ASSESSMENT OF THE METAL AND TRACE ELEMENT CONTENTS IN TISSUES OF FOUR COMMERCIAL FISH SPECIES FROM THE DANUBE RIVER, BELGRADE

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PROCENA SADRŽAJA METALA I ELEMENATA U TKIVIMA 4 KOMERCIJALNE VRSTE RIBA IZ DUNAVA KOD BEOGRADA

Apstrakt

Cilj ovog istraživanja je bio da se proceni akumulacija elemenata u četiri komercijalne vrste riba na dva lokaliteta na Dunavu, u Beogradu. U ovom istraživanju, određene su koncentracije 11 elemenata (As, Cd, Cr, Co, Cu, Fe, Hg, Mn, Ni, Se, i Zn) u tkivima mišića, jetre i škrga sledećih vrsta: mrena (*Barbus barbus*), deverika (*Abramis brama*), štuka (*Esox lucius*) i smuđ (*Sander lucioperca*). Rezultati su ukazali da je distribucija metala i elemenata u tragovima u različitim tkivima specifična za vrstu. Koncentracije As, Cd, Fe, Hg, i Zn u mišićima riba su bile ispod maksimalno dozvoljenih koncentracija MDK, utvrđenih od strane EU i Republike Srbije. Na osnovu dobijenih rezultata, neophodno je uspostaviti program stalnog monitoringa na Dunavu u Beogradskom regionu.

Ključne reči: Dunav, riba, metal, teški metali, ICP-MS
Keywords: Danube River, fish, metal, trace element, ICP-MS.

INTRODUCTION

The Danube River is polluted from various sources, such as industry, agriculture and energetics (Milanović et al. 2010). Metals and trace elements are considered to be among major pollutants in the Danube Basin (Teodorović 2009). Fish, due to their position at the top of the aquatic food chains, are able to accumulate metals and trace elements at such levels that could pose a threat not only to the fish but also to the human population (Yilmaz et al.2007). Therefore, fish are considered as one of the best pollution indicators of aquatic ecosystems. Accumulation levels and distribution patterns of metals and trace elements differ depending on fish species as well as on fish tissue (Višnjić-Jeftić et al. 2010, Jarić et al. 2011). Previous studies of metal and trace element concentrations (Jovičić et al. 2014; Lenhardt et al. 2012) indicated great importance of fish tissue contamination monitoring, given that toxic chemicals can produce adverse effects on consumers.

In the present study, 11 elements were analyzed in the muscle, liver and gills of the four fish species from the Danube River. The main aim of the present study was to assess potential impacts of accumulation patterns in fish tissues in the Danube River.

MATERIAL AND METHODS

Sample collection

Fish specimens were collected from the two localities on the Danube River on the teritory of the city of Belgrade, Serbia (downstream Danube section 44° 52' 29'' N, 20° 22'01'' E; upstream Danube section 44° 49' 57'' N, 20° 27' 47''E). Samples were collected between November 2012 and September 2013. Samples of the muscle, gills and liver of the following fish species were collected: barbel (*Barbus barbus*), freshwater bream (*Abramis brama*), northern pike (*Esox lucius*) and pike-perch (*Sander lucioperca*).

Sample preparation and analysis

Samples were dried by Freeze Dryers Rotational-Vacuum-Concentrator, GAMMA 1-16 LSC, Germany, and sample portions of approximately 0.3 g (dry weight) were afterwards processed in a microwave digester (speedwave™ MWS-3$^+$; Bergof Products + Instruments GmbH, Eningem, Germany), using 6 ml of 65% HNO_3 and 4 ml of 30% H_2O_2 (Merck suprapure) at a food temperature program (100 – 170°C). Potential presence of trace elements in chemicals used in digestion was resolved by using a number of blank samples. Following a cooling to 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 mass spectrometry (ICP-MS). Instrument was Thermo Scientific (Bremen, Germany), model iCAP Q. The following isotopes were measured: ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{77}Se, and ^{111}Cd. The quality of the analytical process was controlled by the analysis of reference material in each sample batch (NIST CRM 1577c). All concentrations were expressed as μg g^{-1} dry weight (dw).

Mercury (Hg) was measured using cold vapor technique by atomic absorption spectrometer Varian "SpectrAA 220" with VGA 77 hydride system. The wavelenght line for Hg was 257.3 nm. The quality of the analytical process was controlled by the analysis of reference material in each sample batch (BCR-186R).

Concentrations in fish meat (i.e., muscle samples) were compared with the maximum allowed concentrations (MAC) for the utilization in human diet, established by the European Union (EU) and the national legislation (European Commission Regulation, 2006, Official Gazette of RS 2011).

Normality of distribution of analyzed data samples was tested by Kolmogorov-Smirnov test. Assessment of the differences among the groups was performed by means of the Kruskal-Wallis H test, which was followed by comparisons of particular pairs of samples by Mann-Whitney U test ($p<0.05$ was used as a threshold value).

RESULTS

Metal and trace element concentrations in analyzed tissues of the four fish species on the two studied sites are presented in Table 1 and Table 2. There were only a few differences between elemental concentrations among the two studied localities.

In the muscle of the barbel, As and Ni concentrations were higher in the upstream Danube section, while Se concentrations were higher in the downstream section. Concentrations of these elements in the liver were higher in the downstream Danube section. Gills had a higher As concentrations in downstream section, while Ni and Se concentrations were lower (Table 1 and 2).

Table 1. Heavy metal and trace element concentrations in muscle, liver and gills of the four fish species at the upstream Danube section (means ± standard deviation). Concentrations are expressed as μg g^{-1} dry weight, nd indicates the values below the detect on threshold.

Tissue	**Element**	**Barbel**	**Freshwater Bream**	**Northern Pike**	**Pike-Perch**
Muscle	As	0.289±0.151	0.325±0.145	0.263±0.090	0.548±0.282
	Cd	nd	nd	0.010±0.011	0.010±0.367
	Cr	0.165±0.056	0.183±0.103	0.416±0.786	0.152±0.079
	Co	nd	0.030±0.023	nd	0.010±0.022
	Cu	1.579±0.365	1.973±1.055	2.477±1.054	1.237±0.439
	Fe	16.193±8.552	25.784±25.788	23.307±13.914	59.059±91.085
	Hg	0.962±0.359	0.447±0.391	1.351±0.644	1.790±0.367
	Mn	1.131±0.479	1.665±0.845	1.116±0.604	4.174±7.435
	Ni	0.410±0.371	0.220±0.145	0.340±0.315	0.210±0.234
	Se	1.118±0.624	1.884±0.881	1.089±0.237	1.604±0.907
	Zn	19.430±5.503	36.675±9.827	48.429±22.487	47.312±19.871
Liver	As	3.043±0.397	7.522±4.008	2.338±1.526	7.617±5.319
	Cd	0.060±0.031	0.180±0.216	0.040±0.050	0.340±0.470
	Cr	0.111±0.061	0.094±0.106	0.175±0.048	0.230±0.255
	Co	0.050±0.019	0.170±0.063	0.060±0.010	0.430±0.334
	Cu	22.069±8.607	69.331±51.031	27.451±10.203	17.066±7.012

	Fe	139.626±26.007	258.193±165.011	126.522±64.167	425.626±421.694
	Hg	0.246±0.119	0.156±0.149	0.350±0.358	0.901±0.435
	Mn	2.052±0.473	16.195±26.174	11.431±13.850	6.473±4.671
	Ni	0.350±0.611	0.460±0.698	0.530±0.168	0.520±0.565
	Se	0.325±0.105	0.310±0.141	0.491±0.298	3.442±1.591
	Zn	72.689±19.463	133.630±70.709	151.546±31.563	120.439±74.104
Gills	As	2.566±0.746	3.197±0.933	1.786±0.855	1.665±0.420
	Cd	0.030±0.011	0.020±0.002	0.020±0.007	0.030±0.024
	Cr	0.326±0.088	0.332±0.122	0.267±0.068	0.385±0.343
	Co	0.060±0.010	0.220±0.080	0.040±0.011	0.050±0.041
	Cu	2.590±0.161	2.550±0.668	2.053±0.293	1.708±0.654
	Fe	279.985±74.612	353.657±109.036	194.362±49.913	136.979±102.696
	Hg	0.161±0.048	0.070±0.045	0.143±0.137	0.413±0.245
	Mn	12.627±7.102	37.221±12.816	28.383±13.795	11.372±7.463
	Ni	0.370±0.244	0.820±0.768	0.520±0.303	0.580±0.474
	Se	0.253±0.067	0.267±0.095	0.179±0.075	0.936±0.640
	Zn	73.368±10.987	107.995±36.395	494.037±79.150	76.629±36.325

Higher Ni concentrations in the muscle of the freshwater bream were observed in the downstream section, while concentrations of Se were higher in the upstream section. Concentrations of As and Mn in the liver were higher in the downstream section, while concentrations of the Ni and Se were lower at this locality. Gills had higher As and Mn concentrations in the downstream section, while Cr, Ni and Se concentrations were higher in the upstream Danube section (Table 1 and 2).

Northern pike muscle had higher As concentrations at the downstream section. Liver had higher As, Cu and Mn concentrations and lower Se concentrations were also observed in the downstream section. Concentrations of As and Mn in gills were higher in specimens from downstream section, while Se concentrations were lower (Table 1 and 2).

Table 2. Heavy metal and trace element concentrations in muscle, liver and gills of the four fish species at the downstream Danube section (means ± standard deviation). Concentrations are expressed as μg g^{-1} dry weight, nd indicates the values below the detection threshold.

Tissue	Element	Barbel	Freshwater Bream	Northern Pike	Pike-Perch
Muscle	As	0.289±0.151	0.325±0.145	0.263±0.090	0.548±0.282
	Cd	nd	nd	0.010±0.011	0.010±0.367
	Cr	0.165±0.056	0.183±0.103	0.416±0.786	0.152±0.079
	Co	nd	0.030±0.023	nd	0.010±0.022
	Cu	1.579±0.365	1.973±1.055	2.477±1.054	1.237±0.439

	Fe	16.193±8.552	25.784±25.788	23.307±13.914	59.059±91.085
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	Mn	1.131±0.479	1.665±0.845	1.116±0.604	4.174±7.435
	Ni	0.410±0.371	0.220±0.145	0.340±0.315	0.210±0.234
	Se	1.118±0.624	1.884±0.881	1.089±0.237	1.604±0.907
	Zn	19.430±5.503	36.675±9.827	48.429±22.487	47.312±19.871
Liver	As	3.043±0.397	7.522±4.008	2.338±1.526	7.617±5.319
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	Fe	139.626±26.007	258.193±165.011	126.522±64.167	425.626±421.694
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Gills	As	2.566±0.746	3.197±0.933	1.786±0.855	1.665±0.420
	Cd	0.030±0.011	0.020±0.002	0.020±0.007	0.030±0.024
	Cr	0.326±0.088	0.332±0.122	0.267±0.068	0.385±0.343
	Co	0.060±0.010	0.220±0.080	0.040±0.011	0.050±0.041
	Cu	2.590±0.161	2.550±0.668	2.053±0.293	1.708±0.654
	Fe	279.985±74.612	353.657±109.036	194.362±49.913	136.979±102.696
	Hg	0.161±0.048	0.070±0.045	0.143±0.137	0.413±0.245
	Mn	12.627±7.102	37.221±12.816	28.383±13.795	11.372±7.463
	Ni	0.370±0.244	0.820±0.768	0.520±0.303	0.580±0.474
	Se	0.253±0.067	0.267±0.095	0.179±0.075	0.936±0.640
	Zn	73.368±10.987	107.995±36.395	494.037±79.150	76.629±36.325

Muscle of the pike-perch had Fe, Mn and Se concentrations higher in specimens from the downstream section, while As and Ni concentrations were lower. Concentrations of As and Cu were higher in the liver tissue from the downstream section specimens, while Ni concentrations were higher in specimens from the upstream section. In gills, concentrations of As were higher in specimens inhabiting the downstream Danube, but they had lower Cr concentrations (Table 1 and 2). Concentrations of As, Cd, Fe, Hg, and Zn in the fish muscle were below the MAC established by both the EU and the Republic of Serbia.

DISCUSSION

Distribution of metal and trace elements in different tissues showed species-specific patterns. In the present study, the species with the highest overall accumulation in the muscle tissue in the downstream section was the pike-perch. Barbel had the highest concentrations of the metal and trace elements in the muscle at the upstream section. However, concentrations of the As, Cd, Cu, Fe and Zn were below MAC in all analyzed muscle samples, which indicates that the meat of the studied fish species can be utilized in the human diet. The gills, due to their direct contact with the water, are considered to be the main site of the metal uptake and can indicate the water as a main source of the contamination (Storelli et al. 2007). In the current study, gills had the highest Cr, Zn and Fe concentrations.

Previous research (Lenhardt et al. 2012) in the Belgrade Danube section, showed some differences from our findings. Since freshwater bream was analyzed in both studies, we observed that overall metal and trace element concentration in liver and muscle was higher in the current study, which can be potentially explained by recent pollution emissions. Additionally, there were no significant differences in the overall gill metal and trace element concentrations.

Since liver is considered as a tissue with the highest accumulation ability (Uysal et al. 2009) and our study showed increase in metal and trace element concentrations, we strongly emphasize the necessity to establish continuous monitoring activities in the future, due to possible environmental deterioration. It is important to establish permanent monitoring of metals and trace elements in these localities, as well as to establish a system of suitable indicator species.

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