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Ecological risk assessment of heavy metals in brown trout (*Salmo trutta* m. *fario*) from the military training area Boletice (Czech republic)

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Abstract

OBJECTIVES: This study to assess the environmental pollution status in streams (Loutecky, Spicak, Olsina, Trebovicky, Polecnicky and Luzny) from the Boletice area.

DESIGN: Were determined of some metal (Hg, Pb, Cd) concentrations in the muscle and correlations among selected metals as well as standard length and total weight in brown trouth – Salmo trutta morpha fario.

RESULTS: The contents of the analyzed metals in muscles were Hg 0.19–0.72, Pb 0.01–0.6 and Cd 0.020–0.083 mg/kg wet weight basis and these concentrations did not exceed the limits admissible in the Czech Republic.

CONCLUSIONS: The Czech republic permissible limit for Hg (0.5 mg/kg to omnivors, 1 mg/kg to predators), Pb (0.3 mg/kg) and Cd (0.05 mg/kg) defined in the Codex Alimentarius for safe human consumption exceeded in 6%, 3%, and 0% of analyzed samples for Hg, Pb and Cd respectively. On an average, the order of metal concentrations in the fish muscle was: Hg>Pb>Cd.

Abbreviations:

T	- Trebovicky Brook
O	- Olsina Brook
S	- Spicak Brook
L	- Loutecky Brook
P	- Polecnicky Brook
LU	- Luzny Brook
SD	- standard deviation
SL	- standard length
TW	- total weight

ORIGINAL ARTICLE

INTRODUCTION

The military training areas and military shooting ranges has resulted in increasing pollution by heavy metals representing a significant environmental hazard for invertebrates, fish, and humans (Robinson *et al.* 2008; Voie & Mariussen 2010). Military areas and shooting ranges can be seriously contaminated by heavy metals, and metalloids. The composition of the small arms ammunition may vary, but consists typically of lead (Pb), cadmium (Cd), and mercury (Hg). Metals from the ammunition residues may then leach into the soil and surrounding watercourses where they pose a threat to exposed wildlife (Bennet *et al.* 2007; Kähkönen *et al.* 2008; Voie & Mariussen 2010).

Heavy metals are inert in the sediment environment and are often considered to be conservative pollutants (Andreji *et al.* 2012; Authman *et al.* 2015), although they may be released into the water column in response to certain disturbances (Agarwal *et al.* 2005) and become potential threat to ecosystems (Hope 2006). The effects of pollutants may be also detected on land as a result of their bioaccumulation and bioconcentration in the food chain (Zhang *et al.* 2004; Cervený *et al.* 2014).

One of the major contaminants is mercury in the form of methylmercury (MeHg). This neurotoxic form mercury affecting mostly aquatic organisms (Voie & Mariussen 2010; Maceda-Veiga *et al.* 2012). Methylmercury is primarily responsible for bioaccumulation in the muscle tissue of fish with the methyl mercury to total mercury ratio of 83–90% (Kruzikova *et al.* 2008; Dvorak *et al.* 2015). Lead (Pb), in the bivalent form, is a stable element that is mainly bioaccumulated by aquatic organisms. The primary mode of lead contamination in freshwater fish is through the gills into the bloodstream. The effect of lead poisoning depends on the life stage of the fish, pH, water hardness, and the presence of organic materials (Widinarko *et al.* 2000; Authman *et al.* 2015). Cadmium (Cd) is a nonessential element that causes severe toxic effects in aquatic organisms in very low concentrations. Cadmium can damage the gills, which represents the key mechanism of acute toxicity; (Piačková *et al.* 2003).

The purpose of this study was to determine the concentration of selected metals in water, sediment and the muscle of brown trouth (*Salmo trutta* m. *fario*) in the brooks Trebovicky, Olsina, Spicak, Loutecky, Polecnicky and Luzny (the Boletice military training area – Czech Republic), which may pose risk to human health and environmental hazard. Furthermore, correlations among the concentrations of metals and standard length body fish were subject to this analysis.

Tab.1. Mean concentration of analyzed metals i water and sediment.

Site	water (mg/l)			sediment (mg/kg dry mass)		
	Hg	Pb	Cd	Hg	Pb	Cd
[T]	0.05	1.14	<0.05	0.078	19	<0.5
[O]	<0.05	0.51	<0.05	0.027	11	<0.5
[S]	b.d.	<0.5	<0.05	0.013	11	<0.5
[L]	b.d.	<0.5	<0.05	0.015	11	<0.5
[P]	b.d.	b.d.	<0.05	0.019	6,5	<0.5
[LU]	<0.05	<0.5	<0.05	0.032	10	<0.5

b.d. – below detectable limit

MATERIALS AND METHODS

Samples of water and sediment (Table 1) were obtained during the seasons of 2015 from 6 sites of the south Boletice – Trebovicky (T) Olsina (O), Spicak (S), Loutecky (L), Polecnicky (P) and Luzny (LU) brooks (Figure 1), but samples of fish muscle were obtained from only 4 sites (T, O, S, L). The fish were obtained by electrofishing (220–250 V,1.5–2.5 A, 63 Hz). As the reference species were chosen brown trouth (*Salmo trutta* m. *fario*) due to their occurrence in all of the evaluated fishing grounds. The fish (n=28) were evaluated by standard methods used in ichthyology (standard length – SL and total weight – TW measurements). Upon recording the biometric data (Table 2), samples of fish muscles were obtained from the dorsal part of their body. The collected tissue and sediment samples were kept at −18 °C.

The total mercury (THg) content was determined directly in the sample units by the selective mercury analyser (Advanced mercury analyser, AMA-254, detection limit 1 µg/kg, recovery 82±6%) based on atomic absorption spectroscopy (AAS). Other toxic metals (Pb and Cd) were measured by the means of electrothermal (flameless) atomic absorption spectrometry with Zeeman background correction (graphite furnace atomic absorption spektrometry (GF-AAS, SpectrAA 220Z, Varian) after microwave mineralisation of the samples (EN13 804, 13805 and 14084). The concentrations of all target analytes in the samples were determined and expressed in wet weight (w.w.) and compared with the Czech nationwide regulation no. 305/2004 (Czech Republic, 2004) setting the maximum residue levels in foodstuff.

For statistical analysis, the Anova One-Way test, Multiple Range test (LSD method), Kruskal-Wallis test, and Linear Model of Simple Regression (least squares fit) were used together with the computer program Statgraphics Centurion XV.

RESULT AND DISCUSSION

<u>Content of analyzed metals in fish</u>

The cadmium concentration in fish muscle tissue varied broadly from <0.02 to 0.03±0.02 mg/kg wet weight. The highest cadmium concentration (0.083 mg/kg) was found in the muscle tissue brown trout at site Spicak. On the other hand lowest cadmium concentrations was found in fishes at Trebovicky, Loutecky and Olsina brooks.

Noel *et al.* (2013) showed that lower concentrations of cadmium are found in the muscle of carnivorous fish species, such as pike (0.001 mg/kg), compared with nonpredatory fish, such as bream or roach (0.004 and 0.005 mg/kg) respectively. This assertion was not confirmed in a study by Dvorak *et al.* (2014) that found levels of cadmium in the muscle samples of the elderly chub and roach in concentrations of from 0.00 to 0.15 and from 0.00 to 0.05 mg/kg w.w. respectively. Any metal-induced disturbance of energy production, allocation, or consumption is reflected in fish growth rate. Significant reduction of growth was observed already at 0,47 mg/kg Cd in salmon (Rombough & Garside 1982). These findings too do not correspond with our muscle tissue results, in which accumulation of cadmium due to the trophic position o was recorded.

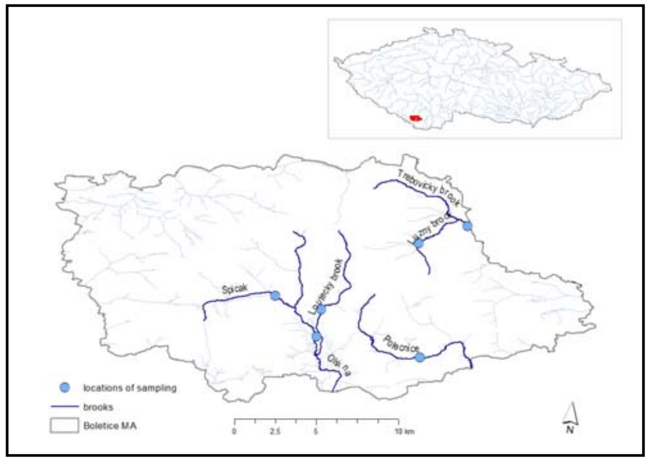

Fig. 1. Map of the rivers studies with the location of sampling sites indicated.

Tab. 2. Characteristics of analyzed specimens of brown trout (*Salmo trutta* m. *truta*)

Site	N	Age (years)	SL (mm) Mean ± SD	TW (g) Mean ± SD
[T]	7	1–3	207±25.47	176±44.31
[O]	7	1–2	138±8.14	24±13.87
[S]	7	1–2	127±9.06	16±6.11
[L]	7	1–3	156±17.01	48±17.13

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

Tab. 3. Contents of analyzed metals in muscle of brown trouth (in mg/kg wet weight)

Site	Hg Mean ± SD	Pb Mean ± SD	Cd Mean ± SD
[T]	0.25±0.09[a]	<0.10[a]	<0,02[a]
[O]	0.61±0.09[c]	<0.10[a]	<0.02[a]
[S]	0.61±0.09[c]	0.22±0.04[b]	0.03±0.02[a]
[L]	0.38±0.05[b]	<0.10[a]	0.02±0.01[a]

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

Tab. 4. Correlations among analyzed metals in brown trout

	Hg	Pb	Cd
Hg	–		
Pb	0.1143[–]	–	
Cd	0.0325[–]	0.0800[–]	–
SL	–0.7485[***]	–0.2939[–]	–0.1859[–]

[–] $p>0.05$, [***] $p<0.001$

Detected lead concentrations in muscle of brown trout ranged from 0.1 to 0.6 mg/kg w.w., with mean value of 0.14 mg/kg w. w. At individual sampling sites varied this mean value from 0.1 mg/kg w.w. sites (T), (O), (L) to 0.2 mg/kg w.w. the site (S). Vitek *et al.* (2007) reported higher concentrations of lead in the muscle tissue of brown trout from the upper course of the Loucka River in concentrations 0.390±0.311 mg/kg. Voie and Mariusen (2010) found higher lead concentrations in fish from rivers in areas of outdoor shooting ranges in Norwege. Watanable *et al.* (2003) found lead content (0.025–0.896 mg/kg) in fish muscle tissue from the lower Mississippi River. Some other authors reported values over 0.100 mg/kg w.w. (Valova *et al.* 2010; Schmitt *et al.* 2007). Valova *et al.* (2013) reported higher concentrations of lead in the muscle of chub from the Bečva River.

Mercury in muscle of analyzed fish specimens was in relatively close range. Its mean concentration achieved the values from 0.46 mg/kg w.w., with highest value at site (O) 0.77 mg/kg w.w. Lower mercury contamination was found in brown trout muscle of the localities (T, L). These values are in accordance with the studies of Dvorak *et al.* (2014; Dyje river basin) and Valova *et al.* (2013; River Morava) from muscle tissue chub. Andrei *et al.* (2012) and Akoto *et al.* (2013) reported that low mercury content in fish muscle tissue from the exposed locations may affect the restocking from generally uncontaminated pond breeding facilities. Despite a very close range of recorded values, statistically significant differences ($p<0.05$) among site were noted (Figure 2.)

Correlations

For all analyzed metals a positive correlations among them were noted (Table 4), but without statistical significance ($p>0.05$). Opposite relationships have been recorded between metals and standard length. With increasing standard length the metal concentration

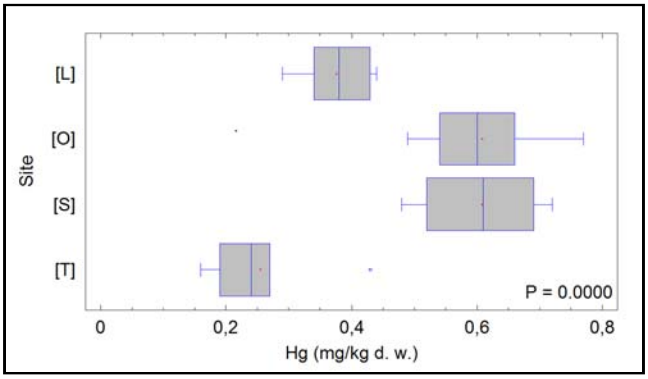

Fig. 2. Content of analyzed mercury of brown trout in relation to sites collection

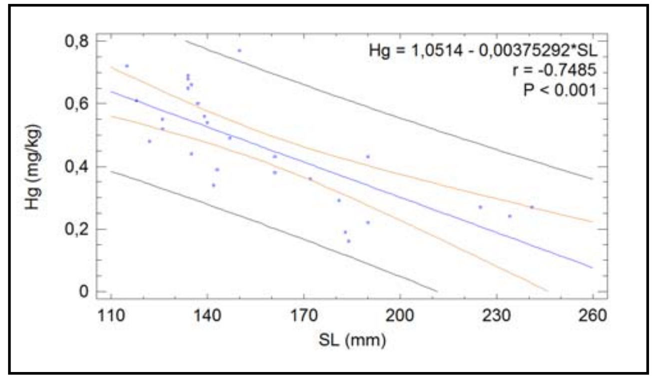

Fig. 3. Content of analyzed mercury in relation to SL (mm) of browny trout

decreased, in the case of mercury also with statistical significance (p<0.001). This pattern decreasing metal concentrations could be attributed to metal-associated mortality, metals elimination, or growth dilution (Smylie *et al.* 2016). On the other hand, there are known studies, where metal concentrations increase with length and/or weight of fish (Dvorak *et al.* 2014, 2015; Yi & Zhang 2012; Andreji *et al.* 2012; Burger & Campbell 2004). These disproportions as well as relationships among metals are not well understood. Except main factors such as fish species, food, status in trophic level, sex, age, concentration of contaminants, time of exposure, season, physico-chemical properties of water, they depend on the metabolic activities, migration possibilities from/to polluted/unpolluted sites, half-life of metals and their forms (Van Walleghem *et al.* 2013; Smylie *et al.* 2015; Trudel & Rasmussen 1997; Roesijadi & Robinson 1994; Di Giulio & Hinton 2008; Sanchez-Chardi *et al.* 2007; Yılmaz *et al.* 2007).

CONCLUSION

In most cases, fish from metal-contaminated water are safe for human consumption due to low metal accumulation (except for mercury) in the muscle tissue. However, such fish may constitute a potential risk for predatory fishes, birds and mammals feeding on contaminated fish. The results of this study provided valuable information about the metal contents in sediment and the muscle of fishes in the brooks T, O, S, L, P and LU (the military training area Boletice). Many researches shows that military activities could release toxic metals into the environment. The behavior of elements in the environment depends on their chemical form. In conclusion, the toxic effect of heavy metals in the muscle of fish, sediments and watter have not been demonstrated in this study. Generally, the order of analyzed metal concentrations was: Hg>Pb>Cd. The hygienic limits for mercury, lead and cadmium are defined as 0.5 (resp. predators 1 mg/kg), 0.3 and 0.05 mg/kg wet weight in Codex alimentarius. Analyses in sediment and watter demonstrated no increase heavy metals (Hg, Pb, Cd) in none monitored stream of military training area Boletice.

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