sava Lake represents an important recreational area, with more than 150.000 visitors per day during the high season, and concentrations of metals and trace elements accumulated in fish tissues are good indicators of water contamination and pollution. Moreover, these two fish species are used for human consumption.

## Material and methods

### *Study area*

This study was conducted in Sava Lake (44°47'17.08'' N, 20° 24' 35.75'' E), formerly a right-hand branch of the Sava River, located next to the river island Ada Ciganlija (Fig. 1). This 86 ha lake is located in the city of Belgrade, within the urban area (18).

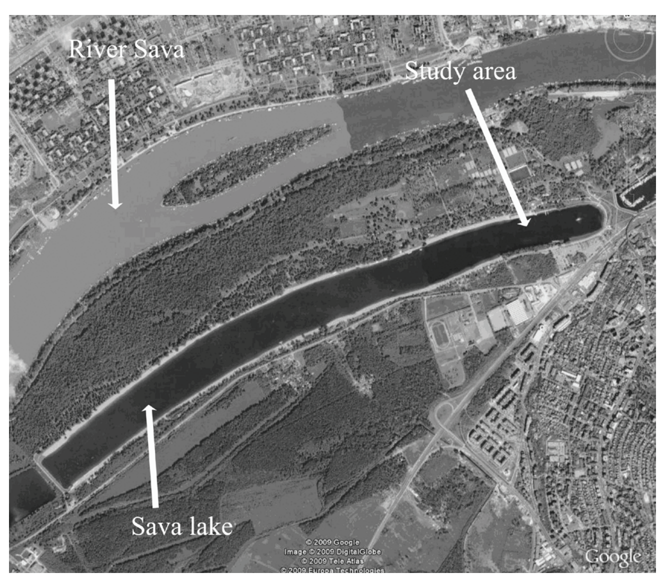


**Figure 1:** Satellite image of Sava Lake with the study area marked

There are 20 different fish species present in the lake. Relative to the total fish biomass in the lake, European perch is represented with 6.0% and black bullhead with 5.7%. According to (19) the total biomass of the European perch and black bullhead populations in the lake was 7.5 kg $ha^{-1}$ and 9.5 kg $ha^{-1}$, respectively, while the annual production was 11.9 kg $ha^{-1}$ and 15.2 kg $ha^{-1}$, respectively.

### *Sampling and sample preparation*

All European perch and black bullhead specimens were sampled in March and September 2010. Samples were collected using 15 double fyke nets (8 mm mesh-size), which were left in the water for 3 nights and checked daily.

Nine European perch and 10 black bullhead specimens were collected and their total length (TL) and weight (W) were measured to the nearest 0.1 cm and 0.1 g accuracy, respectively. Individuals were anaesthetised by administering clove oil in the water until they were determined to be unconscious (i.e., by the loss of reflexes) (20). Specimens were consequently dissected with a plastic laboratory set. Samples of gills, muscle, and liver were quickly removed, washed with distilled water, and stored at -18°C prior to analysis. All animal procedures were in compliance with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes, and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

### *Element analysis*

The following elements were analysed: aluminium (Al), arsenic (As), boron (B), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), strontium (Sr), and zinc (Zn). All samples were dried by Freeze Dryers Rotational-Vacuum-Concentrator, GAMMA 1-16 LSC, Germany, and sample portions between 0.2 and 0.5 g (dry weight) were subsequently processed in a microwave digester (speedwave™ MWS-3$^+$; Berghof Products + Instruments GmbH, Eningem, Germany), using 6 ml of 65% $HNO_3$ (Merck suprapure) and 4 ml of 30% $H_2O_2$ (Merck suprapure) at a food temperature program (100–170°C). After cooling to a room temperature, digested samples were diluted with distilled water to a total volume of 25 ml. The analysis was performed by inductively-coupled plasma optical spectrometry (ICP-OES; Spectro Genesis EOP II, Spectro Analytical Instruments DmbH, Kleve, Germany), comprising the assessment of concentrations of 17 metals expressed as μg $g^{-1}$ dry weight (dw). The following wavelength lines of the ICP-OES analysis were used (nm): Al 394.401, As 189.042, B 249.773, Ba 233.527, Cd 228.802, Co 228.616, Cr 205.552, Cu 324.754, Fe 259.941, Li 460.289, Mn 259.373, Mo 202.095, Ni 231.604, Pb 220.353, Se 196.090, Sr 460.733, and Zn 206.191. The following detection limits were obtained during the analysis (mg $l^{-1}$): Al 0.00158, As 0.00282, B 0.000931, Ba 0.000531, Cd 0.000132, Co 0.00024, Cr 0.000366, Cu 0.000588, Fe 0.000562, Li 0.042, Mn 0.000403, Mo 0.000784, Ni 0.00114, Pb 0.00343, Se 0.00197, Sr 0.00138 and Zn 0.000391. 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.

### *Statistical analysis*

To compare heavy metal and trace element distribution and concentrations between European perch and black bullhead specimens, as well as between different tissues of the two species, the principal component analysis (PCA) was applied. To compare particular pairs of samples, the Mann-Whitney U test was used. In order to compare heavy metal and trace element concentrations in studied fish with maximum allowable concentrations (MAC) in fish meat for the utilization in human diet, established by the national legislation (21), concentrations were recalculated to the μg $g^{-1}$ wet tissue weight (ww).

## Results

The average total length of European perch and black bullhead specimens was 17.4 ± 1.9 cm and 19.6 ± 1.7 cm, respectively, while the average weight was 69.6 ± 25.1 g and 79.6 ± 21.9 g, respectively. Concentrations of B, Ba, Cd, Co, Cr, Li, Ni, Pb, and Se were below the detection limits in all analysed samples, and consequently, concentrations of these elements were not subjected to statistical analysis. Concentration of Al was below the detection limit in muscle and liver, as well as of Fe and Mn in muscle of both fish species. Cu was detected only in black bullhead liver samples. Heavy metal and trace element concentrations in each of the three analysed tissues of the two fish species are presented in Table 1.

Results of the PCA conducted on gill samples indicated that the black bullhead samples were grouped based on the increased concentrations of Al, Fe, Sr, and Zn (Fig. 2). In muscle samples, European perch specimens were differentiated by higher concentrations of Sr (Fig. 3). In liver samples, black bullhead specimens were differentiated by higher Cu, Mn, and Zn concentrations, and European perch specimens by higher As, Mo, and Sr concentrations (Fig. 4).

Accumulation of the elements in gills had the following trend: Fe>Zn>Sr>Al>As>Mo>Mn in European perch, and Fe>Al>Zn>Sr>As>Mn>Mo in black bullhead. The trend of elemental accumulation in muscle was Sr>Zn>As>Mo in European perch, and Zn>Sr>As>Mo in black bullhead. Elemental ranking in the liver was Fe>Zn>Sr>As>Mo in European perch, and Fe>Zn>Sr>Cu>As>Mo>Mn in black bullhead. Results indicate that the distribution of investigated elements among different tissues had a consistent pattern among the two studied fish species.

Mann-Whitney U Test indicated that Fe and Zn concentrations in both liver and gills significantly differed ($p<0.05$) between the two fish species, as well as Al, Mn, and Sr in gills and As and Mo in liver (Table 1). Sr was the only element whose concentrations significantly differed between the two species in muscle.

The national MAC for Fe, prescribed by the National Regulation of the Republic of Serbia (for canned fish meat, 30.0 $\mu g\ g^{-1}$ wet weight) (21), was exceeded in gills and liver, with average values of 33.9 and 42.7 $\mu g\ g^{-1}$ ww in gills of European perch and black bullhead, respectively, and of 32.2 and 110.5 $\mu g\ g^{-1}$ ww in liver of European perch and black bullhead, respectively. Arsenic concentrations exceeded the national MAC (for canned fish meat, 2.0 $\mu g\ g^{-1}$ ww) and the lower limit set by FAO (0.1 $\mu g\ g^{-1}$) only in liver of European perch (3.96 $\mu g\ g^{-1}$ ww). Concentrations of Cu and Zn were below the national MAC of 30 $\mu g\ g^{-1}$ and 100 $\mu g\ g^{-1}$, respectively, in all analysed tissues and in both fish species. There is no national MAC established for Al, Mn, Mo, and Sr.

**Table 1:** Concentrations of chemical elements in different tissues of European perch and black bullhead (mean ± standard deviation). Concentrations are expressed as $\mu g\ g^{-1}$ dw; significant differences between the two species within the same tissue are marked with an asterisk (*) ($p<0.05$ Mann-Whitney U Test); ND indicates the values below the detection limit

| | Gills | | Muscle | | Liver | |
|---|---|---|---|---|---|---|
| | European perch | Black bullhead | European perch | Black bullhead | European perch | Black bullhead |
| Al | 21.0 ± 37.4* | 212 ± 65.6* | ND | ND | ND | ND |
| As | 3.82 ± 0.54 | 4.83 ± 2.07 | 3.89 ± 0.30 | 4.16 ± 0.68 | 8.05 ± 4.01* | 4.82 ± 1.80* |
| Cu | ND | ND | ND | ND | ND | 10.91 ± 8.28 |
| Fe | 126 ± 53.0* | 246 ± 84.7* | ND | ND | 150.84 ± 141* | 451 ± 276* |
| Mn | 1.49 ± 1.07* | 3.29 ± 2.02* | ND | ND | ND | 2.37 ± 1.78 |
| Mo | 1.77 ± 0.27 | 2.30 ± 1.02 | 1.85 ± 0.16 | 1.99 ± 0.33 | 4.23± 2.08* | 2.69 ± 0.90* |
| Sr | 34.7 ± 6.42* | 51.7 ± 11.4* | 30.7 ± 4.60* | 22.0± 3.59* | 41.3 ± 21.7 | 25.5 ± 9.44 |
| Zn | 49.7 ± 10.1* | 73.6 ± 9.99* | 29.8 ± 8.26 | 31.5 ± 8.82 | 42.7 ± 15.0* | 88.5 ± 16.2* |

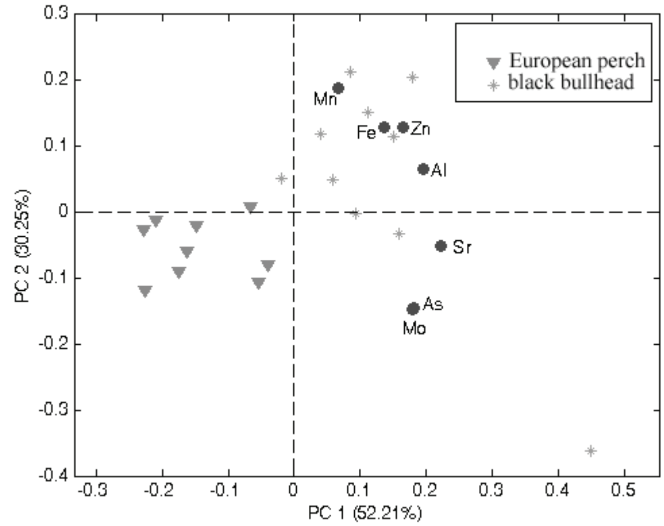


**Figure 2:** PCA of elemental concentrations in gills of European perch and black bullhead (untreated data were used as input variables)

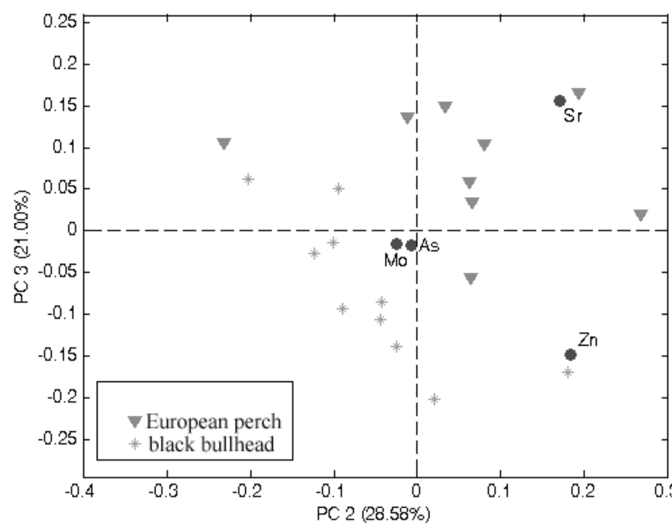


**Figure 3:** PCA of elemental concentrations in muscle of European perch and black bullhead (untreated data were used as input variables)

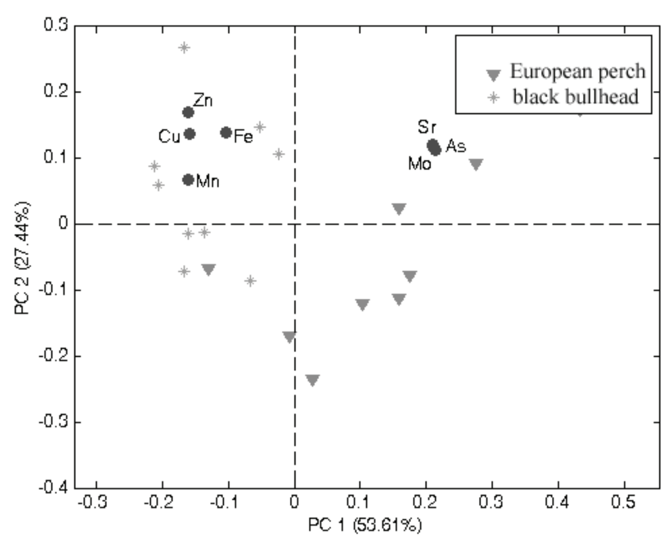


**Figure 4:** PCA of elemental concentrations in liver of European perch and black bullhead (untreated data were used as input variables)

## Discussion

In the present study, elemental concentrations were the lowest in muscles and the highest in liver and gills, which are metabolically more active tissues. This is in line with the findings of other authors (11). Similar results obtained for a number of fish species show that muscle is not an active tissue in accumulating metals (22). As stated by (23), fish muscle tissue accumulates lower concentrations of metals because it is well protected by the activity of other organs.

Cu was below detection limits in muscle and gills of both fish species, which is in accordance with the findings of (24). Tendency of Cu to accumulate in liver, as is the case of black bullhead in the present study, has also been observed by other authors in several fish species (8,17). Concentration of Cu in European perch liver was below detection limits, which differs from the findings of other authors (16, 25). Tkatcheva et al. (25) have found high concentrations of Cu in European perch in the area where the level of Cu is unusually high due to the local geological features, while the area studied by (16) is significantly polluted and eutrophic. On the other hand, as shown by (26), Zn can inhibit the accumulation of Cu in animal tissues and, consequently, offer certain protection against its toxic effects.

Fe was not detected in muscle, but high concentrations were found in gills and liver of both fish species, which is similar to observations made in 20 fish species inhabiting various freshwater habitats in Lithuania (1). High Fe concentrations in fish liver were also reported by other authors (3,8,24). Lowest tendency of Fe to accumulate in muscle was observed by (21) as well.

Mn was detected only in gills of both species and in liver of the black bullhead, but in all cases in very low concentrations in comparison to other elements, which corresponds to findings of (24). The tendency of Mn to accumulate primarily in gills was also observed in perch, as well as in other fish species (3,23).

Determined concentrations of Mo were higher in all three tissues, both in European perch and black bullhead, than in sterlet (*Acipenser ruthenus*) (17), and in Pontic shad (*Alosa immaculata*) (15), where Mo concentrations were below the detection limits. Low Mo concentrations were also detected in muscle, gills, liver and gonads of five fish species from the Danube River (27), in muscle and liver of five fish species from the Persian Gulf (11), and in muscle of sturgeons from the Caspian Sea (28).

This study revealed that both fish species had high Zn concentrations in all three analysed tissues. However, they were below the national MAC of 100 μg $g^{-1}$(21), and also below the lower FAO limit of 40 μg $kg^{-1}$ (31). Petkovšek et al. (9) also found that the overall concentration of Zn was the highest in comparison to other metals detected in gills, muscle, and liver of ten analysed fish species in three lakes in Slovenia. Although Zn is an essential element for human nutrition, it can be harmful when present in high concentrations (29). The mean Zn concentrations in analysed European perch and black bullhead specimens were lower in muscle than in gills, which is in line with the findings of (24), but contrary to the findings of (29).

European perch specimens had higher concentrations of As, Fe, Mo, Sr, and Zn in liver than in muscle, which is in line with the findings of (25), and (16), while Al, Cu, and Mn were below the detection limit in both tissues. There is a deficiency of the published data regarding heavy metal concentrations in black bullhead. The only available reference is the one by (30) who analysed elemental concentrations in homogenates of various fish species from Malibu Creek and Malibu Lagoon (Los Angeles, California), including black bullhead. According to their findings, assessed elements had the following trend: Fe>Zn>Al>Sr>Mo>As>Mn>Cu, which is in line with the results from the present study.

Similar heavy metal accumulation patterns in both species observed in the present study might be explained by a relatively similar diet of the two species. However, European perch had higher concentrations of Sr in muscle and of As, Mo, and Sr in liver, while black bullhead had higher concentrations of all analysed elements in gills, as well as of Cu, Fe, Mn, and Zn in liver (Table 1). This might be caused by different liver physiology and metabolic activity, habitat preferences, and predator-prey relationship between the two species, since recent field studies on Sava Lake indicated that the black bullhead preys on juvenile European perch specimens, while the opposite case was not recorded (Personal unpublished data). It is also well known that molluscs and crustaceans, which are important food components for subadult European perch and for both juvenile and adult black bullhead, contain higher levels of several metals, particularly Cu and Zn, than do fish (16,25), which suggest that these prey organisms are the source of Cu and Zn in fish.

## Conclusions

To conclude, concentrations of all analysed elements in both fish species were below MAC in all muscle samples; therefore, the consumption of these fish does not pose a risk to human health. Only Fe concentrations exceeded MAC in gills and liver of both fish species, and As in liver of European perch, which is in accordance with the previous studies on the Danube fish (17). Since fish species are good bioindicators of water contamination and pollution, the obtained results are important because of the large number of visitors that use the lake for recreational purposes. In addition, these two fish species are also used for human consumption.

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# KONCENTRACIJE ELEMENTOV V RAZLIČNIH TKIVIH EVROPSKEGA OSTRIŽA IN ČRNE PISANKE IZ JEZERA SAVA (SRBIJA)

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**Povzetek:** Preučili smo porazdelitev 17 kemijskih elementov v škrgah, mišicah in jetrih evropskega ostriža (Perca fluviatilis) in črnega somiča (Ameiurus melas) iz jezera Sava (Srbija). Želeli smo ugotoviti vzorce bioakumulacije glede na vrstno specifično prehrano in glede na nivo v prehranski verigi. Koncentracije Ba, Cd, Co, Cr, Li, Ni, Pb, B in Se so bile pod mejo detekcije. Koncentracije Al, Fe, Mn, Sr in Zn so bile višje v škrgah črnega somiča. As, Mo in Sr so bile višji v jetrih evropskega ostriža; Fe in Zn pa v jetrih črnega somiča. V mišicah je bila edina razlika med vrstama v koncentraciji Sr. Baker smo ugotovili samo v jetrih črnega somiča. Glede na to, da nismo odkrili bistvenih razlik v koncentraciji merjenih elementov med vrstama, sklepamo, da imata obe relativno podobno prehrano. Razlika v koncentraciji elementov v škrgah pa nakazuje, da imata vrsti različne preference do življenjskega okolja.

**Ključne besede:** težka kovina; onesnaževanje; ostriži; ameriški somič; Donava