Metal Concentration in Muscle Tissue of Carp and Pike from Different Fish Ponds in Belgrade Area

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Abstract: This study was conducted to determine the contamination data of some heavy metals in 10 fish tissue samples of two different fish species—common carp and Northern pike 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. In various fish ponds, metal concentrations in the carp muscle tissue varied depending on the metal type. Between compared fish ponds, there were significant differences ($P < 0.01$) in the concentration in muscle carp tissue, except for cadmium. Metal concentrations in the pike muscle tissue also varied among fish ponds, depending on the type of metal. In all compared cases, there were significant differences between metal concentration in pike muscle tissue ($P < 0.01$). Lead and cadmium concentration in pike muscle tissue were significantly higher ($P < 0.01$, $P < 0.05$, respectively), and mercury and arsenic concentration were significantly lower ($P < 0.01$) than concentration of this heavy metals in carp muscle tissue in all compared fish ponds. The results obtained in this study indicate variation in a range of examined metal concentration in muscle tissue, which seems to be typical for the Serbian fish ponds, but is lower than those in rivers with considerable anthropogenic impact.

Key words: Common carp, fish pond, metal concentration, Northern pike.

1. 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 [1]. 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 [2-4]. 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 the maximum levels of heavy metals in

foodstuffs fixed by Commission Regulation (EC) No. 1881/2006 [5] amended by EC 629/2008 [6]. 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 As in the near future, as the methodology for the determination of As and its speciation improves [1].

In aquaculture, management practices, such as supplementary feeding or fertilisation of water, are generally considered to improve fish yield in water bodies. Nevertheless, in semi-natural systems where many water bodies are situated in a cultural landscape, this is much less evident for certain fish farmers, because fish production systems are often quite extensive, and fish production is only one economic activity among others for these fish farmers [7].

Although many studies have examined the relationship between metal exposure, accumulation

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and toxicity under laboratory conditions, prediction of toxic effects based on environmental or tissue concentrations remains difficult under natural exposure conditions [8, 9]. Data from the literature indicated 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 various biotic and abiotic factors, such as, fish biological habitat, as well as by fish weight and body length (evaluated from the condition factor), fish age, gender, physiological conditions, spawning status or migration, even in the same area [1, 8, 10-14].

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.

2. Materials and Methods

To determine heavy metals in 10 fish tissue samples of two different fish species—common carp (*Cyprinus carpio*) and Northern pike (*Esox lucius*) were collected. The samples were caught by professional fishermen 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 samples 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 [15]. The total weight (g) of fishes was measured and transported in refrigerator at 5 °C daily to the laboratory. The samples were dissected to obtain muscle samples, then were mixed

homogeneously and immediately frozen and stored at -20 °C.

The analysis was performed by the assessment of concentrations of the following elements: As, Al, Zn, Fe, Cu, As, Sd, Hd and 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 [6], the MAC for Cd, Hg and Pb are 0.05, 0.05 and 0.30 µg/g 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 ww, respectively [16].

All samples were collected and analyzed in duplicate and the results are expressed as the means ± standard deviation (SD). Statistical analysis of the results was elaborated using software GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA¹. 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, and the significant level of 0.01 and 0.05 was applied.

3. Results

Concentrations of different metals in carp muscle tissue from four different fish ponds are shown in Table 1. The Pb concentration in carp muscle tissue from fishpond C was significantly higher ($P < 0.01$) than that from other fish ponds. In various fishponds, metal concentration varied depending on the type of metal. Thus, Hg concentration in carp muscle tissue from fishpond C was significantly higher ($P < 0.01$) than that from other fish ponds. Also, Hg concentration in carp muscle tissue from fishpond A was significantly higher ($P < 0.01$) than that from

¹ www.graphpad.com.

fishpond D. Completely different results were obtained for As concentration in carp fishponds. In carp fish pond D, As concentration in muscle tissue of fish was significantly lower ($P < 0.01$) than that from other fish ponds. Also, the As concentration in carp muscle tissue from fishpond C was significantly higher ($P < 0.01$) than that from fishpond B. There were no significant differences between Cd concentrations in carp muscle tissue from compared fishponds.

Metal concentration in pike muscle tissue differed from the fishpond, depending on the type of metal. The Pb concentration in pike muscle tissue from fishpond D was significantly lower ($P < 0.01$) than that from other fish ponds. Also, the Pb concentration in pike muscle tissue from fishpond A was significantly higher ($P < 0.01$) than that from fish pond B. The Cd concentration of muscle tissue in pike was the highest in fish pond C, and it was significantly higher ($P < 0.01$) than that from other fish ponds. The muscle tissue of pike from fishpond A has significantly higher ($P < 0.01$) Cd concentration than that from fishpond D. A similar situation was observed with a concentration of Hg in muscle tissue

of pike, which was significantly higher ($P < 0.01$) in the fish pond C compared to other fish ponds. The Hg concentration in pike muscle tissue from fishpond D was significantly lower ($P < 0.01$) than that from fishpond A and B. The As concentration in pike muscle tissue was also significantly higher ($P < 0.01$) in fish pond C than that from other fish ponds (Table 2).

The Pb concentration in muscle tissue of pike from all observed fish ponds was significantly higher ($P < 0.01$) than the Pb concentration in carp muscle tissue (Fig. 1).

The Cd concentration in pike muscle tissue was significantly higher ($P < 0.01$) than the Cd concentration in the carp muscle tissue of all compared fish carps (Fig. 2).

As opposed to concentration of Pb and Cd, the Hg concentration in muscle tissue of pike from all observed fish ponds was significantly lower ($P < 0.01$) than the Hg concentration in carp muscle tissue (Fig. 3).

The As concentration was significantly higher ($P < 0.01$) in carp muscle tissue than the As concentration in pike muscle tissue (Fig. 4).

Table 1 Metal concentration in carp muscle tissue.

Fishpond	Metal concentration ($\mu\text{g/g}$)			
	Pb	Cd	Hg	As
	Mean $\pm$ SD			
A	0.0189 ^A $\pm$ 0.0031	0.067 $\pm$ 0.0038	0.401 ^{A, B} $\pm$ 0.0105	0.353 ^A $\pm$ 0.0256
B	0.0186 ^B $\pm$ 0.0039	0.061 $\pm$ 0.0038	0.393 ^C $\pm$ 0.0111	0.343 ^{B, C} $\pm$ 0.0267
C	0.0301 ^{A, B, C} $\pm$ 0.0043	0.065 $\pm$ 0.0043	0.485 ^{A, C, D} $\pm$ 0.0082	0.378 ^{B, D} $\pm$ 0.0085
D	0.0204 ^C $\pm$ 0.0017	0.058 $\pm$ 0.0145	0.387 ^{B, D} $\pm$ 0.0063	0.252 ^{A, C, D} $\pm$ 0.0040

^{A-D} Means with the same superscript letter in a column are significantly different at $P < 0.01$.

Table 2 Metal concentration in pike muscle tissue.

Fishpond	Metal Concentration ($\mu\text{g/g}$)			
	Pb	Cd	Hg	As
	Mean $\pm$ SD			
A	0.069 ^{A, B} $\pm$ 0.0031	0.071 ^{A, B} $\pm$ 0.0031	0.294 ^{A, B} $\pm$ 0.0058	0.224 ^{A, B, C} $\pm$ 0.0096
B	0.063 ^{A, C} $\pm$ 0.0039	0.067 ^C $\pm$ 0.0030	0.289 ^{C, D} $\pm$ 0.0067	0.206 ^{A, D, E} $\pm$ 0.0087
C	0.065 ^D $\pm$ 0.0014	0.079 ^{A, C, D} $\pm$ 0.0046	0.313 ^{A, C, E} $\pm$ 0.0064	0.249 ^{B, D, F} $\pm$ 0.0068
D	0.057 ^{B, C, D} $\pm$ 0.0006	0.063 ^{B, D} $\pm$ 0.0045	0.217 ^{B, D, E} $\pm$ 0.0048	0.159 ^{C, E, F} $\pm$ 0.0074

^{A-F} Means with the same superscript letter in a column are significantly different at $P < 0.01$.

Fig. 1 Pb concentration in carp and pike muscle tissue.

Same letter A means the significant difference at $P < 0.01$.

Fig. 2 Cd concentration in carp and pike muscle tissue.

Same letter A means the significant difference at $P < 0.01$; same letter a means the significant difference at $P < 0.05$.

Fig. 3 Hg concentration in carp and pike muscle tissue.

Same letter A means the significant difference at $P < 0.01$.

Fig. 4 As concentration in carp and pike muscle tissue.

Same letter A means the significant difference at $P < 0.01$.

4. Discussion

Knowledge about heavy metal concentrations in fish is important both with respect to nature management and human health. The toxicity of metal most commonly involves the brain and the kidney, but other manifestations occur, and some metals are

clearly capable of causing cancer. An individual with metals toxicity, even if high dose and acute, typically has very general symptoms, such as weakness or headache [17].

The accumulation of pollutants in organisms includes complex processes controlled by different endogenous and exogenous factors. Exogenous factors

reflect environmental parameters, such as metal speciation or pH, temperature and alkalinity of ambient aquatic surroundings, whereas endogenous factors include species, age, size, physiological state and type of feeding. Mean concentrations of heavy metals in muscle tissue of two different fish species showed great variations. Statistical comparisons revealed that metal concentrations in muscle tissue were significantly different in each fish ponds.

Pb concentrations in carp tissues were lower than MAC [6], and the authors found the significant differences in concentration between different fish ponds, and the same results were reported by Čelechovská et al. [18].

Cd distribution in muscle tissues is shown in Fig. 2. Significantly differences were found between Cd concentration in different fish ponds (A, B, C, D). Due to a very long elimination half-life, Cd gets accumulated in muscle tissues [19]. Cd concentrations in carp and pike muscle tissue were higher than MAC, according to EU legislation [6] (Fig. 2). The same results were reported by different authors from their experimental Cd contamination laboratory studies [20-22].

Hg is a highly toxic and the most closely monitored contaminant in fish [18]. Four homogeneous groups (A, B, C, D) of Hg concentrations were found at the $P < 0.05$ level of significance. Hg concentrations of carp and pike fish are given in Fig. 3. Because of its cycle in nature, Hg remains a topical issue. From the hygienic point of view, Hg concentrations found in carp and pike muscle tissue were lower than MAC [6]. The same results were reported by Čelechovská et al. [18]. Also, significant differences were found between Hg concentration in muscle tissue of carp and pike from different fish pond.

As distribution in fish muscle tissues from four different fish ponds (A, B, C, D) were significantly different ($P < 0.01$). The highest As concentrations were found in muscle tissue from carp in C fish ponds (0.378 $\mu\text{g/g}$). As concentrations in muscle tissue were

lower than MAC [15]. The same results were reported by Čelechovská et al. (median 0.086 mg/kg, mean 0.092 mg/kg) [18].

In this research, high levels of Zn, Cd and Pb concentrations were measured for human consumption in some seasons. The general body of the literature on Pb toxicity indicates that, depending on the dose, Pb exposure in children and adults can cause a wide spectrum of health problems, ranging from convulsions, coma, renal failure and death at the high end to subtle effects on metabolism and intelligence at the low end exposures [23]. In contrast to children, adults are generally allowed by regulations to be exposed to higher amounts of Pb. The health implications of Cd exposure are exacerbated by the relative inability of human beings to excrete Cd. Acute high dose exposures can cause severe respiratory irritation. The occupational levels of Cd exposure prove to be a risk factor for chronic lung disease and testicular degeneration [24, 25].

5. Conclusions

This study provides information about heavy metals content in muscle tissue of carp and pike from four fish ponds. Analysis of carp and pike muscle tissue showed higher concentration of Cd than MAC, but other heavy metals (Pb) in the same samples were under levels of EU legislation. Metal concentration in the muscle tissue of carp and pike varied considerably between the examined fish ponds. The results for the four elements in fish muscle show variation in a range, which seems to be typical for the Serbian fish ponds, but is lower than those in rivers with considerable anthropogenic impact.

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