

## **CONCENTRATIONS OF 16 ELEMENTS IN TISSUES (LIVER, MUSCLE, SCALES) OF PRUSSIAN CARP (*CARASSIUS GIBELIO*, BLOCH, 1782) IN MEDJUVRŠJE RESERVOIR, SEASONAL ASPECT**

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## **KONCENTRACIJA 16 ELEMENATA U TKIVIMA (JETRA, MIŠIĆ, ŠKRGE) BABUŠKE (*CARASSIUS GIBELIO*, BLOCH, 1782) AKUMULACIJE MEĐUVRŠJE, SEZONSKI ASPEKT**

### *Apstrakt*

Akumulacija Međuvršje nalazi se u izlaznom delu Ovčarsko-kablarske klisure. Dužina akumulacije iznosi 9.3km, a površina 1.5km<sup>2</sup>. Najveća širina iznosi 272m, dok je maksimalna dubina do 12m zabeležena neposredno ispod brane. Dno akumulacije je najvećim delom muljevito, i u manjoj meri peskovito. Aktivnost HE Međuvršje dovodi do oscilovanja u litoralnoj zoni od 20 do 30 cm. Stalno taloženje rečnog nanosa dovodi do stvaranje malih zaravni koje usporavaju tok i menjaju izgled rečnog korita. Kako je akumulacija oivičena branama HE Ovčar i HE Međuvršje, od kojih ni jedna ne poseduje objekte za prelaz riba (tzv. riblje staze), uzvodne i nizvodne migracije i kontakti ribljih populacija su praktično onemogućeni što akumulaciju čini jedinstvenim zatvorenim sistemom.

Prema podacima koji su prikupljeni tokom terenskih istraživanja 2012. godine, akumulaciju Međuvršje naseljavaju 19 vrsta riba iz ukupno 6 familija. Akumulacija Međuvršje ima sastav ribljeg naselja koji ukazuje da je to ribolovna voda sa osobinama i elementima gornjeg toka šaranskih riba ili region rečne mreže (epipotamon) i srednjeg toka šaranskih riba ili region deverike (metapotamon). Riblju zajednicu akumulacije Međuvršje karakteriše dominacija uklije (*A.alburnus*), gavčice (*Rodeus sericeus*) i ba-

buške (*Carasius gibelio*), uz zadovoljavajuću brojnost bodorke (*Rutilus rutilus*), skobalja (*Ch.nasus*), grgeča (*Perca fluviatilis*) i klana (*S.cephalus*). Nepovoljnoj strukturi ihtiofaune doprinose „novi članovi“ – alohtone vrste, babuška, cverglan (*Ameiurus melas*), čebačok (*Pseudorasbora parva*) i sivi tolstolobik (*Hypophthalmichthys nobilis*) (Simović 2001).

Babuška (*Carassius gibelio* bloch 1782) živi u stajaćim i sporotekućim nizijskim vodama. Naseljava deo Azije i srednju Evropu, sa izuzetkom Italije, Švajcarske i južne Francuske. Nalažena je i u Sibiru sve do reke Lene. U naše predele introdukovana je iz jugoistočne Azije. Ima je u rekama Dunavskog sliva, a najbrojnija je u plavljenim zonama Dunava, Save, Tise, Begeja, Tamiša, u kanalskoj mreži Dunav-Tisa-Dunav, starim koritima Velike Morave i šljunkarama pored Dunavskih pritoka.

Ribe su često izložene visokom stepenu zagađenja u vodi, što može dovesti do čitavog niza različitih promena, od biohemijskih na nivou ćelija, do promena na nivou celih populacija (Bernet et al. 1999). S obzirom da se ribe nalaze na vrhu lanaca ishrane u vodenoj sredini, često u organizmu akumuliraju velike količine pojedinih teških metala (Yilmaz et al. 2007). Takođe se smatraju i jednim od najosetljivijih akvatičnih organizama na prisustvo toksičnih materija u vodi (Alibabić i sar. 2007).

Do sada je većina istraživanja pretežno bila usmerena na akumulaciju teških metala u mišićnom tkivu riba, pošto je to osnovni deo ribe koji se koristi u ishrani (Storelli et al. 2006; Keskin et al. 2007). Kao rezultat mehanizama absorpcije, regulacije, skladištenja i ekskrecije, tkiva se međusobno razlikuju po stepenu akumulacije, kao i po svojoj ulozi u ovim procesima (Storelli et al. 2006). Mišićno tkivo ne predstavlja uvek dobar indikator celokupne kontaminacije organizma, pa je stoga važno u analizu uključiti i druge organe, kao što su jetra i škrge (Has-Schön et al. 2006).

Tokom ihtioloških istraživanja na akumulaciji Međuvršje 2012. godine ispitivao se nivo akumulacije 16 metala u tkivima (mišić, jetra, škrge) babuske. Škrge su bile centar akumulacije Ba, Mn, Sr, Zn i B, dok je jetra centar akumulacije gvožđa i bakra. Mišići, su generalno imali niži nivo akumulacije u odnosu na druga dva tkiva. U mišićima su zabeležene koncentracije žive iznad maksimalno dozvoljenih evropskom i nacionalnom legislativom.

*Ključne reči: babuška, teški metali, koncentracija, akumulacija Međuvršje*

*Keywords: Prussian carp, heavy metals, concentrations, Međuvršje reservoir*

## INTRODUCTION

The heavy metals presence in aquatic ecosystems, in recent times, is paid considerable attention due to their toxicity and effects on living organisms (Dural et al. 2006). Contamination of aquatic ecosystems (e.g. lakes, rivers, streams) with heavy metals and trace elements has been receiving increased worldwide attention (Mansour and Sidky, 2002). Metallic elements are environmentally ubiquitous, readily dissolved in and transported by water and readily taken up by aquatic organisms. These elements enter in aquatic environment by atmospheric deposition, by erosion of the geological matrix, or through anthropogenic sources, such as industrial effluents and mining wastes, agriculture (Alam et al., 2002, Kumar Singh et al., 2007). They are a serious threat because of toxicity, bioaccumulation effects, long persistence, and biomagnifications in the food chain (Erdogrul and Ates, 2006).

Fish are at the top of the food chain in aquatic ecosystems, which is the reason why some heavy metals accumulate in their body (Yilmaz et al., 2007, Mansour and Sidky, 2002). Fish is also considered as one of the most sensitive groups of organisms in the presence of toxic substances in the water (Alibabić et al., 2007). Fish meat is an essential component of the human diet, there is therefore a great interest in the scientific community for the presence of heavy metals in water and their accumulation in fish tissues of (Dural et al., 2006; Storelli et al., 2006; Alibabić et al., 2007 ; Erdogrul and Erbilir, 2007; Keskin et al., 2007). Most of the research, so far, has been mainly focused on the muscle tissue of fish (Storelli et al., 2006; Keskin et al., 2007). But muscles are not always the best indicators of overall contamination in the body, and therefore it is important to include other organs such as liver and gills in the analysis (Has-Schön et al., 2006). Monitoring of heavy metal contamination of commercially important fish species is of great importance (Erdogrul and Erbilir, 2007; Yilmaz et al., 2007).

Prussian carp is a non-indigenous, invasive fish species, introduced in Europe (European part of USSR) in 1948 (Maletin et al., 1981). In Serbian open water prussian carp appeared at the beginning of the 1960s (Plancic, 1967), and in Medjuvsrje reservoir it was registered for the first time in 1984 (Simović, 2001). After that, prussian carp has been mass present in all fish fauna inventories (Marković, 2003, 2007; Skorić and Đikanić, 2012), and it is the most common species in the catch of recreational fishermen on the reservoir.

Medjuvsrje reservoir is located in the output section of Ovčarsko-Kablarska gorge. It is one of the oldest reservoirs in Serbia, formed in 1953 by damming the Zapadna Morava River. Water quality of Medjuvsrje reservoir is greatly influenced by many pollutants of industrial, municipal and rural wastewater.

## MATERIAL AND METHODS

Collection of fish samples was performed during 2012. with seasonally dynamic (April, June, November) using a set of standing gill-nets with buds (mesh) diameter of 30 - 50 mm. The total weight (g) and total body length (cm) of each fish specimen were measured. Samples of muscle, liver and gills were removed on field and frozen prior to analysis. In laboratory, 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-3b; Bergof Products Instruments GmbH, Eningem, Germany), using 6 ml of 65% HNO<sub>3</sub> and 4 ml of 30% H<sub>2</sub>O<sub>2</sub> (Merck suprapure) at a food temperature program (100–170 °C). Potential presence of analyzed elements was resolved by using a number of blank samples. Following a cooling to a room temperature, the 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). It included the assessment of concentrations of the following 16 elements: As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sr and Zn. The quality of the analytical process was controlled by the analysis of BCR-185R reference material of bovine liver, as well as IAEA-336 Lichen reference material. All elemental concentrations were expressed as mg g<sup>-1</sup> dry weight (dw).

Statistical analysis included comparisons of elemental concentrations between three tissues of the prussian carp individuals (Discriminant Analysis - Multivariate Exploratory Techniques), as well as among different elemental concentration in tissues per season (ANOVA - Tukey test).

## RESULTS AND DISCUSSION

During this study 30 individuals of prussian carp have been analysed. Mean body weight of individuals was  $184.51 \pm 92.43$  g (mean  $\pm$  standard deviation, range 66 – 446 g), total body length  $22.07 \pm 3.57$  cm (range 16 – 30 cm). The determined concentrations of heavy metals and trace elements in three tissues analyzed are shown in Table 1. The analysis showed that the Cd in all samples was below the detection limit.

**Table 1.** Heavy metal and trace elements concentrations in muscle of selected fish species (mean values $\pm$ SD). Concentrations are expressed as  $\mu\text{g/g}$  of dry weight.

		APRIL	JUNE	NOVEMBER
<b>As</b> $\mu\text{g/g}$	liver	0.976 $\pm$ 0.121	0.803 $\pm$ 0.536	1.885 $\pm$ 1.846
	gills	0.375 $\pm$ 0.224	0.357 $\pm$ 0.264	1.748 $\pm$ 1.13
	muscle	0.693 $\pm$ 0.379	0.618 $\pm$ 0.317	2.103 $\pm$ 1.166
<b>B</b> $\mu\text{g/g}$	liver	19.746 $\pm$ 4.039	33.667 $\pm$ 15.950	8.193 $\pm$ 7.894
	gills	29.281 $\pm$ 8.917	18.099 $\pm$ 2.503	10.766 $\pm$ 9.209
	muscle	20.815 $\pm$ 7.737	13.687 $\pm$ 0.745	7.788 $\pm$ 8.018
<b>Ba</b> $\mu\text{g/g}$	liver	4.490 $\pm$ 2.622	7.470 $\pm$ 2.493	6.255 $\pm$ 3.372
	gills	31.662 $\pm$ 12.288	22.169 $\pm$ 4.389	31.652 $\pm$ 8.135
	muscle	3.258 $\pm$ 2.908	0.533 $\pm$ 0.196	0.713 $\pm$ 0.665
<b>Co</b> $\mu\text{g/g}$	liver	0.180 $\pm$ 0.028	0.189 $\pm$ 0.128	0.052 $\pm$ 0.062
	gills	0.210 $\pm$ 0.079	0.167 $\pm$ 0.022	0.169 $\pm$ 0.076
	muscle	0.055 $\pm$ 0.035	0.050 $\pm$ 0.029	0.020 $\pm$ 0.025
<b>Cr</b> $\mu\text{g/g}$	liver	0.284 $\pm$ 0.175	0.628 $\pm$ 0.465	0.487 $\pm$ 0.302
	gills	0.660 $\pm$ 0.281	0.657 $\pm$ 0.297	1.161 $\pm$ 0.335
	muscle	0.068 $\pm$ 0.066	0.118 $\pm$ 0.056	0.105 $\pm$ 0.091
<b>Cu</b> $\mu\text{g/g}$	liver	80.783 $\pm$ 81.257	130.744 $\pm$ 94.97	20.484 $\pm$ 18.737
	gills	15.213 $\pm$ 31.116	3.943 $\pm$ 0.662	5.807 $\pm$ 0.618
	muscle	3.651 $\pm$ 1.013	2.674 $\pm$ 1.002	8.593 $\pm$ 18.833
<b>Fe</b> $\mu\text{g/g}$	liver	255.133 $\pm$ 31.666	399.693 $\pm$ 280.945	236.519 $\pm$ 118.476
	gills	250.6769 $\pm$ 97.911	285.192 $\pm$ 106.438	496.082 $\pm$ 182.12
	muscle	29.866 $\pm$ 12.55	24.890 $\pm$ 15.754	60.399 $\pm$ 111.182
<b>Hg</b> $\mu\text{g/g}$	liver	3.832 $\pm$ 1.297	8.619 $\pm$ 3.906	2.300 $\pm$ 1.545
	gills	5.611 $\pm$ 2.23	4.506 $\pm$ 0.663	2.993 $\pm$ 1.636
	muscle	4.487 $\pm$ 0.986	4.112 $\pm$ 0.24	2.946 $\pm$ 1.641
<b>Li</b> $\mu\text{g/g}$	liver	2.822 $\pm$ 0.990	6.368 $\pm$ 3.125	1.529 $\pm$ 1.349
	gills	4.151 $\pm$ 1.558	3.613 $\pm$ 0.978	3.785 $\pm$ 0.618
	muscle	2.742 $\pm$ 0.422	2.684 $\pm$ 0.219	1.609 $\pm$ 1.48

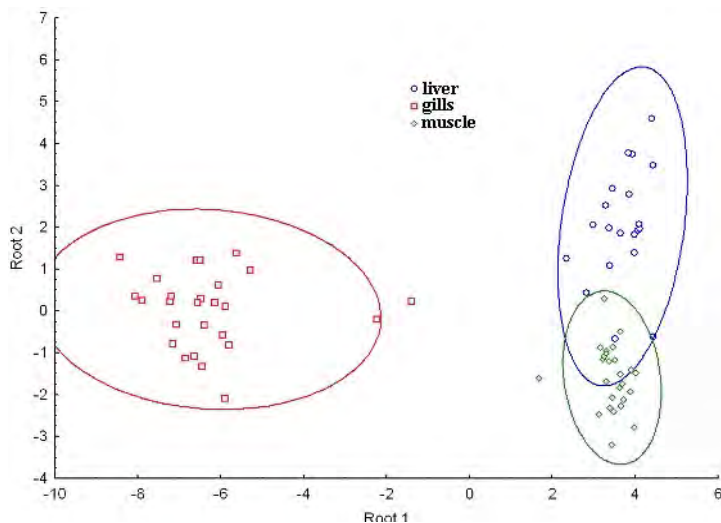
<b>Mn</b> µg/g	liver	2.629±1.520	3.763±1.299	2.783±4.245
	gills	25.344±8.870	23.863±4.006	32.895±6.447
	muscle	1.444±0.431	1.160±0.395	1.262±0.574
<b>Mo</b> µg/g	liver	3.705±0.674	7.406±3.626	3.261±1.454
	gills	4.644±1.963	3.540±0.598	2.946±0.541
	muscle	3.468±0.597	2.864±0.285	2.952±0.384
<b>Ni</b> µg/g	liver	0.038±0.0363	0	0.161±0.225
	gills	0.132±0.172	0.033±0.060	0.954±0.840
	muscle	0.201±0.450	0	0.035±0.110
<b>Pb</b> µg/g	liver	0.649±0.226	1.044±0.728	3.432±2.247
	gills	1.115±0.992	1.078±1.104	11.588±6.771
	muscle	0.535±0.346	0.406±0.284	0.453±0.498
<b>Sr</b> µg/g	liver	0.415±0.69	1.95±0.914	1.118±1.018
	gills	110.56353±22.85251	98.058±24.712	103.753±18.637
	muscle	5.794±10.101	2.443±1.251	3.076±2.033
<b>Zn</b> µg/g	liver	111.46±31.383	111.46±31.383	52.363±13.06
	gills	225.06±73.758	225.06±73.758	278.821±72.334
	muscle	55.536±25.412	55.536±25.412	56.883±18.882

In analyzed fish tissues, As was distributed like liver>muscle>gills, while Cu was staggered muscle>liver>gills. By tissues, Hg was distributed uniformly.

The extent of accumulation significantly differed among the three studied fish tissues (Discriminant Analysis) (Table 1, Figure 1). In this study, gills were the center of accumulation of Ba, Mn, Sr, Zn and B. Liver was the center of accumulation of Fe, Cu while the muscle had the high levels of Fe and Zn. The muscle generally had lower elemental levels when compared with those detected in other tissues. Other elements do not show a clear pattern of accumulation already occurring seasonal variations of concentrations in different tissues. These results are similar with concentration of the same studied elements in research of relation between great cormorants and their prey (prussian carp) (Skoric et al., 2012).

To compare observed heavy metal levels with the maximum acceptable concentrations (MAC) in fish meat provided by both EU and national regulation, all concentrations were recalculated to wet weight. According to the European Commission Regulation (1881/2006/EC), the MAC for Pb, Cd and Hg in fish meat for human consumption are, respectively, 0.3, 0.05 and 0.5 µg/g wet weight. National regulation of Republic of Serbia prescribed 0.3, 0.05, 0.5 and 2 µg/g wet weight as MAC for Pb, Cd, Hg and As in fresh fish meat, respectively (Anonimus, 2011). National MAC for Zn, Cu and Fe are 100.0, 30.0 and 30.0 µg/g wet weight, respectively (for canned fish meat). Hg concentrations in the prussian carp muscle were above MAC for fish meat provided by EU and national regulation of the Republic of Serbia, while the levels of other heavy metals were below the permissible levels.

Remarkably higher concentration of Cu in liver with regard to other analyzed tissues, were registered also in some other researchers (Rashed 2001; Storelli et al. 2006; Wu et al. 2006; Farag et al. 2007; Yilmaz et al. 2007; Uysal et al. 2009).



**Figure 1.** Distribution of elemental concentration in prussian carp tissues (liver, gills, muscle).

Comparison of the extent of accumulation among analyzed fish tissues showed that the tissue concentrations significantly differed ( $p < 0.05$ ; Table 1) for five of the assessed elements (B, Cu, Fe, Sr, Zn), depending of the sampling season. Concentrations of Zn, Fe, Sr were higher in gills with maximum values in November. The highest concentrations of these heavy metals in liver were in June. In muscle concentrations were similar while Fe was the highest in November.

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## REFERENCES

Alam, M. G. M., Tanaka, A., Allinson, G., Laurenson, L. J. B., Stagnitti, F., and Snow, E. T. (2002) A comparison of trace element concentrations in cultured and wild carp (*Cyprinus carpio*) of lake Kasumigaura, Japan. *Ecotoxicology and Environmental Safety*, 53, 348–354.

Alibabić, V., Vlahčić, N., Bajramović, M. (2007) Bioaccumulation of metals in fish of Salmonidae family and the impact on fish meat quality. *Environmental Monitoring and Assessment* 131, 349-364.

Anonimus (2011): Pravilnik o količini pesticida, metala i metaloida i drugih otrovnih supstancija, hemioterapeutika, anabolika i dr. supstancija koje se mogu nalaziti u namirnicama. *Službeni glasnik RS* 25/20110, 28/2011.

Farag, A.M., Nimick, D.A., Kimball, B.A., Church, S.E., Harper, D.D., Brumbaugh, W.G. (2007): Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish in the boulder river watershed, montana, and the role of colloids in metal uptake. *Arch. Environ. Con. Tox.* 52, 397–409.

Dural, M., Goksu, L.Z.M., Ozak, A.A., Derici, B. (2006) Bioaccumulation of some heavy metals in different tissues of *Dicentrarchus labrax* L, 1758, *Sparus aurata* L, 1758 and *Mugil cephalus* L. 1758 from the Camlik Lagoon of the eastern coast of Mediterranean (Turkey). *Environ Monit Assess* 18:65–74.

Erdogrul, Z., Ates, D.A. (2006) Determination of cadmium and copper in fish samples from Sir and Menzelet dam lake Kahramanmaraş, Turkey. *Environ Monit Assess* 117:281–290.

Has-Schön, E., Bogut, I., Strelec, I. (2006): Heavy metal profile in five fish species included in human diet, domiciled in the end flow of River Neretva (Croatia). *Archives of Environmental Contamination and Toxicology* 50, 545-551.

Keskin, Y., Baskaya, R., Özyaral, O., Yurdun, T., Lüleci, N.E., Hayran, O. (2007): Cadmium, lead, mercury and copper in fish from the Marmara Sea, Turkey. *Bulletin of Environmental Contamination and Toxicology* 78, 258-261.

Kumar Singh, R., Chavan, S.L., Sapkale, P.H. (2007): Heavy metal concentrations in water, sediments and body tissues of red worm (*Tubifex* spp.) collected from natural habitats in Mumbai, India. *Environmental Monitoring and Assessment* 129, 471-481.

Maletin, S., Pujin, V., Budakov, Lj. (1981) Variations of morphological characters of *Carassius auratus gibelio* Bloch, 1783 (Ciprinidae) in Vojvodina province waters. *Biosistematika* 7(2): 181-188.

Mansour, S.A. and Sidky, M.M. (2002) Ecotoxicological studies. 3. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Food Chemistry*, 78, 15–22.

Marković, G. (2003) Monitoring of ichthyofauna in Međuvršje reservoir. *Notebook of Ovčarsko-kablarska gorge* 2: 39-47.

Marković, G. (2007) Medium-term program of fisheries management and improvement on fishery catchment area of enormous features „Ovčarsko-kablarska gorge“ for period 2007-2011. Turistic organization of Čačak town.

Plancic, J. (1967): Prussian carp (*Carassius auratus gibelio*) a new member of our ichthyofauna (In Serbian). *Ribarstvo Jugoslavije* XXII:6.

Rashed, M.N.(2001): Monitoring of environmental heavy metals in fish from Nasser Lake, *Environ. Int.* 27, 27–33.

Simović, S. (2001) ecology and cenotic relations between species *Rutilus rutilus* and *Carassius auratus gibelio* Međuvršje and Gruža reservoirs. Phd thesis, Faculty of biology, University of Belgrade.

Skorić, S. and Đikanović, V. (2012) Medium-term program of fisheries management and improvement on fishery catchment area of enormous features „Ovčarsko-kablarska gorge“ for period 2012-2021.

Storelli, M.M., Barone, G., Storelli, A., Marcotrigiano, G.O. (2006): Trace metals in tissues of Mugilids (*Mugil auratus*, *Mugil capito*, and *Mugil labrosus*) from the Mediterranean Sea. *Bulletin of Environmental Contamination and Toxicology* 77, 43-50.

Uysal, K., Köse, E., Bülbül, M., Dönmez, M., Erdoğan, Y., Koyun, M., Ömeroğlu, Ç., Özmal, F. (2009): The comparison of heavy metal accumulation ratios of some fish species in Enne Dame Lake (Kütahya/Turkey). *Environ. Monit. Assess.* 157, 355–362.

Yilmaz, F., Özdemir, N., Demirak, A., Tuna, A.L. (2007): Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. *Food Chemistry* 100, 830-835.

Wu, S.M., Jong, K.J., Lee, Y.J., (2006): Relationships among metallothionein, cadmium accumulation, and cadmium tolerance in three species of fish. *B. Environ. Contam. Tox.* 76, 595–600.