ORIGINAL ARTICLE

Content of selected metals in muscle of cyprinid fish species from the Nitra River, Slovakia

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Abstract

OBJECTIVES: This study presents a rate of bioaccumulation of some metal concentrations in the muscle of five common cyprinid fish species (gudgeon – *Gobio gobio*, Europen chub – *Leuciscus cephalus*, barbel – *Barbus barbus*, roach – *Rutilus rutilus*, and nase – *Chondrostoma nasus*).

DESIGN: Besides, correlations among selected metals as well as standard length and total weight and order of metal accumulation in the fish muscle were determined.

RESULTS: Recorded metal concentrations (mg/kg wet weight basis) ranged as follows: Fe 4.23–22.95, Mn 0.10–0.72, Zn 16.26–166.90, Cu 15.39–25.97, Ni 0.00–3.72, Pb 0.00–1.81, and Cd 0.00–0.11.

CONCLUSIONS: The Slovak permissible limit for Cu (10.0 mg/kg), Ni (0.5 mg/kg), Pb (0.2 mg/kg) and Cd (0.05 mg/kg) defined in the Codex Alimentarius for safe human consumption exceeded in 100%, 12%, 6% and 10% of analyzed samples for Cu, Ni, Pb and Cd respectively. On an average, the order of metal concentrations in the fish muscle was: Zn>Cu>Fe>Mn>Ni>Pb>Cd.

INTRODUCTION

The fish flesh (muscle) is a very important, valuable and recommended food in the human nutrition due to low content of fat and high content of proteins and mineral substances as well as optimal ratio of unsaturated fatty acids with cardio-protective effect. On the other hand, fish muscle may be the depositary for several contaminants, such as heavy metals and metalloids, which occur in the water ecosystem.

Occurrence of contaminants, their frequency and concentration mainly depends on human activities, although sometimes impurities may be of geological origin (Has-Schon *et al.* 2006; Haluzova *et al.* 2010; Mikulikova *et al.* 2011; Plhalova *et* *al.* 2011). River character also plays an important role in metal concentration. Mountain and submountain river parts with higher slope and flow have a transport function in comparison to lowland parts, which have the highest sedimentation (Andreji and Stranai 2007).

It has been shown that water metal concentration is positively correlated with concentrations in fish tissues (Svobodova *et al.* 1996), although metal concentrations in the sediments are the most important factor governing the metal body concentrations of fish (Widinarko *et al.* 2000). The level of metal concentration in fish tissue is affected by biotic and abiotic factors such as habitat, physico-chemical properties of water, age, gender and physiological conditions (Zlabek *et al.* 2002; Piackova *et al.* 2003). The most important toxic factors of metals are their form and way of intake and final effect of their binding on different organs with post-evocation of changes (Stráňai and Andreji 2007).

The purpose of this study was to determine concentration of selected metals in the muscle of five fish species in the river Nitra (Slovak Republic) which may pose risk to human health. Furthermore, correlations among the concentration of metals and order of their accumulation in the fish muscles were analyzed.

MATERIALS AND METHODS

For this study five common cyprinid fish species were used. Two omnivorous benthic species (gudgeon – *Gobio gobio* and barbel – *Barbus barbus*), two omnivorous pelagic species (European chub – *Leuciscus cephalus* and roach – *Rutilus rutilus*) and one herbivorous benthic species (nase – *Chondrostoma nasus*) were collected by electrofishing from the upper Nitra River (Slovak Republic) near the village of Opatovce nad Nitrou, on the 138^{th}–139^{th} river kilometre (Figure 1). This part of the Nitra River forms a fishing territory under government of the Slovak Angling Union, division of the Prievidza, with more than 2.400 anglers. Nearly till to the end of 20^{th} century was this part affected by the mining industry and absence of sewage treatment plants.

Fish (n=50) were evaluated by standard methods used in ichthyology (standard length and total weight measurements, age determination by scales). After the biometric data analysis (Table 1) the samples of fish muscles were obtained from the dorsal part of fish body, without skin and bones. After collection, the tissue samples were kept at –18 °C.

For analysis, two grams of each muscle sample were dissolved in a solution of nitric acid p.a. (HNO_3:H_2O = 2:1) at 130°C for 2 h. Undissolved particles were filtered off and the solution diluted to 25 ml. The digested samples were analyzed for the presence of Fe, Mn, Zn, Cu, Ni, Pb and Cd by an atomic absorption spectrophotometer (AAS) Perkin-Elmer 4100 ZL described by Kolesarova *et al.* (2008) and Kramarova *et al.* (2005). Values of monitored heavy metals are presented on a wet weight (w.w.) basis in mg/kg and compared with Codex Alimentarius of the Slovak Republic.

For statistical analysis, the Anova One Way test, Kruskal-Wallis test, and Linear Model of Simple Regression were used with computer program Statgraphics Centurio XV.

RESULTS AND DISCUSSION

Content of selected metals in muscle

Contents of selected metals in muscle of cyprinid fish species from the Nitra River are given in Table 2. Statistically significant differences contend of manganese ($p<0.05$) copper ($p<0.001$) and cadmium ($p<0.001$) among fish species were recorded.

Iron concentration in muscle of five fish species ranged from 4.23 to 22.95 mg/kg wet weight. Lower iron concentrations (3.03–8.19 µg/g w.w.) were presented by Alhas *et al.* (2009) in two cyprinid fish species (*Barbus xanthopterus* and *Barbus rajanorum mystaceus*) from the Atatürk Dam Lake and by Erdogrul and Erbilir (2007) from the Sir Dam Lake (0.00–2.87 mg/kg w.w.) in thorn bream – *Acanthobrama marmid*, nase carp – *Chondrostoma regium* and common carp – *Cyprinus*

Fig.1 The site of sample collection (Nitra River, Slovak Republic)

carpio (both Turkey). On the other hand, higher concentrations were found in other three cyprinid fish species (mullet – *Mugil auratus*, sefid – *Rutilus frisii kutum* and common carp – *Cyprinus carpio*) from the Iranian coastal waters of the Caspian Sea (Zeynali *et al.* 2009).

The concentration of manganese in muscle varied from 0.10 to 0.72 mg/kg. Comparative results (0.20–0.89 mg/kg w.w.) were presented in our two previous studies from the middle and lower part of Nitra River (Andreji *et al.* 2005, 2006).

From all analyzed metals in the muscle, zinc was cumulated in highest amounts. In our previous two studies from the lower and middle Nitra River higher zinc concentrations (3.51–42.82 mg/kg w.w.) were recorded in omnivorous (cyprinids) fish species in comparison to carnivorous ones (3.72–5.37 mg/kg w.w.) (Andreji *et al.* 2005, 2006). Considerably lower mean values of zinc concentrations were presented by by Yilmaz and Doğan (2008) for himri – *Carasobarbus luteus* from the Orontes River (Turkey).

Detected copper concentrations in the muscle of analyzed fish species varied between 16.39–25.97 mg/kg wet weight. Considerably lower copper values are reported for himri – *Carasobarbus luteus* from the Orontes River (3.06–5.23 µg/g w.w.) and Anatolian khramulya – *Capoeta tinca* from the Yesilirmak (0.65–3.1 µg/g) River, Turkey (Yilmaz and Doğan, 2008; Mendil *et al.* 2005), as well as for the roach – *Rutilus rutilus* and bream – *Abramis brama* from the Lot River (France) (Shinn *et al.* 2009) or for chub – *Leuciscus cephalus* from the Jihlava River (Czech Republic) (Spurny *et al.* 2002).

In comparison with previous metals, nickel accumulates in lower-order amounts. Compare with other authors, lower nickel concentrations in the fish muscle (0.04–0.16 mg/kg w.w.) are published by Spurny *et al.* (2002) for the chub from the Jihlava River (Czech Republic) and higher values by Mendil *et al.* (2005) for the Anatolian khramulya – *Capoeta tinca* from the Yesilirmak River (Turkey). Our findings showed that detected nickel concentrations ranged to 3.72 mg/kg wet weight in muscle of five fish species.

The concentration of lead in analyzed fish muscle varied from 0.00 to 1.81 mg/kg wet weight. Earlier study from the same river section (Stranai 1998) presented mean lead concentrations in the range of 0.94–1.0 mg/kg w.w. in two fish species (European chub and nase), which are multi-fold higher values. Similar higher mean value of lead concentration was detected in our two previous studies (Andreji *et al.* 2005, 2006). Comparable results are presented by Valova *et al.* (2010) in European chub from the Morava River (Czech Republic); in three fish species (bream, perch, roach) from the Lot River (France) (Shinn *et al.* 2009), as well as in himri from Orontes River (Turkey) (Yilmaz and Doğan, 2008).

The mean cadmium content observed values ranged from 0.00 to 0.11 mg/kg wet weight in analyzed fish samples. Comparable results (0.03–0.08 mg/kg w.w.) to our values were detected in muscle of five fish species (carp – *Cyprinus carpio*, tench – *Tinca tinca*, sval – *Leuciscus svallizi*, grey mullet – *Mugil cephalus*, eel – *Anguilla anguilla*) from the Neretva River (Croatia) (Has-Schon *et al.* 2006), in muscle of two fish species (European chub and brown trout) from the Morava River (Czech Republic) (Valova *et al.* 2010), as well as in fish (himri – *Carasobarbus lutheus* – 0.08–0.16 µg/g w.w.) from the Orontes River (Turkey) (Yilmaz and Doğan, 2008). On the other side, higher values are presented in the fish (roach – *Rutilus rutilus*, bream – *Abramis brama* and perch – *Perca fluviatilis*) muscle from the Lot River (France) (Shinn *et al.* 2009), in the muscle of chub from the Jihlava River (Czech Republic) (Spurny *et al.* 2002), and cyprinid fishes from the Nitra River (Slovakia) (Stranai 1998; Andreji *et al.* 2005, 2006) in comparison to our results.

Tab. 1. Characteristics of analysed fishes.

Species	N	Age	SL (mm) Mean ± SD	TW (g) Mean ± SD
gudgeon	10	3–5	114±12.83	25±10.11
European chub	10	3–6	223±22.87	207±74.77
barbel	10	2–5	246±40.05	222±96.18
roach	10	2–3	128±23.48	53±33.16
nase	10	3–4	270±32.97	317±99.28

SL – standard length, TW – total weight, SD – standard deviation, N – number of individuals

Tab. 2. Contents of selected metals in muscle of analysed fish (in mg/kg wet weight).

Species	Fe	Mn	Zn	Cu	Ni	Pb	Cd
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
gudgeon	9.14±4.31[a]	0.24±0.13[a]	56.36±38.93[a]	23.54±0.97[c]	0.15±0.18[a]	0.10±0.08[a]	0.05±0.03[b]
chub	11.58±3.58[a]	0.42±0.14[b]	88.88±19.62[b]	17.25±1.50[a]	0.75±1.36[a]	0.04±0.05[a]	0.01±0.01[a]
barbel	10.35±5.11[a]	0.41±0.17[b]	87.68±29.80[b]	22.10±1.94[b]	0.20±0.42[a]	0.04±0.04[a]	0.01±0.02[a]
roach	12.73±5.20[a]	0.31±0.16[ab]	81.21±24.55[ab]	24.00±0.77[c]	0.37±1,18[a]	0.06±0.05[a]	0.01±0.01[a]
nase	9.77±4.05[a]	0.27±0.13[a]	99.31±28.95[b]	21.95±0.84[b]	0.07±0.11[a]	0.23±0.56[a]	0.01±0.01[a]

The values with identical superscript in the column are not significant at the $p<0.05$ level.

Correlation between metals

The correlation between age and accumulated metals for individual fish species were not detected, however are known the cases, when the bioaccumulation of metals with size and age in fish were noted (Burger *et al.* 2001, 2002). It is caused probably due to absence of some age categories and/or low count of analyzed samples. Positive correlations between SL and/or TW and accumulated metals were confirmed in European chub and roach for Pb and Cu (Tables 4 and 6). Also Burger and Campbell (2004) confirmed positive correlations between total length and/or weight and lead for white bass (*Morone chrysops*) from the Clinch River (USA).

Generally, the iron shows negative correlations among analyzed metals except the manganese, where a positive correlation was recorded; in the four analyzed fish species also with statistical significance (Tables 3–7). These findings are also confirmed by our previous study from the lower Nitra River (Andreji *et al.* 2006), but inverse results were detected in fish *Capoeta tinca* from the Yesilirmak River, Turkey (Mendil *et al.* 2005).

The manganese has with other metals in majority negative correlation, particularly in the cases of copper, lead and cadmium where values were statistically significant, too (Tables 3–7). Adverse results were published

Tab. 3. Correlations among monitored metals in gudgeon.

	Fe	Mn	Zn	Cu	Ni	Pb	Cd
Fe	–						
Mn	0.886***	–					
Zn	0.332	0.010	–				
Cu	0.453	0.159	0.908***	–			
Ni	–0.487	–0.641*	0.476	0.382	–		
Pb	–0.820**	–0.752*	0.018	–0.168	0.640*	–	
Cd	–0.558	–0.588	0.377	0.209	0.708*	0.811**	–
SL	–0.422	–0.346	–0.236	–0.441	–0.012	0.393	–0.059
TW	–0.369	–0.303	–0.362	–0.517	–0.072	0.288	–0.244

Significant differences *$p<0.05$; **$p<0.01$; ***$p<0.001$

Tab. 4. Correlations among monitored metals in European chub.

	Fe	Mn	Zn	Cu	Ni	Pb	Cd
Fe	–						
Mn	0.684*	–					
Zn	–0.566	–0.295	–				
Cu	–0.407	–0.153	0.229	–			
Ni	–0.104	0.258	–0.173	0.540	–		
Pb	–0.665*	–0.734*	0.349	0.113	–0.130	–	
Cd	–0.011	0.203	–0.214	0.323	0.663*	0.278	–
SL	–0.277	–0.585	0.161	–0.012	–0.418	0.742*	0.128
TW	–0.388	–0.636*	0.300	0.051	–0.350	0.784**	0.122

Significant differences *$p<0.05$; **$p<0.01$; ***$p<0.001$

Tab. 5. Correlations among monitored metals in barbel.

	Fe	Mn	Zn	Cu	Ni	Pb	Cd
Fe	–						
Mn	0.540	–					
Zn	–0.422	–0.494	–				
Cu	–0.708*	–0.241	–0.100	–			
Ni	–0.193	0.167	0.220	0.286	–		
Pb	–0.063	–0.428	0.200	0.017	0.332	–	
Cd	–0.361	–0.550	0.462	0.198	0.055	–0.090	–
SL	–0.464	–0.010	0.459	0.074	0.481	0.242	–0.104
TW	–0.401	0.085	0.353	0.028	0.520	0.279	–0.199

Significant differences *$p<0.05$; **$p<0.01$; ***$p<0.001$

Tab. 6. Correlations among monitored metals in roach.

	Fe	Mn	Zn	Cu	Ni	Pb	Cd
Fe	–						
Mn	0.888***	–					
Zn	0.489	0.349	–				
Cu	–0.127	–0.157	0.163	–			
Ni	–0.549	–0.414	–0.527	0.088	–		
Pb	–0.465	–0.362	–0.627	–0.034	0.728*	–	
Cd	–0.319	–0.331	–0.412	0.075	0.898***	0.772**	–
SL	0.233	0.262	0.282	0.635*	–0.038	–0.238	–0.095
TW	0.252	0.323	0.349	0.677*	–0.095	–0.231	–0.130

Significant differences *$p<0.05$; **$p<0.01$; ***$p<0.001$

Tab. 7. Correlations among monitored metals in nase.

	Fe	Mn	Zn	Cu	Ni	Pb	Cd
Fe	–						
Mn	0.860**	–					
Zn	–0.014	–0.323	–				
Cu	–0.050	–0.334	0.018	–			
Ni	–0.694*	–0.611	–0.098	0.136	–		
Pb	–0.370	–0.372	–0.092	0.163	0.848**	–	
Cd	0.089	0.014	0.808**	–0.398	–0.338	–0.321	–
SL	–0.328	–0.240	0.260	–0.541	0.179	0.243	0.472
TW	–0.309	–0.188	0.196	–0.612	0.074	0.129	0.438

Significant differences *$p<0.05$; **$p<0.01$; ***$p<0.001$

by Burger *et al.* (2002) in fish from the Savanah River (USA), but as a whole for all 11 analyzed fish species.

Zinc did not have any significant relationship to other analyzed metals. In majority, the zinc had a positive correlation and statistical significance was confirmed only between Zn-Cu in gudgeon and Zn-Cd in nase (Tables 3–7). Positive correlations were also detected in fish *Capoeta tinca* from the Yesilirmak River, Turkey (Mendil *et al.* 2005).

In relation to other analyzed metals, copper showed in majority a positive correlation,with statistical significance between Cu-Zn in gudgeon. Negative significant correlation was noted between Cu-Fe in barbell (Tables 3–7). Comparable results were reported by Mendil *et al.* (2005) from the Yesilirmak River (Turkey), where negative correlation was detected only between Cu-Fe.

Generally, nickel had to other analyzed metals positive correlation relationship. Significant positive correlations were detected between Ni-Pb in gudgeon, roach and nase, and between Ni-Cd in gudgeon, European chub and roach. Negative significant correlations were observed between Ni-Mn in gudgeon and between Ni-Fe in nase (Tables 3–7). The results presented by Mendil *et al.* (2005) for *Capoeta tinca* from the Yesilirmak River (Turkey) have in majority comparable values to our results, except the relation Ni-Zn, where the positive correlation was recorded.

In majority, lead had a negative correlation relationship to other analyzed metals, with the statistically significant differences in the relationship between Pb-Fe and Pb-Mn for gudgeon and European chub and between Pb-Ni for gudgeon, European chub, roach and nase. Negative correlation with statistic significance was observed in relation between Pb-Mn for European chub (Tables 3–7). Oposite results were recorded by Mendil *et al.* (2005) in fish from the Yesilirmak River (Turkey), where positive correlations among analyzed metals were recorded in majority of cases.

In relation to other analyzed metals, cadmium has in majority positive correlations, in the case of gudgeon and roach for lead and nickel, and in the case of European chub only for nickel (Tables 3–7). These findings were also recorded by Mendil *et al.* (2005) in fish Capoeta tinca from the Yesilirmak River (Turkey), as wel as by Burger and Campbell (2004) in fish muscle of white bass from the Clinch River (USA) and by Burger *et al.* (2002) in fish from the Savanah River (USA).

<u>*Hygienic limits*</u>

The limit for iron, manganese, zinc in Codex Alimentarius is not defined. The hygienic limits for copper, nickels, lead and cadmium are defined as 10.0, 0.5, 0.2 and 0.05 resp. mg/kg wet weight in Codex Alimentarius. Limit for copper was exceeded (1.5 to 2.6 times) in all analyzed samples (100%). The hygienic limit for nickel was exceeded in 6 samples (12%) and values were 2.7–18.6 times higher. The limit for lead was exceeded in 3 samples (6%) and values were 1.01–9.10 times higher than the permissible limit. In 5 samples (10%) the hygienic limit for cadmium was exceeded and the results were 1.12–2.26 times higher.

Heavy metals load and their relationships in fish reflect trophic levels. A Trophic-level difference in metal levels have been reported for number of contaminants. In general, carnivorous species have higher levels than omnivorous, or planktivorous species (Phillips *et al.* 1980). However, bottom-dwelling fish can sometimes have higher levels than carnivores, particularly when they are ingesting sediment, and herbivorous sometimes have higher levels than carnivorous (Tayel and Shriadah, 1996; Burger *et al.* 2002). It was also confirmed by our study, where the inter-species differences in metal loads were detected.

Some metals in some fish are known to bioaccumulate with size and age (Phillips *et al.* 1980; Burger *et al.* 2001, 2002). However, relationships are not clear for the other metals and in some cases the relationship can be the invers (Burger *et al.* 2002). Moreover, the relationship may not exist where food is limiting and fish stop growing but continue to accumulate contaminants (Downs *et al.* 1998; Burger and Campbell 2004). Those knowledge were confirmed also in our study, when by comparison with previous studies were recorded invers results in relationships among analyzed metals for this same fish species.

Next also important role in the heavy metal loads and their relationships in fish tissues play endoparasites. It is known, that the some species of parasites and their count in fish have negative correlation relationship with accumulated metals (Brázová *et al.* 2012).

CONCLUSIONS

Fish is a very sound and nutritious food and on the other hand it may raise a hidden risk in the form of accumulated contaminants. This risk is higher in the free-water fish, which are not under veterinary supervision. This is also confirmed by our results, where the range of individual metals exceeded the hygienic limits in 6–100% of the analyzed samples. From this point of view, fish from this site are not suitable for consumption. This work revealed significant interspecies differences in accumulation of manganese, copper and cadmium, and relationships of essential elements, such as zinc and copper, and heavy metals, mainly cadmium, to standard length and/or total weight of fish. Generally, the order of analyzed metal concentrations was: Zn>Cu>Fe>Mn>Ni>Pb>Cd. In individual fish species this order was almost identical, only in European chub and nase the higher concentrations for nickel than manganese were recorded. The accumulation of metals and their relationships in the muscle tissue of fish is a very complex process that can not be considered only in the relation to a single biotic and/or abiotic factor and further investigation should be realized in the future.

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