Mercury Speciation and Safety of Fish from Important Fishing Locations in the Czech Republic

Renáta KENŠOVÁ, Kamila KRUŽÍKOVÁ and Zdeňka SVOBODOVÁ

Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

Abstract

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The aim of the study was to describe the distribution of total mercury in the tissues of fish originating from important Czech fishing locations and to determine the level of methylmercury as a percentage of total mercury (Hg speciation). At six important fishing locations a total of 144 fishes of 13 species were caught and analysed. Samples of muscle, liver, gonads and scales were analysed for total mercury and in the case of muscles for methylmercury concentrations. Total mercury was determined by AAS using an AMA 254 analyser, and methylmercury determination was performed by GC/ECD using a GC-2010A chromatograph. Total mercury concentrations in muscle and other tissues of all the fish from all fishing locations were below 0.5 mg/kg, with the exception of 6 asps (*Aspius aspius*). A significant ($P < 0.05$) correlation was found between the total mercury concentrations in scales and other tissues. Methylmercury made up about 46–100% of total mercury in muscle. The overall results confirmed the good hygienic quality of fish from important Czech fishing locations.

Keywords: total mercury; methylmercury; muscle; liver; gonads; scales; MeHg/THg ratio; fish consumption

The rising living standards of the human population take their toll in increasing loads of various contaminating substances in the environment that occur naturally at very low concentrations, or do not naturally occur at all. Besides metal manufacturing and processing, the sources of environmental contamination with metals are industrial waste products, the burning of fossil fuels, and the use of metals by consumers. The increase in metal concentrations in the environment is further enhanced by their poor degradability as a result of which metals accumulate in soil, water sediments and, last but not least, in the food chain (Svobodová *et al.* 1987). One of the most closely monitored potentially toxic metals is mercury. Mercury belongs to elements which attract a lot of attention from the aspect of the aquatic environment contamination. It is a global pollutant occurring in the environment that has a negative impact on living organisms. Its most toxic form, organic methylmercury (MeHg), is produced from inorganic mercury in aquatic sediments through the action of microorganisms (Eisler 2006). Methylmercury tends to accumulate in aquatic organisms and is transported along the food chain to its last link, i.e. the humans. The rate of mercury methylation depends not only on bacterial activity but also on total mercury concentrations in aquatic sediments

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(Hamasaki *et al.* 1995). The occurrence of mercury in the aquatic environment receives a lot of attention (Mason *et al.* 1995; Žlábek *et al.* 2005; Maršálek *et al.* 2006; Kružíková *et al.* 2008a). The majority of studies focus on the determination of total mercury concentrations, and information on mercury speciation is less abundant. Attention is drawn mainly to contaminated sites, but from the food safety aspect, it is desirable to focus in the near future on those fishing sites that anglers visit most frequently. Havelková *et al.* (2008) believed that mercury in fish from highly contaminated locations is deposited in the liver, and mercury in fish from less contaminated locations is preferentially deposited in muscle tissues, which negatively affects the safety of fish consumption. A comparison of measured concentrations and maximum total mercury (THg) limits does not, however, suffice for a realistic assessment of health hazards. It implies that the concentrations of the most toxic form of mercury, i.e. methylmercury (MeHg), should be taken into account. Comparing methylmercury concentrations found in muscle tissues of fish analysed against provisional tolerable weekly intake (PTWI) values for methylmercury set for humans by the WHO (1.6 μg/kg bodyweight per week) (WHO 1990), it is possible to determine the amounts of muscle tissues of fish from particular locations that can be eaten per week. The maximum levels of total mercury in fishery products and in certain selected fish species have been set at 0.5 and 1 mg/kg, respectively (Commission Regulation (EC) No. 1881/2006).

The aim of the present study was to assess mercury contamination in important fishing locations. With that purpose in mind, the distribution of total mercury in analysed fish tissues (muscle, liver, gonads, scales) was determined, the levels of methylmercury as a percentage of total mercury in muscle tissue (Hg speciation) were calculated, and the amounts of muscle tissue of fish from particular sites monitored that can be eaten from the food safety aspect were determined. The analysis of fish scales for THg was performed because scales are easy to collect and could possibly be used in mercury contamination assessments and subsequent fish safety assessments.

MATERIAL AND METHODS

The aim of the present study was to determine concentrations of total mercury (THg) and methylmercury (MeHg) in fish caught in some Czech fishing locations abundantly frequented by anglers (Figure 1).

Fish sampling. Both predatory and non-predatory fish species were caught using an electric generator at six important fishing locations (the Lužnice River – Soběslav; the Berounka River before the junction with the Vltava River; Jordán Water Reservoir, Trnávka Water Reservoir, the Otava River – Strakonice; the Otava – Sušice). A total of 144 fishes of 13 species were captured and analysed. The fish were weighed and samples of their tissues (muscle, liver, gonads, scales) were collected for

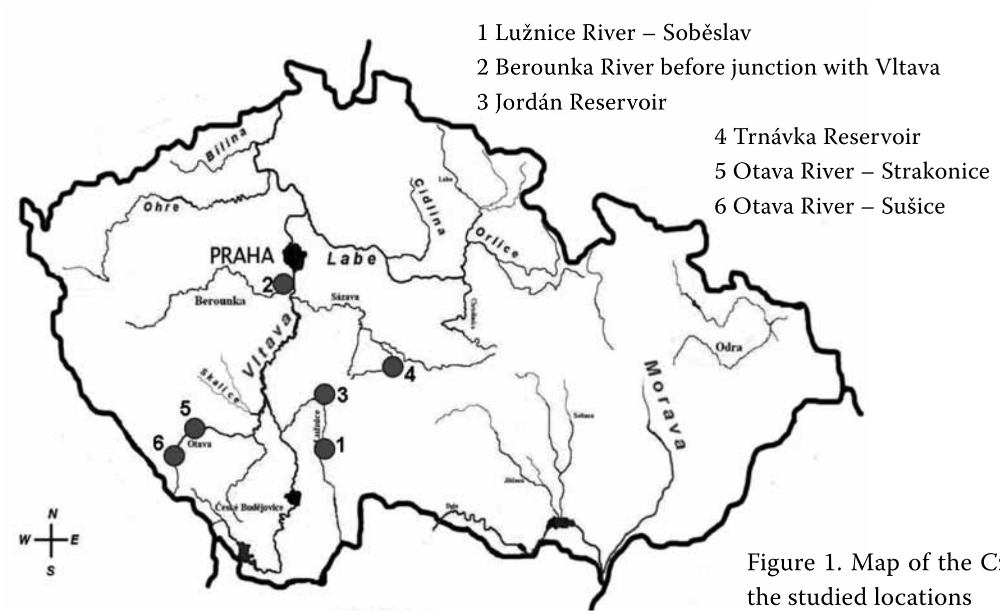

Figure 1. Map of the Czech Republic showing the studied locations

Table 1. Characteristics of the analysed fish and methylmercury (MeHg) levels as percentages of total mercury (THg)

Locations	Fish species	Taxonomic name	*n*	Weight (g) (mean ± SD)	Age (years) (min – max)	Ratio of MeHg/THg (%)
Lužnice River – Soběslav	chub	*Leuciscus cephalus*	5	778 ± 307	6–9	78
	pike	*Esox lucius*	5	1 449 ± 176	4–4	94
	bream	*Abramis brama*	5	982 ± 433	5–9	–
	roach	*Rutilus rutilus*	5	171 ± 51	4–5	90
	asp	*Aspius aspius*	4	1673 ± 813	4–6	94
	carp	*Cyprinus carpio*	3	1693 ± 246	3–4	88
Berounka River before the junction with Vltava	eel	*Anguilla anguilla*	3	582 ± 255	–	–
	pPike	*Esox lucius*	5	1572 ± 358	3–5	71
	bream	*Abramis brama*	5	1144 ± 305	6–9	99
	roach	*Rutilus rutilus*	5	174 ± 45	4–5	79
	asp	*Aspius aspius*	4	971 ± 358	3–5	97
	carp	*Cyprinus carpio*	4	1893 ± 165	3–4	97
Jordán Reservoir	eel	*Anguilla anguilla*	5	507 ± 154	–	77
	perch	*Perca fluviatilis*	4	268 ± 178	3–5	70
	bream	*Abramis brama*	5	483 ± 215	4–6	61
	roach	*Rutilus rutilus*	5	319 ± 67	4–5	53
	asp	*Aspius aspius*	5	808 ± 292	4–6	97
	carp	*Cyprinus carpio*	5	2573 ± 944	4–5	69
Trnávka Reservoir	carp	*Cyprinus carpio*	4	1543 ± 147	3–3	87
	roach	*Rutilus rutilus*	5	413 ± 121	3–5	74
	bream	*Abramis brama*	5	558 ± 115	4–6	46
Otava River – Strakonice	chub	*Leuciscus cephalus*	5	480 ± 363	4–9	96
	pike	*Esox lucius*	4	1109 ± 167	3–4	92
	bream	*Abramis brama*	5	741 ± 555	4–8	75
	roach	*Rutilus rutilus*	5	322 ± 116	3–6	99
	perch	*Perca fluviatilis*	5	133 ± 23	3–4	100
	tench	*Tinca tinca*	5	467 ± 220	3–4	97
Otava River – Sušice	brook trout	*Salvelinus fontinalis*	5	442 ± 48	3–4	95
	grayling	*Thymallus thymallus*	4	315 ± 41	4–5	99
	rainbow trout	*Oncorhynchus mykiss*	5	430 ± 110	2–4	99
	brown trout	*Salmo trutta*	5	224 ± 43	4–5	100

THg analysis and in the case of muscles also for MeHg analysis. Scales were used to determine the age of the fish. The characteristics of the analysed fish are given in Table 1.

Determination of THg and MeHg. Total mercury was determined by atomic absorption spectrophotometry using an AMA 254 analyser (Altec, Prague, Czech Republic) with 0.01 ng/kg detection limit. The MeHg analysis was performed by gas chromatography with ECD detection using a GC2010A chromatograph (Shimadzu Co., Kyoto, Japan) with a detection limit of 1 μg/kg and a limit of quantification of 21 μg/kg (Maršálek & Svobodová 2006).

Determination of maximum safe consumption of fish. Calculations of permitted consumption of fish tissue were based on the provisional tolerable weekly intake (PTWI) of MeHg for humans, i.e. 1.6 μg/kg bodyweight, and the results were subsequently converted to the number of servings (1 serving = 170 g muscle tissue).

Statistical analysis. Statistical analysis of the data was performed using the Statistica 8.0 for Windows program (StatSoft, Prague, Czech Republic). Values of mercury in fish tissues were tested for normal distribution by the Kolmogorov-Smirnov test and data were log-transformed to improve the homogeneity of variance. A one-way analysis of variance (ANOVA) was applied to differences in mercury content between sampling locations. Tukey's HSD test was used and $P < 0.05$ was chosen as the level of significance. The relationship between mercury content in different tissues was assessed using Spearman's correlation coefficient (r_s).

RESULTS AND DISCUSSION

Comparison of THg tissue concentrations in fish from monitored sites

THg concentrations in tissues of particular fish species caught at important fishing locations are shown in Figures 2–7. Only those fish that reached a catchable size were used in the study, and thus the age and weight of particular fish species from monitored locations were comparable (Table 1). The age and weight of fish are two important parameters affecting mercury concentrations in fish tissues (Burger & Gochfeld 2007). Mercury contamination levels in fishing locations can be compared using THg concentrations found there in non-predatory fish species, i.e. in bream and roach, which were captured in all of the locations with the exception of the Otava River – Sušice. Results of a comparison of THg muscle and scale concentrations among the particular locations are shown for bream and roach in Figures 8 and 9, respectively. The highest THg concentrations in these fish species were found in the Otava – Strakonice location.

At the same location, i.e. the Otava – Strakonice, the highest THg concentrations were also found in pike (*Esox lucius*) and in perch (*Perca fluviatilis*) representing predatory fish species. The town of Strakonice is an important centre of mechanical engineering and textile industries, which is probably also reflected in THg concentrations in muscle and other tissues of all the fish from all the six important fishing locations that were below 0.5 mg/kg. The only exception was THg concentrations in asp muscle from the Lužnice – Soběslav (2 asps) and from the Berounka (4 asps).

The THg concentrations found there are comparable with values found at some other locations in the Czech Republic, specifically in the basins of the Elbe River (the Elbe and its tributaries) (Svobodová & Hejtmánek 1978; Žlábek *et al.* 2005; Maršálek *et al.* 2006; Kružíková *et al.* 2008c) and of the Morava River (Svitava and Svratka Rivers, and Nové Mlýny Water Reservoirs) (Kružíková *et al.* 2008b, 2009a; Kenšová *et al.* 2010). The only exception was the Skalka Water Reservoir because it was polluted with mercury-containing waste water from a chemical factory for several decades. There, the THg concentrations in the muscle of predatory and non-predatory fish species were 1–3 mg/kg and about 0.9 mg/kg, respectively (Maršálek *et al.* 2005).

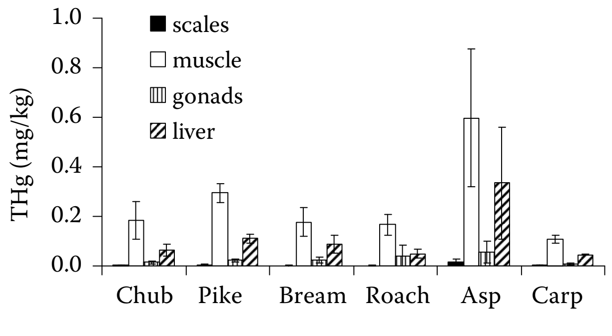

Figure 2. Total mercury (THg) concentrations in tissues of fish from the Lužnice – Soběslav location

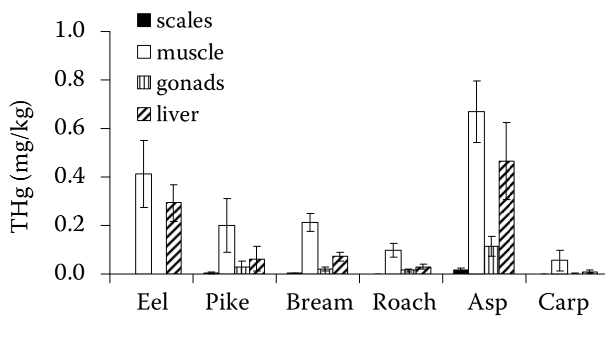

Figure 3. Total mercury (THg) concentrations in tissues of fish from the Berounka River before the junction with the Vltava

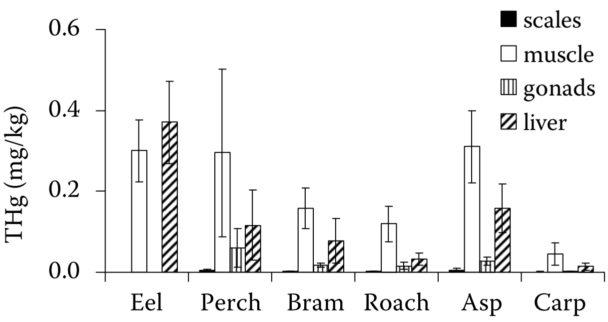

Figure 4. Total mercury (THg) concentrations in tissues of fish from the Jordán Reservoir

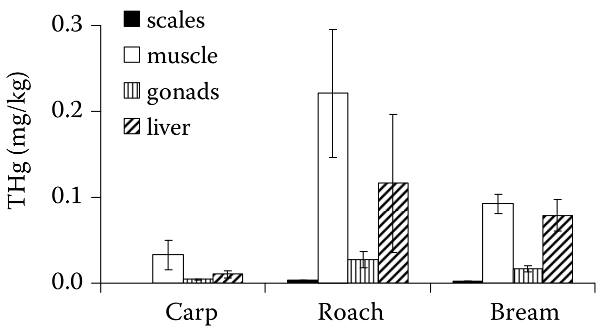

Figure 5. Total mercury (THg) concentrations in tissues of fish from the Trnávka Reservoir

Comparing THg concentrations in different fish species

Our findings regarding mercury concentrations in tissues of different fish species in fish from the studied locations correspond to data reported in the literature (Lacerda *et al.* 1994; Marrugo-Negrete *et al.* 2008). It means that the highest THg concentrations were found in the muscle of predatory fish species, i.e. of asp, eel, pike and perch (Figures 2–6). Among non-predatory fish species, the lowest concentrations were measured in carp and tench (Figures 2–6). THg concentrations in these fish species were below 0.1 mg/kg in most cases. They are the species that are stocked into rivers and reservoirs from uncontaminated fishponds at the age of two years or, sometimes, even later. The fish species at the Otava – Sušice location differed from those at the rest of the studied locations. Higher THg concentrations were found in brown trout and grayling compared with brook trout and rainbow trout (Figure 7). The latter two fish species are stocked into running waters from uncontaminated fish farms at the age of about 15–20 months.

Comparing THg concentrations among different fish tissues

THg concentrations decreased in particular fish tissues in the following order: muscle > liver > gonads > scales. The Otava – Sušice site and the salmonid fish captured there were an exception. The order of tissues according to their total mercury concentrations there was liver > muscle > gonads > scales. A similar trend was reported in the study by Svobodová *et al.* (1996) where, among others, mercury concentrations in rainbow trout from an intensive farming facility were monitored in muscle, liver, kidneys and soft row. According to Staniskiene *et al.* (2006), the distribution of metals in fish tissues depends on the fish species. The distribution of mercury in muscles and internal organs of fish depends, *inter alia*, on the degree of contamination of the monitored site. Havelková *et al.* (2008) demonstrated that in contaminated locations, total mercury concentrations in the liver were significantly higher compared with muscle tissue. On the other hand, in fish captured in uncontaminated locations, mercury concentrations in muscle tissues are usually higher than those

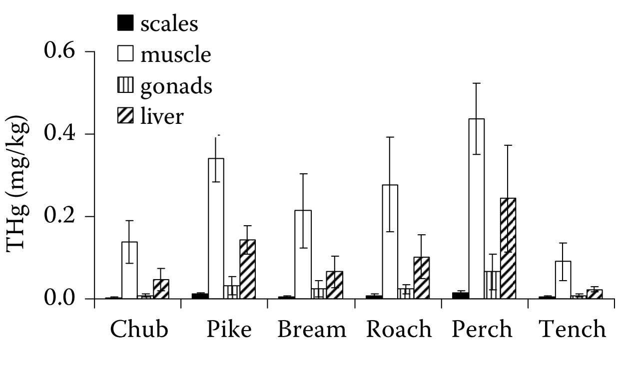

Figure 6. Total mercury (THg) concentrations in tissues of fish from the Otava – Strakonice location

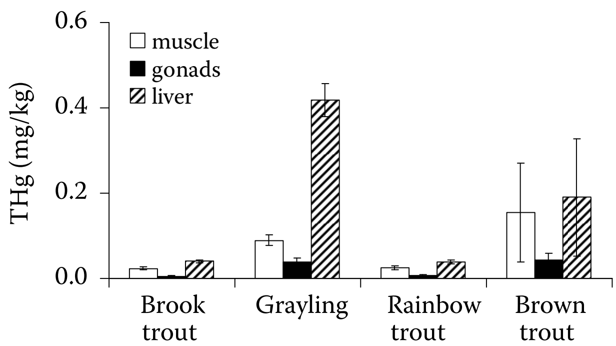

Figure 7. Total mercury (THg) concentrations in tissues of fish from the Otava – Sušice location

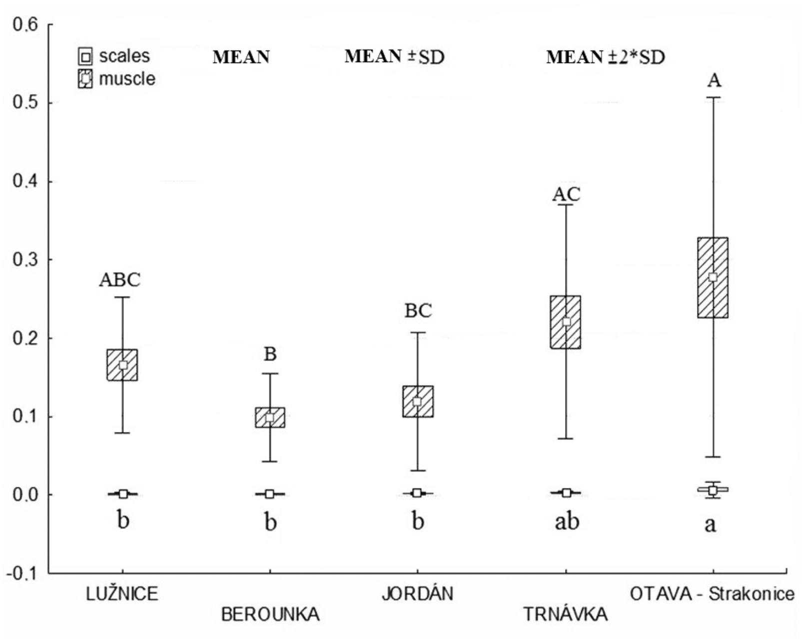

Figure 8. Total mercury (THg) concentrations in muscles and scales of bream from monitored sites

in the liver. In our study, the highest mercury concentrations in the analysed fish were found in muscle tissue, which may lead to a conclusion that the monitored fishing locations belong to uncontaminated locations.

THg concentrations in fish scales

Skin derivatives are used as indicators of contamination with certain toxic metals (arsenic, lead, mercury) (Morton *et al.* 2002; Kružíková *et al.* 2009b). For that reason, we also analysed fish scales for THg. Figure 2–6 clearly show that THg concentrations in the scales were very low. Nevertheless, Figures 8 and 9 show the relationship between THg concentrations in muscle and scales of bream and roach. In the next step, a correlation between tissue THg and scale THg concentrations was calculated using 116 samples of fish, i.e. fish irrespective of the species or location. A significant correlation ($P < 0.05$) was determined between THg concentrations in the scales and muscle tissues ($r_s = 0.64$), between THg concentrations in the scales and liver ($r = 0.69$), and between THg concentrations in the scales and

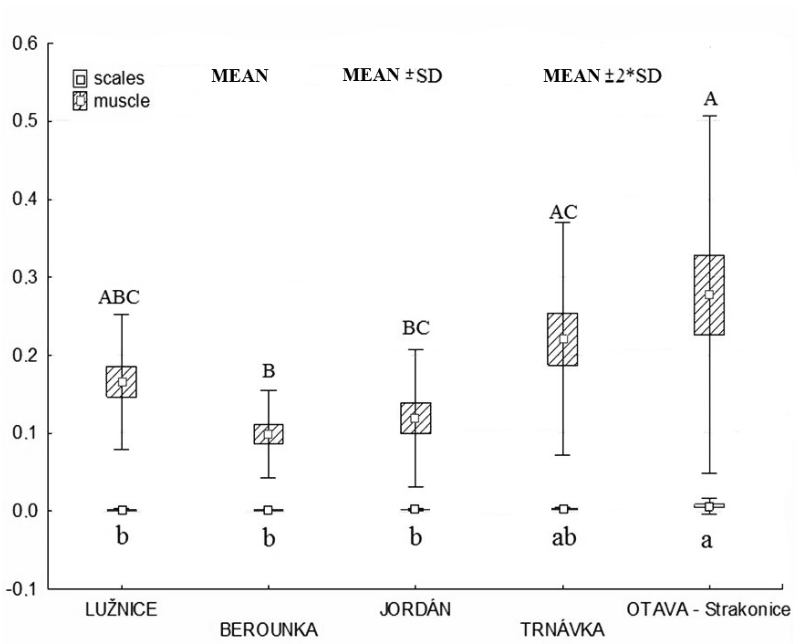

Figure 9. Total mercury (THg) concentrations in muscles and scales of roach

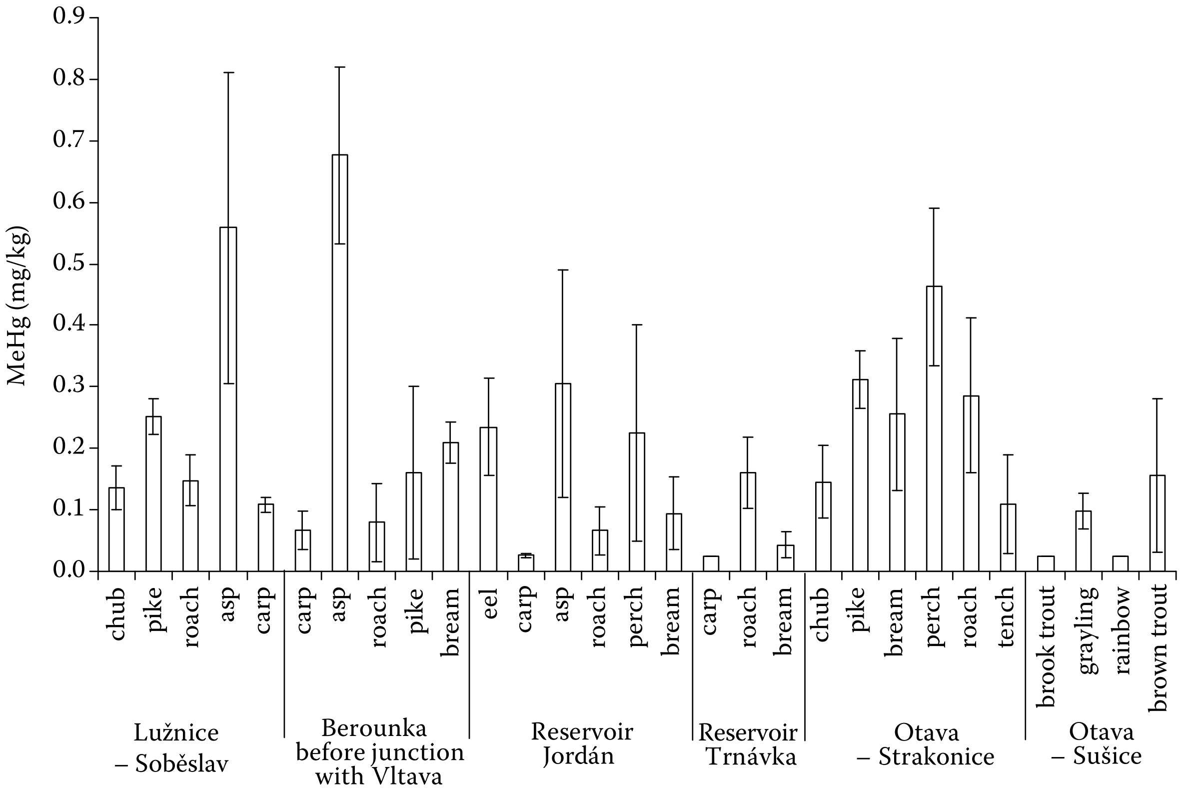

Figure 10. Methylmercury (MeHg) concentrations in muscles of fish from monitored sites

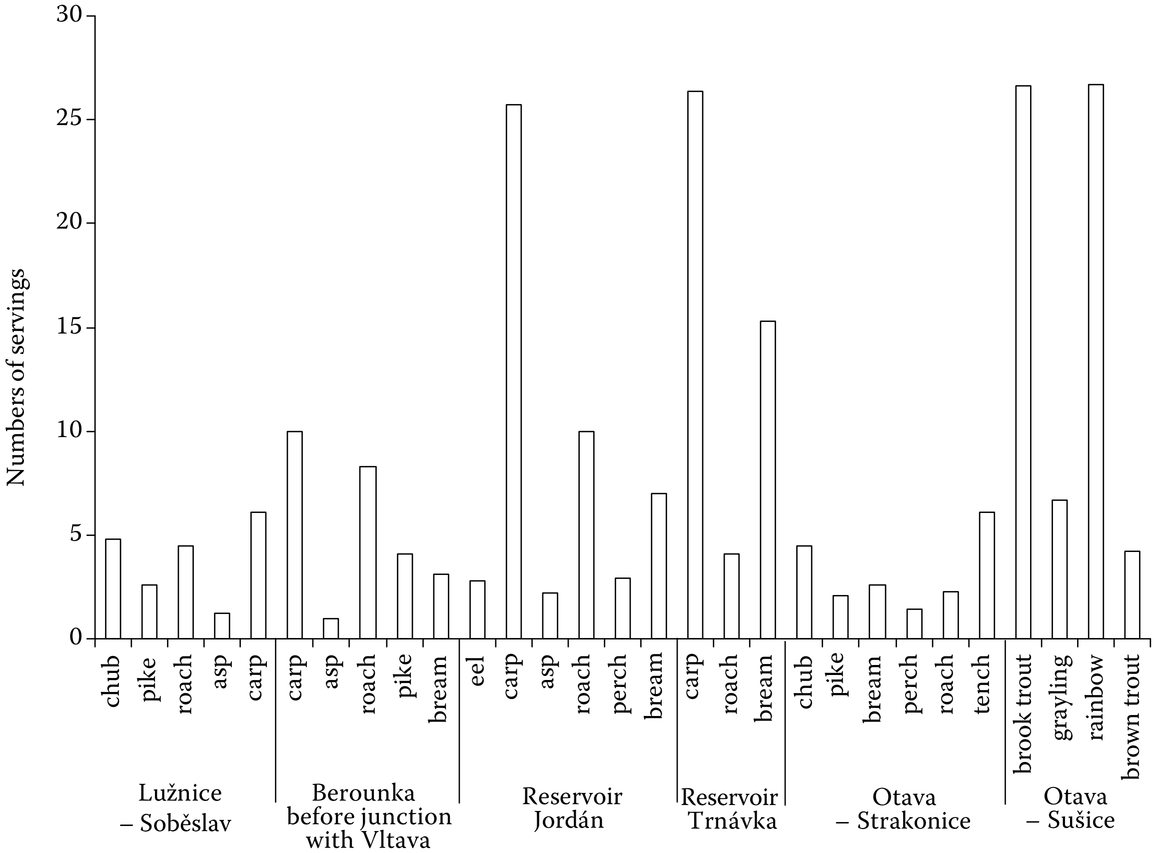

Figure 11. Numbers of servings of the muscle of fish from the studied locations that can be eaten per person per week

gonads (r_s = 0.60). This relationship can be used in mercury contamination assessments of different locations because it eliminates the need to analyse THg concentrations in fish muscle, and scales can be used instead. However, this correlation will need to be further confirmed by an analysis from any highly contaminated environment, such as the Skalka Water Reservoir (Maršálek *et al.* 2005).

Comparison of MeHg concentrations in muscles of fish from monitored sites

The predominant type of mercury occurring in muscle tissues is methylmercury, i.e. the organic type of mercury. MeHg concentrations were measured only in muscle tissues. Methylmercury made up about 46–100% of THg (Table 1). MeHg concentrations found in particular fish species at the monitored locations are shown in Figure 10. Their values practically copy total mercury concentration data. The highest values were found in predatory fish species (asp, perch, pike, eel), and the lowest in fish stocked into fishing locations from fishponds or fish farming facilities (carp, brook trout, rainbow trout).

Determination of maximum safe consumption of fish

One of the aims of the study was to determine quantitative limits for the weekly consumption of muscle of particular species of fish from different fisheries. In the calculation, we used MeHg muscle concentrations in fish from the studied locations and the PTWI value for humans (1.6 µg/kg bodyweight). Resulting values were converted to the number of servings of the muscle of fish species from a specific location that can be eaten within a week (Figure 11).

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Corresponding author:

Mgr. Renáta Kenšová, Veterinární a Farmaceutická Universita Brno, Fakulta veterinární hygieny a ekologie, Ústav veřejného veterinarního lékařství a toxikologie, Palackého 1–3, 612 42 Brno, Česká republika
tel. + 420 541 562 780, e-mail: r.kensova@seznam.cz