ELEMENT CONCENTRATIONS IN MUSCLE TISSUE OF TWO FISH SPECIES FROM DIFFERENT TROPHIC LEVELS (BLEAK AND PIKE) IN THE DANUBE NEAR BELGRADE

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KONCENTRACIJE ELEMENATA U MIŠIĆNOM TKIVU DVE VRSTE RIBA RAZLIČITOG TROFIČKOG NIVOA (UKLIJA I ŠTUKA) U DUNAVU KOD BEOGRADA

Apstrakt

Industrijski i komunalni efluenti uzrok su visokih koncentracija nekih elemenata u rečnoj vodi. Procenjuje se da se u Srbiji oko 90% industrijskih otpadnih voda ispušta u vodotokove bez prethodne obrade. Koncentracije elemenata u tkivima riba pod uticajem su kako prisustva zagađenja staništa, tako i trofičkog položaja i veličine ribe. Koncentracije 15 elemenata analizirane su upotrebom ICP-OES u uzorcima mišićnog tkiva uklije i štuke uhvaćenih na ušću Save u Dunav, između oktobra 2011. i marta 2012. godine. Elementi Cd, Co, Li i Pb nisu detektovani u uzorcima. Jednofaktorska ANOVA je pokazala da postoje značajne razlike između mišićnog tkiva uklije i štuke u odnosu na koncentracije Ba, Cu, Fe i Zn. U mišićnom tkivu štuke, koja predstavlja piscivornu vrstu, detektovana je viša koncentracija Hg nego kod uklije, što ukazuje na biomagnifikaciju kroz lance ishrane, dok je kod uklije, kao obligatnog zooplanktivora, detektovana viša koncentracija Zn, što potvrđuje tendenciju negativne korelacije Zn sa trofičkim nivoom ribe. U mišiću uklije, od analiziranih elementa samo je kod Se uočena pozitivna korelacija sa totalnom dužinom tela (TL), dok je kod štuke uočena negativna korelacija Se sa težinom (W) i pozitivna korelacija koncentracije Al sa TL i W. Kod obe vrste ukupno je nađeno 20 korelacija (14 pozitivnih i 6 negativnih) između koncentracija elemenata u mišićnom tkivu. Kod uklije, najveći broj korelacija sa drugim elementima uočen je kod Mn i Sr. Kod štuke, pozitivna korelacija uočena je između Fe, Se i Zn, dok su sva tri elementa bila negativno korelisana sa Al, a Se i Zn pozitivno sa Mn i Sr.

Ključne reči: uklija, štuka, ICP-OES, mišićno tkivo, toksični metali
Keywords: bleak, pike, ICP-OES, muscle tissue, toxic metals

INTRODUCTION

High concentrations of pollutants in the aquatic environments are often the result of industrial and municipal effluent discharges into the surface waters (Asuquo and Ewa-Oboho 2004). In the Danube River basin, this is especially pronounced around industrial facilities in the vicinity of the cities of Novi Sad, Belgrade, Pančevo, and Bor (Stanić *et al*. 2006), as it is estimated that approximately 90% of all industrial wastewaters in Serbia are discharged into waterbodies without a previous treatment (Matijašević *et al*. 2010). Element concentrations in fishes depend not only on the contamination of the environment, but also on several ecological factors, such as trophic position (Kehrig *et al*. 2013) and size (Al-Yousuf *et al*. 2000; Canli and Atli 2003) of the fish.

MATERIALS AND METHODS

Nine bleak (*Alburnus alburnus*) and six pike (*Esox lucius*) specimens were caught at the confluence of the Sava River with the Danube River, from October 2011 to March 2012. Caught specimens were dissected with a plastic laboratory set and samples of muscle tissue were taken, rinsed with distilled water, and stored at -18 ºC prior to analysis. Samples were freeze-dried using a rotational vacuum concentrator (GAMMA 1-16 LSC Germany) and sample portions between 0.2 and 0.5 g were processed in a microwave digester (speedwave™ MWS-3+; Berghof Products +Instruments GmbH, Eningem, Germany), using a 6 ml of 65% HNO_3 (Suprapur®, Merck) and 4 ml of 30% H_2O_2 (Suprapur®, Merck) at a food temperature program (100-170 ºC). After cooling to room temperature, the digested samples were diluted with distilled water to a total volume of 25 ml. The analysis of concentrations of Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Se, Sr, and Zn in samples was performed by ICP-OES (Spectro Genesis EOP II, Spectro Analytical Instruments DmbH, Kleve, Germany). The results are presented as µg/g dry weight.

We tested the normality of variable distribution with the Shapiro-Wilk test as well as the homogeneity of variance with the Levene's test. Since the assumption of a normal distribution of variables and the homogeneity of variances was satisfied, we applied the parametric one-way ANOVA test to compare the element concentrations in muscle tissue between the two fish species. A correlation analysis (bivariate correlations with Pearson's correlation coefficients) was used to check for significant relationships between the fish total length (TL) and weight (W) with element concentrations. Statistical tests were done using the SPSS 20.0 software.

RESULTS

The mean total length of the analyzed bleak individuals was 16.3 ± 1.0 cm and weight was 35.9 ± 8.4 g, while the mean total length of the analyzed pike individuals was 56.6 ± 4.5 cm and weight was 1263.2 ± 289.6 g.

Elements Cd, Co, Li, and Pb were not detected in both bleak and pike muscle samples. The concentrations of B and Ni were below the detection threshold in the bleak muscle, while the concentration of Mo was below the detection threshold in the pike muscle. The one-way ANOVA test showed significant differences between the bleak and pike muscle tissue with regard to the concentrations of Ba, Cu, Fe, and Zn (Table 1).

Table 1. Mean concentrations and standard deviations of 11 elements in muscle tissue of bleak (*A. alburnus*) and pike (*E. lucius*) (in µg/g dry weight). Significant difference in concentrations of elements between species is marked by an asterisk.

Element	*A. alburnus* (bleak)	*E. lucius* (pike)
Al	2.60 ± 3.38	4.58 ± 3.09
As	1.06 ± 0.21	1.03 ± 0.32
Ba	0.90 ± 0.42 *	0.16 ± 0.13 *
Cr	0.15 ± 0.32	0.26 ± 0.19
Cu	1.71 ± 0.25 *	0.48 ± 0.28 *
Fe	22.30 ± 7.77 *	8.89 ± 5.25 *
Hg	1.47 ± 0.45	2.32 ± 1.15
Mn	1.37 ± 0.46	0.99 ± 1.01
Se	0.34 ± 0.19	0.49 ± 0.21
Sr	3.81 ± 2.44	2.03 ± 3.45
Zn	69.52 ± 24.67 *	18.87 ± 12.26 *

The results of the correlation analysis are presented in Tables 2 and 3. Correlations significant at 0.01 level (two-tailed) are indicated by bold numbers and correlations significant at 0.05 level (two-tailed) are indicated by italic numbers.

Table 2. Correlation matrices between the total length (TL) and weight (W) and element concentration in bleak (*A. alburnus*) muscle (0.01 level significance in bold; 0.05 level significance in italic).

	TL	W	Al	As	Ba	Cr	Cu	Fe	Hg	Mn	Se	Sr	Zn
TL	1												
W	**0.96**	1											
Al	0.06	0.12	1										
As	0.69	0.67	- 0.56	1									
Ba	- 0.21	- 0.05	0.29	- 0.11	1								
Cr	- 0.43	- 0.31	- 0.19	- 0.29	- 0.28	1							
Cu	- 0.24	- 0.28	- 0.58	0.22	- 0.44	0.44	1						
Fe	- 0.49	- 0.39	- 0.39	- 0.15	- 0.29	**0.96**	0.65	1					
Hg	- 0.47	- 0.29	0.56	*- 0.75*	0.43	0.48	- 0.41	0.29	1				
Mn	- 0.20	- 0.11	*0.76*	*- 0.77*	0.30	0.16	- 0.52	- 0.06	**0.86**	1			
Se	*0.73*	0.67	- 0.02	0.62	- 0.07	- 0.27	- 0.05	- 0.27	- 0.42	- 0.30	1		
Sr	- 0.35	- 0.22	*0.83*	*- 0.74*	0.63	- 0.01	- 0.54	- 0.17	*0.83*	**0.88**	- 0.31	1	
Zn	- 0.69	- 0.63	0.22	- 0.63	0.61	0.19	- 0.06	0.19	0.62	0.51	- 0.26	0.66	1

Table 3. Correlation matrices between the total length (TL) and weight (W) and element concentration in pike (*E. lucius*) muscle (0.01 level significance in bold; 0.05 level significance in italic).

	TL	W	Al	As	Ba	Cr	Cu	Fe	Hg	Mn	Se	Sr	Zn
TL	1												
W	**0.96**	1											
Al	*0.88*	**0.94**	1										
As	0.52	0.54	0.38	1									
Ba	- 0.40	- 0.39	- 0.23	- 0.55	1								
Cr	- 0.62	- 0.60	- 0.61	- 0.36	- 0.38	1							
Cu	0.20	0.06	- 0.01	0.55	- 0.61	0.10	1						
Fe	- 0.63	- 0.71	- *0.85*	- 0.43	0.37	0.34	- 0.30	1					
Hg	- 0.49	- 0.40	- 0.51	- 0.30	0.39	0.21	- 0.73	0.74	1				
Mn	- 0.64	- 0.62	- 0.77	0.06	0.26	0.22	- 0.18	0.77	0.75	1			
Se	- 0.76	- *0.82*	- *0.90*	- 0.15	0.41	0.23	0.00	*0.83*	0.52	*0.89*	1		
Sr	- 0.62	- 0.60	- 0.74	0.11	0.29	0.16	- 0.15	0.73	0.71	**1.00**	*0.89*	1	
Zn	- 0.64	- 0.69	- *0.85*	- 0.19	0.33	0.27	- 0.22	**0.95**	0.76	**0.92**	*0.91*	*0.90*	1

DISCUSSION

The higher concentration of Hg was detected in pike muscle tissue than in bleak. The same result was obtained by Zrnčić *et al*. (2012) in the Danube River in Croatia, which is consistent with the observed biomagnification of this element through the food chain (Barbosa *et al*. 2003), considering that pike is a piscivorous predator and bleak an obligatory zooplanktivore. On the other hand, a higher concentration of Zn was detected in bleak muscle tissue. The trend of a higher Zn concentration in tissues of non-predatory than in predatory species is also shown by Mazej *et al*. (2010), which can be attributed to the tendency of Zn to correlate inversely with the trophic position of the fish (Papagiannis *et al*. 2004).

In bleak muscle, only Se is significantly and positively correlated with TL, while in pike muscle Se is significantly and negatively correlated with W, and Al is significantly and positively correlated with both TL and W. Although Zn is negatively correlated with both TL and W, which is consistent with observations made by Hogstrand (2011), this correlation was not significant.

In total, 20 correlations (14 positive and 6 negative) were observed between elements in muscle tissues of bleak and pike. In bleak, Mn and Sr had the largest number of correlations with other elements (four). In pike, a significant positive correlation was observed between Fe, Se, and Zn, while all three were negatively correlated with Al and Se and Zn positively with Mn and Sr. Klavins *et al*. (2009) found a positive correlation between Fe and Zn in muscle tissue of another piscivorous fish (perch, *Perca fluviatilis*), which was also observed in pike samples in our study.

CONCLUSIONS

Statistical differences between bleak and pike muscle tissue were observed with regard to concentrations of Ba, Cu, Fe, and Zn, and bleak had higher concentrations of these ele-

ments. A higher concentration of Hg in muscle tissue of the predatory species (pike) than in non-predatory (bleak) was also observed, which indicates the biomagnification of this element through the food chain. On the other hand, a higher concentration of Zn was detected in bleak muscle tissue, which can be attributed to the tendency of Zn to correlate inversely with the trophic position of the fish. In bleak muscle, only Se is significantly and positively correlated with TL, while in pike muscle, Al was positively correlated with TL and W, and there were similarities between Se and Zn regarding the correlation with other elements.

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