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Heavy Metals in the Common Carp (*Cyprinus carpio* L.) from Three Reservoirs in the Czech Republic

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Abstract

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Toxic metals (cadmium, lead, and mercury) in the tissues of common carp fished from three reservoirs (Pilská, Domaninský, and Matějovský) in the Bohemian-Moravian Highlands were measured in the period from April 2013 to September 2014 and the risk of consuming the fish from these localities was evaluated. During this project 25 fish specimens from each locality were analysed. The concentration of metals in muscle tissue and liver was determined by atomic absorption spectrometry. A variation in the content of heavy metals in fish samples and fish tissues was demonstrated and discussed. Higher concentrations of cadmium in the fish tissue were found in 50 samples of carp liver. Because the fish liver is not commonly consumed, common carp from the Moravian-Moravian Highlands does not pose any health risk for consumers. Concentrations of the other monitored toxic metals in fish tissue were low and were complying with the maximum residue limit in all cases.

Keywords: atomic absorption spectrometry; cadmium; fish; lead; mercury

Fish are a lean, low-calorie food, and are considered as an integral component of healthy diet, a source of high-quality protein, vitamins, minerals, omega-3 fatty acids, and a wide range of other important nutrients. On the other hand, fish generally accumulate contaminants from the aquatic environment and can transfer toxic metals to humans via the food chain. Fish are consumed worldwide and for this reason the contents of heavy metals in fish tissues are highly important for human health and safety. Water pollution results in fish contamination with toxic metals. This pollution has different causes and different sources e.g. accidental spillage of chemical waste, periodic precipitation contaminated with airborne pollutants, discharge of industrial or sewerage effluents, agricultural drainage, domestic wastewater, and gasoline from fishing boats (Dominogo *et al*. 2007; Has-Schön *et al*. 2015). Heavy metals enter the bodies of fish in three ways: through the body surface, the gills, and the digestive tract (Čelechovská *et al*. 2007) and they are accumulated in organs such as liver, kidneys, spleen, and gonads (Spurný *et al*. 2002). For this reason, the content of heavy metals in fish tissue is often used as a bioindicator of contaminated localities in environmental studies.

Great attention is paid to hazardous elements such as mercury, lead, and cadmium (Čelechovská *et al*. 2007), which are of particular interest for fisheries and consequently may pose a health risk to humans (Berg *et al*. 2000).

When contaminant levels in fish products are unsafe, consumption advisories may recommend that people should limit or avoid eating certain species of fish caught in certain places.

The European Commission (2001), Food and Agriculture Organization (1983), and Food and Drug Administration (2003) have recommended maximum limits (ML) for heavy metal residues in fish meat for human consumption. For cadmium, lead, and mercury the ML are 0.05, 0.30, and 0.50 mg/kg fish and fish products, respectively. These ML are included in the European Commission Regulation (EC) No. 629/2008

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and European Commission Regulation (EC) No. 1005/2015 which are also obligatory for the Czech Republic (FAO 1983; FDA 2003).

The Czech Republic is an important producer of high-quality freshwater fish, but in spite of this, fish consumption is very low compared to other EU countries. The predominant fish farmed in Czech ponds and reservoirs is the common carp and it provides 87–90% of all fish consumed in the Czech Republic (Čelechovská *et al.* 2007, Kenšová *et al.* 2010; Cerveny *et al.* 2014).

Apart from industrial fish production, there are about 320 000 anglers registered in the Czech Republic. The families of these anglers consume higher amounts of freshwater fish than the general population. Many studies of the contents of toxic elements in fish meat and organs of domestic fish species have been conducted in the Czech Republic (Spurný *et al.* 2002; Havelková *et al.* 2008; Kružíková *et al.* 2013; Cerveny *et al.* 2014;). Carp and trout caught in Czech rivers and reservoirs have been predominantly investigated (Svobodová *et al.* 2002; Čelechovská *et al.* 2007).

The first studies focused on the quality of fish meat were carried out in Czechoslovakia in the 1970s. Mercury and other heavy metals including lead, cadmium, and arsenic were determined (Svobodová *et al.* 1975, 1982; Mašek *et al.* 1977; Hruška 1979; Svobodová & Boháčková 1979) in fish tissues from contaminated localities. Subsequent investigations of fish meat quality in the Czech Republic were conducted in the known fishing areas or in the places known to be contaminated. Southern Bohemia with its ponds was one of the most frequently monitored areas (Svobodová *et al.* 2002; Čelechovská *et al.* 2007; Kružíková *et al.* 2013; Cerveny *et al.* 2014), followed by Southern Moravia (Houserova *et al.* 2006; Čelechovská *et al.* 2007; Kenšová *et al.* 2010; Kružíková *et al.* 2013; Cerveny *et al.* 2014). Besides that, the reservoirs in Northern Moravia (Svobodová *et al.* 2004; Čelechovská *et al.* 2007; Havelková *et al.* 2008; Kružíková *et al.* 2013; Cerveny *et al.* 2014), Western Bohemia (Svobodová *et al.* 2002), Eastern Moravia (Čelechovská *et al.* 2007; Vítek *et al.* 2007; Cerveny *et al.* 2014) and Eastern Bohemia (Kružíková *et al.* 2013) were monitored.

Reservoirs in other parts of Bohemia and Moravia were also monitored.

Our study is focused on the recreational localities of the Bohemian-Moravian Highlands, which have many lakes and ponds, similar to those in Southern Bohemia. The analysed samples of fish meat and liver originated from common carp (*Cyprinus carpio* L.) caught by anglers from three reservoirs: Pilská, Domaninský, and Matějovský. Cadmium, mercury, and lead were determined in a dorsal muscle due to their important role in human nutrition and for ensuring the safety of consumers. The liver was analysed as an organ where main metabolism and major injuries take place (Pourang 1995). The obtained results were assessed and compared.

The goal of the study was to evaluate the health and safety of common carp fished for human consumption in the above-mentioned localities.

MATERIAL AND METHODS

Fish sampling. Specimens of *Cyprinus carpio* L. were fished from April 2013 to September 2014 in three reservoirs located near the town of Jihlava in the Bohemian-Moravian Highlands (Figure 1), Czech Republic, and samples of muscle tissues and liver were separated and stored for analysis.

The suitable fish were measured from top to tail and weighed and the data from individual fish were recorded. After killing, muscle tissue from the dorsal side of the body, which is the most frequently consumed part of fish, and the liver, known as the organ accumulating toxic metals, were taken for analysis. Tissue samples were kept in a plastic bag at –20°C in the freezer.

Description of the localities. The Pilská reservoir is located 41 km northeast of Jihlava near busy traffic roads. The Domaninský reservoir is located 58 km northeast of Jihlava. Waters from the vicinity of uranium mines flow into this reservoir. The Matějovský reservoir is located 36 km northeast of

Figure 1. Map of sampling sites

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Jihlava between woods and meadows and can be considered as an environmentally clean locality.

Sediment and water sampling. Sediment and water were sampled on September 13, 2014 in Pilská, Domaninský, and Matějovský reservoirs in the Bohemian-Moravian Highlands. Three locations at each reservoir were chosen and three surface and near-surface sediment samples for each location were gathered with a hand-operated grab sampler from a small boat. Water samples were taken in the same locations and both sediment and water samples were collected into cleaned plastic containers. The samples were stored at an adequately low temperature (~ 4°C) during transport to the laboratory. Water was filtered (0.45 µm) and sediments were air-dried before analysis.

Determination of cadmium and lead. The fish samples were lyophilised (Power Dry LL 3000; Thermo Scientific, Pardubice, Czech Republic) for 7 days before analysis and subsequently mineralised in a microwave oven. For mineralisation, 0.4 g of lyophilised tissue and 8 ml of nitric acid (1 : 1) were used. The concentrations of the studied metals in carp tissues mentioned in the results and discussion chapter are expressed in fresh weight (FW). The same procedure was used for the mineralisation of reference material (DORM-4). The microwave oven was also used for sediment decomposition. For mineralisation, 0.5 g of sediment, 3 ml of nitric acid (65%), and 9 ml of hydrochloric acid (37%) were used. Mineralisation procedures followed the instructions of the Milestone producer (Sorisole, Italy). For the preparation of reagents and standards, deionised water and high-quality suprapure (Merck, Darmstadt, Germany) concentrated (65%) nitric acid were used. Cadmium and lead were determined by electrothermal atomic absorption spectrometry (HR-CS AAS, CONTRAA 700; Analytik Jena, Germany) based on the recommendations of the spectrometer manufacturer. The wavelength of 228.8 nm was selected for cadmium measurement and 283.3 nm for lead. Temperature programmes for cadmium and lead determination are shown in Table 1. The standard solutions for calibration were prepared from cadmium and lead stock solutions, respectively, both containing 1.000 ± 0.002 g/l (Analytika Praha, Czech republic). The standards were diluted appropriately and used for calibration (calibration range: Cd 0–100 µg/l, Pb 0–250 µg/l).

The detection limits for cadmium were 0.51 and 0.62 µg/kg FW for muscle and liver, respectively, for lead 4.93 and µg/kg FW for muscle and liver, respectively. The detection limits for cadmium were 1.09 and 0.02 µg/l for sediment and water, respectively. The detection limits for lead were 19.1 and 0.38 µg/l for sediment and water, respectively.

Determination of mercury. An AMA 254 atomic absorption spectrometer (Altec Praha, Czech republic) was used for determination of total mercury. The homogenised solid samples (fish tissues and sediments) (100 ± 0.1 mg) and water (100 µl) were directly weighed into pre-cleaned combustion boats, and inserted into the AMA 254 analyser. The samples were dried at 120°C for 60 s and thermally decomposed at 550°C for 150 s under oxygen flow. The selectively trapped mercury was released from the amalgamator by a brief heat-up and finally quantified (measuring cycle, 57 s) as Hg° by the cold-vapour AAS technique at 253.7 nm.

The detection limit for mercury was 0.11 µg/kg for muscle, liver, water, and sediment.

Statistical evaluation. Statistical analysis of metal content in tissues was done using one-way analysis

Table 1. Temperature programme for the analysis of cadmium and lead

Step	Process	Cadmium				Lead			
		temperature (°C)	ramp (°C/s)	hold time (s)	time (s)	temperature (°C)	ramp (°C/s)	hold time (s)	time (s)
1	drying	80	6	20	29.2	80	6	20	29.2
2	drying	90	3	20	23.3	90	3	20	23.3
3	drying	110	5	10	14.0	110	5	10	14.0
4	pyrolysis	350	50	20	24.8	350	50	20	24.8
5	pyrolysis	600	300	10	10.8	800	300	10	11.5
6	adaption of gas	600	0	5	5.0	800	0	5	5.0
7	atomisation	1200	1400	3	3.4	1500	1400	3	4.5
8	cleaning	2450	500	4	6.5	2450	500	4	5.9

doi: 10.17221/100/2016-CJFS

Table 2. Certified and found values of elements in DORM-4 (mg/kg dry weight) (n = 5)

	Certified values	Found values
Cadmium	0.306 ± 0.015	0.304 ± 0.012
Lead	0.416 ± 0.053	0.415 ± 0.025
Mercury	0.410 ± 0.055	0.408 ± 0.018

n – number of samples

of variance (ANOVA) and statistical significance was declared when the P-value was equal to or less than 0.05. The significance of differences between the average values of the individual localities was tested at a significance level P = 0.05 by Tukey's HSD (Honestly Significant Difference) test. The paired t-test analysis was carried out with the muscle tissue and liver samples; the analysed metals showing differences were identified. The results are presented as means ± standard deviation (SD). All results were evaluated by the statistical software Statistica.cz (Version 10).

Method validation. The reference material DORM-4 (fish protein, Canada) was used for the method validation. The obtained results are presented in Table 2.

The determined values were in good agreement with the certified values.

In this study, concentrations of all studied metals in carp tissues were expressed in fresh weight (FW).

RESULTS AND DISCUSSION

Content of analysed metals in tissues of Cyprinus carpio* L.*, sediment, and water. A total of 75 common carps were analysed, 25 from each reservoir. The minimum length of *Cyprinus carpio* L. is 35 cm for anglers according to Act No. 99/2004 on Fishery, Decree No. 197/2004 of the Ministry of Agriculture. The measured lengths and weights of the studied fish are in Table 3.

The concentrations of metals (μg/kg FW) in dorsal muscles and liver of *Cyprinus carpio* L. in Domaninský, Pilská, and Matějovský reservoirs are summarised in Table 5.

It is evident that cadmium was predominantly accumulated in the liver. The liver concentrations (Pilská 81.0 ± 26.5 μg/kg FW, Domaninský 258.1 ± 47.2 μg/kg FW, Matějovský 49.9 ± 34.0 μg/kg FW) were statistically higher (P < 0.05) than those in dorsal muscles (Pilská: 3.6 ± 2.9 μg/kg FW, Domaninský 38.0 ± 21.0 μg/kg FW, Matějovský 0.5 ± 0.1 μg/kg FW). Analogous results of cadmium were presented in other studies dealing with carp tissues from Czech reservoirs. Čelechovská *et al.* (2007) studied 125 specimens of common carp caught in ten reservoirs in South Bohemia in the Czech Republic during the period 2000–2007. They found that the highest content of cadmium was in the liver (19.0 ± 1.0 μg/kg FW). The same results were obtained by Kenšová *et al.* (2010), who analysed carp from three reservoirs in the South Moravian Region (muscle 5.0 ± 3.0 μg/kg FW, liver 22.0 ± 4.0 μg/kg FW). Svobodová *et al.* (2002) reported that the main organs for cadmium accumulation were liver and kidneys. The liver has the ability to accumulate large quantities of pollutants from the external environment, and also plays an important role in storage, redistribution, detoxification, and transformation of pollutants (Evans *et al.* 1993). Higher concentrations of cadmium in fish tissues were found in 50 samples of carp liver mainly from Domaninský (17 samples, Cd 456.0 ± 204.4 μg/kg FW), Pilská (19 samples, Cd 115.0 ± 7.4 μg/kg FW) and Matějovský (14 samples, Cd 117.1 ± 1.2 μg/kg FW) reservoirs. As carp liver is not intended for human consumption, these higher values do not pose any health risk to fish consumers.

Lead concentration in common carp tissues, dorsal muscle, and liver was highest in the Pilská reservoir (dorsal muscle 253.3 ± 56.1 μg/kg FW, liver 262.7 ± 15.2 μg/kg FW) in comparison with the other reser-

Table 3. The minimum, maximum, and mean weight (kg) and length (cm) of *Cyprinus carpio* L. in Domaninský, Pilská, and Matějovský reservoirs, Bohemian-Moravian Highlands, Czech Republic

	Reservoir	Minimum	Maximum	Mean
Weight	Domaninský	1.92	3.01	2.4 ± 0.3
	Pilská	1.91	3.09	2.5 ± 0.3
	Matějovský	2.12	3.05	2.5 ± 0.2
Length	Domaninský	45.1	50.8	47.6 ± 1.5
	Pilská	42.6	52.4	48.2 ± 2.3
	Matějovský	42.3	51.3	47.2 ± 2.4

doi: 10.17221/100/2016-CJFS

Table 4. Concentrations of metals in water (μg/l) ($n = 9$) and sediment (μg/kg dry weight) ($n = 9$) in Domaninský, Pilská, and Matějovský reservoirs, Bohemian-Moravian Highlands, Czech Republic

	Reservoir	Cd	Pb	Hg
Water	Pilská	< LOD	0.94 ± 0.62	0.29 ± 0.05
	Domaninský	0.19 ± 0.06	< LOD	4.53 ± 0.31 (0.32 ± 0.04)*
	Matějovský	< LOD	< LOD	0.15 ± 0.09
Sediment	Pilská	1.10 ± 0.01	98.1 ± 3.6	23.7 ± 1.3
	Domaninský	2.38 ± 0.13	14.3 ± 1.0	13.0 ± 4.4 (12.5 ± 1.2)*
	Matějovský	0.95 ± 0.03	6.25 ± 0.56	12.0 ± 2.5

Data are presented as mean ± SE; n – number of samples; *sampled on October 20, 2014

Table 5. Concentrations of metals (μg/kg fresh weight) in dorsal muscles ($n = 25$) and in the liver ($n = 25$) of *Cyprinus carpio* L. in Domaninský, Pilská, and Matějovský reservoirs, Bohemian-Moravian Highlands, Czech Republic

Tissues	Reservoir	Cd	Pb	Hg
Muscle	Pilská	$3.6 \pm 2.9^{a***}$	$253.3 \pm 56.1^{a***}$	$30.6 \pm 5.0^{a***}$
	Domaninský	$38.0 \pm 21.0^{b***}$	66.1 ± 29.8^{b}	$72.4 \pm 19.9^{b***}$
	Matějovský	$0.5 \pm 0.1^{a***}$	13.84 ± 7.500^{c}	$18.9 \pm 2.0^{a***}$
Liver	Pilská	81.0 ± 26.5^{A}	262.7 ± 15.2^{A}	11.5 ± 2.5^{A}
	Domaninský	258.1 ± 47.2^{B}	78.0 ± 33.6^{B}	18.9 ± 7.6^{B}
	Matějovský	49.9 ± 34.0^{A}	14.8 ± 1.9^{C}	8.2 ± 2.0^{C}

Data are presented as mean ± SE; n – number of samples; different letters in the column for dorsal muscle ($^{a–c}$) and liver ($^{A–C}$) indicate significant differences at $P < 0.05$ (ANOVA); significance of differences between dorsal muscle and liver from the paired t-test is indicated alongside letters in the columns (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

voirs Domaninský (dorsal muscle 66.1 ± 29.8 μg/kg FW, liver 78.0 ± 33.6 μg/kg FW) and Matějovský (dorsal muscle 13.8 ± 7.5 μg/kg FW, liver 14.8 ± 1.9 μg/kg FW). This observation corresponded with the contamination load of this area located near a busy highway. Although the unleaded petrol is used at present, dust particles containing lead from leaded petrol in the past are still in the environment. It is generally known that the amount of lead in soil is traffic-related (Asgari & Amini 2011). The content of lead in the muscles, liver, kidney, spleen, gills, ovaries, and testes of carp from South Bohemia did not statistically differ (Čelechovská *et al*. 2007) like in the Domaninský and Matějovský reservoirs. Cerveny *et al*. (2014) studied 27 localities in the Czech Republic during 2006–2010. The content of lead was analysed in 86 samples of common carp. No differences were found in the lead content between muscles and liver.

Statistically significant differences ($P < 0.05$) in mercury concentrations were found between the tested tissues. Significantly higher ($P < 0.05$) content of mercury was measured in dorsal muscles (Pilská 30.6 ± 5.0 μg/kg FW, Domaninský 72.4 ± 19.9 μg/kg FW, Matějovský 18.9 ± 2.0 μg/kg FW) than in the liver (Pilská 11.5 ± 2.5 μg/kg FW, Domaninský 18.9 ± 7.6 μg/kg FW, Matějovský 8.2 ± 2.0 μg/kg FW). This fact indicated the studied reservoirs (Pilská, Domaninský, and Matejkovsky reservoirs) as clean localities, in accordance with the studies by Svobodová *et al*. (1995), Foster *et al*. (2000), and Linde *et al*. (2004). These authors demonstrated that in fish from heavily contaminated localities mercury was deposited preferentially in the liver, while in slightly contaminated localities it was preferentially deposited in muscle. Higher contents of mercury in muscles than in the liver were also found in carp tissues from locations in Bohemia during 2003–2011 (Kružíková *et al*. 2013) and 2004 (Houserova *et al*. 2006), and in a study during 1991–1996 (Havelková *et al*. 2008) focused on clean and contaminated rivers in Bohemia. The highest amount of mercury (methylmercury) was found in the particularly contaminated localities in the Czech Republic such as the Skalka reservoir, where the regular consumption of fish is not recommended, in the middle reaches of the Elbe River (near Neratovice) and in the Odra River (near

doi: 10.17221/100/2016-CJFS

Ostrava) (Dušek *et al*. 2005; Havelková *et al*. 2008; Cerveny *et al*. 2014).

The samples of carp tissues analysed in this study originated from fish caught by anglers. These included fish which met the permitted size limit (min. length of common carp 35 cm) for angling. They were of comparable age and were kept in the reservoirs for the same period. As expected, the content of individual metals did not significantly differ within the given range of weight and length in individual fish.

Nevertheless, statistical differences were found when the amounts of cadmium, lead, and mercury in carp tissues fished from the three studied reservoirs were compared (Table 4). The highest contents of cadmium and mercury were found in tissues of carps from the Domaninský reservoir. These results could be associated with the higher contents of heavy metals in water and sediments of the Domaninský reservoir (Table 4). A surprisingly high concentration of mercury in water (4.5 μg/l) of the Domaninský reservoir sampled on September 13, 2014, may be connected with a recent clearing of the Skalsky reservoir, which is the main tributary of the Domaninský reservoir. There was no information about the situation before the clearing of the Skalsky reservoir, so it is possible that this extremely high mercury concentration in water was only a temporary concentration. Recent analyses of mercury in water from the Domaninský reservoir, sampled on October 20, 2014, corresponded with the average value found in the studied localities.

The lowest concentrations of the studied metals in carp tissues, water, and sediments were found in the Matějovský reservoir. The highest concentrations of lead were found in the Pilská reservoir, as mentioned before.

CONCLUSION

Our results complete the long-term studies of heavy metals in Czech reservoirs and rivers which focused on the quality and safety of carp meat from important fishing localities in the Czech Republic, in particular reservoirs in the Bohemian-Moravian Highlands.

It can be summarised that the contents of cadmium, mercury, and lead in meat of the studied carps were in compliance with the maximum residue limits. So it is possible to come to the conclusion that common carp fished in these reservoirs (Pilská, Domaninský, and Matějovský reservoir) should not pose any health risk for human consumption.

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