METAL CONCENTRATION IN MUSCLE TISSUE OF FISH FROM DIFFERENT FISH PONDS

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KONCENTRACIJA METALA U MIŠIĆNOM TKIVU RIBA IZ RAZLIČITIH RIBNJAKA

Apstrakt

Riba predstavlja važan deo ljudske ishrane, a takođe, riba je dobar pokazatelj kontaminacije životne sredine od strane jednog broja supstanci, uključujući i tragove metala u slatkovodnim sistemima, posebno zbog toga što se ribe, kao tercijelni potrošači, nalaze na vrhu lanca ishrane u vodenom ekosistemu (Noël i sar., 2013). Mnogo pažnje posvećeno je elementima kao što su olovo (Pb), kadmijum (Cd), Živa (Hg) i Arsen (As) i efekti izloženosti ovim elementima su sveobuhvatno istraženi (Castro-González and Méndez-Armenta, 2008; Has-Schön et al., 2008). Zbog svoje toksičnosti, otpornosti i bioakumulacije u vodi i sedimentima, kada se nalaze u visokim koncentracijama, ovi elementi predstavljaju opasnost za sve žive organizme. U pogledu bezbednosti javnog zdravlja, kod riba, prate se koncentracije olova, kadmijuma i žive, gde postoje jasno definisani maksimalni nivoi teških metala u namirnicama koji su određeni od strane Evropske Komisije, br. 1881/2006 (EC, 2006), izmenjena i dopunjena EC 629/2008 (EC, 2008). Za arsen, maksimalni nivo još nije uspostavljen na evropskom nivou, ali se očekuje da će granične vrednosti za arsen biti postavljene u bliskoj budućnosti, kao i metode za određivanje arsena (Noël et al., 2013). Za utvrđivanje koncentracije teških metala u tkivu riba prikupljeno je deset uzoraka dve različite vrste riba - šarana (*Cyprinus carpio*) i evropske ili severne štuke (*Esox lucius*). Uzorci su ulovljeni od strane profesionalnih ribara tokom rane jeseni 2013. godine iz četiri različita ribnjaka na području Beograda. Dobijeni rezultati su izraženi kao srednja vrednost ± standardna devijacija. Statistička analiza je urađena je korišćenjem Studentovog t-testa i analizom varijanse (ANOVA) sa višestrukim poređenjem Turkey test za utvrđivanje značajnih razlika između srednjih vrednosti. Primenjen je nivo značajnosti od 0.01 i 0.05. U različitim ribnjacima, koncentracija metala u mišićnom tkivu šarana je varirala u zavisnosti od vrste metala. Između sva četiri poređena ribnjaka nije utvrđena statistički značajna razlika jedino u koncentraciji kadmijuma u mišićnom tkivu. U ostalim slučajevima poređenja utrvđene su statistički značajne razlike ($p<0.01$). Koncentracija metala u mišićnom tkivu štuke razlikovala se između ribnjaka, zavisno od vrste metala. U svim slučajevima poređenja utvrđene su statistički značajne razlike ($p<0.01$). Koncentracija olova u mišićnom tkivu štuke u svim poređenim ribnjacima bila je statistički značajno veća ($p<0,01$) u odnosu na koncentraciju olova u mišićnom tkivu šarana. Koncentracija kadmijuma u mišićnom tkivu štuke bila je statistički značajno veća ($p<0,01$; $p<0,05$) od koncentracije kadmijuma u mišićnom tkivu šaran u svim poređenim ribnjacima. Za razliku od koncentracije olova i kadmijuma, koncentracija žive u mišićnom tkivu štuke u poređenim ribnjacima bila je statistički značajno niža ($p<0,01$) u odnosu na koncentracije žive u mišićnom tkivu šarana. Koncentracija arsena bila je statistički značajno veća ($p<0,01$) u mišićnom tkivu šarana od koncentracije arsena u mišićnom tkivu štuke. Rezultati koncentracija ispitanih elemenata u mišićnom tkivu riba pokazuju varijacije u opsegu koji se čini tipičan za ribnjake u Srbiji, ali su ove koncentracije niže od onih u rekama sa značajnim antropogenim uticajem.

Ključne reči:koncentracija metala, ribnjak, šaran, štuka
Keywords: metal concentration, fish pond, Common carp, Northern pike

INTRODUCTION

Fish is an important part of the human diet, but also a good indicator of environmental contamination by a number of substances, including trace metals in freshwater systems, notably because fish are at the top of the food chain in the water ecosystem (Noël et al., 2013). Much attention has been paid to hazardous elements such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) and the effects of exposure to these elements have been comprehensively studied (Castro-González and Méndez-Armenta, 2008; Has-Schön et al., 2008). Indeed, due to their toxicity, persistence and bioaccumulation in water and sediment, when occurring in high concentrations, these elements become severe poisonous for all living organisms. In terms of public health food safety, Pb, Cd and Hg, are monitored in fish with maximum levels of heavy metals in foodstuffs fixed by Commission Regulation (EC) No. 1881/ 2006 (EC, 2006) amended by EC 629/2008 (EC, 2008). For As, no maximum level has yet been established at European level, but discussions are ongoing on this topic and it is anticipated that limits will be set for arsenic in the near future, as the methodology for the determination of arsenic and its speciation improves (Noël et al., 2013). Although many studies have examined the relationship between metal exposure, accumulation and toxicity under laboratory conditions, prediction of toxic effects based on environmental or tissue concentrations remains difficult under natural exposure conditions (Visnjic-Jeftic et al., 2010; Skorić et al., 2012). Data from the literature indicate that the contents of these elements in fish varied as a function of the different localities, but depended on species and feeding behaviour and also, by various biotic and abiotic factors (Has-Schön et al., 2006; Noël et al., 2013). This study was undertaken to determine the contamination data of Pb, Cd, Hg and As in the muscle of two fish species (common carp-*Cyprinus carpio* and Northern pike- *Esox lucius*) from four different fish ponds and to compare the level of contamination and the differences in the concentration of these elements between these two fish species and between compared fish ponds.

MATERIALS AND METHODS

To determine heavy metals in fish tissue ten samples of two different fish species Common carp (*Cyprinus carpio*) and Northern pike (*Esox lucius*) were collected. The samples were caught by professional fishermen's during early autumn of 2013 from four different fishponds in Belgrade area. All individuals were identified to species level and a random sub-sample of 10 individuals per species at each location was used for metal analysis. Fish were dissected, and a sample of muscle (ca. 200 g) below the dorsal were stored for metal analyses in polypropylene vials previously pre-cleaned with nitric acid (10%) and rinsed three times in water. Muscle was selected to determine the risk posed by metal pollution to humans (Miller et al., 1992). The total weight (g) of fishes was measured and transported in refrigerator at 5 ºC daily to the laboratory. The samples were dissected to obtain muscle samples, then were mixed homogeneously and immediately frozen and stored at -20 ºC. The analysis was performed by included the assessment of concentrations of the following elements: As, Al, Zn, Fe, Cu, As, Sd, Hd, Pb. The 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 the utilization in human diet. As established by the Europea Union (EU) and the national legislation. According to the EU legislation (European Commission Regulation, 2006), MAC for Cd, Hg and Pb are 0.05, 0.05 and 0.30 μg g-1 ww, respectively. The national legislation prescribed MAC for As, Cd, Hg, Pb, Cu, Fe and Zn in fish meat at 2.0, 0.1, 0.5, 1.0, 30.00, 30.00 and 100.00 μg g-1 ww. respectively (Official Gazette of RS 2011). All samples were collected and analyzed in duplicate and the results are expressed as the means ± standard deviation. Statistical analysis of the results was elaborated using software GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The statistical analysis was performed using Student's t-test and analysis of variance (ANOVA) with the multiple comparison Turkey's test to determine the significant differences between means. Significant level of 0.01 and 0.05 was applied.

RESULTS

Concentrations of different metals in carp muscle tissue from four different fish ponds are shown in Table 1. The lead concentration in carp muscle tissue from fishpond C was significantly higher ($p<0.01$) than the lead concentration in carp muscle tissue from other fish ponds. In various fishponds, metal concentration was varied depending on the type of metal. Thus, mercury concentration in carp muscle tissue from fishpond C was significantly higher ($p<0.01$) than mercury concentration muscle tissue of carp from other fish ponds. Also, mercury concentration in carp muscle tissue from fishpond A was significantly higher ($p<0.01$) than the mercury concentration in muscle tissue of carp from fishpond D. Completely different results were obtained for arsenic concentration in carp fishponds. In carp fish pond D arsenic concentration in muscle tissue of fish was significantly lower ($p<0.01$) than the concentration of the same metal in muscle tissue of carp from other fish ponds. Also, the arsenic concentration in carp muscle tissue from fishpond C was significantly higher ($p<0.01$) than the arsenic concentration in carp muscle tissue from fishpond B.

Table 1. Metal concentration in carp muscle tissue

Fishpond	Metal Concentration (μg/g)			
	Lead $\bar{X}$±Sd	Cadmium $\bar{X}$±Sd	Mercury $\bar{X}$±Sd	Arsenic $\bar{X}$±Sd
A	0.0189^{A}±0.0031	0.067±0.0038	$0.401^{A,B}$±0.0105	0.353^{A}±0.0256
B	0.0186^{B}±0.0039	0.061±0.0038	0.393^{C}±0.0111	$0.343^{B,C}$±0.0267
C	$0.0301^{A,B,C}$±0.0043	0.065±0.0043	$0.485^{A,C,D}$±0.0082	$0.378^{B,D}$±0.0085
D	0.0204^{C}±0.0017	0.058±0.0145	$0.387^{B,D}$±0.0063	$0.252^{A,C,D}$±0.0040

Legend: same letters ^{A,B,C,D} - $p<0.01$;

Metal concentration in pike muscle tissue differed from the fishpond, depending on the type of metal. The lead concentration in pike muscle tissue from fishpond D was significantly lower ($p<0.01$) than the lead concentration in pike muscle tissue from other fish ponds. Also, the lead concentration in pike muscle tissue from fishpond A was significantly higher ($p<0.01$) than the lead concentration in pike muscle tissue from fish pond B. The cadmium concentration of muscle tissue in pike was the highest in fish pond C, and it was significantly higher ($p<0.01$) than the cadmium concentration in pike muscle tissue from other fish ponds. The muscle tissue of pike from fishpond A has significantly higher ($p<0.01$) cadmium concentration than the muscle tissue of pike from fishpond D. A similar situation was observed with a concentration of mercury in muscle tissue of pike, which was significantly higher ($p<0.01$) in the fish pond C compared to other fish ponds. The mercury concentration in pike muscle tissue from fishpond D was significantly lower ($p<0.01$) than the mercury concentration in pike muscle tissue from fishpond A and B. The arsenic concentration in pike muscle tissue was also significantly higher ($p<0.01$) in fish pond C than the arsenic concentration in pike muscle tissue from other fish ponds (Table 2).

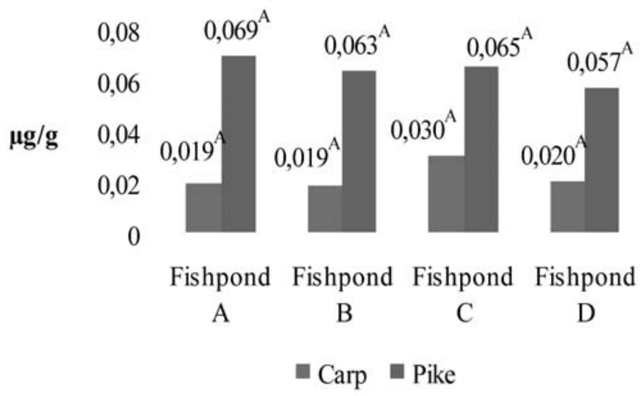
Table 2. Metal concentration in pike muscle tissue

Fishpond	Metal Concentration (μg/g)			
	Lead $\bar{X}$±Sd	Cadmium $\bar{X}$±Sd	Mercury $\bar{X}$±Sd	Arsenic $\bar{X}$±Sd
A	$0.069^{A,B}$±0.0031	$0.071^{A,B}$±0.0031	$0.294^{A,B}$±0.0058	$0.224^{A,B,C}$±0.0096
B	$0.063^{A,C}$±0.0039	0.067^{C}±0.0030	$0.289^{C,D}$±0.0067	$0.206^{A,D,E}$±0.0087
C	0.065^{D}±0.0014	$0.079^{A,C,D}$±0.0046	$0.313^{A,C,E}$±0.0064	$0,249^{B,D,F}$±0.0068
D	$0.057^{B,C,D}$±0.0006	$0.063^{B,D}$±0.0045	$0.217^{B,D,E}$±0.0048	$0.159^{C,E,F}$±0.0074

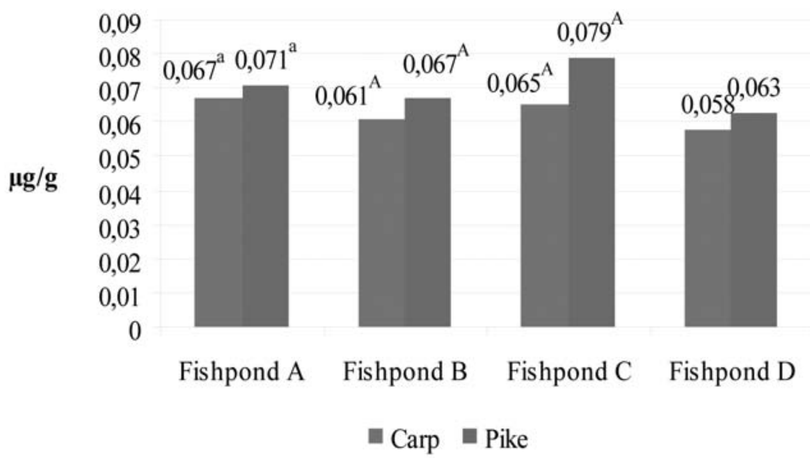
Legend: same letters ^{A,B,C,D,E,F} - $p<0.01$;

DISCUSSION

The lead concentration in muscle tissue of pike from all observed fish ponds was significantly higher ($p<0.01$) than the lead concentration in carp muscle tissue (Fig. 1). The cadmium concentration in pike muscle tissue was significantly higher ($p<0.01$; $p<0.05$) than the cadmium concentration in the carp muscle tissue of all compared fish carps (Fig. 2).

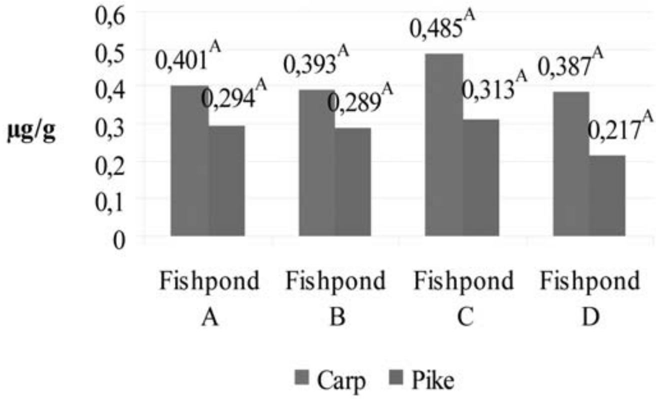


Legend: same letter ^{A} - p<0.01;
Figure 1. Lead concentration in carp and pike muscle tissue



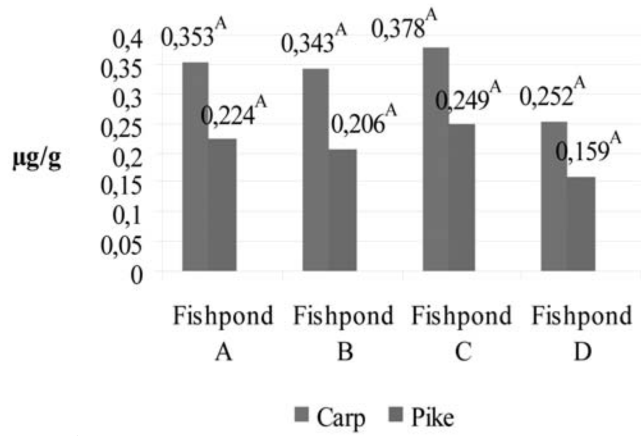
Legend: same letter ^{A} - p<0.01; ^{a} – p<0.05;
Figure 2. Cadmium concentration in carp and pike muscle tissue

As opposed to concentration of lead and cadmium, the mercury concentration in muscle tissue of pike from all observed fish ponds was significantly lower (p<0.01) than the mercury concentration in carp muscle tissue (Fig. 3). The arsenic concentration was significantly higher (p<0.01) in carp muscle tissue than the arsenic concentration in pike muscle tissue (Fig. 4).



Legend: same letter A - p<0.01;

Figure 3. Mercury concentration in carp and pike muscle tissue



Legend: same letter A - p<0.01;

Figure 4. Arsenic concentration in carp and pike muscle tissue

The results for the four elements in fish muscle show variation in a range that seems to be typical for the Serbian fish ponds, but are lower than those in rivers with considerable anthropogenic impact.

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