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Levels of selected contaminants in fish muscle from upper Nitra River

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O R I G I N A L A R T I C L E

Abstract

OBJECTIVES: This study determines the levels of selected contaminants in the muscle of three common fish species from the upper course of the Nitra River.
DESIGN: Were detected levels of Zn, Cu, Ni, Cr, Pb, Cd and Hg in the muscle and correlations among selected metals as well as standard length and total weight in brown trout (*Salmo trutta* morpha *fario*), Alpine bullhead (*Cottus poecilopus*) and grayling (*Thymallus thymallus*).
RESULTS: The content of analysed metals (mg.kg^{-1} wet weight) ranged as follows: brown trout – Zn 5.86–12.97, Cu 0.51–0.76, Ni 0.00–0.37, Cr 0.18–0.41, Pb 0.00–0.34, Cd 0.03–0.13, Hg 0.04–0.07; Alpine bullhead – Zn 7.02–13.68, Cu 0.34–0.62, Ni 0.00–1.13, Cr 0.19–0.24, Pb 0.00–0.37, Cd 0.03–0.09, Hg 0.06–0.18; grayling – Zn 3.38–6.36, Cu – 0.46–0.62, Ni 0.04–0.22, Cr 0.13–0.22, Pb 0.00–0.25, Cd 0.02–0.09, Hg 0.05–0.12, respectively.
CONCLUSIONS: The Slovak permissible limits for safe consumption, defined in the Commission Regulations No. 1881/2006 and 629/2008, in the case of Pb, Cd and Hg were exceeded in 10%, 63% and 0%, respectively.

INTRODUCTION

Occurrence of contaminants, their frequency and concentration mainly depends on human activities, although sometimes impurities may be of geological origin (Has-Schön *et al.* 2006; Haluzova *et al.* 2010; Mikulikova *et al.* 2011). The Nitra River is known by presence of inorganic pollutants, which entered the Nitra River mainly by sewage waters in middle and upper part of the river (Andreji *et al.* 2006). Additional pollution originates from power plant factory, chemical factory, leather works, alcohol and starch factory and lignite coal mines (Curlik and Matusova 1994). As a principal source of contaminants can by labeled the fly ash, which in 1965th leaked from the fly ash deposition into the riverbed in volume of cca 1.5 mil m^3 and contaminated downstream approximately 130 km long river course. Due to the Nitra River belongs to the most contaminated rivers in the Slovak Republic, mainly by heavy metals and metalloids (Andreji *et al.* 2012; Andreji *et al.* 2018).

This environmental status is known over 50 years but, the first published data about fish contamination from the Nitra River as whole were published at the end of 20th century (Stranai 1998). Further information, mostly from the middle and lower parts of the Nitra River, are dated at the start of 21st century (Andreji *et al.* 2005; Stranai & Andreji 2005; Andreji *et al.* 2006; Andreji & Stranai 2007; Stranai & Andreji 2007).

Nowadays, the Nitra River represents the fishing territory administrated by 6 local organizations of the Slovak Angling Federation, covering about 11,000 members. From this aspect, the fish can pose a health risk to local and visiting anglers and their families as consumers, who eat fish more often.

The aim of this study was to determine the levels of selected contaminants (Zn, Cu, Ni, Cr, Pb, Cd and Hg) in the muscle of three common fish species from the upper course of the Nitra River (Slovak Republic) and their comparison with permissible limits for safe consumption. Furthermore, correlations among the metal concentrations, standard length, total weight and age of fish were analysed.

MATERIALS AND METHODS

In this study, three common fish species (brown trout – *Salmo trutta* m. *fario*, Alpine bullhead – *Cottus poecilopus* and grayling – *Thymallus thymallus*) inhabiting salmon/grayling zones were collected by electrofishing in September and October 2007 from upper Nitra River (Slovakia) between 152.3–156.7 river kilometer, near the Poluvsie and Nitrianské Pravno villages (Figure 1).

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

Fish (n=30, 10 for each fish species) were evaluated by standard methods used in ichthyology (standard length – SL, total length – TL and weight – W measurements, age determination by scales and vertebras). After the biometric data recording, 2–3 g samples of fish muscle were obtained from the dorsal part of fish body, without skin and bones. After collection, the muscle samples were kept at −18 °C.

For analysis, two grams of muscle sample was mineralised by microwave digestion (MARS X-press, CEM USA) according to EN 13804 and EN 13805. Digested samples were analysed for the presence of Zn, Cu, Ni, Cr, Pb and Cd by fast sequential flame atomic absorption spectrophotometer (FSF–AAS) Varian AA 240FS (Agilent, USA). The total mercury content was determined directly in the sample units by the selective mercury analyser (Advanced mercury analyser, AMA-254, Altec, Czech Republic) based on atomic absorption spectroscopy. Values of monitored heavy metals are presented on a wet weight (w.w.) basis in mg.kg^{-1} and compared with hygienic limits presented in Commission Regulations (EC) No. 1881/2006 and (EC) No. 629/2008.

For statistical analysis, One-way ANOVA test, Multiple Range test (LSD method), Kruskal-Wallis test, t-test, Kolmogorov-Smirnov test and linear model of simple regression (least square fit) were used together with the computer program Statgraphics Centurion 18 Professional (Statgraphics Technologies Inc., USA).

RESULTS AND DISCUSSION

Content of analysed metals in fish

Content of zinc (Zn) in fish muscle tissue varied broadly from 3.38 to 13.68 mg.kg^{-1} w.w., with statistically significant ($p<0.05$) lowest mean value (5.13 mg.kg^{-1}) in grayling and highest mean value (9.64 mg.kg^{-1}) in Alpine bullhead (Table 2). Lower median values were detected in muscle of brown trout (*Salmo trutta*) from the South Canterbury rivers, New Zealand (Stewart *et al.* 2011). Zn is together with Fe and Cu one of the most cumulative element mainly in cyprinids, compare to other fish species (Stranai & Andreji 2005; Andreji *et al.* 2005, 2006, 2012). Consequently, in contrast to Cu and Fe, Zn does not form free radical ions, and has antioxidant properties (Powell 2000). Zn is essential due to its vital structural and/or catalytic importance in more than 300 proteins that play important roles in piscine growth, reproduction, development, vision and immune function (Bury *et al.* 2003; Malekpouri *et al.* 2011). Zn is a competitive ion for Cd and prevents the toxic effect of this metal (Shaffi *et al.* 2001; Malekpouri *et al.* 2011). On the other hand, higher level of Zn in body may cause intoxication. Hygienic limit for

Zn content in fish muscle is not set in Commission Regulations.

The copper (Cu) concentration in analysed fish muscle samples ranged from 0.34 to 0.76 mg.kg^{-1} w.w., also with statistically significance ($p<0.05$) among analysed fish species. Lowest mean value 0.50 mg.kg^{-1} in Alpine bullhead and highest mean value 0.63 mg.kg^{-1} in brown trout (Table 2) were detected. Similar mean value (518 µg.kg^{-1} w.w.) of accumulated Cu was presented in muscle of relative fish species *Salmo trutta macrostigma* from the Munzur Stream, Tunceli, Turkey (Can *et al.* 2012). Opposite, lower results (0.21–0.48 µg.g^{-1} w.w.) in comparison to our findings have been published by Stewart *et al.* (2011) from the South Canterbury rivers, New Zealand. Cu is an essential elements, which acts as a cofactor for a number of key proteins (Bury *et al.* 2003; El Basuini *et al.* 2017). However, in excess, Cu is toxic. In tissues where Cu accumulates, the primary toxic action is predominantly the production of free radicals. Dietary Cu toxicity in the gut includes inhibition of digestive enzymes and reduced gut motility (Kim *et al.* 2018). Similar to Zn, the hygienic limit for Cu content in fish muscle is not set in Commission Regulations too.

Nickel (Ni) values fluctuated in analysed samples at levels of 0.00–1.13 mg.kg^{-1} w.w., but statistically significant differences among fish species were not observed ($p>0.05$). The highest mean concentration in Alpine bullhead (0.27 mg.kg^{-1}) and lowest mean concentration in grayling were recorded (Table 2). Lower Ni concentrations have been reported in muscle of brown trout (*Salmo trutta*) from the South Canterbury rivers, New Zealand (Stewart *et al.* 2011). Unlike Cu, the essentiality of Ni in fish remains unsubstantiated (Pyle and Couture 2012). Nickel acts primarily as a respiratory toxicant (Pane *et al.* 2004). It has been reported that Ni induces severe morphological and histopathological damage to vital organs in fish where accumulates, mainly in the gill, liver and kidney (Driessnack *et al.* 2017). In addition, Ni also causes reproductive toxicity in fish – significant reduction in egg production and/or egg hatchability (Alsop *et al.* 2014). For Ni the hygienic limit in fish muscle is not set by Commission Regulations.

The chromium (Cr) accumulation rate reached values 0.13–0.41 mg.kg^{-1} w.w. in analysed fish species muscle (Table 2), with statistically significant differences among fish ($p<0.05$). The highest mean concentration in brown trout and lowest mean concentration in grayling were noted. Comparable results to our findings have been presented in previous study carried out by Stranai (1998) at the same sampling site for brown trout (0.25–0.35 mg.kg^{-1} w.w.) and grayling (0.20–0.28 mg.kg^{-1} w.w.). Concentrations below limit of detection are known for brown trout from the work of Stewart *et al.* (2011). Cr is considered to be essential for normal carbohydrate and lipid metabolism and as a cofactor for insulin activity (Reid 2012). The chromium is not typical contaminant which generally accumulates and/or biomagnificates in fish in comparison to other ones. According to Palaniappan and Karthikeyan (2009), Cr may be bioaccumulated by fish, but only at extremely high exposure concentrations, and did not increase concentrations through various trophic levels (Seenayya & Prahalad 1987). Its toxicity depends on the oxidation state (Ride 2012). Also for Cr the hygienic limit in fish muscle is not defined by Commission Regulations.

The lead (Pb) content in fish muscle achieved values from 0.00–0.37 mg.kg^{-1} w.w. (Table 2) with highest

Tab. 1. Characteristics of analyzed fishes.

Species	N	Age	SL (mm)		TW (g)	
			mean ± SD	range	mean ± SD	range
brown trout	10	1–2	167.4±11.37	150–182	80.8±16.96	49–59
Alpine bullhead	10	4–5	90.7±8.47	80–103	17.5±4.25	11–24
grayling	10	1–2	174.6±39.78	110–220	83.6±45.93	18–142

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

Tab. 2. Content of selected metals (mean ± SD and minimum – maximum in parenthesis) in muscle of analysed fishes (mg.kg^{-1} w.w.)

Species	Zn	Cu	Ni	Cr	Pb	Cd	Hg
brown trout	$8.21^{b}\pm2.26$ (5.86–12.97)	$0.63^{b}\pm0.10$ (0.51–0.76)	$0.14^{a}\pm0.14$ (0.00–0.37)	$0.34^{b}\pm0.07$ (0.18–0.41)	$0.10^{a}\pm0.13$ (0.00–0.34)	$0.07^{a}\pm0.03$ (0.03–0.13)	$0.06^{a}\pm0.01$ (0.04–0.07)
Alpine bullhead	$9.64^{b}\pm1.95$ (7.02–13.68)	$0.50^{a}\pm0.10$ (0.34–0.62)	$0.27^{a}\pm0.33$ (0.00–1.13)	$0.21^{a}\pm0.01$ (0.19–0.24)	$0.13^{a}\pm0.13$ (0.00–0.37)	$0.06^{a}\pm0.02$ (0.03–0.09)	$0.09^{b}\pm0.04$ (0.06–0.18)
grayling	$5.13^{a}\pm1.10$ (3.38–6.36)	$0.53^{a}\pm0.06$ (0.46–0.62)	$0.11^{a}\pm0.06$ (0.04–0.22)	$0.18^{a}\pm0.03$ (0.13–0.22)	$0.06^{a}\pm0.08$ (0.00–0.25)	$0.05^{a}\pm0.02$ (0.02–0.09)	$0.07^{ab}\pm0.02$ (0.05–0.12)

The values with identical superscript in the column are not significant at the $p<0.05$ level mean concentration in Alpine bullhead and lowest mean concentration in grayling. Statistically significant differences among anylsed fish species were not confirmed ($p > 0.05$). Lower mean values of accumulated Pb in the muscle of brown trout were reported by Valova *et al.* (2010) from the upper Morava River, Czech Republic, as well as from the South Canterbury rivers, New Zealand (Stewart *et al.* 2011). On the other hand, higher mean Pb concentrations have been presented in previous study (Stranai 1998) for brown trout and grayling from the upper Nitra River and for brown trout from the brooks of the military training area of Boletice and from the upper course of the Loučka River, both Czech Republic (Dvorak *et al.* 2016, Vitek *et al.* 2007). Lead has unknown biological function and there exists no evidence that it is required, or otherwise beneficial, for life (Mager 2012; Rubio-Franchini *et al.* 2016). Furthermore, Pb is toxic even at low doses, but does not biomagnify along the food web, however some trophic transfer assuredly takes place for some species (Farag *et al.* 1998; Mager 2012). In the case of Pb, the permissible limit in fish muscle is set to 0.3 mg.kg^{-1} w.w. This limit was exceeded in 3 samples (10 %) – two in brown trout and one in Alpine bullhead, but mean values for fish species were below this limit.

Tab. 3. Correlations among monitored metals in brown trout.

	Zn	Cu	Ni	Cr	Pb	Cd	Hg
Zn	–						
Cu	0.338	–					
Ni	−0.016	0.516	–				
Cr	0.116	0.509	0.721**	–			
Pb	−0.303	0.477	0.811**	0.549	–		
Cd	−0.176	0.040	0.496	0.392	0.472	–	
Hg	−0.029	−0.153	−0.147	−0.435	−0.037	0.137	–
SL	0.292	0.484	−0.013	−0.179	−0.025	0.107	0.010
TW	0.402	0.626	0.181	0.024	0.082	0.266	0.028
Age	0.084	0.640*	0.277	−0.040	0.242	−0.146	−0.005

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

Tab. 4. Correlations among monitored metals in Alpine bullhead.

	Zn	Cu	Ni	Cr	Pb	Cd	Hg
Zn	–						
Cu	−0.194	–					
Ni	0.245	0.003	–				
Cr	0.104	−0.360	0.073	–			
Pb	−0.427	−0.197	−0.264	−0.176	–		
Cd	0.1467	0.233	0.396	0.001	−0.701*	–	
Hg	0.638*	−0.062	−0.299	−0.174	−0.315	0.458	–
SL	−0.039	0.247	−0.623	−0.471	0.399	−0.379	0.475
TW	−0.066	0.195	−0.600	−0.312	0.356	−0.458	0.421
Age	−0.216	0.438	−0.476	−0.519	0.306	−0.118	0.417

Significant differences *$p < 0.05$

Tab. 5. Correlations among monitored metals in grayling.

	Zn	Cu	Ni	Cr	Pb	Cd	Hg
Zn	–						
Cu	0.680*	–					
Ni	0.423	0.620	–				
Cr	−0.184	−0.137	−0.267	–			
Pb	0.107	0.475	0.332	0.031	–		
Cd	−0.925***	−0.641*	−0.451	0.123	−0.088	–	
Hg	0.157	−0.008	0.333	0.160	−0.255	−0.418	–
SL	−0.844**	−0.767**	−0.525	0.291	0.059	0.826**	−0.197
TW	−0.871***	−0.823**	−0.556	0.346	−0.057	0.853**	−0.161
Age	−0.753*	−0.706**	−0.505	0.393	0.135	0.663*	−0.022

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

Content of cadmium (Cd) in muscle of analysed fish varied closely from 0.02 to 0.13 mg.kg^{-1} w.w., with highest mean concentration (0.07 mg.kg^{-1}) recorded in brown trout and lowest mean concentration (0.05 mg.kg^{-1}) recorded in grayling (Table 2). Statistically significant differences in Cd accumulation among fish species were not confirmed ($p > 0.05$). In the previous study from the upper Nitra River (Stranai 1998) mean values of 0.04–0.1 mg.kg^{-1} w.w. and 0.05–0.06 mg.kg^{-1} w.w. have been presented for the brown trout and grayling, respectively. Lower Cd concentrations in muscle of brown trout from upper Morava River (Valova *et al.* 2010) and from the brooks of the military training area of Boletice (Dvorak *et al.* 2016) were presented. Cd like Pb is regarded as a non-essential element and lacks the essential nutrient properties of other transition metals, such as Cu, Zn, Co, Mn, and Mb (McGeer *et al.* 2012). Cd interacts with Cu chaperones, can also bind to structural elements such as zinc-finger proteins, and readily binds to metallothioneins (Waldron *et al.* 2009). Cd bioaccumulates and bioconcentrates in aquatic organisms and the rate depends on the site of exposure (waterborne/dietborne). Cd accumulates in nearly all tissues and organs, with liver, kidney, and gill (or gut) reaching relatively high levels and muscle tissue being generally much lower (Stranai & Andreji 2005; Andreji & Stranai 2007; McGeer *et al.* 2012). On the other hand, toxicity of Cd for aquatic species is generally dependent on concentrations of its bioavailable forms (species), as defined by the total dissolved concentration in combination with the underlying water chemistry (Di Toro *et al.* 2001). Toxicity of Cd is unequivocally linked to ionoregulatory disturbance, production of reactive oxygen species, reduction of survival and growth, disruption of immune system, hatching disruption, occurrence of developmental abnormalities and tissues degradations

(Livingstone 2001; Lizardo-Daudt & Kennedy 2008). According to Commission Regulations, the hygienic limit for Cd is set at the value of 0.05 mg.kg^{-1} w.w. 19 samples analysed (63%) exceeded this limit – 7 in the case of brown trout as well as Alpine bullhead and 5 in the case of grayling. Mean values for each fish species also exceeded this limit.

The mercury (Hg) accumulated in relatively small rate (0.04–0.18 mg.kg^{-1} w.w.). The highest mean Hg concentration (0.09 mg.kg^{-1}) were detected in muscle of Alpine bullhead and lowest mean (0.06) observed in muscle of brown trout (Table 2). Similar results were reported by Valova *et al.* (2010) for brown trout muscle from the upper Morava River, Czech Republic. Higher Hg concentrations in brown trout muscle have been presented by Dvořák *et al.* (2016) from the brooks of the military training area of Boletice, Czech Republic and from the South Canterbury rivers, New Zealand (Stewart *et al.* 2011). Lower Hg muscle concentration has been presented by Can *et al.* (2012) for relative *Salmo trutta macrostigma* from the Munzur Stream, Tunceli, Turkey. Mercury, in either its inorganic [Hg(II)] or its organic [MeHg(I)] form, is not known to have any positive and essential role in growth, reproduction, or survival of fish (Kidd & Batchelar 2012). Aquatic organisms can obtain methylmercury from food, water, and sediment, and they bioaccumulate methylmercury with continued exposure because elimination is very slow relative to the rate of uptake. Concentrations of MeHg(I) are an important predictor of those in fish tissues because it is this form that is bioconcentrated into lower trophic levels and then biomagnified up through the food web (Wiener *et al.* 2003; Kidd & Batchelar 2012). The hygienic limit for Hg content in fish muscle defined in Commissions Regulations is 0.5 mg.kg^{-1} w.w. No analysed samples exceeded this border.

Metal relationships

Generally, among analysed metals, as well as between total weight and accumulated metals dominated a positive correlations, in some cases also with statistical significance ($p<0.05$). Opposite relationships have been detected between metals accumulation and standard length and age, with statistical significant differences as well (Tables 3–5).

There are known studies focused on inter-metal relationships, as well as on metal concentration and length or weight of fish, with different results (Burger *et al.* 2002; Andreji *et al.* 2005, 2006; 2012; Mendil *et al.* 2005; Dvorak *et al.* 2014, 2015). These disproportions and relationships among metals are not well understood (Burger & Campbell 2004; Dvorak *et al.* 2016). Metal bioaccumulation and internal dynamics vary considerably among species; it is difficult to predict accumulation beyond a particular organism in a particular environment, or group of organisms in similar environments; and accumulated contaminant fractionates into metabolically reactive and detoxified pools. Detoxification responses are dynamic and linked to the damage-repair-acclimation process (Adams *et al.* 2011; McGeer *et al.* 2012).

CONCLUSION

Current information about metal concentration in muscle of common three fish species from upper Nitra River was presented. Althoug the studied site is located in submountain zone outside intensive industrial and/or agricultural areas, in the case of Pb and Cd we found several samples exceeding the permissible limits defined in Commission Regulations (EC) No. 1881/2006 and (EC) No. 629/2008, respectively. From this point of view, the fish from the upper course of the Nitra River are not safe for direct human consumption.

Questionable is source of contamination by Pb and Cd. As a main source of contamination can considered air pollution from the nearby power station (direct distance cca 20 km), contaminating air from low-quality brown coal combustion. According to some authors (Bencko *et al.* 1995; Kacik 2003), during this burning process fly ash is forming, which concentrates 100–1,000 times higher amounts of contaminants than coal. This hypothesis should be confirmed or rejected by the further investigation. On a small scale it is also possible that fish migration from polluted to unpolluted sites during higher water levels contributed to our findings.

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