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CONCENTRATIONS OF METALS AND TRACE ELEMENTS IN DIFFERENT TISSUES OF NINE FISH SPECIES FROM THE MEĐUVRŠJE RESERVOIR (WEST MORAVA RIVER BASIN, SERBIA)

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Abstract: Element concentrations in selected fish species from different trophic levels were analyzed. The following fish species were analyzed: common nase (*Chondrostoma nasus*), roach (*Rutilus rutilus*), freshwater bream (*Abramis brama*), barbel (*Barbus barbus*), Prussian carp (*Carassius gibelio*), chub (*Squalius cephalus*), European perch (*Perca fluviatilis*), wels catfish (*Silurus glanis*) and northern pike (*Esox lucius*). Fish were collected from the Međuvršje Reservoir (West Morava River Basin, western Serbia) during 2012, and samples of liver, muscle and gills were analyzed for As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sr, and Zn using inductively coupled plasma optical emission spectrometry (ICP-OES). The liver and gills had the highest measured element concentrations, with Cu, Zn, Fe and Sr being the most prominent. The bioaccumulation of metals was species-specific, with the accumulation exhibiting the following trends: Prussian carp > northern pike > freshwater bream > European perch > chub > common nase > barbel > roach > wels catfish; Li>Pb>Zn>Cu>Fe>Ba>Mn>Sr>Cr>As>Mo>Ni>B, in all examined species; for Co, Cd and Hg, the bioaccumulation factor (BAF) was 0. Results of this study point to the tissue-specific differences in element concentrations, and to distinct differences between fish species regarding the accumulation patterns: common nase, with the highest accumulation observed in the liver, and Prussian carp, with the highest accumulation observed in the gills.

Key words: fish; metal bioaccumulation; trace element concentration; water reservoir; gills

INTRODUCTION

Contamination of aquatic ecosystems (e.g. lakes, rivers, streams) with heavy metals and trace elements is a serious problem receiving world-wide attention [1,2]. Metals from natural and anthropogenic sources are environmentally ubiquitous, released in and transported by water, and taken up by aquatic organisms. These elements enter aquatic ecosystems by atmospheric precipitation, soil and rock erosion, and through anthropogenic sources, such as industrial effluents, traffic, mining wastes and agriculture [3,4]. Metals are a serious threat because of their toxicity, persistence, capacity for bioaccumulation and biomagnification in the food chain [5]. Certain trace elements such as Fe, Cu, Zn, and Mn are essential in small amounts, but can be toxic and adversely affect aquatic life when present above certain concentrations. Hg and Cd are non-essential metals that are toxic even in traces [6]. Metal accumulation analyses are the best indicator of aquatic ecosystem pollution [2]. Aquatic organisms have the ability to accumulate metals from various sources. The toxic effects of metals range from complete loss of biota to effects on reproduction rate, growth and behavior of organisms [7].

Fish are sensitive to increases in concentrations of different contaminants, such as metals and organic pollutants in water. The accumulation level of metals in fish organs and tissues depends on the fish species, their age patterns, and on the physical and biochemical characteristics and chemical status of their environment [8].

In this work, we analyzed 16 elements in the liver, muscle and gills of nine fish species from different trophic groups: phytophagous (examined in common nase), benthivorous (barbel, freshwater bream), omnivorous (Prussian carp, roach, chub, European perch) and piscivorous species (wels catfish and northern pike). Fish feeding preferences and meal size are factors that determine the trend of element accumulation in fish tissues among species [9]. Sampling was carried out in 2012 in the Međuvršje Reservoir in western Serbia that receives large amounts of untreated industrial and communal waters, with the water quality in the reservoir influenced by various pollutants. The main objectives of the study were to highlight the importance of species and tissue selection in biomonitoring by comparing the accumulation patterns among different fish tissues and species, and comparing element concentrations in fish tissues with their concentrations in the water.

MATERIALS AND METHODS

Study area

A field study was carried out in the Međuvršje Reservoir (West Morava River Basin); coordinates: N – 43°54'43.07"; E – 20°14'12.71"; 277 m a.s.l. (Fig. 1). The reservoir was formed in 1953 after the construction of a 31-m high dam built for water-level control and management [10].The reservoir is located at the exit of the Ovčar-Kablar Gorge. The length of the reservoir is 9.3 km, surface area 1.5 km^2, maximum width 272 m, maximum depth12 m (directly below the dam). The quality of the water is influenced by numerous contaminants in the catchment area. Within the 3165-km^2 catchment area, there is intense emission of industrial, urban and rural wastewater. Neither the settlements nor most of the industrial plants in the area possess facilities for wastewater purification.

Sample preparation

Sampling of nine fish species (10 individuals per species) was carried out in June and August in 2012. Collection of fish samples was performed using a set of standing gill-nets with a mesh diameter of 10-60 mm, as well as by electrofishing (HONDA 1.2 кW, 6 A). The total weight (g) and total body length (cm) of each fish specimen were measured. Fish species were determined according to Simonović [11]. Samples of muscle, liver and gills were removed and frozen until analysis. Water samples were collected at a depth of 20-30 cm below the water surface in 50-ml polyethylene demineralized containers, and conserved with 0.25 ml of concentrated HNO_3 solution. Until analysis the water samples were stored in a fridge (kept at 4°C).

Fig. 1. Field study area: the Međuvršje Reservoir in the West Morava River Basin.

Sample analysis

In the laboratory, the samples were dried using a GAMMA 1-16 LSC plus Freeze Dryers Rotational-Vacuum-Concentrator (Germany), and sample portions between 0.2 and 0.4 g dry weight were subsequently processed in a microwave digester (Speed wave MWS-3; Berghof Products Instruments GmbH, Eningen, Germany), using 6 ml of 65% HNO_3 and 4 ml of 30% H_2O_2 (Merck Suprapur) at a food temperature program (100-170°C).

The potential presence of analyzed elements was resolved using a number of blank samples. After reaching 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. The following wavelength lines were used: As 189.042 nm, B 249.773 nm, Ba 233.527 nm, Cd 228.802 nm, Co 228.616 nm, Cr 205.552 nm, Cu 324.754 nm, Fe 259.941 nm, Hg 184.950 nm, Li 460.289 nm, Mn 259.373 nm, Mo 202.095 nm, Ni 231.604 nm, Pb 220.353 nm, Sr 460.733 nm, and Zn 206.191 nm. All elemental concentrations were expressed as $\mu g\ g^{-1}$ dry weight (dw). Metal concentrations in fish meat (i.e., muscle samples) were also recalculated to the wet tissue weight (WW) and compared with the maximum allowed concentrations (MAC) in fish meat for utilization in the human diet established by the European Union (EU) and the national legislation. According to EU legislation [12], MAC for Cd, Hg and Pb are 0.05, 0.50 and 0.30 $\mu g\ g^{-1}$ w/w, respectively. National legislation prescribes MAC for As, Cd, Hg, Pb, Cu, Fe and Zn in fish meat at 2.0, 0.1, 0.5, 1.0, 30.0, 30.0 and 100.0 $\mu g\ g^{-1}$ w/w, respectively [13].

Statistical analysis

To compare the total metal content in fish species and their tissues, the metal pollution index (MPI) was used, obtained using the equation [14]: MPI = $(As \times B \times Ba \times Cd \times Co \times Cr \times Cu \times Fe \times Hg \times Li \times Mn \times Mo \times Ni \times Pb \times Sr \times Zn)^{1/16}$. When the elemental concentration was equal to zero (not detected), the value equal to half of the spectrometer sensitivity (ICP-OES) for the corresponding element was used. Assessment of the differences among groups was performed by ANOVA two-factor analysis (StatSoft, Inc. 2007). Relationships between fish size, weight and trace element concentrations in different tissues were assessed by Spearman's non-parametric correlation test. The bioaccumulation factor (BAF), the ratio of the concentrations of the chemicals in the organism (CB) to that in the water (CWT), was calculated according to the equation: BAF= CB/CWT [15].

RESULTS

The concentrations of 16 metals in muscle, liver and gills of nine selected fish species from different trophic levels are presented in Table 1. The lowest concentrations of all analyzed metals were found in muscle tissue (Table 1). The highest concentrations in liver were found for As, Cd, Cu and Fe in common nase, freshwater bream, European perch, northern pike, wels catfish, for B in common nase, northern pike, European perch, wels catfish, for Mo in common nase, roach, Prussian carp, barbel, chub, for Co in European perch, wels catfish, for Hg in chub, European perch, for Zn in common nase, freshwater bream, European perch and for Li in wels catfish. In the gills, the highest concentrations of Sr, Pb, Ba, Cr, Mn, Ni were observed in all species except northern pike, of Li in all species except wels catfish, of Hg in common nase, roach, Prussian carp, freshwater bream, barbel, northern pike and wels catfish, of Co in roach, Prussian carp and freshwater bream, of B in roach, Prussian carp, freshwater bream, barbel and chub, of Mo in freshwater bream, European perch, wels catfish and northern pike (Table 1). The three most abundant elements were Zn, Cu, and Fe (Table 1).

According to comparison of tissues, different fish species and different tissues of the same species, the MPI values (ANOVA two-factor analysis) of fish species significantly differed ($p=0.000001$) (Fig. 2b); the MPI values of tissues significantly differed ($p=0.000001$) (Fig. 2c), and the MPI value of tissues and fish species also significantly differed ($p=0.000001$) (Fig. 2a). The MPI value was highest in the gills in most of the analyzed species. Prussian carp, common nase and freshwater bream were distinguished from others by their higher MPI values (Table 2).

There were only a few significant correlations between the overall elemental accumulation and fish size and weight: As and Prussian carp weight correlation coefficient $R= -0.6$, $p<0.05$; As and freshwater bream total length $R=0.553$, $p<0.05$; Cr and chub weight and total length, $R=-0.613$ and $R=-0.578$, respectively, $p<0.05$; Pb and roach weight and total length $R=-0.553$, $p<0.05$; Mo and wels catfish weight $R=-0.685$, $p<0.05$. There were no differences in the distribution of Fe, Li, Mn and Sr among tissues. Two fish species

Table 1. Concentrations of 16 metals and trace elements in different tissues of 9 selected fish species. All element concentrations are presented as μg g^{-1} dry weight (dw).

		Common nase	Roach	Prussian carp	Freshwater bream	Barbel	Chub	European perch	Northern pike	Wels catfish
		mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD	mean±SD
As μg/g	liver	4.26±0.95	2.62±0.53	3.25±1.73	2.80±1.63	2.01±0.42	3.24±0.37	2.97±0.17	1.34±0.02	1.9±0.46
	muscle	3.50±0.66	2.1 ±0.43	3.16±0.29	0.24±0.36	1.8±0.5	3.22±0.43	3.13±0.42	1.76±0.37	1.88±0.28
	gills	3.96±0.93	2.13±0.91	2.78±0.34	0.41 ±0.29	1.5±0.71	2.47±0.32	2.36±0.39	0.92±0.07	2.64±3.26
B μg/g	liver	0.81±0.09	0.54±0.11	0.76± 0.38	1.01± 0.88	0.50±0.24	0.3± 0.15	0.67±0.19	0.48±0.28	1.225±0.488
	muscle	0.003±0.00	0.29±0.11	0.27± 0.20	0.25±0.19	0.29±0.23	0.26±0.27	0.19± 0.06	0.21±0.08	0.512±0.13
	gills	0.618±0.25	0.66± 0.27	2.11±0.98	1.04± 0.69	0.62± 0.23	0.33±0.18	0.37 0.05	0.25±0.02	0.687±0.561
Ba μg/g	liver	5.99±1.11	6.23±6.31	5.71±3.70	3.09±1.05	2.99±0.61	1.39±0.94	0.29±0.18	0.47±0.09	0.61±0.26
	muscle	1.14±0.56	1.95±1.14	0.21±0.14	0.66±0.24	0.6±0.53	0.29±0.09	0.15±0.19	0.11±0.00	0.39±0.04
	gills	24.91±2.88	38.80±11.65	24.49±3.6	21.36±8.97	14.27±3.09	6.32±3.67	1.24±0.3	1.97±0.35	0.91±0.62
Cd μg/g	liver	0.36±0.22	ND*	ND*	0.01±0.01	0.02±0.04	ND*	0.06±0.06	ND*	0.01±0.02
	muscle	0.03±0.05	ND*	ND*	ND*	ND*	ND*	ND*	ND*	ND*
	gills	0.01 ±0.01	ND*	ND*	ND*	ND*	ND*	ND*	ND*	0.06±0.11
Cu μg/g	liver	269.36±128.37	30.43±22.34	13.07±9.67	44.75± 22.75	14.12±6.55	25.07±7.68	17.41±7.48	13.88±7.98	11.1±4.21
	muscle	2.29±1.16	1.74±1.39	14.7±26.55	1.45±1.32	1.38±0.15	0.74±0.43	0.02±0.04	0.84±0.02	0.79±0.12
	gills	5.74±5.23	3.1±1.78	5.95±0.71	2.36±0.63	3.03±1.1	1.85±0.37	0.99±0.35	1.56±0.54	2.02±1.73
Co μg/g	liver	ND*	ND*	ND*	ND*	ND*	ND*	0.13± 0.09	ND*	0.089±0.114
	muscle	ND*	ND*	ND*	ND*	ND*	ND*	ND*	ND*	ND*
	gills	ND*	0.01±0.02	0.12±0.08	0.05± 0.04	ND*	ND*	ND*	ND*	ND*
Cr μg/g	liver	0.27±0.17	0.14±0.07	0.41±0.29	0.38±0.47	0.26± 0.04	0.00	0.12±0.06	0.17±0.03	0.23±0.07
	muscle	0.05±0.07	0.14±0.04	0.05±0.05	0.11±0.05	0.16±0.04	0.07±0.09	0.10±0.06	0.18±0.05	0.24±0.03
	gills	0.48 ±0.34	0.33±0.17	1.03±0.39	0.37±0.21	0.44±0.18	0.11±0.11	0.21±0.11	0.30±0.03	0.51±0.52
Fe μg/g	liver	351.91±122.22	177.23±55.17	240.99±161.79	428.11±511.91	147.32±41.75	139.81±55.8	355.067±136.664	261.383±123.928	529.772±258.273
	muscle	27.8±15.01	25.51±11.15	99.36±154.97	21.13±12.75	41.72±42.93	12.35±4.81	9.236±3.315	18.304±3.999	11.537±6.014
	gills	244.69±136.74	180.98±56.06	626.52±175.01	389.61±130.22	228.48±55.26	141.07±65.83	138.52±41.22	96.69±43.71	171.49±132.62
Hg μg/g	liver	1.84 ±0.41	0.95±0.10	1.3±0.65	1.05±0.31	1.33±0.30	1.67±0.25	1.85±0.18	0.81±0.18	1.06±0.27
	muscle	1.56±0.29	0.95±0.21	1.44±0.11	1.22±0.23	2.21±0.6	1.94±0.21	2.06±0.34	1.04±0.01	1.59±021
	gills	2.02±0.32	1.27±0.24	1.45±0.28	1.31±0.19	1.67±0.37	1.63±0.23	1.8±0.14	1.27±0.06	1.14±0.89
Li μg/g	liver	0.00±0.00	0.37±0.63	0.27±0.29	0.77±1.50	0.12±0.21	ND*	0.64± 0.4	0.52±0.03	0.82±0.722
	muscle	ND*	0.08±0.17	0.28±0.63	0.05± 0.11	ND*	ND*	0.07±0.16	ND*	0.024±0.042
	gills	0.59±0.47	1.33±1.08	3.88± 0.53	2.33± 0.70	1.56± 0.55	1.35± 0.73	2.09 ±0.64	2.68± 0.11	0.477±0.255
Mo μg/g	liver	4.74±0.86	3.7 ±1.81	3.47±2.14	2.63±0.75	2.38±0.11	3.01± 0.27	0.26± 0.56	2.06 0.15	2.273±0.398
	muscle	3.03± 0.43	2.86±1.33	2.9± 0.38	2.17±0.34	2.17± 0.16	2.67± 0.30	3.06±0.48	2.44±0.10	2.445±042
	gills	3.91±1.11	3.44±0.62	2.56± 0.46	2.98±1.30	1.97±0.25	2.48± 0.38	3.73± 2.06	2.30±0.61	2.555±2.798

Table 1. continued:

Mn µg/g	**liver**	5.36±0.77	5.05±0.93	1.52±0.68	5.82±2.3	3.04±0.53	3.02±0.62	2.1±0.39	1.85±1.07	2.13±1.21
	muscle	1.74±1.37	1.02±0.45	0.91±0.45	0.82±0.59	2.29±1.68	0.58±0.05	0.45±0.14	1.249±0.1	0.63±0.04
	gills	37.4±21.97	20.57±4.22	33.29±9.13	21.45±2.71	25.32±10.13	8.68±1.7	12.29±6.47	26.93±2.21	5.6±5.33
Ni µg/g	**liver**	0.09±0.12	ND*	0.29± 0.26	0.05±0.06	0.08±0.13	ND*	ND*	0.07±0.11	ND*
	muscle	ND*	ND*	0.07±0.16	ND*	ND*	ND*	ND*	ND*	ND*
	gills	0.24±0.41	0.08±0.18	1.44±0.99	0.12±0.09	0.34±0.31	0.02±0.05	ND*	ND*	0.2±0.24
Pb µg/g	**liver**	0.12±0.18	0.05±0.10	4.07±2.98	0.02±0.04	0.07±0.12	0.19±0.29	0.02±0.05	ND*	0.12±0.03
	muscle	0.03±0.07	0.00	0.05±0.11	0.08±0.12	0.04±0.06	0.1±0.16	ND*	ND*	0.19±0.08
	gills	0.56±0.48	0.17±0.37	15.44±7.97	0.09±0.12	0.13±0.23	0.51±0.86	ND*	ND*	ND*
Sr µg/g	**liver**	0.41±0.27	0.47± 0.49	1.07±1.29	0.75±0.73	0.68± 0.93	0.2± 0.04	0.27± 0.09	ND*	0.03±0.00
	muscle	1.41±0.96	5.83±4.81	1.41±0.97	2.12 ±1.77	4.47± 4.12	1.51±0.37	0.57±0.45	1.3±0.176	2.02±0.17
	gills	71.52±9.51	115.16±25.60	89.39± 11.16	88.21±9.81	55.36± 16.37	46.06± 11.86	25.24±5.34	43.44±5.08	19.79±23.76
Zn µg/g	**liver**	145.77±21.97	80.51±16.37	49.64±11.52	91.81±27.17	63.24±10.44	100.36±8.85	71.80±7.78	90.72±25.50	54.53±23.3
	muscle	51.49±14.33	47.77±7.26	63.32±15.95	27.30±7.49	20.21±2.44	34.78±4.75	22.69±7.68	48.57±11.08	38.56±26.42
	gills	96.43±10.15	196.69±71.20	322.393±64.820	68.46±7.34	68.58± 4.99	365.03±109.89	71.2±4.27	558.112± 199.87	58.80±40.47

*ND indicates values below the detection threshold

Table 2. Metal pollution index (MPI) based on the concentrations of 16 elements in the liver, gills and muscle of 9 fish species (means±standard deviation).

	Wels catfish	European perch	Chub	Roach	Common nase	Prussian carp	Barbel	Freshwater bream	Northern pike
Liver	0.58±0.23	0.47±0.14	0.22±0.06	0.30±0.09	0.89±0.25	0.60±0.30	0.39±0.24	0.50±0.41	0.25±0.04
Muscle	0.20±0.01	0.06±0.02	0.12±0.04	0.17±0.01	0.19±0.14	0.16±0.10	0.16±0.04	0.16±0.04	0.12±0.00
Gills	0.41±0.28	0.35±0.03	0.56±0.23	0.74±0.40	1.03±0.47	3.44±1.09	0.82±0.26	1.15±0.52	0.41±0.04

Table 3. Bioaccumulation factor (BAF) of liver, muscle and gills of 9 species (mg kg^{-1} wet weight).

Water (mg/l)		**As**	**B**	**Ba**	**Cr**	**Cu**	**Fe**	**Li**	**Mn**	**Mo**	**Ni**	**Pb**	**Sr**	**Zn**
		0.015	**0.058**	**0.03**	**0.002**	**0.0369**	**0.315**	**0.0001**	**0.039**	**0.028**	**0.021**	**0.0006**	**0.150**	**0.06**
Barbel	liver	34.227	2.262	26.192	33.009	99.775	121.644	218.083	20.460	22.365	0.903	27.909	1.172	267.895
	muscle	35.424	1.525	6.104	23.883	11.275	39.749	0.000	17.751	23.483	0.000	16.468	8.952	98.777
	gills	44.334	4.801	216.216	96.810	37.004	326.529	4870.723	294.789	32.085	7.168	90.431	166.405	502.843
Chub	liver	55.215	1.339	12.197	5.315	177.083	115.445	0.000	20.290	28.249	0.000	76.320	0.346	425.172
	muscle	63.359	1.366	2.950	9.415	6.067	11.767	0.000	4.464	28.905	0.000	44.370	3.034	170.027
	gills	72.778	2.604	95.801	24.774	22.665	201.601	4225.822	101.052	40.290	0.482	357.401	138.451	2676.459
Common nase	liver	72.474	3.634	52.451	33.741	1903.115	290.576	391.243	36.084	44.474	1.064	49.961	0.706	617.513
	muscle	68.840	1.442	11.510	7.535	18.631	26.484	0.000	13.535	32.877	0.000	14.741	2.819	251.661
	gills	116.592	4.824	377.588	105.177	70.127	349.699	1857.155	435.795	63.554	4.988	393.588	214.975	707.054

Table 3. continued:

European perch	liver	50.630	3.029	2.579	14.846	123.008	293.187	1150.655	14.125	30.606	0.000	9.344	0.461	304.174
	muscle	61.410	0.982	1.518	14.900	0.157	8.799	154.878	3.487	33.205	0.000	0.000	1.143	110.915
	gills	69.587	2.851	18.835	44.947	12.137	197.969	6563.824	143.065	60.601	0.000	0.000	75.875	522.035
Freshwater bream	liver	47.705	4.541	27.022	47.915	316.185	353.500	1402.082	39.154	24.740	0.539	7.019	1.309	388.940
	muscle	45.110	1.321	6.674	15.989	11.804	20.134	99.652	6.367	23.550	0.000	35.150	4.252	133.454
	gills	54.561	8.092	323.752	81.281	28.854	556.808	7292.576	249.758	48.480	2.586	62.919	265.165	501.922
Northern pike	liver	22.785	2.174	4.107	23.522	98.031	215.830	949.156	12.448	19.338	0.893	0.000	0.181	384.307
	muscle	34.529	1.109	1.123	25.444	6.848	17.440	0.000	9.697	26.471	0.000	0.000	2.598	237.396
	gills	27.003	1.980	29.794	66.409	19.055	138.187	8390.663	313.524	37.464	0.000	0.000	130.564	4092.127
Prussian Carp	liver	55.283	3.423	50.034	51.542	92.318	198.993	496.040	10.200	32.543	3.462	1639.883	1.864	210.280
	muscle	62.069	1.387	2.151	7.798	119.815	94.663	591.828	7.038	31.420	0.971	23.155	2.831	309.492
	gills	82.068	16.492	371.251	223.878	72.790	895.379	12157.310	387.575	41.552	30.072	10772.299	268.690	2363.813
Roach	liver	44.538	2.440	54.555	18.202	215.017	146.339	675.754	33.985	34.758	0.000	18.566	0.813	341.044
	muscle	41.236	1.513	19.743	20.573	14.215	24.307	157.714	7.913	30.962	0.000	0.000	11.688	233.495
	gills	44.698	3.434	454.680	43.617	55.111	214.801	2622.070	154.084	42.492	0.000	178.504	248.992	926.186
Wels catfish	liver	29.574	5.087	5.042	26.350	71.869	403.173	1312.800	13.326	19.564	0.000	42.815	3.146	211.841
	muscle	36.930	2.663	3.979	35.378	6.469	16.709	50.991	4.866	26.500	0.000	86.395	4.040	188.467
	gills	100.471	6.630	16.301	140.042	31.045	299.234	1783.179	73.092	53.242	5.304	0.000	64.726	510.775

Table 4. Concentrations in fish meat (i.e., muscle samples) recalculated to the wet tissue weight (ww) and compared with the maximum allowed concentrations (MAC) established by the European Union (EU), FAO and the national legislation (SER).

Metal		**As**	**Cd**	**Cu**	**Fe**	**Hg**	**Pb**	**Zn**
EU MAC	mg/kg		0.05			0.5	0.3	
FAO MAC	mg/kg		2.0	10.0			4.0	50.0
SER MAC	mg/kg	2.0	0.050	30.0	30.0	0.5	0.30	100
Common nase	mg/kg	0.15±0.03	ND*	0.11±0.06	1.33±0.81	0.07±0.01	ND*	2.35±1.05
Roach	mg/kg	0.11±0.02	ND*	0.09±0.07	1.39±0.59	0.05±0.01	ND*	2.66±0.53
Prussian Carp	mg/kg	0.1±0.03	ND*	0.54±1.01	3.57±5.92	0.05±0.01	ND*	2.02±0.6
Freshwater bream	mg/kg	0.12±0.02	ND*	0.07±0.06	1.05±0.5	0.06±0.01	ND*	1.4±0.42
Barbel	mg/kg	0.09±0.03	ND*	0.06±0.01	0.98±0.2	0.1±0.03	ND*	1.44±0.62
Chub	mg/kg	0.13±0.02	ND*	0.03±0.02	0.5±0.23	0.08±0.01	ND*	1.39±0.2
European perch	mg/kg	0.12±0.02	ND*	0.00	0.36±0.14	0.08±0.02	0.03±0.06	0.86±0.22
Northern pike	mg/kg	0.14±0.03	ND*	0.07±0.01	1.43±0.36	0.08±0.01	ND*	3.8±0.99
Wels catfish	mg/kg	0.08±0.01	ND*	0.03±0.01	0.75±0.29	0.07±0.01	0.04±0.05	1.67±1.25

*ND indicates values below the detection threshold

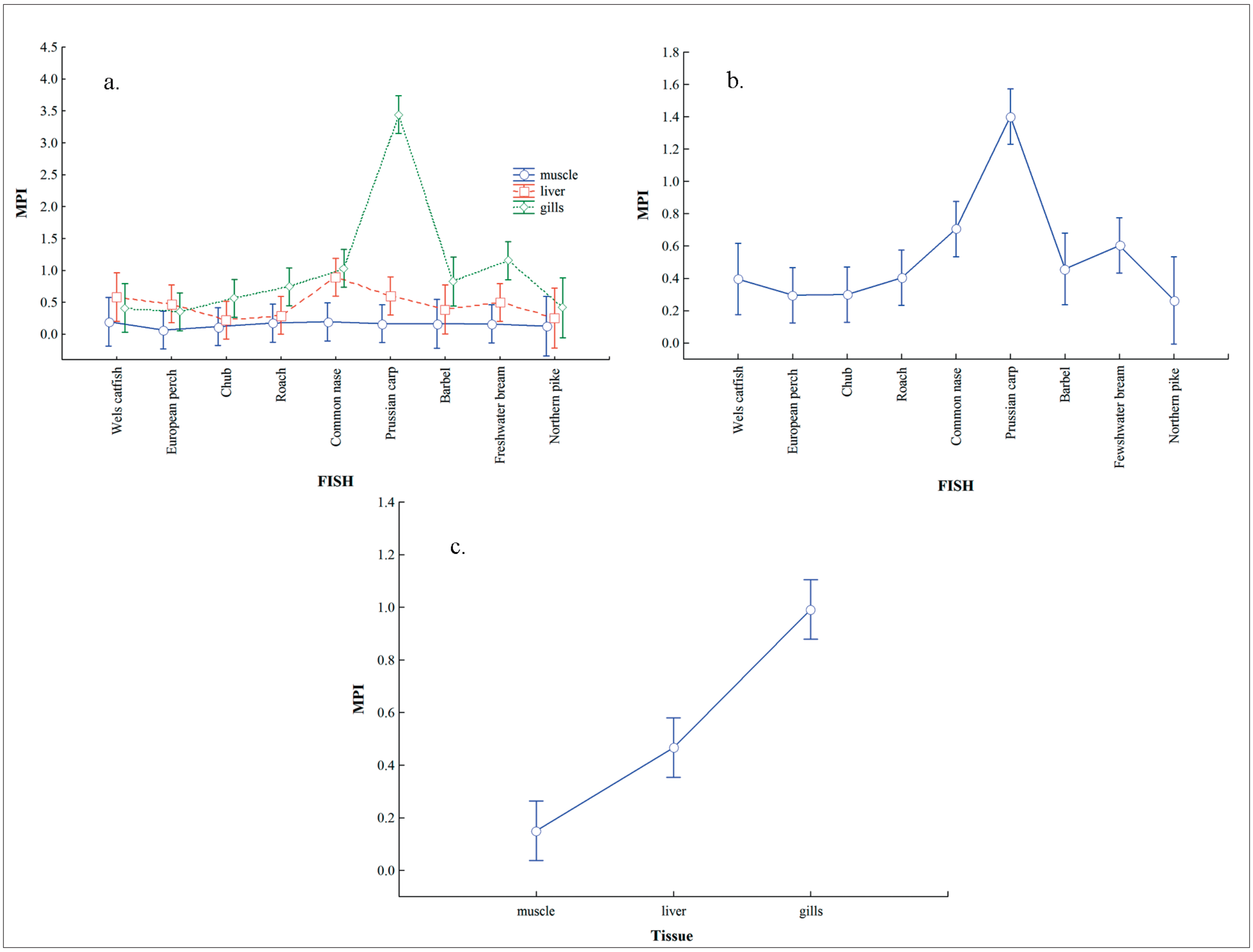

Fig. 2. Differences among **a** tissues and fish species (p=0.000001); **b** fish species (p=0.000001) and **c** tissues (p=0.000001) based on the metal pollution index (MPI). The analysis was performed using ANOVA two-factor analysis, Statistica 8.0. Vertical bars denote 0.95 confidence intervals.

were differentiated from other species by the tissue metal distribution: in common nase, As and Mo had the highest concentrations in all analyzed tissues, and Cd, Zn and Cu had the highest concentrations in the liver; in the Prussian carp, Pb, Ni, Li, Cr and B had the highest concentrations in all analyzed tissues, with the concentrations of Pb and Fe being the highest in the gills.

The BAF was not calculated for Cd, Co and Hg because these metals were not detected in the water. The highest BAF values were observed in the gills of the majority of analyzed fish species (Table 3).

In three different tissues of the nine studied fish species almost all of the 16 metals and trace elements were detected. The concentrations of the metals in muscle did not exceed the MAC prescribed by the EU regulations [12], international standards [16] and the Regulation of the Republic of Serbia [13] (Table 4).

DISCUSSION

The analysis of metal bioaccumulation in different tissues of fish with different diet requirements showed a high level of differentiation, as well as significant differences in the distribution of elements in the body. In many studies, the highest metal bioaccumulation was found in the liver, and this was consistent over a wide range of different fish species [2,17-20]. Muscle is generally considered a tissue with little potential for bioaccumulation and is a tissue with the lowest metal content [21,22]. The liver is a metabolically active and eliminative organ, due to the activities of metallothioneins, proteins with the ability to bind to specific metals, such as Cu, Cd and Zn, thereby reducing their toxicity and allowing for the accumulation of high metal concentrations [20,22,23]. In our study, the highest concentrations of most of the analyzed metals were detected in the gills (Zn, Sr, Pb, Ni, Mn, Li, Cr, Ba, and B). When comparing MPI values between fish species and tissues, only the wels catfish and European perch had higher values of MPI in the liver than in the gills. This could be explained by the fact that the gills are the first organ to come into contact with metals and trace elements in the water. The Međuvršje Reservoir is greatly influenced by many pollutants from upstream sources (industrial, municipal and rural wastewater) [24].

Metal accumulation analyses in different fish tissues have been conducted in some localities of Serbia. An ecotoxicological investigation of common nase, freshwater bream and bleak in the Međuvršje Reservoir was conducted by Lazić et al. [25]. In this study, the concentrations of selected metals, which were also examined in the present study, were close to the maximal allowed values, except for Hg, whose concentration was higher. During similar investigations [26] of tissues of Prussian carp in Old Begej (a Special Nature Reserve in the Vojvodina province), As and Li were not detected, Mn and Mo were not found in the muscle tissue and Cu was present only in the liver. The concentrations of Ba in Prussian carp gills were higher than in the present study, while the concentration of Mo was lower. Substantially higher concentrations of Zn and Fe, up to 10 times higher than those detected in fish from the Međuvršje Reservoir, were detected in the liver and gills [26]. An examination of metal concentrations in tissues of barbel from the Belgrade section of the Danube River did not report the presence of Cr, Cd, Pb, Co, Ni and Li [27]. In this section of the Danube ecotoxicological studies were also performed on freshwater bream and wels catfish. Seventeen selected elements were investigated: B, As, Ba, Cu and Mo were not detected in muscle, As, B and Ba were measured in the liver, As and Cu and As and Ba were not detected in the gills of freshwater bream and catfish, respectively [28]. The level of Cr and Hg in the liver was higher in piscivorous fish (*Sander lucioperca* and wels catfish) [29], whereas in our study the concentrations of these metals were higher in omnivorous (Prussian carp) and benthivorous fish (barbel and freshwater bream). The concentrations of B and Fe were higher in the gills of omnivorous species, *Lota lota* and *Cyprinus carpio* [29], as was also observed in the present study. Muscle samples from Prussian carp from the Gruža Reservoir (West Morava River Basin) exhibited the highest tendency of element accumulation (Fe, Cd, and Cu) [30], whereas in the Međuvršje Reservoir this tendency was not observed for Cu, Zn, Fe and Li in the same species. Remarkably higher concentrations of Cu in Prussian carp liver in comparison to other analyzed tissues, were also registered by other authors [16,21,22].

The presence of metals and trace elements in fish is dependent on species, body size, physiological state, feeding patterns and tissue type [17]. According to our results, common nase and Prussian carp differed from the other analyzed species by the high metal concentrations in their tissues. Therefore, these fish have the potential to be used as bioindicators for monitoring purposes. They are two of the species most frequently caught by anglers in the Međuvršje Reservoir.

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